The reward system and binge eating disorder.

Thèse

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Résumé

Le trouble de la frénésie alimentaire (TFA) se caractérise par une frénésie et un état compulsif, c'est le désordre alimentaire le plus fréquent et la prévalence est plus élevée chez les femmes que chez les hommes, mais son mécanisme est mal connu. L'imagerie par résonance magnétique (IRM) fonctionnelle chez l'humain a fait avancer notre compréhension des régions cérébrales impliquées dans ces TFA. Cependant, nous manquons encore d'informations en ce qui concerne les sous-types de neurones qui jouent un rôle dans cette maladie. Bien que certaines études ont constaté une augmentation de l'activité dans les régions cérébrales en lien avec le traitement de la récompense suite à l'attente ou à la réception d'un stimulus de récompense, d'autres études ont découvert une diminution de l'activité de ces régions par rapport aux sujets contrôles et donc ces études mènent à des résultats mitigés concernant l'activité de ces régions cérébrales pendant les TFA, même s'il est évident que les TFA sont associés à des altérations du système de récompense. Les patients qui ont une frénésie expriment souvent une sensation de soulagement pendant les épisodes de frénésie. Cependant, ce sentiment de soulagement est temporaire et l'état du patient avant la frénésie est rétabli après la frénésie, préparant le patient pour la prochaine frénésie. Les mécanismes concernant cette sensation de soulagement ne sont pas bien étudiés.

En utilisant l'expression de deltaFosB (ΔFosB), un marqueur de stimulation neuronale chronique, nous avons découvert chez les rates (femelles) présentant un comportement ressemblant à la frénésie alimentaire, une diminution de l'activité dans les régions liées au système de récompense, plus particulièrement dans le cortex préfrontal médial (mPFC), le noyau accumbens (Acb), et l'aire tegmentale ventrale (VTA). Ceci suggère que ΔFosB pourrait être responsable de la désensibilisation observée dans ces régions chez certains patients atteints de TFA. De plus, nous avons constaté que la proportion des sous-types de neurones dans ces régions joue un rôle important dans les TFA, car malgré une proportion comparable des sous-types neuronaux exprimant le ΔFosB dans les régions du mPFC et du noyau Acb chez les rats sujets à la frénésie alimentaire (BEP; de l'anglais binge-eating prone) et chez les rats résistants à la frénésie alimentaire

(BER, de l'anglais binge-eating resistant), la proportion des neurones GABAergiques exprimant ΔFosB était plus élevée dans la région VTA chez les rats BER que les rats BEP. Ceci suggère un rôle crucial de la VTA dans les TFA. En étudiant en temps réel l'activité des neurones, nous avons observé dans les régions du mPFC et de la VTA (mais pas dans le noyau Acb) des taux de décharges plus élevés avant et pendant la consommation de sucrose chez les rats BER que chez les rats BEP. De plus, nous avons constaté qu'une proportion plus importante de neurone était excitée et une faible proportion était inhibée au début de la consommation de sucrose chez les rats BER en comparaison au rats BEP. Cela est cohérent avec nos résultats ΔFosB et suggère que les patients avec des TFA pourraient se gaver pour réactiver un système de récompense hypofonctionnel. Par ailleurs, nous avons constaté que le taux de décharges des neurones du mPFC, Acb, et VTA était augmenté pendant et entre les groupes de coups de langues (licking clusters) chez les rats BEP. Ceci pourrait être sous-jacent à la sensation de soulagement chez les patients avec TFA pendant la consommation d'aliments appétents puisque qu'il a été montré que la stimulation de ces régions de façon individuelle a un effet antidépresseur et la dépression est une comorbidité des TFA chez l'homme et les animaux. Nous avons également constaté que le potentiel lié à l'événement chez les rats BEP était d'amplitude plus faible dans toutes les structures étudiées; et dans le mPFC, il était largement retardé par rapport aux animaux BER. Cette étude suggère que l'activité réduite des neurones dans le mPFC, l'Acb et le VTA sous-tend les TFA. D'autres études visant à l'activation ciblée de ces structures pourraient fournir un traitement potentiel pour ce trouble.

Abstract

Binge eating disorder (BED), characterized by bingeing and compulsivity, is the most prevalent eating disorder and is also more prevalent in females than males, yet its underlying mechanisms are poorly understood. Human functional magnetic resonance imaging (fMRI) studies have improved our understanding of the brain regions implicated in BED. However, we still lack information on the neuronal subtypes involved in BED. Moreover, while some of these studies found an increase in activity in brain reward processing regions following the expectation or reception of a rewarding stimulus, others found a decrease in activity in these regions compared to healthy controls, and this has yielded mixed results on the activity of brain regions during BED, even though it is clear that BED is associated with alterations in the reward system. Patients who binge often express a sense of relief during the binge episode. However, this sense of relief is temporary, and the state of the patient prior to the binge is restored after the binge, preparing the patient for the next binge. The mechanisms underlying this sense of relief has not been well investigated.

Using the expression of deltaFosB (ΔFosB), a marker for chronic neuronal stimulation, we found a decrease in activity in regions in the reward system in female binge-like eating rats, specifically the medial prefrontal cortex (mPFC), nucleus accumbens (Acb), and ventral tegmental area (VTA). This suggests that ΔFosB might be responsible for the desensitization observed in these regions in some BED patients. Additionally, we found that the proportions of neuron subtypes in these regions play a significant role in binge-like eating, since, even though the proportions of neuron subtypes expressing ΔFosB were similar in the mPFC and Acb of binge-like eating (BEP) and nonbinge-like eating (BER) rats, the proportions were different in the VTA, where the proportion of GABAergic ΔFosB-expressing neurons was higher in BER than BEP rats. This suggests that the VTA may play a crucial role in BED. By investigating real-time activity of neurons, we observed that the firing rate of identified mPFC and VTA, but not Acb, neurons in BER rats was higher both before and during sucrose consumption than in BEP rats. Additionally, we found that

a greater proportion of neurons were excited and a lower proportion were inhibited at the start of sucrose consumption in BER rats compared to BEP rats. This is consistent with our ΔFosB results and this suggests that BED patients may binge to reactivate a hypofunctioning reward system. Additionally, we found that the firing rate of mPFC, Acb, and VTA neurons increased in BEP rats during and between lick clusters. This may underlie the feeling of relief in BED patients during the consumption of palatable food since stimulating these regions individually has been shown to produce an antidepressant effect, and depression is a comorbidity of BED in humans and animals. We also found that the event-related potential in BEP rats was of lower amplitude in all investigated structures, and in the mPFC it was largely delayed as compared to BER animals. This study suggests that reduced activity of neurons in the mPFC, Acb, and VTA underlies BED. Further studies aiming at the targeted activation of these structures may provide potential treatment for this disorder.

Table of contents

List of figures

Introduction

Chapter 1

Chapter 2

Conclusion

List of abbreviations

Acb: Nucleus accumbens
AcbC: Nucleus accumbens core
AcbSh: Nucleus accumbens shell
ACTH: Adrenocorticotropic hormone
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AN: Anorexia nervosa
BED: Binge eating disorder
BEP: Binge eating prone
BER: Binge eating resistant
BN: Bulimia nervosa
BNST: Bed nucleus of the stria terminalis
cAMP: Cyclic adenosine monophosphate
CE: Lick cluster end
Cl: Cluster
CREB: cAMP response element-binding protein
CRF: Corticotropin-releasing factor
CRFR1: Corticotropin-releasing factor receptor 1
CRFR2: Corticotropin-releasing factor receptor 2
CS: Lick cluster start
DSM-5: Diagnostic and Statistical Manual of Mental Disorders fifth edition
ED: Eating disorder
EDs: Eating disorders
fMRI: Functional magnetic resonance imaging
GABA: Gamma-aminobutyric acid
GABAergic: Gamma-aminobutyric acidergic
GAD: Glutamate decarboxylase
GAD67: Glutamate decarboxylase 67
GRs: Glucocorticoid receptors
HPA axis: Hypothalamic-pituitary-adrenal axis
IC: Insular cortex
ICI: Intercluster interval
ICI firing rate: Intercluster interval firing rate
IL: Infralimbic cortex
LC: Locus coeruleus
LCE: Lick cluster excited
LCI: Lick cluster inhibited
LCN: Lick cluster nonresponsive
mPFC: Medial prefrontal cortex
MRs: Mineralocorticoid receptors
MSN: Medium spiny neuron
NMDA: N-methyl-D-aspartate
NS: Non-stress session
NTS: Nucleus of the solitary tract
PBN: Parabrachial nucleus

PBNl: Parabrachial nucleus, lateral
PBNm: Parabrachial nucleus, medial
PFC: Prefrontal cortex
PrL: Prelimbic cortex
PVN: Paraventricular nucleus of the hypothalamus
PVNm: Paraventricular nucleus of the hypothalamus, magnocellular
PVNp: Paraventricular nucleus of the hypothalamus, parvocellular
S: Stress session
Th: Thalamus
TH: Tyrosine hydroxylase
TRC: Taste receptor cells
VTA: Ventral tegmental area
ΔFosB: DeltaFosB

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Preface

This thesis is divided into five main chapters.

Introduction

The first chapter is a thorough literature review of the most important topics needed to fully understand the scope of my PhD research and the studies described in this thesis.

Chapters 1 and 2 are submitted manuscripts.

Chapter 1:

Richard Quansah Amissah, Sandrine Chometton, Juliane Calvez, Geneviève Guevrémont, Elena Timofeeva, Igor Timofeev. Differential expression of deltaFosB in reward processing regions between binge eating prone and resistant female rats. (PLoS One; submitted on 25th October, 2019; revision requested).
In this paper, RQA performed the experiments, acquired the data, performed data analysis, and wrote and edited the manuscript. SC supervised the experiments, wrote and edited the manuscript, and performed data analysis. JC performed part of the experiment and performed data analysis. GG performed part of the experiment and performed data analysis. ET acquired the grant for the experiment, conceptualized the experiment, and supervised the experiment. IT acquired the grant for the experiment, conceptualized the experiment, supervised the experiment, and wrote and edited the manuscript.

Chapter 2:

Richard Quansah Amissah, Diellor Basha, Olga Bukhtiyarova, Igor Timofeev. Neuronal activities during palatable food consumption in the reward system of binge-like eating female rats. (Physiology and Behavior)
In this paper, RQA performed the experiments, acquired the data, performed data analysis, and wrote and edited the manuscript. DB performed data analysis, and wrote and edited the manuscript. OB performed data analysis. IT acquired the grant

for the experiment, conceptualized the experiment, supervised the experiment, and wrote and edited the manuscript.

Conclusion:

This chapter discusses and summarizes the findings in chapters 1 and 2 of the thesis and some limitations of the presented studies.

Introduction

1.1 Eating Disorders

Eating disorders (EDs) are psychological disorders which cause severe disturbances to our eating habits. EDs are a major public health concern and have been reported to affect about 8.4% of women and 2.2% of men worldwide (Galmiche et al., 2019). The main types are Anorexia Nervosa (AN), Bulimia Nervosa (BN), and Binge Eating Disorder (BED). AN is characterized by very low bodyweight, an intense fear of weight gain, and the fear of fat resulting in extreme food restriction and weight loss (Mayer et al., 2012). While BN and BED both involve the consumption of large quantities of food, BN involves compensatory behaviors like the abuse of laxatives, self-induced vomiting, fasting, and excessive exercising, while BED does not (Dingemans et al., 2002). Other types of EDs exist and are referred to as Other Specified Feeding or Eating Disorders (OSFEDs). These are EDs that do not meet all the criteria for each of the main types of EDs but result in significant impairment to social life (Galmiche et al., 2019).

EDs are associated with impairments in psychological and physical functioning and quality of life (Schmidt, 2003). These EDs were previously thought to be “culture bound syndromes,” mainly associated with developed countries where highly palatable and high caloric foods are cheap and ubiquitous. However, they are now being observed across ethnicities in underdeveloped and developing countries due to the adoption of Western standards of attractiveness and eating habits associated with body sizes considered attractive (King, 1993).

EDs occur due to factors which may be psychological, neurobiological, societal, and interpersonal (Hetherington, 2000). Some psychological factors which may lead to EDs include depression, anger, and isolation, while disturbances in the neurotransmitter and neuroendocrine systems are some examples of the neurobiological factors which contribute to the development of EDs (Collier, 2004). Other factors that may contribute to the development of BED include the female

gender and genetic factors. In fact, in a case control study in a family in Boston, BED heritability was estimated to range between 30 and 77% (Javaras et al., 2008).

A study conducted from 2001 to 2003 among adults reported a lifetime prevalence rate of 0.6% for AN (0.9% of women and 0.3% of men), 1% for BN (1.5% of women and 0.5% of men), and 3% for BED (3.5% of women and 2.0% of men) (Hudson et al., 2007), which suggests that females are more prone to EDs than males, even though others report that this might be because females seek help more often than males (Dingemans et al., 2002), and that BED is the most prevalent eating disorder (Kessler et al., 2013). Despite this high prevalence of BED, its underlying pathophysiology is poorly understood (Schienle et al., 2009; Sinclair et al., 2015).

1.1.1 Binge Eating Disorder: What, Why, When, and How?

The first description of binge eating disorder (BED) was made by Stunkard (1959). It is characterized by the consumption of a large amount of food than would normally be consumed in a discrete amount of time and a loss of sense of control during the bingeing episode (American Psychiatric Association, 2013). This is different from overeating, which occurs in response to hunger and involves the consumption of large amounts of food, which may include palatable foods. Moreover, feelings of shame, guilt, anxiety, and disgust often accompany BED (Hutson et al., 2018).

BED typically occurs during adolescence (Swanson et al., 2011) but can also occur in early adulthood (Hudson et al., 2007) and among the elderly (Guerdjikova et al., 2012). Although a significant number of people with BED have normal weight, the disorder has been associated with obesity (Fairburn et al., 2000; Munsch and Herpertz, 2011). During bingeing episodes, foods which contain high sugar and fat content are consumed (Zellner et al., 2006), even when the individual is sated, which suggests that it is driven by hedonic, and not metabolic, factors (Smail-Crevier et al., 2018). Generally, even though binge eating exists on a continuum among the population, some people may be more prone to binge than others (Klump et al., 2011). Binge-like eating is usually triggered by some form of stressful event (Wolff et al., 2000; Pendleton et al., 2001; Hilbert et al., 2011) or negative affect (Womble

et al., 2001). In fact, people who binge often exhibit a sense of relief during the binge episode, since bingeing temporarily reduces any negative emotions they might have been feeling prior to the episode (Heatherton and Baumeister, 1991; Stickney and Miltenberger, 1999). This confirms the affect regulation model of binge eating which posits that binge eating is triggered by negative emotions and, therefore, individuals binge to mitigate these negative emotions (Hawkins and F. Clement, 1984). However, following the binge episode, the level of negative affect increases, restoring the individual to his previous state prior to the binge, priming the individual for the next binge episode (Deaver et al., 2003).

1.1.2 Neurobiological basis of BED

Food intake is controlled by two main circuits in the brain: the homeostatic brain circuit and the hedonic brain circuit (reward system). The homeostatic circuit is generally activated when the energy stores in the body are low, while the reward system is activated as a result of the rewarding aspects of food (Berthoud, 2012). Considering that BED involves the consumption of palatable food in the absence of hunger, the system of interest during BED is likely to be the reward processing system in the brain (Schwartz et al., 2000; Bake et al., 2013; Wierenga et al., 2014). Additionally, BED is usually triggered by some form of stressful event, therefore, the stress processing system may be implicated (Wolff et al., 2000; Pendleton et al., 2001; Hilbert et al., 2011). Moreover, BED involves a loss of control during bingeing (American Psychiatric Association, 2013), which suggests the involvement of the inhibitory control system of the brain. Therefore, understanding how these systems work in healthy individuals may help to shed more light on malfunctions in these systems during BED.

In human BED patients, a number of regions in the brain have been identified to exhibit abnormal activity. A number of studies, which involved the use of functional magnetic resonance imaging (fMRI), have reported that BED patients, compared to controls, exhibit an elevated brain activity in response to images of palatable foods (Bodell et al., 2018). The study by Simon et al. (2016) showed that in patients with bulimic-type eating disorder (BED and BN patients), there is a reduction in activity in

the posterior cingulate cortex when food reward was expected and an increase in activity in the medial prefrontal and orbitofrontal cortices upon the reception of food rewards. Similar increases in brain activity were reported by Schienle et al. (2009) in the same regions. The study by Filbey et al. (2012) also showed an increase in activity in the orbitofrontal cortex, insula, and striatal regions, in compulsive overeaters, in response to the receipt of a food reward, compared to healthy controls. Another neuroimaging study found a decrease in activity in regions including the insula, lateral orbitofrontal cortex, thalamus, precentral gyrus, and middle frontal gyrus in individuals with binge-type EDs upon the reception of a food reward (Frank et al., 2011). Lee et al. (2017) reported an increase in ventral striatum activity in BED patients compared to healthy controls in response to images of food. In studies involving non-food rewards such as monetary rewards, while the study by Balodis et al. (2013) and Balodis et al. (2014) showed decreased activity in the prefrontal and insular cortices and the ventral striatum in BED patients in response to a monetary reward compared to healthy patients, the study by Simon et al. (2016) reported no differences in activity in these regions, in BED patients compared to controls, upon the anticipation or receipt of a monetary reward. Additionally, Balodis et al. (2013a) reported that there was a decreased activity in the orbitofrontal and the insular cortices in BED patients in response to outcome processing during a monetary incentive delay task. One study involving single-photon emission computed tomography reported increased regional cerebral blood flow in the left frontal and prefrontal cortices in BED patients in the presence of real food following overnight fasting (Karhunen et al., 2000).

To summarize, while the studies of Karhunen et al. (2000), Filbey et al. (2012), and Lee et al. (2017) support increased activity in the reward system in BED patients compared to controls during the anticipation or expectation of a reward, the studies of Balodis et al. (2013), Balodis et al. (2014), and Reiter et al. (2017) support decreased activity in the reward system during reward expectation and anticipation. These mixed findings regarding the activity of the reward system during BED may be attributable to a number of factors including the sex of the participants recruited for the studies, since while the study by Filbey et al. (2012) involved only males, the

study by Karhunen et al. (2000) involved females, while that by Reiter et al. (2017) involved both males and females. Another possible source of discrepancy may be the sample of patients used for the studies. Among the three aforementioned studies, while the study by Reiter et al. (2017) involved BED patients, the study by Filbey et al. (2012) involved patients with high body mass indices, while that by Karhunen et al. (2000) involved obese patients with and without BED. Taken together, the aforementioned studies agree that there is an alteration in activity in these regions, specifically regions involved in reward processing during BED. However, whether these alterations are a result of increased or decreased activity in these regions is still unclear.

1.1.3 Rat as a model for BED

As with most neuropsychiatric diseases, in order to understand the underlying mechanisms which initiate and maintain BED, animal models are employed. The ideal animal model must meet all the criteria specified in the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM-5) for the diagnosis of BED. The criteria are as follows:

1. Recurring episodes of binge eating characterized by the consumption of a large amount of food than would normally be consumed in a discrete amount of time and by a sense of lack of control during the bingeing episode
2. The binge eating episode must be associated with at least three of the following characteristics:
    a. Eating more rapidly than normal
    b. Eating until one feels uncomfortably full
    c. Eating large amounts of food even when not feeling physically hungry
    d. Eating alone due to the embarrassment one feels as a result of the amount of food consumed
    e. The feeling of disgust, depression, and guilt
3. The presence of marked distress associated with bingeing
4. Bingeing occurs at least once a week over a period of three months

5. No compensatory methods are used following the binge episodes (American Psychiatric Association, 2013)

Currently, no animal model of BED exists which fulfills all the criteria listed in the DSM-5 (Corwin and Buda-Levin, 2004). Therefore, well-characterized models of BED are still needed to advance our understanding of the neurobiological underpinnings of BED. However, several animal models of BED meet quite a number of these criteria. As mentioned earlier, BED does not necessarily occur in response to hunger, therefore rodent models which involve food restriction may not be ideal for studying BED, even though they fulfill the criteria of eating a large amount of food than would normally be consumed in a discrete amount of time. These models include the Boggiano model (Boggiano et al., 2007), the Bello model (Bello et al., 2009), and the Hoebel model (Colantuoni et al., 2002). Another advantage of the Boggiano model over other models is that rats are subjected to stress, which is similar to the situation in humans. The Corwin model (Corwin et al., 1998) only involves the use of intermittent access to palatable food to generate the binge eating phenotype in rats. This model is relevant because people with BED usually binge on palatable foods and binge when not feeling physically hungry. Another rat model of BED developed by Boggiano (Boggiano et al., 2007) involved the separation of rats into binge eating prone and binge eating resistant rats based on the amount of palatable food they consumed within a specific amount of time. The binge eating prone rats in this model fulfilled the criteria of the consumption of a large amount of food and a loss of sense of control since these rats endured foot-shock stress in order to obtain palatable food.

In our laboratory, we developed a rat model of binge-like eating using intermittent access to 10% sucrose solution and foot-shock stress. The protocol for developing our rat model of binge-like eating has been described elsewhere (Calvez and Timofeeva, 2016) as shown in Figure 1-1. In brief, naïve adolescent female rats were first exposed to sucrose solution for 24 hours one week after their arrival. They were then given intermittent access to the 10% sucrose solution until their intake of the sucrose solution stabilized. After stabilization, they were subjected to foot-shock

stress three times, with each session of foot-shock stress followed by one-hour access to the sucrose solution. Since our rat model of binge-like eating is a stress-induced binge-like eating model, only the consumption of sucrose by the rats following foot-shock stress was used in the classification of rats as either binge eating prone or resistant. Following the stress sessions, rats were grouped into three tertiles (upper tertile, middle tertile, and lower tertile) according to their consumption of sucrose. Rats that appeared at least two times in the upper tertile but never in the lower tertile were referred to as the binge eating prone (BEP) rats, while those that appeared at least two times in the lower tertile but never in the upper tertile were referred to as binge eating resistant (BER) rats. Our model replicates the finding that, among humans, stress could either result in an increase or a decrease in food intake (WIllenbring et al., 1986).

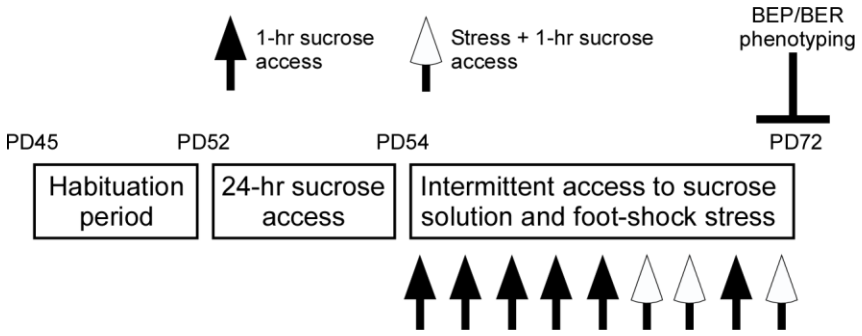

Figure 1 - 1 Protocol for developing our binge-like eating rat model.

Rats arrived on postnatal day (PD) 45. They were allowed one week to habituate to housing conditions and were subsequently given 24-hr access to 10% sucrose solution on PD52. They were given five 1-hr intermittent accesses to 10% sucrose solution starting on PD54. The rats then underwent foot-shock stress followed by 1-hr access to 10% sucrose solution. Rats were then separated into binge eating prone (BEP) and binge eating resistant (BER) rats based on their sucrose consumption after each foot-shock stress. Filled arrows: 1-hour sucrose access, open arrows: foot-shock stress followed by 1-hr access to sucrose solution.

Our BER/BEP model has high face validity for BED as described in humans, since it models several key features of BED. First, BEP rats consistently consumed large amounts of sucrose solution within a period of one hour, which is similar to the

consumption of a large amount of food within a discrete amount of time described in human BED patients. Second, BEP rats were given access to palatable food during both light and dark cycles, and they consumed similar amounts of sucrose solution. This fulfills the criteria of eating when not feeling physically hungry, since rats are nocturnal, and therefore, they feed during the dark cycle and usually sleep during the light cycle. Therefore, consuming a similar amount of sucrose in the light phase suggests bingeing even when the rats were not hungry. Third, human BED patients usually eat more rapidly during the binge episode. This was replicated in our BEP rats through the increase in licking rate/frequency during the consumption of sucrose solution following stress. Finally, BEP rats were compulsive, in that, they displayed a sense of loss of control in the modified light/dark box test. In the modified light/dark box test, BEP rats were not deterred by the aversive light but continued to consume sucrose, while the BER rats remained in the dark zone of the modified light/dark box (Calvez and Timofeeva, 2016).

1.2 The reward system

A reward is any stimulus that has the ability to produce a pleasurable effect, induce the motivation to acquire it, and lead to the learning of associations between the behaviors and cues necessary to obtain it (Berridge and Robinson, 2003). Therefore, by extension, the reward system is the system in the brain responsible for processing all these different aspects of a reward. The reward system comprises of several brain structures but the most important are the main structures that make up the mesocorticolimbic dopamine system such as the ventral tegmental area (VTA), nucleus accumbens (Acb), and prefrontal cortex (PFC) (Kelley and Berridge, 2002; Wise, 2005), as shown in Figure 1-2.

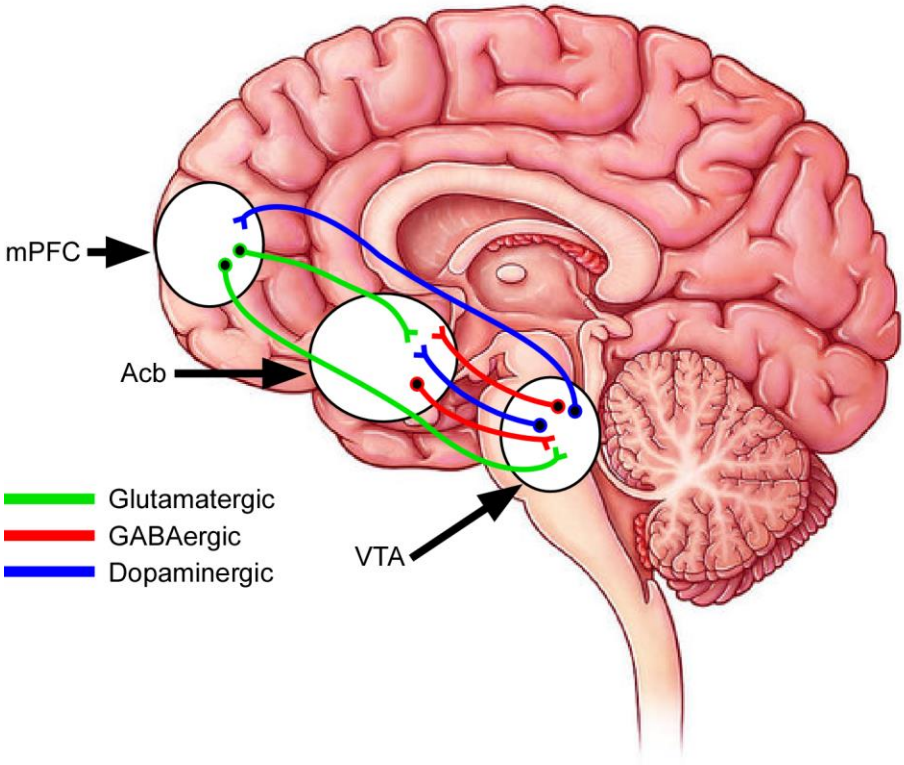

Figure 1 - 2 The mesocorticolimbic dopamine system.

Within the mesocorticolimbic dopamine system, the VTA sends projections to both the PFC and Acb. In addition, the PFC projects to the Acb and VTA, while the Acb projects to the VTA but is targeted by neurons in the PFC. VTA: Ventral tegmental area, Acb: Nucleus accumbens, PFC: Prefrontal cortex, Glutamatergic: Projections of glutamatergic neurons, GABAergic: Projections of GABAergic neurons, Dopaminergic: Projections of dopaminergic neurons. Adapted from http://biobunch.blogspot.com/2016/03/biology-behind-addiction.html.

In the mesocorticolimbic dopamine system, the VTA projects predominantly, through the medial forebrain bundle, to the Acb and PFC, where the dopamine it releases interacts with dopamine receptors located on neurons (Volkow and Morales, 2015).

1.2.1 The Ventral Tegmental Area (VTA)

The VTA is a midbrain structure located close to the midline and is the main source of dopamine in the mesocorticolimbic system. The VTA has been implicated in

several functions including incentive salience, decision making, working memory, positive and negative reinforcement, stimulus salience, and aversion (Adcock et al., 2006; Brischoux et al., 2009; Bromberg-Martin et al., 2010).

1.2.1.1 Neuron types in the VTA

The VTA comprises of three main neuronal populations namely dopaminergic, which form the majority, followed by gamma aminobutyric acidergic (GABAergic) and glutamatergic neurons (Nair-Roberts et al., 2008). However, neurons which respond to opioid and serotonin receptor agonists were also identified in the VTA (Cameron et al., 1997). The VTA also contains dopaminergic neurons that co-release glutamate (Oliva and Wanat, 2016).

In the VTA, several subtypes of dopaminergic and GABAergic neurons exist based on the neurotransmitters they express. These neuron subtypes have also been shown to coexpress cholecystokinin and parvalbumin (Hokfelt et al., 1980; Olson and Nestler, 2007). Additionally, even though majority of the neurons identified to express the calcium-binding proteins calbindin and calretinin are dopaminergic neurons, a few GABAergic neurons that also express these proteins were observed (Olson and Nestler, 2007).

1.2.1.2 Morphological properties of VTA neurons

VTA dopaminergic neurons with four morphologically different shapes have been identified, even though none of these shapes were exclusive to a particular cell subtype. They are the fusiform, round, multipolar, and elliptical dopaminergic neurons (Margolis et al., 2006). The fusiform-shaped dopaminergic neurons had an elliptical soma with two opposing dendrites along the major axis. The elliptical-shaped dopaminergic neurons were similar to the fusiform-shaped dopaminergic neurons. However, the dendrites of elliptical-shaped dopaminergic neurons are numerous (Margolis et al., 2006).

GABAergic VTA neurons are characteristically multipolar, with at least two dendrites noticeable in a single plane of a section. These neurons are targeted by neurons

which form either symmetric or asymmetric synapses with them (Steffensen et al., 1998).

1.2.1.3 Electrophysiological properties of VTA neurons

Considering that the activity of neurons recorded in anesthetized rats or in *in vitro* slice preparations could be different from those recorded *in vivo* (Zilberter et al., 2010; Belle et al., 2018) and that the experiment in this research project involved *in vivo* single unit recordings in awake behaving rats, electrophysiological properties of neurons obtained from *in vivo* and *in vitro* studies will be discussed.

Kiyatkin and Rebec (1998) failed to identify VTA neurons of different neurochemical properties (that is, dopaminergic, glutamatergic, and GABAergic) according to their electrophysiological properties using single unit activity. They identified units with biphasic and triphasic spikes of varying durations. The triphasic spikes formed the majority of identified units. They subcategorized units into long-spike type I, II, and III cells, and short-spike cells. Long-spike type I cells were spontaneously active and discharged irregularly at a slow and stable rate. Some of these neurons showed burst-firing patterns, with bursts comprising two to five spikes. These neurons had a spike duration of 2.27 ± 0.09 ms (1.94 – 2.91 ms) and a spontaneous firing rate of 6.03 ± 0.47 Hz (range: 0.42 – 15.78 Hz). Long-spike type II neurons, unlike long-spike type I neurons, had a high spontaneous firing rate which was 22.82 ± 2.02 Hz (4.42 – 59.67 Hz) and a spike duration of 2.19 ± 0.05 ms (range: 1.79 – 2.64 ms). Burst activity was common among this group of neurons, with bursts comprising of 10 – 14 spikes. The long-spike type III group of cells comprise neurons that discharged at rates higher than that of type I cells but lower than that of type II cells. The spontaneous firing rate of these cells was 22.70 ± 3.33 Hz (range: 13.88 – 39.47 Hz). Other cells in this group were either totally silent or discharged at low sporadic rates. Yet another subgroup of long-spike type III cells had a firing rate similar to that of long-spike type II cells. Short-spike cells had very short interspike intervals (2.9 - 3.8 ms), short spike duration (1.44 ± 0.06 ms; range 1.13 – 1.70 ms) and a firing rate of 8.16 ± 2.39 Hz (2.17 – 34.13 Hz). This group of cells comprised of bursting and nonbursting cells.

In vitro, dopaminergic neurons in the VTA are characterized by the presence of a hyperpolarization-activated nonselective conductance. VTA dopaminergic neurons have an initial resting membrane potential of -44 ± 1 mV, an action potential threshold of -24 ± 2 mV, and an action potential amplitude of 89 ± 3 mV. The input resistance of dopaminergic neurons is 390 ± 40 MΩ. The action potential duration at half-width is 1.09 ± 0.08 ms (Margolis et al., 2006). The spontaneous firing rate of dopaminergic neurons in the medial VTA is 2.9 ± 0.4 Hz, while dopaminergic neurons in the lateral VTA have a firing rate of 1.1 ± 0.3 Hz (Hnasko et al., 2012).

In general, GABAergic neurons in the VTA have a spontaneous firing rate of 19.1 ± 1.4 Hz, a spike duration of 310 ± 10 µs (50% amplitude), and refractory period typically lasting for 0.6 ± 0.1 ms. They have a resting membrane potential of -61.9 ± 1.8 mV, an action potential amplitude of 68.3 ± 2.1 mV, and an excitatory post synaptic potential amplitude of 7.6 ± 0.3 mV (Steffensen et al., 1998).

Glutamatergic VTA neurons have an initial membrane potential of -59.1 ± 1.6 mV, a spontaneous firing rate of 3.7 ± 0.8 Hz, an action potential threshold of -41.3 ± 0.9 mV, and an action potential duration (time between action potential threshold and when the membrane potential re-crossed the threshold) of 2.6 ± 0.2 Hz (Hnasko et al., 2012).

1.2.1.4 Afferents and efferents of the VTA

The VTA receives inputs from several regions in the brain (Figure 1–3) including the PFC, Acb, subthalamic nucleus (Kita and Kitai, 1987), pedunculopontine tegmental and laterodorsal tegmental nuclei (Clements et al., 1991), bed nucleus of the stria terminalis (BNST) (Georges and Aston-Jones, 2002), and superior colliculus (Dommett et al., 2005). Other regions of the brain that contain neurons which target VTA dopaminergic and GABAergic neurons include the central amygdala, deep cerebellar nuclei, dorsal raphe nucleus, dorsal striatum, extended amygdala area, lateral hypothalamus, globus pallidus, lateral habenula, medial habenula, parabrachial nucleus (PBN), paraventricular hypothalamic nucleus, nucleus of the solitary tract (NTS), and ventral pallidum (VP) (Beier et al., 2015). GABAergic

neurons in the VP serve as inhibitory inputs to both dopaminergic and nondopaminergic neurons in the VTA (Hjelmstad et al., 2013; Root et al., 2015). While activating these VP GABAergic neurons results in an increase in the activity of dopaminergic neurons in the VTA, its effect on the nondopaminergic neurons is unknown (Floresco et al., 2003).

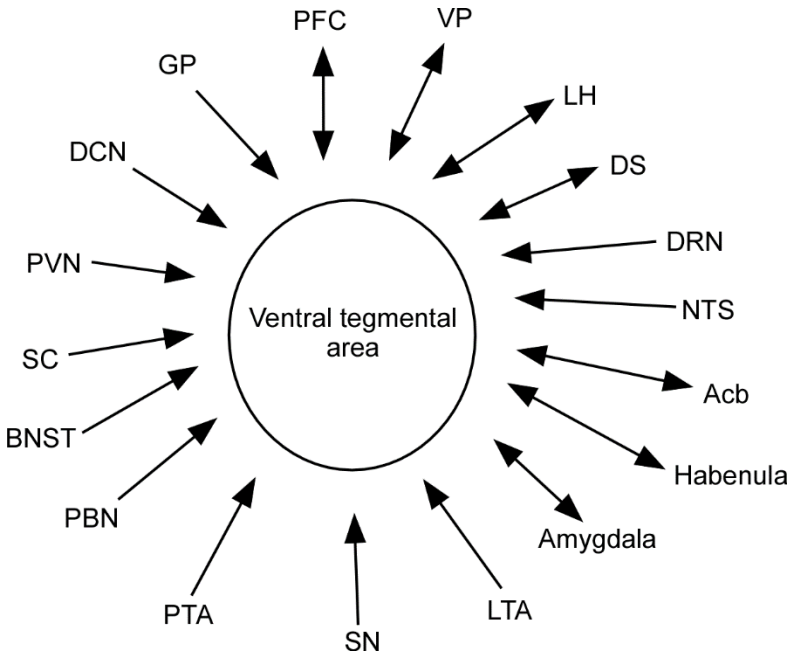

Figure 1 - 3 Afferents and efferents of the ventral tegmental area.

Acb: Nucleus accumbens, NTS: Nucleus of the solitary tract, DRN: Dorsal raphe nucleus, DS: Dorsal striatum, LH: Lateral hypothalamus, VP: Ventral pallidum, PFC: Prefrontal cortex, GP: Globus pallidus, PVN: Paraventricular hypothalamic nucleus, PBN: Parabrachial nucleus, SN: Subthalamic nucleus, DCN: Deep cerebellar nucleus, SC: Superior colliculus, BNST: Bed nucleus of the stria terminalis, PTA: Pedunculopontine tegmental area, LTA: Laterodorsal tegmental area. Bi-directional arrow: Reciprocal connection, Unidirectional arrow pointing towards the ventral tegmental area: afferents of the ventral tegmental area, Unidirectional arrow pointing away from the ventral tegmental area: efferents of the ventral tegmental area. Adapted from Morales and Margolis (2017).

VTA dopamine neurons project to the Acb, dorsal striatum, PFC, and the lateral habenula (Beier et al., 2015), as shown in Figure 1-2. Interestingly, VTA dopaminergic neurons that project to the lateral part of the Acb receive more inputs from the anterior cortex, dorsal striatum, and Acb core (AcbC), while VTA dopaminergic neurons that project to the medial part of the Acb receive more inputs from the dorsal raphe nucleus (Beier et al., 2015). GABAergic neurons in the VTA, even though function as local interneurons (Johnson and North, 1992), where they directly influence the activity of dopaminergic neurons (van Zessen et al., 2012), also send projections to regions including the ventral pallidum, lateral hypothalamus, lateral habenula, amygdala (Taylor et al., 2014), PFC (Carr and Sesack, 2000), and the Acb (Brown et al., 2012). Some VTA dopaminergic neurons have been shown to synthesize GABA (Kim et al., 2015), which is co-released with dopamine into the Acb (Tritsch et al., 2012). Glutamatergic neurons in the VTA, in addition to forming synapses with local dopaminergic neurons, also project to the Acb, ventral pallidum, lateral habenula, PFC, and amygdala (Yamaguchi et al., 2011; Hnasko et al., 2012). A small proportion of VTA glutamatergic neurons, which project to the prefrontal cortex and Acb, also express dopamine (Yamaguchi et al., 2011).

1.2.1.5 The VTA and reward

As discussed earlier, the VTA is a heterogeneous structure containing dopaminergic, GABAergic, and glutamatergic neurons that target and are targeted by several structures within and outside the mesocorticolimbic dopamine system. These inputs to the VTA modulate the activity of neurons in the VTA, which results in the regulation of behavior.

VTA dopaminergic neuron activity has been shown to increase in response to an unpredicted reward, shift when cues that predict a reward are presented, and decrease when an anticipated reward is omitted (Matsumoto and Hikosaka, 2009; Cohen et al., 2012), and the optogenetic stimulation of VTA dopamine neurons enhances positive reinforcement (Tsai et al., 2009; Kim et al., 2012). Aversive stimuli and cues that predict these stimuli have also been shown to affect the activity of VTA dopaminergic neurons. It was shown that these stimuli could either increase or

decrease the firing of dopaminergic neurons in the VTA (Ungless et al., 2004; Brischoux et al., 2009; Mileykovskiy and Morales, 2011). Interestingly, the inhibition of the activity of VTA dopamine neurons is aversive (Chang et al., 2016).

The stimulation of glutamatergic inputs from the laterodorsal tegmental nucleus to the VTA produced conditioned place preference, while the stimulation of glutamatergic neurons in the lateral habenula, which projected to mPFC-projecting VTA dopamine neurons, causes conditioned place aversion (Lammel et al., 2012; Steidl et al., 2017). Additionally, the selective activation of glutamatergic neurons in the dorsal raphe nucleus that target the VTA results in a conditioned place preference (Qi et al., 2014). The activation of medium spiny neurons in the Acb by dopaminergic inputs from the VTA promotes positive reinforcement in rats (Steinberg et al., 2014).

The activity of GABAergic neurons in the VTA have been shown to increase in response to reward-predicting cues and the receipt of a reward. These neurons transiently increase their activity following the presentation of an aversive stimulus (Cohen et al., 2012; Tan et al., 2012). To establish a cause and effect relationship, van Zessen et al. (2012) optogenetically stimulated VTA GABAergic neurons during the consumption of a reward. They reported that doing so disrupted reward consummatory behavior, even though another study found that the stimulation of VTA GABAergic neurons that project to the Acb was neither rewarding nor aversive (Brown et al., 2012). Tsunekawa et al. (2019) also reported that $GABA_B$ receptor signaling in the VTA, and other regions in the reward system, suppressed palatable food consumption in mice.

Even though the role of the lateral hypothalamus in reward processing has been known for a while, it was not until recently that Nieh et al. (2016), by using optogenetics, showed that the stimulation of lateral hypothalamus GABA neurons which target the VTA produced place preference and decreased VTA GABAergic neuron activity, resulting in the release of dopamine in the Acb. On the other hand, the stimulation of glutamatergic lateral hypothalamus neurons that target nondopaminergic VTA neurons caused conditioned place avoidance (Nieh et al.,

2016). Additionally, while the optogenetic stimulation of BNST glutamatergic neurons that target the VTA resulted in aversive behavioral phenotypes, the stimulation of BNST GABAergic neurons that target the VTA produced rewarding behavioral phenotypes (Jennings et al., 2013).

1.2.2 The Nucleus Accumbens (Acb)

Based on differences in the cytoarchitecture, neurotransmitter characteristics, and afferent and efferent connections, the Acb can be subdivided into two distinct areas: the core and shell (Zaborszky et al., 1985). The Acb core (AcbC) is situated laterally in the Acb and is encompassed by the Acb shell (AcbSh), which is situated more ventromedially in the Acb.

1.2.2.1 Neuron types in the Acb

The main neuron type in the Acb is the GABAergic medium spiny neuron (MSN), which makes up approximately 90-95% of the neuron population in this region. There are two main types of MSNs in the Acb based on the dopamine receptors and neuropeptides they express (Gerfen and Surmeier, 2011): MSNs that express D1-like receptors and those that express D2-like receptors. D1-like receptor-expressing MSNs co-express substance P, dynorphin, and M4 cholinergic receptors while D2-like receptor-expressing MSNs co-express enkephalin and neurotensin receptors (Le Moine and Bloch, 1995). The D1-like receptor subtypes are coupled to Gs proteins that activate adenylyl cyclase, leading to the production of cAMP and the subsequent activation of cAMP-dependent protein kinase and other cAMP-dependent proteins, while D2-like receptor subtypes are coupled to Gi protein coupled receptors that inhibit adenylyl cyclase and the production of cAMP (Missale et al., 1998; Vallone et al., 2000). The remaining 5-10% of the neuron population are made up of interneurons. Three types of GABAergic interneurons have been identified in the Acb namely interneurons that express parvalbumin, those that express, calretinin, and those that coexpress neuronal nitric oxide synthase, somatostatin, and neuropeptide Y (Tepper et al., 2010). Acetylcholine interneurons have also been identified in the Acb (Calabresi et al., 2000).

1.2.2.2 Morphological properties of Acb neurons

MSNs generally have round somas. Their dendrites are aspiny proximal to the soma but the density of spines increases as the distance of dendrites from the soma increases. The thickness of dendrites decreases proximodistally. The dendrites extend in all directions and branch regularly. Additionally, the axons seem to arise either from the soma or primary dendrites. The sizes of the somas of neurons in the AcbC and AcbSh are similar. The dendrites of neurons in both the AcbC and AcbSh are of similar lengths and thicknesses. However, some differences in the morphologies of MSNs in the AcbC and AcbSh have been reported. MSNs in the AcbC have more spiny dendrites and are less sparsely distributed than those in the AcbSh. MSNs in the lateral part of the AcbSh have dendrites which are longer, branch more often, and have more spines than those in the medial part of the AcbSh (Meredith et al., 1992).

Parvalbumin-expressing interneurons have either a round or oval soma with three to eight smooth primary dendrites, while both nitric oxide-synthase positive interneurons and cholinergic interneurons are either fusiform or polygonal in shape, and have two to five primary dendrites. Parvalbumin-positive interneurons also have more restricted dendritic fields and more dense axonal collaterals compared to the other subtypes of interneurons. Additionally, interneurons which are positive for choline acetyltransferase are larger in diameter compared to the other subtypes of interneurons (Kawaguchi, 1993).

1.2.2.3 Electrophysiological properties of Acb neurons

In vivo, neurons within the Acb had a spontaneous firing rate ranging from 0.1–18 Hz (4.08 ± 3.86 Hz). According to Callaway and Henriksen (1992), it was impossible to separate cells into subgroups based on their firing rates, suggesting that the activity of these neurons *in vivo* was similar. They had unimodally distributed interspike intervals, which means that the neurons were nonbursting neurons.

According to *in vitro* studies, MSNs can be distinguished from the other types of interneurons within the Acb based on their electrophysiological properties. MSNs

have a resting membrane potential of -81.1 ± 4.4 mV, an input resistance of 193 ± 77 MΩ, a spike width at half amplitude of 1.05 ± 0.20 ms, and an amplitude of afterhyperpolarization of -13 ± 2.4 mV (Kawaguchi, 1993). MSNs fire spontaneously at a rate of 3.3 ± 3.5 Hz with a mean interspike interval of 515.6 ± 624.4 ms (Mahon et al., 2006).

Based on electrophysiological properties identified using *in vitro* studies, three types of interneurons were identified in the Acb. They are the fast-spiking cells (FS), persistent and low-threshold spike cells (PLTS), and the long-lasting afterhyperpolarization (LA) cells. The FS cells were found to express parvalbumin, the LA cells express choline acetyltransferase, while the PLTS cells were positive for nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase and nitric oxide-synthase (Kawaguchi, 1993). FS interneurons have a resting membrane potential of -79.9 ± 3.2 mV, an input resistance of 86 ± 38 MΩ, a spike width at half amplitude of 0.29 ± 0.04 ms, and an amplitude of afterhyperpolarization of -23 ± 3.8 mV. The resting membrane potential, input resistance, spike width at half amplitude, and amplitude of afterhyperpolarization of PLTS and LA interneurons are -56.4 ± 15.7 mV, 638 ± 245 MΩ, 1.00 ± 0.41 ms, and -19.9 ± 6.5 mV, and -57.3 ± 5.0 mV, 433 ± 231 MΩ, 0.82 ± 0.19 ms, and -21.6 ± 5.4 mV, respectively (Kawaguchi, 1993).

1.2.2.4 Afferents and efferents of the Acb

The AcbC receives glutamatergic projections from both the dorsomedial (prelimbic and anterior cingulate cortices) and dorsolateral (anterior insula) prefrontal cortices (Brog et al., 1993); GABAergic projections from the ventral pallidum (Stefanik et al., 2013), VTA (Taylor et al., 2014), lateral septum (Brog et al., 1993), and parvalbumin projection neurons in the PFC (Lee et al., 2014); and dopaminergic inputs from the VTA (Stefanik et al., 2013), as shown in Figure 1-4. The AcbC also receives serotonergic and nonserotonergic inputs from the dorsal raphe nucleus (Brown and Molliver, 2000) and cholinergic inputs from the laterodorsal tegmental area (Dautan et al., 2014). The AcbC sends projections to dopaminergic neurons in the VTA and the dorsolateral ventral pallidum (Heimer et al., 1991).

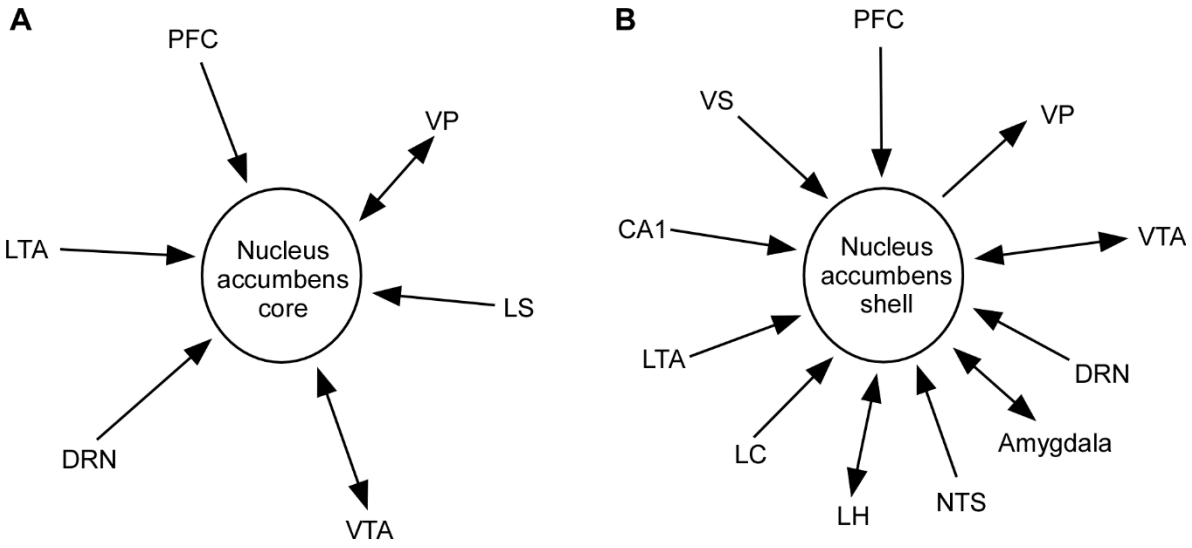

Figure 1 - 4 Afferents and efferents of the nucleus accumbens core and shell.

PFC: Prefrontal cortex, VP: Ventral pallidum, LS: Lateral septum, VTA: Ventral tegmental area, DRN: Dorsal raphe nucleus, LTA: Laterodorsal tegmental area, NTS: Nucleus of the solitary tract, LH: Lateral hypothalamus, LC: Locus coeruleus, CA1: Cornu ammonis 1, VS: Ventral subiculum. Bi-directional arrow: Reciprocal connection, Unidirectional arrow pointing towards the nucleus accumbens: afferents of the nucleus accumbens, Unidirectional arrow pointing away from the nucleus accumbens: efferents of the nucleus accumbens.

The medial part of the AcbSh receives glutamatergic inputs from the ventromedial (infralimbic cortex, ventral prelimbic cortex, and orbitofrontal cortex) and ventrolateral PFC (Groenewegen et al., 1999), the ventral subiculum, and the ventral Cornu Ammonis 1 (CA1) region (Groenewegen et al., 1987; Strange et al., 2014), while the ventrolateral part of the AcbSh is targeted by glutamatergic projections from the basolateral amygdala (Brog et al., 1993; Groenewegen et al., 1999). Both the medial and lateral parts of the AcbSh receive dopaminergic inputs from the VTA (Beckstead et al., 1979). The AcbSh is also targeted by noradrenergic projections from the locus coeruleus and NTS (Delfs et al., 1998) and serotonergic and nonserotonergic inputs from the dorsal raphe nucleus (Brown and Molliver, 2000). The caudal dorsomedial part of the AcbSh receives inputs from the lateral hypothalamus (Brog et al., 1993). Neurons in the AcbSh innervate the lateral

hypothalamus and the extended amygdala, while no such projections exist for the AcbC (Heimer et al., 1991). The medial AcbSh projects to the ventromedial part of the ventral pallidum and the lateral hypothalamus (Heimer et al., 1991; Heimer et al., 1997), while both the medial and lateral AcbSh project directly to the VTA (Watabe-Uchida et al., 2012).

1.2.2.5 The Acb and reward

The Acb mediates reward-related processing and goal-directed behaviors associated with natural rewards (Carelli, 2002). During a Go No-Go task in monkeys, Apicella et al. (1991) reported an increase in the firing of a distinct population of neurons in the Acb following reward delivery, while a different group of neurons increased their firing during reward anticipation. Another study also found similar increases in activity of neurons in rats just before the reception of a reward and either an increase or decrease in activity following the reception of the reward (Roop et al., 2002). Additionally, Hollander et al. (2002) showed that the activity of Acb neurons decreases during extinction, but increases following reinstatement. These studies suggest that the Acb is involved in the processing of goal-directed behavior for natural rewards.

As described earlier, the Acb can be subdivided into the core and shell, and these subregions project and receive inputs from similar and different regions in the brain, which may explain why they have been found to perform different functions (Bassareo and Di Chiara, 1999; Corbit et al., 2001; Corbit and Balleine, 2011).

Bassareo and Di Chiara (1999) examined dopamine transmission in the AcbC and AcbSh of rats during the consummatory and appetitive phases of behavior motivated by palatable food. They reported that appetitive food stimuli induced dopamine transmission phasically in the AcbC but not the AcbSh, and the unpredicted consumption of palatable food resulted in the preferential release of dopamine into the AcbSh in rats pre-exposed to the palatable food. From their experiments and those of other research groups, it appears that the AcbC may mediate processes involved in the evaluation of the incentive value of an instrumental outcome during

the performance of goal-directed actions, while the AcbSh may mediate the excitatory effects of reward-predicting stimuli during goal-directed behavior (Corbit et al., 2001).

Krause et al. (2010) reported that during consumption, MSN neurons in the AcbC and AcbSh decrease their activity, and the subsequent stimulation of these neurons resulted in a disruption of intake. The infusion of either muscimol or baclofen into the AcbSh also resulted in an increase in feeding in satiated rats (Stratford and Kelley, 1997; Basso and Kelley, 1999). This suggests that a decrease in the activity of GABAergic AcbSh neurons may be necessary for consumption. However, other studies suggest that this function may be more specific to the rostral, and not the entire, AcbSh. This is because, the activation of $GABA_A$ receptors with muscimol in the rostral shell of the Acb resulted in increased feeding, place preference, and positive reactions to the taste of sucrose, while the infusion of muscimol in the caudal shell of the Acb caused negative fearful behavior, negative affective reactions to taste, and place avoidance (Reynolds and Berridge, 2002). The activation of GABAergic neurons mid-way between the rostral and caudal AcbSh resulted in positive and negative motivational effects.

An increase in the intake of palatable food was observed following the stimulation of μ-opioid receptors in the Acb suggesting that opioid receptors in the AcbSh may also be implicated in reward processing during the consumption of palatable foods (Zhang and Kelley, 1997). Additionally, the knocking out of mu opioid receptors in mice resulted in a decrease in palatable food consumption (Awad et al., 2019). However, the infusion of the μ-agonist DAMGO into the rostrodorsal part of the medial AcbSh resulted in an increase in “liking,” while a similar infusion in all other areas within the medial AcbSh resulted in an increase in “wanting” (Pecina and Berridge, 2005). This suggests that the opioid microcircuitry for enhancing motivated “wanting” is more widespread in the medial AcbSh compared to that for enhancing hedonic “liking” (Castro et al., 2015).

1.2.3 The Prefrontal Cortex (PFC)

The prefrontal cortex (PFC) is defined as the region of the brain that has strong reciprocal connections with the mediodorsal thalamus (Rose and Woolsey, 1948). The rat PFC can be divided into the medial, orbital, and lateral aspects (Ongur and Price, 2000). The medial prefrontal cortex (mPFC) of rodents comprises of the dorsal and ventral regions, which can be subdivided into medial agranular and anterior cingulate cortices and prelimbic (PrL) and infralimbic (IL) cortices, respectively (Ray and Price, 1992; Ongur and Price, 2000). The ventral mPFC, which comprises the PrL and IL, are functionally and anatomically associated with the limbic system (Vertes, 2004).

1.2.3.1 Neuron types in the mPFC

Based on the neurotransmitters expressed, two main types of neurons have been identified in the mPFC: the glutamatergic neurons and GABAergic interneurons. Approximately 75-80% of neurons in the mPFC are glutamatergic pyramidal projection neurons, while the remaining 20-25% are GABAergic local circuit interneurons (Beaulieu, 1993; Gabbott et al., 2005). GABAergic interneurons in the mPFC include parvalbumin-, vasoactive intestinal peptide-, and somatostatin-expressing interneurons (Kawaguchi and Kubota, 1997; Lewis et al., 2005).

1.2.3.2 Morphological properties of mPFC neurons

Glutamatergic neurons are projecting neurons with a pyramid-shaped soma, a single apical dendrite, and several basal dendrites. They usually have long, slender axons which target both cortical and subcortical regions within the brain (Elston, 2003). Glutamatergic neurons are located in layers 2-6 of the mPFC; those in layers 2 and 3 are targeted by cortico-cortical projections while those in layers 5 and 6 target subcortical regions (Giustino and Maren, 2015).

Parvalbumin-positive neurons can be classified into three groups based on their morphology: class 1, class 2, and class 3 cells. Class 1 cells form the majority of the parvalbumin-positive cells. They are multipolar cells with radial dendrites that rarely bifurcate. These cells can be found in all layers of the mPFC. Class 2 cells are less

common compared to class 1 cells. They are bitufted cells and can be found in layer 2 through to layer 6a. Class 3 cells are rare. They are bipolar and have dendrites which spread horizontally along the boundary between layer 6b and the white matter (Gabbott et al., 1997). These interneurons target the soma and axon initial segments of pyramidal neurons (Kawaguchi and Kubota, 1997).

Somatostatin-positive interneurons are usually located in layers 2/3 and 5. They are either multipolar or bitufted cells. Their axons were observed to originate either from the cell body or the dendrite. Their axons subsequently ascended and emitted collaterals in several directions (Kawaguchi and Kubota, 1996). Somatostatin-positive cells comprised of Martinotti cells. Somatostatin interneurons target the apical dendrites and spines of pyramidal neurons (Tremblay et al., 2016). Vasoactive intestinal protein-containing cells comprised cells of different morphologies. They comprised of bipolar, double bouquet, small basket, and arcade cells. These cells were usually located in layers 2/3 and 5. The bipolar and double bouquet cells had long descending axons with several collaterals. Their axon travelled downward, horizontally, or even upwards (Kawaguchi and Kubota, 1996). These interneurons usually target other interneurons, specifically somatostatin-positive interneurons. Interestingly, these are the only cells in the mPFC which express mu opioid receptors (Ferezou et al., 2007; Tremblay et al., 2016).

1.2.3.3 Electrophysiological properties of mPFC neurons

According to their electrophysiological properties, three main pyramidal neurons were identified. They are the regular spiking (RS) cells, which comprises the slow-adapting and fast-adapting regular spiking cells, the intrinsic (inactivating) bursting cells, and the non-inactivating bursting cells (Yang et al., 1996; Degenetais et al., 2002).

Slow-adapting RS neurons have a resting membrane potential of -68.7 ± 5.3 mV, an input resistance of 34.6 ± 10.6 MΩ, a spike amplitude of 61.0 ± 7.0 mV, a spike duration of 1.8 ± 0.42 ms, a spike threshold of -50.8 ± 5.8 mV, and a rheobase of 0.30 ± 0.15 nA. These neurons have a mean spontaneous firing rate of 0.90 ± 1.23

Hz. The resting membrane potential, input resistance, spike amplitude, spike duration, spike threshold, and rheobase of fast-adapting RS neurons are -70.5 ± 2.6 mV, 33.8 ± 15.2 MΩ, 54.9 ± 4.6 mV, 1.92 ± 0.24 ms, -48.5 ± 3.9 mV, and 0.42 ± 0.20 nA. These neurons did not show spontaneous firing. For the intrinsic bursting cells and the non-inactivating bursting cells the resting membrane potential, input resistance, spike amplitude, spike duration, spike threshold, and rheobase are -68.5 ± 2.8 mV, 23.5 ± 6.9 MΩ, 66.6 ± 8.3 mV, 2.10 ± 0.50 ms, -55.5 ± 5.4 mV, and 0.25 ± 0.13 nA, and -68.4 ± 5.0 mV, 42.3 ± 12.3 MΩ, 60.1 ± 6.8 mV, 1.91 ± 0.53 ms, -51.7 ± 5.3 mV, and 0.23 ± 0.16 nA, respectively. The spontaneous firing rate and mean spike frequency during a burst of intrinsic bursting cells are 3.1 ± 2.6 Hz and 77 ± 21 Hz, respectively, while those for non-inactivating bursting cells are 2.8 ± 3.2 Hz and 92 ± 39 Hz, respectively (Degenetais et al., 2002).

Based on electrophysiological properties, four types of interneurons were identified (Kawaguchi, 1993, 1995). These are the fast-spiking (FS) cells, late-spiking (LS) cells, low-threshold spiking (LTS) cells, and the regular-spiking non-pyramidal (RSNP) cells. The FS cells are parvalbumin-expressing interneurons. Vasoactive intestinal protein was expressed by most RSNP interneurons. FS cells have a resting membrane potential of -77.4 ± 2.9 mV, an input resistance of 157 ± 42 MΩ, a spike threshold of -41 ± 5 mV, and a spike width of 0.43 ± 0.06 ms. The resting membrane potential, input resistance, spike threshold, and spike width of LS, LTS, and RSNP cells are -67.2 ± 5.5 mV, 297 ± 118 MΩ, -38 ± 4 mV, and 0.77 ± 0.19 ms; -57.5 ± 7.4 mV, 460 ± 144 MΩ, -46 ± 3 mV, and 0.94 ± 0.15 ms; and -60.6 ± 5.1 mV, 414 ± 207 MΩ, -45 ± 4 mV, and 0.75 ± 0.13 ms, respectively (Kawaguchi, 1995).

1.2.3.4 Afferents and efferents of the mPFC

The mPFC receives and sends projections from and to a number of brain regions (Figure 1–5). Projections from the PrL target the IL, AcbC, AcbSh, central and basolateral amygdala, VTA, dorsal raphe nucleus, periaqueductal gray, and the reuniens nucleus of the thalamus, while those from the IL target the PL, IL, medial, basomedial, cortical, and central nuclei of the amygdala, dorsomedial and lateral hypothalamus, PBN, and the reuniens nucleus of the thalamus (Vertes, 2004).

mPFC GABAergic neurons with long range projections have been shown to target the Acb (Lee et al., 2014).

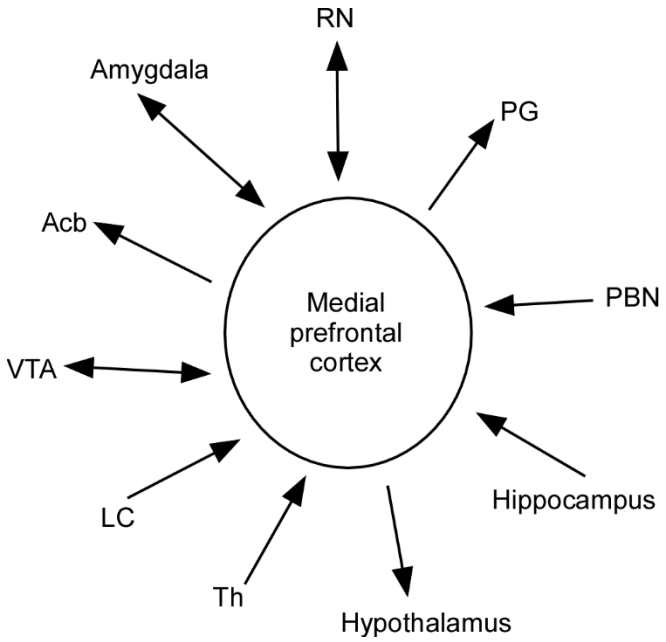

Figure 1 - 5 Afferents and efferents of the medial prefrontal cortex.

LC: Locus coeruleus, PBN: Parabrachial nucleus, VTA: Ventral tegmental area, Acb: Nucleus accumbens, RN: Raphe nucleus, PG: Periaqueductal gray, Th: Thalamus. Bi-directional arrow: Reciprocal connection, Unidirectional arrow pointing towards the medial prefrontal cortex: afferents of the medial prefrontal cortex, Unidirectional arrow pointing away from the medial prefrontal cortex: efferents of the medial prefrontal cortex.

Glutamatergic inputs to the mPFC originate from the amygdala, hippocampus, and mediodorsal and centromedial thalamic nuclei. The mPFC also receives projections from serotonergic neurons in the dorsal and medial raphe nuclei (Celada et al., 2013), noradrenergic neurons in the locus coeruleus (Chandler et al., 2014), and dopaminergic neurons in the VTA (Van Eden et al., 1987). Additionally, the mPFC receives cholinergic inputs from basal forebrain structures including the nucleus basalis magnocellularis (Lehmann et al., 1980).

1.2.3.5 The mPFC and reward

The mPFC is involved in several functions including value-based decision making, working memory, habitual behavior, and attention (Fuster, 1973; Killcross and Coutureau, 2003; Botvinick, 2007; Gilmartin and Helmstetter, 2010). Within the reward system the mPFC sends glutamatergic projections to the other important regions namely the Acb and VTA, and in turn, receives dopaminergic projections from the VTA (Van Eden et al., 1987; Vertes, 2004). Stimulation of the mPFC results in the activation of VTA dopaminergic neurons (Gariano and Groves, 1988), leading to the release of dopamine in the Acb (Taber et al., 1995).

A number of studies showed that the consumption of a reward results in the activation of neurons within the rat mPFC (Horst and Laubach, 2013; Jezzini et al., 2013). Ishikawa et al. (2008) showed that the inactivation of the PrL decreased the number of lever presses by rats to obtain palatable food and the inactivation of the PrL reduced food seeking behavior in rats (Sangha et al., 2014). These studies suggest that the inactivation of the PrL caused a decrease in the motivation of rats to obtain palatable food. In contrast to the aforementioned studies, the study by Parent et al. (2015) reported that the inactivation of the PrL rather increases motivation since they observed an increase in the number of licks of rats during the consumption of sucrose. The activation of the adjacent structure, the IL, following the infusion of a GABA-A receptor agonist resulted in a decrease in the intake of chow in rats (Richard and Berridge, 2013).

The inactivation of neuronal ensembles within the ventral mPFC decreased the number of lever presses for palatable food (Warren et al., 2016) while the activation of glutamatergic neurons in the same region increased the number of lever presses for palatable food (Warthen et al., 2016). A number of studies reported results that were somewhat different from the aforementioned studies. Inactivation of the ventral mPFC was also shown to result in an increase in the consumption of a highly palatable liquid (Mena et al., 2011; Baldo et al., 2016; Corwin et al., 2016).

Even though the exact role of the mPFC in palatable food consumption is unclear, that is whether it promotes or inhibits palatable food seeking and consumption, the findings of the aforementioned studies highlight the role of the mPFC in reward seeking and consumption. One possible reason for these discrepancies might be the definition of the boarders and subregions within the mPFC, since different studies define the mPFC differently. Another reason could be how widespread the drugs infused within the mPFC are. If these drugs are not restricted to the region of interest, they might result in mixed results. In the same vein, lesions which were not restricted to the region of interest could result in discrepancies in the findings of these studies.

1.2.3.6 The mPFC and inhibitory control

One of the main functions of the mPFC is inhibitory control (Rossetti and Boutrel, 2019). Inhibitory control refers to the ability to control one's attention, thoughts, behavior, and/or emotions, to inhibit a strong internal predisposition, in order to do what is more appropriate or needed (Diamond, 2013). Dysfunction in inhibitory control may manifest as an impulsive or compulsive behavior. Impulsive behaviors involve making decisions rashly, without prior consideration of the consequences, while compulsive behaviors involve persistent, repetitive actions despite adverse consequences (Kessler et al., 2016). Inactivation of the mPFC following lesions or the infusion of a GABA agonist resulted in an increase in compulsive and impulsive behaviors in rats performing a five-choice task (Murphy et al., 2012; Feja and Koch, 2014), while activation of the mPFC reduced impulsivity (Warthen et al., 2016). Sinclair et al. (2019) also reported that a decrease in mPFC activity is associated with proneness to compulsive eating. Interestingly, Mushquash et al. (2019) reported that impulsivity could predict future binge eating in humans.

The mPFC, like the Acb, is also involved in the regulation of goal-directed behaviors. Specifically, Corbit and Balleine (2003) showed that lesioning the PrL resulted in the inability of rats to distinguish between actions that would result in the receipt of a preferred reward and those that would not. On the other hand, lesioning the IL results in the loss of inhibitory control and a decrease in the influence of prior learning on a reflexive response (habit) following changes in favor of that reflexive response

(Killcross and Coutureau, 2003). Taken together, these studies suggest that the PrL is involved in the regulation of responses according to the value of an outcome, which may sometimes include response inhibition, while the IL is implicated in the expression of habitual behaviors (Gourley and Taylor, 2016).

1.3 The Stress System

Stress is the state of real or perceived threat to homeostasis, usually induced by aversive stimuli, and the response of the endocrine, nervous, and immune systems to re-establish homeostasis following an aversive stimulus is referred to as the stress response (Chrousos and Gold, 1992). Behaviorally, the stress response results in an increase in awareness, improved cognition, and enhanced analgesia, while physiologically, it leads to an increase in heart rate, respiratory rate, and metabolism, and a decrease in feeding, digestion, and reproduction (Habib et al., 2001; Charmandari et al., 2005). The hypothalamic-pituitary-adrenal (HPA) axis is the main system in the body responsible for the regulation of stress. However, the brain stem noradrenergic neurons and the parasympathetic system also mediate stress response (Habib et al., 2001).

1.3.1 The HPA axis

The main structures in the HPA axis are the paraventricular hypothalamic nucleus (PVN), the anterior lobe of the pituitary gland, and the adrenal gland, as shown in Figure 1-6. Following the occurrence of a stressful event, the PVN releases corticotropin-releasing factor (CRF) into the hypophysial portal vessels, where it travels to the anterior pituitary gland. Here, it binds to CRF receptors, which activates adenylyl cyclase and the production of cAMP leading to the release of adrenocorticotropic hormone (ACTH). Two types of CRF receptors have been identified: the CRF type 1 receptor (CRFR1) and the CRF type 2 receptor (CRFR2). It was shown that deficiency of CRFR1 leads to the attenuation of HPA response to stress (Smith et al., 1998), while deficiency of CRFR2 results in an amplified stress response (Bale et al., 2000). The PVN neurons also synthesize and release vasopressin, which amplifies the effects of CRF on the release of ACTH by the

anterior pituitary (Rivier and Vale, 1983). The ACTH stimulates the adrenal gland to release glucocorticoids; cortisol in humans and corticosterone in rodents. This occurs by the ACTH binding to melanocortin type 2 receptors in parenchymal cells of the adrenocortical zona fasciculata, which promotes the conversion of cholesterol into the precursor for glucocorticoid synthesis (Simpson and Waterman, 1988; Raffin-Sanson et al., 2003). Even though the effect of glucocorticoid is beneficial, too much or too little could result in the development of pathological conditions (Munck et al., 1984).

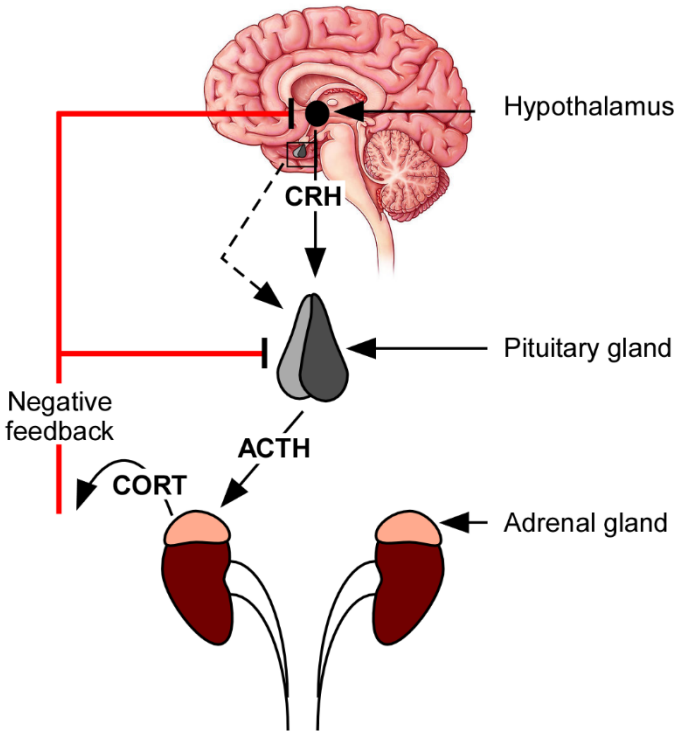

Figure 1 - 6 The hypothalamic-pituitary-adrenal axis.

The hypothalamic-pituitary-adrenal axis comprises the paraventricular nucleus of the hypothalamus, the pituitary gland, and the adrenal glands. Stress leads to the release of CRH by the paraventricular nucleus. This CRH stimulates the pituitary gland to release ACTH, which subsequently stimulates the adrenal gland to release CORT. This CORT inhibits the further release of CRH and ACTH by the pituitary and paraventricular hypothalamus. CRH: Corticotropin releasing hormone, ACTH: Adrenocorticotropic hormone, CORT: Cortisol. Red lines: Feedback inhibition of the pituitary gland and the paraventricular hypothalamic nucleus by cortisol.

As mentioned earlier, excessive levels of glucocorticoids can have negative consequences. Therefore, to prevent this, the glucocorticoids act as feedback to inhibit the HPA axis from releasing excess ACTH (Keller-Wood and Dallman, 1984), which would stimulate the adrenal gland to release more glucocorticoids. This inhibition of the HPA axis by glucocorticoids is mediated through the glucocorticoid receptors (GRs) and the mineralocorticoid receptors (MRs). Due to the high affinity of MRs to glucocorticoids, they bind to glucocorticoids when there are basal levels of glucocorticoids, while GRs bind to glucocorticoids when the levels are elevated (Reul and de Kloet, 1985). This suggests that the feedback inhibition by the glucocorticoid is mainly mediated by GRs (Dallman et al., 1989). Additionally, there is a high concentration of GRs on PVN neurons, and the administration of glucocorticoid on these neurons reduces PVN activity (Sawchenko, 1987) and attenuates the hypersecretion of ACTH by the adrenal gland (Kovacs and Makara, 1988).

The HPA axis is modulated by a number of structures within the limbic system such as the hippocampus, amygdala, and prefrontal cortex (Jacobson and Sapolsky, 1991; Feldman et al., 1995). These limbic structures have very limited direct projections to the HPA axis and therefore modulate the HPA axis indirectly through intermediary neurons in the BNST, hypothalamus, and brain stem (Herman et al., 2004; Herman et al., 2005). The hippocampus modulates the activity of the HPA axis by projecting to the BNST, which in turn projects directly to the parvocellular nucleus of the hypothalamus. The stimulation of the hippocampus decreases PVN neuron activity leading to the attenuation of the release of glucocorticoids, while its lesioning results in the increase in the levels of CRF in the PVN, prolonged ACTH release, and elevated levels of glucocorticoids (Knigge, 1961; Sapolsky et al., 1984; Sapolsky et al., 1991; Herman et al., 1992; Herman et al., 1995). Even though lesions of the PrL have been shown to result in an increase in the release of ACTH and glucocorticoids following stress, suggesting an inhibitory effect (Figueiredo et al., 2003), no direct projection to the BNST was observed from tracing studies. This suggests that the PrL might modulate the activity of the HPA axis through its

projections to other regions including the preoptic area and the dorsomedial hypothalamic nucleus (Sesack et al., 1989).

1.3.2 The locus coeruleus-noradrenergic stress system

The noradrenergic system in the brain is involved in several processes including emotion, learning and memory, attention, sleep/wakefulness, reproduction, and stress response (Berridge and Waterhouse, 2003). Through its action on the CRF-expressing PVN neurons, it is considered as an important regulatory system for the HPA axis (Itoi et al., 2004).

Among the noradrenergic nuclei in the brainstem, the locus coeruleus (LC) is the largest (Itoi and Sugimoto, 2010). It comprises of a well-delineated group of neurons situated adjacent to the fourth ventricle (Berridge and Waterhouse, 2003). Using retrograde labeling, Mason and Fibiger (1979) showed that the neurons in the LC that projected to the hippocampus and the septum were located in the dorsal LC, those that projected to the thalamus and hypothalamus were located in the caudal and rostral poles of the LC, while those that projected to the cerebellum were located in the dorsal and ventral LC. In addition to the PVN and the central nucleus of the amygdala, the LC receives projections from the insula cortex, medial, lateral, and magnocellular preoptic areas, the BNST, and the dorsomedial and lateral hypothalamic areas (Cedarbaum and Aghajanian, 1978). Additionally, it is targeted by neurons located in the reticular formation, raphe nucleus, NTS, vestibular nucleus, and the lateral reticular nucleus.

The LC is considered to be involved in the stress response because the activity of neurons in this region increased after a stressful stimuli (Rasmussen and Jacobs, 1986; Rasmussen et al., 1986). Additionally, *c-fos* (Pirnik et al., 2004) and tyrosine hydroxylase mRNA (Chang et al., 2000) expression in the LC increased after exposure to foot-shock stress. Moreover, the expression of tyrosine hydroxylase mRNA by LC neurons was dependent on the level of glucocorticoids after immobilization stress (Makino et al., 2002). The LC receives CRF innervation from regions including the central nucleus of the amygdala and the PVN (Valentino et al.,

1992; Van Bockstaele et al., 1999), and Jedema and Grace (2004) demonstrated that the administration of CRF onto LC neurons resulted in the activation of these neurons.

1.3.3 Stress and palatable food intake

The activation of the HPA axis following stress causes an increase in the synthesis of glucocorticoids and the availability of glucose, which may be necessary to support the metabolic needs for behavioral and physiological stress response (Ulrich-Lai et al., 2015). Stress can result in either an increase or a decrease in food intake in both humans and animals (Levine and Morley, 1981; WIllenbring et al., 1986) and like humans, following the occurrence of a stressful event, rats prefer palatable food to normal chow, when given a choice between the two (Pecoraro et al., 2004; Dallman et al., 2005), possibly due to the positive reinforcement property of palatable foods (Sclafani and Ackroff, 2003).

The type of stress, that is, whether it is acute or chronic, may affect the consumption of palatable food, when available (Maniam and Morris, 2012). Acute stress can lead to a decrease in palatable food consumption, while chronic stress can either increase or decrease palatable food consumption (Timofeeva and Calvez, 2014). Stresses may also be classified as either physical or emotional. Pijlman et al. (2003) in their study found that physical stress (foot-shock) reduced preference for and consumption of saccharin in rats, while emotional stress (where rats were placed in the same cage as another rat which was undergoing foot shock stress) increased preference for and consumption of saccharin. Another study found an increase in palatable food consumption in fasted rats following foot-shock stress (Boggiano et al., 2005), while chronic stress increased the preference for and consumption of palatable food (Pecoraro et al., 2004). In humans, the administration of glucocorticoids intravenously resulted in an increase in food intake (Tataranni et al., 1996).

In line with the affect model posited by Hawkins and F. Clement (1984), both rats (Prasad and Prasad, 1996; Buwalda et al., 2001) and humans, following the

occurrence of a stressful event, consume palatable food in an attempt to mitigate the effects of that stressful event (Epel et al., 2001). This theory has been subsequently confirmed by several studies. Pecoraro et al. (2004) demonstrated that HPA axis response to stress is blunted following palatable food consumption. The intermittent consumption of sucrose has been shown to reduce CRF mRNA expression in the PVN, decrease HPA axis activity, and reduce tachycardic response following acute stress (Ulrich-Lai et al., 2007; Ulrich-Lai et al., 2011). Prasad and Prasad (1996) showed that the consumption of palatable food reduces stress-induced anxiety, and Bell et al. (2002) showed that post-stress plasma corticosterone concentration decreases following palatable food consumption.

Sucrose solution may have rewarding properties which may be sufficient to relieve stress because when consumed, HPA axis activity was dampened, but when delivered through a gavage, this property was not observed (Ulrich-Lai et al., 2010). The repeated consumption of palatable food, which results in the repeated stimulation of the reward pathways, leading to elevated sucrose intake and the release of dopamine in rats (Rada et al., 2005), may result in neurobiological adaptations that initiate the loss of control observed in some EDs (Volkow and Wise, 2005).

1.4 The taste system

The taste system (Figure 1–7) is one of the systems that may be implicated in binge eating. This system is engaged when food is placed in the mouth. Within the mouth is the tongue on which are found several types of taste receptor cells (TRCs). There are three types of TRCs: Type I, II, and III (Chaudhari and Roper, 2010). These TRCs express receptors that are responsible for identifying different types of tastes including sweet, salty, sour, bitter, umami, and fat. Generally, sweet tastes are indicative of energy dense foods, while bitter tastes are usually associated with toxic foods. Some examples of receptors expressed by these TRCs include T1R2/T1R3 receptors, which detect sweet taste (Nelson et al., 2001; Jiang et al., 2004), T1R1/T1R3 receptors, which detect umami taste (Nelson et al., 2002), and T2R receptors, which detect bitter taste (Chandrashekar et al., 2000). These receptors

are G-protein coupled receptors. Interestingly, T1Rs have also been detected in the gastrointestinal tract (Treesukosol et al., 2011).

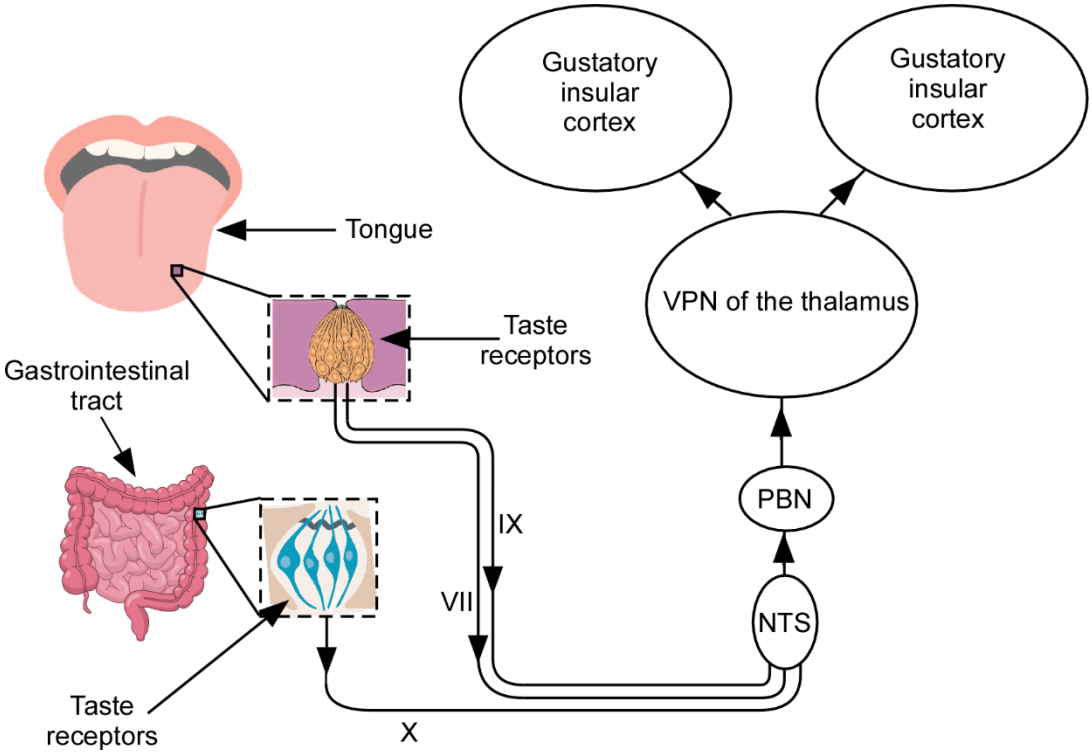

Figure 1 - 7 The taste system.

Activation of taste receptors in the tongue and gastrointestinal tract by a stimulus such as sugar results in a signal being transferred via the cranial nerves VII, IX, and X to the NTS, then to the PBN, subsequently to the VPN of the thalamus, and finally to the gustatory insular cortex. VPN: Ventral posteromedial nucleus, PBN: Parabrachial nucleus, NTS: Nucleus of the solitary tract, VII: Cranial nerve VII (facial nerve), IX: Cranial nerve IX (Glossopharyngeal nerve), X: Cranial nerve X (Vagus nerve). Adapted from Timofeeva and Mitra (2013).

Upon the binding of a tastant, for example sucrose, to these receptors, a conformational change occurs which activates alpha-gustducin, a G-protein, and the subsequent activation of adenylyl cyclase and phospholipase C, leading to the influx of free calcium (Ishimaru and Matsunami, 2009). This increase in calcium leads to the activation of the transient receptor potential channel M5, resulting in the release

of adenosine triphosphate as a neurotransmitter and the activation of the primary afferent neurons (Ishimaru and Matsunami, 2009).

Taste information is transmitted to the brain via the facial nerve (cranial nerve VII), the glossopharyngeal nerve (cranial nerve IX), and the vagus nerve (cranial nerve X) (Kamath et al., 2015). Information from these nerves are relayed by the NTS (Travers and Norgren, 1995) mainly to the PBN in the pons (Norgren and Leonard, 1971), but also to the PVN, paraventricular thalamic nucleus, central amygdala (Timofeeva et al., 2005), and the VTA (Richard et al., 2015). The PBN then targets the parvocellular part of the ventral posteromedial nucleus of the thalamus, and other regions including the paraventricular thalamic nucleus, VTA, and the medial hypothalamus (Cechetto and Saper, 1987; Alden et al., 1994; Krout and Loewy, 2000; Geisler et al., 2007). Information from the PBN targeting the paraventricular thalamic nucleus is sent to the prefrontal cortex. The targeting of the reward system by afferents from the taste system, specifically the PBN and the NTS may lead to the activation of the reward system, comprising the Acb, mPFC, and the VTA, through the release of dopamine (Hernandez and Hoebel, 1988; Hajnal et al., 2004; Taha and Fields, 2005; de Araujo et al., 2008; Krause et al., 2010). The information from the parvocellular part of the ventral posterior thalamic nucleus is then sent to the anterior dysgranular insular cortex (Wise, 2006).

1.5 Interaction between the stress, taste, and reward systems following a stressful event and the subsequent consumption of palatable food

Considering that people resort to eating palatable food, in an attempt to mitigate the effects of a stressful event, the possible neuronal systems that might be implicated include the reward, taste, and stress systems. The knowledge of how these systems interact normally (Figure 1–8) may help in the understanding of how some EDs develop and in the identification of possible treatment strategies for them.

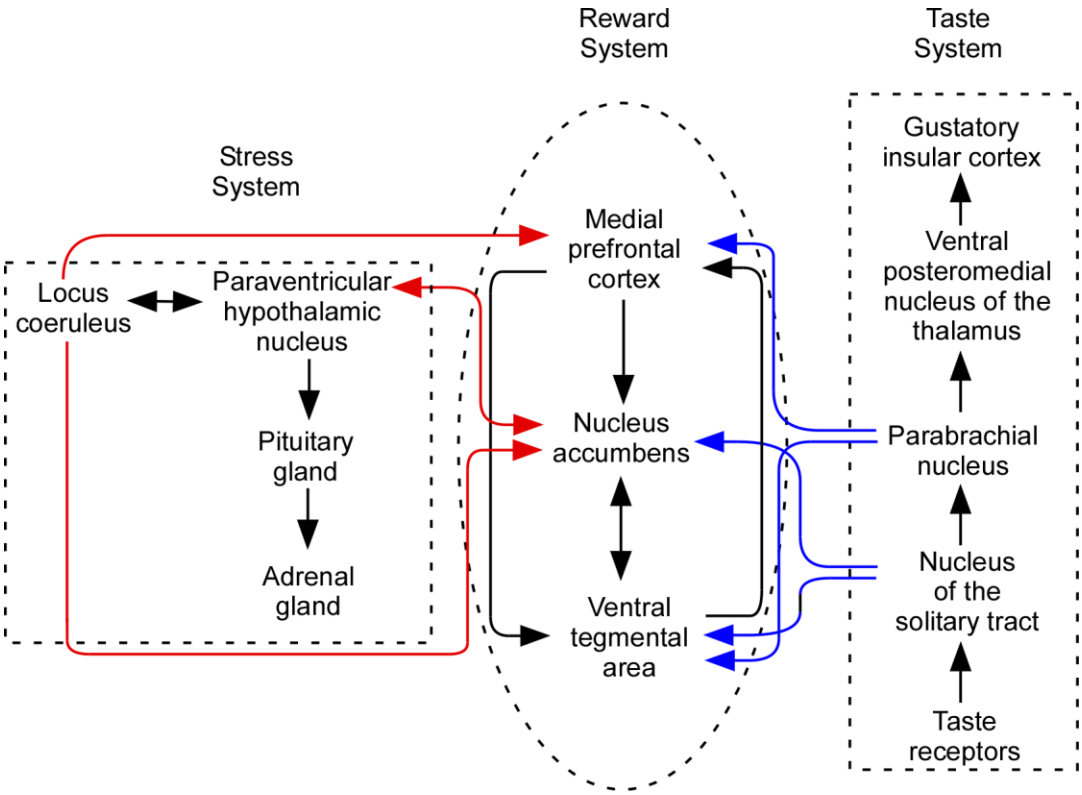

Figure 1 - 8 Interaction between the reward, stress, and taste systems.

The brain reward system receives projections from and sends projections to the regions in the stress and taste systems. Black arrows: Interaction with each system, Red arrows: Interaction between the stress system and the reward system, Blue arrows: Interaction between the taste system and the reward system.

Based on the earlier discussion of the systems that might be involved, one possibility of the interaction between these systems comes to mind. This hypothesis, seeks to give a general idea of the interaction between these systems. A stressful event in humans, results in the activation of the PVN neurons in the hypothalamus, leading to the release of CRF onto the anterior part of the pituitary gland. This CRF stimulates the pituitary gland to release ACTH onto the adrenal gland, stimulating it to release cortisol into the blood stream. As discussed earlier, the activation of the PVN results in a decrease in the activity of the prefrontal cortex (Figueiredo et al., 2003). The consumption of palatable food following stress, results in the activation of the taste receptors in the taste buds on the tongue. The changes in conformation of the receptors result in the subsequent release of the neurotransmitter ATP onto

neurons which form the facial, glossopharyngeal, and the vagus nerves (Ishimaru and Matsunami, 2009). These nerves then transmit the information to the NTS (Travers and Norgren, 1995), where it is relayed to the PBN (Norgren and Leonard, 1971). The NTS also targets neurons in the AcbSh (Delfs et al., 1998). The PBN sends projections to the VTA (Geisler et al., 2007). The activation of the VTA results in the release of dopamine into regions within the reward system including the mPFC and the Acb (Hajnal et al., 2004). The activation of the prefrontal cortex decreases activity of parvocellular neurons in the PVN since Figueiredo et al. (2003) reported that lesioning the prefrontal cortex leads to the expression of c-fos mRNA in the PVN. This might cause the reduction in the amount of CRF produced by PVN neurons, leading to a lesser stimulation of the pituitary gland which would result in a decrease in the amount of ACTH produced. The low level of ACTH might cause a lower stimulation of the adrenal gland leading to a decrease in the level of cortisol circulating in the blood. This may occur since it has been shown that consuming palatable foods reduces HPA axis activity and the plasma glucocorticoid level (Ulrich-Lai et al., 2007; Ulrich-Lai et al., 2010; Ulrich-Lai et al., 2011).

As mentioned earlier, this is just a hypothesis, and since none of the systems discussed works in isolation, the interaction between these systems is obviously more complex. However, this might give a general idea of how these systems interact normally in humans, and possibly animals as well.

1.6 Identification of regions implicated in binge-like eating in rats

Throughout the course of life, animals are continuously bombarded by stimuli from their external environment. In order to adapt to their environments, these stimuli are converted to intracellular signals in the brain through gene expression. Immediate early genes (IEGs), such as *c-fos*, zinc finger protein 225 (*Zif268*), and activity regulated cytoskeleton-associated protein (*Arc*), are transiently expressed in response to acute external stimuli, but rapidly degrade and return to basal levels once the stimuli cease. These IEGs encode transcription factors including Jun and Fos (Herdegen and Leah, 1998). Upon translation of transcription factor proteins in the cytoplasm, they are translocated back into the nucleus (Silver, 1991), where they

dimerize with other transcription factors, subsequently binding to target sites on promoters and enhancers to initiate the expression of target genes (Herdegen and Leah, 1998). In response to persistent stimuli, other transcription factors which mediate long-term neuronal plasticity are expressed in the brain. One such transcription factor is deltaFosB (ΔFosB), which is expressed in neurons in response to chronic stress (Perrotti et al., 2004), chronic treatments with drugs (Perrotti et al., 2008), and chronic sucrose consumption (Wallace et al., 2008; Munoz-Escobar et al., 2019). ΔFosB is therefore considered to be an inducible transcription factor since it is expressed in response to an external stimulus (Herdegen and Leah, 1998).

1.6.1 DeltaFosB

In the Acb, the release of glutamate and dopamine has been shown to activate N-methyl-D-aspartate (NMDA) and D1-like receptors, respectively, on medium spiny neurons (Broussard, 2012). In the case of the NMDA receptors, their activation results in the influx of calcium into the neuron. This activates calcium-dependent kinases including calcium/calmodulin-dependent kinases (Bito et al., 1996) and mitogen-activated protein kinases (Dolmetsch et al., 2001). These kinases in turn activate c-AMP response element-binding protein (CREB) (Bito et al., 1996), a constitutive transcription factor, meaning that it exists prior to the occurrence of an external stimuli (Herdegen and Leah, 1998). CREB is subsequently phosphorylated by protein kinase A, for G-protein coupled receptors such as D1-like receptors (Gonzalez and Montminy, 1989), or calcium/calmodulin, for NMDA receptors (Dash et al., 1991). CREB then becomes associated with the CREB binding protein, resulting in the acetylation and activation of genes including the *c-fos and fosB*. Following repeated stimulation, ΔFosB protein expression is upregulated (Figure 1–9), while c-fos protein is downregulated (Renthal and Nestler, 2009).

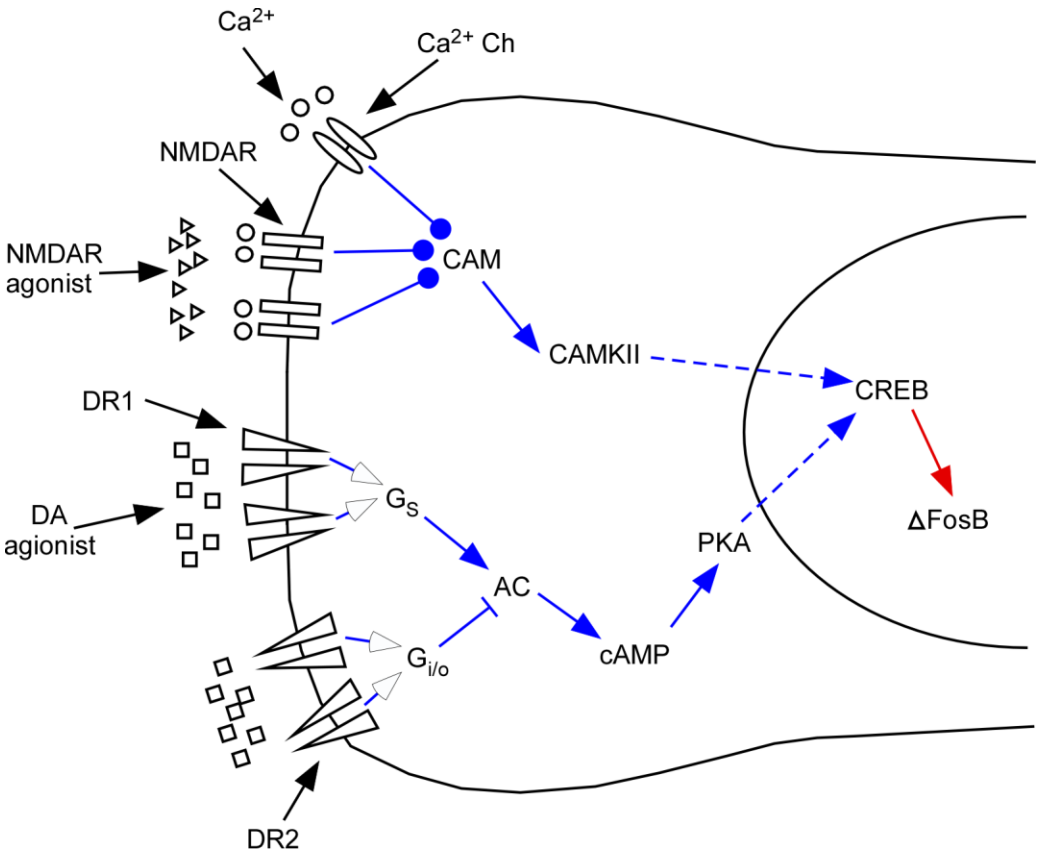

Figure 1 - 9 DeltaFosB expression in a neuron in response to NMDA and dopamine receptor agonists.

Upon the release of neurotransmitters onto a neuron in response to palatable food consumption, a number of signaling pathways are activated subsequently leading to an increase in the expression of deltaFosB. DR1: D1-like dopamine receptor, DR2: D2-like dopamine receptor, NMDAR: N-methyl-D-aspartate receptor, CAM: calmodulin, CAMKII: Calcium calmodulin-dependent protein kinase II, cAMP: Cyclic adenosine monophosphate, PKA: Protein kinase A, AC: Adenylyl cyclase, CREB: cAMP-response element binding protein, Ca^{2+}: Calcium, Ca^{2+}Ch: Calcium ion channel, $G_{i/o}$: $G_{i/o}$ protein-coupled receptor, G_s: G_s protein-coupled receptor. Blue open arrow: Activation of G proteins, Blue dashed arrow: Phosphorylation, Blue filled arrow: Activation/interaction, Blue arrow with round head: Interaction between calcium and CAM. Red filled arrow: ΔFosB expression. Adapted from https://en.wikipedia.org/wiki/FOSB.

ΔFosB, a truncated version of FosB, is a member of the Fos family of transcription factors. It is 101 amino acids shorter than the FosB protein and has a molecular weight ranging from 33 to 37 kD. While the 33-35 kD isoforms of ΔFosB degrade rapidly, the 37 kD isoform is very stable and has been shown to be present in neurons more than one month after last exposure to a stimulus (Nestler et al., 2001). This high stability of ΔFosB *in vivo* was shown to be due to the lack of the degrons at the C-terminus which regulate protein degradation (Carle et al., 2007) and the phosphorylation of ΔFosB at serine 27 by casein kinase 2 (Ulery et al., 2006).

The expression of ΔFosB may represent tolerance to persistent stimulation by causing the reduction of the responsiveness of neurons to these stimulations (Nestler et al., 1999). ΔFosB expression in the Acb resulted in an increase in the response to (Olausson et al., 2006) and consumption of (Wallace et al., 2008) reward. Its expression in neurons in the ventral striatum has been shown to result in a decrease in the responsiveness of neurons to stimulation. Being a transcription factor, ΔFosB regulates the expression of several genes including Ca^{2+}/calmodulin-dependent protein kinase II (CAMKII), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptor sub-unit 2 (GluR2), N-methyl-D-aspartate (NMDA) Z1, and glutamate decarboxylase (GAD) (McClung and Nestler, 2003). ΔFosB overexpression in MSN neurons resulted in the increased expression of GluR2 (Figure 1–10). AMPA receptors containing these GluR2 subunits were less permeable to calcium and had lower receptor conductance, compared to GluR2-lacking AMPA receptors, suggesting that neurons which express ΔFosB are less excitable compared to non-ΔFosB-expressing neurons in the ventral striatum (Vialou et al., 2010). Furthermore, ΔFosB overexpression in CA1 neurons in the hippocampus is correlated with increased interspike interval, decreased spike amplitude, decreased number of spikes generated, and increased rheobase of ΔFosB-expressing neurons. In the CA1, it was suggested that ΔFosB operates by regulating NMDA receptor expression since its inhibition resulted in a decrease in the NMDA current (Eagle et al., 2018).

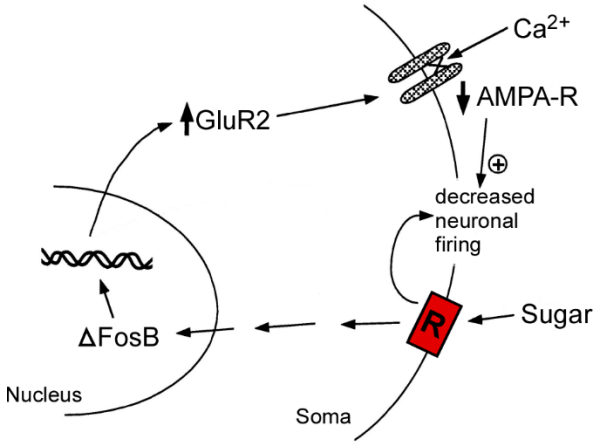

Figure 1 - 10 Effect of deltaFosB expression on nucleus accumbens neuron activity.

Repeated sucrose consumption results in the expression of deltaFosB in the neuron. This leads to the upregulation of the GluR2 receptor subunit of the AMPA receptor. In the end, overall AMPA current and calcium current is decreased, leading to a reduction in the firing of the neuron. R: Receptor, AMPA-R: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, Ca^{2+}: Calcium. Adapted from Nestler et al. (2001).

1.7 Methodological consideration

1.7.1 Why 10% sucrose solution?

As discussed earlier, people with BED usually binge on palatable foods. These foods are usually high in sugar and fat content (Hadigan et al., 1989; Guertin and Conger, 1999). These foods are usually preferred probably because of their ability to mitigate the effect of a stress response. In order to avoid any confounding effect from the consumption of foods that are high in both fat and sugar, the palatable food chosen for the series of experiments involving rats in this research project is 10% sucrose, since fat and sugar are different chemically and may have different effects (Avena et al., 2009). Additionally, when rats consume solid foods, they break these foods into crumps while eating. This makes it difficult to accurately quantify how much of the solid food they consumed. This can result in inaccurate conclusions especially in experiments which require precise quantification of food consumed. Moreover, in electrophysiological experiments, chewing during consumption of food can introduce artefacts in the signal being recorded (Hernan et al., 2017). In order to avoid all these

issues, 10% sucrose solution was the palatable food of choice. Both humans and rodents have been shown to have a preference for 10% sucrose solutions (Mc and Eaton, 1947; Perez et al., 1994). For example, most of the sweetened beverages consumed contain between 8 and 12% sugar (Ventura et al., 2011). Therefore, we believe that using sucrose as the palatable food of choice will help tease out the neurobiological underpinnings of binge eating disorder.

1.7.2 Licking microstructure of rats during sucrose solution consumption

In experiments involving rats, sucrose solution is used for two main reasons: 1) It encourages rats to approach and consume the sucrose solution when they are placed in the operant chamber and 2) It motivates rats to continue consuming the sucrose solution after its tongue has made contact with the spout (Davis and Smith, 1992).

Rats consume sucrose solution through licking. The licking of fluids is characterized by rhythmic tongue and jaw movements controlled by the central pattern generator (Travers et al., 1997), located in the lateral medullary reticular formation (Chen et al., 2001). The pattern of licks exhibited by rats depends on several factors including the occurrence of stress (Vajnerova et al., 2003) and the palatability of the fluid which is dependent on the type of liquid, the concentration of the solution, and the motivation of the rat to drink the liquid (Weiss and Di Lorenzo, 2012). In the laboratory, it can also be affected by how accessible the drinking spout is (Hernandez-Mesa et al., 1985).

Rats ingest fluids in clusters or bouts comprising of licks at a frequency of approximately 7 Hz (Davis, 1989). A burst is defined as a group of licks separated by an interlick interval (ILI) less than 250 ms, and a cluster is a group of licks separated by ILIs less than 500 ms. The ILI is the interval between two individual licks. Interburst interval (IBI) is the interval between bursts (250 ms ≤ ILI ≤ 500 ms), while the intercluster interval (ICI) is the interval between clusters (ILI > 500 ms) (Davis and Smith, 1992).

In the study by Davis and Smith (1992), where they analyzed the licking pattern in response to varying concentrations of sucrose solution, they reported that the size of the lick clusters (number of licks in clusters) reflects the palatability of the sucrose solution to the rats. They showed that the cluster sizes increased when the concentration of the sucrose increased and vice versa. Additionally, another characteristic of the lick pattern which reflects palatability is the duration of the licking. Similarly, as the concentration of the sucrose solution increases, the duration of continuous licking increases. They observed that during licks of long durations, the tongue of rats usually remained in contact with the spout longer, probably because of the concentration of the sucrose solution. Other studies have also shown that a high number of lick clusters reflect greater motivation to consume sucrose (Calvez and Timofeeva, 2016; Johnson, 2018).

1.7.3 Why use foot-shock as a stressor?

Yerkes and Dodson (1908) were the first to report the relationship between a stimulus and the rapidity of habit formation. Following this report, a number of research groups have used different stimuli to induce stereotypic behaviors in animals (O'Kelly and Steckle, 1939; Richter, 1949) for the study of human diseases including anxiety, depression, and post-traumatic stress disorder (Glanzman et al., 1989; Fendt, 2000; Shimizu et al., 2004).

In the field of stress, several types of stimuli (stressors) have been used to develop animal models of human diseases. These stressors include immobilization, exposure to cold and hot temperatures, exposure to loud noise, exposure to bright light, and handling. However, animals exhibit adaptation to these types of stressors but not to foot-shock stress (Grissom and Bhatnagar, 2009). Therefore, we adopted this form of stress for the development of our binge-eating rat model. An advantage of foot-shock is that its intensity, frequency, and duration can be precisely regulated compared to the other types of stressors (Bali and Jaggi, 2015b).

As discussed earlier, foot-shock stress effectively activates the HPA axis and the sympathetic adrenomedullary system leading to the release of glucocorticoids and

catecholamines (norepinephrine) into the blood stream (Bali and Jaggi, 2015a). In the studies discussed in this thesis, foot-shock stress was only used to generate the rat model of binge-like eating and occurred only three times during the stress sessions as described by Calvez and Timofeeva (2016). The parameters of the foot-shock including its intensity (0.6 mA), frequency (four times), inter-shock interval (15 s), and duration (3 s) are similar to that described by Hagan et al. (2002).

1.7.4 Extracellular electrophysiology

Several techniques have been developed to study the activity of neurons within the brain in response to several stimuli. These include the use of imaging techniques such as magnetic resonance imaging, computed tomography, and positron emitted tomography. These methods are especially very useful for studying whole brain activity non-invasively in humans and animals. However, to study the activity of neurons on a single-neuron resolution more invasive techniques are necessary including intracellular and extracellular recordings of neuronal activity. Among these invasive techniques, the technique selected depends on the type of question that is to be investigated. If the question involves studying neuronal activity in a non-behaving animal, the most appropriate technique might be the intracellular recording of neurons. This may be done in a slice preparation, in an anesthetized animal, or a head restrained animal. Extracellular recordings can also be made in slices, head restrained, and anesthetized animals. However, to study neuronal activity at a single neuron-resolution in an awake behaving animal, the best option might be the recording of single unit activity in these rats in response to a specific stimulus.

In the study described in chapter 2, the technique used was the recording of single-unit activity in awake behaving rats. This was because the aim of the study was to investigate the activity of neurons in structures in the reward system including the mPFC, Acb, and VTA, while the animal was consuming sucrose solution. This study involved the implantation of tetrodes into the regions of interest during surgery.

1.8 Problem definition

Among the main EDs described in the DSM-5, the most prevalent is BED. However, BED is understudied and there still remains several gaps in our understanding of the underlying neurobiological mechanisms which lead to its development and maintenance. This makes it difficult to develop treatments for BED. Additionally, BED is generally more prevalent in females compared to males. However, most of the studies which have been published in the literature have involved the use of male rodents as animal models, (Corwin, 2004a; Corwin et al., 2016; Anastasio et al., 2019), and data from male humans (Swanson et al., 2011; Sweeting et al., 2015; Hammerle et al., 2016), which may produce inconsistent results that may be different from the actual situation in human female BED patients. In fact, differences in binge eating patterns have been identified in male and female rats, which further supports the need to study binge eating in females, instead of males (Klump et al., 2013). Moreover, to study BED, using a rodent BED model is necessary. However, some studies used normal, non-bingeing rodents to study BED, which might result in discrepancies in the available literature on the underlying neurobiological underpinnings of BED.

In humans, several imaging studies have reported contradictory results regarding the regions in the brain that are implicated during BED. While some studies have suggested that bingeing decreases the rewarding value of the palatable food consumed, which leads to the consumption of an even larger amount of the food to produce the same effect, suggesting decreased reward sensitivity (Smail-Crevier et al., 2018), others suggest that it is rather the opposite situation in bingeing individuals, that is, bingeing causes hypersensitivity to rewards (Wojnicki et al., 2010; Simon et al., 2016). Some studies also reported a decrease in activity in regions including the mPFC, Acb, and VTA (Balodis et al., 2013; Balodis et al., 2013a; Balodis et al., 2014; Reiter et al., 2017), while others reported an increase in activity in these regions (Karhunen et al., 2000; Filbey et al., 2012; Lee et al., 2017). As much as fMRI studies are helpful in studying BED, they provide information on the

overall activity of neurons in specific regions of the brain. We therefore still lack information concerning the neuronal subtypes implicated during BED.

Most studies conducted so far investigated individual regions within the brain reward system (Krause et al., 2010; van Zessen et al., 2012; Jennings et al., 2013). However, since BED involves the consumption of palatable food, implicating the entire reward system, studying the activity simultaneously in more than one region might provide more useful information on how these regions interact to produce this eating disorder.

1.8.1 Hypotheses and objectives

For this research project I hypothesized that the reward, taste, and stress systems are implicated in binge eating disorder and that differences in activity in brain regions within the reward system underlie at least some of the characteristics exhibited by binge eating disorder patients.

The main objectives of my research project are to elucidate the role of the reward system in binge eating disorder and to identify possible differences in activity in the reward system during binge eating disorder using a binge-like eating female rat model.

Study 1: Differential expression of deltaFosB in reward processing regions between binge eating prone and resistant female rats

Hypotheses

1. Regions in the reward system, stress system, and taste system are implicated in the development of binge eating disorder.
2. Activity in these regions are different in the two female rat phenotypes developed to study binge eating disorder.

Objectives

1. To identify regions of the brain which are implicated in binge eating disorder

2. To identify possible differences in activity in these regions in the two phenotypes
3. To identify subtypes of neurons implicated in the regions which show differences in activity in the two phenotypes

Study 2: Neuronal activities during palatable food consumption in the reward system of binge-like eating female rats

Hypotheses

1. Neuronal activity in the reward system is decreased in binge eating prone rats compared to binge eating resistant rats.
2. Changes in activity in the reward system in binge eating prone rats might underlie the sense of relief expressed during bingeing.

Objectives

1. To investigate the real-time activity of neurons in the reward system before and during sucrose consumption in binge eating prone and resistant female rats
2. To investigate acute effects of palatable food consumption on neuronal activity in the reward system during sucrose consumption
3. To investigate possible differences in population activity of neurons in regions in the reward system between binge eating prone and resistant female rats

Chapter 1 Differential expression of deltaFosB in reward processing regions between binge eating prone and resistant female rats

2.1 Résumé

Le trouble de la frénésie alimentaire (TFA) est très répandu; cependant, sa physiopathologie est inconnue. Nous voulions identifier les régions et types de neurones impliqués dans le développement du TFA en quantifiant les neurones exprimant ΔFosB chez les rats sujets (BEP) et résistants (BER) au TFA. Le nombre de neurones ΔFosB+ était plus élevé chez les rats BEP dans le cortex préfrontal médian (mPFC), le noyau accumbens (Acb), l'aire tegmentale ventrale (VTA) et le noyau paraventriculaire, mais similaire dans le cortex insulaire, le noyau parabrachial et le locus coeruleus. Dans le mPFC et l'Acb, les proportions de neurones GABAergiques et non-GABAergiques / ΔFosB+ étaient similaires pour les deux phénotypes. Dans la VTA, la proportion de neurones dopaminergiques exprimant ΔFosB était similaire, mais celle de neurones GABAergiques-ΔFosB+ était plus élevée chez les rats BER. Nos résultats suggèrent que les régions de traitement des récompenses sont importantes pour le développement du TFA.

2.2 Abstract

Binge eating disorder (BED) is characterized by bingeing and compulsivity. Even though BED is the most prevalent eating disorder, little is known about its pathophysiology. We aimed to identify brain regions and neuron subtypes implicated in the development of binge-like eating in a female rat model. We separated rats into binge eating prone (BEP) and binge eating resistant (BER) phenotypes based on the amount of sucrose they consumed following foot-shock stress. We quantified deltaFosB (ΔFosB) expression to assess chronic neuronal activation during phenotyping. The number of ΔFosB-expressing neurons was: 1) higher in BEP than BER rats in reward processing areas (medial prefrontal cortex (mPFC), nucleus accumbens (Acb), and ventral tegmental area (VTA)); 2) similar in taste processing areas (insular cortex and parabrachial nucleus); 3) higher in the paraventricular nucleus of BEP than BER rats, but not different in the locus coeruleus, which are stress processing structures. To study subtypes of ΔFosB-expressing neurons in the reward system, we performed *in situ* hybridization for glutamate decarboxylase 65 and tyrosine hydroxylase mRNA after ΔFosB immunohistochemistry. In the mPFC and Acb, the proportions of gamma-aminobutyric acidergic (GABAergic) and non-GABAergic ΔFosB-expressing neurons were similar in BER and BEP rats. In the VTA, while the proportion of dopaminergic ΔFosB-expressing neurons was similar in both phenotypes, the proportion of GABAergic ΔFosB-expressing neurons was higher in BER than BEP rats. Our results suggest that reward processing brain regions, particularly the VTA, are important for the development of binge-like eating.

2.3 Introduction

Eating disorders, namely Anorexia Nervosa (AN), Bulimia Nervosa (BN), and Binge Eating Disorder (BED), cause severe disturbances to eating habits. Hudson et al. (Hudson et al., 2007) reported a lifetime prevalence rate of 0.6% for AN (0.9% of women and 0.3% of men), 1% for BN (1.5% of women and 0.5% of men), and 3% for BED (3.5% of women and 2.0% of men), which suggests that females are more prone to eating disorders than males and that BED is the most prevalent eating disorder (Kessler et al., 2013). Despite this high prevalence, the pathophysiology of BED is still poorly understood (Sinclair et al., 2015).

BED is characterized by eating a large amount of palatable food than would normally be consumed in a discrete amount of time and a loss of sense of control during the bingeing episode (American Psychiatric Association, 2013). Bingeing episodes are usually triggered by stressful events (Wolff et al., 2000). While a number of neuroimaging studies, using functional magnetic resonance imaging in humans, showed that BED is associated with an increase in fMRI activity in reward processing brain regions (Karhunen et al., 2000; Schafer et al., 2010; Tanofsky-Kraff et al., 2013; Lee et al., 2017), others reported a decrease in fMRI activity in similar regions (Filbey et al., 2012; Balodis et al., 2013; Halpern et al., 2013; Balodis et al., 2014; Reiter et al., 2017). It is therefore unclear whether BED is associated with an increase or a decrease in neuronal activity in these regions.

To study BED, rodent models developed using intermittent access to palatable food and either food restriction or stress, or both, were proposed (Corwin and Babbs, 2012). Even though each model has its strengths and weaknesses, none of these BED models meets all the criteria defined in the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM V). Since BED involves the consumption of palatable food and is triggered by a stressful event, we recently developed a binge-like eating rat model using intermittent access to sucrose solution and foot-shock stress without food restriction, resulting in binge eating prone (BEP; 30% of rats) and binge eating resistant (BER; 30% of rats) rat phenotypes (Calvez and Timofeeva, 2016).

We hypothesized that reward-, taste-, and stress- mediating brain regions are involved in the development of the binge eating phenotype. The goal of the study was to identify brain regions implicated in the development of binge-like eating, possible differences in neuronal activity between BEP and BER rats in these regions, and the neuron types implicated in these regions. Most studies used c-fos expression to evaluate the effect of acute neuronal stimulation by palatable food consumption in binge-like eating rodents (Bello et al., 2009; Sinclair et al., 2015). However, c-fos is transiently expressed and degrades rapidly (Herrera and Robertson, 1996). We therefore opted for deltaFosB (ΔFosB) because it persists for long periods of time due to its high stability (Nestler et al., 2001) and would last throughout the phenotyping period in our study. ΔFosB accumulates in neurons after chronic stress (Perrotti et al., 2004), chronic treatment with drugs (Cunningham et al., 2008; Perrotti et al., 2008), and chronic sucrose consumption (Wallace et al., 2008). Since binge eating involves the consumption of palatable food and is triggered by stress, our aim was to analyze ΔFosB expression in brain regions which process reward (medial prefrontal cortex (mPFC), nucleus accumbens (Acb), and ventral tegmental area (VTA) (Richard et al., 2013b)), taste (parabrachial nucleus (PBN) and insular cortex (IC) (Lundy and Norgren, 2004)), and stress (paraventricular hypothalamic nucleus (PVN) and locus coeruleus (LC) (Ziegler et al., 1999)). The results showed that reward processing brain regions are implicated in the development of binge-like eating. We also found that the VTA is the main reward processing region with differential neuronal activation in BEP and BER rats.

2.4 Materials and Methods

All experiments were performed in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Université Laval Committee on Ethics and Animal Research.

2.4.1 Animals

Forty naïve 45-day-old female Sprague-Dawley rats (body weight: 151-175 g) were purchased from the Canadian Breeding Laboratories (St-Constant QC, Canada) for

this study. Each rat was housed individually and maintained on a 12-hour light/dark cycle with the dark cycle starting at 14:00 h in a housing facility with an ambient temperature of 23 ± 1 ºC. Unless otherwise stated, all rats had ad libitum access to tap water and standard rat chow (2018 Teklad Global 185 Protein Diet; 3.1 kcal/g, Harlan Teklad, Montreal, QC). We allowed seven days for acclimatization of rats to the environmental conditions followed by 24-hour access to 10% sucrose solution, one week before the start of experiments, to prevent neophobia to the taste of sucrose solution.

2.4.2 Generation of binge-like eating rat phenotypes

We generated the binge-like eating rat phenotype as described in a previous study (Calvez and Timofeeva, 2016). Briefly, naïve female Sprague-Dawley rats were given one-hour ad libitum access to 10% sucrose solution (non-stress session), just at the start of the dark phase, until sucrose consumption was considered stable. This usually requires four to five accesses to 10% sucrose solution. The interval between any two non-stress sessions was at least two days. Afterwards, they underwent three stress sessions which consisted of an unpredictable foot-shock stress immediately followed by one-hour access to sucrose solution. Each foot-shock stress comprised of four foot-shocks with a direct current of 0.6 mA, lasting for 3 s. The equipment for delivering the foot-shock comprises a chamber with a metal grid floor through which electrical current was sent. The inter-shock interval was 15 s. Consecutive stress sessions were separated by at least three days. Since this is a stress-induced binge-like eating model, only consumption of sucrose during stress sessions was used to classify rats as either BEP or BER. For each stress session, using the tertile approach: rats considered high consumers were placed in the upper tertile, average consumers were placed in the middle tertile, and low consumers were placed in the lower tertile. Any rat which appeared at least twice in the upper tertile and never in the lower tertile was considered BEP, while BER rats were rats which appeared at least twice in the lower tertile and never in the upper tertile (Calvez and Timofeeva, 2016). In this model, the proportions of rats identified to be BEP, BER, and intermediate (rats considered neither BEP nor BER) are approximately 30%, 30%,

and 40%, respectively. In this study, 11 BEP and 12 BER female rats were obtained. They were subsequently divided into two cohorts (n = 6 and 5 for BEP, and n = 6/cohort for BER).

2.4.3 Test for compulsivity

A modified light/dark box was used to test compulsivity in the first cohort and this test was conducted according to a previously published study (Calvez and Timofeeva, 2016). It consists of a dark zone and a light zone. The light zone comprises a 30 cm x 30 cm box made of white Plexiglas while the black zone comprises a 30 cm x 30 cm box made of black Plexiglas. These two zones are connected by a 10-cm wide open door. The light zone was brightly illuminated with a light of 300 lx considered aversive to rats (Kaplan et al., 1965). The dark zone was covered with a lid to allow as minimum amount of light as possible to enter (<5 lx). In the light zone, rats had free access to a 10% sucrose solution in a pre-weighed bottle. The experiment was conducted during the dark phase. Rats were first placed in the light compartment facing the spout of the sucrose bottle. The duration of the test session was 10 minutes. In order to distinguish the activity of rats around the sucrose bottle from activity elsewhere in the light zone, a demarcation (14 cm x 8 cm) around the sucrose bottle, called the zone of sucrose, was made. Rats which, despite the obvious aversive light condition, consumed high amounts of sucrose were considered compulsive (Dalley et al., 2011). The sucrose bottle was weighted before and after the 10 minute-experiment to determine the quantity of consumed sucrose.

2.4.4 DeltaFosB immunohistochemistry

Three to four days after last access to sucrose solution, rats were anaesthetized using ketamine (160 mg/kg) and xylazine (20 mg/kg). After confirming that rats had no reflex upon pinching, they were intracardially perfused with 100 ml of ice-cold isotonic saline followed by 200 ml of 4% paraformaldehyde (PFA) solution. The rat brains were kept in 4% PFA at 4 ºC for one week. They were then transferred into 20% sucrose in 4% PFA overnight. Using a sliding microtome (Histoslide 2000,

Heidelberger, Germany), we cut 30-µm thick coronal sections of brains and kept them at –30 ºC in a sterile cryoprotecting solution made of sodium phosphate buffer (50 mM), ethylene glycol (30%), and glycerol (20%) until they were processed for immunohistochemistry.

The primary antibody used for ΔFosB immunohistochemistry in this study stains both FosB and ΔFosB, but since FosB is known to degrade with time leaving the shorter 37 kD ΔFosB isoform after chronic stimulation (Nestler, 2004), we can confidently say that only a minority of the detected staining were contributed by FosB, similar to the antibody used in other studies (Cunningham et al., 2008; Cunningham et al., 2012).

Brain sections were first washed in 1% potassium phosphate buffered saline (PPBS) solution followed by treatment with 30% H_2O_2 diluted in methanol (1:10). They were washed again in 1% PPBS and blocked for one hour in a solution comprising 0.4% Triton-X, 2% bovine serum albumin, and 1% PPBS. The sections were incubated overnight at 4 °C in the primary rabbit anti-ΔFosB antibody diluted in the blocking solution (sc-48; Santa Cruz Biotechnology, Santa Cruz, CA, 1:1000). The next day, sections were rinsed in 1% PPBS solution, followed by incubation for one hour at room temperature in 1:1500 biotinylated goat anti-rabbit immunoglobulin (Vector Laboratories Inc., Burlingame, CA) diluted in blocking solution. The sections were then rinsed and transferred into a complex of horseradish peroxidase (HRP)-avidin solution (Vector Laboratories Inc., Burlingame, CA) for one hour at room temperature. They were washed with 1% PPBS and then with tris-imidazole solution. To detect staining, a solution containing tris-imidazole, diaminobenzidine (DAB; 0.12 mg/ml), and 0.3% H_2O_2 was used. The sections were kept in DAB solution for 10 minutes, rinsed with PPBS, mounted on slides, and cover-slipped with DPX mounting medium.

2.4.5 Double-labeling for neuron subtypes

To study neurochemical subtypes of neurons which express ΔFosB, we used a glutamic acid decarboxylase 65 (GAD65) probe to identify GABAergic neurons and

a tyrosine hydroxylase (TH) probe to identify dopaminergic neurons in brains of the second rat cohort. *In situ* hybridization was performed as described previously (Mitra et al., 2011). Following ΔFosB immunohistochemistry, sections were mounted on poly L-lysine coated slides and left to dry overnight under vacuum. The sections were subsequently fixed in 4% PFA for 20 minutes, exposed to proteinase K (10 µg/ml in 100 mM Tris-HCl containing 50 mM ethylenediaminetetraacetic acid (EDTA), pH 8.0) for 25 minutes to break down contaminating proteins, acetylated with acetylate anhydride (0.25% in 0.1 M triethanolamine, pH 8.0), and dehydrated by exposure to ethanol solutions of increasing concentration (50, 70, 95, and 100 %). Afterwards, the slides were vacuum dried for at least two hours, followed by addition of 90 µl of the hybridization solution to the slides. This solution contains an antisense 35S-labeled cRNA probe against GAD65 or TH. Cover slips were placed on the slides followed by overnight incubation at 55 ºC. After removal of cover slips the following day, the slides were washed in standard saline citrate (SSC; 0.6 M, 60 mM trisodium citrate buffer, pH 7.0), and exposed for 30 minutes to RNase-A at 37 ºC (20 µg/ml in 10 mM Tris-500 mM NaCl containing EDTA). They were then washed in decreasing concentrations of SSC (2X, 10 minutes; 1X, 5 minutes; 0.5X, 10 minutes; 0.1X, 30 minutes at 60 ºC), followed by dehydration in graded concentrations of ethanol. After vacuum drying for two hours, the slides were defatted in xylene and later dipped in NTB2 nuclear emulsion. The slides were exposed for seven days and then developed in D19 developer for 3.5 minutes at 14-15 ºC. They were later fixed in rapid fixer (Eastman Kodak, Rochester, NY, USA) for five minutes. The slides were then washed for one hour under running water, followed by counterstaining with Thionin (0.25%) and dehydration in graded concentrations of ethanol. They were cleared in xylene and cover-slipped following application of DPX mounting medium.

2.4.6 Quantification of immunoreactive cells

In order to estimate the number of ΔFosB-positive cells in the various regions of interest, we used the Image-Pro Plus Software version 10.0 (Media Cybernetics, Silver Spring, USA). By comparing each brain section with the corresponding section

in the Paxinos rat brain atlas (Paxinos and Watson, 2009), the outlines of regions of interest which are relatively small in size (PVN, VTA, LC, and PBN) were made under the 20x objective of the Olympus BX61 microscope (Olympus Canada, Richmond Hill, ON Canada). For these regions, we analyzed the actual brain sections. For regions of interest which are relatively large in size (mPFC, Acb, and IC), sections were first scanned using the TISSUEScope 4000 scanner (Huron Digital Pathology, St. Jacobs, ON, Canada) to obtain high-quality images of sections for subsequent analysis. ΔFosB-expressing neuron quantification was performed automatically. To do this, the Image-Pro Plus software was used to identify objects within the regions of interest. Subsequently, the software was fine-tuned continuously by the experimenter until majority of the objects considered to be neurons in the region of interest were identified by the software. The parameters used were color, area (in pixels: 90–1500), and size (length: 10–90; width: 5–60). At this point, the value of each parameter was noted and applied to all sections containing regions of interest for analysis. The software was then used to automatically identify all similar objects and the number of objects identified was considered as the number of neurons obtained. To verify the results obtained with the automatic counting, we also performed manual cell counting on some brain sections. The results of both automatic and manual counting were similar. For each brain, the number of neurons identified to express ΔFosB was obtained by averaging the number of ΔFosB-expressing neurons per section in regions of interest in both hemispheres of the brain. The regions of interest were the mPFC (prelimbic (PrL) and infralimbic (IL) cortices; +3.72 mm to +2.72 mm from the bregma), Acb (core and shell; +2.28 mm to +0.96 mm), VTA (-4.80 mm to -5.04 mm), IC (+4.2 mm to +0.12 mm), PBN (medial and lateral parts; -8.88 mm to -9.24 mm), PVN (magnocellular and parvocellular parts; -1.72 mm to -1.92 mm), and LC (-9.60 mm to -9.96 mm).

To quantify double-labeled cells, all sections were scanned using the TISSUEScope 4000 scanner to obtain high-quality images of sections and the regions of interest (an example of a typical GAD/ΔFosB-labeled section is shown in Figure 2-1A). ΔFosB-expressing cells were then identified in all outlined regions of interest (Figure 2-1B), as previously described. The Image-Pro Plus software defined the

coordinates of all identified ΔFosB-expressing cells using the parameters Center X and Center Y. The coordinates of all cells were then exported into Excel files. Similarly, GAD or TH mRNA expression obtained by *in situ* hybridization, which appears as dark silver grains, were also identified based on specific parameters ((in pixels) area: 1–90; size (length): 1–20; size (width): 1–20; Figure 2-1C) and their coordinates were exported into Excel files. By using a custom-written MATLAB script, double-labeled cells were identified when there was an overlap of ΔFosB expression and mRNA expression at the same location as shown in Figure 2-1D. The least number of dark silver grains required for a cell to be considered as double-labeled was set to 5. In addition to the number of double-labeled cells, cells expressing ΔFosB only were also identified using the MATLAB script.

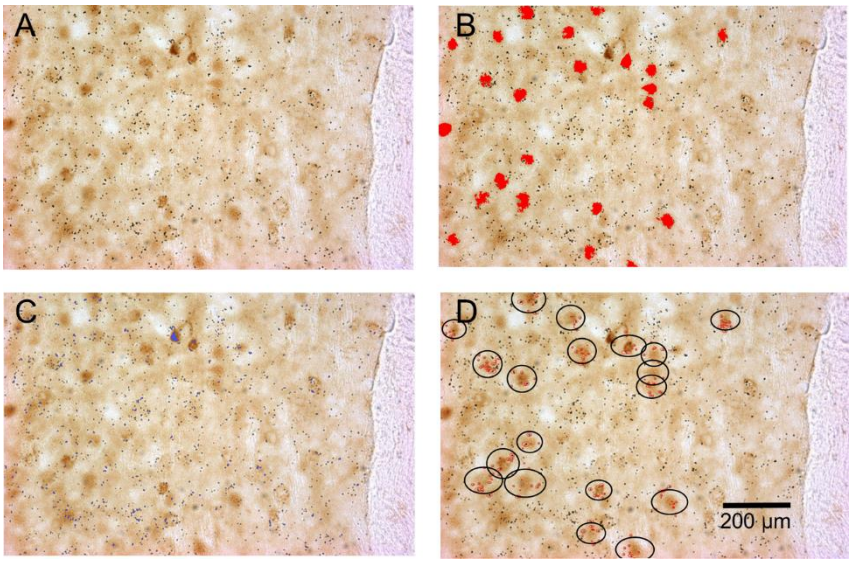

Figure 2 - 1 Identification of double-labeled cells (cells expressing both ΔFosB and GAD65 mRNA in this example) using the Image-Pro Plus software and a custom-written MATLAB script

A) An example of a scanned rat brain section showing ΔFosB-expressing cells (dark brown staining) and GAD65 mRNA expression (dark silver grains). B) Identified ΔFosB-expressing cells (red color) using the Image-Pro Plus software. C) Detected GAD65 mRNA expression (blue color) using the Image-Pro Plus software. D) Identified double-labeled cells using a custom-written MATLAB script. In the black circles are ΔFosB-expressing cells that contain more than five dark silver grains (red dots).

2.4.7 Statistical Analysis

The two-tailed, unpaired student's t-test was used to compare sucrose intake and time spent in the light zone, dark zone, and zone of sucrose between BEP and BER rats during the 10-minute light/dark box test. Additionally, the two-tailed, unpaired student's t-test was used to compare the difference in means of the number of ΔFosB-expressing and double-labeled (ΔFosB/GAD65 mRNA and ΔFosB/TH mRNA) neurons in the regions of interest in BEP and BER rats. The ordinary two-way analysis of variance (ANOVA) test followed by the Bonferroni post-hoc test to correct for multiple comparisons was used to compare the total quantities of sucrose solution consumed (dependent variable) by BEP and BER rats (independent variable 1) during sessions with and without foot-shock stress (independent variable 2). The interaction between these two independent variables was also assessed. Data are expressed as mean ± standard error of mean (SEM). Differences in means were considered significant when $p < 0.05$. The statistical tests were performed using GraphPad version 6.01 (GraphPad Software Inc., La Jolla CA, USA).

2.5 Results

2.5.1 Sucrose intake during phenotyping

Similar to the sucrose intake of BEP and BER rats generated in the study of Calvez and Timofeeva (Calvez and Timofeeva, 2016), during one hour sessions, BER rats consistently consumed smaller amounts of 10% sucrose solution compared to BEP rats both during non-stress and stress sessions (Figure 2-2). BEP rats increased their intake of sucrose solution after foot-shock stress, while the BER rats consumed similar amounts of sucrose solution both during sessions with and without foot-shock stress ($p > 0.9999$). There was an effect of phenotype ($F_{1,42} = 47.41$, $p < 0.0001$) and interaction between phenotype and treatment ($F_{1,42} = 5.611$, $p = 0.0225$), but not treatment ($F_{1,42} = 3.294$, $p = 0.0767$) on sucrose consumption in BEP and BER rats.

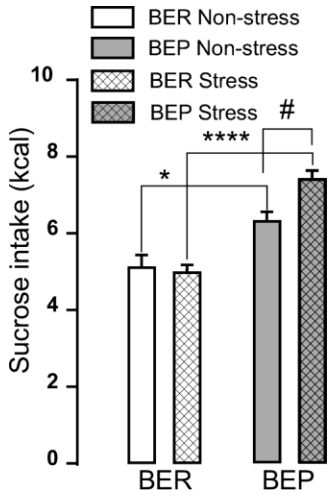

Figure 2 - 2 Sucrose intake during phenotyping.

The graph shows the 10% sucrose consumption in kilocalories by binge eating prone (BEP) and binge eating resistant (BER) rats during a one-hour access without foot-shock stress (non-stress) and after foot-shock stress (stress). Comparison between phenotypes - *: $p < 0.05$; ****: $p < 0.0001$; Comparison within phenotype - #: $p < 0.05$.

2.5.2 Compulsive sucrose consumption in BER and BEP rats

We used the light/dark box test (Figure 2-3A) to assess compulsivity in rats. Rats were allowed to explore the box for 10 minutes with *ad libitum* access to 10% sucrose solution in the light zone. BEP rats consumed more sucrose than BER rats during the 10-minute *ad libitum* access to sucrose in the light/dark box (Figure 2-3B). The zones of interest within the light/dark box were the dark zone, light zone, and zone of sucrose. BER and BEP rats spent similar amounts of time in both the light (Figure 2-3C) and dark (Figure 2-3D) zones of the box. However, BEP rats spent significantly more time within the zone of sucrose than BER rats (Figure 2-3E).

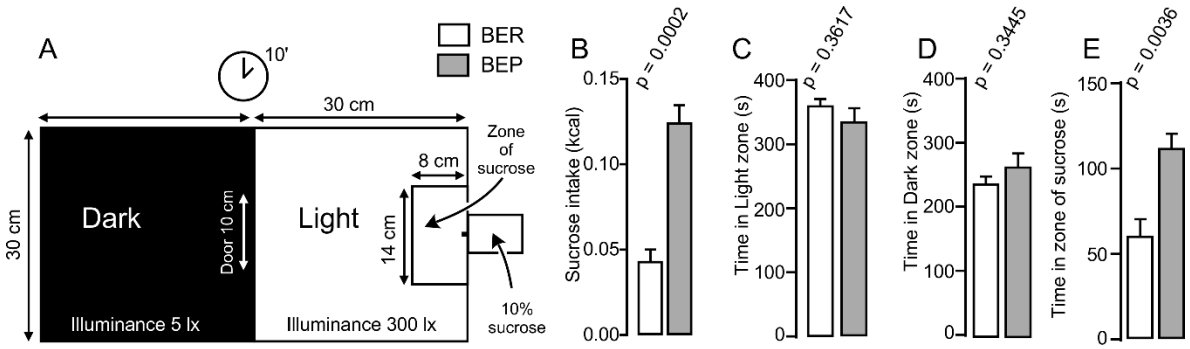

Figure 2 - 3 Light/dark box experiment.

A) An illustration of the modified light/dark box used during the behavioral experiment. B) Amount of sucrose in kilocalories consumed by binge eating resistant (BER) and binge eating prone (BEP) rats during the 10-minute session in the light/dark box. C) Amount of time in seconds spent by BER and BEP rats in the light zone of the light/dark box. D) Amount of time in seconds spent by BER and BEP rats in the dark zone of the light/dark box. E) Amount of time in seconds spent by BER and BEP rats in the zone of sucrose of the light/dark box. lx: luxes, **: $p < 0.01$, ***: $p < 0.001$.

2.5.3 ΔFosB expression in reward, taste, and stress systems

The number of ΔFosB-expressing cells in all investigated reward processing regions in BEP rats was significantly higher than that in BER rats (Figure 2-4). A significant difference in the number of ΔFosB-expressing cells was observed in the mPFC, with a higher number of ΔFosB-expressing cells in the PrL and IL of BEP rats compared to BER rats (Figure 2-4A~D). ΔFosB expression was significantly higher in BEP than in BER rats in the AcbC and AcbSh (Figure 2-4E~H). There were also more ΔFosB-expressing cells in the VTA of BEP rats compared to BER rats (Figure 2-4I~K).

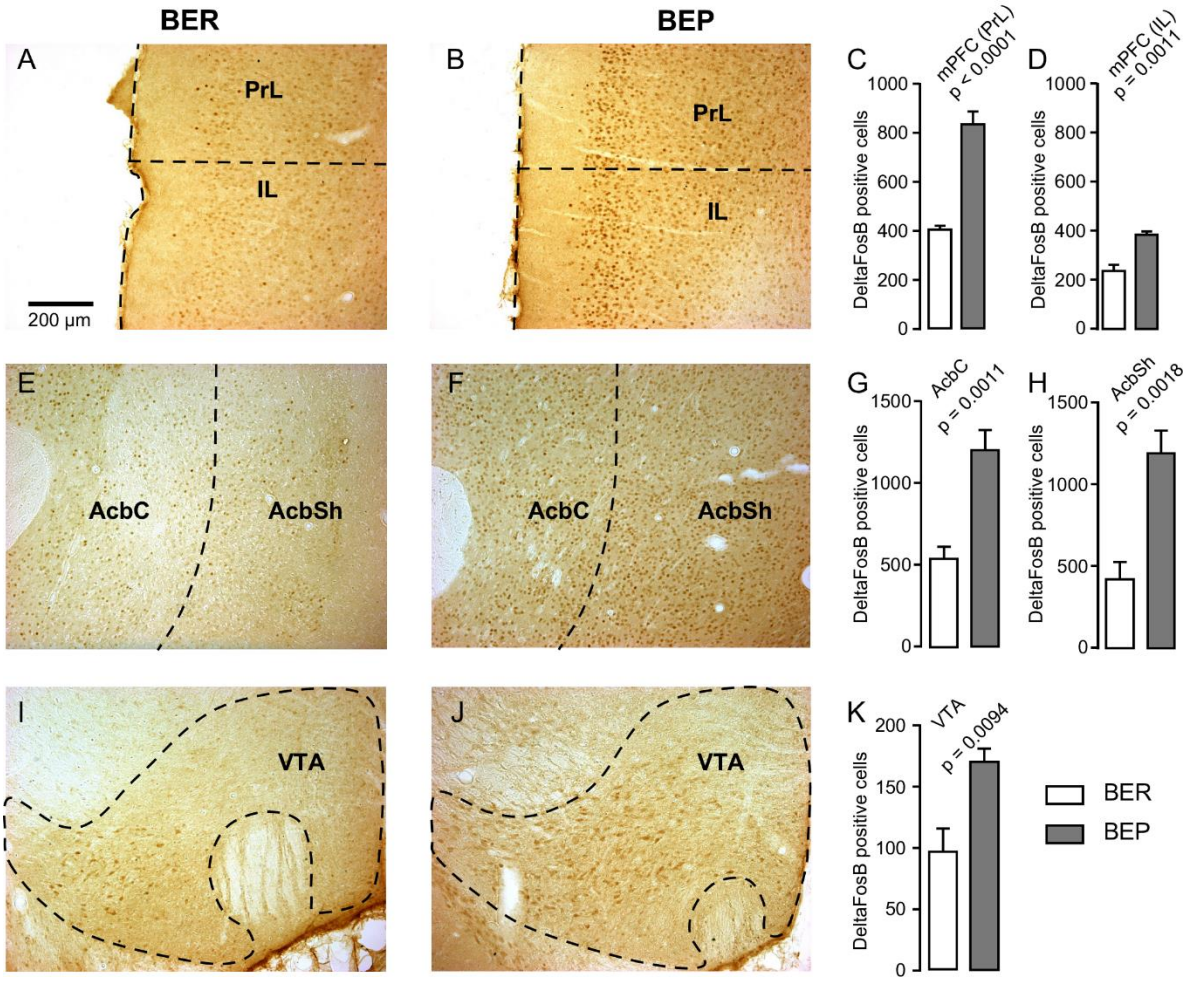

Figure 2 - 4 ΔFosB expression in neurons in reward processing regions.

A) and B) Images showing ΔFosB-expression in neurons in the prelimbic (PrL) and infralimbic (IL) cortices of the medial prefrontal cortex (mPFC) in binge eating resistant (BER) and binge eating prone (BEP) rats. Inset: A schematic showing the location from which the images in A and B were acquired. C) and D) The number of ΔFosB-positive cells in the PrL and IL of the mPFC in BEP and BER rats. E) and F) Images showing ΔFosB-expression in neurons in the nucleus accumbens core (AcbC) and shell (AcbSh) in BER and BEP rats. G) and H) The number of ΔFosB-positive cells in the AcbC and AcbSh in BEP and BER rats. I) and J) Images showing ΔFosB-expression in neurons in the ventral tegmental area (VTA) in BEP and BER rats. K) The number of ΔFosB-expressing cells in the VTA of BEP and BER rats. **: $p < 0.01$, ****: $p < 0.0001$. Scale bar: 200 µm.

ΔFosB expression was also analyzed in a subset of taste processing regions including the IC and PBN. Similar numbers of ΔFosB-expressing cells were identified in both the medial and lateral parts of the PBN in BEP and BER rats (Figure 2-5A~D), as well as in the IC (Figure 2-5E~G).

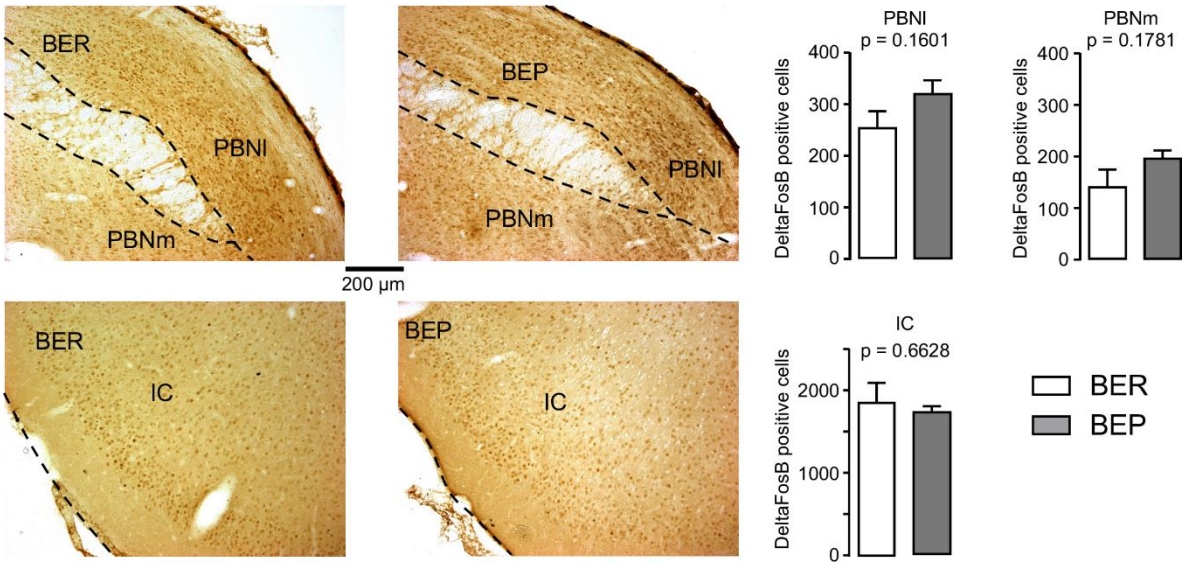

Figure 2 - 5 ΔFosB expression in neurons in taste processing regions.

A) and B) Images showing ΔFosB-expression in neurons in the lateral (PBNl) and medial (PBNm) parts of the parabrachial nucleus (PBN) of binge eating prone (BEP) and binge eating resistant (BER) rats. C) and D) The number of ΔFosB-positive cells in the PBNl and PBNm in BEP and BER rats. E) and F) Images showing ΔFosB-expression in neurons in the insular cortex (IC) of BEP and BER rats. G) The number of ΔFosB-positive cells in the IC of BEP and BER rats. Scale bar: 200 µm.

We also analyzed ΔFosB expression in two stress processing regions: the LC and PVN (Figure 2-6). Our analyses revealed that there was a significantly higher number of ΔFosB-expressing cells in both the magnocellular and parvocellular parts of the PVN of BEP rats (Figure 2-6A~D). However, the expression of ΔFosB in the LC of BEP and BER rats (Figure 2-6E~G) was similar.

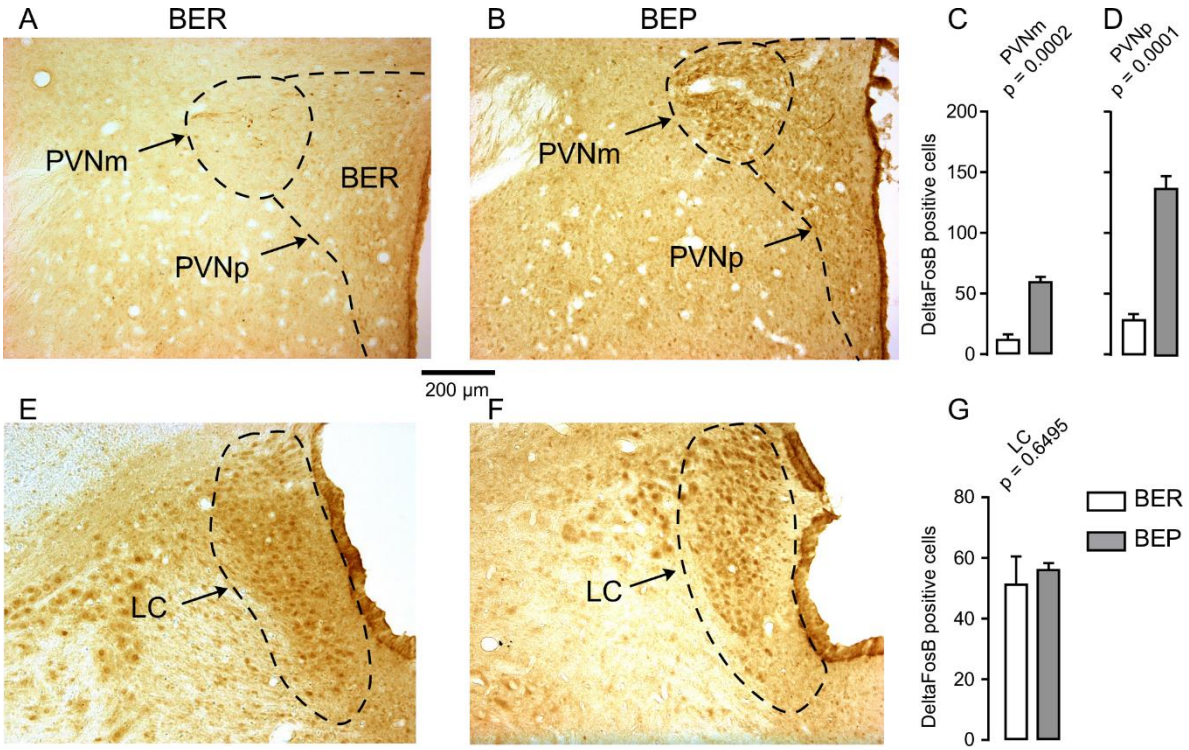

Figure 2 - 6 ΔFosB expression in neurons in stress processing regions.

A) and B) Images showing ΔFosB-expression in neurons in the magnocellular (PVNm) and parvocellular (PVNp) parts of the paraventricular nucleus of the hypothalamus (PVN) of binge eating prone (BEP) and binge eating resistant (BER) rats. C) and D) The number of ΔFosB-positive cells in the PVNm and PVNp in BEP and BER rats. E) and F) Images showing ΔFosB-expression in neurons in the locus coeruleus (LC) of BEP and BER rats. G) The number of ΔFosB-positive cells in the LC of BEP and BER rats. ***: $p < 0.001$. Scale bar: 200 µm.

These results show an increase in ΔFosB expression in reward processing areas and in one of the analyzed stress regions, but not in taste processing areas in BEP rats as compared to BER rats.

2.5.4 Neuronal types implicated in binge-like eating in reward processing regions of the brain

2.5.4.1 Nucleus Accumbens

About 95% of neurons in the Acb are GABAergic cells (Self, 2010). Therefore, we investigated whether the increase in ΔFosB expression during our phenotyping was due to the activation of these cells exclusively or other types of neurons. We found that the number of cells expressing both GAD65 mRNA and ΔFosB was higher in the AcbC (Figure 2-7A) and AcbSh (Fig 2-7C) in BEP compared to BER rats. For both phenotypes, the proportion of ΔFosB-expressing cells that also expressed GAD65 mRNA was 85-90% in the AcbC (Figure 2-7B) and AcbSh (Figure 2-7D), suggesting that ΔFosB expression occurred mainly in GABAergic cells but also in non-GABAergic cells in the Acb of both BEP and BER rats.

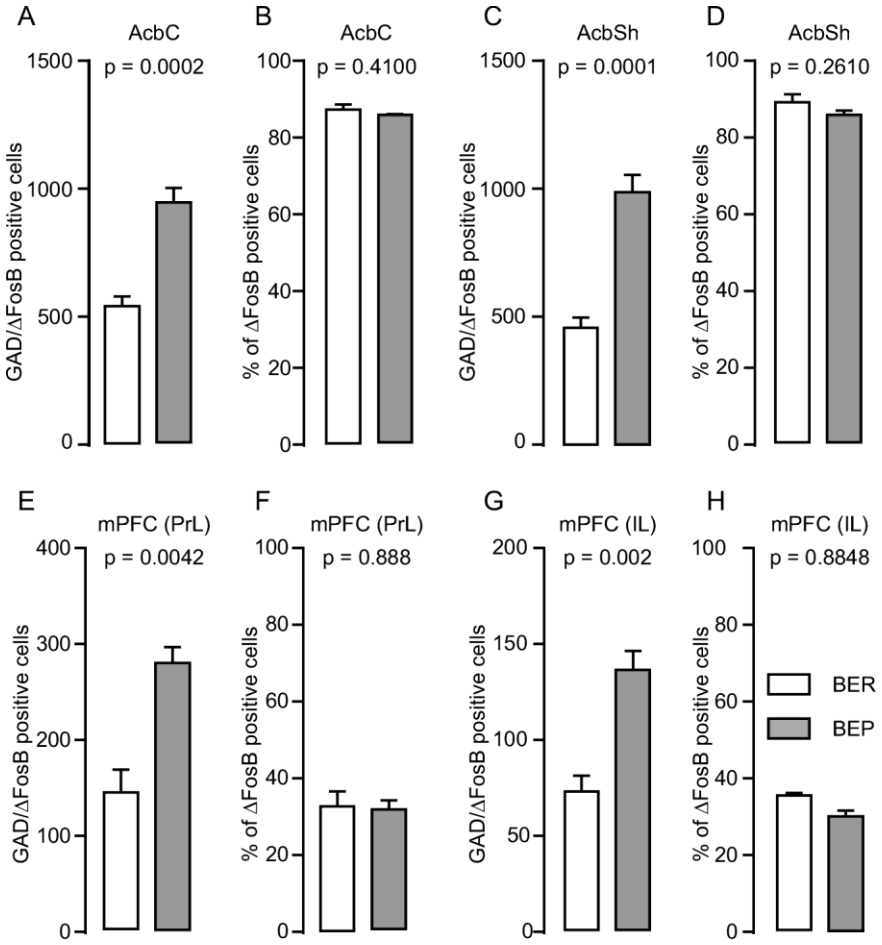

Figure 2 - 7 ΔFosB and GAD65 mRNA expression in neurons in the nucleus accumbens (Acb) and medial prefrontal cortex (mPFC) in binge eating prone (BEP) and binge eating resistant (BER) rats.

A) and B) The number and percentage of double-labeled cells (ΔFosB cells that express GAD65 mRNA) in the nucleus accumbens core (AcbC) in BEP and BER rats. C) and D) The number and percentage of double-labeled cells in the nucleus accumbens shell (AcbSh) in BEP and BER rats. E) and F) The number and percentage of double-labeled cells in the prelimbic cortex (PrL) of the medial prefrontal cortex (mPFC) in BEP and BER rats. G) and H) The number and percentage of double-labeled cells in the infralimbic cortex (IL) of the mPFC in BEP and BER rats. **: $p < 0.01$, ***: $p < 0.001$.

2.5.4.2 Medial Prefrontal Cortex

Gabbott et al. (Gabbott et al., 1997) reported that about 20% of mPFC neurons are GABAergic, while the remaining are glutamatergic. We found that a higher number of neurons co-expressed ΔFosB and GAD65 mRNA in the PrL of BEP compared to BER rats (Figure 2-7E). The findings were similar in the IL where more ΔFosB-expressing neurons also expressed GAD65 mRNA in BEP compared to BER rats (Figure 2-7G). The percentage of ΔFosB-positive neurons that expressed GAD65 mRNA were similar in the PrL (Figure 2-7F) and IL (Figure 2-7H) of both BEP and BER rats and was about 30% of the population of the ΔFosB-expressing neurons.

2.5.3 Ventral Tegmental Area

Majority of the neurons in the VTA (65%) are dopaminergic neurons, followed by GABAergic neurons which make up 30%, and then glutamatergic neurons which make up about 5% of the total neuron population (Nair-Roberts et al., 2008). In the VTA, ΔFosB-positive cells which also expressed GAD65 mRNA were observed (Figure 2-8A, B, D, and E). There was no difference in the number of ΔFosB-expressing cells which co-expressed GAD65 mRNA identified in BEP and BER rats (Figure 2-8C). Interestingly, the percentage of double-labeled cells in the VTA was significantly higher in BER rats compared to BEP rats (Figure 2-8F).

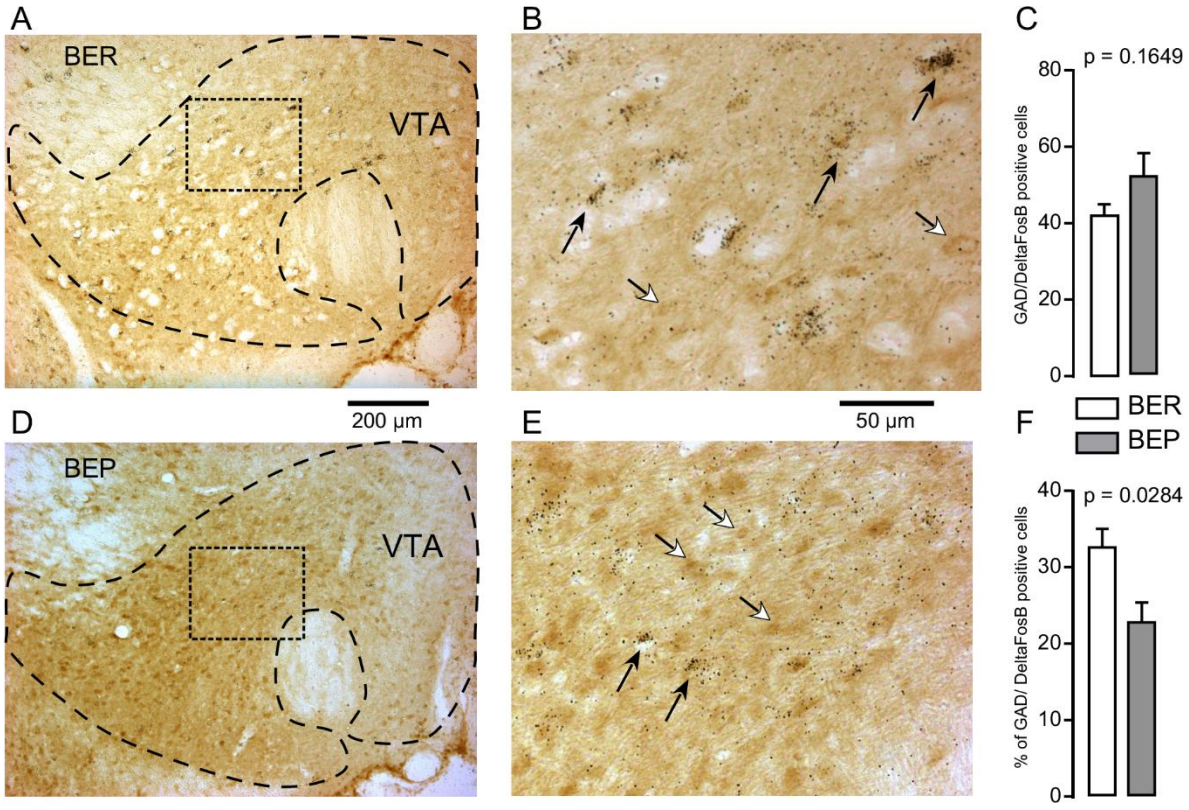

Figure 2 - 8 ΔFosB and GAD65 mRNA expression in neurons in the ventral tegmental area (VTA) in binge eating prone (BEP) and binge eating resistant (BER) rats.

A) and B) Images showing the labeling of ΔFosB (dark brown) and GAD65 mRNA (dark silver grains) in neurons in the VTA in BER rats. C) The number of ΔFosB/GAD65 mRNA-expressing cells in the VTA in BEP and BER rats. D) and E) Images showing the labeling of ΔFosB and GAD65 mRNA in neurons in the VTA in BEP rats. F) The percentage of double-labeled cells (ΔFosB cells that express GAD65 mRNA) in the VTA of BER and BEP rats. Black arrows point to double labeled neurons, white arrows point to ΔFosB only labelled cells. Scale bar: (A and C) = 200 µm, (B and D) = 50 µm.

Cells which co-expressed ΔFosB and TH-mRNA were observed in the VTA (Figure 2-9A, B, D, and E). The number of cells which expressed both ΔFosB and TH-mRNA

in the VTA of BEP rats was significantly higher than that in BER rats (p = 0.0430; Figure 2-9C). However, there was no difference in the percentage of ΔFosB-expressing cells which were also positive for TH mRNA (p = 0.2962; Figure 2-9F) in BEP and BER rats.

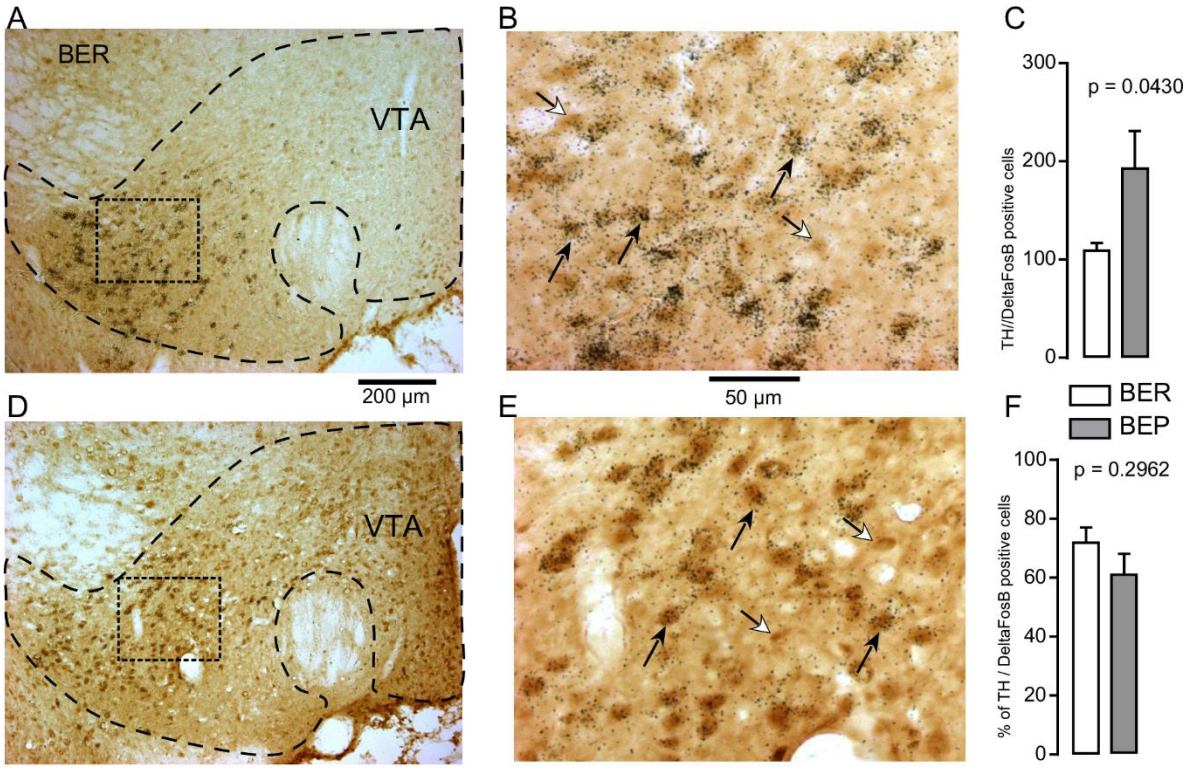

Figure 2 - 9 ΔFosB and TH mRNA expression in neurons in the ventral tegmental area (VTA) in binge eating prone (BEP) and binge eating resistant (BER) rats.

A) and B) Images showing the labeling of ΔFosB (dark brown) and TH mRNA (dark silver grains) in neurons in the VTA in BER rats. C) The number of ΔFosB/TH mRNA-expressing cells in the VTA in BEP and BER rats. D) and E) Images showing the labeling of ΔFosB and TH mRNA in neurons in the VTA in BEP rats. F) The percentage of double-labeled cells (ΔFosB cells that express TH mRNA) in the VTA of BER and BEP rats. Black arrows point to double labeled neurons, white arrows point to ΔFosB only labelled cells. Scale bar: (A and C) = 200 µm, (B and D) = 50 µm.

2.6 Discussion

BED involves the consumption of a large amount of palatable food and it is usually triggered by stress, which suggests that reward, taste, and stress processing brain regions may be involved (Wolff et al., 2000; American Psychiatric Association, 2013). To verify these hypotheses, we evaluated ΔFosB expression in neurons in these regions in our binge-like eating rat model (Calvez and Timofeeva, 2016). ΔFosB was expressed after repeated neuronal stimulation, and our binge-like eating rat model was developed using repeated accesses to sucrose and several foot-shock stresses. Our results show that the main brain regions implicated in BED are the reward processing regions (mPFC, Acb, and VTA). In BEP rats, the number of ΔFosB-positive neurons was higher in these regions than in BER rats. Additionally, even though the proportions of non-GABAergic and GABAergic neurons in the mPFC, GABAergic neurons in the Acb, and dopaminergic neurons in the VTA were similar in BEP and BER rats, the proportion of VTA GABAergic neurons involved in binge-eating development was different between the two phenotypes.

ΔFosB expression in the Acb resulted in a reduction in the activity of medium spiny neurons (Vialou et al., 2010). We observed high ΔFosB expression in the Acb of BEP compared to BER rats. This suggests that there was a significant reduction in the excitability of medium spiny neurons (Vialou et al., 2010) in the Acb of BEP compared to BER rats. In the literature, studies showed that a decrease in neuronal firing in the Acb induced an increase in food consumption whereas stimulation decreased it (Maldonado-Irizarry et al., 1995; Krause et al., 2010; O'Connor et al., 2015). It was also shown that inhibition of the Acb leads to an increase in the response to (Olausson et al., 2006) and consumption of (Wallace et al., 2008) a reward. Additionally, the brain stimulation of the Acb in mice alleviates binge eating (Halpern et al., 2013). The high ΔFosB expression in the Acb can be linked to the high sucrose intake observed in our BEP group.

It has been shown that ΔFosB expression in the hippocampus also decreases neuronal activity (Eagle et al., 2018). We therefore conclude that the activity of ΔFosB-expressing neurons in both the mPFC and VTA would also decrease, and

this decrease is greater in BEP than in BER rats. Our results extend the findings of neuroimaging studies which revealed a reduction of activity in the mPFC (Filbey et al., 2012; Balodis et al., 2013; Reiter et al., 2017), VTA (Bello and Hajnal, 2010; Cordeira et al., 2010), and Acb (Balodis et al., 2014) in BED patients. Furthermore, a decrease in mPFC activity is associated with compulsivity (Sarica et al., 2018). BED patients display compulsive behavior, which is associated with a loss of inhibitory control due to hypoactivity in the mPFC (Balodis et al., 2013; Reiter et al., 2017). Our light/dark box test showed that BEP rats spent more time in the zone of sucrose and consumed more sucrose than BER rats despite the exposure to the aversive light zone, as was previously shown (Calvez and Timofeeva, 2016). It is not surprising that rats consume more palatable food when it is available, but BEP rats consumed more sucrose despite the adverse condition (the light zone) which is abnormal (Oswald et al., 2011). BEP rats in our study, therefore, exhibit compulsive-like behavior, one of the symptoms of binge eating disorder (American Psychiatric Association, 2013).

Neurons in the taste processing regions (IC and PBN) expressed ΔFosB, and the number of ΔFosB-expressing neurons in these regions was similar in the two phenotypes, which suggests that both phenotypes processed the sucrose taste similarly, even though BEP rats consumed more sucrose than BER rats.

Neurons in the LC, a structure with multiple functions, including stress processing, also expressed ΔFosB. Acute stressful stimuli caused an increase in single unit activity in LC neurons and plasma norepinephrine level (Abercrombie and Jacobs, 1987). Acute stress activates the LC (Borodovitsyna et al., 2018) but as the number of stresses increases, ΔFosB accumulates which reduces the activity of LC neurons. We did not find a difference at the level of LC ΔFosB expression likely because we used repeated foot-shock stresses in our study.

Stress also activates the hypothalamic-pituitary-adrenal (HPA) axis. ΔFosB expression in the PVN was higher in BEP than in BER rats, therefore the activity of these neurons was reduced in BEP rats. We showed previously that these BEP rats displayed a blunted stress-induced activation of the HPA axis, with disruption in the

levels of corticosterone and corticotropin-releasing factor (CRF) (Calvez et al., 2016a). This may be due to the high number of ΔFosB-expressing neurons in the PVN of BEP rats observed after repeated stresses.

Our ΔFosB results show that reward processing brain regions are important for developing binge-like eating behavior. Under normal conditions, VTA dopaminergic neurons are activated when a reward is received (Cohen et al., 2012). These dopaminergic neurons subsequently release dopamine in the mPFC (Phillips et al., 2004) and Acb (van Zessen et al., 2012). The released dopamine activates both glutamatergic and GABAergic neurons in the mPFC (Santana et al., 2009) and activates GABAergic medium-spiny neurons in the Acb (Chuhma et al., 2004). However, following several stresses (foot-shocks in our experiment), vulnerable rats become binge-eaters (Calvez et al., 2016b). To identify the neuronal subtypes of ΔFosB-expressing neurons in reward processing regions, we performed double-labeling experiments for ΔFosB/GAD65 mRNA to characterize GABAergic neurons and ΔFosB/TH mRNA to characterize VTA dopaminergic neurons. In the Acb, because approximately 95% of neurons are GABAergic medium spiny neurons (Self, 2010), most of the ΔFosB-expressing neurons were GABAergic in both phenotypes. Since ΔFosB expression reduces neuronal activity (Vialou et al., 2010) and since more ΔFosB/GABAergic neurons were observed in BEP rats than in BER rats, we conclude that the activity of GABAergic neurons in BEP rats was significantly reduced compared to that in BER rats, and this reduction of inhibitory drive can explain their high sucrose consumption (Krause et al., 2010; Richard et al., 2013a). In the mPFC, there were more GABAergic ΔFosB-expressing neurons in BEP rats than in BER rats. However, as the number of neurons which expressed only ΔFosB was also high in BEP rats, the proportion of ΔFosB/GAD65 mRNA-positive neurons was similar in both phenotypes. Since ΔFosB reduces neuronal activity, we conclude that mPFC neuronal activity in BEP rats was significantly reduced compared to that in BER rats, but the proportion of GABAergic and non-GABAergic cells involved are similar for both phenotypes.

Acb medium spiny neurons and both non-GABAergic and GABAergic mPFC neurons express D1 and D2 dopamine receptors and receive projections from VTA dopaminergic neurons (Santana et al., 2009). In the VTA, the number of dopaminergic ΔFosB-expressing neurons was higher in BEP rats compared to BER rats, but the proportions of these neurons were similar between the two phenotypes. However, the number of GABAergic ΔFosB-positive neurons was similar in BEP and BER rats. While the expression of ΔFosB in BEP rats was higher, the proportion of GABAergic ΔFosB-expressing neurons was lower in BEP than in BER rats. This difference shows that the VTA is important for the development of binge-like eating. In this region, GABAergic neurons inhibit the activity of dopaminergic neurons. ΔFosB reduces the activity of ΔFosB-expressing neurons (Vialou et al., 2010; Eagle et al., 2018). As the proportion of GABAergic ΔFosB-expressing neurons was lower in BEP rats, it suggests that the GABAergic neuronal activity is less reduced in BEP rats compared to BER rats. In other words, the GABAergic neuronal activity in VTA is more present in BEP rats than in BER rats. This implies an overall decrease in the activity of the other neurons in the VTA, and of the other structures that receive VTA GABAergic projections in BEP rats, as previously reported in the VTA of human BED patients (Bello and Hajnal, 2010; Cordeira et al., 2010).

2.7 Conclusion

In conclusion, these experiments were designed to analyze, for the first time, ΔFosB expression in different brain regions during the development of binge-like eating in a rat model. We found that the reward system is very important for the development of binge-like eating. In this reward system, the proportions of neuron subtypes involved were similar in the mPFC and Acb, but different in the VTA in BEP and BER rats. The results suggest that these differences in proportion in the VTA may play an important role in bingeing.

2.8 Acknowledgement

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2.9 References

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Chapter 2 Neuronal activities during palatable food consumption in the reward system of binge-like eating female rats

3.1 Résumé

Le trouble de la frénésie alimentaire (TFA) est le plus répandu; cependant, sa physiopathologie est inconnue. Il est associé à une désensibilisation du système de récompense (cortex préfrontal médian (mPFC), noyau accumbens (Acb) et aire tegmentale ventrale (VTA)). Nous avons effectué des enregistrements électrophysiologiques in vivo chez des rats sujets (BEP) et résistants (BER) au TFA, puis analysé les décharges neuronales dans les trois structures. Nous avons observé chez les rats BEP des taux de décharges inférieurs dans le mPFC et la VTA avant et pendant la consommation de sucrose, ainsi qu'une désynchronisation de l'activité du mPFC, pouvant potentiellement contribuer au TFA. Ceci est cohérent avec des études sur des patients avec TFA ayant rapporté une diminution de l'activité du système de récompense. Nous proposons que l'augmentation de l'activité neuronale dans le mPFC, l'Acb ou la VTA pourrait sous-tendre le sentiment de soulagement exprimé par les patients pendant les frénésies.

3.2 Abstract

Binge eating disorder (BED), characterized by bingeing episodes and compulsivity, is the most prevalent eating disorder; however, little is known about its neurobiological underpinnings. In humans, BED is associated with desensitization of the reward system, specifically, the medial prefrontal cortex (mPFC), nucleus accumbens (Acb), and ventral tegmental area (VTA). Additionally, BED patients feel relieved during bingeing, suggesting that bingeing helps to decrease the negative emotions they were feeling prior to the binge episode. However, the mechanisms that underlie this feeling of relief in BED patients have not been well investigated.
To investigate neuronal activity before and during palatable food consumption during BED, we performed *in vivo* electrophysiological recordings in a binge-like eating rat model (bingeing, n=12 and non-bingeing, n=14) and analyzed the firing rate of neurons in the mPFC, Acb, and VTA before and during access to sucrose solution. We also investigated changes in the firing rate of neurons in these regions during and between active bingeing, which may underlie the feeling of relief in BED patients. We found that neuronal firing rates of mPFC and VTA neurons in bingeing rats were lower than those in non-bingeing rats before and during sucrose consumption. Palatable food consumption increased neuronal firing rates during and between active bingeing in bingeing rats. Our results suggest a desynchronization in the activity of reward system regions, specifically in the mPFC, in bingeing rats, which may also contribute to BED. These results are consistent with those of functional magnetic resonance imaging studies that reported decreased activity in the reward system in BED patients. We propose that increased neuronal activity in the mPFC, Acb, or VTA produces an antidepressant effect in rats, which may underlie the sense of relief patients express during bingeing episodes.

3.3 Introduction

Binge eating disorder (BED) is the most prevalent eating disorder, yet its underlying neurobiological mechanisms are not well understood. It is characterized by the consumption of large amounts of palatable food when not feeling physically hungry and a loss of sense of control during the binge episode (American Psychiatric Association, 2013), and is usually triggered by stress (Torres and Nowson, 2007). BED patients express a sense of relief during a binge episode (Abraham and Beumont, 1982; Kaye et al., 1986; Elmore and de Castro, 1990). This suggests that bingeing helps to decrease the negative emotions they were feeling as a result of the stressful event (Heatherton and Baumeister, 1991; Stickney and Miltenberger, 1999), and since depression and anxiety are comorbidities of BED (Yanovski et al., 1993; Wilfley et al., 2000; Hudson et al., 2007; Godfrey et al., 2019), BED patients may be less anxious or depressed following the binge episode, which is consistent with the affect regulation model that states that people binge to mitigate the effects of negative emotions (Hawkins and Clement, 1984). However, the mechanisms that underlie the feeling of relief have not been well investigated.

In human functional magnetic resonance imaging (fMRI) studies, BED patients displayed decreased activity in reward processing regions (Balodis et al., 2013; Balodis et al., 2013a; Balodis et al., 2014; Reiter et al., 2017). Therefore, bingeing might be a response to the need to reactivate a hypofunctioning reward system (Wang et al., 2001; Geiger et al., 2009). Bingeing on palatable food chronically stimulates, and eventually desensitizes, the reward system, mainly comprising of the medial prefrontal cortex (mPFC), nucleus accumbens (Acb), and ventral tegmental area (VTA) (O'Connor and Kenny, 2016; Moore et al., 2018). Chronic palatable food consumption leads to the expression of deltaFosB in the Acb, mPFC, and VTA (Wallace et al., 2008; Munoz-Escobar et al., 2019; Quansah Amissah et al., 2019).

DeltaFosB expression correlates with reduced excitability of neurons (Nestler et al., 2001; Vialou et al., 2010; Eagle et al., 2018) and deltaFosB is abundantly expressed in the reward system of bingeing rats (Munoz-Escobar et al., 2019; Quansah Amissah et al., 2019). Reduced mPFC activity was also reported in binge eating

prone rats and may underlie the loss of control during the consumption of palatable food in BED patients (Sinclair et al., 2019). The aforementioned studies suggest that the desensitization of the reward system, which may underlie the decrease in activity in the reward system, may be due to deltaFosB expression.

The present study was conducted to investigate possible changes in neuronal firing rates and local field potential (LFP) activities in reward processing regions in a binge-like eating rat model. Based on findings on increased deltaFosB expression in reward processing regions in BEP rats, we hypothesized that neuronal firing in these structures in these animals should be reduced. Specifically, we investigated the acute effects of palatable food consumption on real-time neuronal activity in the mPFC, Acb, and VTA of binge-like eating female rats developed using the same protocol as our previous studies (Calvez and Timofeeva, 2016; Quansah Amissah et al., 2019). Desynchronization in the activity of regions in brain systems underlie several neuropsychological disorders including schizophrenia, autism, and epilepsy (Uhlhaas and Singer, 2006). To investigate this possibility in BED, we also analyzed the phases of the LFP event-related potentials, the response of the neuron population within a specific brain region to a stimulus or event (Blackwood and Muir, 1990), during sucrose consumption in our binge-like eating rat model.

We found that the firing rate of mPFC and VTA neurons in binge eating prone rats was lower than that in binge eating resistant rats both before and during sucrose access. Additionally, in binge eating resistant rats, a larger proportion of neurons were activated and a smaller proportion of neurons were inhibited compared to binge eating prone rats during sucrose consumption. We also found a reduction in lick-triggered evoked potential amplitude in reward processing regions, specifically the mPFC, suggesting neuronal desynchronization that may contribute to BED.

3.4 Methods

All experiments were performed in accordance with the guidelines of the Canadian Council on Animal Care and approved by the Université Laval Committee on Ethics and Animal Research.

3.4.1 Animals

Fifty-one naïve 42-day-old female Sprague-Dawley rats (body weight: 151-175 g) were purchased for this study from a reputable breeder supplier. All rats were housed individually in plastic cages lined with wood shavings. Upon arrival, the rats were allowed one week to acclimatize to the housing facility. They were housed in a facility with a 12-h light/dark cycle (lights on at 10:00 pm). The ambient temperature in the housing facility was 23 ± 1 ^{o}C. Rats were given *ad libitum* access to standard laboratory chow (Teklad Global 185 Protein Diet; 3.1 kcal/g, Harlan Teklad, Montreal, QC) and tap water.

3.4.2 Generating binge-eating rat phenotypes

Binge-like eating phenotypes were generated according to the protocol described by Calvez and Timofeeva (2016). Sucrose consumption following foot-shock stress was used to classify rats as either binge eating prone (BEP) or binge eating resistant (BER). Briefly, one week after their arrival, rats were given *ad libitum* access to 10% sucrose solution for 24 hours to prevent neophobia to the sucrose solution. Rats were then given unpredictable access to 10% sucrose solution for one hour, just at the start of the dark phase. The interval between any two non-stress sessions was at least two days. This access to sucrose was repeated several times until the amount of sucrose consumed was stable. This usually requires four to five access sessions to 10% sucrose solution. Afterwards, the rats underwent unpredictable foot-shock stresses three times, each followed by immediate, one-hour access to palatable food (stress sessions). Each foot-shock stress was comprised of four foot-shocks separated by 15 s, with a direct current of 0.6 mA, lasting for 3 s, delivered through a metal grid on the floor of the plastic cage. Consecutive stress sessions were separated by at least three days. Since this is a stress-induced binge eating model, only consumption of sucrose after foot-shock stress was used to classify rats as either BEP or BER. Using the tertile approach, rats were ranked according to their sucrose consumption. Rats that consumed large amounts of sucrose were placed in the upper tertile, those that consumed average amounts of sucrose were placed in

the middle tertile, and those that consumed low amounts of sucrose were placed in the lower tertile. Any rat that appeared at least twice in the upper tertile and never in the lower tertile was considered BEP, while rats that appeared at least twice in the lower tertile and never in the upper tertile were considered BER.

3.4.3 Stereotaxic surgery

Following the generation of the two rat phenotypes, rats were anesthetized using isoflurane (1-3%) and oxygen was added to the respiration gas mixture. The heads of rats were shaved and rats were placed in the stereotaxic apparatus. Eye gel was applied to their eyes to prevent drying during the surgery. Subsequently, the skin on the head was washed with three alternating passages of chlorhexidine and alcohol (70%) and was cut open to expose the skull of the rat. Small craniotomies were made for the placement of the reference, anchor screws, and tetrodes. The tetrodes were constructed in-house out of four 12.5-µm, gold-plated nickel-chromium microwires with an impedance of 400 kΩ. Three movable probes each comprised of a tetrode sheathed within a cannula were inserted into the mPFC (AP: +3.24 mm, ML: 0.5 mm, DV: 2.5 mm), Acb (AP: +1.8 mm, ML: 1.0 mm, DV: 5.5 mm), and VTA (AP: -5.28 mm, ML: 1.0 mm, DV: 7.5 mm), as per the coordinates of the Paxinos rat atlas measured from bregma. The probes were initially inserted 1 mm above the target position, with the tetrodes retracted within the cannula to prevent damage during insertion. The tetrodes were then lowered to their target position. Anchor screws were inserted into the skull to stabilize the implant. The reference was soldered to a screw inserted just above the cerebellum. The non-movable parts of the probe were encased in a plastic tube and covered with dental cement. Rats had access to a heated carpet for two days following surgery. The rats underwent post-operative care consisting of lactate ringer injection and meloxicam for two days and were allowed one week to recover from the surgery before recordings begun.

3.4.4 Multiunit and local field potential recordings

After recovery from surgery, rats were placed in a behavioral cage with two photo-beam lickometers (Med Associates Inc., VT, USA). One of the lickometers provided access to the spout of a bottle containing water while the other provided access to

the spout of a bottle containing 10% sucrose solution. Extracellular multiunit and local field potential activity in the mPFC, Acb, and VTA as well as lickometer activity were recorded simultaneously using a TDT multichannel acquisition system (Tucker Davis Technologies, FL, USA) for 20 minutes. Even though the entire recording session lasted for 20 mins, rats were given access to sucrose only after the first 10 mins.

3.4.5 Histology

Upon the completion of recordings, rats were anesthetized deeply and subsequently intracardially perfused with saline followed by phosphate-buffered paraformaldehyde (4%). Their brains were retrieved and sectioned using a vibratome into 100-µm thick sections. The sections were mounted on gelatinized slides, stained with cresyl violet (Nissl stain), cover-slipped and visualized under a microscope to identify the locations of tetrode placement in the brain. Following histology, three BER rats and one BEP rat were excluded because the probes were located outside the regions of interest.

3.4.6 Analysis of neuronal firing

Signals were exported into MATLAB (R2015a, The MathWorks^{TM}) and pre-processed using a custom-written MATLAB script for artefact removal. Artefacts arising from movement, whereby the amplifier voltage was saturated, were removed. This type of noisy signal was mostly observed before sucrose access. After pre-processing, the original data (Figure 3-1A) were imported into Spike2 software version 8.0 (Cambridge Electronic Design) and the signals were filtered between 500 Hz and 10 kHz for spike analysis (Figure 3-1B). Spikes were detected when input signals from four channels of the tetrode crossed a threshold level which was determined automatically according to signal-to-noise ratios within each channel. The minimum threshold used was two standard deviations above the background noise level in the signal. The built-in template matching algorithm in Spike2 was then used to sort spike waveforms. Briefly, a template was formed from a detected spike and a template width was estimated (twice the mean difference between the template and the spike that created it). Each new spike was then compared against

the template and added to it if the spike's sample points fell within the template width. The template was modified with the addition of each new spike up to a maximum of eight spikes after which the template was checked against confirmed templates. If a match was found, the newly generated template was merged with the existing confirmed template. Otherwise, a new confirmed template was generated.

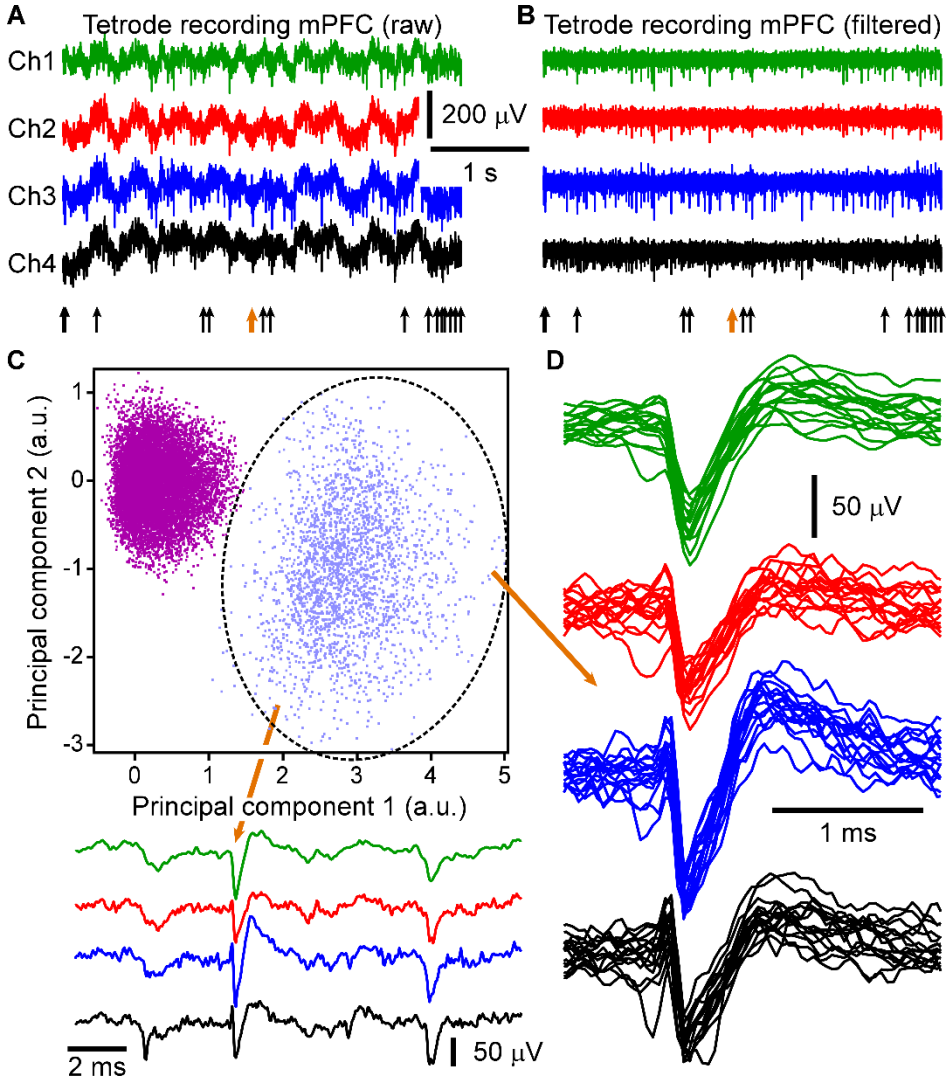

Figure 3 - 1 Example of raw recorded signal, filtered signal, and spike identification.

A. Raw signal from a tetrode located in the medial prefrontal cortex of a rat. Ch1, Ch2, Ch3, and Ch4 represent the signals recorded from each of the electrodes in the tetrode. B. Filtered raw signal between 500 Hz and 10 kHz. Brown arrow: The location of the spike in the recorded signal for the unit shown in C. Black arrows: The timing of other spikes recorded in the same tetrode as the unit shown in C. C. Unit

identification following principal component analysis. Two clearly isolated units were identified. a.u.: arbitrary units. D. Fifteen spikes from the same unit identified on the four electrodes. Circled cluster: cluster from which the spikes shown in C and D were identified.

After template matching, spike features were extracted from the detected waveforms and principal component analysis (PCA) was performed in Spike2 to cluster waveforms into putative units (Figure 3-1C). This is an automatic procedure that was performed to extract the three most important features in the data that contribute the most to the differences in the waveforms that make up the recorded spikes. Well separated clusters derived from the PCA were considered as single-units. All other units were discarded and not used for subsequent analysis. Examples of spikes from presumably one neuron, which were recorded simultaneously with the four electrodes of the tetrode are shown in Figure 3-1C and D.

The time stamps at which spikes occurred in all putative units and the time stamps of licks were then imported into NeuroExplorer (Nex Technologies, Dallas, TX, USA) to characterize neuronal activity around licks. This was done by aligning the activity of putative units with lick cluster start and lick cluster end (Fig. 3-2 C). Peri-event histograms were plotted using IgorPro software (Lake Oswego, Oregon, USA). The firing rate in between lick clusters was considered as intercluster interval firing rate, while the firing rate during lick cluster was considered as cluster firing rate. Peri-event histograms of the intercluster interval firing rate and the cluster firing rate of the identified neurons were plotted. Since both the lick clusters and intercluster interval during licking varied in duration, the lick cluster durations and the intercluster intervals were first normalized by dividing the cluster/intercluster interval durations into 50 equal bins (see Fig. 3-5). The firing rate within each bin was then calculated and averaged. The intercluster interval firing rate and the cluster firing rate were obtained by averaging the intercluster interval firing rate and the cluster firing rate for all identified mPFC, Acb, and VTA neurons for BER and BEP rats. The firing rate of neurons within 3 s before lick cluster start (baseline firing rate) was compared to

their firing rate within 3 s after lick cluster start (response firing rate) in order to classify neurons as either lick cluster excited (LCE; neurons whose response firing rate is significantly higher than the baseline firing rate), lick cluster inhibited (LCI; neurons whose response firing rate is significantly lower than the baseline firing rate), and lick cluster nonresponsive neurons (LCN; neurons whose response firing rate was not different from the baseline firing rate). This was done using the Wilcoxon signed rank test ($p < 0.05$). A non-parametric test was used because the normality test (Kolmogorov-Smirnov, $p < 0.05$) did not show a normal distribution of the frequency counts. The peri-event histograms showing the LCE, LCI, and LCN neurons were plotted using a bin size of 100 ms. To investigate the activity of neurons in anticipation of sucrose consumption, we compared the firing rate of neurons between -2 to -1 s and -1 to 0 s (anticipation period) before the lick cluster start, as previously described by Mitra et al. (Mitra et al., 2015). To examine the average neuronal activity per lick, peri-event histograms showing the activity of neurons from 500 ms before the lick to 500 ms after the lick were plotted using 10 ms bins. Additionally, the average firing rate of neurons before sucrose access (first 500 s) during the 20-minute recording session was analyzed. The average activity of neurons during sucrose access (last 500 s) was also analyzed to determine the overall activity of neurons during sucrose consumption in BER and BEP rats.

3.4.7 Event-related potential analysis

To analyze the peri-lick event-related potentials (ERPs) at the time of lick of mPFC, Acb, and VTA neurons, the activity of the neuron population from 500 ms before to 500 ms after each lick in identified lick clusters was isolated. These ERPs were then averaged for all licks in each recording session for each rat. The Hilbert transform was applied to extract the phase of the neural activity at the time of lick. The ERP phase for each lick from the recording electrodes was averaged for each rat. The mean resultant vector was taken as a measure of the ERP phase preference of licks. The average ERPs and the ERP phases for all BER and BEP rats were then compared. ERP analysis was performed using custom-written MATLAB scripts.

3.4.8 Analysis of sucrose lick microstructure

Two lickometers were used to detect the licks of rats during the consumption of 10% sucrose and water in the behavioral cage during the recording of neuron activity. Lick activity for water consumption was not analyzed because most of the rats consumed little or no water during the recording sessions. Licks were detected whenever the tongues of rats crossed an infrared beam emitted by the lickometer. Lick activity during the last 10 minutes of the 20-minute recording session, when rats had access to 10% sucrose solution, was recorded.

A cluster was defined as a group of licks separated by pauses (inter-cluster interval; ICI) which were at least 3 s long. Based on previous findings (Spector et al., 1998), we considered a cluster as a group of at least nine licks. The total number of licks in clusters, mean number of licks per cluster, number of lick clusters, lick frequency, and mean and total cluster duration were determined using a custom-written MATLAB script. The total cluster duration was calculated as the total time the rat spent licking. The lick frequency was calculated by dividing the total number of licks during the entire session for each rat by the total cluster duration.

3.4.9 Circular Statistics

Statistical analysis of the phase of the event-related potential at the time of lick was performed using the circular statistics tool box developed by Berens (2009). The parameters of interest included the mean resultant vector and the mean resultant vector length (evaluates the circular spread of the data points) for all the ERP phases identified in each region of interest. The Rao's spacing test was also performed to determine the uniformity in the phase preference of ERP. The phases were converted to radians from degrees before analysis. The Watson-Williams test was performed to compare the mean ERP phase preference at lick onset between BEP and BER rats for all regions of interest. Polar plots showing the phases, mean phases, and resultant vector lengths of the ERPs were plotted using MATLAB.

3.4.10 Statistical analysis

The statistical analyses were performed using GraphPad version 6.01 (GraphPad Software Inc., La Jolla CA, USA). The unpaired Student's t-test was used to compare

the means of amount of sucrose consumed and lick microstructure parameters between BEP and BER rats. The unpaired Student's t-test was also performed to compare the properties of the ERPs (ERP amplitude at first peak and first peak latency) generated in response to licks. Additionally, the properties of the spike activity of neurons around individual licks were compared between BER and BEP rats using the unpaired Student's t-test. We also compared the response firing rate of neurons between lick cluster start and lick cluster end using the unpaired Student's t-test. The two-way analysis of variance (ANOVA) was performed to compare sucrose intake before and after foot-shock stress, firing rate of neurons before and during sucrose access, baseline and response firing rates at the lick cluster start, and firing rates during the anticipation period. All two-way ANOVA tests were followed by a post-hoc Bonferroni test. All data were presented as mean ± standard error of mean (SEM). Statistical significance was set at a p value < 0.05.

3.5 Results

3.5.1 Sucrose intake

During the phenotyping period, the sucrose intake by BEP (n = 12) and BER (n = 14) rats were similar to that already reported in the study of Calvez and Timofeeva (2016) as shown in Figure 3-2A. During non-stress sessions, BER rats consumed significantly lower amounts of sucrose than BEP rats. Additionally, BEP rats consumed more sucrose compared to BER rats during stress sessions. Moreover, while BER rats did not change their sucrose intake following foot-shock, BEP rats significantly increased their sucrose intake following foot-shock. Even though there was no effect of treatment on sucrose consumption in BEP and BER rats ($F_{1,40} = 2.685$, $p = 0.1092$), there was an effect of phenotype ($F_{1,40} = 62.58$, $p < 0.0001$) and the interaction between phenotype and treatment ($F_{1,40} = 7.226$, $p = 0.0104$) on sucrose consumption in both phenotypes.

During the recording session, BEP rats consumed significantly more sucrose solution during the 10-minute sucrose access in the recording chamber as shown in Figure 3-2B.

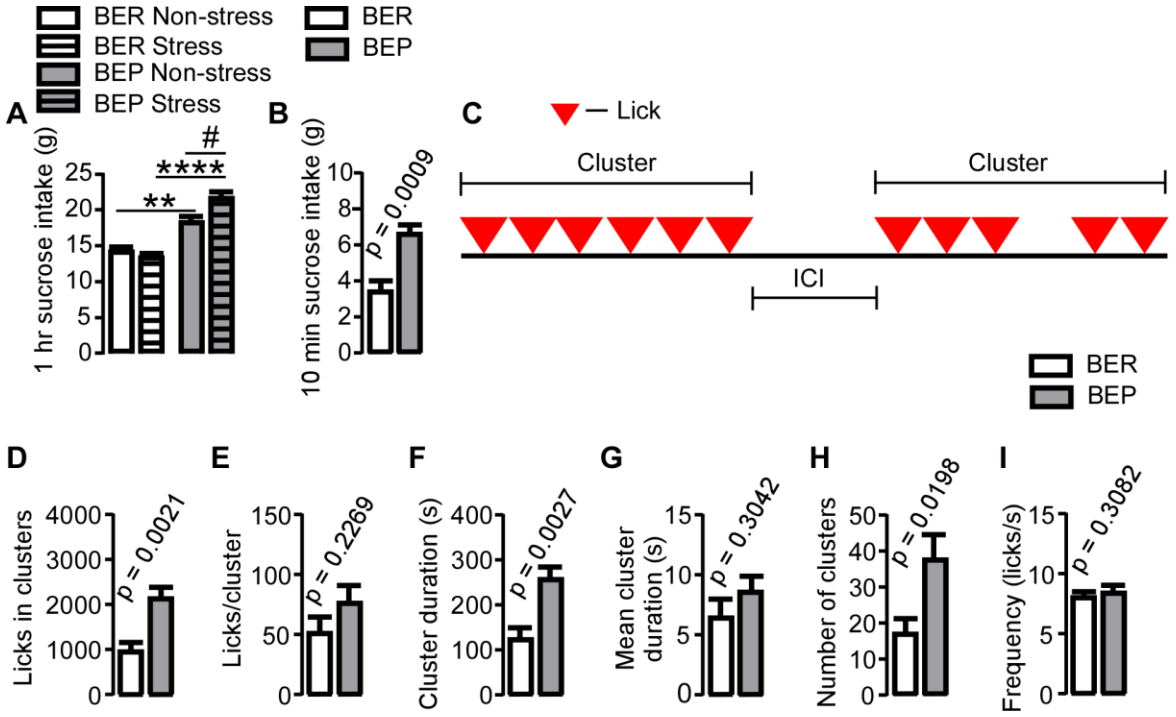

Figure 3 - 2 Sucrose intake and lick microstructure.

A. The 1-hr sucrose intake of binge eating resistant (BER) and binge eating prone (BEP) rats during the phenotyping period. B. The 10-min sucrose intake of BEP and BER rats during the 20-minute recording session. C. Schematic showing the definition of a lick cluster and the intercluster interval (ICI). The lick parameters of interest were the D. Licks in all identified clusters, E. Number of licks per cluster, F. Total duration of clusters, G. Mean cluster duration, H. Number of clusters, and I. Lick frequency. NS: Sucrose intake during the non-stress session, S: Sucrose intake during the stress session. Comparison between phenotypes - **: $p < 0.01$, ****: $p < 0.0001$; comparison within phenotype - #: $p < 0.05$.

3.5.2 Lick microstructure

The lick microstructure of rats during sucrose consumption was also analyzed (Figure 3-2C). The total number of licks in clusters (Figure 3-2D), total cluster duration (Figure 3-2F), and number of clusters (Figure 3-2H) were higher in BEP rats compared to BER rats. The number of licks per cluster (Figure 3-2E), mean cluster duration (Figure 3-2G), and lick frequency (Figure 3-2I) were similar in both BER and BEP rats. In conclusion, the number of licks and the duration per cluster were similar

between groups. The increase in sucrose intake observed in the BEP rats is due to the larger number of clusters, inducing a larger total number of licks and total duration.

3.5.3 Identified mPFC, Acb, and VTA neurons and their firing rates in BER and BEP rats

As stated earlier, tetrodes were implanted in the mPFC, Acb, and VTA of BER and BEP rats. Example images of the locations of the probes in these regions are shown in Figure 3-3.

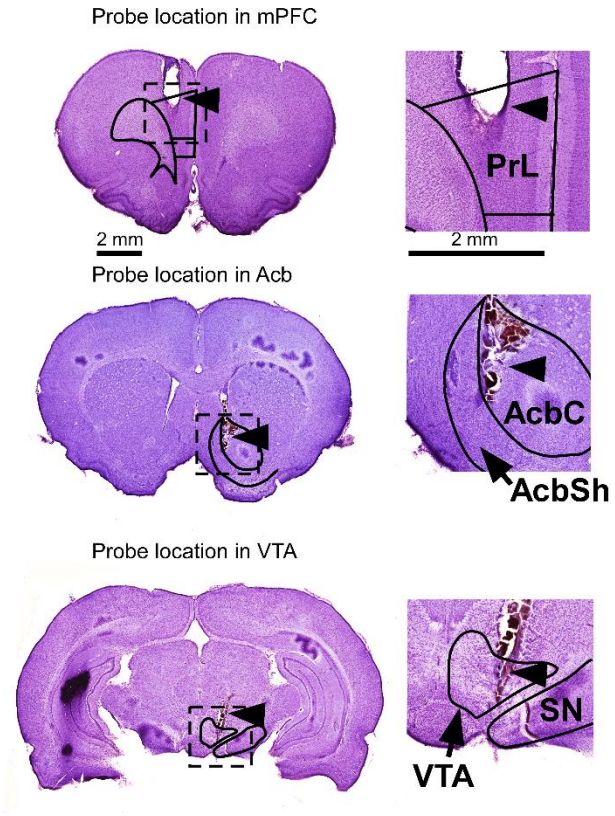

Figure 3 - 3 Target locations of tetrodes in binge eating resistant (BER) and binge eating prone (BEP) rats.

During stereotaxic surgery, the tetrodes were inserted into the medial prefrontal cortex (A), nucleus accumbens (B), and ventral tegmental area (C) of rats. SN: Substantia nigra, AcbC: nucleus accumbens core, AcbSh: nucleus accumbens shell, PrL: Prelimbic cortex. Scale bar: 2 mm.

Following spike detection, a number of units (neurons) were identified in the mPFC, Acb, and VTA (Figure 3-4A and B). The average firing rates of these neurons before and during sucrose access were compared in BEP and BER rats (Figure 3-4C, D, and E).

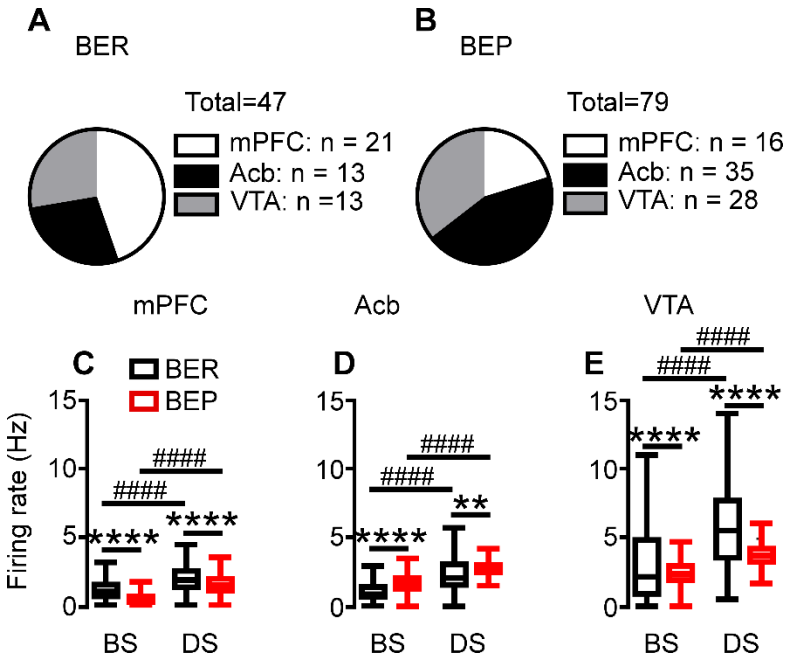

Figure 3 - 4 Identified medial prefrontal cortex (mPFC), nucleus accumbens (Acb), and ventral tegmental area (VTA) neurons and their firing rates.

A and B. Pie charts showing the number of identified neurons in the mPFC, Acb, and VTA of BER and BEP rats, respectively. C, D, and E. The results of two-way analysis of variance comparing the average firing rates of identified neurons in the mPFC, Acb, and VTA of binge eating resistant (BER) and binge eating prone (BEP) rats during the first 500 s before sucrose access (BS) and during the last 500 s of sucrose access (DS). ICI: Intercluster interval. Comparison between phenotypes - ****: $p < 0.0001$; comparison within phenotype - ##: $p < 0.01$, ####: $p < 0.0001$.

mPFC neurons in both BER and BEP rats increased their firing rate during access to sucrose solution. Additionally, mPFC firing rate in BER rats was significantly higher both before and during access to sucrose solution compared to that in BEP rats (Figure 3-4C). The effects of the phenotype ($F_{1,1996} = 127.3$, $p < 0.0001$),

treatment (access or no access to sucrose; $F_{1,1996}$ = 276.0, $p < 0.001$), and the interaction between these factors ($F_{1,1996}$ = 6.081, p = 0.0137) on the firing rate of mPFC neurons were significant. In both phenotypes, even though Acb neurons also increased their firing rate following access to sucrose solution, the firing rate of Acb neurons in BER rats before and during sucrose access was significantly lower than that for BEP rats (Figure 3-4D). There was no effect of interaction on the firing rate of Acb neurons ($F_{1,1996}$ = 1.566, p = 0.2110). However, there were significant effects of phenotype ($F_{1,1996}$ = 32.86, $p < 0.0001$) and treatment ($F_{1,1996}$ = 440.2, $p < 0.0001$) on the firing rates of these neurons. Similar to the other regions, VTA neurons in both phenotypes also increased their firing rate in response to sucrose intake. However, unlike the Acb but similar to the mPFC, the firing rate of VTA neurons before and during access to sucrose solution was higher in BER rats compared to BEP rats (Figure 3-4E). There were also significant effects of the phenotype ($F_{1,1996}$ = 172.0, $p < 0.0001$), treatment ($F_{1,1996}$ = 366.9, $p < 0.0001$), and the interaction between the phenotype and treatment ($F_{1,1996}$ = 32.51, $p < 0.0001$) on the firing rate of VTA neurons during the recording session.

3.5.4 Effects of sucrose intake on firing rate of identified neurons during lick cluster and intercluster interval

During the last 10 minutes of the recording session, when rats had access to sucrose solution, we analyzed the firing rates of neurons only when rats where actively licking (cluster firing rate) and during the intercluster interval (ICI; ICI firing rate) (Figure 3-5A).

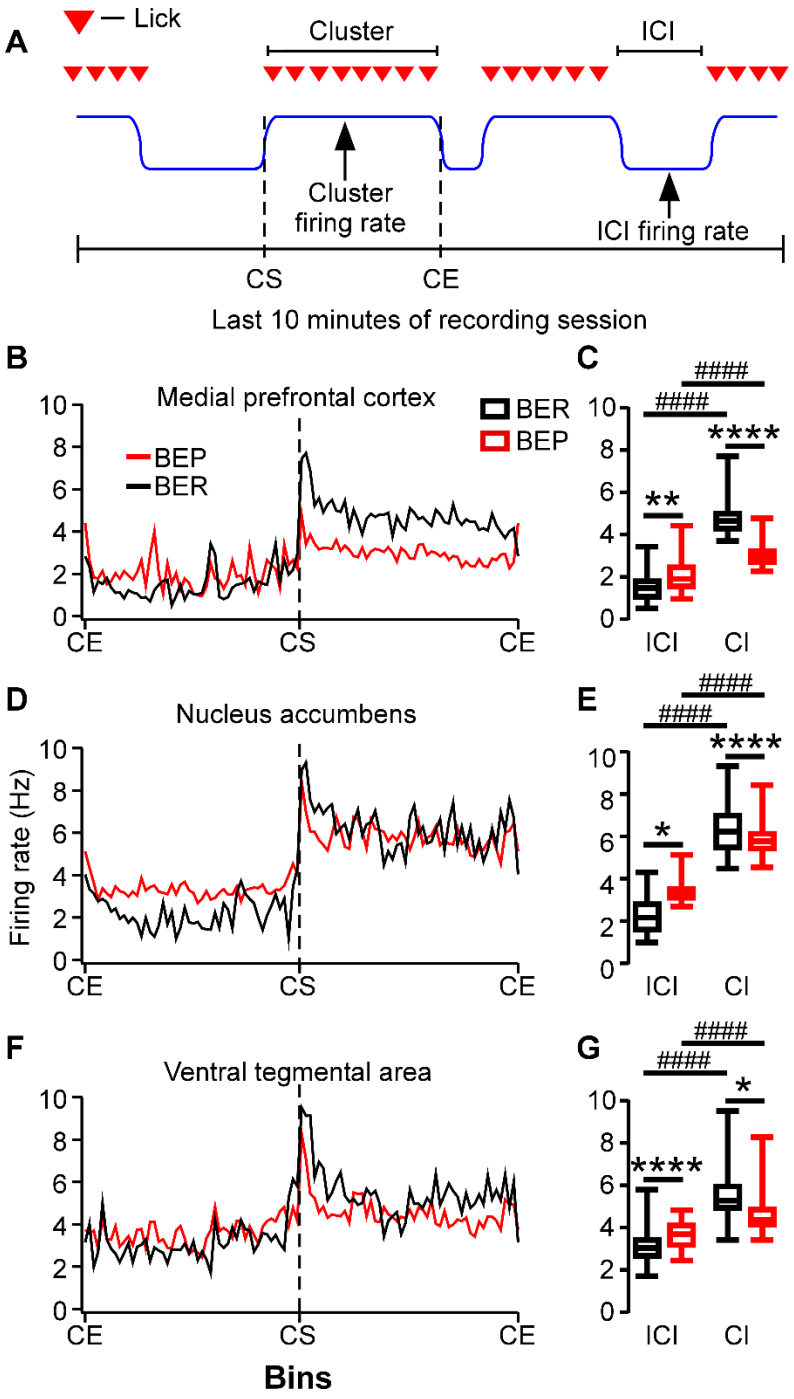

Figure 3 - 5 The firing rate of identified neurons during active licking and during the intercluster interval.

A. A schematic showing the period of interest during the last 10 minutes of the recording session. B, D, and F. Peri-event histograms showing the average firing rate of identified nucleus accumbens (Acb), medial prefrontal cortex (mPFC), and ventral tegmental area (VTA) neurons during the intercluster interval (ICI firing rate)

and the cluster (Cluster firing rate) in binge eating resistant (BER) and binge eating prone (BEP) rats after normalization of cluster duration. Each histogram comprises of 100 bins (first 50 bins: intercluster interval firing rate; last 50 bins: cluster firing rate). C, E, and G. Comparison between the ICI firing rate and cluster firing rate in BER and BEP rats. CS: Lick cluster start, CE: Lick cluster end, ICI: Intercluster interval, ICI firing rate: intercluster interval firing rate, Cl: cluster. Comparison between phenotypes - *: $p < 0.05$, **: $p < 0.001$, ****: $p < 0.0001$; comparison within phenotype - ####: $p < 0.0001$.

In all three regions, firing rate analysis revealed that the identified neurons increased their activity during the transition from the intercluster interval to the lick cluster in both BER and BEP rats (Figure 3-5B, D, and F). Additionally, during the intercluster interval, the ICI firing rate of identified mPFC, Acb, and VTA neurons in BER rats was significantly lower than that in BEP rats (Figure 3-5C, E, and G). However, during the lick cluster, the cluster firing rate of neurons in the mPFC, Acb, and VTA in BER rats was higher than that in BEP rats. In the mPFC, there was an effect of the time interval (whether intercluster interval or lick cluster; $F_{1,196} = 475.2$, $p < 0.0001$) on the firing rate of the identified neurons. There were also effects of the phenotype ($F_{1,196} = 47.2$, $p < 0.0001$) and the interaction between the phenotype and time interval ($F_{1,196} = 140.4$, $p < 0.0001$) on the firing rate of the identified neurons. Additionally, in the Acb, there were effects of the phenotype ($F_{1,196} = 10.42$, $p = 0.0015$), time interval ($F_{1,196} = 921.2$, $p < 0.0001$), and the interaction between the phenotype and the time interval ($F_{1,196} = 50.87$, $p < 0.0001$) on the firing rate of the identified neurons. However, in the VTA, even though there was no effect of phenotype on the firing rate of the identified neurons ($F_{1,195} = 2.813$, $p = 0.0951$), the time interval ($F_{1,195} = 184.1$, $p < 0.0001$) and the interaction between the time interval and the phenotype ($F_{1,195} = 31.89$, $p < 0.0001$) significantly affected the firing rate of the identified neurons.

3.5.5 Firing rate of identified mPFC, Acb, and VTA neurons during the anticipation period

Anticipation was calculated by comparing firing rate between -2 to -1 s and -1 to 0 s (anticipation period) before lick cluster start. Analysis revealed that during the anticipation period, the firing rate of mPFC neurons in BER rats did not change (Figure 3-6A). However, in BEP rats the firing rate of mPFC neurons increased significantly during this period. Additionally, there were also significant effects of the phenotype ($F_{1,36} = 10.58$, $p < 0.0025$), time interval ($F_{1,36} = 11.21$, $p < 0.0019$), and the interaction between the time interval and phenotype ($F_{1,36} = 4.730$, $p < 0.0363$) on the firing rate of mPFC neurons. In the Acb, the firing rate of neurons in BER rats did not increase in anticipation of sucrose consumption but it increased in BEP rats (Figure 3-6B). The firing rates of Acb neurons were significantly affected by the phenotype ($F_{1,35} = 59.08$, $p < 0.0001$), time interval ($F_{1,35} = 32.50$, $p < 0.0001$), and interaction between the phenotype and time interval ($F_{1,35} = 4.364$, $p < 0.0440$). The situation was also similar in the VTA, where the firing rate of VTA neurons in BER rats did not change during the anticipation period but changed in BEP rats (Figure 3-6C). There were also significant effects of the phenotype ($F_{1,36} = 37.88$, $p < 0.0001$), time interval ($F_{1,36} = 12.65$, $p < 0.0001$), and interaction between the phenotype and time interval ($F_{1,36} = 4.481$, $p < 0.0413$) on the firing rates of identified VTA neurons.

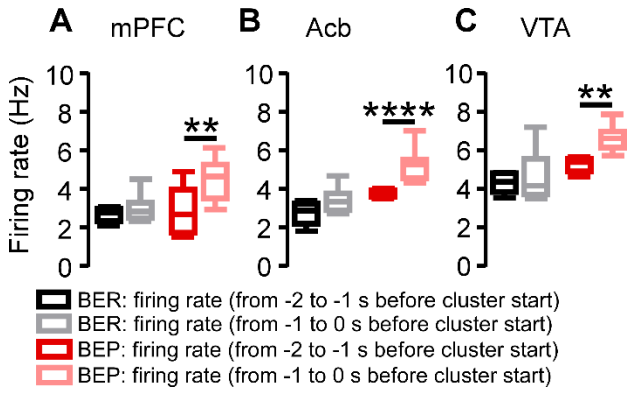

Figure 3 - 6 Firing rate of neurons during the anticipation period (-1 to 0 s).

The firing rates of neurons in the medial prefrontal cortex (mPFC, A), nucleus accumbens (Acb, B), and ventral tegmental area (VTA, C) in binge eating prone

(BEP) rats, but not binge eating resistant (BER) rats increased significantly during the anticipation period. Comparison within phenotype - **: $p < 0.001$, ****: $p < 0.0001$.

3.5.6 Firing activity of identified mPFC, Acb, and VTA neurons in BER and BEP rats around each lick

The average firing activity of all identified neurons in the mPFC of both phenotypes of rats was evaluated (Figure 3-7A (first row), B, and C).

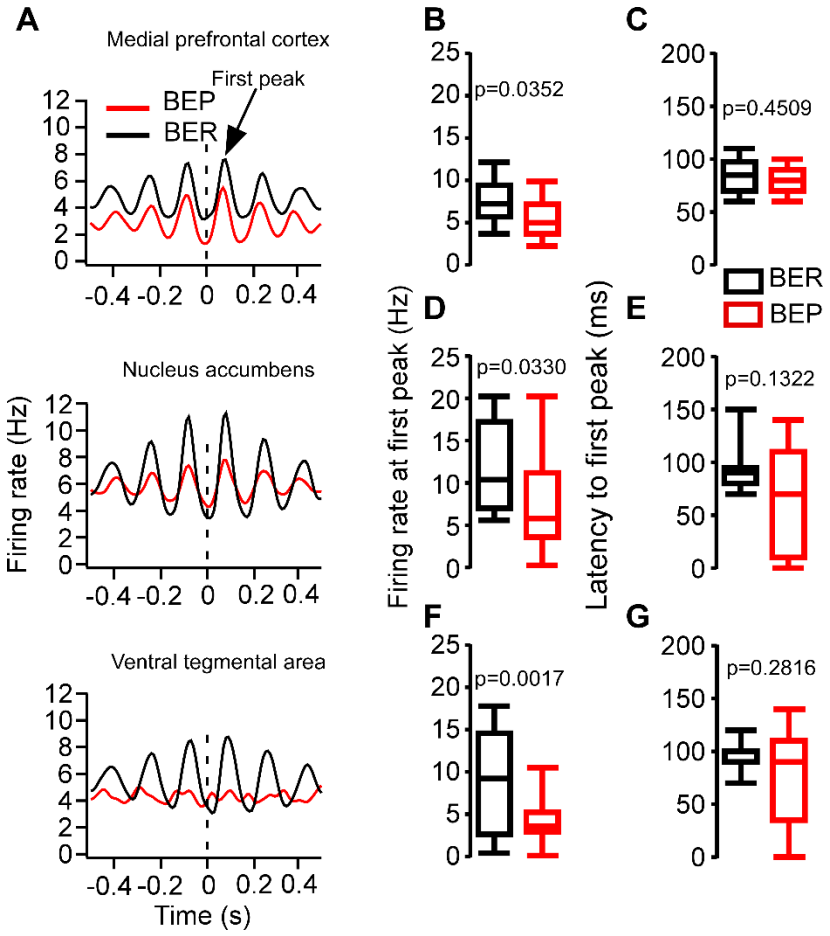

Figure 3 - 7 The average activity of identified medial prefrontal cortex (mPFC), nucleus accumbens (Acb), and ventral tegmental area (VTA) neurons around each lick.

A. First row: Graphical representation of the activity of mPFC neurons around each lick. B. Second row: Graphical representation of the activity of Acb neurons around each lick. C. Third row: Graphical representation of the activity of VTA neurons around each lick. For activity around each lick, the interval of interest was between

500 ms before lick and 500 ms after lick. B, D, and F. Firing rate at first peak after lick of mPFC, Acb, and VTA neurons, respectively. C, E, and G. Latency to first peak of mPFC, Acb, and VTA neurons, respectively. BEP: binge eating prone, BER: binge eating resistant.

Analysis revealed that the average firing rate of mPFC neurons immediately following a lick was significantly higher in BER rats compared to BEP rats, even though the times at which these activities occurred were not significantly different between the two phenotypes (Figure 3-7B and C). Similar to the mPFC, the firing rate at first peak of Acb neurons immediately after a lick was significantly higher in BER rats compared to BEP rats (Figure 3-7D) and there was no difference in latency to first peak following the lick in BER and BEP rats (Figure 3-7E). Additionally, the average firing rate of VTA neurons just after the lick was significantly higher in BER compared to BEP rats, in which licking only moderately affected firing activity (Figure 3-7F). Moreover, the times at which the first peaks occurred were similar in both BER and BEP rats (Figure 3-7G).

3.5.7 Identity of identified neurons based on activity around cluster start and end

Based on the activity of neurons before and after lick cluster start, three subtypes of neurons were identified: the lick cluster excited (LCE; Figure 3-8A, first row), lick cluster inhibited (LCI; Figure 3-8A, second row), and lick cluster nonresponsive (LCN; Figure 3-8A, third row). The proportions of the neuron subtypes (Figure 3-8B) in the mPFC, Acb, and VTA were evaluated. Interestingly, no LCI neuron was identified in BER rats. The percentage of LCE neurons in BER rats was significantly higher than that in BEP rats (Figure 3-8C). However, no difference was found in the percentage of LCN neurons in BER and BEP rats (Figure 3-8D).

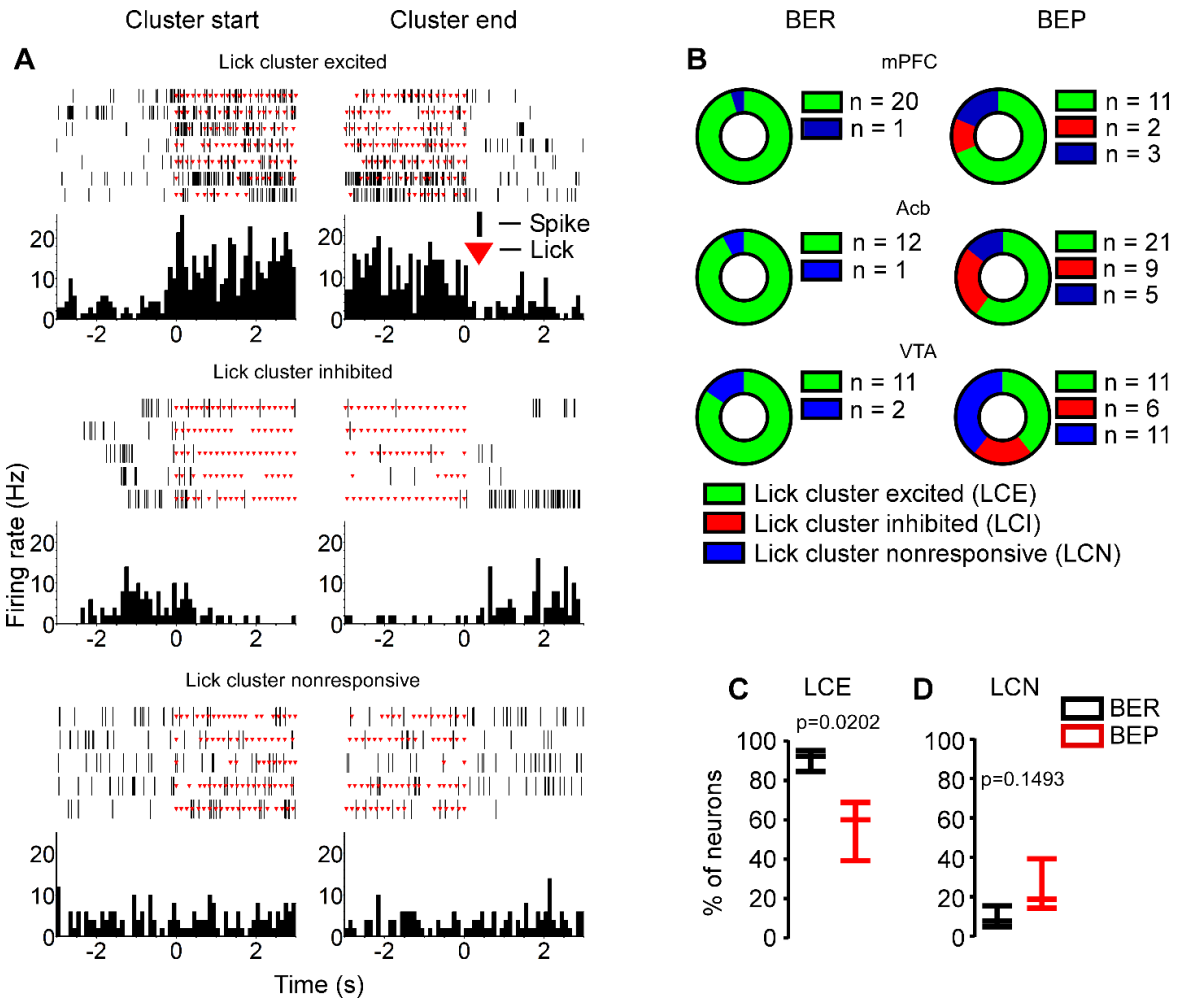

Figure 3 - 8 Subtypes of neurons were identified based on the activity of neurons around lick cluster start.

A. First row: The activity of a lick cluster excited (LCE) neuron at lick cluster start and lick cluster end; Second row: The activity of a lick cluster inhibited (LCI) neuron at lick cluster start and lick cluster end; Third row: The activity of a lick cluster nonresponsive (LCN) neuron at lick cluster start and lick cluster end. B. First row: Pie chart showing the numbers of neuron subtypes identified in the mPFC of BER and BEP rats; Second row: Pie charts showing the numbers of neuron subtypes identified in the Acb of BER and BEP rats; Third row: Pie charts showing the numbers of neuron subtypes identified in the VTA of BER and BEP rats. C and D. Comparison of the percentage of LCE and LCN neuron subtypes in BER and BEP rats using the Student's t-test.

To determine if subtypes of neurons still exist based on the time at which the peak firing rate of the identified LCE, LCI, and LCN neurons around lick cluster start occurred, we plotted histograms for the time of occurrence of the peak firing rates of these neurons. Histograms showing the peak firing rate times of these three subtypes of neurons (Figure 3-9A~C) revealed that majority of the LCE neurons had their maximal firing rate after lick cluster start, while majority of the LCI neurons had their maximal firing rate before lick cluster start.

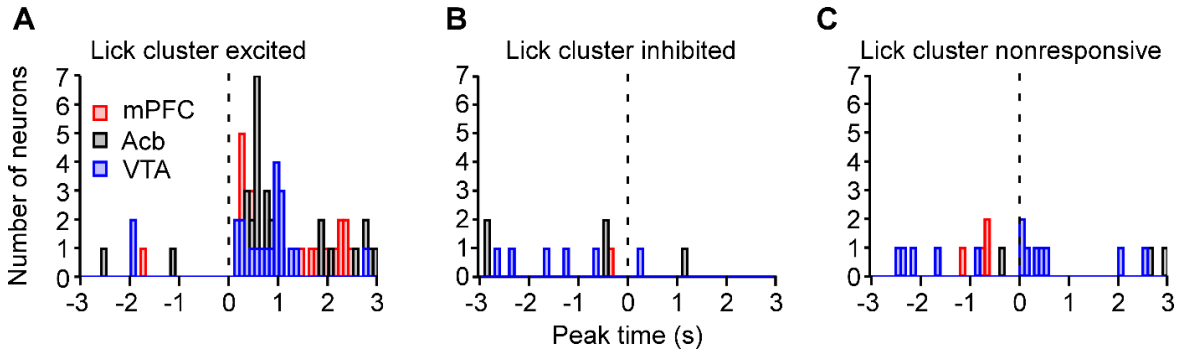

Figure 3 - 9 Histogram showing peak firing rate times.

A, B, and C. Histograms showing the times at which the peak firing rates of lick cluster excited, lick cluster inhibited, and lick cluster nonresponsive neurons in the medial prefrontal cortex (mPFC), nucleus accumbens (Acb), and ventral tegmental area (VTA) occurred in binge eating prone and binge eating resistant rats. The peak firing rate was calculated as the highest firing rate detected during the period between -3 s from lick cluster start to 3 s after lick cluster start. Red bars: peak times of neurons in the mPFC, Grey bars: peak times of neurons in the Acb, Blue bars: peak times of neurons in the VTA. Dashed line: lick cluster start. The histograms were plotted using a bin size of 100 ms.

3.5.8 Event-related potentials (ERPs)

For this analysis, the event was a lick. We compared the first depth-positive peak amplitude of the ERPs and the first peak latency following the lick between BEP and BER rats in the mPFC, Acb, and VTA (Figure 3-10).

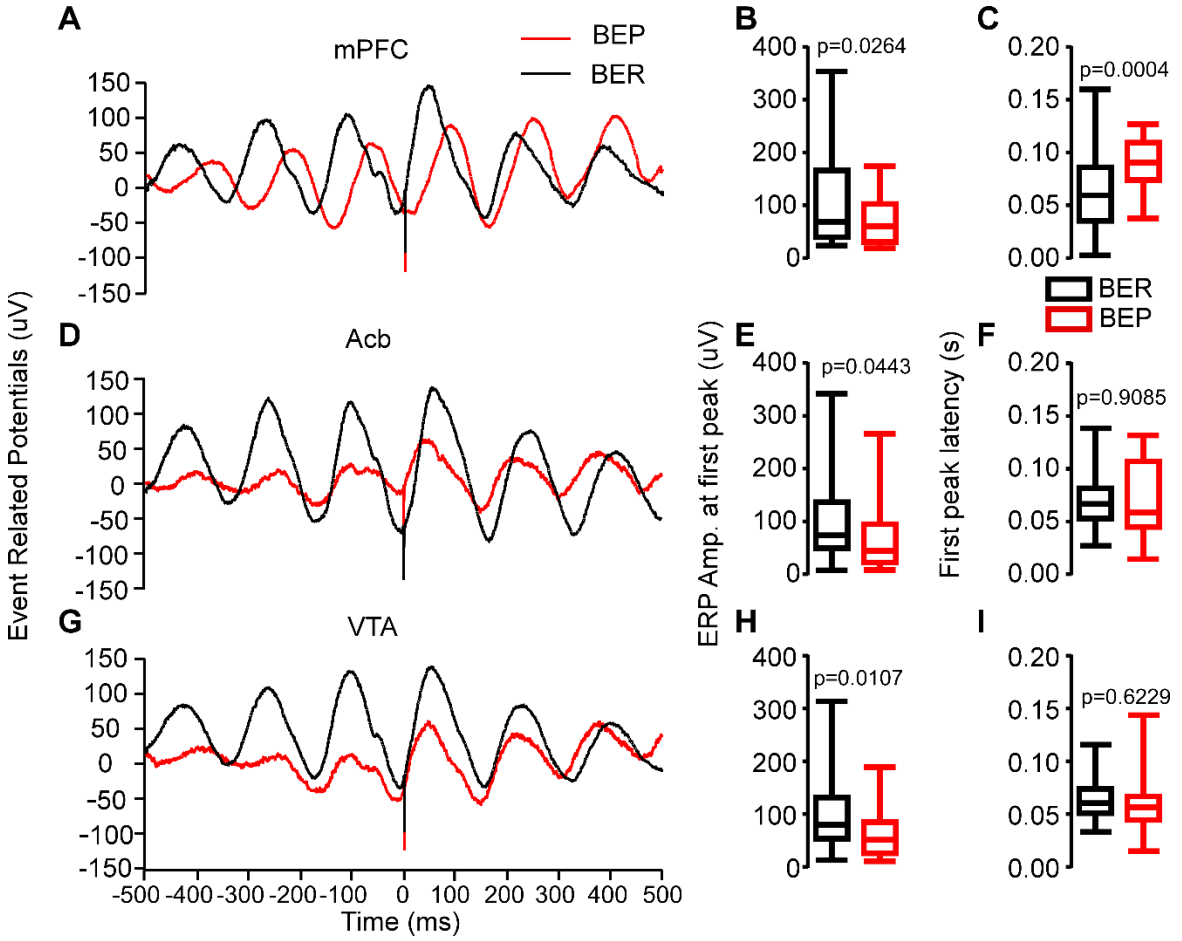

Figure 3 - 10 Averaged event-related potentials (ERP) per lick in binge eating prone (BEP) and binge eating resistant (BER) rats.

The event-related potential of BEP (red) and BER (black) from 500 ms before to 500 ms after each lick during the recording session in the medial prefrontal cortex (mPFC; A), nucleus accumbens (Acb; D), and ventral tegmental area (VTA; G). The ERP amplitude at the first peak just after each lick in BEP and BER rats for the mPFC (B), Acb (E), and VTA (H). The first peak latency in the mPFC (C), Acb (F), and VTA (I) of BER and BEP rats.

In all three regions, the ERP amplitude at the first peak following the lick was significantly higher in BER rats compared to BEP rats (Figure 3-10A, B, D, E, G, and H). Moreover, with the exception of the mPFC which showed a difference in first peak latency between BER and BEP rats (Figure 3-10C), the first peak latencies

were similar in both the Acb and VTA of BER and BEP rats (Figure 3-10F and I). In the mPFC, the first peak occurred earlier in BER rats than that in BEP rats.

By using polar plots, the ERP phases in the mPFC, Acb, and VTA at the time of lick was measured and summed as a polar histogram (Figure 3-11A). No significant difference in lick ERP phase preference was observed between the mPFC, Acb, and VTA of BER rats (Watson-Williams test, $p = 0.4748$; Figure 3-11A~C). However, this was not the case in the BEP rats in which the lick phase preference was significantly different between the mPFC, Acb, and VTA ($p < 0.0001$; Figure 3-11D~F). From the plots, it is obvious that this difference was due to the difference in the mPFC mean ERP phase preference (Figure 3-11A and D) in BEP rats. The ERP phase in the mPFC at the time of lick was much earlier in BER than in BEP rats ($p < 0.0001$). The Rao's spacing test was also performed to assess the uniformity in the distribution of the phases of the ERP for each region. According to this test, with the exception of the mPFC in BER rats which showed uniform distribution of the ERP phases ($p = 0.05$), the ERP phases in the mPFC of BEP rats ($p = 0.001$), and those in the Acb (BER: 0.001, BEP: 0.001) and VTA (BER: 0.001; BEP: 0.010) of both phenotypes were not uniformly distributed.

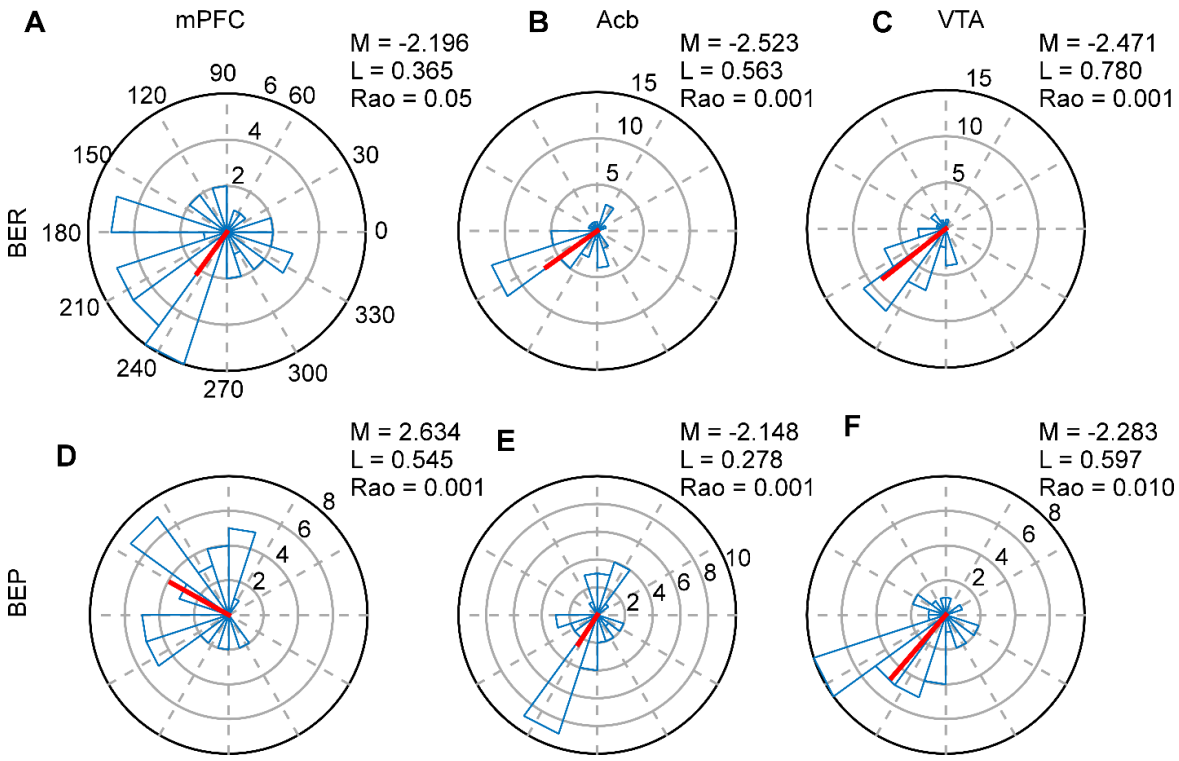

Figure 3 - 11 Polar plots of the event-related potential (ERP) phases in regions of interest.

Polar plots showing the ERP phases at time of lick from all electrodes in the medial prefrontal cortex (mPFC), nucleus accumbens (Acb), and ventral tegmental area (VTA) in binge eating resistant (BER; A, B, and C) and binge eating prone (BEP; D, E, and F) rats. Bin size: 20°, concentric circles: number of electrodes (channels), thick red line: line indicating the mean ERP phase preference in a specific region, M: mean ERP phase preference, L: length of resultant vector (length of thick red line), Rao: Results of Rao's spacing test.

3.6 Discussion

Our results showed that even though BEP rats consumed more sucrose than BER rats, the firing rates of neurons in the mPFC and VTA, but not the Acb, of BEP rats were lower than those in BER rats both before and during sucrose consumption. We also observed an increase in firing rate in all regions during sucrose consumption. Additionally, there was an increase in firing rate in between binges in BEP rats compared to BER rats. The results also showed that during the anticipation period,

the firing rate of mPFC, Acb, and VTA neurons increased in BEP rats but not in BER rats. Finally, we observed a reduction in ERP suggesting desynchronization in activity in the reward regions of BEP rats but not BER rats.

In the present study, the number of licks in clusters, cluster duration, and number of clusters were significantly higher in BEP compared to BER rats. The number and duration of licks reflect the palatability of sucrose (Davis and Smith, 1992) and since the number and duration of licks of BEP rats was larger and longer, respectively, compared to BER rats, BEP rats found the 10% sucrose solution more palatable. Additionally, since the number of lick clusters of BER rats was significantly lower than that of BEP rats, BER rats were less motivated to consume the 10% sucrose solution during the recording sessions (Davis and Perez, 1993; Spector et al., 1998; Lardeux et al., 2013). One of the criteria for BED is eating more rapidly than normal (American Psychiatric Association, 2013), which may be assessed using the lick frequency of rats. In this study, there was no difference in lick frequency between BER and BEP rats. This result is similar to that of Calvez and Timofeeva (2016), who reported that under non-stressful conditions, there is no difference in lick frequency between BER and BEP rats during 10% sucrose consumption.

Our results showed that before and during sucrose intake, the firing rate of mPFC and VTA, but not Acb, neurons in BEP rats was lower than that in BER rats (Figure 8). This finding is consistent with the decrease in activity in these regions observed in humans with BED (Balodis et al., 2013; Balodis et al., 2013a; Balodis et al., 2014; Reiter et al., 2017) and in animals (Moore et al., 2019; Quansah Amissah et al., 2019). The overall decrease in activity in the reward processing regions in BED patients may be due to the large proportion of neuron subtypes that are inhibited. In bingeing rats, deltaFosB is abundantly expressed in the mPFC, Acb, and VTA (Munoz-Escobar et al., 2019; Quansah Amissah et al., 2019). This may explain the low firing rate of neurons in the mPFC and VTA of BEP rats before sucrose access. Additionally, neurons in both BER and BEP rats increased their firing rate during sucrose consumption. This result is not surprising since sucrose consumption increases the activity of neurons in the mPFC, Acb, and VTA (Roop et al., 2002;

Matsumoto and Hikosaka, 2009; Cohen et al., 2012; Horst and Laubach, 2013). A decrease in mPFC activity has been associated with deficits in inhibitory control in humans (Balodis et al., 2013a). In animals, mPFC inactivation results in an increase in compulsive and impulsive behaviors (Murphy et al., 2012; Feja and Koch, 2014; Sinclair et al., 2019), while mPFC stimulation reduces these behaviors (Warthen et al., 2016). In the VTA, Rada et al. (2010) reported a decrease in VTA activity in bingeing rats. Surprisingly, in the Acb, the activity of identified neurons in BEP rats before and during sucrose access was higher than that in BER rats. This is surprising because stimulation of the Acb has been shown to decrease consumption (O'Connor et al., 2015) while its inhibition increases consumption (Wallace et al., 2008). Even though our finding is consistent with that of a neuroimaging study that reported an increase in Acb activity in BED patients (Lee et al., 2017), it may be due to the properties of the Acb neurons we identified in our study since the identified Acb neurons (n = 35) in the BEP rats, represent only a small subpopulation of neurons in the Acb. The temporary increase in the firing rate of neurons in the reward processing regions of BEP rats during sucrose consumption may underlie the fleeting feeling of relief that BED patients experience during the consumption of palatable food. This is because stimulation of neurons in the mPFC (Fuchikami et al., 2015; Hare et al., 2019) and VTA (Tye et al., 2013; Settell et al., 2017) has been shown to produce antidepressant effects, and depression is a comorbidity of BED (Yanovski et al., 1993; Wilfley et al., 2000; Hudson et al., 2007; Godfrey et al., 2019). This is also consistent with the hypothesis that BED patients binge to reactivate a hypofunctioning reward system (Wang et al., 2001; Geiger et al., 2009). However, further studies will be required to confirm this possibility using binge-like eating rats.

In all three regions, even though the firing rates of the identified neurons increased significantly during sucrose consumption, the intercluster interval firing rate of neurons in BER rats was significantly lower than that in BEP rats, while the cluster firing rate of BER rats was significantly higher than that in BEP rats. The high intercluster interval firing rate of BEP neurons during sucrose consumption may be due to the anticipation of BEP rats for the sucrose solution and may also contribute to the feeling of relief BED patients feel during bingeing since the anticipation for

sucrose solution increases neuronal activity in the reward regions (Roop et al., 2002; Matsumoto and Hikosaka, 2009; Horst and Laubach, 2013). To confirm this, we compared the firing rate of neurons between -2 to -1 s and -1 to 0 s before lick cluster start, which revealed that the firing rate during the latter period was significantly higher than that during the former period in BEP rats. This suggests that the BEP rats were expecting and anticipating the consumption of the sucrose solution while BER rats were not. Our results also showed that immediately following a lick, the activity of the identified neurons increased. However, this increase was much higher in BER rats compared to BEP rats. This was also expected since a decrease in activity was observed in the mPFC, Acb, and VTA of BED patients (Balodis et al., 2013; Balodis et al., 2013a; Balodis et al., 2014; Reiter et al., 2017) and animals (Moore et al., 2019; Quansah Amissah et al., 2019; Sinclair et al., 2019) and it has been shown that neurons in these regions express deltaFosB, which reduces neuronal excitability (Nestler et al., 2001; Vialou et al., 2010; Eagle et al., 2018). However, there were no differences in the latency to first peak in these regions between BER and BEP rats. One explanation may be that these identified neurons respond similarly in BEP and BER rats.

In our study, there was a higher proportion of LCE and lower proportion of LCI neurons in BER rats compared to BEP rats. Consistent with our previous deltaFosB measurements, this result suggests that less neurons were activated, and more neurons were inhibited in BEP rats compared to BER rats. DeltaFosB is a marker for chronic neuronal stimulation, and it was abundantly expressed in the mPFC, Acb, and VTA of BEP rats. DeltaFosB expression is negatively correlated with neuronal excitability (Nestler et al., 2001; Vialou et al., 2010; Eagle et al., 2018). We previously also showed that the proportion of deltaFosB-expressing neurons (inhibited neurons) was higher in BEP rats compared to BER rats, suggesting that the proportions of deltaFosB-expressing neuron subtypes may be relevant to the development of BED. Overall, the present and previous study (Quansah Amissah et al., 2019) support the hypothesis of the decrease in activity in the reward regions in BED patients (Balodis et al., 2013; Balodis et al., 2013a; Balodis et al., 2014).

We observed, based on LFP activity, that the ERP amplitude at first peak immediately after a lick was significantly higher in the mPFC, Acb, and VTA of BER rats compared to BEP rats, and that this ERP amplitude occurred earlier in the mPFC of BER rats compared to that in the mPFC of BEP rats. The major regions of the reward system are the mPFC, Acb, and VTA (Kelley and Berridge, 2002; Wise, 2005). The VTA is the main source of dopamine in the reward system and is activated upon the receipt of palatable food (Cohen et al., 2012), leading to the release of dopamine into the mPFC (Phillips et al., 2004) and Acb (van Zessen et al., 2012). The dopamine released by the VTA activates GABAergic medium spiny neurons in the Acb (Chuhma et al., 2004) and both glutamatergic and GABAergic neurons in the mPFC (Santana et al., 2009). Since the Acb and mPFC receive projections from the VTA, and the amount of dopamine released is affected by the extent of VTA activation. As a consequence, the extent of Acb and mPFC activity may depend on the extent of activation of the VTA (Yun et al., 2004; Lohani et al., 2019). Sucrose intake results in an increase in the levels of dopamine in the mPFC (Bassareo et al., 2002) and Acb (Hajnal et al., 2004), suggesting that the VTA may modulate activity in the Acb and mPFC. Our results show that the activity of neurons in the mPFC and Acb of BER rats was high when the VTA activity was high, while the mPFC and Acb activity in BEP rats was low when the VTA activity was low. A study in bingeing rats showed a decrease in the amount of dopamine released into the Acb, suggesting a decrease in VTA activity (Rada et al., 2010). Furthermore, our results in BEP rats are consistent with the reported decrease in activity in these reward regions in BED patients (Balodis et al., 2013; Balodis et al., 2013a; Balodis et al., 2014; Reiter et al., 2017). Altogether, the results suggest that impaired activity in the VTA may affect the quantity of dopamine released in the mPFC and Acb, inducing binge eating disorder.

In the present study, our results showed that while the ERP phase preference in the mPFC, Acb, and VTA of BER rats at the time of lick was not different, that of BEP rats was different, suggesting a desynchronization in the reward system of BEP rats. Synchrony in the activity of regions within brain systems is critical for their proper functioning. Diseases such as schizophrenia, epilepsy, and autism are associated

with abnormalities in neuronal synchronization in certain regions of the brain (Uhlhaas and Singer, 2006). The desynchronization we observed in BEP rats was mainly due to the mPFC, which is involved in inhibitory control, and previous studies from our lab showed that our BEP rats are compulsive (Calvez and Timofeeva, 2016; Quansah Amissah et al., 2019). This suggests that desynchronization in the mPFC may be an important part of BED and treatments which can restore synchronization may be beneficial for treating this disorder.

In conclusion, these results show that activity in mPFC and VTA is low in binge-like eating rats regardless of whether they are bingeing or not. However, during and between binges, reward system activity in bingeing rats increases, and may underlie the sense of relief patients express during bingeing episodes since individually stimulating these regions produces antidepressant effects and depression is a comorbidity of binge eating disorder. We also found that the decrease in synchrony in the activity of regions in the reward system, specifically the mPFC, may contribute to binge eating disorder.

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3.9 Declarations of interest

None.

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Conclusion

EDs are psychological disorders that affect our eating habits. There are several types, however, the most prevalent is BED, which is more prevalent in females than in males (Hudson et al., 2007; Kessler et al., 2013). However, most of the studies on binge eating disorder involved either males (Swanson et al., 2011; Sweeting et al., 2015; Hammerle et al., 2016) or nonbingeing rats, which may have contributed to a number of mixed results. To address this issue we used a female binge-like eating rat model. The high prevalence of BED in the general population, and the consequent effects on the quality of life of people, and its high socioeconomic cost (Paxton et al., 2012; Agh et al., 2016) warrant research into BED for the development of efficient treatments. In order to do this, we must understand the neurobiological mechanisms that underlie BED. This is the overarching objective of my research topic.

BED is characterized by the consumption of a large amount of palatable food than would normally be consumed in a discrete amount of time, and a loss of sense of control during the binging episode (American Psychiatric Association, 2013). BED is usually triggered by some form of negative event (Torres and Nowson, 2007), which leads to the consumption of palatable food, suggesting the involvement of the reward system, taste system, and stress system. In humans, fMRI studies have reported contradictory results on the regions implicated in BED. While some studies support an increase in activity (Karhunen et al., 2000; Filbey et al., 2012; Lee et al., 2017), others reported a decrease in activity (Balodis et al., 2013; Balodis et al., 2013a; Balodis et al., 2014; Reiter et al., 2017) in regions of interest in BED patients. In order to clearly identify the implicated systems, I conducted an experiment in binge-like and nonbinge-like eating rats to investigate the activity of neurons in these systems. My experiment revealed that among the systems in the brain, which were hypothesized to be implicated, the reward system is the most important. This was done using the expression of deltaFosB, a marker for chronic neuronal stimulation (Perrotti et al., 2004; Perrotti et al., 2008; Wallace et al., 2008).

Palatable food consumption results in the stimulation of neurons in the reward system (Bassareo et al., 2002; Hajnal et al., 2004; Cohen et al., 2012). More specifically, palatable food consumption activates taste receptors, which then transfers this signal via the facial, vagus, and glossopharyngeal nerves (Kamath et al., 2015) to the NTS (Travers and Norgren, 1995). This information reaches the PBN via projections from the NTS (Norgren and Leonard, 1971). Neurons in both the PBN and NTS send projections to the VTA (Beier et al., 2015; Richard et al., 2015). These inputs may lead to the activation of the VTA, which comprises mostly of dopaminergic neurons. Upon activation, these dopaminergic neurons release dopamine in a number of structures. However, in the reward system, the most important structures targeted by VTA dopaminergic neurons are the mPFC (Phillips et al., 2004) and Acb (van Zessen et al., 2012). This results in the activation of neurons in these structures (Chuhma et al., 2004; Santana et al., 2009). Repeated sucrose consumption leads to the repeated stimulation of neurons in these regions. These neurons eventually become desensitized, resulting in a decrease in activity in these regions (O'Connor and Kenny, 2016; Moore et al., 2018). Among the neuronal markers available, ΔFosB was shown to be expressed as a result of chronic stimulation of neurons (Perrotti et al., 2004; Perrotti et al., 2008; Wallace et al., 2008; Munoz-Escobar et al., 2019). Interestingly, neurons which express ΔFosB become less excitable (Nestler et al., 2001; Vialou et al., 2010; Eagle et al., 2018). This suggests that the ΔFosB expressed in the mPFC, Acb, and VTA in our study might play a role in this desensitization observed following chronic sucrose consumption.

ΔFosB, upon its expression, modulates the expression of a number of genes, including those for the brain-derived neurotrophic factor (BDNF), cholecystokinin, and calcium calmodulin-dependent protein kinase II (CAMKII) (McClung and Nestler, 2003). The gene from which the GluR2 receptor is expressed (Kelz et al., 1999) is also modulated by ΔFosB. Specifically, ΔFosB binds to the GluR2 promoter resulting in an increase in the expression of GluR2 mRNA in neurons which express AMPA receptors (Vialou et al., 2010). The overexpression of GluR2 receptors has been shown to decrease the excitability of neurons in which this ΔFosB is expressed (Vialou et al., 2010; Eagle et al., 2018). This evidence suggests that ΔFosB may

underlie the desensitization observed in the reward processing regions in BED patients.

In the first study in this research project (Figure 4-1A), the activity of mPFC glutamatergic neurons and GABAergic interneurons decreased as a result of the increased expression of ΔFosB. However, this decrease in activity was more prominent in the mPFC of binge eating prone rats compared to that of binge eating resistant rats. In the Acb, which contains 95% GABAergic medium spiny neurons, activity was also significantly decreased in BEP rats compared to BER rats due to the abundant expression of ΔFosB. In the VTA as well, there was a decrease in activity in BEP rats compared to BER rats.

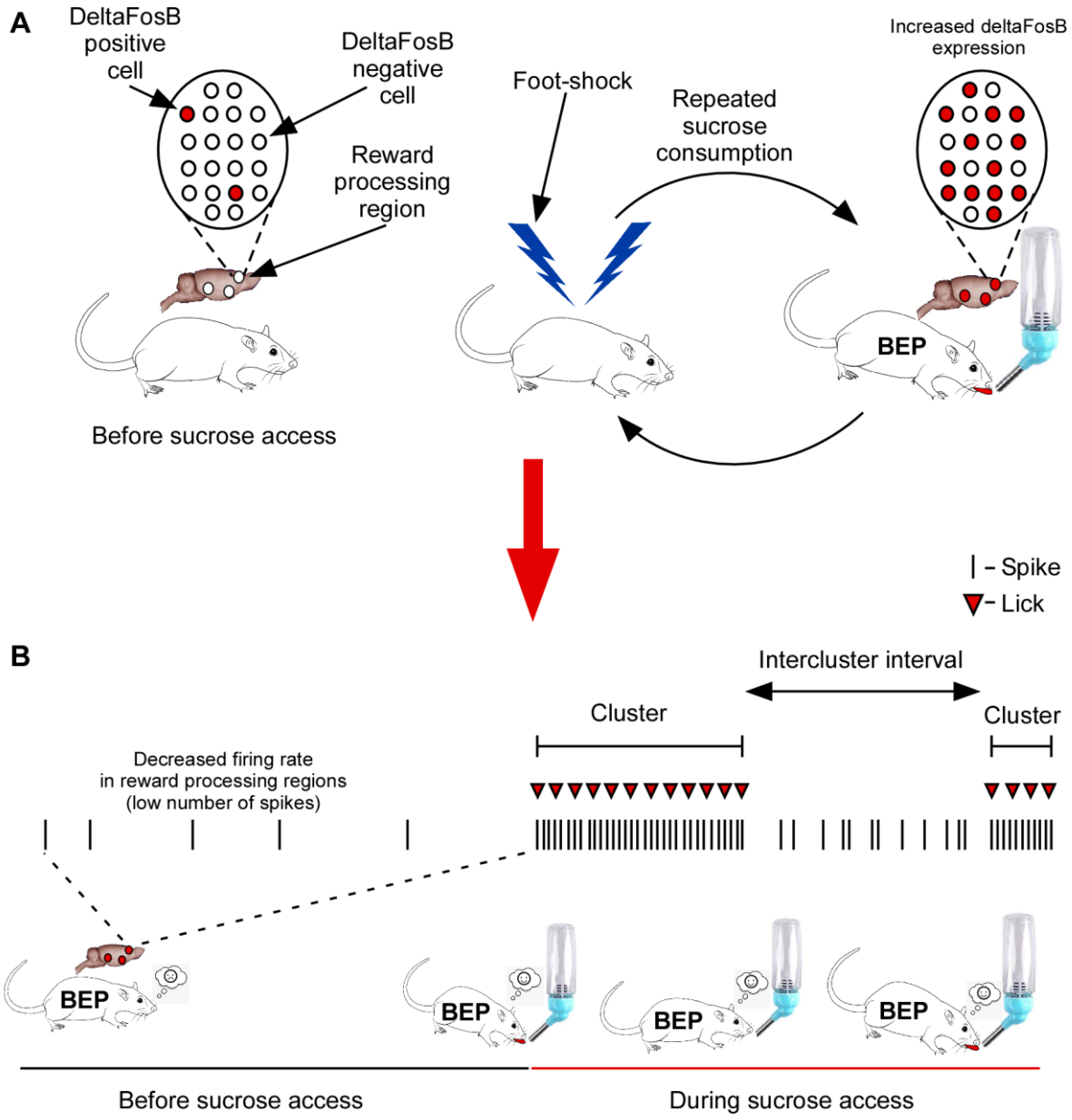

Figure 4 - 1 Role of the reward system in a female binge-like eating rat model.

A. Chronic consumption of palatable food results in the abundant expression of ΔFosB in the reward processing regions which include the medial prefrontal cortex, nucleus accumbens, and ventral tegmental area in binge eating prone rats. ΔFosB expression results in a decrease in activity (firing rate) of neurons in which they are expressed, eventually leading to the overall decrease of activity in these regions. B. Consumption of palatable food reactivates the previously hypofunctioning reward regions, which may underlie the sense of relief during bingeing. This reactivation is

sustained, even though at a lower amplitude, in between binges. However, this reactivation ceases following the termination of bingeing, preparing the rat for the next binge.

These results are consistent with studies which reported a decrease in activity in the reward system (Balodis et al., 2013; Balodis et al., 2013a; Balodis et al., 2014; Reiter et al., 2017). Additionally, a decrease in activity in the dopaminergic neurons in the VTA was reported in bingeing animals (Rada et al., 2010). Inhibition of the Acb has been shown to increase response to and consumption of food reward (Olausson et al., 2006; Wallace et al., 2008). The proportion of neuron populations were found to be similar in the mPFC of both BEP and BER rats and the Acb of both BEP and BER rats. However, in the VTA, the proportion of GABAergic neurons that expressed ΔFosB was higher in the BER rats compared to that in BEP rats. This suggests that the proportions of neurons in the VTA might play a significant role in the development of BED in humans. Decreased mPFC activity was reported to underlie the loss of sense of control observed in BED patients (Balodis et al., 2013a; Hege et al., 2015) since the mPFC is responsible for inhibitory control (Aron et al., 2004). Additionally, several animal studies have shown that inhibiting the mPFC increased compulsive and impulsive behaviors (Murphy et al., 2012; Feja and Koch, 2014), while stimulating neurons in the mPFC reduces these behaviors (Warthen et al., 2016).

Several studies suggested that the reward system is implicated in binge eating disorder (Schwartz et al., 2000; Bake et al., 2013; Wierenga et al., 2014). However, most studies involving electrophysiology targeted individual structures within this system (Krause et al., 2010; van Zessen et al., 2012; Jennings et al., 2013). Even though the results from such studies may be relevant, it will be better to study the entire system simultaneously in order to detect any abnormalities that might be revealed at the system level but missed at the individual structure level. In order to confirm the results of the first study in real-time in awake behaving rats, we conducted a second study (Figure 4-1B) involving the *in vivo* recording of neuronal activity in the most important regions in the reward system such as the mPFC, Acb, and VTA. BED patients may binge in order to reactivate an already hypofunctioning

reward system (Wang et al., 2001; Geiger et al., 2009), since my previous study showed that activity in the reward system in bingeing rats was decreased compared to nonbingeing rats. This hypothesis was derived from the observation that BED patients express a sense of relief following the binge episode (Heatherton and Baumeister, 1991; Stickney and Miltenberger, 1999), which suggests that people binge in order to reduce a particular negative emotion they were feeling prior to the binge episode. This relief may be attributable to a decrease in their state of depression following the bingeing episode. This is because BED patients have also been found to be depressed (Yanovski et al., 1993; Wilfley et al., 2000; Hudson et al., 2007; Godfrey et al., 2019), which might be the reason why these patients can be treated using antidepressants (Carter et al., 2003; McElroy, 2017). However, the relief following palatable food consumption is temporary since immediately after the binge, the level of negative affect in these binge eating disorder patients is restored to its level prior to the binge, preparing the patients for the next binge episode (Deaver et al., 2003). To assess this possibility, we investigated neuron firing rate during the lick intercluster interval. The results of the second study confirmed that real-time firing rate of neurons in the mPFC and VTA, but not the Acb, in binge eating prone rats was lower than that in binge eating resistant rats both before and during sucrose access, even though for both phenotypes the firing rate of neurons increased following sucrose consumption. This increase in firing rate in neurons in the reward system of binge eating prone rats may underlie the fleeting sense of relief they express during palatable food consumption. We also found that a greater percentage of neurons were inhibited and a lower percentage of neurons were excited at the beginning of sucrose consumption in binge eating prone rats. The high proportion of inhibited neurons and low proportion of activated neurons in the reward processing regions of binge eating prone rats may contribute to the overall decrease in activity in these regions, as observed in a number of fMRI studies (Balodis et al., 2013; Balodis et al., 2013a; Balodis et al., 2014; Reiter et al., 2017). In between sucrose consumption, the firing rate of neurons in binge eating prone rats was much higher than that in binge eating resistant rats. This may also underlie the feeling of relief attributed to the consumption of palatable food by human patients, since the

consumption of palatable food increases the firing rate of neurons in the reward system (Bassareo et al., 2002; Hajnal et al., 2004; Cohen et al., 2012). This result is consistent with the results of studies which showed that stimulation of either the mPFC (Fuchikami et al., 2015; Hare et al., 2019), Acb (Schlaepfer et al., 2008), or the VTA (Tye et al., 2013; Settell et al., 2017) produced antidepressant effects, and it has been shown that one of the comorbidities of binge eating disorder is depression (Yanovski et al., 1993; Wilfley et al., 2000; Hudson et al., 2007).

Another interesting result from the second study is that there was a desynchronization in the activity of regions in the reward system of binge eating prone rats, which was evaluated as a reduction in the mean ERP amplitude and phase preference at lick onset in the mPFC. Desynchronization in the activity of neurons in the brain systems in patients with schizophrenia, autism, and epilepsy has been reported (Uhlhaas and Singer, 2006). This may be the case in BED as well. However, further studies to investigate this possibility in both humans and animals are required to confirm these results. This is because, a few studies have hypothesized that binge eating disorder is a disorder of inhibitory control (Volkow et al., 2013), highlighting the significant role of the mPFC, since it is one of the main structures in the brain responsible for inhibitory control.

In conclusion, the series of studies in this research project demonstrated that in our binge-like eating rat model, there is hypofunctioning of the brain reward system and that this decrease in activity may be due to the expression of ΔFosB, which reduces neuronal excitability, in the reward system. It also suggests that the desensitization of these regions due to repeated stimulation may be due to the ΔFosB expressed. These results were also confirmed using real-time activity of neurons in the reward processing regions recorded *in vivo* in awake behaving rats. We also found an increase in activity of the reward system during and between binges in bingeing rats. This may underlie the feeling of relief expressed by binge eating disorder patients during and between binges, since stimulation of these regions individually have been shown to produce antidepressant effects and depression is a comorbidity of binge eating disorder. Another interesting finding was the lack of synchrony in the reward

processing regions, specifically due to the medial prefrontal cortex, in bingeing rats. This may have also contributed to the binge-like eating in these rats, since desynchronization within brain systems have been associated with schizophrenia, autism, and epilepsy.

Limitations

It would be interesting to know exactly when rats become binge eating prone based on activity in these regions since a limitation in my research is that I only studied activity in these regions of interest following the development of the binge eating model. However, information on the exact time when the binge eating disorder is initiated as a result of the decrease in activity in these regions might help develop an intervention before full-blown binge eating disorder sets in.

Additionally, even though my studies showed a decrease in activity in these regions, I did not confirm in this model whether this decrease in activity is a consequence of the bingeing or is the cause of the bingeing. This can be done in future using optogenetic or chemogenetic approaches. Simultaneously activating or inhibiting regions in both bingeing and nonbingeing rats should affect bingeing.

Finally, knowing that desynchronization might be associated with binge eating disorder, the existing treatments can, in addition to using pharmacological approaches to restore normalcy, also attempt approaches that will help restore synchrony in these regions, specifically the medial prefrontal cortex. If synchrony is indeed key to the development of binge eating disorder then studies can first attempt to identify the exact time when this desynchrony occurs. Following that, desynchronization can be induced in these three brain regions in normal rats to study its consequences and later restored to investigate if the disorder can be reversed.

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