Compulsive eating of binge- like eating prone rats under conditioned fear and exploration of the neural mechanism with c-fos expression

Thèse

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Résumé

Le trouble de l'hyperphagie boulimique (THB) est un trouble de l'alimentation défini de manière autonome dans la 5e édition du Manuel diagnostique et statistique des troubles mentaux (DSM-5) en mai 2013. Le THB est caractérisé par des épisodes d'ingestion d'une quantité anormalement élevée de nourriture dans une courte période de temps sans comportements compensatoires tels que des vomissements auto-induits. La prévalence du THB a augmenté très rapidement en raison de la grande disponibilité d'aliments riches en calories et du stress croissant dans la vie moderne. Malheureusement, l'étiologie du THB est encore mal comprise et les traitements cliniques actuels du THB sont principalement limités à la thérapie cognitive comportementale, dont le pronostic est également assez limité. Afin d'étudier le THB, notre laboratoire a précédemment développé un modèle de THB chez le rat en utilisant une combinaison de stress causé par des chocs électriques aux pattes et d'un accès intermittent d'une heure à une solution de sucrose à 10%. Dans notre modèle de rat THB, les rats sujets à la frénésie alimentaire (BEP; de l'anglais binge-eating prone) ont consommé plus de sucrose que les rats résistants à la frénésie alimentaire (BER, de l'anglais binge-eating resistant) à la fois dans des conditions normales et ces rats ont augmenté davantage leur consommation après avoir vécu le stress. Nous avons également observé une alimentation compulsive dans les rats BEP avec notre test modifié de boîte claire / sombre. Une alimentation compulsive est la caractéristique la plus obstinée du THB. Mon projet de thèse se concentre sur l'observation de l'alimentation compulsive dans le modèle de THB chez le rat avec un test concurrentiel, dans lequel les comportements d'alimentation et d'immobilité ont été surveillés en présence d'un stimulus auditif conditionné de manière aversive. Les rats BEP ont montré une consommation persistante élevée de sucrose et ont montré une réponse inhibée à la peur induite en situation stressante en comparaison aux rats BER, indiquant respectivement un déficit de dévaluation de l'appétence et une réponse anxiolytique plus forte au sucrose. Après l'observation de l'alimentation compulsive dans nos rats BEP, nous avons analysé les activités cérébrales de ces rats avec l'hybridation *in situ* de l'ARNm c-fos. Nous avons trouvé que, dans les rats BEP, le sucrose réduisait l'activité c-fos du noyau paraventriculaire de l'hypothalamus, tout en augmentant l'activité dans la zone hypothalamique latérale face au stimulus conditionné aversif. La résistance à la dévaluation de l'appétence de la nourriture pourrait être le résultat d'un recrutement atténué de la réponse

du cortex préfrontal médian et d'une réponse persistante du noyau accumbens à la consommation de sucrose. Ces résultats suggèrent que le système de récompense a pris le dessus sur les systèmes homéostatiques et répondant au stress. Étonnamment, l'apport de sucrose sous la peur conditionnée n'a pas inhibé l'activité de l'amygdale centrale, mais l'a plutôt activée à la place. Cette étude a exploré le mécanisme de l'alimentation compulsive dans un modèle de THB et a fourni certaines cibles cérébrales, telles que le noyau accumbens, pour de futures recherches thérapeutiques.

Abstract

The binge eating disorder (BED) is an eating disorder that was defined in the 5th edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-5) in May 2013. The BED is characterized by episodes of ingestion of abnormally large amounts of food in a short period of time without compensative behaviors such as self-induced vomiting. The prevalence of the BED is on the rise due to the availability of high-calorie food and the stressors of modern life. Unfortunately, the etiology of the BED is still poorly understood, and current clinical treatments of the BED are mostly limited to cognitive behavioral therapy, of which the prognosis is also quite limited. In order to study the BED, our lab previously developed a rat model of the BED with combination of foot-shock stress and intermittent 1 h access to a 10% sucrose solution. In our BED rat model, the binge-like eating prone rats (BEPs) consumed more sucrose than the binge-like eating resistant rats (BERs) in normal conditions and consumed more sucrose in response to stress. We also observed compulsive eating in the BEPs with our modified light/dark box test. Compulsive eating is the most obstinate feature of the BED. My PhD project focuses on the observation of compulsive eating in the BED rat model with a conflicting test, in which the feeding and freezing behaviors were monitored in the presence of an aversively conditioned auditory stimulus. The BEPs showed persistently high sucrose intake and inhibited fear response under stress when comparted with BERs, respectively indicating a deficiency in palatability devaluation and stronger anxiolytic response to sucrose. After the observation of compulsive eating in the BEPs, we further analyzed the brain activities of the BEPs and BERs by analyzing the expression of *c-fos* mRNA using *in situ* hybridization. In the BEPs, we found that sucrose reduced *c-fos* expression in the paraventricular nucleus of the hypothalamus (PVN) in response to an aversively conditioned stimulus (CS), but enhanced activities in the lateral hypothalamic area (LHA) in response to the CS. The resistance to devaluating the palatable food could be a result of attenuated recruitment of the medial prefrontal cortex (mPFC) and persistent nucleus accumbens (Acb) response to the sucrose intake. These findings suggest that the rewarding system overrode the homeostatic and the stress-responding systems. Surprisingly, the sucrose intake under fear conditions did not inhibit the activity of the central amygdala, but further activated it instead. Current study explored the mechanism of compulsive eating in the BED, and suggests that the mPFC and Acb should be examined for further therapeutic research.

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List of abbreviation

3V	3rd ventricle
5-HT	5-hydroxytryptamine, serotonin
5-HT1A	5-hydroxytryptamine receptor 1A
5-HT1B	5-hydroxytryptamine receptor 1B
5-HT2C	5-hydroxytryptamine receptor 2C
5-HT4	5-hydroxytryptamine receptor 4
5-HTIAA	5-hydroxyindoleacetic acid
ac	anterior commissure
aca	anterior part of the anterior commissure
Acb	nucleus accumbens
AcbC	nucleus accumbens core
AcbSh	nucleus accumbens shell
ACh	acetylcholine
ACTH	adrenocorticotrophic hormone
AgRP	agouti-related protein
AMPA	a-amino-3hydoxy-5-meghyl-4isoxazolepropionate
AP-1	activating protein complex 1
ARC	arcuate nucleus of the hypothalamus
$ARC_{AgRP/NPY}$	AgRP neurons in the ARC
ARC_{POMC}	POMC neurons in the ARC
ARC_{OXTR}	Oxytocin receptor expressing neurons in the ARC
BED	binge eating disorder
BEP	bigne-like eatign prone
BEPs	bigne-like eatign prone rats
BER	binge-like eating resistant
BERs	binge-like eating resistant rats
BIS-11	Barratt impulsiveness scale
BMI	body mass index
BNST	bed nucleus of the stria terminalis
CaMK	calcium/calmodulin-dependent protein kinase
Ce	central amygdala
CGRP	calcitonin gene-related peptide
Cl	claustrum
CNS	central neverous system
CREB	cAMP responsive element binding protein
CRH	corticotrophin releasing hormone
CS	conditioned stimulus
D2R	dopamine receptor D2

DA	dopamine
DAT	dopamine transporter
db	diabetes
DNA	deoxyribonucleic acid
f	fornix
fmi	forceps minor of the corpus callosum
GHS-R1A	growth hormone secretagogue receptor 1A, a ghrelin receptor
HPA axis	hypothalamic-pituitary-adrenal axis
IEG	immediate early gene
IL	infralimbic part of the medial prefrontal cortex
LHA	lateral hypothalamic area
LRG	late response gene
L-VSCCs	L type voltage-sensitive calcium channels
MAPK	Ras-mitogen-associated protein kinase
MC4R	melanocortin-4 receptor
MEF2	myocyte enhancer factor 2
MG	medial geniculate nucleus
mPFC	medial prefrontal cortex
mRNA	messenger RNA
NMDA	N-methyl-D-aspartate
NPY	neuropeptide Y
NTS	nucleus tractus solitaries
ob	obese
ob-R	leptin receptor
opt	optic tract
PAG	periaqueductal gray area
PBN	parabrachial nucleus
PBN_{CGRP}	PBN CGRP neurons
PeFLH	perifornical part of the LHA
PFC	prefrontal cortex
PIN	posterior intralaminar nuclei
PLH	peduncular part of the LHA
POMC	proopiomelanocortin
PrL	prelimbic part of the medial prefrontal cortex
PV	paralvumin
PVN	paraventricular nucleus of the hypothalamus
PVNm	magnocellulair part of the PVN
PVNmpd	dorsolateral part of the medial parvocellular PVN

PVNp	parvocellulair part of the PVN
PVN_{MC4R}	MC4R expressing neurons in the PVN
RNA	Ribonucleic acid
SERT	serotonin transporter
SON	supraoptic nucleus
SRF	serum response factor
SST	somatostatin
STD	dorsal part of the BNST
STV	ventral part of the BNST
TFs	transcription factors
UPPS-P	urgency, premeditation, perseverance, sensation seeking, and positive urgency
US	unconditioned stimulus
VIP	vasoactive intestinal peptide
vlPAG	ventrolateral PAG
VMH	ventromedial nucleus of the hypothalamus
VTA	ventral tegmental area

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Foreword

The manuscript presented in Chapter 1 has not been submitted yet. I am the first author of this manuscript. I was involved in the experiments presented in all figures. The experiment was designed by Dr. Elena Timofeeva, Dr. Juliane Calvez and me. The procedures were conducted by me, with assistance from Geneviève Guèvremont. The manuscript was written by me, and revised by Dr. Sandrine Chometton and Dr. Igor Timofeev. The programs used in the Med Associates system were written by me, the MATLAB programs used to analysis the licking events and optical density of the *c-fos* mRNA signals were adapted from Christoph Longlos and modified by me to our particular purposes.

The results were obtained using unbiased approaches. Licking events were detected using a lickometer of Med Asociates system, recorded with TDT system, and analyzed with customized MATLAB program. As for the measurements of *c-fos* mRNA, images were taken using an optical microscope with the StereoInvestigator software, and the optical density of the areas of interest on the images were quantified using customized MATLAB program.

Introduction

1.1 Two forms of food intake regulation

1.1.1 Homeostatic eating

For animals, food is the main source of all kinds of nutritions, such as proteins, carbohydrates, fat, and vitamins. The amount of energy intake is controlled via the regulation of feeding behaviors by complicated neural and endocrine systems (Figure 1-1). The cooperation of these systems ensures a stable bodyweight by making a dynamic balance between energy intake and expenditure over a long period, which is called 'energy homeostasis'. The concept of energy homeostasis was first proposed by Kennedy in 1953 (Kennedy 1953, Morton and Schwartz 2001). Sometimes, the energy homeostasis could be disrupted, and the circuits regulating the normal food intake could be replaced by some 'emergency feeding circuits'. When the energy intake is larger than the expenditure, the bodyweight will increase as a way to store the extra energy.

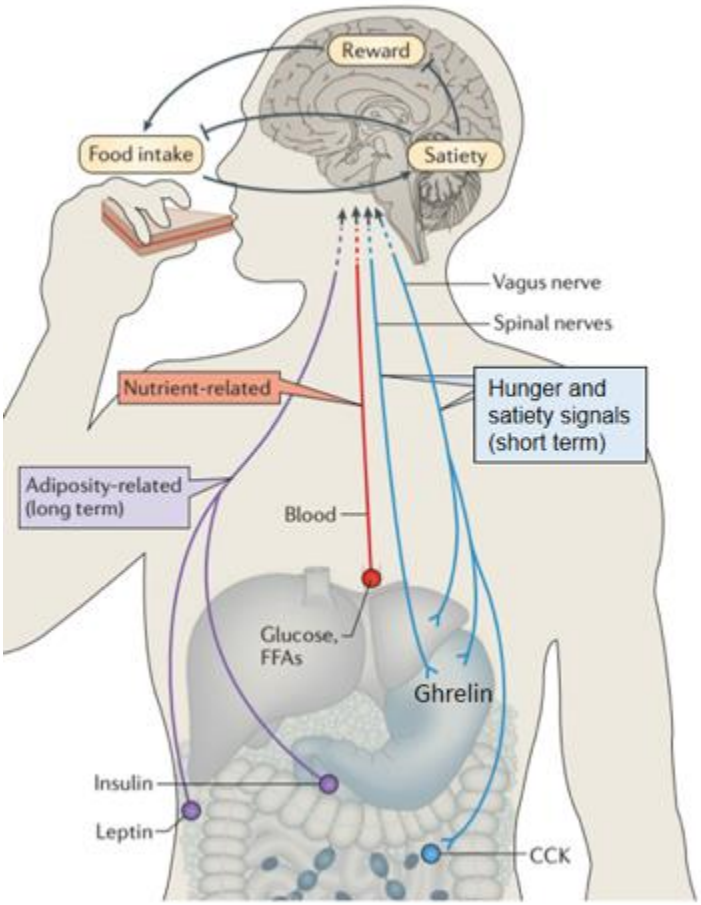

Figure 1- 1. Homeostatic eating regulating system.

The CNS integrates input from long-term energy stores (for example, leptin) and short-term meal-related signals (nutrients and gut-derived satiety signals) to regulate food intake and energy expenditure in a manner that maintains stable body fat stores over time. Positive energy balance induced by overfeeding inhibits the rewarding properties of food while enhancing meal-induced satiety, thereby reducing food intake. In response to energy deprivation, CNS adaptive responses are engaged that both increase the rewarding properties of food and reduce the response to satiety signals, collectively resulting in increased food consumption until deficient fat stores are replenished. CCK, cholecystokinin; FFAs, free fatty acids; GLP1, glucagon-like peptide. Adapted from (Morton, Meek, and Schwartz 2014).

Feeding behaviors are regulated by delicate feedback systems, which are modulated not only by the stored and circulating nutrients but also by various physical, psychological and environmental factors. The initiation and cessation of food intake is basically controlled by hunger and satiety signals generated in peripheral tissues such as the digestive tract (e.g. ghrelin and cholecystokinin) and adipose tissue (e.g. leptin) as well as the central nervous system (Figure 1-1). The sensation of hunger and the motivation to seek food are the central events of feeding behaviors, while the satiety signals mediate the termination of food ingestion largely through inhibiting the effects of hunger signals (Nakamura and Nakamura 2018).

The discovery and identification of appetite regulating signals started with the realization of the importance of satiety peptides (e.g. leptin), in controlling the termination of food intake. It has long been recognized that endocrine factors play critical roles in the regulation of appetite and food ingestion. Until now, more than 20 regulatory adiposity and gastrointestinal hormones have been found with a wider variety of functions related to feeding (Suzuki, Simpson et al. 2010). More metabolism and appetite regulating factors from different sources are being discovered, such as some bone-derived hormones (Mera, Ferron et al. 2018). Several hunger and satiety peptides revealed numerous new knowledge in appetite regulation and pharmacological treatment of eating disorders, such as anorexia nervosa and binge eating disorder (BED), which will be discussed in detail in the following part of the introduction.

1.1.2 Hedonic eating/Eating for reward

Hedonia is the sensation of pleasant stimuli, which is a subjective perception. The rewarding feature of the palatable food can motivate the animal to consume more food, and the hedonic response to palatable foods is very effective to trigger eating even when food ingestion is not necessary (Tepper and Yeomans 2017). This type of eating has been termed as 'non-homeostatic eating', because its purpose is not to restore the energy depletion. Schwartz argued that the regulation of response to the rewarding food is integral to how energy homeostasis is achieved, because the levels of hedonic response to foods are influenced by the metabolic status of the body, and accordingly lead to different levels of motivation for food consumption (Morton, Meek et al. 2014). Nevertheless, an undeniable truth is that food consumption motivated by hedonic responses to palatable foods can result in excessive

caloric intake, which plays an important role in the increasingly high rates of obesity in modern society (Timofeeva and Mitra 2013).

The taste of palatable food provokes its intrinsic reinforcing properties, while the interaction between taste, metabolism and reward systems makes this effect more dynamic. The taste system has different thresholds for different tastants, the taste-provoking chemical molecules. Largely, the threshold concentrations of the taste system are much higher than that of the olfactory system. The perceptual threshold is 2 mM for citric acid, 10 mM for salt (NaCl), and 20 mM for sucrose. It is not surprising that a higher sensitivity for poisonous substances is vitally important to for animals. For example, the perceptual threshold is 0.08 mM for quinine, and 0.0001 mM for the deadly substance strychnine (Hoegg and Alba 2006). Rats can identify an aversive tastant with relatively few licks, and change their licking pattern as a result (Weiss and Di Lorenzo 2012).

One possible reason for the higher threshold for nutritious components is that the body needs more nutrients, such as carbohydrates and salt, to maintain the homeostasis of energy and electrolyte. The blunted sensitivity to nutritious components can promote adequate ingestion of specific food likely by being proportional to the requirement of the body. Considering the short history of the prevalence of sweeteners, such as sugar and honey, it is reasonable to speculate that the decreasing sensitivity to sweet taste could be one of the reasons of increasing ingestion of sugar in the modern world.

1.2 Endocrine and neuronal signals involved in food intake regulation

Fat and glucose are two main form of energy in the animal body. The quantity of them ingested, stored and circulating in the body is controlled to keep the energy homeostasis in the body (Figure 1-1). To maintain the energy homeostasis the regulating systems need to detect, integrate and respond to various internal and external signals, which involves the interaction between the nervous system and a variety of long-term and short-term signals related to the metabolic status of the organism and nutrient content of individual meals.

1.2.1 Hunger-related signals

1.2.1.1 Decreased plasma glucose

As the main form of direct energy supply for the body, plasma glucose also signals to the brain about the quantity of immediately available fuel (Figure 1-1). The deficiency of

circulating glucose in the plasma during hypoglycemia can trigger the feeling of hunger and subsequent brain responses to motivate physical and behavioral activities, such as food seeking and eating, to restore the energy availability and storage (Morton, Meek et al. 2014).

1.2.1.2 Orexin

Orexin is a neuropeptide well known for its functions in regulating arousal, wakefulness and appetite (Davis, Choi et al. 2011). Two subtypes of the orexins, orexin A and B, derive from the same precursor, a 130 amino acid named prepro-orexin. Both orexin A and B are produced in the lateral and posterior hypothalamus and projected widely into many brain regions. The receptors for orexins belong to the G-protein coupled receptor family (Sakurai, Amemiya et al. 1998). Animal studies showed that fasting could activate the orexin neurons and that intracerebroventricular injection of orexin A or B had prominent orexigenic effects (Sakurai, Amemiya et al. 1998). More interestingly, orexin participates in the regulation of food intake not only via the metabolic pathway. The Orexin/hypocretin receptor 1 has been found to be necessary for the Palvovian appetitive conditioning and extinction, which means it is also very important for learned feeding-related behaviors (Keefer, Cole et al. 2016).

Since the discovery of orexin numerous studies have suggested a dichotomy in orexin functions, in regulation of both arousal and feeding (Harris and Aston-Jones 2006). Harris and Aston-Jones hypothesized that orexin neurons in different sub-regions of the hypothalamus have different functions. They proposed that orexin neurons in the lateral hypothalamus are involved in the process of reward information related to food intake and drug abuse, whereas those in the dorsomedial and perifornical hypothalamus mediate arousal and stress response (Harris and Aston-Jones 2006). Furthermore, Saper proposed a role of orexin in coordinating the sleep/awake cycle with the need of eating. ‘After all, animals can only eat when they are awake’. He came up with a flip-flop model, in which the orexin plays a critical role in switching between sleep and wakefulness. After integrating the information about the energy storage, carried by circulating signals such as leptin and ghrelin, the dorsomedial hypothalamus (DMH) relays to the lateral hypothalamic area (LHA), and modulates the arousal states according to the need of food ingestion (Saper 2006).

1.2.1.3 Ghrelin

Synthesized and released by specialized gastrointestinal endocrine cells (Figure 1-1), ghrelin (the “hunger hormone”) is a 28-amino acid acylated peptide functioning as a gut-brain

hunger hormone that typically stimulates food ingestion (Wren, Seal et al. 2001). Ghrelin levels in the blood fluctuate with a circadian rhythm in harmony with eating schedules, peaking before and descending rapidly after meals (Cummings, Purnell et al. 2001). Studies in animals have demonstrated that administration of ghrelin is sufficient to increase food intake, as well as to enhance appetite for palatable foods such as fat and sucrose (Schellekens, Finger et al. 2012).

The facilitating effect of ghrelin on food intake may be via increasing the motivation for food, because it can enhance dopamine transmission in the VTA (Abizaid, Liu et al. 2006, Jerlhag, Egecioglu et al. 2007). Indeed, the ventral tegmental area (VTA), a central component of reward system, has been found with high levels of ghrelin receptors (50-60%) (Abizaid, Liu et al. 2006, Zigman, Jones et al. 2006). Administration of ghrelin into the VTA leads to increased DA turnover in the nucleus accumbens (Acb) (Abizaid, Liu et al. 2006). Consistently, suppression of drug induced reward related activities (Jerlhag, Egecioglu et al. 2010) and reduced alcohol consumption was observed after the blockage of ghrelin signaling by an antagonist (Landgren, Simms et al. 2012). Interestingly, ghrelin levels are positively correlated with the neuronal activities induced by the view of palatable food, indicating its participation in cue-induced motivation for food (Kroemer, Krebs et al. 2013), and ghrelin antagonism with a ghrelin receptor antagonist GHRP-6 [D-Lys3] can reduce the appetitive response to food-associated cues (Dailey, Moran et al. 2016).

Additionally, the dysfunction of the ghrelin receptors has been found to induce a decrease in reward seeking activities. For example, mice with abnormal ghrelin receptor (GHS-R1A) gene, and rats treated with a GHS-R1A antagonist both have lower intake of palatable food in a free choice model (Egecioglu, Jerlhag et al. 2010).

1.2.1.4 Neuropeptide Y (NPY)

NPY is a 36 amino acid peptide functioning via specific receptors, Y1-Y6 (Krysiak, Obuchowicz et al. 1999). NPY has a major role in appetite regulation as an orexigenic neural transmitter (Gehlert 1999), and intraventricular administration of NPY was found to significantly enhance feeding and drinking in rats during light phase when they usually ingest small amounts of food (Levine and Morley 1984). A recent study found that the small-molecule NPY receptor antagonist can suppress biting and blood-feeding of mosquitos on

live hosts (Duvall, Ramos-Espiritu et al. 2019), suggesting that the appetite-regulating effect is a highly conserved function of NPY in the processes of evolution.

Interestingly, NPY positive neurons in the arcuate nucleus (ARC) are activated by orexin-A administration, and NPY antagonism can partially inhibit the orexin-induced feeding behavior. These findings indicate that the NPY system is possibly one of the downstream pathways in the feeding-enhancing effects of orexin-A (Yamanaka, Kunii et al. 2000).

1.2.2 Satiety-related signals:

The sensation of satiety is composed of the generation of satiety signals in response to food ingestion, as well as the suppression of hunger signals. The transmission of satiety signals, such as gastrointestinal hormones (e.g. cholecystokinin), includes a pathway from the periphery to the brain via the vagus nerve and/or the circulation of blood (Figure 1-1).

1.2.2.1 Insulin

Insulin is synthesized and released by the β-cells of the pancreas in response to increased blood glucose (Figure 1-1). Peripherally, insulin decreases the blood sugar concentration by promoting energy storage and glucose metabolism in insulin sensitive tissues, such as liver, muscle and adipose tissue. It can pass through the blood brain barrier and induce satiety via the insulin receptors in the brain (Baskin, Lattemann et al. 1999), and studies in rats found that intracerebroventricular admission of insulin could reduce the sucrose intake (Sipols, Bayer et al. 2002). Moreover, the metabolic and satiety signaling functions of insulin are possibly mediated by different receptor systems. For example, in mice the global deletion of nucleobindin-2 protein caused insulin resistance in obesity, without affecting satiety (Ravussin, Youm et al. 2018).

In this way, insulin is involved in two main aspects of energy homeostasis. Insulin in the blood is increased following a meal to promote the storage of excess glucose in the form of glycogen thus making a balance between the circulating and stored energy. Dynamic level of insulin in the blood can also work as a signal of the glucose accessibility, and regulate the feeding behaviors by provoking satiety (Baskin, Lattemann et al. 1999). Studies have demonstrated that fasting leads to a larger meal size, partially due to greatly reduced insulin penetrating into the brain (Strubbe, Porte Jr et al. 1988).

1.2.2.2 Leptin

Before the finding of leptin, there had already been a hypothesis about the existence of a feedback loop keeping a dynamic homeostasis of energy intake and expenditure, and in this modulating system, the hypothalamus plays as the monitor of energy storage by sensing a product of fat tissue circuiting in the plasma. This hypothesis is also called the lipostasis theory (Mayer 1955).

Zhang et al found that the obesity of ob/ob (obese) mice resulted from a mutation of the ob gene. After locating and sequencing this ob gene with exhausting work of positional cloning, they revealed that it coded for a hormone synthesized and secreted by the white adipose tissue. They latter dubbed the product of ob gene as 'leptin', derived from 'leptos' (Greek, 'thin'), and predicted it as the signaling molecule in the lepostasis theory.

Leptin is a 16 kDa peptide, synthesized by in fat tissue (Figure 1-1), and emitted into the blood in proportion to the quantity of adipose storage. The concentration of leptin in the blood directly represents energy storage. It was found that food deprivation significantly lowered the leptin level, which could be reversed by refeeding the animals. Leptin has a strong appetite-stimulating effect via its receptor (ob-R), which was first isolated by Tartaglia (Tartaglia, Dembski et al. 1995). In this way, the CNS centers can monitor the energy storage by detecting the level of the circulating leptin and regulating the energy intake and expenditure accordingly to keep an energy homeostasis in the body. Shortly after the finding of leptin, it was confirmed that the abnormal feeding behavior and bodyweight of the ob/ob mice could be reversed by administration of exogenous leptin (Campfield, Smith et al. 1995, Halaas, Gajiwala et al. 1995).

Leptin inhibits food consumption by enhancing the response to other satiety signals (Morton, Blevins et al. 2005) and inhibiting the reward value of foods (Davis, Choi et al. 2010). The incentive value of foods can be assessed with behaviors such as conditioned place preference (CPP) and the breaking points of lever-pressing for food rewards. For example, ob/ob mice show higher CPP for a high fat diet, which can be decreased by leptin treatment independent of obesity (Shimizu, Son et al. 2017). Thus, leptin has been proposed to suppress the hedonic response to palatable food. Interestingly, the inhibition of reward system by leptin may have more functions other than inhibiting the food consumption. For example, leptin antagonism

enhances cocaine-induced CPP, accompanied by upregulated level of dopamine in the Acb (Shen, Jiang et al. 2016). Considering the crucial roles of leptin in regulating the energy homeostasis, it is not surprising that a malfunction of the leptin/leptin receptor system will result in many health problems such as obesity and diabetes. Obese individuals have high level of leptin, but there is no corresponding inhibition of food intake or reduction in bodyweight, possibly due to a deficit of leptin receptors instead of leptin itself. Approximately one year after the identification of leptin, a mutation in the long form of the leptin receptor was proved to be underlying the phenotype of db/db (diabetes) mouse (Chen, Charlat et al. 1996).

1.2.2.3 Serotonin (5-HT)

Studies using brain microdialysis found increased release of 5-HT in the hypothalamus of rats during feeding and pre-ingestive events (Schwartz, Hernandez et al. 1990). Furthermore, it was also found that the hypothalamic release of 5-HT after ingestion of a meal may be underlying the generation of satiety signals to terminate the meal (Haleem 1993).

The caudal 5-HT receptor-expressing neurons in the nucleus tractus solitarius (NTS) send glutamatergic projections to the parabrachial nucleus (PBN). Inhibition of these 5-HT receptors can reverse the feeding inhibiting effects induced by ablation of agouti-related protein (AgRP) neurons in the arcuate nucleus (Wu, Clark et al. 2012). Pharmacological agents which can increase the release of 5-HT (e.g. d-fenfluramine) (Gibson, Kennedy et al. 1993) or selectively inhibit 5-HT reuptake (e.g. fluoxetine) (Tao, Fray et al. 2002) have also a function of suppressing feeding in humans and experimental animals (Heisler, Kanarek et al. 1999, Halford, Harrold et al. 2007). Some researchers have proposed that postsynaptic 5-HT1B and 5-HT2C receptors in the hypothalamus may be underlying the feeding inhibiting effect of these 5-HT related agents (Kennett and Curzon 1988).

Besides the hypothalamus, 5-HT signals in the Acb have also been found involved in the regulation of food intake. The Acb has long been recognized to play critical roles in the perception of food-related rewards and modulation of motivation to eat (Georgescu, Sears et al. 2005). The 5-HT4 receptors are richly expressed in the Acb, and inhibition of these receptors in mice can reduce feeding behavior in fed mice, although not in food-deprived

ones (Jean, Conductier et al. 2007), suggesting a role of hypothalamus 5-HT4 receptors in inhibiting the motivation for food in sated animals.

Taken together, the 5-HT/ 5-HT receptors system in the hypothalamus controls food intake by enhancing the satiety signals, while Acb 5-HT modulates the motivation for food possibly via interaction with the dopamine system.

1.2.2.4 Cholecystokinin (CCK)

CCK is synthesized and secreted by enteroendocrine cells in the duodenum (Figure 1-1 and Figure 1-3) and causes the release of digestive enzymes and bile from the pancreas and gallbladder, respectively. It acts as a hunger suppressant and is the first gut peptide that is proposed to act as a satiety signal. As a peptide hormone, CCK mediates satiety by acting on the CCK receptors distributed widely throughout the central nervous system.

A study in rats found that 48h food deprivation attenuated the CCK-induced satiety, which could be prevented by leptin replacement (McMinn, Sindelar et al. 2000). This indicates that the fasting-induced leptin deficiency impairs the efficiency of CCK signaling for satiety, and that leptin regulates food intake particularly through sensitivity modulation to satiety signals such as CCK.

1.2.2.5 Oxytocin

Oxytocin is mainly produced in the hypothalamus and released via the posterior pituitary. A single dose of intranasal oxytocin is enough to significantly decrease food intake in humans. The effects of oxytocin are more prominent in obese individuals (Lawson 2017). Oxytocin seems to enhance the hindbrain response to gut-derived satiety signals such as CCK, leading to the consumption of smaller meals. In support of this concept, leptin-responsive oxytocin neurons in the paraventricular nucleus of the hypothalamus (PVN) project to the NTS and leptin-induced anorexia requires oxytocin signaling (Blevins, Schwartz et al. 2004). It has been recently found that oxytocin expressing glutamatergic neurons (ARC$_{OXTR}$) in the arcuate nucleus of the hypothalamus (ARC) plays a very important role in facilitating the acute food intake inhibiting effect of leptin (Fenselau, Campbell et al. 2017), which will be discussed later in details. Another possibility is that oxytocin can decrease the motivation for eating by inhibiting the hedonic responses to palatable foods. For example, studies using fMRI found that oxytocin administration in men significantly

decreased functional connectivity between the VTA with other food intake-related brain regions, such as the insula, oral somatosensory cortex and primary visual cortex in response to viewing pictures of palatable foods (Kerem, Hadjikhani et al. 2019).

1.2.3 Reward-related signals in hedonic eating

1.2.3.1 Dopamine (DA)

The dopamine system is the most thoroughly investigated component of the reward system (Volkow, Wang et al. 2011), with primary importance for incentive motivation. The dopamine system involved in food intake regulation is mainly composed of a mesoaccumbal projection from the VTA to the Acb, as well as projections from the VTA to the prefrontal cortex, amygdala and hypothalamus (Egecioglu, Skibicka et al. 2011). A study in human found a positive correlation between DA release in the dorsal striatum and the self-reported level of pleasure induced by ingestion of palatable food (Small, Jones-Gotman et al. 2003). The dopaminergic projections from the VTA to the Acb is activated upon first exposure to new or unexpected access to palatable foods (Norgren, Hajnal et al. 2006). However, after repeated exposure to the same palatable food, the DA response habituates to the stimulus of the food itself, and gradually shifts to the stimuli (e.g. smell) associated with the food, or cues predicting the availability (Epstein, Temple et al. 2009, Schultz 2010). Thus, the DA release in the Acb also signals a 'reward prediction error' in the absence of expected food reward or receiving unexpected food reward (Nieh, Matthews et al. 2015).

Interestingly, a recent study found that dopamine also participates the timing of feeding behavior. In this study, D1 dopamine receptor (Drd1) signaling in response to high fat diet was found to perturb circadian feeding rhythms, and Drd1-knockout mice are resistant to this effect. Drd1 rescue within the suprachiasmatic nucleus, the central circadian clock, completely abolished this perturbing influence of high fat diet on the circadian feeding rhythms (Grippo, Tang et al. 2020).

1.2.3.2 Opioid

The endogenous opioid system is another major component of the reward system in the brain, and involved in both hedonic perception and motivational responses to pleasant/rewarding stimuli, including consumption of palatable food (Berridge, Ho et al. 2010, Nummenmaa and Tuominen 2018). Administration of μ-opioids into the mesolimbic system, such as the VTA

is hedonic, and rats can learn to 'work' (lever-pressing) for intracranial self-administration of opioid (Bozarth and Wise 1981). Ingestion of palatable food can stimulate the release of opioid in the Acb, and administration of opioid agonist and antagonist into the Acb can respectively increase and decrease the consumption of palatable food (Woolley, Lee et al. 2006). Moreover, genetic ablation of μ-opioids receptors has recently been found sufficient to reduce palatable food intake in binge-like eating mice (Awad, Roeckel et al. 2019).

1.3 Brain circuits involved in food intake regulation

1.3.1 Brain regions involved in homeostatic eating

1.3.1.1 The Lateral hypothalamic area (LHA)

The hypothalamus accounts for only ~3% of the brain volume, but it directly controls many critical homeostatic functions. The hypothalamus is divided into many regions such as the supraoptic nucleus (SON), paraventricular nucleus of the hypothalamus (PVN), arcuate nucleus (ARC) and ventromedial nucleus (VMH), based on their functions, gene expression patterns, or classical anatomical boundaries. Nevertheless, the lateral hypothalamic area composes a large portion of the hypothalamus and consists of a large area of neurons and fibers, with much less clear anatomical definition.

The LHA is located anterior to the VTA, and posterior to the preoptic area. It integrates information from cortical and subcortical regions, such as the amygdala and basal forebrain networks, and consequently mediates some specific behaviors via projecting to downstream circuits involved in reward (e.g. the VTA, in Figure 1-2) and feeding regulation (e.g. brain stem motor pattern generator in Figure 1-2 and the NTS in Figure 1-3) (Rossi and Stuber 2018).

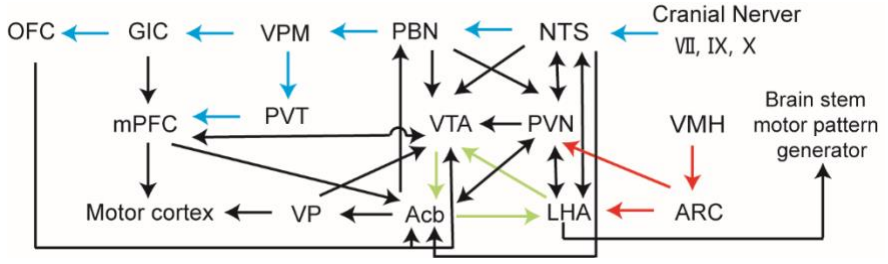

Figure 1- 2. Brain circuits involved in the regulation of food intake.

This diagram of brain connections includes the neural pathways underlying the taste information procession (pathways with blue arrows), homeostatic eating regulation (pathways with red arrows), and reward value perception (pathways with green arrows). Acb, nucleus accumbens. ARC, arcuate

nucleus of the hypothalamus. GIC, gustatory insular cortex. LHA, lateral hypothalamic area. NTS, nucleus tractus solitarius. OFC, orbitofrontal cortex. PBN, parabrachial nucleus. PFC, prefrontal cortex. PVN, paraventricular nucleus of the hypothalamus. PVT, paraventricular nucleus of thalamus. VMH, ventromedial hypothalamus. VPM, ventral posteromedial nucleus. VP, ventral pallidum.

The LHA is a highly heterogeneous brain area, with the glutamatergic and GABAergic neurons in the LHA playing opposing roles in modulating the homeostatic and hedonic feeding behaviors (Stuber and Wise 2016, Qualls-Creekmore and Münzberg 2018). The appetite-promoting effect of the LHA is mediated by excitation of its GABAergic neurons, which inhibit the GABAergic interneurons in the VTA. The final effect is the disinhibition of the dopaminergic neurons in the VTA, and augmentation of the hedonic response to food intake (Nieh, Vander Weele et al. 2016, Stuber and Wise 2016). The activation of glutamatergic neurons in the LHA can inhibit feeding by projecting to the lateral habenula (LHb). For example, mice with genetic ablation of glutamatergic neurons in the LHA showed higher daily caloric intake and larger weight gain when they had access to a high-fat diet compared with the wild-type controls. Moreover, optogenetic stimulation of the glutamatergic LH--LHb projections can acutely inhibit food consumption in mice, and optogenetic inhibition of these projections produced the opposite effect (Stamatakis, Van Swieten et al. 2016).

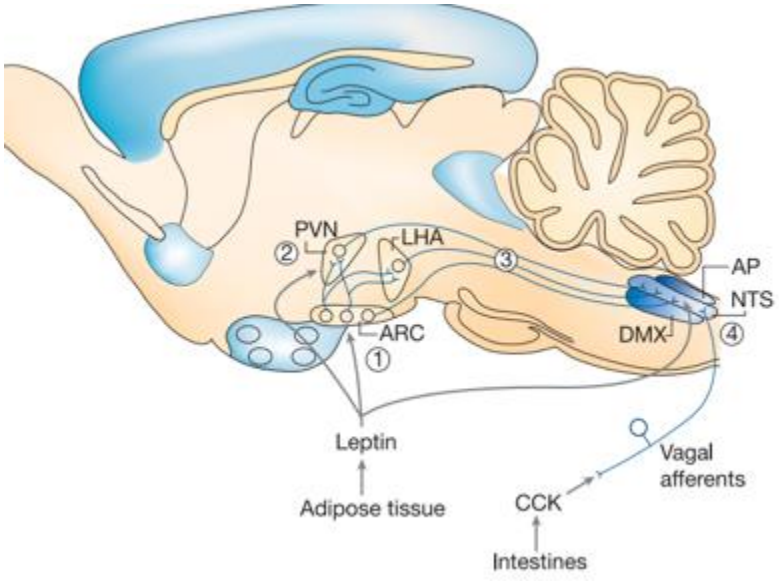

Figure 1- 3. Brain circuits integrating information about adiposity and satiety

Afferent input in proportion to body fat mass (for example, leptin) enhances the response to satiety signals such as CCK that are generated in response to food consumption and lead to meal termination. Whereas the hypothalamus is a key target for leptin action, the satiety effect of CCK involves activation of vagal afferent fibres that terminate in the NTS. Integration of these inputs can involve the actions of leptin in the ARC (1) or other hypothalamic areas (2) involving neurons that project to the NTS (3) and influence the response to CCK (4). In addition, leptin can act directly in the NTS. AP, area postrema; DMX, dorsal motor nucleus of the vagus nerve; LHA, lateral hypothalamic area; NTS, nucleus of the solitary tract; PVN, paraventricular nucleus. Adapted from (Morton, Cummings et al. 2006).

1.3.1.2 The Arcuate nucleus of the hypothalamus (ARC)

The ARC of the hypothalamus is a small sub-region of the mediobasal hypothalamus, adjacent to the median eminence and third ventricle. The ARC is the main site where the leptin signals enter the brain (Figure 1-3). Briefly, leptin inhibits food intake via inhibiting the AgRP/NPY (agouti-related peptide/neuropeptide Y) neurons ($ARC_{AgRP/NPY}$ neurons) and activating the POMC (proopiomelanocortin) neurons (ARC_{POMC}) in the ARC. Moreover, these $ARC_{AgRP/NPY}$ neurons send GABAergic projections to ARC_{POMC} neurons (Newton, Hess et al. 2013).

$ARC_{AgRP/NPY}$ neurons are GABAergic and co-express two inhibitory neuropeptides, NPY and AgRP. The intrinsic firing rate of $ARC_{AgRP/NPY}$ neurons increases after food deprivation, but this modulation depends on the presence of leptin (Takahashi and Cone 2005). Optogenetic activation of $ARC_{AgRP/NPY}$ neurons was sufficient to stimulate voracious food consumption in sated mice without training, by decreasing the latency to take the food and increasing the duration of meal (Atasoy, Betley et al. 2012). This effect is very rapid, usually within minutes, and disappears immediately after the end of the stimulation. The intensity of this regulation depends on the frequency and duration of the photo stimulation (Aponte, Atasoy et al. 2011). Ghrelin can stimulate eating by activating $ARC_{AgRP/NPY}$ neurons. Ghrelin receptor (GSH) mRNA is expressed in 94% NPY expressing ARC neurons (Tannenbaum, Lapointe et al. 1998). ARC_{POMC} neurons are recognized as the counterpoint to $ARC_{AgRP/NPY}$ neurons (Palmiter 2017). However, unlike $ARC_{AgRP/NPY}$ neurons, activation of ARC_{POMC} neurons is extremely slow to take effect to inhibit food consumption (many hours). Long-term (24h) excitation of ARC_{POMC} neurons in this region was sufficient to inhibit the food intake and decrease the bodyweight. In complementary to the ARC_{POMC} neurons, oxytocin receptor expressing glutamatergic neurons in the ARC (ACR_{OXTR}) rapidly promote satiety when chemo- or optogenetically activated (Fenselau, Campbell et al. 2017, Palmiter 2017). The same study found that these oxytocin responding neurons project to melanocortin-4 receptor expressing satiety neurons (PVN_{MC4R}) in the paraventricular hypothalamus (PVN), which also converge the projections from GABAergic $ARC_{AgRP/NPY}$ neurons. Interestingly, transmission across these glutamatergic ARC_{OXTR}--PVN_{MC4R} projections is potentiated by the ARC_{POMC} neurons (Fenselau, Campbell et al. 2017).

$ARC_{AgRP/NPY}$ neurons promote food intake mainly through projections to the LHA and PVN, and optogenetic stimulation of the ARC--LHA and ARC--PVN projections (Figure 1-2, Figure 1-3) can induce comparable feeding behaviors as to activation of $ARC_{AgRP/NPY}$ neurons themselves (Betley, Cao et al. 2013). ARC_{POMC} neurons also densely innervate the LHA and PVN, as well as other feeding related regions such as the VMH and dorsomedial hypothalamus (DMH) (Waterson and Horvath 2015).

1.3.1.3 The Parabrachial (PBN)

Campos et al. found that parabrachial CGRP (calcitonin gene-related peptide) neurons (PBN_{CGRP} neurons) relay satiety information from the NTS, and initiate meal termination by projecting to the central amygdala (Ce). Inactivation of PBN_{CGRP} neurons in mice leads to impaired ability to terminate meals. It was also found that PBN_{CGRP} neurons receive inhibiting projections from the orexigenic $ARC_{AgRP/NPY}$ neurons. Simultaneous inactivating PBN_{CGRP} neurons and activating $ARC_{AgRP/NPY}$ neurons leads to voracious feeding (Campos, Bowen et al. 2016).

Since the feeding behaviors are influenced by a large number of factors, including the physical conditions of the organism itself, environmental conditions and stimuli, the body needs a complicated system composed of a large number of signals to present these factors and maintain proper feeding behaviors. Based on aforementioned signals and brain regions, we can see that the energy homeostasis is achieved via a system where the circulating signals transfer the information about circulating and stored energy to the brain, and after integrating all related information the brain makes corrective adjustment to food intake.

1.3.2 Brain circuits involved in reward-induced feeding

Feeding behavior is also modulated by the rewarding properties of foods, other than homeostatic requirements. As most of subjective perceptions, it is susceptible to the experience, physical status and environmental situations etc. This hedonic regulation of food intake has been found to be largely modulated by the mesolimbic reward system and influenced by hypothalamic inputs. Taste information is perceived by oral taste receptors, projected in turn to the NTS by sensory fibers and subsequently transmitted to multiple structures in the hindbrain (e.g. the PBN), midbrain (e.g. the VTA), and forebrain (e.g. the Acb), thalamus and cerebral cortex (e.g. the mPFC and OFC), which assigns the rewarding

value to the taste via integrating and discriminating different tastes and textures (Morton, Cummings et al. 2006) (Figure 1-2). The evaluation of reward is mediated by the release of dopamine from the VTA to the Acb, striatum and some other brain regions. This dopamine signal is not directly responsible for the hedonic perception itself ('liking'), but potently drives the motivation to obtain a rewarding stimulus ('wanting'). Instead, the opioid signaling in the Acb and other adjacent forebrain regions, partially activated by dopamine release, is underlying the hedonic experience (Morton, Cummings et al. 2006).

1.3.2.1 The Ventral tegmental area (VTA)

The post-ingestive rewarding effect of nutritious food also enhances the craving for energy-dense food. Neurons in the LHA, which co-express orexin and glutamate, send excitatory projections to the VTA dopaminergic neurons. The activities of these LHA neurons can be influenced by peripheral signals of energy state, such as blood glucose (Sheng, Santiago et al. 2014). Moreover, the LHA also send GABAergic projections into the VTA, and activation of these projections significantly increases food intake (Nieh, Matthews et al. 2015). Dopaminergic neurons in the VTA synthesize dopamine (DA) and sent it to the Acb to facilitate its functions (Ikemoto 2007). Moreover, the projections directly or indirectly from the frontal lobe (e.g. the mPFC in Figure 1-2) and the amygdala to the VTA bias the responses towards reward and this effect is modulated by the DA signaling (Gottfried, O'Doherty et al. 2003, Stefani and Moghaddam 2006).

1.3.2.2 The Nucleus accumbens (Acb)

The name of the nucleus accumbens septi was derived from latin meaning 'nucleus adjacent to the septum'. The Acb is made up by two components, the core (AcbC) and the shell (AcbSh). It is located between the caudate and putamen, without a specific demarcation from either of them. While the caudate nucleus and putamen comprise the dorsal striatum, the Acb and the olfactory tubercle comprise the ventral striatum. The striatum is part of the basal ganglia, and the majority of striatal neurons are medium spiny neurons (MSNs) (Yager, Garcia et al. 2015). Roitman et al. found a very precise and temporally discrete striatal dopamine release in trained animals during sucrose consumption (Roitman, Stuber et al. 2004). Carlezon proposed that the effect of reward has predominant association with a decreased activity of neurons in the Acb, such as the medium spiny neurons (Carlezon Jr and Thomas 2009).

There is a paucity of evidence suggesting that rostral part of the Acb is responsible for the 'liking' of rewards, while the caudal part works as a hedonic cold spot. Moreover, as the environment shifts between safe and stressful, some changes may occur on the functional map of the Acb. Reynolds and colleagues found that a stressful environments recruited ~90% of the Acb shell in the caudal, whereas the fear-generating zones shrink in the home environment, while the appetitive-generating zones expended caudally to fill ~90% of the Acb shell (Reynolds and Berridge 2008).

In the Acb, multiple separate, but interacting neurochemical systems are involved in the modulation of discrete aspects of appetitive motivation. For example, the GABAergic projections from the Acb shell to the hypothalamus has a direct influence on the feeding motor patterns. The dopamine signal in the Acb is involved in the control of general motoric and arousal process underlying pleasant response and response selection. The hedonic impact of palatable food is mediated by the enkephalinergic neurons that distributed widely in the Acb (Kelley, Baldo et al. 2005).

The special connections of the Acb with other brain regions involved in the regulation of food intake and energy homeostasis makes it a good candidate of integrating point of feeding related information. For example, the Acb receives taste information from the brain stem and gastrointestinal sensory information about the ingested food via direct connection with the NTS. Moreover, the Acb also has a bidirectional connections with the lateral hypothalamus, which plays essential roles in maintaining energy homeostasis (Erlanson-Albertsson 2005).

1.4 The binge eating disorder (BED)

1.4.1 What is BED

Under normal conditions, the homeostasis is capable of maintaining intake and expenditure of energy in a dynamic balance over time. However, when the homeostatic regulation of feeding behaviors is disrupted, various eating disorders may consequently occur. Eating disorders are manifested as a disturbance of eating habits or weight-control behaviors. Nevertheless, not all abnormal eating behaviors can be attributed to eating disorders. A basic criterion for the diagnosis of eating disorders is that the behavioral disturbance or associated core eating features should stem directly from significantly impaired physical health or

psychosocial functioning, rather than a result of general medical disorders or psychiatric condition.

Before the fifth edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-5), eating disorders were classified into three diagnostic categories: Anorexia nervosa, bulimia nervosa, and atypical eating disorders not otherwise specified, the last of which included the binge eating disorder. In May 2013, the binge eating disorder was defined as an autonomous eating disorder in the DSM-5.

Binge eating disorder is characterized by discrete episodes of consuming more food than would normally be consumed under similar conditions during a similar period of time (Bogduk 2013). Binge-eating episodes are associated with three or more of the following:

1) eating until feeling uncomfortably full;

2) eating large amounts of food when not physically hungry;

3) eating much more rapidly than normal;

4) eating alone because one is embarrassed by how much she/he is eating;

5) feeling disgusted, depressed, or guilty after overeating;

6) marked distress or anxiety regarding binge eating.

People with binge eating will eat until uncomfortably full. Not surprisingly, binge eating is often associated with overweight and obesity. About 35% of those who frequently binge eats have the problems of overweight or obesity. Furthermore, binging subjects have higher risk of weight regain after treatment than non-binging ones (Stunkard and Wadden 1992, Swanson, Crow et al. 2011).

Severe eating compulsiveness often results into low mood, negative feelings and feeling of out of control, which in turn lead to more serious psychopathological impairment if not well coped with (Carrard, Crépin et al. 2012). Personality disorders and mood disorders, can be frequently seen among BED patients, and in some cases with substance abuse. All these comorbidities eventually lead to more complicated psychopathological problems and worse prognosis (Carrard, Crépin et al. 2012). Because of the close relation to the BED, these comorbidities were proposed as markers of severity of the BED, instead of simply as

associated conditions. All of these hazards caused by the BED can lead to a tremendous impact on our society and economy, because of reduced life quality, work efficiency, and increased health service cost.

1.4.2 Inducers of the BED

1.4.2.1 Dieting and limited/ intermittent access to palatable food

The attractiveness of food is induced by taste or nutritional value, such as carbohydrate and fat, but the hedonic value of palatable food is also influenced by the nutritional state of the body. Long-term dietary restriction, as well as intermittent feeding, have been found to augment the rewarding value of food and various drugs of abuse (De Vaca and Carr 1998, Carr 2002). In other words, the reward system can be sensitized by food restriction, which in turn increases the craving for food, as well as for drugs and alcohol (Söderpalm and Hansen 1999). As a result, it is not surprising that food restriction can provoke binge eating in both human and animals (Hagan, Chandler et al. 2003).

Sugar dependence has been triggered in rodents with intermittent access to concentrated sugar solutions (Colantuoni, Rada et al. 2002). Binge-like eating animal models developed with intermittent feeding protocol are often used to support the concept of food addiction, and to explore the mechanism underlying it.

1.4.2.2 Stress

An even more common inducer of eating disorders in humans is stress (Corning and Viducich 2019). Stress is a state in which the organism is threatened and the homeostatic balance is disrupted. Under some emergent or stressful situations, the stress-responding circuits override the homeostasis system, and induce some abnormal feeding behavior. The abnormal eating of palatable food induced by stress is also known as ‘non-homeostatic eating’ or ‘emotional eating’.

Most studies in animals demonstrate that stress inhibits feeding, unless animals are given the access to palatable food when they are stressed, which is consistent with reports about feeding responses to stressful situations in human (Pecoraro, Reyes et al. 2004, Tomiyama, Dallman et al. 2011). For example, people tend to eat palatable, high-calorie foods during stress, while maintain or decrease intake of lower-calorie foods, such as vegetables (Zellner, Loaiza et al. 2006). Individuals with stress-induced overeating problems just over-eat palatable food

which they usually tried to avoid for weight-control or health purposes (Zellner, Loaiza et al. 2006).

Psychogenic stressors, such as social defeat and loss of family members, can induce eating disorders in humans. (Over-) eating induced by emotional stressors is also called ‘emotional eating’. A study in male Japanese workers found that obesity and abnormal eating behaviors are associated with psychological stress response of ‘tension/anxiety’ induced by high job demand and/or low job latitudes (Nishitani and Sakakibara 2006). In students, a correlation has been found between academic stress (e.g. exam or high workload) and the intake of high-calorie foods (Michaud, Kahn et al. 1990) or consumption of less healthy diets (Weidner, Kohlmann et al. 1996).

1.4.2.3 Interaction between dieting and stress

Human studies also showed that the direction (increase or decrease) of change in food intake induced by stress is also modulated by dietary restraint. Healthy individuals with history of dieting are more likely to increase their food intake in response to stress (Zellner, Loaiza et al. 2006). The interaction between stress and feeding is complicated. Numerous evidences have demonstrated that stress may enhance eating, leading to obesity in chronic situations (Levine and Morley 1981, Tamashiro, Hegeman et al. 2007). Several studies also found that this feeding response to stress could be significantly modulated by dieting. Individuals classified as restrained eaters respond to stress with enhanced eating, instead of inhibited eating in control/non-restrained eaters. For example, restrained eaters ate more than the controls following an interpersonal stressor , in an intensity dependent way, which means that the greater the restraint, the more restrained eaters ate (Tanofsky‐Kraff, Wilfley et al. 2000).

Studies with animal models showed that food restriction itself only moderately increased the food consumption. However, when food restriction is followed by foot shock stress, and tested with palatable foods, this effect was tremendously enhanced, and animals showed binge-like eating behaviors. An example in human is that restrained eaters ate more than unrestrained after a stressful reaction-time task, whereas the result was opposite when test was done in a relaxation condition (Lattimore and Caswell 2004).

Besides physical stressor, other stressful situations are created by manipulating various environmental factors, such as maternal separation-- a very widely used model of early life stress. Maternal separated rodents display anxiety-like behaviors and depression in adulthood, along with disturbed serotonin levels (Lee, Kim et al. 2007), but without obvious sign of overeating or overweight. Nevertheless, when the maternal separation induced stress is followed by repeated fasting/refeeding protocol during adolescent time, animals start to exhibit binge-like food intake.

1.4.3 Hormones and neurotransmitters involved in the development of the BED

1.4.3.1 Glucocorticoid

It has long been known that HPA axis governs the endocrine response to stress. As a main product of activation of the HPA axis by stress, glucocorticoid is found to be involved in appetite regulation (Wolkowitz, Epel et al. 2001) and energy homeostasis by increasing circulating nutrient via lipolysis and gluconeogenesis (Epel, Lapidus et al. 2001). Acute stress typically induces an increase of cortisol, which in turn influences the releasing and functioning of neurotransmitters and hormones involved in appetite regulation, energy metabolism and feeding behaviors (Piazza, Rougé-Pont et al. 1996, Adam and Epel 2007). It is true that acute increase of glucocorticoid plays a protective role in coping with stress, but repeatedly or persistently elevated levels of glucocorticoid may lead to eating disorders including the BED.

A study in humans explored the relationship between laboratory stress-induced cortisol changes and food intake. This study found that in healthy female participants, after a cognitive stress task, individuals with higher levels of cortisol consumed significantly larger amount of food compared to individuals with lower cortisol levels (Epel, Lapidus et al. 2001). In a study examining the BED individuals, a positive correlation was found between binge eating severity and baseline salivary cortisol concentration (Coutinho, Moreira et al. 2007). In healthy female individuals without any eating disorder, stress-induced cortisol is positively correlated with food consumption (Epel, Lapidus et al. 2001). Studies with BED animal models showed that binge-like eating rats having significantly elevated corticosterone levels compared with non-binging rats (Artiga, Viana et al. 2007).

Human and animal studies both in vitro and in vivo have found that glucocorticoids can increase leptin mRNA expression and secretion in adipose tissue (Hardie, Rayner et al. 1996). Other studies also reported that administration of exogenous glucocorticoid could stimulate NPY expression in the ARC nucleus (Sato, Arima et al. 2005, Goto, Arima et al. 2006). Specifically, in the situation of chronic stress, repeated activation of the HPA axis resulted in persistently high levels of glucocorticoids, which inhibited glycolysis and enhanced gluconeogenesis to fuel the behavioral response to stressor, consequently leading to increased secretion of insulin (Chrousos 2000, Nieuwenhuizen and Rutters 2008).

1.4.3.2 Ghrelin

Besides the functions in food intake regulation, ghrelin has also been found involved in the stress responding process, with most reports demonstrating that stress, such as restraint stress (Zheng, Dobner et al. 2009) and social defeat stress (Lutter, Sakata et al. 2008), significantly increases ghrelin in animal models. Administration of ghrelin, no matter peripheral, central or intraparaventricular injections, is sufficient to activate corticotrophin releasing hormone (CRH)-producing neurons, resulting into hyperactivation of the HPA axis (Asakawa, Inui et al. 2001, Cabral, Suescun et al. 2012), and elevated levels of ACTH and glucocorticoids (Schellekens, Finger et al. 2012). Thus, stress stimuli can induce ghrelin secretion which in turn can influence the HPA axis response to stress. Considering the functions of ghrelin in food intake regulation, it is reasonable to expect a role for ghrelin in the alteration of feeding behavior by stress (e.g., Kristenssson et al., 2006). Studies in rodents showed that social defeat stress could increase plasma ghrelin and intake of palatable fat-rich diet, associated with higher hedonic response to food, assessed by conditioned place preference (CPP) (Chuang, Perello et al. 2011). These findings indicate that stress-induced changes of ghrelin release may be involved in the development of the BED.

1.4.3.3 Orexin

Similar to ghrelin, orexin has also been found involved in the regulation of stress response other than enhancing feeding behaviors. For example, orexin expressing neurons in the LHA are recruited for the cardiovascular responses induced by acute stressors and stimulation of the amygdala and the bed nucleus of stria terminalis (BNST), and both inhibition of orexin expression and antagonism of orexin receptors can attenuate these responses (Kuwaki 2011, Carrive 2017). This is consistent with early findings that 60 min of immobilization stress

increased orexin mRNA levels in the LHA, and that administration of orexin increased the plasma adrenocorticotropic hormone (ACTH), corticosterone levels (Ida, Nakahara et al. 2000, Samson, Taylor et al. 2002), and *c-fos* mRNA expression in the PVN (Sakamoto, Yamada et al. 2004). This evidence indicates that stress can stimulate the expression of orexin, and orexin has a potentiating effect on stress responses.

Recent studies also suggest that orexin is involved in more diverse processes including attention, reward, motivation and emotional regulation (Sargin 2019). The activity of orexin expressing neurons decreases within milliseconds after the feeding initiation, and remain in an inhibited state through the meal (Gonzalez, Jensen et al. 2016). Considering the functions of orexin in both stress response and feeding regulation, this hormone is likely involved in the stress-induced eating.

1.4.3.4 Dopamine (DA)

Changes of the DA signaling is very similar between sugar binging and drug addiction. Extracellular DA concentration in the Acb can be repeatedly increased by drugs of abuse without a habituation effect, while the same DA response to normal feeding are gradually blunted as food loses its novelty after repeated exposure (Bassareo and Di Chiara 1997). However, in the situation of binge eating, the DA response in rats is more similar to that of a drug of abuse, rather than a normal meal, which means that the DA level in the Acb increases upon each episode of binging. Whereas in non-binging rats, such as rats fed with sugar or chow ad libitum, the DA response fades out which is typical as the food loses its novelty (Rada, Avena et al. 2005). Thus, binging or non-binging on sugar can make a significant difference on the neurological response, even if the total sugar consumption is similar in both situations.

There are also findings from studies with other models of sugar overeating in support of these results, in which changes of Acb DA turnover and DA transporter were reported (Hajnal and Norgren 2002, Bello, Sweigart et al. 2003). Hajnal et al. found an increase in Acb dopamine turnover to sucrose with a 7 day paradigm that provokes diet-induced binge eating on a 0.3M (10.26%) sucrose solution (Hajnal and Norgren 2002). Further studies also found that this alternation of Acb DA is accompanied by an increase of dopamine transporter (DAT) in the Acb and VTA, with diminished D2R binding in the Acb core and shell (Hajnal and Norgren

2002, Bello, Sweigart et al. 2003). It indicates that short-term (i.e. 7 days) DA signaling alterations by palatable food consumption could be essential for the development of more protracted diet-induced binge eating schedules. (i.e. ≥21 days).

Peripheral administration of raclopride, a D2-like antagonist, showed differential effects on palatable food intake, with lower doses enhance intake of the binge-like eating rats, and higher doses inhibit intake of the controls. These results indicate differential pre- and post-synaptic D2 signaling might underlie different feeding behaviors between binge and control conditions (Corwin and Wojnicki 2009).

1.4.3.5 Serotonin (5-TH)

Findings of 5-HT dysfunction in eating disorders are complex and mostly from studies from obese humans and animals. Early studies found that bulimic patients with a binge eating history showed lower levels of 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5-HT, and that there is a negative correlation between the 5-HIAA level and binge frequency (Jimerson, Lesem et al. 1992). It was suggested that this low central 5-HT activity may be partially underlying the blunted satiety responses in the BED.

Further studies have implicated several 5-HT receptor subtypes, such as 5-HT2A and 5-HT4 in the pathophysiology of overweight and obesity, as well as eating disorders. For example, it was found that the Body Mass Index (BMI) is positively correlated with the 5-HT2A receptor binding in the cerebral cortex, and it is strongly associated with the cerebral serotonin transporter (SERT) binding and 5-HT4 receptor density in the ventral pallidum, the Acb, and OFC (Erritzoe, Frokjaer et al. 2010, Haahr, Rasmussen et al. 2012). A decreased level of SERT binding was found in obese binge-eating women, compared with obese controls (Kuikka, Tammela et al. 2001).

In genetic or diet-induced obese rodents, the 5-HT release in the hypothalamus is decreased. Along with this finding, decreased SERT binding and increased 5-HT2A and 5-HT4 receptor binding was found, especially in the reward related Acb shell (De Fanti, Gavel et al. 2000, Ratner, Ettrup et al. 2012). Moreover, chronic overeating increased hypothalamic expression of 5-HT1B, 5-HT2A and 5-HT2C receptors, and inactivation of 5-HT2A receptors showed an effect of inhibiting overeating and obesity in mice (Nonogaki, Nozue et al. 2006). Elevated 5-HT2A receptors and down-regulated SERT are proposed to be a compensatory mechanism

for the decreased 5-HT level in overweight individuals, and finally lead to increased food intake (Erritzoe, Frokjaer et al. 2009). Furthermore, 5-HT4 receptors have also been found involved in the regulation of food intake, and genetic or pharmacological manipulation of them in reward-related brain structures can alter food intake (Jean, Conductier et al. 2007).

1.5 Stress response

1.5.1 What is stress response

For the survival of animals, appropriate responses to emergent and stressful stimuli are just as important as feeding. All of us are exposed to various stress throughout our life, from prenatal period until death. In response to stress, including environmental and homeostatic challenges, the survival and well-being of an organism requires an appropriate behavioral and physiological response. Adaptation under stressful conditions is a priority for all organisms, which gives the organism a better chance of surviving and prospering (Ulrich-Lai and Herman 2009). Stress can be acute or chronic. The broad definition of stress includes actual as well as anticipated threat to the well-being or disruption of homeostasis. Stressors may include childhood maltreatment, adverse physical environment, emotional disruption, social problems, and change in diet that disturbs homeostasis (Grilo and Masheb 2001, Hancock and Grant 2009, Jahng 2011). Both human studies and animal experiments found that stress exposure, especially during early life time, leads to various changes in behavioral performance and alternates brain development (Heim and Nemeroff 1999, Heim and Binder 2012).

Different species has their specific patterns of responses to stressors. One of the principles of animal behavior analysis is that it presumes that an animal has a series of genetically determined and highly stereotyped behaviors that it displays when confronted with a particular functional problem, such as feeding (Fanselow 1994). If the problem is highly challenging and demands immediate responses, the behavioral repertoire of this animal is quite likely to be restricted to that set of stereotyped behaviors.

Based on this fact, Bolles (1970) developed his species-specific defense reaction (SSDR) theory. In rats, the SSDRs are those innately determined defensive behaviors, such as freezing, flight and fighting. For example, when a rat comes into a threatening environment, or stimulated with an abrupt stressor, its behavioral repertoire will be restricted to it SSDRs.

Nevertheless, the displayed behaviors indeed may have some flexibility, anyway, there are several SSDRs from which the animal can choose. An assumption of the adaptationism is that the behavior selected form this limited pre-determined repertoire is the most effective response for this challenging situation, thus offers the optimized opportunity for the animal to survive (Fanselow 1994).

When rats are stressed, the most salient behavior in the response repertoire is the freezing response. The behavior of freezing is defined as cessation of all physical movements except breathing, and it has long been recognized as a typical response to fear (Small 1899). For example, in the observation of Darwin in 1872, he described that the frightened subject 'stands like a statue motionless and breathless, or crouches down as if instinctively to escape observation'. The freezing behavior should not be mistakenly taken for catatonia or tonic immobility. During freezing, the rats are highly alert, reveal considerable muscle tone, and freezing rats can conduct a potentiated startle response when presented with a sudden loud sound (Leaton and Borszcz 1985).

1.5.2 Fear conditioning paradigm

1.5.2.1 What is fear conditioning

A well-known learned stress response, fear conditioning, has been widely used to study the mechanism of stress response, as well as related disorders such as depression disorder and PTSD (post-traumatic stress disorder) (Careaga, Girardi et al. 2016, Rabinak, Mori et al. 2017). Pavlovian fear conditioning refers to the learning of associations between nonthreatening environmental stimuli and painful, dangerous or threatening stimulation. In the beginning, when an emotionally neutral stimulus is presented, such as a tone or indicator light, the animal will not show fear response to it. However, if we pair these neutral stimuli with some physically aversive stimuli, such as foot shock, and after some repetitions, the animal will response to these originally neutral stimuli with responses which are typically observed when the animal is in fear.

For example, in auditory fear conditioning, a previously neutral sound, such as a tone with specific frequency is paired with an aversive stimulus, such as a mild electric foot shock, and after a few repetitions, presentation of the tone alone will generate similar visceral and behavioral reactions as induced by the foot shock itself. In this fear conditioning procedure,

we name this tone as ‘conditioned stimulus (CS)’, and the foot shock as ‘unconditioned stimulus (US)’.

As a result, after fear conditioning learning, presentation of CS itself will elicit a series of reactions indicating of fear. In animals, these reactions typically include freezing (Bouton and Bolles 1980), startle reflex (Brown, Kalish et al. 1951) and suppression of ongoing instrumental behaviors (Davis 1990), during the presentation of the CS. In humans, besides physiological indices of fear, in parallel with those in animals, such as skin conductance, heart beat rate, we can also assess the fear expression directly through verbal self-reports (Lipp 2006).

Generally speaking, Pavlovian fear conditioning is largely a highly adaptive strategy that helps to detect threatening signals for impending dangers based on previous aversive experiences. In this sense, if a signal in the environment is usually followed by aversive, or life-threatening events, it will be entirely reasonable for an animal to be feared in presence of that signal (Frijda 1986). Moreover, in laboratory studies, almost every object will learn the association between the US and CS, especially when the CS reliably predicts an aversive physical stimulus.

The fear conditioning procedure aims to mimic real-life experiences, in which the challenging US causes pain or other harms, while the CS occurs in temporal or logical correlation with the CS. For example, after a rat escaped from the attack of a cat, it will be scared when it hears again the sound of a cat.

1.5.2.2 Conditioned fear responses

There are variety of reactions to the cues signaling the potential threat, measured as fear conditioned responses, including autonomic changes such as hyperthermia and increased blood pressure (Carrive and Gorissen 2008), as well as behavioral responses such as freezing and ultrasonic vocalization(Lee, Choi et al. 2001). Many of these responses are species-specific. For example, in the presence of a tone, which was previously coupled with an eyelid electric shock, the heart rate of rabbits will decrease (Schreurs, Smith-Bell et al. 2011); while when rats hear the tone signaling the foot-shock, they will have a raised heart rate.

In fact, fear is a mood, a subjective feeling, but here we use this term to refer specifically to the combination of measurable fear related responses to stressful events. The measurement

of conditioned fear includes quantifying conditioned responses elicited by the auditory CS, in absence of the US during a memory test trial (Johansen, Cain et al. 2011). When we choose the methods to measure and quantify the conditioned fear in rats, we have some criteria to follow:

- This fear response should be expressed by all rats, rather than only in some specific ones.
- This response can be quantified or at least be discriminated into different levels.
- This response should not have a low ceiling effect, which mean it cannot saturate too quickly.
- The measurement should not be invasive. Invasion of the animal body could possibly influence its following perception and response to the stimulus.

Defecation can discriminate between no fear and some level of fear, and it is used quite often in studies with rodents, but the measure saturates very quickly (Fanselow 1986). Freezing has a very low threshold for fear, which means rats will show freezing behavior when they are experiencing a low level of fear. It has very good sensitivity and temporal resolution, and very importantly, it does not saturate like the defecation (Fanselow and Bolles 1979). Freezing is the most widely used measure to quantify fear response in rats and mice now (Fanselow and Sterlace 2014), and it also can be found in human (Roelofs, Hagenaars et al. 2010). Thus, the freezing behavior was measured to quantify the fear levels of rats in this study instead of defecation.

1.5.3 Fast and slow neural responses to stress

Depending on the features and duration of stressors, animals need a tuned fast and/or slow, short-term and/or long-term response to them, in order to maintain or reinstate homeostasis during stress. The autonomic nervous system (ANS), both sympathetic and parasympathetic, conducts the most rapid response to stressors, through its innervation of end organs (Uylings, Van Eden et al. 2000). Nevertheless, the influence of the ANS diminishes also quickly, because of the reflex parasympathetic activation, which results in a short-lived response (Kandel, Schwartz et al. 2000).

The hypothalamic-pituitary-adrenocortical axis (HPA axis) modulates the main slow stress responses, and the effects last longer than the ANS response (Myers, McKlveen et al. 2012).

The HPA axis is essentially composed of the paraventricular nucleus of the hypothalamus (PVN), the anterior pituitary, and the adrenal cortex. Exposure to stressor activates the PVN, which consequently releases the CRH and arginine vasopressin (AVP) into the portal circulation of the median eminence. The CRH, along with other co-released secretagogues, stimulates the anterior pituitary to secrete adrenocorticotropic hormone (ACTH) into the blood circulating system, which functions on the adrenal cortex to enhance the synthesis and release of glucocorticoid hormones, such as corticosterone in rodents and cortisol in primates. The circulating glucocorticoids in turn facilitate the recruitment of stored energy to support the stress response. Glucocorticoids have more functions, including potentiate some functions mediated by sympathetic system. Moreover, the sympathetic nervous system can also regulate the HPA axis activation indirectly via modulation of cardiovascular tone and directly via innervation into the adrenal cortex (Jasper and Engeland 1997). Thus, the sympathetic system and the HPA axis play largely complementary roles, and interact with each other, in the regulation of stress response (Uylings, Van Eden et al. 2000).

1.5.4 Brain circuits underlying stress response

Generally, stress-related information is collected by all major sensory systems, including interoceptive signals such as blood pressure, and exteroceptive cues, such as the view of a predator. All major sensory systems participate in the perception of stress-related information and convey it to the central nervous system (CNS). After complicate processing of the stress information, and computing the potential cost and benefits, the CNS will recruit the neural and neuroendocrine to respond correctly to the specific stress situation, to minimize the damage to the animal (Ulrich-Lai and Herman 2009). The physiological and behavioral responses to stress recruit an efficient and highly conserved set of neural systems and aim to maintain the physiologic integrity and homeostatic balance even in the most stressful situations (Ulrich-Lai and Herman 2009).

1.5.4.1 Thalamic sensory-related structures

Thalamic sensory regions such as the posterior intralaminar nuclei (PIN) and medial geniculate nucleus (MG), along with primary, secondary and associative auditory cortices, provide sensory input to the lateral amygdala (LA) (Figure 1-4) (LeDoux 2000, Davis and Whalen 2001). It has been suggested that the ventral part of the MG and the auditory cortex

may play an important role in the discrimination of neutral and fearful auditory stimuli. In consistent with the function of MG in fear conditioning, lesions after fear conditioning learning leads to diminished expression of conditioned fear (Romanski and LeDoux 1992). Inactivation and lesion of auditory cortex can also reduce the effects of fear conditioning, especially when the CS is a complex acoustic stimulus (Boatman and Kim 2006, Sacco and Sacchetti 2010). However, lesions of aforementioned thalamic or cortical areas before fear conditioning training show no effect on the acquisition, suggesting a compensation effect by other brain circuits (Romanski and LeDoux 1992). In conclusion, these thalamic and cortical areas responsible for auditory information processing are very important for fear conditioning acquisition and expression.

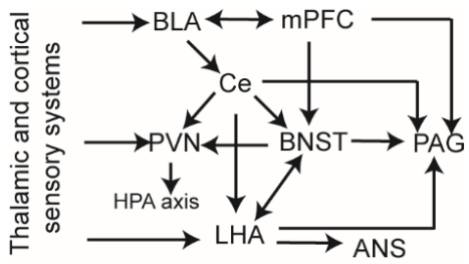

Figure 1- 4. Brain circuits involved in the stress response.

Different neural pathways mediate different aspects of stress responses, with different output sites. For example, the HPA axis mediates the glucocorticoid release, the vlPAG controls the freezing behavior. ANS, autonomic nervous system. BLA, basolateral amygdala. BNST, bed nucleus of stria terminalis. Ce, central amygdala. HPA axis, hypothalamic-pituitary-adrenal axis. LHA, lateral hypothalamic area. mPFC, medial prefrontal cortex. PAG, periaqueductal gray area. PVN, paraventricular nucleus of the hypothalamus.

Information about the tone and foot shock are signaled through the PIN-LA pathway, in parallel with the insular cortex-LA one (Shi and Davis 1999). The periaqueductal gray area (PAG) is also found to be involved in the processing of aversive stimulus. After pairing a tone with PAG stimulation, without any physical aversive stimulus, the presentation of the tone was also sufficient to induce reactions observed in the classical fear-conditioning paradigm, and this effect is dependent on the LA neurons as well (Kim, Horovitz et al. 2013).

1.5.4.2 The paraventricular nucleus of the hypothalamus (PVN)

The PVN is the initiating site of the HPA axis (Figure 1-4), and neurosecretory neurons in the dorsolateral part of the medial parvocellular PVN (PVNmpd) are mainly responsible for the release of CRH through median eminence in response to stress (Swanson, Sawchenko et al. 1987). Magnocellular component of the PVN (PVNm) release vasopressin and oxytocin into the systemic circulation upon stress exposure (Plotsky, Bruhn et al. 1985). The main

functions of vasopressin and oxytocin include modulating fluid and electrolyte balance, blood pressure (Swanson and Sawchenko 1983), but evidence also suggested that they also play an important role in modulating the ACTH release in some circumstances, in synergy with the CRH (Verbalis and Dohanics 1991). Nevertheless, the mechanism is not clear about how the magnocellular PVN modulates the functions of the HPA axis under stress exposure. Neurons in the dorsal, ventromedial and lateral parvocellular subregions of the PVN send dense projections to the brainstem and spinal cord ANS and are believed to integrate sympathetic/parasympathetic outflow (Swanson and Sawchenko 1983). Even though the CRH secreting parvocellular neurons in the PVN (PVNp) dominate the release of ACTH, the PVNm populations are still consider to make important contributions to the modulation of stress response (Herman, Cullinan et al. 2002)

The primary source of monosynaptic projections to the PVN are deep brain structures, such as hypothalamic and brain stem nuclei. The limbic forebrain structures includes the amygdala, hippocampus and prefrontal cortex. They play critical roles in processing the psychological stress information, but barely send direct projections to the PVN. Instead, these structures relay information in deep brain structures, which will finish sending the information in to the PVN (Bains, Cusulin et al. 2015). For example, the infralimbic cortex and central amygdala innervate the NTS to activate the HPA axis and ANS (van der Kooy, Koda et al. 1984).

The PVN receives heavy inhibitory GABAergic inputs, some of which come directly from neurons in the peri-PVN regions. This peri-PVN region is innervated by several limbic regions, enabling the limbic modulation of the HAP axis (Ulrich-Lai and Herman 2009). Whereas the CRH releasing parvocellular neuroendocrine cells (PNCs) are targeted by various inputs, including glutamatergic and GABAergic, as well as monoaminergic and peptidergic inputs (Bains, Cusulin et al. 2015).

1.5.4.3 The amygdala

The basolateral amygdala (BLA) and LA are cortical–like structures consisting of GABAergic interneurons and glutamatergic cells (Sah, Faber et al. 2003), but they are not layered as they are in the cortex. It has been found that LA is the primary site in the amygdala receiving the sensory information of both US and CS in fear conditioning learning process

(LeDoux 2000, Davis and Whalen 2001). Interestingly, single neurons in the LA can receive information from somatosensory, auditory and nociceptive systems. Moreover, after fear-conditioning learning, the synapses from the auditory thalamus and cortex to the LA are strengthened. Taken together, these findings depict a neural mechanism underlying the fear conditioning acquisition, the local plasticity induced by integration of the CS and US in the LA facilitates phasic neural spiking in response to the CS after learning, and consequently produces fear memories (Herry and Johansen 2014).

The central nucleus of the amygdala (Ce), which can be further divided into two main subregions, the lateral (CEl) and medial (CEm) part of the Ce, has be suggested to relay information from the basal amygdala (BA) into the hypothalamic, midbrain and brainstem systems (LeDoux, Iwata et al. 1988, Petrovich and Swanson 1997). There are also some small clusters of GABAergic neurons surrounding the BLA, named as intercalated neurons (ITCs). Lateral cluster of the intercalated neurons provides feedforward inhibition to the BLA (Asede, Bosch et al. 2015). The LA projects to the Ce directly and indirectly via BA and a medial cluster of the intercalated neurons (Asede, Bosch et al. 2015). After the formation of condition fear memories, the signals of CS is then sufficient to activate the LA-Ce projections, and downstream Ce projections, for example to the brain stem, mediate the defensive behavior such as freezing, as well as autonomic and endocrine responses.

In addition to the BA-CEm circuit, following studies using refined techniques, such as large-scale recording and optogenetic manipulations identified a complementary pathway of direct projections from the CEl neurons to the ventrolateral part of the periaqueductal gray area (PAGvl), which regulates the fear behaviors. It has also been found that the dorsal part of the medial prefrontal cortex is also involved in the conditioned fear expression, possibly by its connection to the BA, which provides a regulatory effect from higher cognitive system (Sotres-Bayon and Quirk 2010).

1.5.4.4 The medial prefrontal cortex (mPFC)

The choice of appropriate defensive behavior (e.g. fight/flight) in a dangerous situation is very important for a better chance of surviving. It has been found that the medial prefrontal cortex is involved in shifting from one strategy to another in various kinds of tasks, including

selecting proper defensive responding strategies in stressful situations (Rich and Shapiro 2009).

The contribution of mPFC to the stress responses is quite complex, differing between subregions. In response to psychogenic stress, the prelimbic component of mPFC (PrL) preferentially inhibits the activity of HPA axis (Radley, Arias et al. 2006). Moreover, it tends to regulate the duration instead of the peak value of glucocorticoid response to stress, suggesting a role in terminating the responses. Consistent with the role of the PrL in depressing the ANS activity in response to stress, PrL inhibition or local injection of noradrenaline significantly increased the heart rate in response to psychological stimuli (Akana, Chu et al. 2001). Opposite to the PrL, the infralimbic mPFC (IL) is suggested involved in stimulating the ANS and HPA responses to stress (Radley, Arias et al. 2006). In conclusion, the PrL and IL play different roles, and collaborate in organizing the stress responses.

The mPFC also send projections to the amygdala (Figure 1-4). For example, the IL sends inhibitory projections to the BA, and excitatory projections to the intercalated cells of the amygdala. Taken together, the total effect of IL projections to the amygdala is to inhibit the CEm neurons, and as a result, to inhibit the fear responses.

A large number of studies with various techniques have indicated the involvement of the mPFC, particularly the IL, in the retention and expression of fear extinction. Single unite recording in the mPFC found increased neural activity in response to the CS following fear extinction training, accompanied with induction of synaptic plasticity (Herry and Garcia 2002, Milad and Quirk 2002, Hugues, Chessel et al. 2006). Visualization of the mPFC activity via metabolic mapping found that the extinction training increased the uptake of a labeled glucose analog in prefrontal tissue of rodents (Barrett, Shumake et al. 2003). Moreover, retention and expression of fear extinction can be facilitated by microstimulation of the mPFC during extinction training, which also reduces the conditioned freezing response to the CS during the training process (Milad and Quirk 2002, Milad, Vidal-Gonzalez et al. 2004).Studies in humans showed that the expression of fear extinction is associated with increased BOLD response in the ventromedial mPFC as revealed by fMRI, as well as

enlarged thickness of the prefrontal cortex as assessed with structural MRI (Milad, Quinn et al. 2005).

1.5.4.5 The bed nucleus of stria terminalis (BNST)

The BNST is composed of a large number of subregions with different functions in stress responding. The anteroventral BNST is highly involved in HPA axis activation. Lesions of this subregion diminishes the PVN activation and compresses the HPA axis response to restrain the impacts of stress (Gray, Piechowski et al. 1993, Choi, Furay et al. 2007). The anterolateral BNST CRH-expressing neurons projecting to the PVN, indicating a central modulation action of CRH on the HPA axis (Ulrich-Lai and Herman 2009). In contrast, the HPA response to stress is facilitated by lesions of the posteromedial BNST, demonstrated as increased ACTH and corticosterone secretion, PVN Fos and CRH mRNA expression (Choi, Furay et al. 2007). In consistent with this finding, tracing studies found that the PVN-projecting neurons in this subregion are largely GABAergic (Cullinan, Herman et al. 1993). Moreover, previous studies also suggest that the BNST is involved in the modulation of cardiovascular response to stress, possibly via the parasympathetic nervous system (Alves, Crestani et al. 2007, Crestani, Alves et al. 2008).

1.5.4.6 The lateral hypothalamic area (LHA)

Hypothalamus plays as a relaying point between limbic sites and HPA axis, as well as ANS (Figure 1-4). In this way, the hypothalamus can influence responses to stress depending on the ongoing physiological status. When activated by stress, the LHA can modulate the responses of the HPA axis and ANS (Buijs and Van Eden 2000). Various types of neurons, such as GABAergic, Glutamatergic and peptidergic neurons, are highly intermingled in the LHA. As a result, it is difficult to determine its net function in stress response (Swanson, Sanchez-Watts et al. 2005).

Some types of conditioned stress responses could be disrupted by lesions of the perifornical hypothalamus and the LHA (Iwata, Ledoux et al. 1986, Furlong and Carrive 2007). It raised the hypothesis that the limbic-hypothalamic relay areas has a function of integrating the conditioned HPA and ANS activation, which means that both limbic and homeostatic information are involved in regulating learned stress responses (Ulrich-Lai and Herman 2009).

The neural circuits underlying fear conditioning are largely composed of 3 parts, sensory components that processes the CS and US, integrating components where the CS becomes associated with the US, and output components that control the expression of conditioned fear (Davis and Whalen 2001). Several brain regions have been found to play important roles in fear inhibition and fear extinction, such as the amygdala, mPFC, sensory cortex (Teich, McCabe et al. 1989, Sotres-Bayon and Quirk 2010), the BNST, PAG (McNally 2005), and the striatum (Rogan, Leon et al. 2005).

1.6 Compulsive eating in spite of aversive consequence

1.6.1 'Food addiction'—a concept under debate

Intake of palatable food, which is also called 'comfort food', has been proposed to activate the reward system (hedonic effect) and decrease the hypothalamic-pituitary-adrenal (HPA) axis response to stress (anxiolytic effect), with lower corticosterone (Pecoraro, Reyes et al. 2004) and cortisol (Tomiyama, Dallman et al. 2011) concentrations. Despite the rewarding and anxiolytic effects of palatable food, there are abundant evidence showing that abstinence from preferred food activates the amygdala stress circuits (Teegarden and Bale 2007), and elicits negative emotions, anxiety, depressive-like behaviors, and impaired reward responding (Avena, Rada et al. 2008, Cottone, Sabino et al. 2008). The resumption to the 'comfort food' ameliorates these aversive responses.

Binging on palatable food is able to transiently reinstate the reward deficit and suppress negative emotions, but dampen them in the long term (Moore, Sabino et al. 2017). This vicious cycle is similar to what happens in drug addiction and alcoholism. Avena previously proposed that binge eating is a salient component of 'food addiction' (Avena 2010). In fact, many behavioral signs of addiction have been reported in animal models of sugar bingeing, such as over eating, somatic signs of withdrawal (Colantuoni, Rada et al. 2002), elevated motivation after withdrawal (Grimm, Fyall et al. 2005) and cross-sensitization to some addictive drugs (Avena and Hoebel 2003) and alcohol (Avena, Carrillo et al. 2004). A study of 79 female patients with BED demonstrated that when the term 'drug use' was changed into 'binge eating', 92% of the participants met the criteria for substance dependence in the DSM-4, indicating an astonishing similarity of the BED with substance abuse disorders (Cassin and von Ranson 2007).

The Yale Food Addiction Scale is a self-report instrument for assessing food addiction based on the diagnostic criteria for substance dependence (Gearhardt, Corbin et al. 2009, Horsager, Færk et al. 2020). Scores on the Yale Food Addiction Scale correlate with greater activation of the anterior cingulate cortex, the medial orbitofrontal cortex and the amygdala, regions associated with motivation, in response to anticipation of palatable food (Gearhardt, Yokum et al. 2011). Consumption of foods that are particularly palatable can activate these same brain regions (Wang, Volkow et al. 2004, Gearhardt, Yokum et al. 2011), which may underlie cognitive aspects of craving for food.

The parallels between the BED and substance used disorders (SUDs) drawn by researchers are largely based on their common effects on the reward system in the brain (N Gearhardt, Davis et al. 2011, Kessler, Hutson et al. 2016). In supporting of this comparison, neuroimaging studies found similar activities in reward system when comparing BED patients with SUDs individuals (Kessler, Hutson et al. 2016). Moreover, the alterations in DA/acetylcholine (ACh) balance observed during withdrawal from drugs, has also been confirmed in rats binging on sugar during withdrawal, demonstrating as specifically decreased DA and increased ACh.

1.6.2 Compulsive eating in human patient with BED

Elevated impulsivity and compulsivity, and altered reward sensitivity/punishment are frequently reported features of the BED (Carrard, Crépin et al. 2012, Schag, Teufel et al. 2013). As clarified by Kessler, 'Impulsivity reflects decision-making occurring with limited forethought and represents a multidimensional construct involving tendencies to act rashly, to have increased reward-related drives, and to have disadvantageous decision-making. In contrast, compulsivity is characterized by the performance of repetitive and persistent actions that are not related to an overall goal or reward and that can persist despite adverse consequences' (Kessler, Hutson et al. 2016).

Binge-eating patients clearly know the aversive consequences of overeating, but they cannot resist the desire to gorge on palatable food, which is exactly the manifestation of the compulsivity of binge eating. People with BED are constantly troubled with the feeling of loss of control. This compulsivity for food in the BED is similar to the craving for drugs in drug addiction, that some researchers developed a theory named 'food addiction' (Appleton

1985). Even though the existence of food addiction is still controversial, it has been getting more attention in recent research. In fact, binge eating animal models have been also used to find the evidences for food addiction (Avena 2010).

In drug addiction, the persistent behavior of drug-seeking or taking represents the compulsive property of this substance dependence disorder. The most prominent symptom shared by binge eating and drug dependence is the compulsive craving for a preferred substance even with full knowledge of the negative consequences (Oswald, Murdaugh et al. 2011). These negative consequences include but not limited to body figure problems, emotional disturbances, psychiatric problems, social impairment, and medical conditions related to obesity. Most obese people have a desire to limit their food intake, and indeed struggle with dieting, but repeatedly return into a hyperphagia. The consequent feeling of loss of control is one of defining features of the BED. In animal models of binge eating disorder, the compulsivity element is observed as relentless seeking and consumption of palatable even if it is associated with an aversive physical or emotional punishment (e.g. eating in a stressful environment, created by a foot shock) (Oswald, Murdaugh et al. 2011).

1.6.3 Impaired decision-making in patients with BED

With an innate positive value, a pleasurable object can spontaneously elicit approaching behavior in animals, and this is a ubiquitous phenomenon in behavioral science. However, in real life, the value of an object is not always fixed, instead it is usually dynamic, and changes according to the physical, psychological, and environmental situations. In conflicting situations, when a stimulus is positive, but approaching is undesirable, the inhibition of the approaching reaction is the result of devaluation of this positive stimulus, which means its rewarding value is diminished in this specific situation. It has been suggested that when a response conflict occurs, a negative affective value will be added onto the originally positive stimulus, making the stimulus less desirable, and consequently decreased the approach tendency. Consistent with this idea, studies have found that pairing an initially desired goal with negative consequence made such a goal less desirable and less likely to trigger motivation directed behaviors (Aarts, Custers et al. 2007).

In fact, both impulsivity and compulsivity are represented by impaired decision-making ability. Not surprisingly, decision-making impairments have also been associated with the

BED. In a test with probabilistic-reward task, BED patients were more likely to make risky decisions than non-BED individuals (Svaldi, Brand et al. 2010). In another study, obese BED patients showed less preference for probabilistic rewards and delayed rewards than normal participants as well as obese individuals without BED (Manwaring, Green et al. 2011).

Impaired decision-making by the BED has also been proven by a task involving choosing between risky or sure monetary choices. Consistent with aforementioned results, the obese BED patients tended to take more risky choices in response to moderate probabilities of benefit and high probabilities of loss. Interestingly, this decision-making pattern was similar to the observation in alcohol-dependent individuals (Voon, Morris et al. 2015). Another interesting finding is that in BED patients, the tendency of making risky decision was not changed by the magnitude of reward, while it decreased in the control participants as the reward magnitude increased along with increased possibility of loss (Voon, Morris et al. 2015).

Nevertheless, loss of control over binge eating is closely associated with the helpless and despair feeling reported by BED patients (Wolfe, Baker et al. 2009). Some researchers even proposed to use the intensity of the subjective feeling of loss of control as the defining criteria of the BED, instead of the objective episodes of binge eating (Wolfe, Baker et al. 2009).

1.6.4 Brain regions involved in decision-making

In all species, a better chance of survival and prosperity requires well planned physiological and behavioral response to homeostatic and environmental challenges, especially in complicated situations. Making decisions in a conflicting situation means choosing the appropriate responses to acquire rewards and to avoid punishment. The collecting and processing of information and decision-making based on it requires the coordinated activation of neural and neuroendocrine systems.

1.6.4.1 The amygdala

Ce is involved in emotion control, and fear response expression. However, studies suggest that the Ce can also influence the feeding behaviors through multiple circuits. Functional studies demonstrate that Ce signaling is involved in the modulation of opioid-induced feeding via the NTS and PVN (Giraudo, Billington et al. 1998, Giraudo, Kotz et al. 1998). Considering the special functions of the Ce in the modulation of both emotion and food

consumption, it is not surprising that the Ce is involved in the regulation of feeding behavior under conditioned stress. Petrovich found that bilateral central amygdala (Ce) lesion was sufficient to abolish the cessation of feeding by aversively conditioned cues, while rats with bilateral lesions of other amygdala subregions, such as basal lateral amygdala (BLA), still ceased feeding under conditioned fear (Petrovich, Ross et al. 2009). In another study the same group found that bilateral electrolytic lesion of the ventrolateral subregion of the periaqueductal gray area (PAGvl), a midbrain target of Ce, after fear-conditioning training, was sufficient to abolish the freezing response to the CS, while leave the cessation of feeding intact (Petrovich, Ross et al. 2009).

More and more studies implicate the amygdala in signaling the information about both arousal and valence (Morrison and Salzman 2010). It was proposed that amygdala puts an emotional tag on the stimulus, and send it back to the reward system (Morrison and Salzman 2010). An example is that anorexia patients show increased amygdala activation when they are shown with their distorted body image, which serves as a threatening, symptom-provoking cue (Seeger, Braus et al. 2002). Moreover, increased amygdala activities has been correlated with elevated anxiety in young women without eating disorders, when looking at pictures of idealized, slim female bodies (Friederich, Uher et al. 2007).

1.6.4.2 The medial prefrontal cortex (mPFC)

Extinction, as well as devaluation, is a form of inhibitory learning, in which the newly learned responses will inhibit previously conditioned responses. Both drug seeking and conditioned fear are conditioned responses, and when they are expressed without control, it will lead to maladaptive behaviors such as addiction and anxiety disorders respectively. It has been found that the mPFC is critically involved in the extinction of both drug seeking (Moorman, James et al. 2015) and conditioned fear responses (Giustino and Maren 2015).

Besides the malfunctioned reward system, altered cognitive function is also proposed to be involved in the compulsivity of binge eating (Voon, Morris et al. 2015). Among the cognitive related brain areas, suppressed activities in the mPFC has been suggested underlying the compulsive eating in the BED. In obese individuals with BED, studies have found higher impulsivity, and impaired response inhibition compared with normal-weight individuals

without BED (Nasser, Gluck et al. 2004, Balodis, Molina et al. 2013, Schag, Teufel et al. 2013, Kessler, Hutson et al. 2016).

1.6.4.3 The nucleus accumbens (Acb)

The dorsal striatum is the central component in the neural circuit of processing the information about the contingencies of the reward stimulus, and controlling goal-directed learning process, such as instrumental conditioning (Balleine, Delgado et al. 2007). In other words, it integrates, and processes all reward related information, and subsequently optimizes the reward related responses. After that, it is the Acb, part of the ventral striatum, that makes the outcome-based predictions (Asaad and Eskandar 2011). The Acb is responsible for predicting the error-based outcome, and constantly updating the predictions about reward and punishment (Burton, Nakamura et al. 2015).

It has been suggested that the shell part of the Acb is responsible for detecting and signaling safe periods between aversive cues. Connections between the hippocampus and the Acb shell mediate the searching behavior, particularly if the outcome is ambiguous, and direction towards reward is not clear (Floresco 2015). In consistent with this function, the Acb shell is also the key structure responsible for inhibition of irrelevant and less profitable actions to keep the attention on current task. There is evidence showing that lesions to the Acb shell induces uninhibited reward seeking behaviors with less circumspection (Stopper and Floresco 2011).

Complementary to the functions of the Acb shell, the Acb core is responsible for learning to discriminate the aversive cues, and mediating the subsequent responses to avoid them (Li and McNally 2015). As a result, with the dichotomized functions of the Acb shell and core, when the conditions and circumstances are ambiguous and unpredictable, the Acb will help to avoid the aversive consequence and approach towards intended goals.

It has been proposed that the DA signaling in the striatum, including the Acb, is composed of compartments, which are regulated by distinct modes of transmission (Grace 1991, Grace, Floresco et al. 2007, Floresco, Onge et al. 2008). Tonic DA signaling changes on a time scales of minutes, while phasic DA signaling represents a more temporally sensitive signal mediated by the activation of DA neurons. Phasic DA signaling receives more attention than the tonic signaling, as a result of findings that dopaminergic neurons in the VTA is involved

in encoding reward prediction errors. It has been found that reward predictive stimuli and unexpected reward can trigger the phasic DA bursts (Schultz, Dayan et al. 1997), which are mediated by excitatory projections from regions such as the mPFC and pedunculopontine tegmental nucleus (PPTg) (Lokwan, Overton et al. 1999, Floresco, West et al. 2003).

There are also many studies showing that manipulations of the Acb neuronal activity and DA signaling (Stopper, Khayambashi et al. 2013) can influence the result of cost/benefit decision-making, which indicate the Acb as a integrating site of signals related to risk/reward decision outcomes. This synaptic plasticity induced by DA bursts paired with activities induced by a rewarded choice may enhance these circuits in a way that they will tend to response in similar patterns at the next decision-making opportunity (Humphries and Prescott 2010). In conclusion, the Acb DA signal system appears to be responsible for integrating various types of information, such as reward uncertainty and changes in reward availability, to guide risk/reward decision-making (Onge, Ahn et al. 2012).

1.6.4.4 The ventral tegmental area (VTA)

The representations of anticipated reward value in the neural system are the core component in all models of the mechanisms of learning (Schultz, Dayan et al. 1997) and decision-making. In these models, the subjective association of predictive cues to their subsequent outcomes, prior to the occurrence of the outcomes, reflects 'anticipation'. In order to represent the value of choices for learning and decision-making, the neural system has to encode the associated information about the affective valence and motivational relevance of the anticipated outcomes. Evidence from numerous studies have indicated the Acb and VTA as the key brain structures to conduct these and other aspects of valuation (Liu and Kanoski 2018). Interestingly, both potential losses and potential gains can increase the activities in the Acb and VTA, and there is a positive correlation between the activations induced by gains and by losses across participants (Watabe-Uchida, Eshel et al. 2017).

In addition to facilitate simple association between behavioral choices and potential rewards, DA signals also promote reward seeking in more complex and dynamic situations, for example, a situation demanding precise evaluation and comparison of the relative value of the outcomes following different actions (Salamone, Cousins et al. 1994, Floresco, Onge et al. 2008, Floresco 2013). In support of this function, brief increases of mesoaccumbens DA

and activation of dopaminergic neurons were observed during the presence of reward-predictive stimuli (Schultz, Dayan et al. 1997, Schultz 1998, Flagel, Clark et al. 2011) and during the decision-making involving reward of varying costs, magnitudes, and uncertainty (Day, Jones et al. 2010, Gan, Walton et al. 2010, Sugam, Day et al. 2012).

1.6.5 Interaction between reward system and the HPA axis in the development of the BED

It has been proposed that stress-induced emotional eating is regulated by subcortical areas in control of stress-induced arousal and energy recruitment (HPA axis and limbic area), reward and motivational drive (Acb and VTA) (Adam and Epel 2007). Based on the findings from recent studies, it has been suggested that non-homeostatic eating of palatable food can activate the reward system in the brain and compensate for the aversive effects induced by stress, and decrease activity of the HPA axis, inhibiting the central response to stress accompanied with a decrease in plasma cortisol (Tomiyama, Dallman et al. 2011) and corticosterone (Pecoraro, Reyes et al. 2004) concentration. For example, it has been found in rats that restraint stress can increase the intake of palatable food (lard or sucrose), and the palatable food intake consequently decreased that plasm corticosterone levels in rats with access to palatable food after stress, compared with those only consuming chow (Bell, Bhargava et al. 2002, la Fleur, Houshyar et al. 2005). This reward related feeding behavior is suggested to provide a negative feedback to diminish the HPA axis response to stress, and in turn to inhibit further CRH release, facilitating the recovery from stress.

In other words, the stress-induced (over-) eating, especially palatable food, is likely as 'self-medication' to relieve the impact of stress. However, repeated activation of the reward system by stress-induced HPA recruitment, consumption of palatable food, or both of them may cause neurobiological and behavioral adaptations that in turn enhance the compulsive component of binge eating (Klatzkin, Baldassaro et al. 2018, Finch, Tiongco-Hofschneider et al. 2019). Moreover, the reward system and HPA axis may interact with each other in response to stress, and in the regulation of stress-induced overeating. For example, stress-induced CRH release can activate VTA dopamine neurons in a dose-dependent manner and this effect can be diminished by CRH-1 receptor antagonist (Wanat, Hopf et al. 2008). These findings are consistent with the fact that stress may induce palatable food consumption in both animals (Foster, Warne et al. 2008) and human (Macht and Mueller 2007).

1.7 Sexual dimorphism in binge eating disorder, stress responses and cue-induced inhibition of feeding behaviors

The prevalence of the BED in female individuals is approximately twice as high as in male (Kessler, Berglund et al. 2013, Cossrow, Pawaskar et al. 2016), which triggered development animal models of BED with female animals (Hagan, Wauford et al. 2002, Oswald, Murdaugh et al. 2011). Similarly, the rates of anorexia nervosa and bulimia nervosa are higher in females compare with males (Timko, DeFilipp et al. 2019). Surprisingly, testosterone exposure protects males from eating pathology as early as from prenatal period and across development, and the level of circulating testosterone is negatively correlated with the rates of eating disorders in males (Klump, Culbert et al. 2017).

Although an aversively conditioned cue can inhibit food intake comparably in female and male rats, this inhibitory effect lasts longer in female than male rats after the cue was disassociated from foot shock (Petrovich and Lougee 2011). Fear extinction is a process of re-learning, instead of de-learning/forgetting. Fear conditioning can induce permanent plasticity in the amygdala, and fear extinction lead to the formation of new plasticity in brain regions such as amygdala and prefrontal cortex, instead of deleting existing connections formed during fear conditioning (Luchkina and Bolshakov 2019). Both conditioning and extinction are adaption strategies to the environment, and different rates of cue-induced inhibition of food intake, and food-enhanced fear extinction may represent different strategies of adaption in female and male animals.

In females, food intake and stress responses are prominently influenced by the hormone fluctuations during menstrual cycles. For example, the within-cycle fluctuations of 2 main ovarian hormones, estradiol and progesterone, can negatively and positively predict shifts in food intake respectively (Roney and Simmons 2017). Interestingly, these patterns are precisely opposite to the predictive effects of the variations of these hormones on self-reported sexual desire from the same sample (Roney 2015). The menstrual cycles in female rodents and naturally cycling women have considerable influences on the retention and recall of fear extinction (Zeidan, Igoe et al. 2011). Sex hormones, including estradiol and progesterone, modulate NMDA receptor function and synaptic plasticity. Estrogen receptors are also expressed in the central amygdala, which is critical for cue-induced inhibition of feeding (Petrovich, Ross et al. 2009).

1.8 Methods used in this study

1.8.1 Rate model of binge-like eating

Binge eating disorder has been increasingly studied both pre-clinically and clinically. Individuals with BED display altered sensitivity to palatable food, and increased impulsivity and compulsivity to eat. Although the pathology of the BED is still not clear, its neurobiological basis is being elucidated gradually with studies in animal models as well as human patients. Stress is a very common trigger of binge eating in human, and has been proven to be as effective to induce binge eating in animal models (Boggiano and Chandler 2006). Many other precursory events that contribute to the development of the BED in humans, such as caloric restriction and intermittent access to palatable food, are also used to elicit binge eating in animal models (Avena and Hoebel 2003, Corwin, Avena et al. 2011). Thus, animal models provide an isomorphic mimic of the eating behavior, by recreating conditions associated with the BED, and this behavior finally provide targets for the exploration of the underlying mechanisms of this eating disorder.

Our laboratory developed a binge-like eating rat model, with combination of intermittent access to a 10% sucrose solution and foot shock stress (Calvez and Timofeeva 2016). Rats are classified into binge-like eating prone (BEP) and binge-like eating resistant (BER) phenotypes according to their sucrose intake after stress. In this rat model, the BEP rats (BEPs) take more sucrose than BER rats (BERs), both in normal condition and after stress. The foot shock stress increased the sucrose intake in the BEPs, but has no significant effect on the sucrose intake of the BERs (Figure 1-5) (Calvez and Timofeeva 2016). Cottone et al. used to dissect compulsive eating into three main elements (Moore, Sabino et al. 2017): (1) habitual overeating, (2) overeating to relieve negative emotion, and (3) overeating despite aversive consequences. During the phenotyping process, the BEPs took more sucrose solution than the BERs under normal condition, which reflected a habitual overeating. They further increased their sucrose intake after foot-shock stress, presumptively to relieve the effect of this aversive experience.

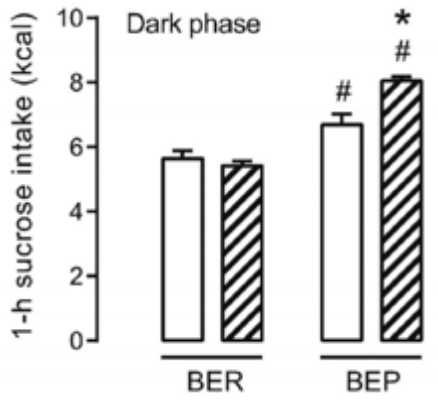

Figure 1- 5. Phenotyping of the BEPs and BERs based on sucrose intake

Sucrose intake at the beginning of dark phase in non-stressful conditions (clear bars) and after foot shock stress (dashed bars) BERs and BEPs. Adapted from (Calvez and Timofeeva 2016).

In this binge-like eating rat model (Calvez and Timofeeva 2016), a modified light/dark box (L/D box) test was used to observe the compulsive eating despite potential aversive consequence (Figure 1-6 A). The light box is white, open on top and intensely illuminated; while the dark box is black, closed and dark inside. On the lateral side of the light box, there is a spout for a 10% sucrose solution, which is not provided in the dark box. By nature, rats should avoid a bright open field, where is easier for the predator to spot them. However, in this modified L/D box test it was found that after stress the BEPs took significantly more sucrose in the light box than the BERs, indicating the compulsive eating in the BEPs (Figure 1-6 B) (Calvez and Timofeeva 2016).

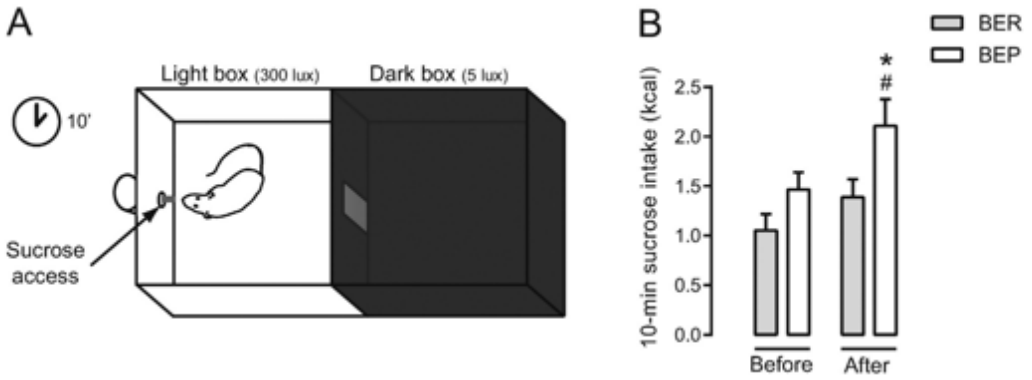

Figure 1- 6. Compulsive-like eating behavior in BER and BEP rats.

(A) Diagram of the modified light-dark box test adapted from (Cottone, Wang et al. 2012). (B) Intake of sucrose during a 10min exposure to the modified light-dark box before and after intermittent access to sucrose and repeated exposure to stress. *, significantly ($p < 0.05$) different from the BERs within the same time window. #, significantly different ($p<0.05$) compared with the value obtained before intermittent access to sucrose and repeated stress exposure within the same phenotype. n = 12 rats/group. Adapted from (Calvez and Timofeeva 2016).

Further biochemical analyses found that the compulsive-like intake of sucrose of the BEPs in the L/D box were associated with a blunted stress-induced plasma corticosterone response (Calvez and Timofeeva 2016), which is consistent with the anti-stress effects of palatable food as described previously. After the foot shock stress, the expression of CRH mRNA in

the PVN was increased only in the BERs, but not the BEPs, while the expression in the bed nucleus of the BNST only increased in the BEPs. These results suggested that dampened response of the HPA axis and higher expression of CRH in the BNST may underlie the development of stress-induced binge-like eating of sucrose in the BEPs (Calvez, de Ávila et al. 2016)

1.8.2 Monitoring food intake in rats

1.8.2.1 Feeding behavior in rats

Richter's classic experiments suggested that water and food intake of all mammals is episodic, rather than continuous. This episodic feeding and drinking is a result of dynamic interactions between complex intertwining signals, with some (hunger and thirst) stimulating these behaviors while others (satiety and satiation) restraining them.

Naturally, the daily food intake of rats comes as series of meals, and the overall food intake can be assessed roughly by two important components, namely the meal size and meal frequency. Both of these components has been manipulated in laboratory conditions separately, suggesting that they are possibly regulated via separate physiological mechanisms (Rosenwasser, Boulos et al. 1981).

Rats drink liquid, such as water, by repeatedly dipping their tongue into the liquid and scoop it into their mouth, which is also called licking. Licking is a repetitive, rhythmic and stereotypic behavior in rats generated by a central pattern generator formed by a network of brainstem neurons located in lateral medullary reticular formation (Chen, Travers et al. 2001). This central pattern generator is under the modulation of signals from the olivocerebellar neurons, which fire in a time-locked way to the licking events (Welsh, Lang et al. 1995, Bryant, Boughter et al. 2010). At the same time, the mammalian brainstem also controls many other rhythmic movements such as respiration and mastication, the well timing of which is vitally importantly for our survival. Although the licking behavior in rats is very different to human drinking behavior, exploration of its mechanism will give us some knowledge about the generation and modulation of ingestive behavior in human. Moreover, it also provides a method to test the behavioral effects of some artificial manipulations, such as physical or social stress and drug treatments.

Different patterns of licking can result in similar intakes. Therefore, we have to look into more detailed licking structures, besides the quantity of ingested liquid. Researchers usually classify bouts of licks into clusters, sustained licks separated by a pause of 500ms (Smith 2001, Dwyer 2012). In this way, we can further analyze the licking frequency, number of clusters, number of licks/cluster and meal size (total number licks in the clusters) in addition to the total number of licks. Previous studies have found that the licking frequency can be influenced by many environmental factors. For example, rats will decrease their licking rate when the spout accessibility become restricted (Hernandez-Mesa, Mamedov et al. 1985), while increase the frequency when they lick under stress (Vajnerova, Bielavska et al. 2003). The mean number of licks in a cluster (cluster size) is related to the palatability of the consumed solution which makes it a good indicator of hedonic reaction to palatable food (Dwyer 2012). The total number of licks in clusters (meal size) is largely controlled by gustatory stimulation and post-ingestive feedback (Davis and Smith 2009), and reflects the nutritious value of the ingested food.

1.8.2.2 Methods of monitoring feeding behavior in animals

With the advantages of techniques, feeding behavior in laboratory animals can be recorded automatically and continuously in various ways, and further, one can synchronize the behavior data with other events, such as neural activities. Moreover, we can also monitor their feeding behaviors in real time, and give them some feedback stimuli accordingly. Automated monitoring can improve the accuracy of the assessment. More importantly, it reduces the stressful impact on the animals caused by human interference. Currently available techniques can simultaneously monitor large numbers of animals over long periods of time without interference from experimenters throughout the process. Unlike manual recording, automatic recording can provide detailed information about the meal size, meal frequency, and microstructures of feeding patterns for each individual animal with good temporal resolution.

A variety of automated monitoring techniques has been employed in studies of feeding behavior in rats, including:

1. Operant methods, in which rats need to finish some required behavioral task, such as pressing a bar ten times, to obtain a specific quantity of food (Anliker and Mayer

1956);

2. Devices that record the entering of part of the animal's body, such as the head or tongue into the feeding port, for example the lickometer, detecting intrusions of the tongue with photo beam (Madrid, Lopez-Bote et al. 1993);
3. Devices that detect the animal's contact with the food, which are commonly known as eatometers (Fallon 1965);
4. Electronic balances that continuously weighing the food and send the information into a control center (Hulsey and Martin 1991)

In this study, a lickometer with a photo-beam detector was used to record the tongue-intrusion of the animals during the sucrose solution consumption. We aimed to observe the intake of palatable food in the situation of free access, so the operant methods were excluded from our options. Another reason is that, when we designed this study, we considered that if we would found some different activities in some brain regions between BEP and BER rats, we would do some electrophysiological recordings in these brain regions to have more details about the brain activities underlying the different feeding and fear responding behaviors between BEP and BER rats. Compared with the eatometers, which will pass a current through the animal body to detect the contact with the food, the lickometer detecting the tongue intrusion with a photo beam is more 'electrophysiologically friendly', because the current may generate some noise to the signals, or even damage the amplifier.

1.8.3 Use of *c-fos* expression as an indicator of neuronal activity

Transcription of genes is the process by which the DNA is read and interpreted as a set of instructions for cells to divide, differentiate, migrate, and mature. As cells function in their respective niches, transcription further allows mature cells to interact dynamically with their external environment while reliably retaining fundamental information about past experiences.

1.8.3.1 What is *c-fos*

Fos transcription was discovered by Greenberg and Ziff in 1984 (Greenberg and Ziff 1984), and its repaid and transient response to the stimulus demonstrated that cells could be activated on molecular level within minutes (Greenberg, Greene et al. 1985, Greenberg, Ziff et al. 1986, Lau and Nathans 1987). Thereafter, it became recognized that trans-synaptic signals

can regulate the synthesis of certain enzymes and neuropeptides (Chen, Dionne et al. 1983, Black, Chikaraishi et al. 1985).

It was later found that the transcription of Fos gene happens widely in many different types of cells , and that the Fos gene family encodes Fos proteins (Curran, Miller et al. 1984). These findings suggested that the expression of the Fos gene could possibly control the cell type specific functions such as long-term potentiation or depression that underlie learning and memory.

Fos is a family of transcription factors (TFs) which includes *c-fos*, FosB and ΔFosB etc (Perez-Cadahia, Drobic et al. 2011). In the following years, a large number of studies were conducted to explore the signal-dependent transcription of the immediate early gene (IEG). IEGs are a class of genes, just like Fos, which are transiently and rapidly activated by extracellular stimuli, without requirement of synthesis of new protein. Many IEGs, including *c-fos* gene, encode sequence-specific DNA-binding transcription factors, regulating a subsequent cascade of expression of late response genes (LRG), which has been known to be cell-type specific and specifically correlated to the function of the cells (Sheng and Greenberg 1990, Mardinly, Spiegel et al. 2016).

1.8.3.2 Mechanism of *c-fos* expressing and functioning

Over decades, a great number of studies has contributed to elucidate the signaling mechanisms underlying the induction of transcriptions of *c-fos* gene, as well as their regulatory functions on other LRGs (Vanhoutte, Barnier et al. 1999, Gallo, Katche et al. 2018). An influx of extracellular Ca^{2+} into the neuron is required for the neurotransmitter-dependent induction of IEGs (Hudson 2018). The consequent surging level of cytoplasmic calcium triggers a cascade of molecular events, including activation of series of enzymes, such as the MAPK (Ras-mitogen-associated protein kinase) and CaMKs (calcium/calmodulin-dependent protein kinase) (Sheng, Thompson et al. 1991, Xing, Ginty et al. 1996, Hardingham, Chawla et al. 1997). Activation of these signaling cascades can mediate various local changes at synapses, such as local mRNA translation, synthesis and internalization of glutamate receptors, and post-translational modifications of proteins (Wayman, Lee et al. 2008, Holt and Schuman 2013). More importantly it can also specifically

induce the transcription of activity regulated genes, which include the *c-fos* gene (Hilgenberg and Smith 2004).

The calcium influx that triggers IEG induction includes pathway through ligand gated ion channel, such as AMPA (a-amino-3hydoxy-5-meghyl-4isoxazolepropionate-type) and NMDA (N-methyl-D-aspartate-type) glutamate receptors, voltage gated calcium channels, and release of calcium from intracellular stores (West, Chen et al. 2001). Particularly, a variety of studies have demonstrated that calcium entry via the L-VSCCs (L type voltage-sensitive calcium channels) preferentially promotes gene transcription (Westenbroek, Ahlijanian et al. 1990).

Considering the extremely rapid induction of IEGs, including the *c-fos*, it has been suggested that their induction is not dependent on synthesis of new proteins. Instead, it relies on transcription factors that are pre-existing and can be activated rapidly. Therefore, these pre-existing TFs are constitutively expressed in the neurons. In fact, these TFs have been identified, such as CREB (cAMP responsive element binding protein), SRF (serum response factor) and MEF2 (myocyte enhancer factor 2), with the former two have been revealed to be in control of Fos transcription (Norman, Runswick et al. 1988, Sheng, Dougan et al. 1988).

These TFs are constitutively expressed, but not in an active status. Their activation is dependent on their induction in response to neuronal activation. Instead, TFs are activated by integrating signals from various Ca^{2+}-dependent pathways and undergoing some post-translational modifications, including phosphorylation in most cases (McKinsey, Zhang et al. 2002, Aizawa, Hu et al. 2004, Flavell, Cowan et al. 2006, Shalizi, Gaudillière et al. 2006).

After translation the *c-fos* proteins are transmitted into the nucleus, and it combines with its partner Jun to form a heterodimer named activating protein complex 1(AP-1). There are also other members of the AP-1 family of TFs, including JunB, JunD, FosB, FosL1 and FosL2. Any of them can replace Jun or Fos respectively to form the AP-1 heterodimer (Sheng and Greenberg 1990). Results from genome-wide assessments indicated that an estimated 104 binding sites are targeted by these activity-dependent TFs, and the number of LRGs regulated by these TFs were estimated to be 300-500 in neurons (Kim, Hemberg et al. 2010, Benito and Barco 2015, Mardinly, Spiegel et al. 2016). The functions of LGRs typically are encoding

effector proteins in regulation of cellular processes such as dendritic growth, synapse elimination, spine maturation (West and Greenberg 2011).

Both cell culture and in vivo studies have found that the activity-dependent transcriptions is neuronal subtype-specific. In a RNA sequencing study in cultured embryonic neurons, it was found that inhibitory and excitatory neurons express almost overlapped sets of genes, immediately (within 60min) after being activated, and these genes are found to be activity-dependent transcription factors, such as Fos, FosB and NPAS4. Nevertheless, after a relatively longer period (120 min after stimulation), inhibitory and excitatory neurons begin to express different genes (Spiegel, Mardinly et al. 2014). Further studies using ribosome tagging to isolate mRNAs from specific subtypes of neurons in vivo, identified some unique activity-dependent gene programs in some inhibitory neuronal subtypes, such as interneurons expressing VIP (vasoactive intestinal peptide), PV (parvalbumin), and SST (somatostatin) (Mardinly, Spiegel et al. 2016).

1.8.3.3 Using *c-fos* expression as an indicator of neuronal activation

Shortly after the finding of activity-dependent induction of *c-fos* transcription, it became a high resolution metabolic marker for tracing polysynaptic, and multi-region pathways in the brain and spinal cord (Sagar, Sharp et al. 1988). For example, early works in rats using *c-fos* as a marker of neuronal activation, found that Fos expression can be used to map the pathways underlying the spread of epileptic seizure. A recent study also used *c-fos* protein as a marker to map the brain regions activated by multi-modal and foot shock stress (Lin, Itoga et al. 2018). The *c-fos* expression is also widely used in the studies of mechanisms underlying the BED. For example, a recent study using *c-fos* as the marker of neuronal activation found that the mPFC may serve as a behavioral 'brake' over excessive intake of palatable food, and 80-90% of all mPFC neurons are activated by palatable food (Gaykema, Nguyen et al. 2014).

The *c-fos* mRNA appears in the cell nuclei 2-5 min after onset of neuronal activation and it disappears within 20 min as it is transferred into the cytoplasm. The accumulation of *c-fos* mRNA in the cytoplasm peaks approximately 30-40 min after the initial activations of the neurons in most brain regions (Saidov, Tiunova et al. 2019), which is the reason most studies using *c-fos* mRNA as the marker of neuronal activation sacrifice the animals 30 min after some specific stimuli, as we did in the presented below study.

1.9 Hypotheses and Objectives

The general objectives of this study are (1) to observe compulsive eating in the binge-like eating rat model and (2) to explore the neural activation underlying the BED.

1.9.1 Hypotheses and Objectives 1

In previous studies, the compulsive eating of the BEPs has been observed with the L/D box test (Calvez and Timofeeva 2016). Nevertheless, stress response in this L/D box is not easy to be quantified. As a result, some questions are still not answered: (1) whether the different sucrose intake in the light box between the BEPs and BERs was due to different sensitivity to stress between these two phenotypes, and (2) whether palatable food intake has comparable anxiolytic effect on them. To answer these questions, this study was designed to observe the palatable food intake and freezing response in the presence of an aversive CS in this binge-like eating rat model. In response to these questions, the hypotheses of this study were that (1) the BEPs and BERs are comparably sensitive to stress stimuli, that (2) the compulsive eating of the BEPs is induced by higher motivation for palatable compared with the BERs, and that (3) sucrose has stronger inhibiting effect on stress response in the BEPs than in the BERs.

Specific aims:

1. To observe if the BEPs and BERs have comparable sensitivity to stress stimulus.
2. To observe the compulsive sucrose consumption despite of potential aversive consequence in this binge-like eating rat model.
3. To observe if palatable food intake has comparable impact on stress response in the BEPs and BERs.

1.9.2 Hypotheses and Objectives 2

To explore the neural mechanisms underlying the compulsive eating of the BED, the BEPs and BERs were sacrificed after the behavioral test. Sections of the whole brain were prepared, and *c-fos* mRNA expression was quantified as a marker of neuronal activation stimulated by palatable food, the CS and their combination. In accordance with the overall relation between the behavioral responses to palatable food and stress stimulus, we hypothesized that (1) the BEPs and BERs have comparable activities in brains regions related to stress response, such as the amygdala, BNST and PVN when they have no access to sucrose, that (2) sucrose intake can decrease the activities in these brain regions in the BEPs, and that (3) the BEPs have enhanced response to sucrose intake in feeding related brain regions such as the LHA and Acb, and (4) less recruitment of brain regions involved in decision-making, such as the mPFC.

Specific aims:

1. To explore the way these brain regions responding to palatable food intake.
2. To explore the way these brain regions responding to an aversive CS.
3. To explore the way these regions responding to palatable food intake in the presence of the CS.
4. To explore if there is different activity in these brain regions between the BEPs and BERs in response to the CS, palatable food intake, and food intake in the presence of CS.

Chapter 1. Compulsive eating in rat model of binge eating disorder (BED) under conditioned fear and exploration of the neural mechanism with *c-fos* mRNA expression

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2.1 Key points summary

- Compulsive eating, eating despite of aversive consequences, is a hallmark of binge eating disorder (BED).
- An aversive conditioned stimulus (CS) failed to inhibit the sucrose intake of binge-like eating prone rats (BEPs), and they showed decreased fear response with access to palatable food.
- Analysis of *c-fos* mRNA expression in brain sections revealed a negative correlation between sucrose intake and the activity of nucleus accumbens (Acb). Persistent Acb response, hypoactivity of the medial prefrontal cortex and paraventricular nucleus of the hypothalamus, and hyperactivity of the lateral hypothalamus possibly contribute to the compulsive eating in the BEPs.
- This study provides some brain targets for further therapeutic research of the BED, and this fear conditioning test may serve as a good tool to test the effects of new treatments.

2.2 Résumé

Une alimentation compulsive est la caractéristique la plus obstinée du trouble de l'hyperphagie boulimique (THB). Dans cette étude, nous avons observé la consommation compulsive induite par le stress dans notre modèle de THB chez rat avec un test contradictoire, avec la présence de sucrose et de stimulus aversif conditionné (SAC) en même temps. Les rats sujets à la frénésie alimentaire (BEP, de l'anglais binge-eating prone) par rapport aux rats résistants à la frénésie alimentaire (BER, de l'anglais binge-eating resistant) ont montré une consommation élevée persistante de sucrose et ont inhibé la réponse à la peur en situation stressante, indiquant respectivement un déficit de dévaluation de l'appétence et une réponse anxiolytique plus forte au sucrose. Nous avons en outre analysé les activités cérébrales avec l'hybridation *in situ* de l'ARNm *c-fos*. Étonnamment, l'accès au sucrose sous la peur conditionnée n'a pas inhibé l'activité de l'amygdale, mais a plutôt activé l'amygdale centrale. Dans les rats BEP, le sucrose a réduit la réponse du noyau paraventriculaire de l'hypothalamus, tout en augmentant l'activité dans la zone hypothalamique latérale au SAC. Nous avons trouvé une forte corrélation négative entre la consommation de sucrose et l'activité du noyau accumbens et cette corrélation était inversée par le SAC dans les rats BER. La résistance à la dévaluation de l'appétence de la nourriture dans les rats BEP pourrait être le résultat d'une réponse persistante du noyau accumbens à l'apport en sucrose et au recrutement atténué du cortex préfrontal médian. Nous interprétons cette découverte comme étant que le système de récompense des rats BEP a vaincu le système homéostatique et le système répondant au stress.

Mots clés: trouble de l'hyperphagie boulimique, alimentation compulsive, dévaluation, *c-fos*, conditionnement de la peur, nucleus accumbens

2.3 Abstract

Compulsive eating is the most obstinate feature of the binge eating disorder (BED). In this study, we observed the compulsive eating in our stress-induced binge-like eating rat model with a conflicting test, with the presence of sucrose and aversively conditioned stimulus (CS) at the same time. The binge-like eating prone rats (BEPs) as compared to binge-like eating resistant rats (BERs) showed persistent high sucrose intake and inhibited fear response in the stressful situation, respectively indicating a deficit in palatability devaluation and stronger anxiolytic response to sucrose. We further analyzed the brain activities with *c-fos* mRNA *in situ* hybridization. Surprisingly, the sucrose access under conditioned fear did not inhibit the activity of amygdala, but further activated the central amygdala. In the BEPs, sucrose reduced the response of the paraventricular nucleus of the hypothalamus (PVN), while enhanced activities in the lateral hypothalamic area (LHA) to the CS. We found a strong negative correlation between sucrose intake and nucleus accumbens (Acb) activity, and it was reversed by the CS in the BERs. The resistance to devaluating the palatable food in the BEPs could be a result of persistent Acb response to the sucrose intake and attenuated recruitment of medial prefrontal cortex (mPFC). We interpret this finding as that the reward system of the BEPs overcame the homeostasis system and the stress-responding system.

Key words: binge eating disorder, compulsive eating, devaluation, *c-fos*, fear conditioning, nucleus accumbens

2.4 Introduction

The binge eating disorder (BED) is characterized by discrete episodes of overeating within a short period of time, usually less than 2 hours, even when not feeling physically hungry (Bogduk 2013). People with BED will eat until feeling physically uncomfortable. In humans, negative consequences associated with overeating include social impairment, emotional disturbances, psychiatric disorders, and life-threatening medical conditions associated with weight gain (Moore, Sabino et al. 2017).

Stress is one of main inducers of the BED in human patients. Evidence from both human and animal studies showed that stress could have bidirectional influence on feeding behaviors, either inhibiting or stimulating food intake (Maniam and Morris 2012). The direction of this changing effect of stress on feeding has been found to be dependent on the palatability of the food after stress (Pecoraro, Reyes et al. 2004). A variety of rodent models of BED has been developed using food deprivation and/or stress to induce the binge eating. Because stress and palatable food are highly involved in the development of the BED, our lab has developed a binge-like eating rat model using intermittent foot-shock stress followed by 1 h sucrose access (Calvez and Timofeeva 2016). In this model, the binge-eating prone rats (BEPs) consume more 10% sucrose solution than the binge-eating resistant rats (BERs) both in normal condition and after stress, and the BEPs further increase their sucrose intake after the stress stimulation.

Another hallmark of the BED is the compulsive eating, demonstrated as repetitively returning into engorging unhealthy food with full knowledge of the hazardous physical and psychological consequences. Compulsive eating has been observed in our BED rat model using a modified Light/Dark box test (Calvez and Timofeeva 2016), as well as in animal models in other studies using unconditioned stimulus such as foot shock (Oswald, Murdaugh et al. 2011). The BEPs consumed larger amount of sucrose than the BERs in the intensively illuminated light box. In response to stress, the BEPs demonstrated a hyporeactivity of the hypothalamic-pituitary-adrenal axis but a higher expression of CRH in the bed nucleus of the stria terminalis (BNST) compared with the BERs (Calvez, de Ávila et al. 2016).

In order to understand better the compulsive eating behavior, we adopted the fear conditioning paradigm in BED rat model. Aversively conditioned stimuli have an inhibitory effect on the feeding behavior, and this effect can be abolished by lesions of the central

nucleus but not the basolateral nucleus of the amygdala (Petrovich, Ross et al. 2009). Thus, the first objective of this study is to observe compulsive eating in our BED rat model by creating a conflicting situation with simultaneous presence of a 10% sucrose solution and an aversively conditioned stimulus. We hypothesize that the abnormally intense craving for palatable food in the BEPs will attenuate the inhibitory effect of the CS on feeding, and they will show less fear response because of the anxiolytic function of the comforting food, sucrose. The second objective of this study was to explore the neural mechanisms underlying the behavioral responses of the BERs and BEPs under conditioned fear, via analyzing the *c-fos* mRNA expression in different brain structures related to feeding, reward processing and stress response. The *c-fos* gene is one of immediate early genes widely used as a marker of early neuronal activation (Kovács 1998), because its expression is correlated with the functional activation of neurons. We hypothesize that different levels of *c-fos* mRNA expression in brain regions involved in stress response, feeding regulation and reward processing will be observed in the BEPs vs the BERs after fear conditioning test.

2.5 Materials and Methods

Ethical Approval. All experiments were carried out according to the guidelines of the Canadian Council on Animal Care in Science and Use of Laboratory Animals, and the experimental protocols were approved by the institutional Université Laval animal care committee.

2.5.1 Animals

Young (PD 45, 151-175 g) female Sprague Dawley rats (n = 170) were purchased from Charles Rivers. All rats were individually housed in transparent plastic cages, lined with wood shavings and crinkle paper. The rats were maintained on a 12h light/dark cycle (lights on from 2:00 to 14:00) and provided with ad libitum access to standard laboratory rat chow (Teklad Global 18% Protein Rodent Diet; 3.1 kcal/g, Harlan Teklad, Montreal, QC) and tap water, unless noted otherwise. All rats were acclimated to the housing conditions and handling procedures for at least one week prior to the experiments.

2.5.2 Classification of the BEPs and BERs

Rats were classified as the BERs or the BEPs according to the procedures previously described (Calvez and Timofeeva 2016). All rats were given a 24h access to a 10% sucrose

solution in their housing-cages, to decrease their neophobia to sucrose. Thereafter, we assessed the consumption of 10% sucrose solution during several intermittent 1 h sessions, with random inter trail intervals (ITIs) of 1 or 2 days, starting at the beginning of the dark phase in home cages. When the sucrose intake became stable for 3 consecutive No Stress sessions, three Stress sessions, with 1 h sucrose access sessions immediately after 4 rounds of mild foot shock (0.6 mA DC impulse, 3s duration, with inter-shock intervals of 15s), were conducted with intervals of 2 or 3 days. Between the second and third *Stress* sessions, we added a *No Stress* session to prevent the rats from associating the sucrose access with foot-shock. During all 1 h sucrose access sessions, the food pellets were removed from the home cage, and put back immediately after each session ended. Sucrose intake of all animals in each Stress session was divided into high, intermediate and low intake tertiles. If the sucrose consumption of a rat fell at least twice into the high tertiles, and never into the low tertiles, this rat would be classified as BEP. On the contrary, a rat would be classified as BER if the sucrose consumption during stress sessions fell at least twice into the low tertiles and never into the high tertiles. Finally, 42 rats were classified as the BEPs and 44 as the BERs, and the other rats were excluded from next experiments.

2.5.3 Fear-Conditioning test

The fear-conditioning test was composed of 3 parts: *Habituation* and *Appetitive* sessions, *Fear Conditioning* sessions, and the *Test* session (Figure 2-1A). All sessions lasted for 15 min, were video monitored (Logitech HD Webcam C270) from the top of the sound-attenuating cubicle (Med Associates inc.; ENV-022V, 55.9 cm ×38.1 cm × 35.6 cm) containing the behavioral test chamber (Med Associates inc.; ENV-007-VP, 30.5 cm x 24.1 cm × 29.2 cm). The test chamber had a grid floor and aluminum sides, except that the back, front and top sides were Plexiglas. The test chamber was illuminated with a light (4 W) placed 25cm above the floor. A speaker (Med Associates inc.; ENV-224AM) was installed on the left wall, 20 cm above the floor. Two photobeam lickometers (Med Associates inc.; ENV-251L) were installed on front and back parts of the right wall, 3cm above the floor, supplied with bottle of 10% sucrose solution and water respectively. A door in front of the sucrose lickometer was controlled by a custom designed program (Med-PC V, Med Associates inc.) to start and end the sucrose access.

2.5.3.1 *Habituation* and *Appetitive* sessions:

For the *Habituation* and *Appetitive* sessions, the test chamber was arranged as context A. In this context, a white plastic panel was installed on the grid floor and apple scented beads were placed in the sound attenuating box. The light was placed on the top right corner of the left wall. Furthermore, the test chamber was cleaned with Percept cleaner between each rat and the animals were transported from the housing room to the test room on a cart. In this context, the rats were habituated to drink 10% sucrose solution in the behavioral test chamber with ITIs of 1 or 2 days at random, until the sucrose intake became stable for 3 sessions. Thus, the last 3 sessions were considered as *Appetitive* sessions (Figure 2-1A).

2.5.3.2 *Fear Conditioning* sessions:

In *Fear Conditioning* sessions, the behavioral cage was modified into context B to be different from the context of *Appetitive* sessions. The plastic floor and apple-scented beads were removed. The light was moved to the top right corner of the right wall. A piece of curved white plastic board was placed to serve as the back and side walls of the test chamber and there was neither sucrose nor water access for the rats. The test chamber was cleaned with Citrosol cleaner between each rat and the BEPs and BERs were transported by the experimenter. In this stage, we divided rats of both phenotypes into 3 groups (*Control*, *Tone* and *Paired* groups; Figure 2-1B).

1) For the *Paired* groups, a mild foot-shock (1 s, 0.6 mA, DC impulses) was delivered through the grid floor during the last second of each of six tones (20 s, 2 kHz, 75 dB), separated by 125 s no-stimulus periods.
2) For the *Tone* groups, the same condition was performed, but no foot shock was delivered in these sessions.
3) For the *Control* groups, no tone or foot shock was applied during each 15min-*Fear Conditioning* session.

Between the second and third *Fear Conditioning* sessions, an *Appetitive* session was given to prevent the rats from losing their phenotype (Figure 2-1A).

2.5.3.3 *Test* session:

During the *Test* session, the context A was used as in the *Appetitive* session. We divided rats of each group in the *Fear Conditioning* sessions into two groups (No Sucrose and Sucrose; Figure 2-1B). *Sucrose* groups had access to 10% sucrose solution throughout the *Test* session, while the *No Sucrose* groups did not. For the *Tone* and *Paired* groups, 6 rounds of the same tones as in the *Fear Conditioning* sessions were presented, but without foot shock stimulus (Figure 2-1A).

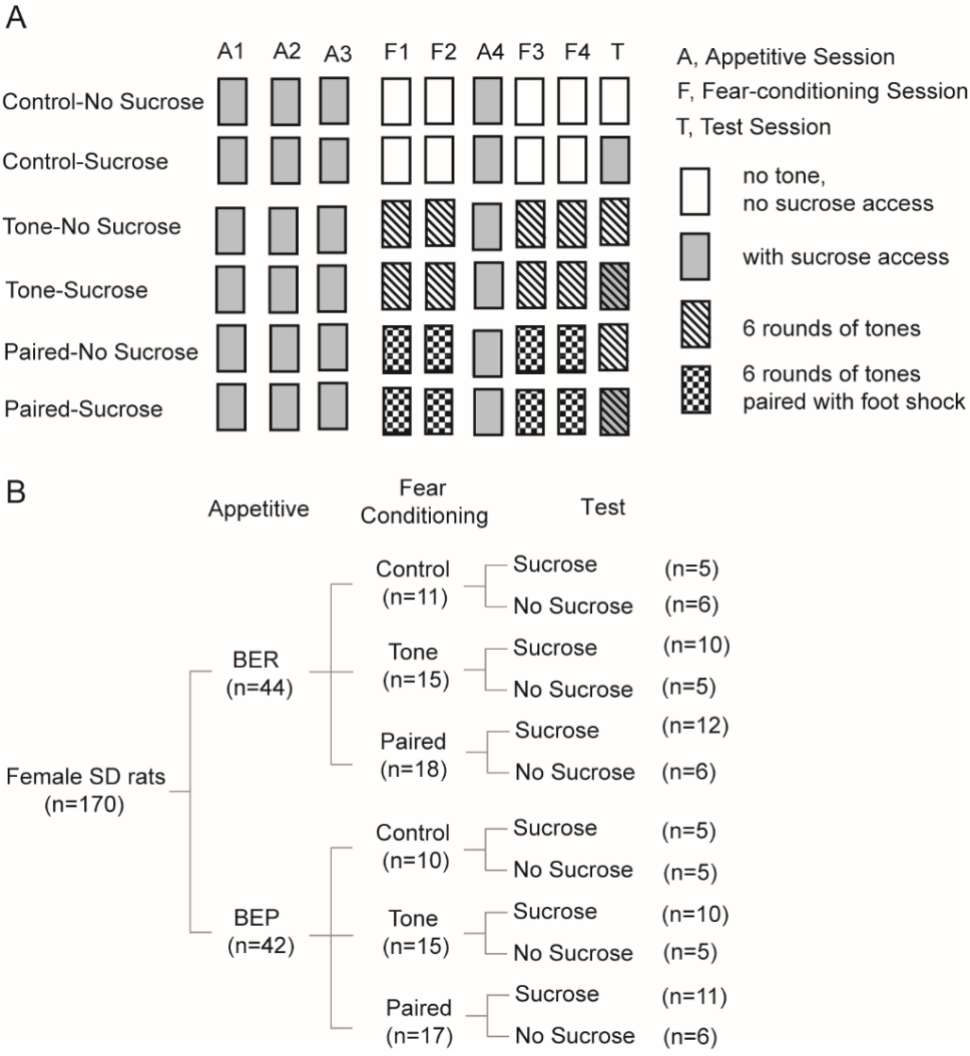

Figure 2- 1. Diagram of treatments and groups in the fear conditioning test.

A, diagram of fear-conditioning test procedures. B, number of animals in each group at each step during the fear-conditioning test

During *Appetitive* sessions and the *Test* session, sucrose intake was assessed by weighing the bottle before and after the experiment, and the licking events were detected by the lickometer

and recorded with a multichannel system (Tucker-Davis Technologies) and the Med Associates system (Med Asscociates inc.). Freezing behavior of the *Paired* groups was observed on video and quantified by the experimenter during the tones in *Fear Conditioning* sessions and the *Test* session.

2.5.4 Licking microstructure analyses

The licking events were analyzed in all 12 groups (Figure 2-1B). Because the licks data were very low or almost inexistent in *No Sucrose* groups, these results were not presented in the paper. The first lick latency was defined as the time in seconds between the beginning of the session and the first lick of sucrose solution. A licking cluster was defined as a burst of 3 or more licks with inter-cluster intervals of 500 ms or longer. The meal duration was calculated as the total duration in seconds of all clusters during the 15 min access to sucrose solution. The cluster size was defined as the average number of licks per cluster, and the cluster duration as the average duration of all clusters in seconds. The total number of licks, the meal duration, number of clusters, cluster size, cluster duration and first lick latency were computed for each session with a custom written MATLAB script (R2014a, The MathWorks, inc.).

2.5.5 Analysis of *c-fos* mRNA expression as a marker for brain activity

2.5.5.1 Brain preparation

Immediately after the *Test* session, each rat was returned to its home cage, with neither access to chow nor water for 30 min. Then, rats were anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (7.5 mg/kg) and intracardially perfused with ice-cold saline followed by 4% paraformaldehyde (PFA) in phosphate buffer. The brains were removed from the skulls and fixed in the 4% PFA for one week before being transferred to a PFA (4%) / sucrose (20%) solution. After freezing, brains were conserved in –80 ℃. Each brain was cut into 30 µm coronal sections using a microtome (Histoslide 2000, ReichertJung, Heidelberger, Germany). All sections from each brain were distributed into a 24 wells plate filled with a cold sterile cryoprotecting solution containing ethylene glycol (30%), glycerol (20%) and sodium phosphate buffer (50 mM, PH 7,2), and stored at –30 ℃.

2.5.5.2 *In situ* hybridization for *c-fos*

The protocol of *in situ* hybridization we used to localize the *c-fos* mRNA in this study was largely adapted from the method described by Simmons et al. (Simmons, Arriza et al. 1989).

The procedures have been described in detail (Poulin and Timofeeva 2008). Briefly, brain sections were mounted onto poly-L-lysine coated slides and conserved in 100% ethanol. After the slides dried up, they were successively fixed in 4% PFA for 20 min, digested with proteinase K (0.01 mg/ml) at 37 ℃ for 25 min, acetylated with acetic anhydride (0.25% in 0.1 M triethanolamine, pH 8.0) and dehydrated through ethanol gradient (50, 70, 95 and 100%). After the slides dried up, 90 µl of the hybridization solution containing an ^{35}S labeled antisense cRNA probe against *c-fos* mRNA (De Ávila, Chometton et al. 2018) was spread on each slide. All slides were then covered with coverslips and incubated overnight in a slide warmer at 60 ℃. After the coverslips were removed, the slides were rinsed four times with 4× saline sodium citrate buffer (SSC, 0.6 M NaCl, 60 mM trisodium citrate buffer, pH 7.0), digested with RNase-A (20 µg/ml in 10 mM Tris- 500 mM NaCl containing 1 mM EDTA) for 30 min at 37 ℃, rinsed in SSC with descending concentrations (2×, 1×, 0.5× and 0.1×) and finally dehydrated through ethanol gradient. Thereafter, the slides were defatted in toluene, dipped in nuclear emulsion (Kodak), and exposed for 7 days before being developed in the developer (Kodak) and fixed in rapid fixer (Kodak). Finally, slides were rinsed in running cold tap water for 1 h, stained with thionin, dehydrated through ethanol gradient, cleared in toluene and cover slipped with DPX.

2.5.5.3 *c-fos* mRNA expression analyses

The slides were analyzed under a light microscope (Olympus) equipped with a camera coupled to a computer with Stereo Investigator software (v1103). The luminosity of the system was set to the maximum. In order to avoid saturation, the exposure time for each region was adjusted with the section which had the strongest hybridization signal. Photos of the areas of interest were taken under dark-field illumination at a magnification of 4×. The *c-fos* mRNA expression was measured in the amygdala (2.16 mm to 3.48 mm caudal to the bregma), the paraventricular nucleus of the hypothalamus (PVN, 1.56 mm to 1.80 mm caudal to the bregma), the lateral hypothalamic area (LHA, 2.28 mm to 3.48 mm caudal to the bregma), the nucleus accumbens (Acb, 2.28 mm to 1.08 mm rostral to the bregma), the bed nucleus of the stria terminalis (BNST, 0.36 mm rostral to 0.36 mm caudal to the bregma) and the medial prefrontal cortex (mPFC, 3.72 mm to 2.52 mm rostral to the bregma). The area of interest was outlined on each photo by the experimenter with Stereo Investigator, and the hybridization signal was quantified by calculating the optical density (OD) in the contour

with a custom designed MATLAB script (R2014a, The MathWorks, Inc). The OD of each area of interest was corrected by subtracting the background signal, which was determined by 3 small contours on the unlabeled areas around the area of interest.

2.5.6 Statistical analyses

Results are presented as mean ± standard deviation (SD). The phenotype effects on first lick latency in *Appetitive* sessions and the *Test* session were analyzed using 1-way ANOVA. Two-way ANOVA with Bonferroni post hoc test was used for all other statistics analysis. A difference was considered significant when p-values < 0.05. Statistical analyses were performed using the Prism 6.04 (GraphPad Software inc., La Jolla, CA), and graphs were made with Prism 6.04, IBM SPSS Statistics 19 and arranged into figures with Adobe Illustrator® CS.

2.6 Results

2.6.1 Classification of the BERs and BEPs

The pattern of sucrose intake during the phenotyping in our study was similar to previously published results (Calvez and Timofeeva 2016). Two-way ANOVA assessed the effect of stress ($F_{1, 168} = 24.53$, $p<0.0001$), phenotype ($F_{1,168} = 168.4$, $p < 0.0001$) and their interaction ($F_{1,168} = 25.22$, $p < 0.0001$) on the 1 h sucrose intake in both non-stressful and after stress situations. The BEPs had significantly higher sucrose intake than the BERs both in non-stressful situation ($p < 0.0001$) and after foot-shock stress ($p < 0.0001$). Moreover, the BEPs consumed even more sucrose after stress ($p < 0.0001$), but the stress showed no significant impact on the sucrose intake of the BERs ($p = 0.9984$) (Figure SD 1. in the Annex A).

2.6.2 Sucrose intake behavior during the *Appetitive* and *Test* sessions

As the BEPs and BERs accommodated to the behavioral test chamber, the 15min sucrose intake of both phenotypes gradually reached a plateau. The last three sessions with stable sucrose consumption of the BEPs and BERs were defined as the *Appetitive* sessions (Figure 2-2A). In order to eliminate the influence of bodyweight on the sucrose intake, the quantity of sucrose intake of each rat was calculated as the energy of the consumed sucrose normalized by its bodyweight (kcal/kg bodyweight). Two-way ANOVA revealed a significant effect of phenotype on the sucrose intake in the *Appetitive* sessions. The BEPs took more sucrose than the BERs in all *Appetitive* sessions, which is consistent with their phenotypes in the home

cage during the binge eating classification (Figure 2-2A; Table 2-1). Analysis of the microstructure of licking behavior showed that the total number of licks was significantly higher in the BEPs than BERs for the three *Appetitive* sessions (Figure 2-2B; Table 2-1). The meal duration was also significantly higher in the BEPs than BERs in the first and second sessions, and close to the significance in the third (Figure 2-2C; Table 2-1). The number of clusters (Figure 2-2D; Table 2-1), the cluster size (Figure 2-2E; Table 2-1) and the cluster duration (Figure 2-2F; Table 2-1) were not significantly different between the BEPs and BERs. Finally, the BEPs showed significantly lower first lick latency in the average of the three *Appetitive* session than the BERs (two-tailed unpaired t test, p = 0.0027,) (Figure SD 2 in the Annex A).

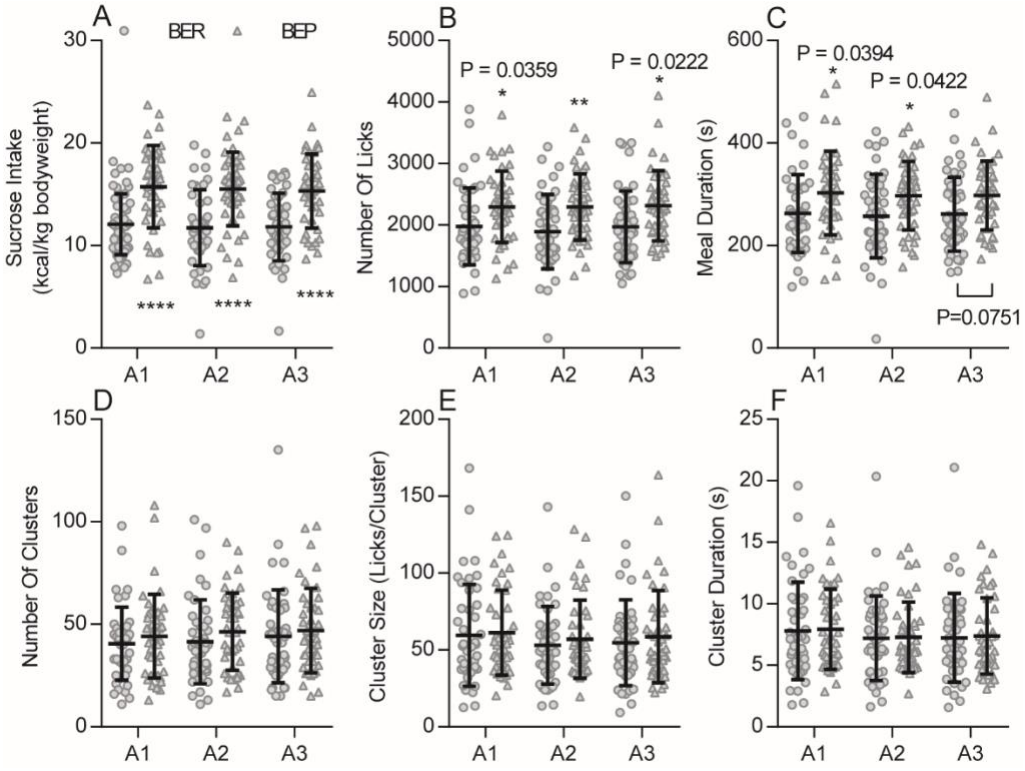

Figure 2- 2. Habitual over-eating of the BEPs during *Appetitive* sessions.

A, sucrose intake normalized to bodyweight. Licking microstructures during the *Appetitive* sessions: total number of licks (B), meal duration (C), number of clusters (D), cluster size (D), cluster duration (F). *, significantly different ($p<0.05$) between the BEPs and BERs in the same session. **, $p<0.01$; ****, $p<0.0001$.

Table 2- 1. Effects of phenotype, *Appetitive* session and their interaction on the feeding behavior in *Appetitive* sessions

	Phenotype		Session		Interaction	
	F	p	F	p	F	p
Sucrose intake	$F_{1,252}$=68,22	<0,0001****	$F_{2,252}$=0,2351	0.7907	$F_{2,252}$=0,03095	0.9695
Number of licks	$F_{1,252}$=23,54	<0,0001****	$F_{2,252}$=0,1934	0.8243	$F_{2,252}$=0,1103	0.8956
Meal duration	$F_{1,252}$=17,4	<0,0001****	$F_{2,252}$=0,1096	0.8936	$F_{2,252}$=0,01791	0.9823
Number of clusters	$F_{1,252}$=2,256	0.1343	$F_{2,252}$=0,5386	0.5842	$F_{2,252}$=0,05674	0.94448
Cluster size	$F_{1,252}$=0,8529	0.3566	$F_{2,252}$=0,811	0.4456	$F_{2,252}$=0,04598	0.9551
Cluster duration	$F_{1,252}$=0,07082	0.7904	$F_{2,252}$=0,8696	0.4204	$F_{2,252}$=0,0031	0.9969

Table 2- 2. Effects of phenotype, fear conditioning and their interaction on the feeding behavior both during tones and between tones in the *Test* session

	During Tones						Between Tones					
	Phenotype		Fear conditioning		Interaction		Phenotype		Fear conditioning		Interaction	
	F	p	F	p	F	p	F	p	F	p	F	p
Number of licks	$F_{1,39}$=0.0332	0.8563	$F_{1,39}$=39.93	<0.0001****	$F_{1,39}$=2.138	0.1517	$F_{1,39}$=0.181	0.6728	$F_{1,39}$=5.295	0.0268*	$F_{1,39}$=4.11	0.0495*
Meal duration	$F_{1,31}$=0.6958	0.4106	$F_{1,31}$=20.28	<0.0001****	$F_{1,31}$=0.6566	0.4240	$F_{1,39}$=0.0351	0.8523	$F_{1,39}$=6.531	0.0146*	$F_{1,39}$=4.999	0.0312*
Number of clusters	$F_{1,39}$=0.0378	0.8467	$F_{1,39}$=10.09	0.0029**	$F_{1,39}$=0.6552	0.4332	$F_{1,39}$=0.0769	0.7830	$F_{1,39}$=2.771	0.1040	$F_{1,39}$=0.058	0.8108
Cluster size	$F_{1,32}$=1.968	0.1702	$F_{1,32}$=9.412	0.0044**	$F_{1,32}$=0.6785	0.4162	$F_{1,39}$=0.1984	0.6585	$F_{1,39}$=0.978	0.3287	$F_{1,39}$=9.773	0.0033**
Cluster Duration	$F_{1,32}$=2.374	0.1332	$F_{1,32}$=11.49	0.019*	$F_{1,32}$=1.074	0.3077	$F_{1,39}$=0.309	0.5815	$F_{1,39}$=1.136	0.2931	$F_{1,39}$=9.873	0.0032**

In the *Test* session, because of the scarcity of licks in the *No-Sucrose* groups, we only considered the sucrose intake of the groups with sucrose access. In order to diminish the individual differences, we normalized the sucrose intake of each rat to its average sucrose intake in the *Appetitive* sessions. Two-way ANOVA revealed a significant effect of stress ($F_{2,47} = 14.18$, $p < 0.0001$) on the sucrose intake in the *Test* session, and a close to significant effect of its interaction with phenotype ($F_{2,47} = 3.117$, $p = 0.0536$) but not of the phenotype itself ($F_{1,47} = 0.4809$, $p = 0.4914$). The result demonstrated that the unconditioned tones failed to change the sucrose intake of either the BEPs or the BERs (Figure 2-3A). When the tones were previously associated with foot-shocks, they prominently decreased the sucrose intake of BER-*Paired* group compared with the BER-*Control* group and the BER-*Tone* group (Figure 2-3A). The aversive CS slightly decreased the sucrose intake of the BEPs, but without reaching a significant level (Figure 2-3A). We also analyzed the first lick latency in the *Test* session, showing that the conditioned fear significantly increased the first lick latency of BER-Paired rats (n=18) compared with the BEP-*Paired* rats (n=17) (two-tailed unpaired t test, p=0.0465) (Figure SD2 in the Annex A).

Thereafter, we analyzed the licking microstructures during tones and between tones separately, in *Tone* and *Paired* groups. During tones, 2-way ANOVA revealed a significant effect of the CS on all licking microstructures, namely number of licks , meal duration ($F_{1,31}$= 20.28, $p<0.0001$), number of clusters ($F_{1,39}$=0.03787, p=0.0029), cluster size and cluster duration, without effect of phenotype or their interaction. Between tones, the CS only showed a significant effect on the number of licks and meal duration, while the interaction between CS and phenotype showed a significant effect on the number of licks, meal duration, cluster size and cluster duration. For the BERs, the conditioned fear significantly decreased the number of licks (Figure 2-3B and 3C), the meal duration (Figure 2-3D and 3E), the cluster size (Figure 2-3H and 3I) and the cluster duration (Figure 2-3J and 3K) both during tones and between tones, and the number of clusters only during tones (Figure 2-3F). In the BEPs, the conditioned fear only significantly decreased the number of licks (Figure 2-3B) and the meal duration (Figure 2-3D) during the tones, the other analyzed licking microstructures were not significantly changed by the conditioned fear (the statistical analyses are presented in Table 2- 2).

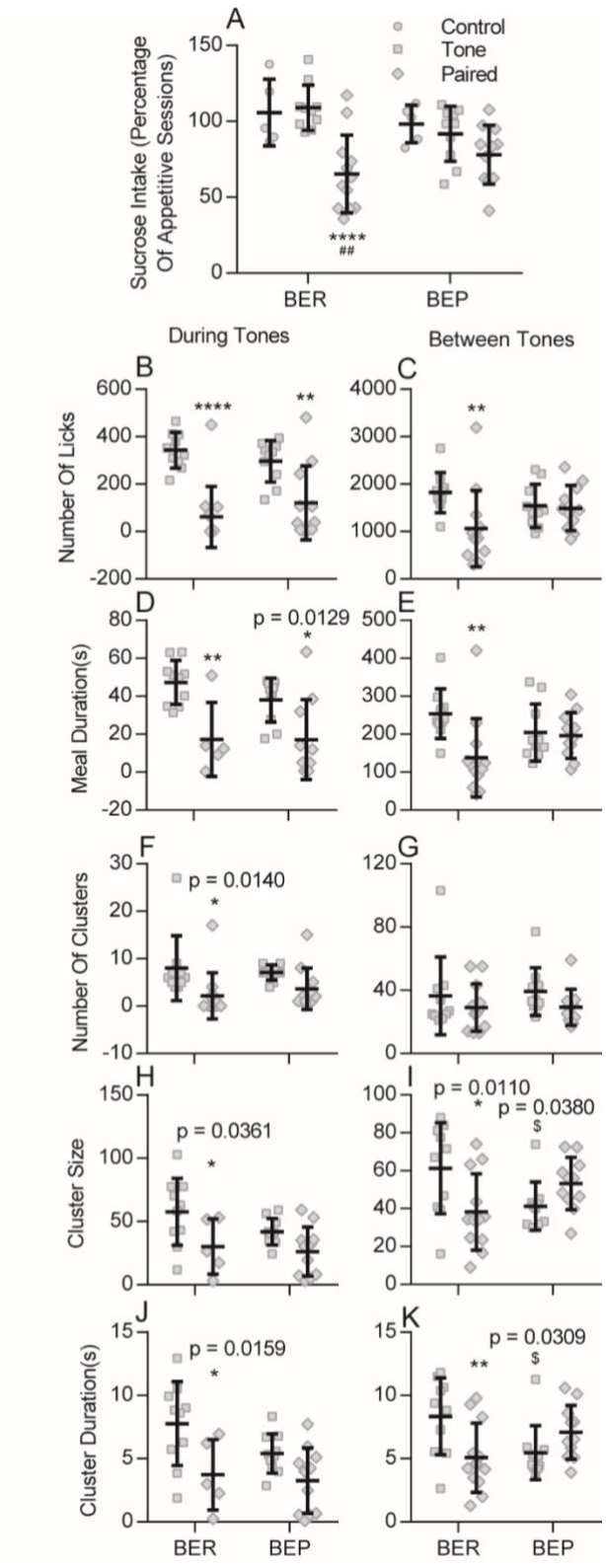

Figure 2- 3. Compulsive eating of the BEPs in the *Test* session.

A, sucrose intake during the *Test* session normalized to the average intake of each animal during all *Appetitive* sessions. ****, significantly different ($p<0.0001$) from the BER-*Control* group. ##, significantly different ($p<0.01$) from the BER-*Tone* group. Licking microstructures during the *Test* session: number of licks (B, C), meal duration (D, E), number of clusters (F, G), cluster size (H, I) and cluster duration (J, K). *, significantly different ($p<0.05$) from the *Tone* group with the same phenotype. **, $p<0.01$; ****, $p<0.0001$. $, significantly different ($p<0.05$) from the BER-*Tone* group.

2.6.3 Freezing behavior during *Fear Conditioning* sessions and the *Test* session

The fear response was calculated for each rat as the average freezing time during six tones in each session. In the *Fear Conditioning* sessions, the BERs and BEPs of the *Paired* groups had similar acquisition efficiency and showed no phenotype difference throughout all 4 sessions (Figure 2-4A). The freezing time significantly increased in the second *Fear Conditioning* session compared with the first and became relatively stable thereafter. Two-way ANOVA revealed significant effect of the session number ($F_{3,99} = 23.75$, $p < 0.0001$) on the freezing behavior, but not the phenotype ($F_{1,33} = 0.3646$, $p = 0.5501$) or their interaction ($F_{3,99} = 0.6594$, $p = 0.5789$).

Again, to diminish individual differences, we normalized the freezing time in the *Test* session to the average freezing time of the last three *Fear Conditioning* sessions (Figure 2-4B). With the absence of foot-shock during the *Test* session, the BER- and BEP-Paired rats slightly reduced their freezing behavior in response to the CS when they had no access to sucrose, and no significant difference between phenotypes was observed. The presence of sucrose decreased the freezing behavior in both BER- and BEP-Paired rats compared with the *No Sucrose* groups, but only the diminution in BEP-Paired rats was statistically significant. Moreover, the freezing time of BEP-Paired rats was significantly different compared with BER-Paired rats when they had access to sucrose solution. Two-way ANOVA revealed significant effect of the sucrose ($F_{1,31} = 15.17$, $p = 0.0005$) on the freezing behavior, but not the phenotype ($F_{1,31} = 2.928$, $p = 0.0970$) or their interaction ($F_{1,31} = 1.429$, $p = 0.2410$).

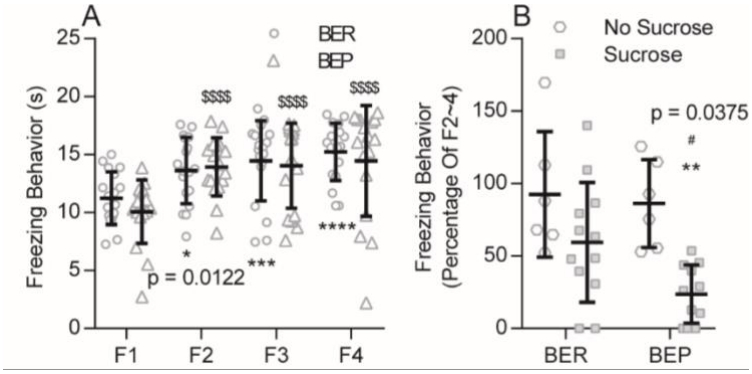

Figure 2- 4. Conditioned fear response of the BERs and BEPs during *Fear Conditioning* sessions and the *Test* session

A, freezing behavior during the Training sessions. *, significantly different ($p<0.0.5$) from the F1 session within BER group. $$$$, significantly different ($p<0.0001$) from the F1 session within BEP group. ***, $p<0.001$; ****, $p<0.0001$. B, freezing behavior during the Training sessions normalized to the average freezing behavior of each animal during F2-4. F1-4, *Fear Conditioning* session 1-4. **, significantly ($p < 0.01$) different from the BEP-*No Sucrose* group. #, significantly ($p < 0.05$) different from the BER- *Sucrose* group.

2.6.4 The *c-fos* mRNA expression

The *c-fos* mRNA expression was detected by *in situ* hybridization and quantified as the optical density (OD). For each region, to analyze the relationship between the *c-fos* mRNA expression and a behavior (sucrose intake or freezing response), we have made the 2-D scatter plots of *c-fos* mRNA expression/sucrose intake and *c-fos* mRNA expression/freezing time. To simplify presentation, we decided to reduce the number of groups. We combined the *Control* and *Tone* groups into *a Non-Paired* group for both phenotypes.

2.6.4.1 Activation of the amygdala by the CS was not inhibited by sucrose intake

Two sub-regions were analyzed in the amygdala: the central nucleus (Ce) and the basolateral nucleus (BLA). In both sub-regions and both phenotypes, the *c-fos* mRNA expression was more prominent in *Paired* groups compared with *Non-Paired* groups, as shown in the representative photos (Figure 2-5A). Linear regression analysis revealed strong negative correlations between sucrose intake and *c-fos* expression in the BLA (Figure 2-5B) and the Ce (Figure 2-5F) of BERs (n = 17; BLA: $r_2 = 0.4573$, p = 0.0029; Ce: $r_2 = 0.4286$, p = 0.0043), but not the BEPs (n = 16; BLA: $r_2 = 0.09616$, p = 0.2424; Ce: $r_2 = 0.08638$, p = 0.2692). No strong correlation was found between freezing behavior and the *c-fos* expression in the BLA (Fig 5C) and Ce (Figure 2-5G). Statistical analysis revealed that the *Paired* group had higher *c-fos* mRNA expression than the *Control* and *Tone* groups in both BLA (Figure 2-5D) and Ce (Figure 2-5H) of both phenotypes no matter with or without sucrose access. Sucrose intake had tendency to increase *c-fos* mRNA expression in the Ce in both BEPs (p = 0.0597) and BERs (p = 0.0604) under conditioned fear (Figure 2-5I), but this effect was not close to be significant in the BLA (Figure 2-5E). Two-way ANOVA analysis results are presented in Table 2-3A-C. Therefore, amygdala only responded to the CS, not influenced by the phenotype or sucrose intake.

2.6.4.2 The LHA was activated by the CS

Two sub-regions were analyzed in the LHA: the perifornical part (PeFLH) and the posterior lateral part (PLH). In both sub-regions, the *c-fos* mRNA expression was higher in the *Paired* groups than *Non-Paired* groups (Figure 2-6A) no matter with or without sucrose access. Linear regression analysis revealed strong negative correlations between sucrose intake and *c-fos* expression in the PeLH (Figure 2-6B) and the PLH (Figure 2-6F) of the BERs (n = 17; PeLH: $r_2 = 0.3375$, p = 0.0145; PLH: $r_2 = 0.2771$, p = 0.0299), but not the BEPs (n = 16; PeLH: $r_2 = 0.01843$, p = 0.6161; PLH: $r_2 = 0.04029$, p = 0.4560). The freezing behavior and *c-fos* expression showed strong negative correlation in the PeLH (Figure 2-6C) of the BEPs (n = 16; $r_2 = 0.2935$, p = 0.0689), and in the PLH (Figure 2-6G) of the BEPs (n = 16; $r_2 = 0.4713$, p = 0.0137) and BERs (n = 17; $r_2 = 0.3327$, p = 0.0390). The *c-fos* mRNA levels in the PLH (Figure 2-6D) and the PLH (Figure 2-6H) demonstrated a significant increase in the *Paired* groups compared with *Non-Paired* groups in both phenotypes, no matter with or without sucrose access in the *Test* session. Sucrose intake increased *c-fos* mRNA expression

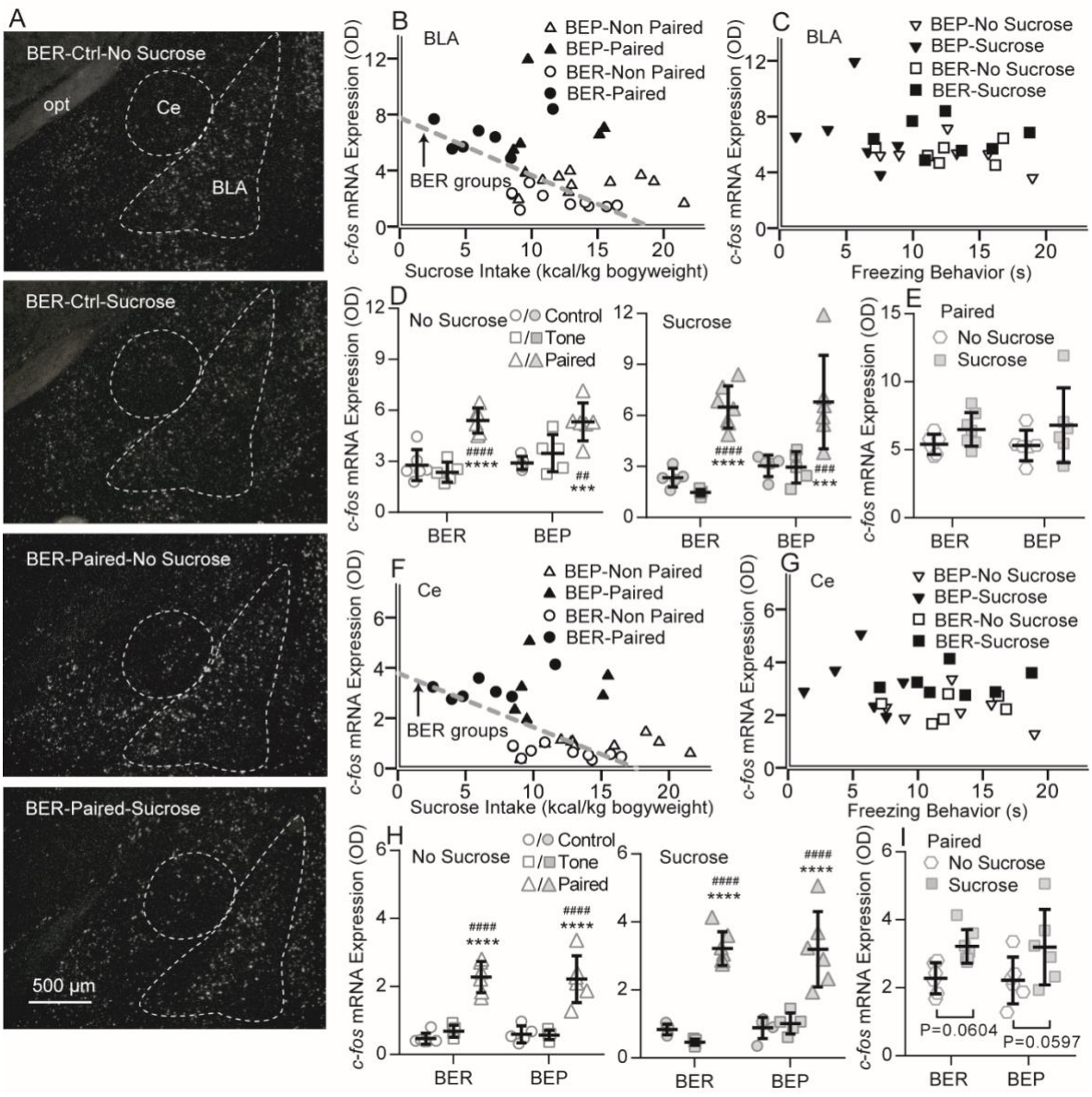

Figure 2- 5. Activation of the amygdala by the CS was unaffected by sucrose intake

A, representative images of the *c-fos* mRNA *in situ* hybridization signals in the Ce and BLA marked with broken contours. 2-D scatter plots of relative *c-fos* mRNA expression/sucrose intake (B, F), and relative *c-fos* mRNA expression/freezing behavior (C, G). Relative *c-fos* mRNA expression of No *Sucrose* and *Sucrose* groups (D, H) and *Paired* groups (E, I). *, significantly different ($p<0.05$) from the *Control* group with the same phenotype. ***, $p<0.001$; ****, $p<0.0001$. #, significantly different ($p<0.05$) from the *Tone* group with the same phenotype. ##, $p<0.01$; ###, $p<0.001$; ####, $p<0.0001$. BLA, basolateral amygdala. Ce, central amygdala. opt, optic tract.

in the PLH of the BEPs ($p = 0.0459$) but not in the BERs under conditioned fear (Figure 2-6I), while this effect was not observed in the PeLH (Figure 2-6E). Two-way ANOVA analysis results are presented in Table 2-3A-C. Thus, we conclude that the CS also had strong

impact on the activity of the LHA, and under the conditioned fear, the PLH responded differently to the sucrose in the BEPs vs BERs.

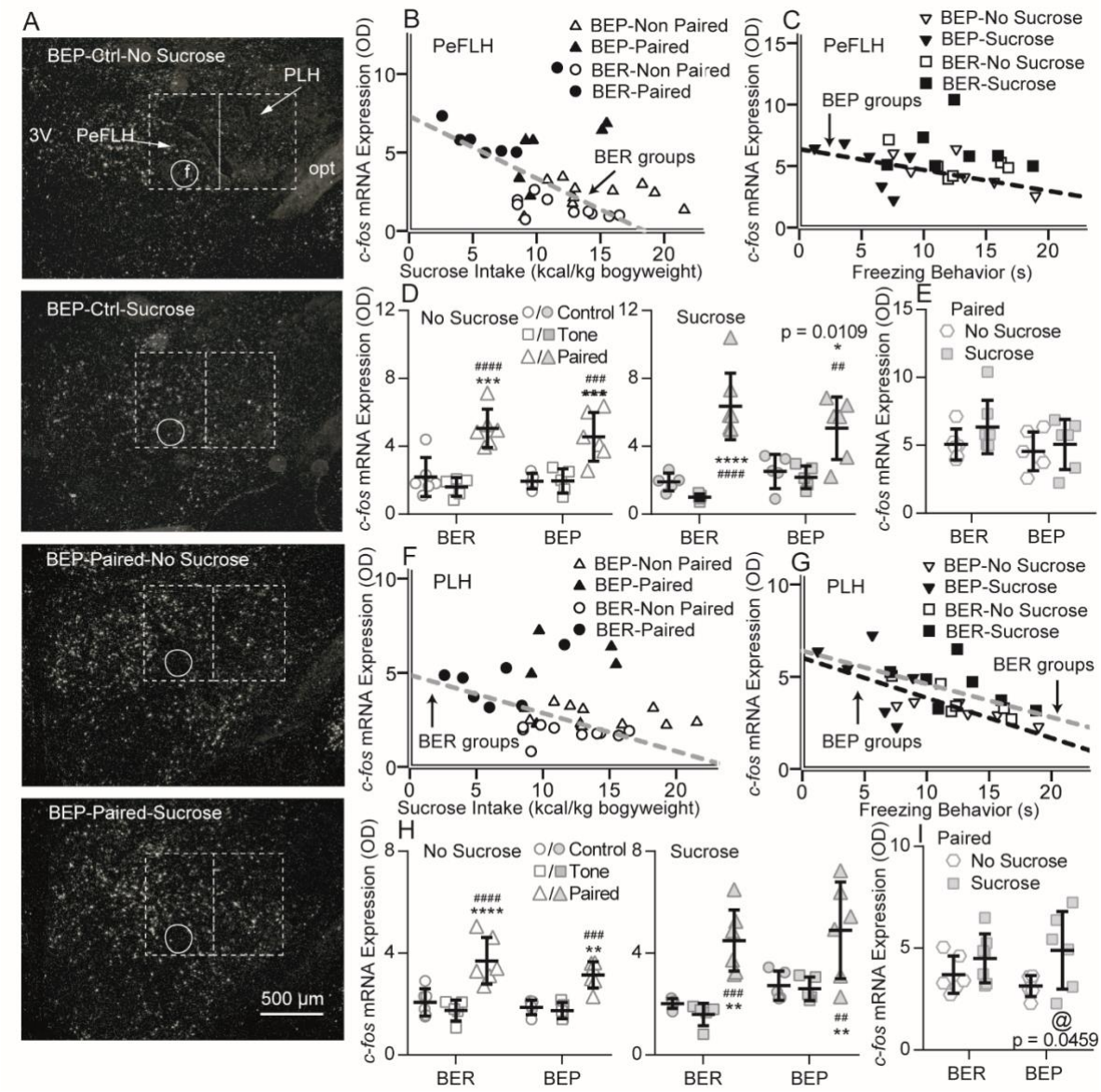

Figure 2- 6. Activation of the LHA by the CS

A, representative images of the *c-fos* mRNA *in situ* hybridization signals with the PeFLH and PLH marked with broken contours. 2-D scatter plots of relative *c-fos* mRNA expression/sucrose intake (B, F), and relative *c-fos* mRNA expression/freezing behavior (C, G). Relative *c-fos* mRNA expression of *No Sucrose* and *Sucrose* groups (D, H) and *Paired* groups (E, I). *, significantly different ($p<0.05$) from the *Control* group with the same phenotype. ***, $p<0.001$; ****, $p<0.0001$. #, significantly different ($p<0.05$) from the *Tone* group with the same phenotype. ##, $p<0.01$; ###, $p<0.001$; ####, $p<0.0001$. @, significantly different ($p<0.05$) from the *No Sucrose* group with the same phenotype. 3V, 3rd ventricle. f, fornix. PeFLH, perifornical part of lateral hypothalamus. PLH, peduncular part of lateral hypothalamus. opt, optic tract.

Table 2-3 A Effects of sucrose, fear conditioning and their Interaction on the relative *c-fos* mRNA expression in related brain regions of all groups

	BER						BEP					
	Sucrose		Fear conditioning		Interaction		Sucrose		Fear conditioning		Interaction	
	F	p	F	p	F	p	F	p	F	p	F	p
BLA	$F_{1,28}$=0.08091	0.7782	$F_{2,28}$=82.54	<0.0001****	$F_{2,28}$=4.751	0,0167*	$F_{1,26}$=0.4863	0.4918	$F_{2,26}$=15.73	<0.0001****	$F_{2,26}$=1.37	0.2719
Ce	$F_{1,28}$=10.38	0,0032**	$F_{2,28}$=173.8	<0.0001****	$F_{2,28}$=9.148	0,0009***	$F_{1,26}$=6.968	0,0138*	$F_{2,26}$=38.29	<0.0001****	$F_{2,26}$=0.9859	0.3866
PeFLH	$F_{1,28}$=0.1015	0.7524	$F_{2,28}$=47.7	<0.0001****	$F_{2,28}$=2.222	0.1272	$F_{1,26}$=1.081	0.308	$F_{2,26}$=19.26	<0.0001****	$F_{2,26}$=0.06141	0.9406
PLH	$F_{1,28}$=0.5725	0.4556	$F_{2,28}$=35.63	<0.0001****	$F_{2,28}$=1.432	0.2558	$F_{1,26}$=12.35	0,0016**	$F_{2,26}$=14.01	<0.0001****	$F_{2,26}$=0.8884	0.4235
PaP	$F_{1,28}$=0.05503	0.8162	$F_{2,28}$=35.08	<0.0001****	$F_{2,28}$=0.9515	0.3983	$F_{1,26}$=1.255	0.2728	$F_{2,26}$=44.83	<0.0001****	$F_{2,26}$=3.068	0.0637
PaM	$F_{1,28}$=0.09133	0.7647	$F_{2,28}$=14.06	<0.0001****	$F_{2,28}$=0.78	0.4681	$F_{1,26}$=0.576	0.4547	$F_{2,26}$=20.69	<0.0001****	$F_{2,26}$=0.09913	0.906
AcbC	$F_{1,28}$=0.04829	0.8278	$F_{2,28}$=3.58	0,0423*	$F_{2,28}$=0.2833	0.7556	$F_{1,26}$=27.46	<0.0001	$F_{2,26}$=10.11	0,0006***	$F_{2,26}$=2.173	0.1341
AcbSh	$F_{1,28}$=2.616	0.1179	$F_{2,28}$=14.93	<0.0001****	$F_{2,28}$=0.0182	0.982	$F_{1,26}$=15.74	0,0005***	$F_{2,26}$=2.432	0.1076	$F_{2,26}$=2.357	0.1146
STD	$F_{1,28}$=3.426	0.0748	$F_{2,28}$=1.57	0.2259	$F_{2,28}$=1.479	0.245	$F_{1,26}$=0.8579	0.3628	$F_{2,26}$=8.07	0,0019**	$F_{2,26}$=0.2179	0.8056
STV	$F_{1,28}$=6.625	0,0156*	$F_{2,28}$=3.303	0.0515	$F_{2,28}$=0.2563	0.7757	$F_{1,26}$=1.011	0.324	$F_{2,26}$=7.93	0,002**	$F_{2,26}$=0.8095	0.456
PrL	$F_{1,28}$=1.233	0.2771	$F_{2,28}$=8.491	0,0015**	$F_{2,28}$=0.7381	0.4878	$F_{1,26}$=5.25	0.303	$F_{2,26}$=3.279	0.0537	$F_{2,26}$=1.069	0.3579
IL	$F_{1,28}$=1.429	0.2427	$F_{2,28}$=8.472	0,0015**	$F_{2,28}$=0.7524	0.4812	$F_{1,26}$=1.1	0.304	$F_{2,26}$=3.959	0,0315*	$F_{2,26}$=0.05213	0.9493

Table 2-3 B Effects of phenotype, fear conditioning and their Interaction on the relative *c-fos* mRNA expression in related brain regions of all groups

	No-Sucrose						Sucrose					
	Phenotype		Fear conditioning		Interaction		Phenotype		Fear conditioning		Interaction	
	F	p	F	p	F	p	F	p	F	p	F	p
BLA	$F_{1,27}$=1.679	0.2061	$F_{2,27}$=31.87	<0.0001****	$F_{2,27}$=1.456	0.2509	$F_{1,27}$=2.804	0.1055	$F_{2,27}$=35.06	<0.0001****	$F_{2,27}$=0.503	0.6103
Ce	$F_{1,27}$=0.0142	0.906	$F_{2,27}$=72.29	<0.0001****	$F_{2,27}$=0.2936	0.7479	$F_{1,27}$=0.9482	0.3388	$F_{2,27}$=72.08	<0.0001****	$F_{2,27}$=0.8992	0.4472
PeFLH	$F_{1,27}$=0.148	0.7034	$F_{2,27}$=31.32	<0.0001****	$F_{2,27}$=0.494	0.6156	$F_{1,27}$=0.133	0.7182	$F_{2,27}$=33.13	<0.0001****	$F_{2,27}$=2.787	0.0794
PLH	$F_{1,27}$=1.672	0.2069	$F_{2,27}$=29.97	<0.0001****	$F_{2,27}$=0.6954	0.5076	$F_{1,27}$=3.646	0.0669	$F_{2,27}$=21.80	<0.0001****	$F_{2,27}$=0.2377	0.7901
PaP	$F_{1,27}$=0.2195	0.6432	$F_{2,27}$=44.78	<0.0001****	$F_{2,27}$=0.1267	0.2979	$F_{1,27}$=2.596	0.1188	$F_{2,27}$=32.43	<0.0001****	$F_{2,27}$=2.242	0.1256
PaM	$F_{1,27}$=1.798	0.1912	$F_{2,27}$=28.71	<0.0001****	$F_{2,27}$=1.066	0.3583	$F_{1,27}$=0.169	0.6842	$F_{2,27}$=13.70	<0.0001****	$F_{2,27}$=0.003	0.9967
AcbC	$F_{1,27}$=7.904	0,0091**	$F_{2,27}$=7.13	0,0033**	$F_{2,27}$=5.615	0,0091**	$F_{1,25}$=7.752	0,0101*	$F_{2,25}$=0.9572	0.3976	$F_{2,25}$=2.218	0.1298
AcbSh	$F_{1,27}$=8.05	0,0085**	$F_{2,27}$=6.482	0,005**	$F_{2,27}$=5.734	0,0084**	$F_{1,25}$=9.071	0,0059**	$F_{2,25}$=2.583	0.0955	$F_{2,25}$=2.480	0.1041
STD	$F_{1,27}$=0.6335	0.433	$F_{2,27}$=3.94	0,0315*	$F_{2,27}$=1.264	0.2986	$F_{1,27}$=0.012	0.9132	$F_{2,27}$=5.288	0,0115*	$F_{2,27}$=1.196	0.3181
STV	$F_{1,27}$=5.328	0,0289*	$F_{2,27}$=5.787	0,0081**	$F_{2,27}$=2.133	0.138	$F_{1,27}$=1.018	0.3219	$F_{2,27}$=2.95	0.0694	$F_{2,27}$=3.735	0.0370*
PrL	$F_{1,27}$=2.953	0.0971	$F_{2,27}$=7.617	0,0024**	$F_{2,27}$=0.6937	0.5084	$F_{1,27}$=1.029	0.3201	$F_{2,27}$=4.413	0,0228*	$F_{2,27}$=1.158	0.3303
IL	$F_{1,27}$=1.743	0.1979	$F_{2,27}$=6.308	0,0057**	$F_{2,27}$=1.733	0.1958	$F_{1,27}$=3.966	0.0575	$F_{2,27}$=5.877	0,0081**	$F_{2,27}$=0.3004	0.7431

Table 2-3 C Effects of phenotype, sucrose and their Interaction on the relative *c-fos* mRNA expression in related brain regions of *Paired* groups

	Phenotype		Sucrose		Interaction	
	F	p	F	p	F	p
BLA	$F_{1,21}$=0.0284	0.8678	$F_{1,21}$=3.82	0.0641	$F_{1,21}$=0.0815	0.7781
Ce	$F_{1,21}$=0.02164	0.8844	$F_{1,21}$=10.83	0,0035**	$F_{1,21}$=0.004245	0.9487
PeFLH	$F_{1,21}$=1.84	0.1893	$F_{1,21}$=1.854	0.1877	$F_{1,21}$=0.3522	0.5592
PLH	$F_{1,21}$=0.02495	0.876	$F_{1,21}$=6.608	0,0178*	$F_{1,21}$=0.9271	0.3466
PVNp	$F_{1,21}$=1.543	0.2278	$F_{1,21}$=0.4463	0.5114	$F_{1,21}$=4.276	0.0512
PVNm	$F_{1,21}$=0.0365	0.8503	$F_{1,21}$=0.7566	0.3942	$F_{1,21}$=0.004	0.9515
AcbC	$F_{1,21}$=12.83	0,0018**	$F_{1,21}$=1.733	0.2022	$F_{1,21}$=2.756	0.1118
AcbSh	$F_{1,21}$=11.46	0,0028**	$F_{1,21}$=0.1425	0.7096	$F_{1,21}$=2.585	0.1228
STD	$F_{1,21}$=0.4926	0.4905	$F_{1,21}$=3.39E-5	0.9954	$F_{1,21}$=0.0026	0.9601
STV	$F_{1,21}$=1.686	0.2082	$F_{1,21}$=0.3984	0.5437	$F_{1,21}$=0.8873	0.3569
PrL	$F_{1,21}$=0.00111	0.9737	$F_{1,21}$=9.559	0,0055**	$F_{1,21}$=0.5893	0.4513
IL	$F_{1,21}$=0.05393	0.8186	$F_{1,21}$=4.318	0.0502	$F_{1,21}$=0.6476	0.43
VTA	$F_{1,21}$=6.23	0,021*	$F_{1,21}$=0.8702	0.3615	$F_{1,21}$=0.5175	0.4798

2.6.4.3 The activation of the PVN by the CS was inhibited by sucrose intake in the BEPs

Two sub-regions were analyzed in the PVN: the parvocellular part (PVNp) and the magnocellular part (PVNm). In both sub-regions and both phenotypes, the *c-fos* mRNA expression was obviously higher in *Paired* groups compared with *Non-Paired* groups, as shown in the representative photos (Figure 2-7A). Linear regression analysis revealed negative correlations between sucrose intake and *c-fos* expression in the PVNp (Figure 2-7B. n= 33; r_2 = 0.1727, p = 0.0162) and the PVNm (Figure 2-7F. n= 33; r_2 = 0.1312, p = 0.0383) when we put together all the BEPs and BERs of *Sucrose* groups. The freezing behavior and *c-fos* expression showed positive correlation in the PVNp (Figure 2-7C) of the BEPs (n = 16; r_2 = 0.4739, p = 0.0133). The *c-fos* mRNA expression in the PVNp (Figure 2-7D) and the PVNm (Figure 2-7H) showed a significant increase in the *Paired* groups in both phenotypes, no matter with or without sucrose access. With access to sucrose, the BEP-*Paired* group had lower *c-fos* mRNA expression than the BER-*Paired* group in the PVNp (Figure 2-7D), while sucrose did not significantly change the *c-fos* expression in the PVNm (Figure 2-7I). Two-way ANOVA analysis results are presented in Table 2-3A-C.

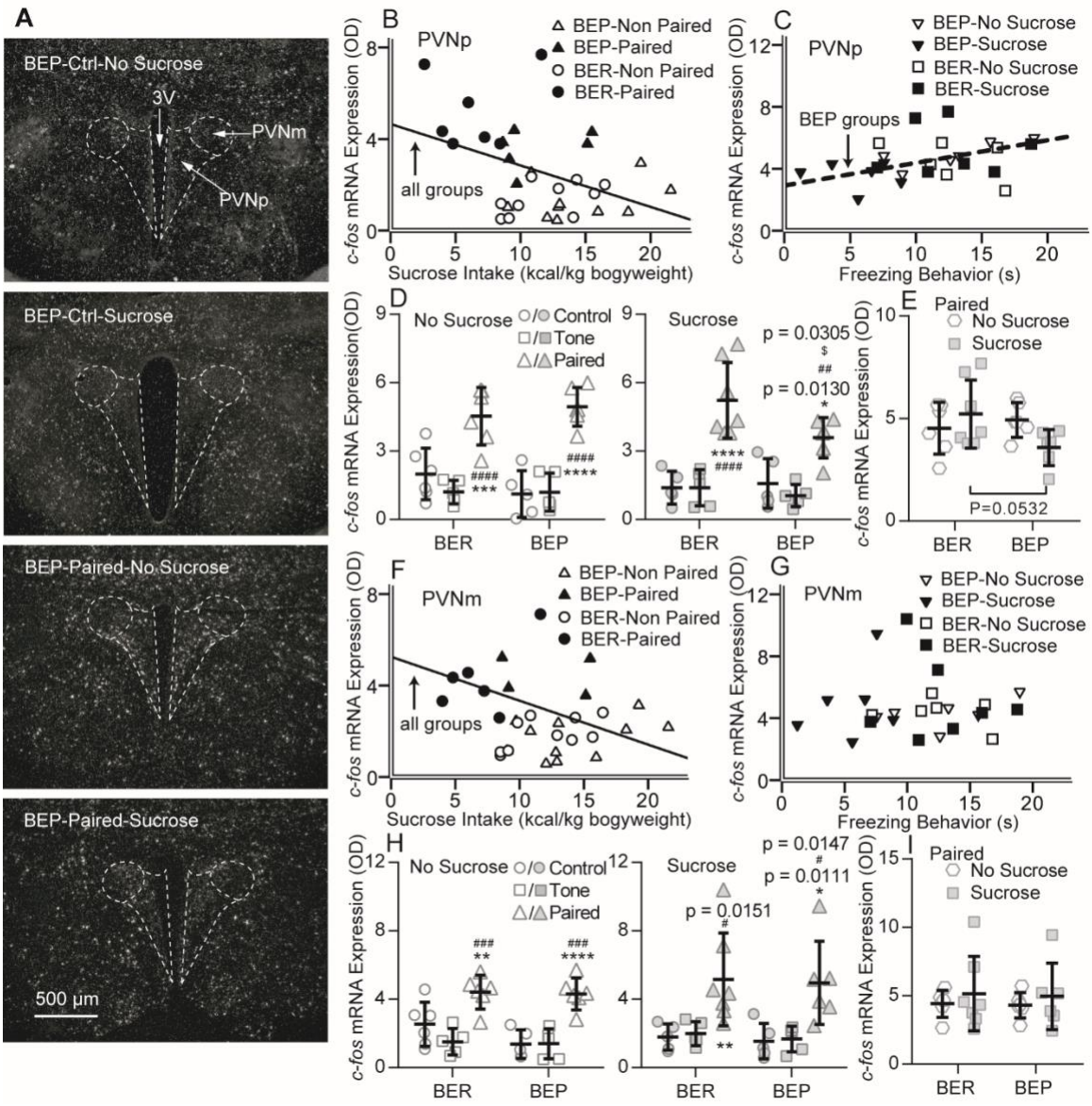

Figure 2- 7. Inhibition of the PVN by the CS by sucrose intake in the BEPs

A, representative images of the *c-fos* mRNA *in situ* hybridization signals with the PVNp and PVNm marked with broken contours. 2-D scatter plots of relative *c-fos* mRNA expression/sucrose intake (B, F), and relative *c-fos* mRNA expression/freezing behavior (C, G). Relative *c-fos* mRNA expression of *No Sucrose* and *Sucrose* groups (D, H) and *Paired* groups (E, I). *, significantly different (p<0.0.5) from the *Control* group with the same phenotype. **, p<0.01***; p<0.001; ****, p<0.0001. #, significantly different (p<0.05) from the *Tone* group with the same phenotype. ##, p<0.01; ###, p<0.001; ####, p<0.0001. $, significantly different (p<0.05) from the BER group with the same treatment. 3V, 3rd ventricle. PVN, paraventricular part of the lateral hypothalamus. PVNm, magnocellulair part of the PVN. PVNp, parvocellulair part of the PVN.

2.6.4.4 Persistent Acb response to sucrose intake in the BEPs under conditioned fear

Two sub-regions were analyzed in the Acb: the core part (AcbC) and the shell part (AcbSh), as shown in Figure 2-8A. Linear regression analysis revealed strong negative correlations between sucrose intake and *c-fos* expression in the AcbC (Figure 2-8B. n= 32; r_2 = 0.3444, p = 0.0004) and the AcbSh (Figure 2-8F. n= 32; r_2 = 0.5314, p < 0.0001) when we put together all the BEPs and BERs of *Sucrose* groups. However, in the BER-*Paired* group, this correlation became not significant in the AcbC (Figure 2-8B. n= 7; r_2 = 0.1904, p = 0.3278), and reversed from negative to positive in the AcbSh (Figure 2-8F. n= 7; r_2 = 0.5856, p = 0.0450). Strong negative correlations between freezing behavior and the *c-fos* expression were found in the AcbC (Figure 2-8B r_2 = 0.4289, p = 0.0151) and AcbSh (Figure 2-8 G r_2 = 0.3384, p = 0.037) of the BERs (n=13), whereas these correlations in the BEPs (n=12) were positive in both sub-regions (AcbC: Figure 2-8B r_2 = 0.4456, p = 0.0177; AcbSh: Figure 2-8G r_2 = 0.2335, p = 0.1115). Without sucrose access, the BEPs had higher *c-fos* mRNA expression in both AcbC and AcbSh than the BERs in the *Non-Paired* groups. Moreover, the CS significantly decreased the *c-fos* mRNA expression in the AcbC and AcbSh of the BEP-*Paired* group compared with the BEP-*Control* group (AcbC: p = 0.0004; AcbSh: p = 0.0065; Figure 2-8D and H). With the presence of the CS, BEPs had significantly lower *c-fos* mRNA expression in AcbC and AcbSh than the BERs when they had access to sucrose (Figure 2-8D, E, H and I). Two-way ANOVA analysis results are presented in Table 2-3A-C.

2.6.4.5 The BNST only responded to the CS in BEPs

Two sub-regions were analyzed in the BNST: the dorsal part (STD) and the ventral part (STV), as shown in Figure 2-9A. Linear regression analysis revealed strong negative correlations between sucrose intake and *c-fos* expression in the STD of BER-*Paired* group (Figure 2-9B n = 7; r_2 = 0.6426, p = 0.0302). No significant correlation between the freezing behavior and the *c-fos* expression was found in the BNST (Figure 2-9C and G). The BEP-*Paired* groups showed significantly lower *c-fos* mRNA expression in the STD (Figure 2-9D) and STV (Figure 2-9H) than the BEP-*Control* groups, no matter with or without sucrose access. For the *Paired* groups, the sucrose did not change the *c-fos* mRNA expression in the STD and STV of both phenotypes (Figure 2-9E and I). Two-way ANOVA analysis results are presented in Table 2-3A-C.

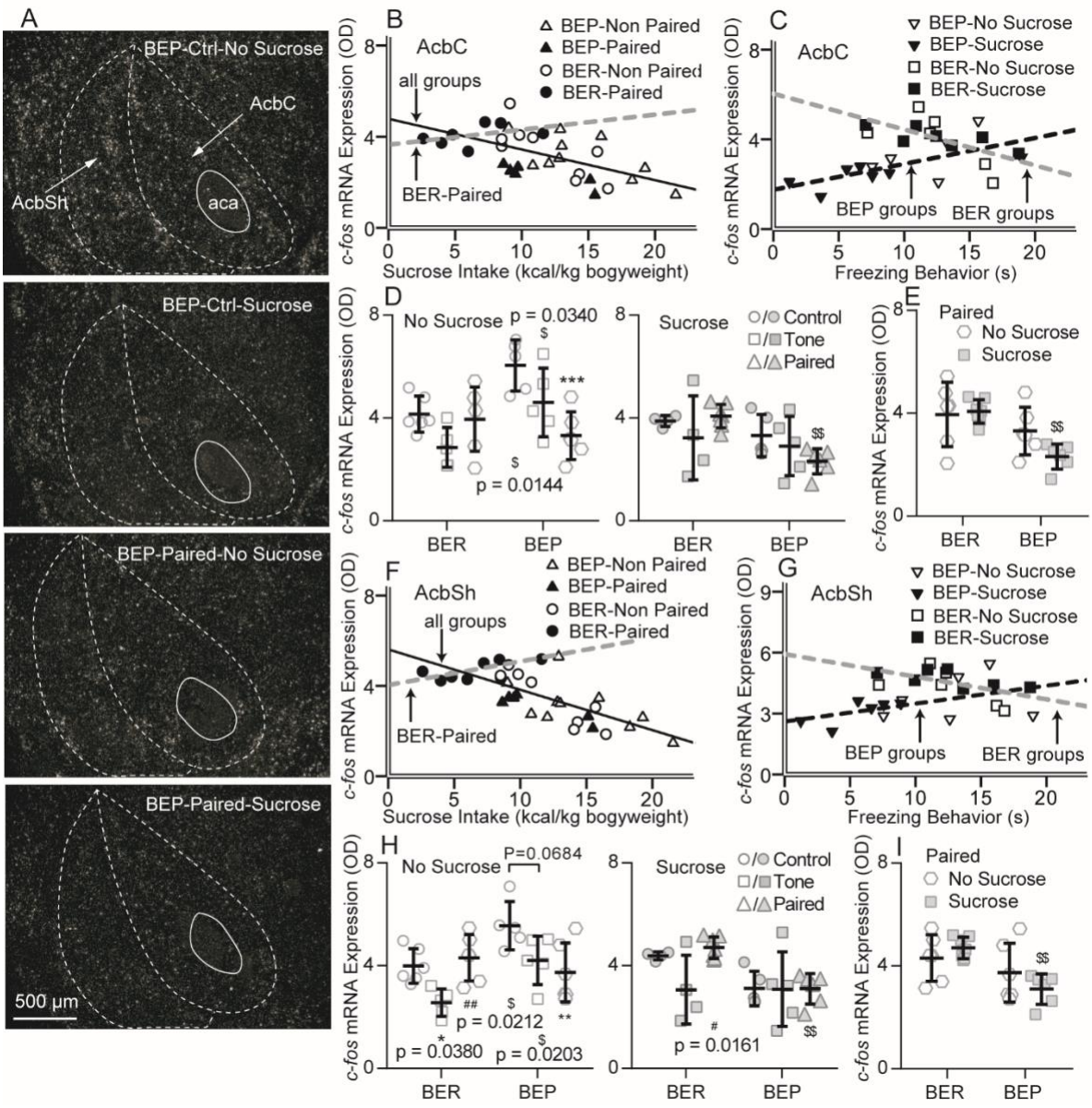

Figure 2- 8. Persistent Acb response to sucrose intake in the BEPs under conditioned fear

A, representative images of the *c-fos* mRNA *in situ* hybridization signals with the AcbC and AcbSh marked with broken contours. 2-D scatter plots of relative *c-fos* mRNA expression/sucrose intake (B, F), and relative *c-fos* mRNA expression/freezing behavior (C, G). Relative *c-fos* mRNA expression of *No Sucrose* and *Sucrose* groups (D, H) and *Paired* groups (E, I). *, significantly different ($p<0.0.5$) from the *Control* group with the same phenotype. **, $p<0.01$; ***; $p<0.001$; ****, $p<0.0001$. #, significantly different ($p<0.05$) from the *Tone* group with the same phenotype. ##, $p<0.01$; ###, $p<0.001$; ####, $p<0.0001$. $, significantly different ($p<0.05$) from the BER group with the same treatment. aca, anterior part of the anterior commissure. AcbC, nucleus accumbens core. AcbSh, nucleus accumbens shell.

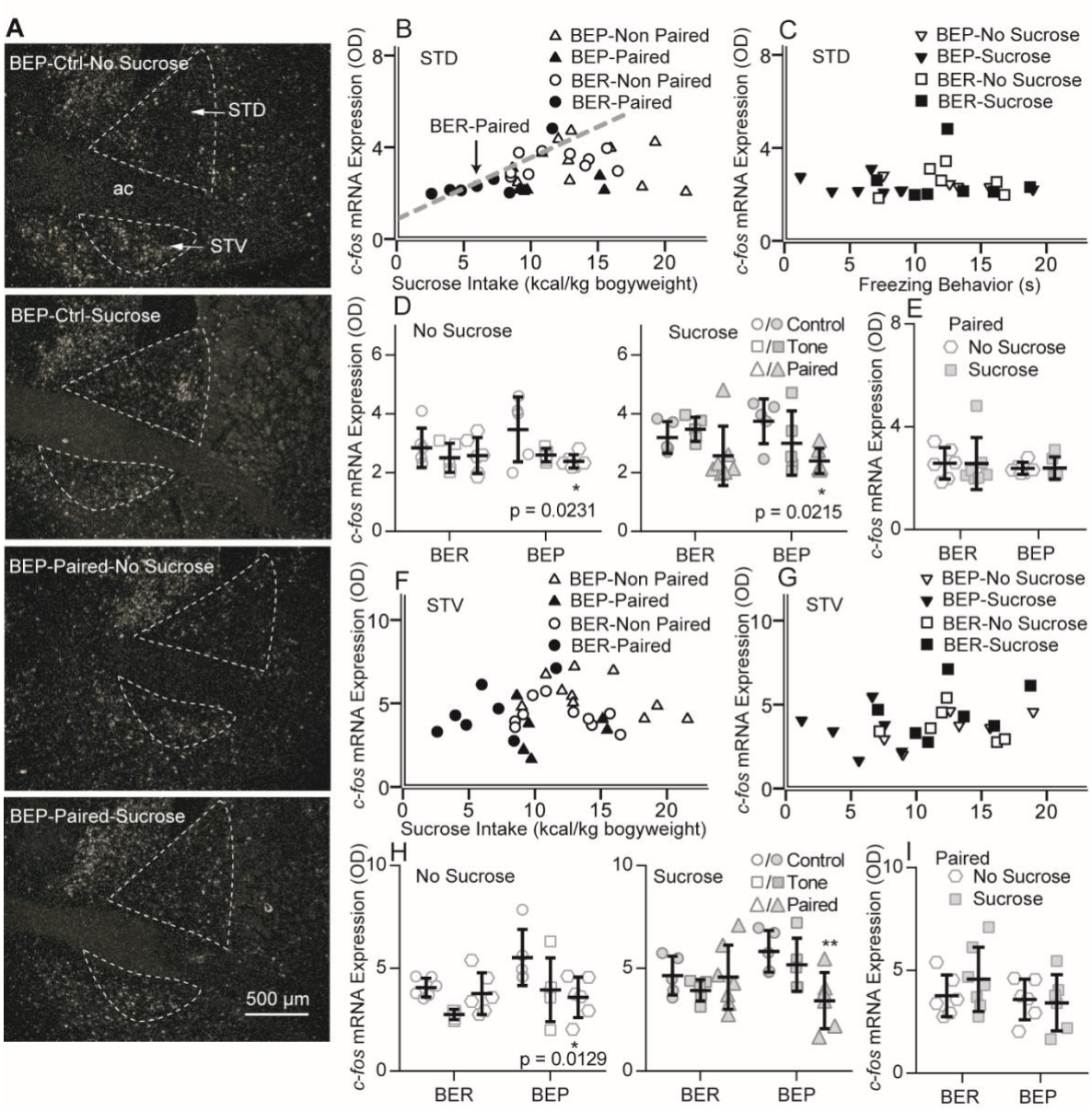

Figure 2- 9. Inhibition of the BNST by the CS in BEPs

A, representative images of the *c-fos* mRNA *in situ* hybridization signals with the STD and STV marked with broken contours. 2-D scatter plots of relative *c-fos* mRNA expression/sucrose intake (B, F), and relative *c-fos* mRNA expression/freezing behavior (C, G). Relative *c-fos* mRNA expression of *No Sucrose* and *Sucrose* groups (D, H) and *Paired* groups (E, I). *, significantly different ($p<0.0.5$) from the *Control* group with the same phenotype. ac, anterior commissure. STD, dorsal part of the bed nucleus of stria terminalis. STV, ventral part of the bed nucleus of stria terminalis.

2.6.4.6 The mPFC is less recruited in response to the CS in BEPs with access to sucrose

Two sub-regions were analyzed in the mPFC: the prelimbic part (PrL) and the infralimbic part (IL), as shown in Figure 2-10A. Linear regression analysis revealed strong negative correlation between sucrose intake and *c-fos* expression in the PrL (Figure 2-10B $r_2 = 0.6786$, $p = 0.0063$) and IL (Figure 2-10F $r_2 = 0.8045$, $p = 0.0010$) of BER-*Non Paired* rats ($n = 9$). This correlation reversed

into positive in the BER-*Paired* group (n = 7; PrL: r2 = 0.5126, p = 0.0704; IL: r2 = 0.4258, p = 0.1121). No significant correlation between freezing behavior and *c-fos* expression was found in the mPFC (Figure 2-10C and G). Without access to sucrose (Figure 2-10C and G), the *c-fos* mRNA expression in both PrL and IL of the BER-*Tone* group was significantly lower than BER-*Control* and -*Paired* groups (Figure 2-10D and H). Finally, with the presence of CS, the BEP-*Sucrose* group had significantly lower *c-fos* mRNA expression than the BEP-*No Sucrose* group in the PrL (p = 0.0280; Figure 2-10E), but not in the IL (Figure 2-10I). Two-way ANOVA analysis results are presented in Table 2-3A-C.

2.7 Discussion

In this study, we found that the fear conditioning failed to inhibit the sucrose intake of the BEPs. Difference of licking behaviors between the BEPs and BERs happened mainly between tones. The sucrose access significantly diminished their fear response to the CS. The analysis of *c-fos* mRNA expression in brain regions involved in regulation of feeding, reward processing and stress responding demonstrated that the CS activated the amygdala, LHA and PVN regardless of the phenotype. Under conditioned fear, the sucrose intake inhibited the neuronal activation in PVN and PrL of BEPs, while increased it in the PLH of the BEPs and the Ce of the BEPs and BERs. We found a negative correlation between sucrose intake and *c-fos* mRNA expression in the BEPs and BERs under non-stressful situation, which was disrupted by the CS in BERs but not in the BEPs. The CS inhibited the *c-fos* mRNA expression in the BNST of the BEPs but not BERs, no matter with or without sucrose. Therefore, out of investigated regions, the main difference in neuronal activity in the two phenotypes was found in the PrL, PVNp, PLH and Acb.

Until now, we have observed all aspects of compulsive eating in the BEPs in our BED rat model: habitual overeating, overeating to relieve negative emotion, and overeating despite aversive consequences. In an acute stressful environment, the animals have to recruit their energy and attention for a swift and proper response, and concurrently inhibit other housekeeping activities, such as food intake, digestion and reproduction. For the BERs, the conditioned fear not only inhibited the licking behavior during tones, but also between tones, a relatively less stressful but uncertain situation. For the BEPs, the licking behavior was not affected between tones, suggesting a deficiency of devaluating the palatable food when confronted with potential dangers. Similarly, both humans and animals with eating disorders show some problems in suppressing food seeking and taking in an emergent situation (Oswald, Murdaugh et al. 2011). Overeating of palatable food

despite aversive consequence happens when an abnormally high motivation for palatable food hijack the stress-responding system (Voon 2015).

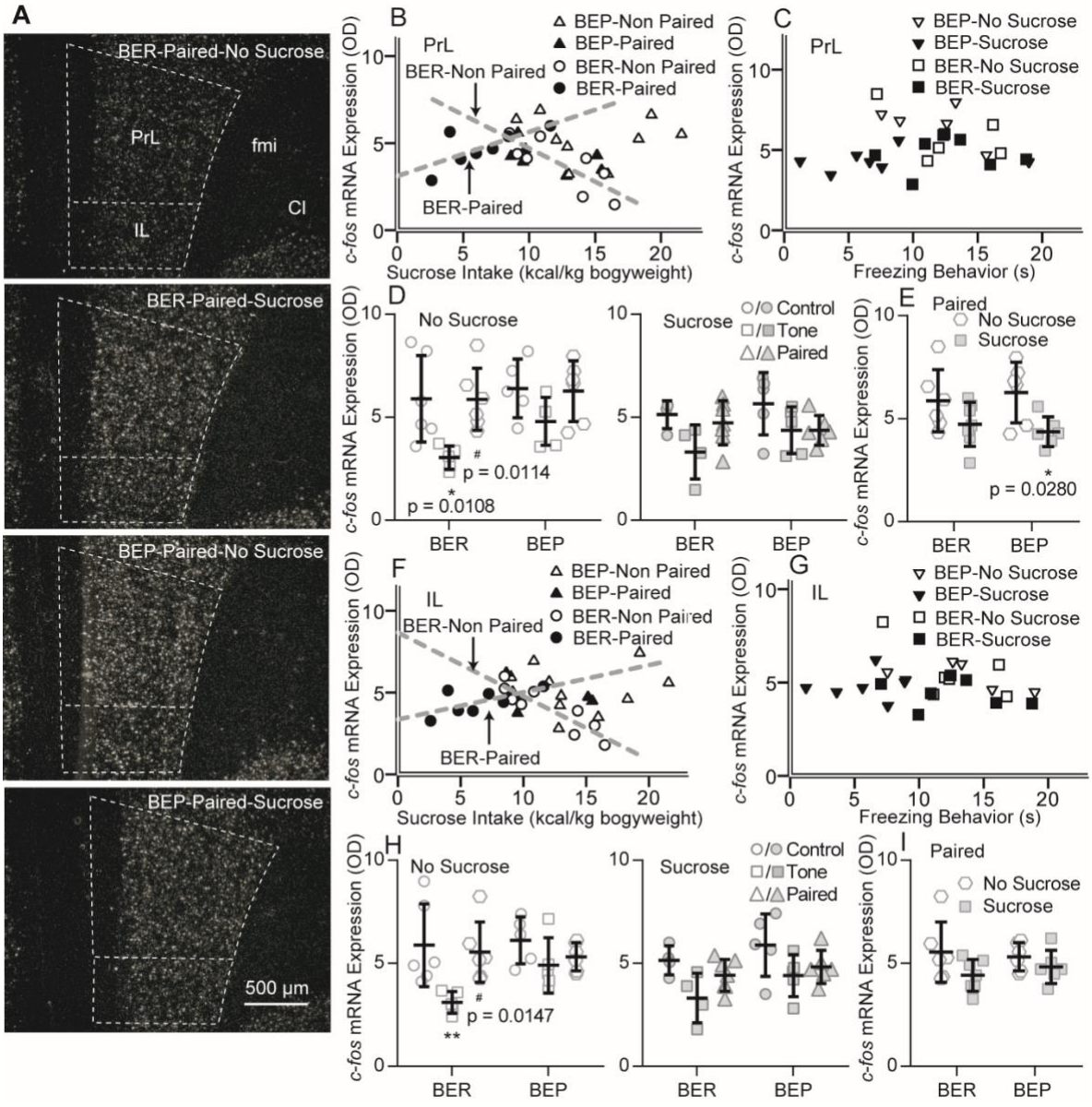

Figure 2- 10. Less recruitment of the mPFC in response to the CS in BEPs with access to sucrose

A, representative images of the *c-fos* mRNA *in situ* hybridization signals with the PrL and IL marked with broken contours. 2-D scatter plots of relative *c-fos* mRNA expression/sucrose intake (B, F), and relative *c-fos* mRNA expression/freezing behavior (C, G). Relative *c-fos* mRNA expression of *No Sucrose* and *Sucrose* groups (D, H) and *Paired* groups (E, I). *, significantly different ($p<0.0.5$) from the *Control* group with the same phenotype. **, $p<0.01$. #, significantly different ($p<0.05$) from the *Tone* group with the same phenotype. Cl, claustrum. fmi, forceps minor of the corpus callosum. IL, infralimbic part of the medial prefrontal cortex. PrL, prelimbic part of the medial prefrontal cortex.

The BEPs and BERs displayed comparable fear response during the *Fear Conditioning* sessions and the *Test* session when they had no access to sucrose, indicating that the CS was equally

stressful for both phenotypes, and that they had similar capacity of fear-conditioning acquisition, consolidation and expression. Thus, we can exclude the possibility that the higher sucrose intake in the BEPs under conditioned stress was a result of lower sensitivity to stress events, thus firmly pin the abnormal motivation on the compulsive eating in our rat model. The sucrose access decreased the freezing behavior during tones in the BEPs, but not in the BERs. Since both BEP- and BER-*Paired* groups decreased their meal duration and number of licks during tones, the lower freezing behavior was more likely a result of a stronger anxiolytic effect of sucrose on the BEPs than BERs.

To understand the neural mechanisms underlying the different feeding and freezing behavior under conditioned fear, we analyzed the *c-fos* mRNA expression in brain regions involved in stress responding, feeding regulation and reward processing.

For many years, the amygdala has been considered as the emotional center, especially for coding the conditioned fear response. Consistent with the functions of the BLA and Ce in the fear-conditioning acquisition and expression respectively, the CS activated both of them without difference between the BERs and BEPs. This is consistent with the comparable fear response of the BEPs and BERs in *Fear Conditioning* sessions and the *Test* session when they had no access to sucrose. Previous study showed that lesion of the Ce, not the BLA, abolished the feeding-inhibiting effect of an aversive CS (Petrovich, Ross et al. 2009). Consistent with this special function of the Ce, this study found that the Ce further increased its activity in response to sucrose access combined with an aversive cue, compared with the cue itself, indicating the involvement of the Ce in the devaluation of sucrose under stressful situations. Surprisingly, the activities of the BLA and Ce were not inhibited by sucrose intake in this study, suggesting that the amygdala is not likely the functioning site of the anxiolytic effects of palatable foods.

Studies with retrograde tracing technique revealed that the BLA and Ce send projections to the ventral and dorsal LHA respectively (Reppucci and Petrovich 2016). Noxious stimulation increases the *c-fos* expression in the LHA (Bullitt 1990), and lesions of the LHA significantly decrease arterial pressure response to a CS (LeDoux, Iwata et al. 1988). The activation of the LHA by CS in this study was likely induced by activating projections from the amygdala, and might play a role in the devaluation of sucrose by projecting to the VTA (Nieh, Matthews et al. 2015, Nieh, Vander Weele et al. 2016). The LHA is downstream of the amygdala in the autonomic system responding to stress, which can possibly explain why the activity of the LHA was more closely correlated to the freezing behavior compared with the amygdala. The LHA is recognized as the feeding center. The LHA receives inputs from the arcuate nucleus (ARC), and changes the

value of the nutrition via projections to the reward areas, such as the VTA (Domingos, Sordillo et al. 2013). The variance of nutritious value is important for the regulation of homeostatic feeding behavior. The post-ingestive rewarding effect of sucrose might be response for the increase of PLH activity of the BEPs under conditioned fear that likely contributed to the inhibiting effect of sucrose on the freezing behavior. We propose that Ce-LHA projections may modulate the metabolic value of sucrose in the LHA, while the LHA-Ce projections influences the affective and emotional significance of sucrose in the amygdala.

As part of the hypothalamus-pituitary-adrenal (HPA) axis, the neuroendocrine neurons of the PVN are responsible for the secretion of CRH and vasopressin in response to stress stimuli. Not surprisingly, in *Paired* groups, the CS significantly upregulated the *c-fos* mRNA expression in the PVN of both BEPs and BERs, which is consistent with many previous IEG-based (immediate early gene-based) mapping studies (Honkaniemi, Kononen et al. 1994). It is also known that palatable food, such as sucrose and lard, could attenuate the stimulating effect of stress on the HPA axis (Foster, Warne et al. 2008). In this study, sucrose ingestion attenuated the PVNp response to stress in the BEPs compared with the BERs. This result is consistent with the freezing behavior, but similar to amygdala, there is no evidence showing that the PVNp directly controls the freezing behavior. Nevertheless, we can still hypothesize that sucrose has stronger anxiolytic effect on the BEPs than the BERs, which can also explain the higher sucrose intake of the BEPs during the phenotype classification. This decreased HPA axis response to stress in the BEPs has been observed in another study with BED rat model, demonstrating attenuated plasm corticosterone, and CRH mRNA expression in the PVN in response to the foot-shock stress (Calvez, de Ávila et al. 2016).

A popular hypothesis is that the hyperpolarization of medial spinal neurons (MSNs) in the Acb is primarily underling the appetitive motivation (wanting), and inhibition of these GABAergic neurons can disinhibit the downstream targets, such as ventral pallidum, VTA and LHA, and promote hedonic responses (liking) and continuation of feeding behaviors (Castro, Cole et al. 2015). Without the stimulation of CS and sucrose, the BEPs showed a higher baseline activity in the Acb compared with the BERs, indicating a stronger motivation for palatable food after a prolonged history of sucrose intake. Extended access to palatable food can induce hyperphagia and compulsive eating in rats, accompanied with gradually worsened responsiveness of reward system, decreased baseline extracellular dopamine in the Acb (van de Giessen, Celik et al. 2014) and downregulated Striatal dopamine D2 receptors (D2Rs) (Johnson and Kenny 2010). Considering the inhibitory function of the D2Rs, these changes could decrease the tonic inhibition

of the Acb, and possibly contributed to the higher baseline Acb activity in the BEPs. Thus, we propose that tonic reward response was diminished in the BEPs, and consequently they had to eat more and faster to activate the reward system. It could possibly drive a higher motivation and expectation for sucrose in BEPs, which could explain the shorter first lick latency of the BEPs when they got access to sucrose. It also indicates that the changes in the Acb possibly played a crucial role in the transition from goal-directed food intake to habitual overeating and from casual to compulsive food seeking and eating in the BEPs.

Among rats with sucrose access, we found a negative correlation between sucrose intake and Acb activity, which is consistent with the result of single unit recording study (Roitman, Wheeler et al. 2005). It is well known that inhibition of the Acb increased, and stimulation decreased, intake of food (Baldo, Gual‐Bonilla et al. 2004, O'Connor, Kremer et al. 2015). Under conditioned fear, the direction of the correlation between sucrose intake and the Acb activity reversed from negative to positive in the BER rats. It indicates that the dynamic function of the Acb depends on the current salience of stimuli, and that the hedonic value of sucrose for BER decreased in response to the CS. We can comprehend this disrupted responding pattern as a direct evidence of devaluation of palatable food in the presence of aversive stimuli. Increased activity of the projection from the AcbSh D1R (dopamine type 1 receptor expressing) MSNs to the LHA GABA neurons has been proven underlying the inhibition of feeding behavior by salient external stimuli (O'Connor, Kremer et al. 2015). On the contrary, the CS did not change the pattern of responding to sucrose in the BEPs, suggesting a resistance to the devaluation of palatable food by potential aversive consequences. The deficits of devaluation has already been observed in long-term access induced binge-like eating rats with progressive ratio schedule test, also known as 'outcome devaluation test' (Furlong, Jayaweera et al. 2014).

We hypothesize that while the LHA works as an integrating spot regulating the 'homeostatic eating', and maintaining a long-term energy balance, the Acb possibly is another integrating region converging information from both stress system and reward system, and modulating the hedonic value of palatable food under different situations. Abnormal activities of the Acb may lead to overeating by overriding the homeostatic feeding system including the LHA.

The BNST is part of the 'extended amygdala', along with the Ce and caudal Acb. Substantial evidences support the involvement of the BNST in the fear and anxiety response to conditioned and unconditioned stimuli. The BNST controls the stress-induced seeking and consumption of drugs and palatable food by receiving stress information from the Ce (Erb, Salmaso et al. 2001) and projecting to the VTA (Kudo, Uchigashima et al. 2012). In human patients, severe obsessive-

compulsive disorder could be alleviated by electrical deep brain stimulation in the BNST (Luyten, Hendrickx et al. 2016).

In this study, sucrose intake inhibited the *c-fos* mRNA expression in the Acb of the BEPs, but not in the BERs. The BEPs showed decreased BNST activity in response to the CS, but not the BERs. Lesions of the BNST did not affect the fear response to an aversively conditioned sound, but decreased the response to another unconditioned sound, indicating a fear-generalization function of the BNST (Duvarci, Bauer et al. 2009). Considering this discrimination inhibitory effect of the BNST, its lower activity in the BEPs during the *Test* session might explain the un-affected licking behavior of BEP-Paired rats between tones compared with BER-Paired rats. This different BNST response to the CS between the BEPs and BERs was observed even without sucrose access, suggesting different stress responding strategies between phenotypes, which might be involved in the development of stress-induced overeating in the BEPs, and drove their compulsive sucrose seeking behavior under the conditioned fear situation.

Considering its reciprocal connections with the amygdala, and dense projections to the LHA and Acb, the mPFC may take part in devaluating the palatable food under stressful situation and modulating value-based decision-making. The sucrose inhibited the PrL activity with the presence of aversive CS in the BEPs, but not the BERs. It recently has been found that deep brain stimulation in the PrL reduced the binge size in a binge eating rat model (Sarica, Ozkan et al. 2018). Thus, this decreased PrL activity in the BEPs was very likely responsible for their persistent sucrose intake under the conditioned fear. The fear conditioning reversed the correlation between sucrose intake and activity of the PrL and IL in the BERs, possibly indicating that the mPFC changed its response to sucrose, and the mode of regulation of feeding behavior under stressful situation.

In the BERs, the un-conditioned cue inhibited the mPFC activity compared with *Control* groups, both with and without sucrose. There are at least two types of decision-making: (1) ‘Value-based decision-making’ the decision-making in common-sense, like deciding what to eat for breakfast and (2) ‘perceptual decision-making’ refers to the subjective judgement about a sensory input, deciding what the stimulus is, and whether it is good or bad (Wallis 2011). The mPFC response to a neutral sound in the BERs in this study was likely coding for a perceptual decision-making, representing uncertainty about a meaningless stimulus.

The correlations between sucrose intake and *c-fos* expression in the Acb and mPFC were reversed by the CS in the BERs, but not in the BEPs, which means that the response of Acb and mPFC to the palatable food is dynamically modulated by current value of the food, and that the Acb and mPFC participate in coding for the devaluation of palatable food in response to potential aversive

consequences. Another difference between the BEPs and BERs is that the correlation between freezing behavior and *c-fos* expression in the Acb was negative in the BERs, but positive in the BEPs.

We suggest that mPFC-Acb-LHA could underlie the devaluation of sucrose in stressful situations, and abnormal activity of this circuit could promote compulsive eating (Nieh, Matthews et al. 2015). The compulsive eating observed in the BEPs was likely facilitated by deficits in devaluating the rewarding effects of sucrose, represented by attenuated recruitment of mPFC, persistent Acb response to sucrose intake and increased LHA activity in stressful situations. The interaction between the rewarding system and the stress responding system facilitates the development of the BEP phenotype, leading to abnormally high motivation for binging on high-sugar and high-fat diet over healthier food. When the hedonic system overrides the homeostasis system and the stress-responding system, the compulsive eating will attenuate the normal response to potential aversive consequences.

This study used female rats, rather than male rats, because that the prevalence of the BED is higher in women compared to men (Cossrow, Pawaskar et al. 2016). However, this choice leads to an inevitable problem: the estrus cycle and hormone fluctuations in female animals. One of the limitations of our study is that the estrous phase of individual animals was not taken into account. It was possible that the fear response and food intake of the female rats in this study were influenced by the estrous phase of individual animals. However, the effects of estrous phase were likely equally affecting rats in all groups, which possibly increased variability of observed measures, but likely did not affect the overall mean values of all measurements.

To our knowledge, this is the first study that combined the fear conditioning and binge eating animal model to observe the impulsivity of binge eating, and explored the neuronal activities underlying the regulation of feeding behavior, fear response and compulsive eating under a stressful situation. The fear-conditioning paradigm has many advantages over direct foot shock. In future experiments we plan to do electrophysiological recording during aversive stimulus, the foot shock will generate major artifact in the signals, and which is not the case with the conditioned cues, such as light and sound. Our findings indicate some potential targets for the treatment of the BED. For example, we can possibly suppress the craving for palatable by decreasing the baseline activity of the Acb and enhance the devaluation of palatable food by increasing the recruitment of the mPFC, and modifying the Acb response to palatable food. The conflicting test with fear conditioning paradigm developed in this study can provide a useful tool to explore the brain and

endocrine mechanisms of the occurrence of the BED and may provide a platform to test and compare the pharmacotherapies to suppress the compulsive eating of palatable foods.

2.8 Additional information

Competing interests. The authors declare no competing interest.

Author contributions. This project was designed by Elena Timofeeva, conducted mainly by Zhi Fei Li, with support from Geneviève Guèvremont. Manuscript was written by Zhi Fei Li and revised by Sandrine Chometton and Igor Timofeev.

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2.9 Reference

Baldo, Brian A, Lisa Gual-Bonilla, Karuna Sijapati, Roger A Daniel, Charles F Landry, and Ann E Kelley. 2004. 'Activation of a subpopulation of orexin/hypocretin-containing hypothalamic neurons by GABAA receptor-mediated inhibition of the nucleus accumbens shell, but not by exposure to a novel environment', European Journal of Neuroscience, 19: 376-86.

Bogduk, Nikolai. 2013. 'Diagnostic and statistical manual of mental disorders', Encyclopedia of pain: 979-82.

Bullitt, Elizabeth. 1990. 'Expression of c-fos-like protein as a marker for neuronal activity following noxious stimulation in the rat', Journal of Comparative Neurology, 296: 517-30.

Calvez, Juliane, Camila de Ávila, Geneviève Guèvremont, and Elena Timofeeva. 2016. 'Stress differentially regulates brain expression of corticotropin-releasing factor in binge-like eating prone and resistant female rats', Appetite, 107: 585-95.

Calvez, Juliane, and Elena Timofeeva. 2016. 'Behavioral and hormonal responses to stress in binge-like eating prone female rats', Physiology & behavior, 157: 28-38.

Castro, Daniel C, Shannon L Cole, and Kent C Berridge. 2015. 'Lateral hypothalamus, nucleus accumbens, and ventral pallidum roles in eating and hunger: interactions between homeostatic and reward circuitry', Frontiers in systems neuroscience, 9: 90.

Cossrow, Nicole, Manjiri Pawaskar, Edward A Witt, Eileen E Ming, Timothy W Victor, Barry K Herman, Thomas A Wadden, and M Haim Erder. 2016. 'Estimating the Prevalence of Binge Eating Disorder in a Community Sample From the United States: Comparing DSM-IV-TR and DSM-5 Criteria', The Journal of clinical psychiatry, 77: e968-74.

De Ávila, Camila, Sandrine Chometton, Christophe Lenglos, Juliane Calvez, Andrew L Gundlach, and Elena Timofeeva. 2018. 'Differential effects of relaxin-3 and a selective relaxin-3 receptor agonist on food and water intake and hypothalamic neuronal activity in rats', Behavioural brain research, 336: 135-44.

Domingos, Ana I, Aylesse Sordillo, Marcelo O Dietrich, Zhong-Wu Liu, Luis A Tellez, Jake Vaynshteyn, Jozelia G Ferreira, Mats I Ekstrand, Tamas L Horvath, and Ivan E de Araujo. 2013. 'Hypothalamic melanin concentrating hormone neurons communicate the nutrient value of sugar', Elife, 2: e01462.

Duvarci, Sevil, Elizabeth P Bauer, and Denis Paré. 2009. 'The bed nucleus of the stria terminalis mediates inter-individual variations in anxiety and fear', Journal of Neuroscience, 29: 10357-61.

Erb, Suzanne, Natalina Salmaso, Demetra Rodaros, and Jane Stewart. 2001. 'A role for the CRF-containing pathway from central nucleus of the amygdala to bed nucleus of the stria terminalis in the stress-induced reinstatement of cocaine seeking in rats', Psychopharmacology, 158: 360-65.

Foster, Michelle T, James P Warne, Abigail B Ginsberg, Hart F Horneman, Norman C Pecoraro, Susan F Akana, and Mary F Dallman. 2008. 'Palatable foods, stress, and energy stores sculpt corticotropin-releasing factor, adrenocorticotropin, and corticosterone concentrations after restraint', Endocrinology, 150: 2325-33.

Furlong, Teri M, Hirosha K Jayaweera, Bernard W Balleine, and Laura H Corbit. 2014. 'Binge-like consumption of a palatable food accelerates habitual control of behavior and is dependent on activation of the dorsolateral striatum', Journal of Neuroscience, 34: 5012-22.

Honkaniemi, Jari, Juha Kononen, Tommi Kainu, Ilkka Pyykönen, and Markku Pelto-Huikko. 1994. 'Induction of multiple immediate early genes in rat hypothalamic paraventricular nucleus after stress', Molecular Brain Research, 25: 234-41.

Johnson, Paul M, and Paul J Kenny. 2010. 'Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats', Nature neuroscience, 13: 635-41.

Kovács, Krisztina J. 1998. 'Invited review c-Fos as a transcription factor: a stressful (re) view from a functional map', Neurochemistry international, 33: 287-97.

Kudo, Takehiro, Motokazu Uchigashima, Taisuke Miyazaki, Kohtarou Konno, Miwako Yamasaki, Yuchio Yanagawa, Masabumi Minami, and Masahiko Watanabe. 2012. 'Three types of neurochemical projection from the bed nucleus of the stria terminalis to the ventral tegmental area in adult mice', Journal of Neuroscience, 32: 18035-46.

LeDoux, Joseph E, J Iwata, PRDJ Cicchetti, and DJ Reis. 1988. 'Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear', Journal of Neuroscience, 8: 2517-29.

Luyten, L, S Hendrickx, S Raymaekers, L Gabriëls, and B Nuttin. 2016. 'Electrical stimulation in the bed nucleus of the stria terminalis alleviates severe obsessive-compulsive disorder', Molecular psychiatry, 21: 1272.

Maniam, Jayanthi, and Margaret J. Morris. 2012. 'The link between stress and feeding behaviour', Neuropharmacology, 63: 97-110.

Moore, Catherine F, Valentina Sabino, George F Koob, and Pietro Cottone. 2017. 'Neuroscience of Compulsive Eating Behavior', Frontiers in Neuroscience, 11.

Nieh, Edward H, Gillian A Matthews, Stephen A Allsop, Kara N Presbrey, Christopher A Leppla, Romy Wichmann, Rachael Neve, Craig P Wildes, and Kay M Tye. 2015. 'Decoding neural circuits that control compulsive sucrose seeking', Cell, 160: 528-41.

Nieh, Edward H, Caitlin M Vander Weele, Gillian A Matthews, Kara N Presbrey, Romy Wichmann, Christopher A Leppla, Ehsan M Izadmehr, and Kay M Tye. 2016. 'Inhibitory Input from the Lateral Hypothalamus to the Ventral Tegmental Area Disinhibits Dopamine Neurons and Promotes Behavioral Activation', Neuron, 90: 1286-98.

O'Connor, Eoin C, Yves Kremer, Sandrine Lefort, Masaya Harada, Vincent Pascoli, Clément Rohner, and Christian Lüscher. 2015. 'Accumbal D1R neurons projecting to lateral hypothalamus authorize feeding', Neuron, 88: 553-64.

Oswald, Kimberly D., Donna L. Murdaugh, Vinetra L. King, and Mary M. Boggiano. 2011. 'Motivation for palatable food despite consequences in an animal model of binge eating', International Journal of Eating Disorders, 44: 203-11.

Pecoraro, Norman, Faith Reyes, Francisca Gomez, Aditi Bhargava, and Mary F Dallman. 2004. 'Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress', Endocrinology, 145: 3754-62.

Petrovich, Gorica D, Cali A Ross, Pari Mody, Peter C Holland, and Michela Gallagher. 2009. 'Central, but not basolateral, amygdala is critical for control of feeding by aversive learned cues', The Journal of Neuroscience, 29: 15205-12.

Poulin, Anne-Marie, and Elena Timofeeva. 2008. 'The dynamics of neuronal activation during food anticipation and feeding in the brain of food-entrained rats', Brain research, 1227: 128-41.

Reppucci, Christina J, and Gorica D Petrovich. 2016. 'Organization of connections between the amygdala, medial prefrontal cortex, and lateral hypothalamus: a single and double retrograde tracing study in rats', Brain Structure and Function, 221: 2937-62.

Roitman, Mitchell F, Robert A Wheeler, and Regina M Carelli. 2005. 'Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output', Neuron, 45: 587-97.

Sarica, Can, Mazhar Ozkan, Husniye Hacioglu Bay, Umit Sehirli, Filiz Onat, and M Ibrahim Ziyal. 2018. 'Prelimbic cortex deep brain stimulation reduces binge size in a chronic binge eating rat model', Stereotactic and functional neurosurgery, 96: 33-39.

Simmons, Donna M, Jeffrey L Arriza, and LW Swanson. 1989. 'A complete protocol for in situ hybridization of messenger RNAs in brain and other tissues with radio-labeled single-stranded RNA probes', Journal of Histotechnology, 12: 169-81.

van de Giessen, Elsmarieke, Funda Celik, Dave H Schweitzer, Wim van den Brink, and Jan Booij. 2014. 'Dopamine D2/3 receptor availability and amphetamine-induced dopamine release in obesity', Journal of Psychopharmacology, 28: 866-73.

Voon, Valerie. 2015. 'Cognitive biases in binge eating disorder: the hijacking of decision making', CNS spectrums, 20: 566-73.

Wallis, Jonathan D. 2011. 'Cross-species studies of orbitofrontal cortex and value-based decision-making', Nature neuroscience, 15: 13.

Discussion and perspectives

3.1 Possibility of an effect of genetic background in the development of the BED

The phenotyping result in this study was consistent with our previous studies (Calvez and Timofeeva 2016). During the phenotyping process, the BEPs had already showed higher consumption of sucrose solution than the BERs before the intermittent stress sessions, an evidence of habitual overeating of palatable food in the BEPs. The foot-shock stress significantly increased the sucrose intake of the BEPs, while kept the BERs intact, which means the effect of stress for enhancing the food intake also depends on the genetic background of animals, possibly an effect of gene-environment interplay during the development of the BED.

It has been suggested that ingestion of palatable food can activate the reward systems in the brain), soothe the stress response (Polivy and Herman 1999) and decrease the response of the hypothalamic-pituitary-adrenal (HPA) axis to aversive stimuli, characterized as attenuated increase of plasma cortisol (Tomiyama, Dallman, and Epel 2011) and corticosterone (Pecoraro et al. 2004) concentration. As we can see in Figure 2-8 D, H, the BEPs had higher baseline activities in the Acb than the BERs, when they were not stressed and had no access to sucrose, indicating possibly divergent sensitivity, anticipation and motivation for the palatable food. Since this difference was observed after the development of binge eating in BEPs, it is difficult to determine whether it was a direct result of gene-environment interaction. Interestingly, the sucrose lost the inhibitory effect on the activity in the Acb of the BERs under conditioned fear. In contrast, the Acb of the BEPs responded to the sucrose intake with decreased activity in the same patter as the *Control* groups despite of the conditioned fear (Figure 2-8 A, F).

In different environmental conditions the animals with different feeding related genes may produce different response of reward system to the palatable food consumption, and consequently influencing the comforting effect of palatable food on the BEPs and BERs. As the association between the intake of “comfort food” and the relief of stress escalated, for BEPs in our model, binging on palatable could possibly become a self-medication to relieve a negative emotional state after the foot-shock stress (Pecoraro et al. 2004; Dallman, Pecoraro, and la Fleur 2005).

3.2 A theory about the mechanisms of stress-induced eating

In nature, the stress-induced eating may be a helpful adaptive strategy for better survival. For example, when an animal is chased by a predator, running to escape usually costs a large amount of energy. In addition, it will hide and stop or at least postpone seeking for food, before making

sure the predator has left. Thus, predatory stress is usually followed by a period of food shortage, leading to hunger or starvation. In this situation, the stress-induced eating can work as a shortcut from predatory stress to eating (the arrow with broken line in Figure 3-1.), which is more effective to motivate the animal to forge for food and restore the energy homeostasis, or even store extra energy to prepare for next rounds of predatory stress and food deprivation.

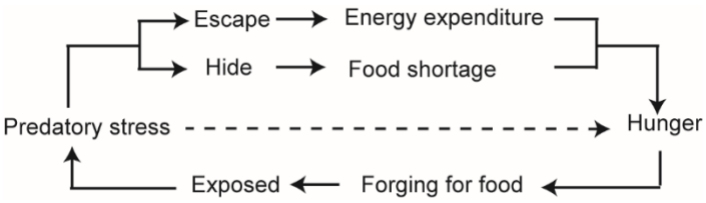

Figure 3- 1. Predatory stress may induce hunger response.

After predatory stress, the energy expenditure and food shortage will lead to energy depletion and hungry feeling because of escaping and hiding respectively, which in turn should enhance the motivation to overcome the anxiety for potential dangers and drive the animal to forge for food. Via stress generalization, the predatory stress may activate the hunger responses directly (the arrow with broken line), before real energy crisis. In this state, the animal has better chance of survival, because of higher efficiency of searching for food and escaping from predators.

This short-cut response could be conserved in the process of evolution, organized into specific genes that expressed as endocrine and neural circuits that directly stimulate food intake in response to stressors, similar to some instincts. Twin studies in humans found that eating habits, including emotional stimuli induced eating, are partially heritable (Sung, Lee et al. 2010). This enhancement of innate association between stress and food intake underlies the stress generalization in stress-induced eating and could possibly be a scenario of the origin of the stress-induced eating.

The stress-induced eating could be efficient in promoting energy consumption and restoration for wild animals and our hunter-gatherer ancestors, when the environment is dangerous and food resources are limited or unpredictable—the ‘feast or famine’ scenario. A good adaptation in this situation is being sensitive to energy deficiency but comparatively tolerant of excess energy. However, with unprecedentedly easy availability of palatable and nutritious food in modern society, the advantages of stress-induced eating are almost ignorable, and its disadvantages have been getting more and more attention.

3.3 Possible pathways for devaluation of palatable food

This study found a strong negative correlation between sucrose intake and the Acb activity, especially in a non-stressful environment. This is speculated to be a result of mainly response to the palatable taste through a short pathway, such as NTS-VTA-Acb, or even direct NTS-Acb projections. The reason is that higher taste sensory circuits integrate diverse information. For

example, when the taste signals reach the OFC, they are integrated with other information about the food, such as color, texture and smell, and finally form the perception of the flavor of the food (Rolls 2011). The taste information is also sent to the mPFC, combined with other higher cognitive information, such as memories of previous experiences about this food, and participates in regulation of feeding behaviors via projecting to the motor cortex (Lin, Wang et al. 2010, Jezzini, Mazzucato et al. 2013, Gonzalez, Kramar et al. 2014). Although both OFC and mPFC have direct projections to the Acb (Schoenbaum, Roesch et al. 2006, Moore, Oelberg et al. 2019), the highly processed taste information is more likely to involve factors influenced by individual difference, and less likely to show a simple correlation with the quantity of sucrose intake.

The sucrose intake showed strong positive correlation with the activities in the Acb and mPFC in the BERs in a stressful situation. This reversed response of Acb activity to sucrose intake could be mediated by the mPFC-Acb projections, where the mPFC works as a 'brake' over excessive palatable food intake (Sinclair, Klump et al. 2019) or feeding behavior in response to potential aversive consequences, and led to the devaluation of sucrose by the aversive CS in the BERs. Another interesting brain region that are proven to be involved in the devaluation of palatable foods is the orbitofrontal cortex, (Thompson, Drysdale et al. 2017) possibly via modulating the rewarding properties of food intake through amygdala-OFC-Acb projections.

3.4 Each animal model has strengths and weaknesses related to validity and use.

The evaluation of an animal model depends on a clear classification and validation criteria. Animal models of binge eating have been proposed to be classified into 4 types: etiologic, isomorphic, predictive and mechanistic. Etiologic models require development with the same underlying causes as it happens in human patients. However, the causes of human binge eating are still not known. As a result, etiologic model of the BED is still not possible to develop. Isomorphic models can be developed by duplicating the human symptoms in animals, without knowing the causes of the BED. All currently existing animal models of BED are isomorphic, but developed with different procedures and vary in their similarities to human symptoms.

There are many difficulties to designing appropriate BED animal models, also many limitations about the existing ones. Firstly, all animal models we developed for the study of the BED are based on its clinical symptoms in human. However, some symptoms are not possible to be verified in animals, such as 'eating alone because one is embarrassed by how much she/he is eating. It means even if we can observe that the binge-like eating animals prefer to eat away from others, we still cannot know the psychological reasons behind this phenomenon. Whether it is because that they

feel shamed about the amount of food they are taking, like in human BED patients, or they just want to protect the food from being robbed by others around, this uncertainty will make a big difference about the validity of the animal model. Moreover, the symptoms and definitions of eating disorders are overlapped in a considerable degree under current diagnostic criterion. For example, the bulimia nervosa and BED share most of their diagnostic symptoms, and the distinction is whether the binge eating behavior is accompanied with compensatory activities, such as self-induced vomiting. However, self-induced vomiting is not easy to reproduce in these animal models. Most importantly, the etiology of the BED is not clear yet, partially because of its complicated and multifactorial nature, which can be influenced by genetic, personal, social and environmental factors (Mathes, Brownley et al. 2009, Wolfe, Baker et al. 2009). Not surprisingly, existing animal models obviously cannot cover all of these factors, but they still have been contributing to help us exploring the mechanism of eating disorders in many ways.

3.5 Using a single time point of *c-fos* mRNA

The *c-fos* transcription peaks 30 min after the activation of neurons in most brain regions (Saidov, Tiunova et al. 2019). Nevertheless, the peak transcription time point may vary in different regions and different neuronal subtypes (Filipkowski, Rydz et al. 2000), and the sacrifice time might not be optimum for all brain regions. This fact could possibly be among the reasons why some expected correlations between behaviors and *c-fos* expression were not observed in some brain regions. For example, a correlation between freezing behavior and *c-fos* expression in the Ce was expected, but not even a weak correlation was observed between them. An explanation was proposed in Chapter 1 that the freezing behavior is not directly controlled only by the Ce. However, a complementary explanation could be that the peaking time of expression in these regions, including the Ce, is not 30 min, and that it also varies between individuals. As a result, the *c-fos* expression in these regions was not accurate enough to reflect the influence of neuronal activation on related behaviors.

3.6 Current treatments of the BED

Until now, there is no approved pharmacological treatment specifically for the BED, and clinicians depend mostly on cognitive behavioral therapy, in combination with the off-label use of some medicine such as anticonvulsants, anti-obesity agents, and selective serotonin reuptake inhibitors (Grilo, Reas et al. 2016, Palavras, Hay et al. 2017).

The treatment of the BED, as well as for other eating disorders, should be based on its etiological background. It is composed of a complex interaction of physiological, psychological and

environmental factors, all of which should be taken into consideration when we plan the treatments (Fassino, Daga et al. 2007, Brambilla, Samek et al. 2009).

Ideally, the therapeutic procedures should lead to a stable reduction of caloric intake and establish a long-lasting maintenance of healthy eating habits (Vocks, Tuschen - Caffier et al. 2010, Iacovino, Gredysa et al. 2012). One of the final target of the BED treatments is to promote an abstinence from episodes of binge eating, and consequently a sustainable weight loss (Grilo, White et al. 2012). In order to achieve these goals, our therapeutic programs should aim at decreasing the abnormal motivation for food, and increasing the insight and abilities to deal with negative emotions and conflicts (Fassino, Daga et al. 2007, Iacovino, Gredysa et al. 2012)

3.7 Outlook:

As mentioned in the introduction part, the hazardous consequences of binge eating do not happen to all individuals with BED. However, in our conflicting test, the CS was always associated with the foot shock, which means the CS predicted 100% occurrence of the foot shock, which is not similar to the situations of binge eating humans.

To solve this problem, we can use partially reinforced conditioning in the training sessions, which means that only a portion of the tones will be followed by a foot shock. With the partial reinforcement, learned behaviors will be acquired slower, but the responses are more resistant to extinction.

Another problem with our methods is that the CS was temporally scheduled, rather than triggered by the binge eating. To solve this problem, we can design a closed-loop program, in which a specific feeding behavior will trigger the delivery of the aversive CS.

The major findings of this project suggest that the Acb and mPFC could be potential target for the treatment of the BED. In follow up studies, we can try to manipulate the activity of the Acb and mPFC with optogenetic methods to reverse the compulsive eating in the BEPs. For example, we can decrease the baseline activity of the Abc, to see if it will decrease the habitual overeating of the BEPs. Moreover, we can also increase the Acb activity when the BEPs take sucrose, with a closed-loop program, during the conflicting test, to see if we can evoke the devaluation of the sucrose in the presence of the aversive CS and decrease the sucrose intake.

Conclusion

Eating is one of the most important life elements for all animals, because food provides the energy that necessary for all activities. Feeding behaviors are influenced by numerous factors from the

living environments, as well as the physical and psychological conditions within the organisms. In order to maintain the energy homeostasis, complex endocrine and neural systems are recruited to regulate the food intake and nutrient metabolism. Malfunctions of the regulating systems usually lead to eating disorders, such as the bulimia nervosa, anorexia nervosa and BED.

Although the etiology of the BED is still not clear, we know that some certain factors may promote the development of the BED. Among those factors, dieting and stress are the most prevalent in modern society. The rat model of BED was developed by combining foot shock stress with intermittent access to sucrose, imitate the development of the BED in humans. The effect of interaction between gene and environment is supposed to play an important role in the development of the BED, which can be explored with this rat model in future studies.

The BEPs in our model demonstrate several BED symptoms observed in human patients:

1. Habitual overeating, during the process of phenotyping, as well as during the *appetitve sessions* in the fear conditioning test. The BEPs took more sucrose than the BERs.
2. Overeating after stress. The BEPs further increased their sucrose intake after foot shock stress during the phenotyping process, but not the BER rats.
3. Compulsive eating despite potential aversive consequences. As a normal response to potential danger, the BERs decreased their sucrose intake in the presence of the CS. However, the BEPs showed persistently high sucrose intake under the same stressful situation.

We found that at the level of brain circuits, the amygdala, LHA and PVN were prominently activated by the CS, which was consistent with their functions respectively in the emotional, autonomic and endocrine responses to stressors. Sucrose access barely changed the activities of these brain structures in the BERs. However, sucrose intake diminished PVN response to the CS in the BEPs. Although the freezing behavior is not directly controlled by the PVN, this form of behavior inhibited HPA axis response along with decreased fear response, suggesting a stronger anxiolytic effect of sucrose for the BEPs compared with the BERs.

With the BERs as the normal controls, in future studies, we can probe the neural responses to the fear conditioning and sucrose intake in normal individuals without BED. For example, in a non-stressful environment, the Acb activity had a negative correlation with sucrose intake, which reversed into a positive correlation in a stressful situation. Similar changes were observed in the mPFC. Such changes in responding mode to sucrose by environmental factors could possibly underlie the assignment of value to a stimulus, and in this study, it might mediate the devaluation

value of sucrose by the CS. The BEPs did not change their responding mode like the BERs, which was possibly underlying their compulsive eating despite of the CS.

Based on the findings in this study, activation of the Acb should decrease compulsive eating in the BEPs and the LTP facilitated by this training will likely alleviate the binge eating behavior. Going further, a new tools able specific activation, like deep brain stimulation in humans (i.e. the Acb) could be applied to effectively treat the BED.

Bibliography

Aarts, H., R. Custers and R. W. Holland (2007). "The nonconscious cessation of goal pursuit: When goals and negative affect are coactivated." Journal of personality and social psychology **92**(2): 165.

Abizaid, A., Z.-W. Liu, Z. B. Andrews, M. Shanabrough, E. Borok, J. D. Elsworth, R. H. Roth, M. W. Sleeman, M. R. Picciotto and M. H. Tschöp (2006). "Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite." The Journal of clinical investigation **116**(12): 3229-3239.

Adam, T. C. and E. S. Epel (2007). "Stress, eating and the reward system." Physiology & behavior **91**(4): 449-458.

Aizawa, H., S.-C. Hu, K. Bobb, K. Balakrishnan, G. Ince, I. Gurevich, M. Cowan and A. Ghosh (2004). "Dendrite development regulated by CREST, a calcium-regulated transcriptional activator." Science **303**(5655): 197-202.

Akana, S., A. Chu, L. Soriano and M. Dallman (2001). "Corticosterone exerts site‐specific and state‐dependent effects in prefrontal cortex and amygdala on regulation of adrenocorticotropic hormone, insulin and fat depots." Journal of neuroendocrinology **13**(7): 625-637.

Alves, F., C. Crestani, L. Resstel and F. M. d. A. Corrêa (2007). "Cardiovascular effects of carbachol microinjected into the bed nucleus of the stria terminalis of the rat brain." Brain research **1143**: 161-168.

Anliker, J. and J. Mayer (1956). "An operant conditioning technique for studying feeding-fasting patterns in normal and obese mice." Journal of Applied Physiology **8**(6): 667-670.

Aponte, Y., D. Atasoy and S. M. Sternson (2011). "AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training." Nat Neurosci **14**(3): 351-355.

Appleton, N. (1985). Lick the sugar habit, Penguin.

Artiga, A., J. Viana, C. Maldonado, P. Chandler-Laney, K. Oswald and M. Boggiano (2007). "Body composition and endocrine status of long-term stress-induced binge-eating rats." Physiology & behavior **91**(4): 424-431.

Asaad, W. F. and E. N. Eskandar (2011). "Encoding of both positive and negative reward prediction errors by neurons of the primate lateral prefrontal cortex and caudate nucleus." Journal of Neuroscience **31**(49): 17772-17787.

Asakawa, A., A. Inui, T. Kaga, H. Yuzuriha, T. Nagata, M. Fujimiya, G. Katsuura, S. Makino, M. A. Fujino and M. Kasuga (2001). "A role of ghrelin in neuroendocrine and behavioral responses to stress in mice." Neuroendocrinology **74**(3): 143-147.

Asede, D., D. Bosch, A. Lüthi, F. Ferraguti and I. Ehrlich (2015). "Sensory inputs to intercalated cells provide fear-learning modulated inhibition to the basolateral amygdala." Neuron **86**(2): 541-554.

Atasoy, D., J. N. Betley, H. H. Su and S. M. Sternson (2012). "Deconstruction of a neural circuit for hunger." Nature **488**(7410): 172.

Avena, N. M. (2010). "The study of food addiction using animal models of binge eating." Appetite **55**(3): 734-737.

Avena, N. M., C. A. Carrillo, L. Needham, S. F. Leibowitz and B. G. Hoebel (2004). "Sugar-dependent rats show enhanced intake of unsweetened ethanol." Alcohol **34**(2): 203-209.

Avena, N. M. and B. Hoebel (2003). "A diet promoting sugar dependency causes behavioral cross-sensitization to a low dose of amphetamine." Neuroscience **122**(1): 17-20.

Avena, N. M., P. Rada and B. G. Hoebel (2008). "Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake." Neuroscience & Biobehavioral Reviews **32**(1): 20-39.

Awad, G., L.-A. Roeckel, D. Massotte, M. C. Olmstead and K. Befort (2019). "Deletion of mu opioid receptors reduces palatable solution intake in a mouse model of binge eating." Behavioural pharmacology.

Bains, J. S., J. I. W. Cusulin and W. Inoue (2015). "Stress-related synaptic plasticity in the hypothalamus." Nat Rev Neurosci **16**(7): 377-388.

Baldo, B. A., L. Gual‐Bonilla, K. Sijapati, R. A. Daniel, C. F. Landry and A. E. Kelley (2004). "Activation of a subpopulation of orexin/hypocretin‐containing hypothalamic neurons by GABAA receptor‐mediated inhibition of the nucleus accumbens shell, but not by exposure to a novel environment." European Journal of Neuroscience **19**(2): 376-386.

Balleine, B. W., M. R. Delgado and O. Hikosaka (2007). "The role of the dorsal striatum in reward and decision-making." Journal of Neuroscience **27**(31): 8161-8165.

Balodis, I. M., N. D. Molina, H. Kober, P. D. Worhunsky, M. A. White, R. Sinha, C. M. Grilo and M. N. Potenza (2013). "Divergent neural substrates of inhibitory control in binge eating disorder relative to other manifestations of obesity." Obesity **21**(2): 367-377.

Barrett, D., J. Shumake, D. Jones and F. Gonzalez-Lima (2003). "Metabolic mapping of mouse brain activity after extinction of a conditioned emotional response." Journal of Neuroscience **23**(13): 5740-5749.

Baskin, D. G., D. F. Lattemann, R. J. Seeley, S. C. Woods, D. Porte Jr and M. W. Schwartz (1999). "Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight." Brain research **848**(1-2): 114-123.
Bassareo, V. and G. Di Chiara (1997). "Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed ad libitum." Journal of Neuroscience **17**(2): 851-861.
Bell, M. E., A. Bhargava, L. Soriano, K. Laugero, S. F. Akana and M. F. Dallman (2002). "Sucrose intake and corticosterone interact with cold to modulate ingestive behaviour, energy balance, autonomic outflow and neuroendocrine responses during chronic stress." Journal of neuroendocrinology **14**(4): 330-342.
Bello, N. T., K. L. Sweigart, J. M. Lakoski, R. Norgren and A. Hajnal (2003). "Restricted feeding with scheduled sucrose access results in an upregulation of the rat dopamine transporter." American Journal of Physiology-Regulatory, Integrative and Comparative Physiology **284**(5): R1260-R1268.
Benito, E. and A. Barco (2015). "The neuronal activity-driven transcriptome." Molecular Neurobiology **51**(3): 1071-1088.
Berridge, K. C., C.-Y. Ho, J. M. Richard and A. G. DiFeliceantonio (2010). "The tempted brain eats: pleasure and desire circuits in obesity and eating disorders." Brain research **1350**: 43-64.
Betley, J. N., Z. F. H. Cao, K. D. Ritola and S. M. Sternson (2013). "Parallel, redundant circuit organization for homeostatic control of feeding behavior." Cell **155**(6): 1337-1350.
Black, I., D. Chikaraishi and E. Lewis (1985). "Trans-synaptic increase in RNA coding for tyrosine hydroxylase in a rat sympathetic ganglion." Brain research **339**(1): 151-153.
Blevins, J. E., M. W. Schwartz and D. G. Baskin (2004). "Evidence that paraventricular nucleus oxytocin neurons link hypothalamic leptin action to caudal brain stem nuclei controlling meal size." American Journal of Physiology-Regulatory, Integrative and Comparative Physiology **287**(1): R87-R96.
Boatman, J. A. and J. J. Kim (2006). "A thalamo‐cortico‐amygdala pathway mediates auditory fear conditioning in the intact brain." European Journal of Neuroscience **24**(3): 894-900.
Bogduk, N. (2013). "Diagnostic and statistical manual of mental disorders." Encyclopedia of pain: 979-982.
Boggiano, M. M. and P. C. Chandler (2006). "Binge eating in rats produced by combining dieting with stress." Current Protocols in Neuroscience: 9.23 A. 21-29.23 A. 28.
Bouton, M. E. and R. C. Bolles (1980). "Conditioned fear assessed by freezing and by the suppression of three different baselines." Animal learning & behavior **8**(3): 429-434.
Bozarth, M. A. and R. A. Wise (1981). "Intracranial self-administration of morphine into the ventral tegmental area in rats." Life sciences **28**(5): 551-555.
Brambilla, F., L. Samek, F. Lovo, L. Cioni and C. Mellado (2009). "Multivariate therapeutic approach to binge-eating disorder: combined nutritional, psychological and pharmacological treatment." International clinical psychopharmacology **24**(6): 312-317.
Brown, J. S., H. I. Kalish and I. Farber (1951). "Conditioned fear as revealed by magnitude of startle response to an auditory stimulus." Journal of experimental psychology **41**(5): 317.
Bryant, J. L., J. D. Boughter, S. Gong, M. S. LeDoux and D. H. Heck (2010). "Cerebellar cortical output encodes temporal aspects of rhythmic licking movements and is necessary for normal licking frequency." European Journal of Neuroscience **32**(1): 41-52.
Buijs, R. M. and C. G. Van Eden (2000). The integration of stress by the hypothalamus, amygdala and prefrontal cortex: balance between the autonomic nervous system and the neuroendocrine system. Progress in brain research, Elsevier. **126:** 117-132.
Bullitt, E. (1990). "Expression of c‐fos‐like protein as a marker for neuronal activity following noxious stimulation in the rat." Journal of Comparative Neurology **296**(4): 517-530.
Burton, A. C., K. Nakamura and M. R. Roesch (2015). "From ventral-medial to dorsal-lateral striatum: neural correlates of reward-guided decision-making." Neurobiology of Learning and Memory **117**: 51-59.
Cabral, A., O. Suescun, J. M. Zigman and M. Perello (2012). "Ghrelin indirectly activates hypophysiotropic CRF neurons in rodents." PLoS One **7**(2): e31462.
Calvez, J., C. de Ávila, G. Guèvremont and E. Timofeeva (2016). "Stress differentially regulates brain expression of corticotropin-releasing factor in binge-like eating prone and resistant female rats." Appetite **107**: 585-595.
Calvez, J. and E. Timofeeva (2016). "Behavioral and hormonal responses to stress in binge-like eating prone female rats." Physiology & behavior **157**: 28-38.

Campfield, L. A., F. J. Smith, Y. Guisez, R. Devos and P. Burn (1995). "Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks." Science **269**(5223): 546-549.
Campos, C. A., A. J. Bowen, M. W. Schwartz and R. D. Palmiter (2016). "Parabrachial CGRP neurons control meal termination." Cell metabolism **23**(5): 811-820.
Careaga, M. B. L., C. E. N. Girardi and D. Suchecki (2016). "Understanding posttraumatic stress disorder through fear conditioning, extinction and reconsolidation." Neuroscience & Biobehavioral Reviews **71**: 48-57.
Carlezon Jr, W. A. and M. J. Thomas (2009). "Biological substrates of reward and aversion: a nucleus accumbens activity hypothesis." Neuropharmacology **56**: 122-132.
Carr, K. D. (2002). "Augmentation of drug reward by chronic food restriction: behavioral evidence and underlying mechanisms." Physiology & behavior **76**(3): 353-364.
Carrard, I., C. Crépin, G. Ceschi, A. Golay and M. Van der Linden (2012). "Relations between pure dietary and dietary-negative affect subtypes and impulsivity and reinforcement sensitivity in binge eating individuals." Eating behaviors **13**(1): 13-19.
Carrive, P. (2017). "Orexin, stress and central cardiovascular control. A link with hypertension?" Neuroscience & Biobehavioral Reviews **74**: 376-392.
Carrive, P. and M. Gorissen (2008). "Premotor sympathetic neurons of conditioned fear in the rat." European Journal of Neuroscience **28**(3): 428-446.
Cassin, S. E. and K. M. von Ranson (2007). "Is binge eating experienced as an addiction?" Appetite **49**(3): 687-690.
Castro, D. C., S. L. Cole and K. C. Berridge (2015). "Lateral hypothalamus, nucleus accumbens, and ventral pallidum roles in eating and hunger: interactions between homeostatic and reward circuitry." Frontiers in systems neuroscience **9**: 90.
Chen, C., F. T. Dionne and J. L. Roberts (1983). "Regulation of the pro-opiomelanocortin mRNA levels in rat pituitary by dopaminergic compounds." Proceedings of the National Academy of Sciences **80**(8): 2211-2215.
Chen, H., O. Charlat, L. A. Tartaglia, E. A. Woolf, X. Weng, S. J. Ellis, N. D. Lakey, J. Culpepper, K. J. More and R. E. Breitbart (1996). "Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice." Cell **84**(3): 491-495.
Chen, Z., S. P. Travers and J. B. Travers (2001). "Muscimol infusions in the brain stem reticular formation reversibly block ingestion in the awake rat." American Journal of Physiology-Regulatory, Integrative and Comparative Physiology **280**(4): R1085-R1094.
Choi, D. C., A. R. Furay, N. K. Evanson, M. M. Ostrander, Y. M. Ulrich-Lai and J. P. Herman (2007). "Bed nucleus of the stria terminalis subregions differentially regulate hypothalamic – pituitary – adrenal axis activity: implications for the integration of limbic inputs." Journal of Neuroscience **27**(8): 2025-2034.
Chrousos, G. (2000). "The role of stress and the hypothalamic – pituitary – adrenal axis in the pathogenesis of the metabolic syndrome: neuro-endocrine and target tissue-related causes." International Journal of Obesity **24**(S2): S50.
Chuang, J.-C., M. Perello, I. Sakata, S. Osborne-Lawrence, J. M. Savitt, M. Lutter and J. M. Zigman (2011). "Ghrelin mediates stress-induced food-reward behavior in mice." The Journal of clinical investigation **121**(7): 2684-2692.
Colantuoni, C., P. Rada, J. McCarthy, C. Patten, N. M. Avena, A. Chadeayne and B. G. Hoebel (2002). "Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence." Obesity research **10**(6): 478-488.
Corning, A. F. and I. M. Viducich (2019). "Stress in Eating Disorders." The Oxford Handbook of Stress and Mental Health: 221.
Corwin, R. L., N. M. Avena and M. M. Boggiano (2011). "Feeding and reward: perspectives from three rat models of binge eating." Physiology & behavior **104**(1): 87-97.
Corwin, R. L. and F. H. Wojnicki (2009). "Baclofen, raclopride, and naltrexone differentially affect intake of fat and sucrose under limited access conditions." Behavioural pharmacology **20**(5-6): 537-548.
Cossrow, N., M. Pawaskar, E. A. Witt, E. E. Ming, T. W. Victor, B. K. Herman, T. A. Wadden and M. H. Erder (2016). "Estimating the Prevalence of Binge Eating Disorder in a Community Sample From the United States: Comparing DSM-IV-TR and DSM-5 Criteria." The Journal of clinical psychiatry **77**(8): e968-974.
Cottone, P., V. Sabino, L. Steardo and E. P. Zorrilla (2008). "Opioid-dependent anticipatory negative contrast and binge-like eating in rats with limited access to highly preferred food." Neuropsychopharmacology **33**(3): 524.
Cottone, P., X. Wang, J. W. Park, M. Valenza, A. Blasio, J. Kwak, M. R. Iyer, L. Steardo, K. C. Rice and T. Hayashi (2012). "Antagonism of sigma-1 receptors blocks compulsive-like eating." Neuropsychopharmacology **37**(12): 2593-2604.

Coutinho, W. F., R. O. Moreira, C. Spagnol and J. C. Appolinario (2007). "Does binge eating disorder alter cortisol secretion in obese women?" Eating behaviors **8**(1): 59-64.
Crestani, C., F. Alves, L. Resstel and F. Corrêa (2008). "Both α 1 and α 2 - adrenoceptors mediate the cardiovascular responses to noradrenaline microinjected into the bed nucleus of the stria terminal of rats." British journal of pharmacology **153**(3): 583-590.
Cullinan, W. E., J. P. Herman and S. J. Watson (1993). "Ventral subicular interaction with the hypothalamic paraventricular nucleus: evidence for a relay in the bed nucleus of the stria terminalis." Journal of Comparative Neurology **332**(1): 1-20.
Cummings, D. E., J. Q. Purnell, R. S. Frayo, K. Schmidova, B. E. Wisse and D. S. Weigle (2001). "A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans." Diabetes **50**(8): 1714-1719.
Curran, A. T., D. Miller, L. Zokas and I. M. Verma (1984). "Viral and cellular fos proteins: a comparative analysis." Cell **36**(2): 259-268.
Dailey, M. J., T. H. Moran, P. C. Holland and A. W. Johnson (2016). "The antagonism of ghrelin alters the appetitive response to learned cues associated with food." Behavioural brain research **303**: 191-200.
Davis, J. D. and G. P. Smith (2009). "The conditioned satiating effect of orosensory stimuli." Physiology & behavior **97**(3-4): 293-303.
Davis, J. F., D. L. Choi and S. C. Benoit (2010). "Insulin, leptin and reward." Trends in Endocrinology & Metabolism **21**(2): 68-74.
Davis, J. F., D. L. Choi and S. C. Benoit (2011). Orexigenic hypothalamic peptides behavior and feeding. Handbook of behavior, food and nutrition, Springer**:** 355-369.
Davis, M. (1990). "Animal models of anxiety based on classical conditioning: the conditioned emotional response (CER) and the fear-potentiated startle effect." Pharmacology & therapeutics **47**(2): 147-165.
Davis, M. and P. J. Whalen (2001). "The amygdala: vigilance and emotion." Molecular psychiatry **6**(1): 13.
Day, J. J., J. L. Jones, R. M. Wightman and R. M. Carelli (2010). "Phasic nucleus accumbens dopamine release encodes effort-and delay-related costs." Biological psychiatry **68**(3): 306-309.
De Ávila, C., S. Chometton, C. Lenglos, J. Calvez, A. L. Gundlach and E. Timofeeva (2018). "Differential effects of relaxin-3 and a selective relaxin-3 receptor agonist on food and water intake and hypothalamic neuronal activity in rats." Behavioural brain research **336**: 135-144.
De Fanti, B. A., D. A. Gavel, J. S. Hamilton and B. A. Horwitz (2000). "Extracellular hypothalamic serotonin levels after dorsal raphe nuclei stimulation of lean (Fa/Fa) and obese (fa/fa) Zucker rats." Brain research **869**(1-2): 6-14.
De Vaca, S. C. and K. D. Carr (1998). "Food restriction enhances the central rewarding effect of abused drugs." Journal of Neuroscience **18**(18): 7502-7510.
Domingos, A. I., A. Sordillo, M. O. Dietrich, Z.-W. Liu, L. A. Tellez, J. Vaynshteyn, J. G. Ferreira, M. I. Ekstrand, T. L. Horvath and I. E. de Araujo (2013). "Hypothalamic melanin concentrating hormone neurons communicate the nutrient value of sugar." Elife **2**: e01462.
Duvall, L. B., L. Ramos-Espiritu, K. E. Barsoum, J. F. Glickman and L. B. Vosshall (2019). "Small-molecule agonists of Ae. aegypti neuropeptide Y receptor block mosquito biting." Cell **176**(4): 687-701. e685.
Duvarci, S., E. P. Bauer and D. Paré (2009). "The bed nucleus of the stria terminalis mediates inter-individual variations in anxiety and fear." Journal of Neuroscience **29**(33): 10357-10361.
Dwyer, D. M. (2012). "Licking and liking: The assessment of hedonic responses in rodents." The Quarterly Journal of Experimental Psychology **65**(3): 371-394.
Egecioglu, E., E. Jerlhag, N. Salomé, K. P. Skibicka, D. Haage, M. Bohlooly - Y, D. Andersson, M. Bjursell, D. Perrissoud and J. A. Engel (2010). "PRECLINICAL STUDY: FULL ARTICLE: Ghrelin increases intake of rewarding food in rodents." Addiction biology **15**(3): 304-311.
Egecioglu, E., K. P. Skibicka, C. Hansson, M. Alvarez-Crespo, P. A. Friberg, E. Jerlhag, J. A. Engel and S. L. Dickson (2011). "Hedonic and incentive signals for body weight control." Reviews in Endocrine and Metabolic Disorders **12**(3): 141-151.
Epel, E., R. Lapidus, B. McEwen and K. Brownell (2001). "Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior." Psychoneuroendocrinology **26**(1): 37-49.
Epstein, L. H., J. L. Temple, J. N. Roemmich and M. E. Bouton (2009). "Habituation as a determinant of human food intake." Psychological review **116**(2): 384.
Erb, S., N. Salmaso, D. Rodaros and J. Stewart (2001). "A role for the CRF-containing pathway from central nucleus of the amygdala to bed nucleus of the stria terminalis in the stress-induced reinstatement of cocaine seeking in rats." Psychopharmacology **158**(4): 360-365.

Erlanson-Albertsson, C. (2005). "How Palatable Food Disrupts Appetite Regulation." Basic & Clinical Pharmacology & Toxicology **97**(2): 61-73.
Erritzoe, D., V. G. Frokjaer, M. T. Haahr, J. Kalbitzer, C. Svarer, K. K. Holst, D. Hansen, T. L. Jernigan, S. Lehel and G. M. Knudsen (2010). "Cerebral serotonin transporter binding is inversely related to body mass index." Neuroimage **52**(1): 284-289.
Erritzoe, D., V. G. Frokjaer, S. Haugbol, L. Marner, C. Svarer, K. Holst, W. F. Baaré, P. M. Rasmussen, J. Madsen and O. B. Paulson (2009). "Brain serotonin 2A receptor binding: relations to body mass index, tobacco and alcohol use." Neuroimage **46**(1): 23-30.
Fallon, D. (1965). "Eatometer: A device for continuous recording of free-feeding behavior." Science **148**(3672): 977-978.
Fanselow, M. S. (1986). "Associative vs topographical accounts of the immediate shock-freezing deficit in rats: implications for the response selection rules governing species-specific defensive reactions." Learning and Motivation **17**(1): 16-39.
Fanselow, M. S. (1994). "Neural organization of the defensive behavior system responsible for fear." Psychonomic bulletin & review **1**(4): 429-438.
Fanselow, M. S. and R. C. Bolles (1979). "Naloxone and shock-elicited freezing in the rat." Journal of comparative and physiological psychology **93**(4): 736.
Fanselow, M. S. and S. R. Sterlace (2014). "Pavlovian Fear Conditioning." The Wiley Blackwell Handbook of Operant and Classical Conditioning: 117.
Fassino, S., G. A. Daga, A. Piero and N. Delsedime (2007). Psychological factors affecting eating disorders. Psychological Factors Affecting Medical Conditions, Karger Publishers. **28:** 141-168.
Fenselau, H., J. N. Campbell, A. M. Verstegen, J. C. Madara, J. Xu, B. P. Shah, J. M. Resch, Z. Yang, Y. Mandelblat-Cerf and Y. Livneh (2017). "A rapidly acting glutamatergic ARC→ PVH satiety circuit postsynaptically regulated by α -MSH." Nature neuroscience **20**(1): 42.
Filipkowski, R. K., M. Rydz, B. Berdel, J. Morys and L. Kaczmarek (2000). "Tactile experience induces c-fos expression in rat barrel cortex." Learning & Memory **7**(2): 116-122.
Finch, L. E., L. Tiongco-Hofschneider and A. J. Tomiyama (2019). Stress-induced eating dampens physiological and behavioral stress responses. Nutrition in the prevention and treatment of abdominal obesity, Elsevier**:** 175-187.
Flagel, S. B., J. J. Clark, T. E. Robinson, L. Mayo, A. Czuj, I. Willuhn, C. A. Akers, S. M. Clinton, P. E. Phillips and H. Akil (2011). "A selective role for dopamine in stimulus–reward learning." Nature **469**(7328): 53.
Flavell, S. W., C. W. Cowan, T.-K. Kim, P. L. Greer, Y. Lin, S. Paradis, E. C. Griffith, L. S. Hu, C. Chen and M. E. Greenberg (2006). "Activity-dependent regulation of MEF2 transcription factors suppresses excitatory synapse number." Science **311**(5763): 1008-1012.
Floresco, S. B. (2013). "Prefrontal dopamine and behavioral flexibility: shifting from an "inverted-U" toward a family of functions." Frontiers in neuroscience **7**: 62.
Floresco, S. B. (2015). "The nucleus accumbens: an interface between cognition, emotion, and action." Annual review of psychology **66**: 25-52.
Floresco, S. B., J. R. S. Onge, S. Ghods-Sharifi and C. A. Winstanley (2008). "Cortico-limbic-striatal circuits subserving different forms of cost-benefit decision making." Cognitive, Affective, & Behavioral Neuroscience **8**(4): 375-389.
Floresco, S. B., A. R. West, B. Ash, H. Moore and A. A. Grace (2003). "Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission." Nature neuroscience **6**(9): 968.
Foster, M. T., J. P. Warne, A. B. Ginsberg, H. F. Horneman, N. C. Pecoraro, S. F. Akana and M. F. Dallman (2008). "Palatable foods, stress, and energy stores sculpt corticotropin-releasing factor, adrenocorticotropin, and corticosterone concentrations after restraint." Endocrinology **150**(5): 2325-2333.
Friederich, H.-C., R. Uher, S. Brooks, V. Giampietro, M. Brammer, S. C. Williams, W. Herzog, J. Treasure and I. C. Campbell (2007). "I' m not as slim as that girl: neural bases of body shape self-comparison to media images." Neuroimage **37**(2): 674-681.
Frijda, N. H. (1986). The emotions, Cambridge University Press.
Furlong, T. and P. Carrive (2007). "Neurotoxic lesions centered on the perifornical hypothalamus abolish the cardiovascular and behavioral responses of conditioned fear to context but not of restraint." Brain research **1128**: 107-119.

Furlong, T. M., H. K. Jayaweera, B. W. Balleine and L. H. Corbit (2014). "Binge-like consumption of a palatable food accelerates habitual control of behavior and is dependent on activation of the dorsolateral striatum." Journal of Neuroscience **34**(14): 5012-5022.
Gallo, F. T., C. Katche, J. F. Morici, J. H. Medina and N. V. Weisstaub (2018). "Immediate early genes, memory and psychiatric disorders: focus on c-Fos, Egr1 and Arc." Frontiers in behavioral neuroscience **12**: 79.
Gan, J. O., M. E. Walton and P. E. Phillips (2010). "Dissociable cost and benefit encoding of future rewards by mesolimbic dopamine." Nature neuroscience **13**(1): 25.
Gaykema, R., X.-M. T. Nguyen, J. M. Boehret, P. S. Lambeth, J. Joy-Gaba, D. M. Warthen and M. M. Scott (2014). "Characterization of excitatory and inhibitory neuron activation in the mouse medial prefrontal cortex following palatable food ingestion and food driven exploratory behavior." Frontiers in neuroanatomy **8**: 60.
Gearhardt, A. N., W. R. Corbin and K. D. Brownell (2009). "Preliminary validation of the Yale food addiction scale." Appetite **52**(2): 430-436.
Gearhardt, A. N., S. Yokum, P. T. Orr, E. Stice, W. R. Corbin and K. D. Brownell (2011). "Neural correlates of food addiction." Archives of general psychiatry **68**(8): 808-816.
Gehlert, D. (1999). "Role of hypothalamic neuropeptide Y in feeding and obesity." Neuropeptides **33**(5): 329-338.
Georgescu, D., R. M. Sears, J. D. Hommel, M. Barrot, C. A. Bolanos, D. J. Marsh, M. A. Bednarek, J. A. Bibb, E. Maratos-Flier and E. J. Nestler (2005). "The hypothalamic neuropeptide melanin-concentrating hormone acts in the nucleus accumbens to modulate feeding behavior and forced-swim performance." Journal of Neuroscience **25**(11): 2933-2940.
Gibson, E. L., A. J. Kennedy and G. Curzon (1993). "d-Fenfluramine-and d-norfenfluramine-induced hypophagia: differential mechanisms and involvement of postsynaptic 5-HT receptors." European journal of pharmacology **242**(1): 83-90.
Giraudo, S. Q., C. J. Billington and A. S. Levine (1998). "Effects of the opioid antagonist naltrexone on feeding induced by DAMGO in the central nucleus of the amygdala and in the paraventricular nucleus in the rat." Brain research **782**(1-2): 18-23.
Giraudo, S. Q., C. M. Kotz, C. J. Billington and A. S. Levine (1998). "Association between the amygdala and nucleus of the solitary tract in μ -opioid induced feeding in the rat." Brain research **802**(1-2): 184-188.
Giustino, T. F. and S. Maren (2015). "The role of the medial prefrontal cortex in the conditioning and extinction of fear." Frontiers in behavioral neuroscience **9**: 298.
Gonzalez, J. A., L. T. Jensen, P. Iordanidou, M. Strom, L. Fugger and D. Burdakov (2016). "Inhibitory interplay between orexin neurons and eating." Current Biology **26**(18): 2486-2491.
Gonzalez, M. C., C. P. Kramar, M. Tomaiuolo, C. Katche, N. Weisstaub, M. Cammarota and J. H. Medina (2014). "Medial prefrontal cortex dopamine controls the persistent storage of aversive memories." Frontiers in behavioral neuroscience **8**: 408.
Goto, M., H. Arima, M. Watanabe, M. Hayashi, R. Banno, I. Sato, H. Nagasaki and Y. Oiso (2006). "Ghrelin increases neuropeptide Y and agouti-related peptide gene expression in the arcuate nucleus in rat hypothalamic organotypic cultures." Endocrinology **147**(11): 5102-5109.
Gottfried, J. A., J. O'Doherty and R. J. Dolan (2003). "Encoding predictive reward value in human amygdala and orbitofrontal cortex." Science **301**(5636): 1104-1107.
Grace, A. A. (1991). "Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia." Neuroscience **41**(1): 1-24.
Grace, A. A., S. B. Floresco, Y. Goto and D. J. Lodge (2007). "Regulation of firing of dopaminergic neurons and control of goal-directed behaviors." Trends in neurosciences **30**(5): 220-227.
Gray, T. S., R. A. Piechowski, J. M. Yracheta, P. A. Rittenhouse, C. L. Bethea and L. D. Van de Kar (1993). "Ibotenic acid lesions in the bed nucleus of the stria terminalis attenuate conditioned stress-induced increases in prolactin, ACTH and corticosterone." Neuroendocrinology **57**(3): 517-524.
Greenberg, M. E., L. Greene and E. Ziff (1985). "Nerve growth factor and epidermal growth factor induce rapid transient changes in proto-oncogene transcription in PC12 cells." Journal of Biological Chemistry **260**(26): 14101-14110.
Greenberg, M. E. and E. B. Ziff (1984). "Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene." Nature **311**(5985): 433.
Greenberg, M. E., E. B. Ziff and L. A. Greene (1986). "Stimulation of neuronal acetylcholine receptors induces rapid gene transcription." Science **234**(4772): 80-83.

Grilo, C., M. White, G. Wilson, R. Gueorguieva and R. Masheb (2012). "Rapid response predicts 12-month post-treatment outcomes in binge-eating disorder: theoretical and clinical implications." Psychological Medicine **42**(4): 807-817.
Grilo, C. M. and R. M. Masheb (2001). "Childhood psychological, physical, and sexual maltreatment in outpatients with binge eating disorder: Frequency and associations with gender, obesity, and eating‐related psychopathology." Obesity Research **9**(5): 320-325.
Grilo, C. M., D. L. Reas and J. E. Mitchell (2016). "Combining pharmacological and psychological treatments for binge eating disorder: current status, limitations, and future directions." Current psychiatry reports **18**(6): 55.
Grimm, J. W., A. M. Fyall and D. P. Osincup (2005). "Incubation of sucrose craving: effects of reduced training and sucrose pre-loading." Physiology & behavior **84**(1): 73-79.
Grippo, R. M., Q. Tang, Q. Zhang, S. R. Chadwick, Y. Gao, E. B. Altherr, L. Sipe, A. M. Purohit, N. M. Purohit and M. D. Sunkara (2020). "Dopamine Signaling in the Suprachiasmatic Nucleus Enables Weight Gain Associated with Hedonic Feeding." Current Biology.
Haahr, M. E., P. M. Rasmussen, K. Madsen, L. Marner, C. Ratner, N. Gillings, W. F. Baaré and G. M. Knudsen (2012). "Obesity is associated with high serotonin 4 receptor availability in the brain reward circuitry." Neuroimage **61**(4): 884-888.
Hagan, M., P. Wauford, P. Chandler, L. Jarrett, R. Rybak and K. Blackburn (2002). "A new animal model of binge eating: key synergistic role of past caloric restriction and stress." Physiology & behavior **77**(1): 45-54.
Hagan, M. M., P. C. Chandler, P. K. Wauford, R. J. Rybak and K. D. Oswald (2003). "The role of palatable food and hunger as trigger factors in an animal model of stress induced binge eating." international Journal of Eating disorders **34**(2): 183-197.
Hajnal, A. and R. Norgren (2002). "Repeated access to sucrose augments dopamine turnover in the nucleus accumbens." Neuroreport **13**(17): 2213-2216.
Halaas, J. L., K. S. Gajiwala, M. Maffei, S. L. Cohen, B. T. Chait, D. Rabinowitz, R. L. Lallone, S. K. Burley and J. M. Friedman (1995). "Weight-reducing effects of the plasma protein encoded by the obese gene." Science **269**(5223): 543-546.
Haleem, D. (1993). "Serotonergic neurotransmission in the regulation of appetite: a receptor approach." Pakistan journal of pharmaceutical sciences **6**(1): 89-96.
Halford, J. C., J. A. Harrold, E. J. Boyland, C. L. Lawton and J. E. Blundell (2007). "Serotonergic drugs." Drugs **67**(1): 27-56.
Hancock, S. and V. Grant (2009). "Early maternal separation increases symptoms of activity-based anorexia in male and female rats." Journal of Experimental Psychology: Animal Behavior Processes **35**(3): 394.
Hardie, L. J., D. V. Rayner, S. Holmes and P. Trayhurn (1996). "Circulating leptin levels are modulated by fasting, cold exposure and insulin administration in lean but not Zucker (fa/fa) rats as measured by ELISA." Biochemical and biophysical research communications **223**(3): 660-665.
Hardingham, G. E., S. Chawla, C. M. Johnson and H. Bading (1997). "Distinct functions of nuclear and cytoplasmic calcium in the control of gene expression." Nature **385**(6613): 260.
Harris, G. C. and G. Aston-Jones (2006). "Arousal and reward: a dichotomy in orexin function." Trends in neurosciences **29**(10): 571-577.
Heim, C. and E. B. Binder (2012). "Current research trends in early life stress and depression: Review of human studies on sensitive periods, gene‐environment interactions, and epigenetics." Experimental neurology **233**(1): 102-111.
Heim, C. and C. B. Nemeroff (1999). "The impact of early adverse experiences on brain systems involved in the pathophysiology of anxiety and affective disorders." Biological psychiatry **46**(11): 1509-1522.
Heisler, L. K., R. B. Kanarek and B. Homoleski (1999). "Reduction of fat and protein intakes but not carbohydrate intake following acute and chronic fluoxetine in female rats." Pharmacology Biochemistry and Behavior **63**(3): 377-385.
Herman, J. P., W. E. Cullinan, D. R. Ziegler and J. G. Tasker (2002). "Role of the paraventricular nucleus microenvironment in stress integration." European Journal of Neuroscience **16**(3): 381-385.
Hernandez-Mesa, N., Z. Mamedov and J. Bureš (1985). "Operant control of the pattern of licking in rats." Experimental brain research **58**(1): 117-124.
Herry, C. and R. Garcia (2002). "Prefrontal cortex long-term potentiation, but not long-term depression, is associated with the maintenance of extinction of learned fear in mice." Journal of Neuroscience **22**(2): 577-583.

Herry, C. and J. P. Johansen (2014). "Encoding of fear learning and memory in distributed neuronal circuits." Nat Neurosci **17**(12): 1644-1654.
Hilgenberg, L. G. and M. A. Smith (2004). "Agrin signaling in cortical neurons is mediated by a tyrosine kinase‐dependent increase in intracellular Ca2+ that engages both CaMKII and MAPK signal pathways." Journal of neurobiology **61**(3): 289-300.
Hoegg, J. and J. W. Alba (2006). "Taste perception: More than meets the tongue." Journal of Consumer Research **33**(4): 490-498.
Holt, C. E. and E. M. Schuman (2013). "The central dogma decentralized: new perspectives on RNA function and local translation in neurons." Neuron **80**(3): 648-657.
Honkaniemi, J., J. Kononen, T. Kainu, I. Pyykönen and M. Pelto-Huikko (1994). "Induction of multiple immediate early genes in rat hypothalamic paraventricular nucleus after stress." Molecular brain research **25**(3-4): 234-241.
Horsager, C., E. Færk, M. B. Lauritsen and S. D. Østergaard (2020). "Validation of the Yale Food Addiction Scale 2.0 and estimation of the population prevalence of food addiction." Clinical Nutrition.
Hudson, A. E. (2018). Chapter Twelve - Genetic Reporters of Neuronal Activity: c-Fos and G-CaMP6. Methods in Enzymology. R. G. Eckenhoff and I. J. Dmochowski, Academic Press. **603:** 197-220.
Hugues, S., A. Chessel, I. Lena, R. Marsault and R. Garcia (2006). "Prefrontal infusion of PD098059 immediately after fear extinction training blocks extinction‐associated prefrontal synaptic plasticity and decreases prefrontal ERK2 phosphorylation." Synapse **60**(4): 280-287.
Hulsey, M. G. and R. J. Martin (1991). "A system for automated recording and analysis of feeding behavior." Physiology & behavior **50**(2): 403-408.
Humphries, M. D. and T. J. Prescott (2010). "The ventral basal ganglia, a selection mechanism at the crossroads of space, strategy, and reward." Progress in neurobiology **90**(4): 385-417.
Iacovino, J. M., D. M. Gredysa, M. Altman and D. E. Wilfley (2012). "Psychological treatments for binge eating disorder." Current psychiatry reports **14**(4): 432-446.
Ida, T., K. Nakahara, T. Murakami, R. Hanada, M. Nakazato and N. Murakami (2000). "Possible involvement of orexin in the stress reaction in rats." Biochemical and biophysical research communications **270**(1): 318-323.
Ikemoto, S. (2007). "Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens‐olfactory tubercle complex." Brain research reviews **56**(1): 27-78.
Iwata, J., J. E. Ledoux and D. J. Reis (1986). "Destruction of intrinsic neurons in the lateral hypothalamus disrupts the classical conditioning of autonomic but not behavioral emotional responses in the rat." Brain Research **368**(1): 161-166.
Jahng, J. W. (2011). "An animal model of eating disorders associated with stressful experience in early life." Hormones and behavior **59**(2): 213-220.
Jasper, M. S. and W. C. Engeland (1997). "Splanchnicotomy increases adrenal sensitivity to ACTH in nonstressed rats." American Journal of Physiology-Endocrinology And Metabolism **273**(2): E363-E368.
Jean, A., G. Conductier, C. Manrique, C. Bouras, P. Berta, R. Hen, Y. Charnay, J. Bockaert and V. Compan (2007). "Anorexia induced by activation of serotonin 5-HT4 receptors is mediated by increases in CART in the nucleus accumbens." Proceedings of the National Academy of Sciences **104**(41): 16335-16340.
Jerlhag, E., E. Egecioglu, S. L. Dickson, A. Douhan, L. Svensson and J. A. Engel (2007). "Preclinical study: ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens." Addiction biology **12**(1): 6-16.
Jerlhag, E., E. Egecioglu, S. L. Dickson and J. A. Engel (2010). "Ghrelin receptor antagonism attenuates cocaine-and amphetamine-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference." Psychopharmacology **211**(4): 415-422.
Jezzini, A., L. Mazzucato, G. La Camera and A. Fontanini (2013). "Processing of hedonic and chemosensory features of taste in medial prefrontal and insular networks." Journal of Neuroscience **33**(48): 18966-18978.
Jimerson, D. C., M. D. Lesem, W. H. Kaye and T. D. Brewerton (1992). "Low serotonin and dopamine metabolite concentrations in cerebrospinal fluid from bulimic patients with frequent binge episodes." Archives of General Psychiatry **49**(2): 132-138.
Johansen, J. P., C. K. Cain, L. E. Ostroff and J. E. LeDoux (2011). "Molecular mechanisms of fear learning and memory." Cell **147**(3): 509-524.
Johnson, P. M. and P. J. Kenny (2010). "Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats." Nature neuroscience **13**(5): 635-641.

Kandel, E. R., J. H. Schwartz, T. M. Jessell, D. o. Biochemistry, M. B. T. Jessell, S. Siegelbaum and A. Hudspeth (2000). Principles of neural science, McGraw-hill New York.
Keefer, S. E., S. Cole and G. D. Petrovich (2016). "Orexin/hypocretin receptor 1 signaling mediates Pavlovian cue-food conditioning and extinction." Physiology & behavior **162**: 27-36.
Kelley, A. E., B. A. Baldo, W. E. Pratt and M. J. Will (2005). "Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward." Physiology & behavior **86**(5): 773-795.
Kennedy, G. (1953). "The role of depot fat in the hypothalamic control of food intake in the rat." Proceedings of the Royal Society of London. Series B-Biological Sciences **140**(901): 578-592.
Kennett, G. and G. Curzon (1988). "Evidence that hypophagia induced bymCPP and TFMPP requires 5-HT 1C and 5-HT 1B receptors; hypophagia induced by RU 24969 only requires 5-HT 1B receptors." Psychopharmacology **96**(1): 93-100.
Kerem, L., N. Hadjikhani, L. Holsen, E. A. Lawson and F. Plessow (2019). "Oxytocin reduces the functional connectivity between brain regions involved in eating behavior in men with overweight and obesity." International Journal of Obesity: 1-10.
Kessler, R. C., P. A. Berglund, W. T. Chiu, A. C. Deitz, J. I. Hudson, V. Shahly, S. Aguilar-Gaxiola, J. Alonso, M. C. Angermeyer and C. Benjet (2013). "The prevalence and correlates of binge eating disorder in the World Health Organization World Mental Health Surveys." Biological psychiatry **73**(9): 904-914.
Kessler, R. M., P. H. Hutson, B. K. Herman and M. N. Potenza (2016). "The neurobiological basis of binge-eating disorder." Neuroscience & Biobehavioral Reviews **63**(Supplement C): 223-238.
Kessler, R. M., P. H. Hutson, B. K. Herman and M. N. Potenza (2016). "The neurobiological basis of binge-eating disorder." Neuroscience & Biobehavioral Reviews **63**: 223-238.
Kim, E. J., O. Horovitz, B. A. Pellman, L. M. Tan, Q. Li, G. Richter-Levin and J. J. Kim (2013). "Dorsal periaqueductal gray-amygdala pathway conveys both innate and learned fear responses in rats." Proceedings of the National Academy of Sciences **110**(36): 14795-14800.
Kim, T.-K., M. Hemberg, J. M. Gray, A. M. Costa, D. M. Bear, J. Wu, D. A. Harmin, M. Laptewicz, K. Barbara-Haley and S. Kuersten (2010). "Widespread transcription at neuronal activity-regulated enhancers." Nature **465**(7295): 182.
Klatzkin, R. R., A. Baldassaro and E. Hayden (2018). "The impact of chronic stress on the predictors of acute stress-induced eating in women." Appetite **123**: 343-351.
Klump, K. L., K. M. Culbert and C. L. Sisk (2017). "Sex differences in binge eating: Gonadal hormone effects across development." Annual review of clinical psychology **13**: 183-207.
Kovács, K. J. (1998). "Invited review c-Fos as a transcription factor: a stressful (re) view from a functional map." Neurochemistry international **33**(4): 287-297.
Kroemer, N. B., L. Krebs, A. Kobiella, O. Grimm, M. Pilhatsch, M. Bidlingmaier, U. S. Zimmermann and M. N. Smolka (2013). "Fasting levels of ghrelin covary with the brain response to food pictures." Addiction biology **18**(5): 855-862.
Krysiak, R., E. Obuchowicz and Z. S. Herman (1999). "Interactions between the neuropeptide Y system and the hypothalamic-pituitary-adrenal axis." European Journal of Endocrinology **140**(2): 130-136.
Kudo, T., M. Uchigashima, T. Miyazaki, K. Konno, M. Yamasaki, Y. Yanagawa, M. Minami and M. Watanabe (2012). "Three types of neurochemical projection from the bed nucleus of the stria terminalis to the ventral tegmental area in adult mice." Journal of Neuroscience **32**(50): 18035-18046.
Kuikka, J. T., L. Tammela, L. Karhunen, A. Rissanen, K. A. Bergström, H. Naukkarinen, E. Vanninen, J. Karhu, R. Lappalainen and E. Repo-Tiihonen (2001). "Reduced serotonin transporter binding in binge eating women." Psychopharmacology **155**(3): 310-314.
Kuwaki, T. (2011). "Orexin links emotional stress to autonomic functions." Autonomic Neuroscience **161**(1-2): 20-27.
la Fleur, S. E., H. Houshyar, M. Roy and M. F. Dallman (2005). "Choice of lard, but not total lard calories, damps adrenocorticotropin responses to restraint." Endocrinology **146**(5): 2193-2199.
Landgren, S., J. A. Simms, P. Hyytiä, J. A. Engel, S. E. Bartlett and E. Jerlhag (2012). "Ghrelin receptor (GHS‐R1A) antagonism suppresses both operant alcohol self‐administration and high alcohol consumption in rats." Addiction biology **17**(1): 86-94.
Lattimore, P. and N. Caswell (2004). "Differential effects of active and passive stress on food intake in restrained and unrestrained eaters." Appetite **42**(2): 167-173.
Lau, L. F. and D. Nathans (1987). "Expression of a set of growth-related immediate early genes in BALB/c 3T3 cells: coordinate regulation with c-fos or c-myc." Proceedings of the National Academy of Sciences **84**(5): 1182-1186.
Lawson, E. A. (2017). "The effects of oxytocin on eating behaviour and metabolism in humans." Nature Reviews Endocrinology **13**(12): 700.

Leaton, R. N. and G. S. Borszcz (1985). "Potentiated startle: its relation to freezing and shock intensity in rats." Journal of Experimental Psychology: Animal Behavior Processes **11**(3): 421.
LeDoux, J. E. (2000). "Emotion circuits in the brain." Annual review of neuroscience **23**(1): 155-184.
LeDoux, J. E., J. Iwata, P. Cicchetti and D. Reis (1988). "Different projections of the central amygdaloid nucleus mediate autonomic and behavioral correlates of conditioned fear." Journal of Neuroscience **8**(7): 2517-2529.
Lee, H. J., J.-S. Choi, T. H. Brown and J. J. Kim (2001). "Amygdalar NMDA receptors are critical for the expression of multiple conditioned fear responses." Journal of Neuroscience **21**(11): 4116-4124.
Lee, J.-H., H. J. Kim, J. G. Kim, V. Ryu, B.-T. Kim, D.-W. Kang and J. W. Jahng (2007). "Depressive behaviors and decreased expression of serotonin reuptake transporter in rats that experienced neonatal maternal separation." Neuroscience research **58**(1): 32-39.
Levine, A. S. and J. Morley (1984). "Neuropeptide Y: a potent inducer of consummatory behavior in rats." Peptides **5**(6): 1025-1029.
Levine, A. S. and J. E. Morley (1981). "Stress-induced eating in rats." American Journal of Physiology-Regulatory, Integrative and Comparative Physiology **241**(1): R72-R76.
Li, S. S. and G. P. McNally (2015). "A role of nucleus accumbens dopamine receptors in the nucleus accumbens core, but not shell, in fear prediction error." Behavioral neuroscience **129**(4): 450.
Lin, P.-Y., S.-P. Wang, M.-Y. Tai and Y.-F. Tsai (2010). "Differential involvement of medial prefrontal cortex and basolateral amygdala extracellular signal-regulated kinase in extinction of conditioned taste aversion is dependent on different intervals of extinction following conditioning." Neuroscience **171**(1): 125-133.
Lin, X., C. A. Itoga, S. Taha, M. H. Li, R. Chen, K. Sami, F. Berton, W. Francesconi and X. Xu (2018). "c-Fos mapping of brain regions activated by multi-modal and electric foot shock stress." Neurobiology of stress **8**: 92-102.
Lipp, O. V. (2006). "Human fear learning: Contemporary procedures and measurement." Fear and learning: From basic processes to clinical implications(2001): 37-51.
Liu, C. M. and S. E. Kanoski (2018). "Homeostatic and non-homeostatic controls of feeding behavior: Distinct vs. common neural systems." Physiology & behavior **193**: 223-231.
Lokwan, S., P. Overton, M. Berry and D. Clark (1999). "Stimulation of the pedunculopontine tegmental nucleus in the rat produces burst firing in A9 dopaminergic neurons." Neuroscience **92**(1): 245-254.
Luchkina, N. V. and V. Y. Bolshakov (2019). "Mechanisms of fear learning and extinction: synaptic plasticity – fear memory connection." Psychopharmacology **236**(1): 163-182.
Lutter, M., I. Sakata, S. Osborne-Lawrence, S. A. Rovinsky, J. G. Anderson, S. Jung, S. Birnbaum, M. Yanagisawa, J. K. Elmquist and E. J. Nestler (2008). "The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress." Nature neuroscience **11**(7): 752.
Luyten, L., S. Hendrickx, S. Raymaekers, L. Gabriëls and B. Nuttin (2016). "Electrical stimulation in the bed nucleus of the stria terminalis alleviates severe obsessive-compulsive disorder." Molecular psychiatry **21**(9): 1272.
Macht, M. and J. Mueller (2007). "Immediate effects of chocolate on experimentally induced mood states." Appetite **49**(3): 667-674.
Madrid, J. A., C. Lopez-Bote and E. Martín (1993). "Effect of neonatal androgenization on the circadian rhythm of feeding behavior in rats." Physiology & behavior **53**(2): 329-335.
Maniam, J. and M. J. Morris (2012). "The link between stress and feeding behaviour." Neuropharmacology **63**(1): 97-110.
Manwaring, J. L., L. Green, J. Myerson, M. J. Strube and D. E. Wilfley (2011). "Discounting of various types of rewards by women with and without binge eating disorder: Evidence for general rather than specific differences." The Psychological Record **61**(4): 561-582.
Mardinly, A., I. Spiegel, A. Patrizi, E. Centofante, J. Bazinet, C. Tzeng, C. Mandel-Brehm, D. Harmin, H. Adesnik and M. Fagiolini (2016). "Sensory experience regulates cortical inhibition by inducing IGF1 in VIP neurons." Nature **531**(7594): 371.
Mathes, W. F., K. A. Brownley, X. Mo and C. M. Bulik (2009). "The biology of binge eating." Appetite **52**(3): 545-553.
Mayer, J. (1955). "Regulation of energy intake and the body weight: the glucostatic theory and the lipostatic hypothesis." Annals of the New York Academy of sciences **63**(1): 15-43.
McKinsey, T. A., C. L. Zhang and E. N. Olson (2002). "MEF2: a calcium-dependent regulator of cell division, differentiation and death." Trends in biochemical sciences **27**(1): 40-47.
McMinn, J. E., D. K. Sindelar, P. J. Havel and M. W. Schwartz (2000). "Leptin Deficiency Induced by Fasting Impairs the Satiety Response to Cholecystokinin**This work was supported by grants from the NIH (DK-12829, DK-52989,

and NS-32272) and by the Royalty Research Fund, the Diabetes Endocrinology Research Center, and the Clinical Nutrition Research Unit of the University of Washington." Endocrinology **141**(12): 4442-4448.
McNally, G. P. (2005). "Facilitation of fear extinction by midbrain periaqueductal gray infusions of RB101 (S), an inhibitor of enkephalin-degrading enzymes." Behavioral neuroscience **119**(6): 1672.
Mera, P., M. Ferron and I. Mosialou (2018). "Regulation of energy metabolism by bone-derived hormones." Cold Spring Harbor perspectives in medicine **8**(6): a031666.
Michaud, C., J. Kahn, N. Musse, C. Burlet, J. Nicolas and L. Mejean (1990). "Relationships between a critical life event and eating behaviour in high - school students." Stress Medicine **6**(1): 57-64.
Milad, M., I. Vidal-Gonzalez and G. Quirk (2004). "Electrical stimulation of medial prefrontal cortex reduces conditioned fear in a temporally specific manner." Behavioral neuroscience **118**(2): 389.
Milad, M. R., B. T. Quinn, R. K. Pitman, S. P. Orr, B. Fischl and S. L. Rauch (2005). "Thickness of ventromedial prefrontal cortex in humans is correlated with extinction memory." Proceedings of the National Academy of Sciences **102**(30): 10706-10711.
Milad, M. R. and G. J. Quirk (2002). "Neurons in medial prefrontal cortex signal memory for fear extinction." Nature **420**(6911): 70.
Moore, C. F., V. Sabino, G. F. Koob and P. Cottone (2017). "Neuroscience of Compulsive Eating Behavior." Frontiers in Neuroscience **11**.
Moore, K. M., W. L. Oelberg, M. R. Glass, M. D. Johnson, L. E. Been and R. L. Meisel (2019). "Glutamate afferents from the medial prefrontal cortex mediate nucleus accumbens activation by female sexual behavior." Frontiers in behavioral neuroscience **13**: 227.
Moorman, D. E., M. H. James, E. M. McGlinchey and G. Aston-Jones (2015). "Differential roles of medial prefrontal subregions in the regulation of drug seeking." Brain research **1628**: 130-146.
Morrison, S. E. and C. D. Salzman (2010). "Re-valuing the amygdala." Current opinion in neurobiology **20**(2): 221-230.
Morton, G., D. Cummings, D. Baskin, G. Barsh and M. Schwartz (2006). "Central nervous system control of food intake and body weight." Nature **443**(7109): 289-295.
Morton, G. and M. Schwartz (2001). "The NPY/AgRP neuron and energy homeostasis." International Journal of Obesity **25**(5): S56-S62.
Morton, G. J., J. E. Blevins, D. L. Williams, K. D. Niswender, R. W. Gelling, C. J. Rhodes, D. G. Baskin and M. W. Schwartz (2005). "Leptin action in the forebrain regulates the hindbrain response to satiety signals." The Journal of clinical investigation **115**(3): 703-710.
Morton, G. J., T. H. Meek and M. W. Schwartz (2014). "Neurobiology of food intake in health and disease." Nat Rev Neurosci **15**(6): 367-378.
Myers, B., J. M. McKlveen and J. P. Herman (2012). "Neural regulation of the stress response: the many faces of feedback." Cellular and molecular neurobiology **32**(5): 683-694.
N Gearhardt, A., C. Davis, R. Kuschner and K. D Brownell (2011). "The addiction potential of hyperpalatable foods." Current drug abuse reviews **4**(3): 140-145.
Nakamura, K. and Y. Nakamura (2018). "Hunger and Satiety Signaling: Modeling Two Hypothalamomedullary Pathways for Energy Homeostasis." BioEssays **40**(8): 1700252.
Nasser, J. A., M. E. Gluck and A. Geliebter (2004). "Impulsivity and test meal intake in obese binge eating women." Appetite **43**(3): 303-307.
Newton, A. J., S. Hess, L. Paeger, M. C. Vogt, J. Fleming Lascano, E. A. Nillni, J. C. Brüning, P. Kloppenburg and A. W. Xu (2013). "AgRP innervation onto POMC neurons increases with age and is accelerated with chronic high-fat feeding in male mice." Endocrinology **154**(1): 172-183.
Nieh, E. H., G. A. Matthews, S. A. Allsop, K. N. Presbrey, C. A. Leppla, R. Wichmann, R. Neve, C. P. Wildes and K. M. Tye (2015). "Decoding neural circuits that control compulsive sucrose seeking." Cell **160**(3): 528-541.
Nieh, E. H., C. M. Vander Weele, G. A. Matthews, K. N. Presbrey, R. Wichmann, C. A. Leppla, E. M. Izadmehr and K. M. Tye (2016). "Inhibitory input from the lateral hypothalamus to the ventral tegmental area disinhibits dopamine neurons and promotes behavioral activation." Neuron **90**(6): 1286-1298.
Nieh, Edward H., Caitlin M. Vander Weele, Gillian A. Matthews, Kara N. Presbrey, R. Wichmann, Christopher A. Leppla, Ehsan M. Izadmehr and Kay M. Tye (2016). "Inhibitory Input from the Lateral Hypothalamus to the Ventral Tegmental Area Disinhibits Dopamine Neurons and Promotes Behavioral Activation." Neuron **90**(6): 1286-1298.
Nieuwenhuizen, A. G. and F. Rutters (2008). "The hypothalamic-pituitary-adrenal-axis in the regulation of energy balance." Physiology & behavior **94**(2): 169-177.

Nishitani, N. and H. Sakakibara (2006). "Relationship of obesity to job stress and eating behavior in male Japanese workers." International journal of obesity **30**(3): 528.
Nonogaki, K., K. Nozue and Y. Oka (2006). "Increased hypothalamic 5-HT2A receptor gene expression and effects of pharmacologic 5-HT2A receptor inactivation in obese Ay mice." Biochemical and biophysical research communications **351**(4): 1078-1082.
Norgren, R., A. Hajnal and S. Mungarndee (2006). "Gustatory reward and the nucleus accumbens." Physiology & behavior **89**(4): 531-535.
Norman, C., M. Runswick, R. Pollock and R. Treisman (1988). "Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-fos serum response element." Cell **55**(6): 989-1003.
Nummenmaa, L. and L. Tuominen (2018). "Opioid system and human emotions." British Journal of Pharmacology **175**(14): 2737-2749.
O'Connor, E. C., Y. Kremer, S. Lefort, M. Harada, V. Pascoli, C. Rohner and C. Lüscher (2015). "Accumbal D1R neurons projecting to lateral hypothalamus authorize feeding." Neuron **88**(3): 553-564.
Onge, J. R. S., S. Ahn, A. G. Phillips and S. B. Floresco (2012). "Dynamic fluctuations in dopamine efflux in the prefrontal cortex and nucleus accumbens during risk-based decision making." Journal of Neuroscience **32**(47): 16880-16891.
Oswald, K. D., D. L. Murdaugh, V. L. King and M. M. Boggiano (2011). "Motivation for palatable food despite consequences in an animal model of binge eating." International Journal of Eating Disorders **44**(3): 203-211.
Palavras, M., P. Hay and A. Claudino (2017). "The efficacy of psychological therapies in reducing weight and binge eating in people with bulimia nervosa and binge eating disorder who are overweight or obese—A critical synthesis and meta-analyses." Nutrients **9**(3): 299.
Palmiter, R. D. (2017). "Fast-acting neurons that suppress appetite." Nature neuroscience **20**(1): 2.
Pecoraro, N., F. Reyes, F. Gomez, A. Bhargava and M. F. Dallman (2004). "Chronic stress promotes palatable feeding, which reduces signs of stress: feedforward and feedback effects of chronic stress." Endocrinology **145**(8): 3754-3762.
Perez-Cadahia, B., B. Drobic and J. R. Davie (2011). "Activation and function of immediate-early genes in the nervous system." Biochemistry and Cell Biology **89**(1): 61-73.
Petrovich, G. and L. Swanson (1997). "Projections from the lateral part of the central amygdalar nucleus to the postulated fear conditioning circuit." Brain research **763**(2): 247-254.
Petrovich, G. D. and M. A. Lougee (2011). "Sex differences in fear-induced feeding cessation: Prolonged effect in female rats." Physiology & behavior **104**(5): 996-1001.
Petrovich, G. D., C. A. Ross, P. Mody, P. C. Holland and M. Gallagher (2009). "Central, but not basolateral, amygdala is critical for control of feeding by aversive learned cues." Journal of Neuroscience **29**(48): 15205-15212.
Petrovich, G. D., C. A. Ross, P. Mody, P. C. Holland and M. Gallagher (2009). "Central, but not basolateral, amygdala is critical for control of feeding by aversive learned cues." The Journal of Neuroscience **29**(48): 15205-15212.
Piazza, P. V., F. Rougé-Pont, V. Deroche, S. Maccari, H. Simon and M. Le Moal (1996). "Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission." Proceedings of the national Academy of Sciences **93**(16): 8716-8720.
Plotsky, P. M., T. O. Bruhn and W. Vale (1985). "Evidence for multifactor regulation of the adrenocorticotropin secretory response to hemodynamic stimuli." Endocrinology **116**(2): 633-639.
Poulin, A.-M. and E. Timofeeva (2008). "The dynamics of neuronal activation during food anticipation and feeding in the brain of food-entrained rats." Brain research **1227**: 128-141.
Qualls-Creekmore, E. and H. Münzberg (2018). "Modulation of feeding and associated behaviors by lateral hypothalamic circuits." Endocrinology **159**(11): 3631-3642.
Rabinak, C. A., S. Mori, M. Lyons, M. R. Milad and K. L. Phan (2017). "Acquisition of CS-US contingencies during Pavlovian fear conditioning and extinction in social anxiety disorder and posttraumatic stress disorder." Journal of affective disorders **207**: 76-85.
Rada, P., N. Avena and B. Hoebel (2005). "Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell." Neuroscience **134**(3): 737-744.
Radley, J. J., C. M. Arias and P. E. Sawchenko (2006). "Regional differentiation of the medial prefrontal cortex in regulating adaptive responses to acute emotional stress." Journal of Neuroscience **26**(50): 12967-12976.

Ratner, C., A. Ettrup, M. Bueter, M. E. Haahr, V. Compan, C. W. Le Roux, B. Levin, H. H. Hansen and G. M. Knudsen (2012). "Cerebral Markers of the Serotonergic System in Rat Models of Obesity and After Roux‐en‐Y Gastric Bypass." Obesity **20**(10): 2133-2141.
Ravussin, A., Y.-H. Youm, J. Sander, S. Ryu, K. Nguyen, L. Varela, G. I. Shulman, S. Sidorov, T. L. Horvath and J. L. Schultze (2018). "Loss of nucleobindin-2 causes insulin resistance in obesity without impacting satiety or adiposity." Cell reports **24**(5): 1085-1092. e1086.
Reppucci, C. J. and G. D. Petrovich (2016). "Organization of connections between the amygdala, medial prefrontal cortex, and lateral hypothalamus: a single and double retrograde tracing study in rats." Brain Structure and Function **221**(6): 2937-2962.
Reynolds, S. M. and K. C. Berridge (2008). "Emotional environments retune the valence of appetitive versus fearful functions in nucleus accumbens." Nature neuroscience **11**(4): 423.
Rich, E. L. and M. Shapiro (2009). "Rat prefrontal cortical neurons selectively code strategy switches." Journal of Neuroscience **29**(22): 7208-7219.
Roelofs, K., M. A. Hagenaars and J. Stins (2010). "Facing freeze: Social threat induces bodily freeze in humans." Psychological Science **21**(11): 1575-1581.
Rogan, M. T., K. S. Leon, D. L. Perez and E. R. Kandel (2005). "Distinct neural signatures for safety and danger in the amygdala and striatum of the mouse." Neuron **46**(2): 309-320.
Roitman, M. F., G. D. Stuber, P. E. Phillips, R. M. Wightman and R. M. Carelli (2004). "Dopamine operates as a subsecond modulator of food seeking." Journal of Neuroscience **24**(6): 1265-1271.
Roitman, M. F., R. A. Wheeler and R. M. Carelli (2005). "Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output." Neuron **45**(4): 587-597.
Rolls, E. T. (2011). "Taste, olfactory and food texture reward processing in the brain and obesity." International Journal of Obesity **35**(4): 550-561.
Romanski, L. M. and J. E. LeDoux (1992). "Equipotentiality of thalamo-amygdala and thalamo-cortico-amygdala circuits in auditory fear conditioning." Journal of Neuroscience **12**(11): 4501-4509.
Roney, J. R. (2015). An evolutionary functional analysis of the hormonal predictors of women’s sexual motivation. The evolution of sexuality, Springer**:** 99-121.
Roney, J. R. and Z. L. Simmons (2017). "Ovarian hormone fluctuations predict within-cycle shifts in women's food intake." Hormones and Behavior **90**: 8-14.
Rosenwasser, A. M., Z. Boulos and M. Terman (1981). "Circadian organization of food intake and meal patterns in the rat." Physiology & Behavior **27**(1): 33-39.
Rossi, M. A. and G. D. Stuber (2018). "Overlapping Brain Circuits for Homeostatic and Hedonic Feeding." Cell Metabolism **27**(1): 42-56.
Sacco, T. and B. Sacchetti (2010). "Role of secondary sensory cortices in emotional memory storage and retrieval in rats." Science **329**(5992): 649-656.
Sagar, S., F. R. Sharp and T. Curran (1988). "Expression of c-fos protein in brain: metabolic mapping at the cellular level." Science **240**(4857): 1328-1331.
Sah, P., E. L. Faber, M. Lopez de Armentia and J. Power (2003). "The amygdaloid complex: anatomy and physiology." Physiological reviews **83**(3): 803-834.
Saidov, K., A. Tiunova, O. Subach, F. Subach and K. Anokhin (2019). "New Tools in Cognitive Neurobiology: Biotin-Digoxigenin Detection of Overlapping Active Neuronal Populations by Two-Color c-fos Compartment Analysis of Temporal Activity by Fluorescent in situ Hybridization (catFISH) and c-Fos Immunohistochemistry." OBM Genetics **3**(1).
Sakamoto, F., S. Yamada and Y. Ueta (2004). "Centrally administered orexin-A activates corticotropin-releasing factor-containing neurons in the hypothalamic paraventricular nucleus and central amygdaloid nucleus of rats: possible involvement of central orexins on stress-activated central CRF neurons." Regulatory peptides **118**(3): 183-191.
Sakurai, T., A. Amemiya, M. Ishii, I. Matsuzaki, R. M. Chemelli, H. Tanaka, S. C. Williams, J. A. Richardson, G. P. Kozlowski and S. Wilson (1998). "Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior." Cell **92**(4): 573-585.
Salamone, J. D., M. S. Cousins and S. Bucher (1994). "Anhedonia or anergia? Effects of haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure." Behavioural brain research **65**(2): 221-229.

Samson, W. K., M. M. Taylor, M. Follwell and A. V. Ferguson (2002). "Orexin actions in hypothalamic paraventricular nucleus: physiological consequences and cellular correlates." Regulatory peptides **104**(1-3): 97-103.
Saper, C. B. (2006). "Staying awake for dinner: hypothalamic integration of sleep, feeding, and circadian rhythms." Progress in brain research **153**: 243-252.
Sargin, D. (2019). "The role of the orexin system in stress response." Neuropharmacology **154**: 68-78.
Sarica, C., M. Ozkan, H. H. Bay, U. Sehirli, F. Onat and M. I. Ziyal (2018). "Prelimbic cortex deep brain stimulation reduces binge size in a chronic binge eating rat model." Stereotactic and functional neurosurgery **96**(1): 33-39.
Sato, I., H. Arima, N. Ozaki, M. Watanabe, M. Goto, M. Hayashi, R. Banno, H. Nagasaki and Y. Oiso (2005). "Insulin inhibits neuropeptide Y gene expression in the arcuate nucleus through GABAergic systems." Journal of Neuroscience **25**(38): 8657-8664.
Schag, K., M. Teufel, F. Junne, H. Preissl, M. Hautzinger, S. Zipfel and K. E. Giel (2013). "Impulsivity in binge eating disorder: food cues elicit increased reward responses and disinhibition." PloS one **8**(10): e76542.
Schellekens, H., B. C. Finger, T. G. Dinan and J. F. Cryan (2012). "Ghrelin signalling and obesity: at the interface of stress, mood and food reward." Pharmacology & therapeutics **135**(3): 316-326.
Schoenbaum, G., M. R. Roesch and T. A. Stalnaker (2006). "Orbitofrontal cortex, decision-making and drug addiction." Trends in neurosciences **29**(2): 116-124.
Schreurs, B. G., C. A. Smith-Bell and L. B. Burhans (2011). "Classical conditioning and conditioning-specific reflex modification of rabbit heart rate as a function of unconditioned stimulus location." Behavioral neuroscience **125**(4): 604.
Schultz, W. (1998). "Predictive reward signal of dopamine neurons." Journal of neurophysiology **80**(1): 1-27.
Schultz, W. (2010). "Dopamine signals for reward value and risk: basic and recent data." Behavioral and brain functions **6**(1): 24.
Schultz, W., P. Dayan and P. R. Montague (1997). "A neural substrate of prediction and reward." Science **275**(5306): 1593-1599.
Schwartz, D. H., L. Hernandez and B. G. Hoebel (1990). "Serotonin release in lateral and medial hypothalamus during feeding and its anticipation." Brain research bulletin **25**(6): 797-802.
Seeger, G., D. F. Braus, M. Ruf, U. Goldberger and M. H. Schmidt (2002). "Body image distortion reveals amygdala activation in patients with anorexia nervosa − a functional magnetic resonance imaging study." Neuroscience Letters **326**(1): 25-28.
Shalizi, A., B. Gaudillière, Z. Yuan, J. Stegmüller, T. Shirogane, Q. Ge, Y. Tan, B. Schulman, J. W. Harper and A. Bonni (2006). "A calcium-regulated MEF2 sumoylation switch controls postsynaptic differentiation." Science **311**(5763): 1012-1017.
Shen, M., C. Jiang, P. Liu, F. Wang and L. Ma (2016). "Mesolimbic leptin signaling negatively regulates cocaine-conditioned reward." Translational psychiatry **6**(12): e972.
Sheng, M., S. T. Dougan, G. McFADDEN and M. E. Greenberg (1988). "Calcium and growth factor pathways of c-fos transcriptional activation require distinct upstream regulatory sequences." Molecular and Cellular Biology **8**(7): 2787-2796.
Sheng, M. and M. E. Greenberg (1990). "The regulation and function of c-fos and other immediate early genes in the nervous system." Neuron **4**(4): 477-485.
Sheng, M., M. A. Thompson and M. E. Greenberg (1991). "CREB: a Ca (2+)-regulated transcription factor phosphorylated by calmodulin-dependent kinases." Science **252**(5011): 1427-1430.
Sheng, Z., A. M. Santiago, M. P. Thomas and V. H. Routh (2014). "Metabolic regulation of lateral hypothalamic glucose-inhibited orexin neurons may influence midbrain reward neurocircuitry." Molecular and Cellular Neuroscience **62**: 30-41.
Shi, C. and M. Davis (1999). "Pain pathways involved in fear conditioning measured with fear-potentiated startle: lesion studies." Journal of Neuroscience **19**(1): 420-430.
Shimizu, Y., C. Son, D. Aotani, H. Nomura, T. Hikida, K. Hosoda and K. Nakao (2017). "Role of leptin in conditioned place preference to high-fat diet in leptin-deficient ob/ob mice." Neuroscience letters **640**: 60-63.
Simmons, D. M., J. L. Arriza and L. Swanson (1989). "A complete protocol for in situ hybridization of messenger RNAs in brain and other tissues with radio-labeled single-stranded RNA probes." Journal of Histotechnology **12**(3): 169-181.
Sinclair, E. B., K. L. Klump and C. L. Sisk (2019). "Reduced medial prefrontal control of palatable food consumption is associated with binge eating proneness in female rats." Frontiers in behavioral neuroscience **13**: 252.

Sipols, A., J. Bayer, R. Bennett and D. Figlewicz (2002). "Intraventricular insulin decreases kappa opioid-mediated sucrose intake in rats." Peptides **23**(12): 2181-2187.
Small, D. M., M. Jones-Gotman and A. Dagher (2003). "Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers." Neuroimage **19**(4): 1709-1715.
Small, W. S. (1899). "Notes on the psychic development of the young white rat." The American Journal of Psychology.
Smith, G. (2001). "John Davis and the meanings of licking." Appetite **36**(1): 84-92.
Söderpalm, A. and S. Hansen (1999). "Alcohol alliesthesia: food restriction increases the palatability of alcohol through a corticosterone-dependent mechanism." Physiology & behavior **67**(3): 409-415.
Sotres-Bayon, F. and G. J. Quirk (2010). "Prefrontal control of fear: more than just extinction." Current opinion in neurobiology **20**(2): 231-235.
Spiegel, I., A. R. Mardinly, H. W. Gabel, J. E. Bazinet, C. H. Couch, C. P. Tzeng, D. A. Harmin and M. E. Greenberg (2014). "Npas4 regulates excitatory-inhibitory balance within neural circuits through cell-type-specific gene programs." Cell **157**(5): 1216-1229.
Stamatakis, A. M., M. Van Swieten, M. L. Basiri, G. A. Blair, P. Kantak and G. D. Stuber (2016). "Lateral hypothalamic area glutamatergic neurons and their projections to the lateral habenula regulate feeding and reward." Journal of Neuroscience **36**(2): 302-311.
Stefani, M. R. and B. Moghaddam (2006). "Rule learning and reward contingency are associated with dissociable patterns of dopamine activation in the rat prefrontal cortex, nucleus accumbens, and dorsal striatum." Journal of Neuroscience **26**(34): 8810-8818.
Stopper, C. M. and S. B. Floresco (2011). "Contributions of the nucleus accumbens and its subregions to different aspects of risk-based decision making." Cognitive, Affective, & Behavioral Neuroscience **11**(1): 97-112.
Stopper, C. M., S. Khayambashi and S. B. Floresco (2013). "Receptor-specific modulation of risk-based decision making by nucleus accumbens dopamine." Neuropsychopharmacology **38**(5): 715.
Strubbe, J., D. Porte Jr and S. Woods (1988). "Insulin responses and glucose levels in plasma and cerebrospinal fluid during fasting and refeeding in the rat." Physiology & behavior **44**(2): 205-208.
Stuber, G. D. and R. A. Wise (2016). "Lateral hypothalamic circuits for feeding and reward." Nature neuroscience **19**(2): 198.
Stuber, G. D. and R. A. Wise (2016). "Lateral Hypothalamic Circuits for Feeding and Reward." Nature neuroscience **19**(2): 198-205.
Stunkard, A. J. and T. A. Wadden (1992). "Psychological aspects of severe obesity." The American journal of clinical nutrition **55**(2): 524S-532S.
Sugam, J. A., J. J. Day, R. M. Wightman and R. M. Carelli (2012). "Phasic nucleus accumbens dopamine encodes risk-based decision-making behavior." Biological psychiatry **71**(3): 199-205.
Sung, J., K. Lee, Y. M. Song, M. K. Lee and D. H. Lee (2010). "Heritability of eating behavior assessed using the DEBQ (Dutch Eating Behavior Questionnaire) and weight‐related traits: the Healthy Twin Study." Obesity **18**(5): 1000-1005.
Suzuki, K., K. A. Simpson, J. S. Minnion, J. C. Shillito and S. R. Bloom (2010). "The role of gut hormones and the hypothalamus in appetite regulation." Endocrine journal **57**(5): 359-372.
Svaldi, J., M. Brand and B. Tuschen-Caffier (2010). "Decision-making impairments in women with binge eating disorder." Appetite **54**(1): 84-92.
Swanson, L., P. Sawchenko, R. Lind and J. H. Rho (1987). "The CRH motoneuron: differential peptide regulation in neurons with possible synaptic, paracrine, and endocrine outputs." Annals of the New York Academy of Sciences **512**(1): 12-23.
Swanson, L. and P. E. Sawchenko (1983). "Hypothalamic integration: organization of the paraventricular and supraoptic nuclei." Annual review of neuroscience **6**(1): 269-324.
Swanson, L. W., G. Sanchez-Watts and A. G. Watts (2005). "Comparison of melanin-concentrating hormone and hypocretin/orexin mRNA expression patterns in a new parceling scheme of the lateral hypothalamic zone." Neuroscience letters **387**(2): 80-84.
Swanson, S. A., S. J. Crow, D. Le Grange, J. Swendsen and K. R. Merikangas (2011). "Prevalence and correlates of eating disorders in adolescents: Results from the national comorbidity survey replication adolescent supplement." Archives of general psychiatry **68**(7): 714-723.

Takahashi, K. A. and R. D. Cone (2005). "Fasting induces a large, leptin-dependent increase in the intrinsic action potential frequency of orexigenic arcuate nucleus neuropeptide Y/Agouti-related protein neurons." Endocrinology **146**(3): 1043-1047.
Tamashiro, K. L., M. A. Hegeman, M. M. Nguyen, S. J. Melhorn, L. Y. Ma, S. C. Woods and R. R. Sakai (2007). "Dynamic body weight and body composition changes in response to subordination stress." Physiology & behavior **91**(4): 440-448.
Tannenbaum, G. S., M. Lapointe, A. Beaudet and A. D. Howard (1998). "Expression of growth hormone secretagogue-receptors by growth hormone-releasing hormone neurons in the mediobasal hypothalamus." Endocrinology **139**(10): 4420-4423.
Tanofsky‐Kraff, M., D. E. Wilfley and E. Spurrell (2000). "Impact of interpersonal and ego‐related stress on restrained eaters." International Journal of Eating Disorders **27**(4): 411-418.
Tao, R., A. Fray, S. Aspley, R. Brammer, D. Heal and S. Auerbach (2002). "Effects on serotonin in rat hypothalamus of D-fenfluramine, aminorex, phentermine and fluoxetine." European journal of pharmacology **445**(1-2): 69-81.
Tartaglia, L. A., M. Dembski, X. Weng, N. Deng, J. Culpepper, R. Devos, G. J. Richards, L. A. Campfield, F. T. Clark and J. Deeds (1995). "Identification and expression cloning of a leptin receptor, OB-R." Cell **83**(7): 1263-1271.
Teegarden, S. L. and T. L. Bale (2007). "Decreases in dietary preference produce increased emotionality and risk for dietary relapse." Biological psychiatry **61**(9): 1021-1029.
Teich, A. H., P. M. McCabe, C. C. Gentile, L. S. Schneiderman, R. W. Winters, D. R. Liskowsky and N. Schneiderman (1989). "Auditory cortex lesions prevent the extinction of Pavlovian differential heart rate conditioning to tonal stimuli in rabbits." Brain research **480**(1-2): 210-218.
Tepper, B. and M. Yeomans (2017). Flavor, satiety and food intake, John Wiley & Sons.
Thompson, J. L., M. Drysdale, C. Baimel, M. Kaur, T. MacGowan, K. A. Pitman and S. L. Borgland (2017). "Obesity-induced structural and neuronal plasticity in the lateral orbitofrontal cortex." Neuropsychopharmacology **42**(7): 1480.
Timko, C. A., L. DeFilipp and A. Dakanalis (2019). "Sex Differences in Adolescent Anorexia and Bulimia Nervosa: Beyond the Signs and Symptoms." Current psychiatry reports **21**(1): 1.
Timofeeva, E. and A. Mitra (2013). "The effects of sucrose on neuronal activity." Sucrose: Properties, Biosynthesis and Health Implications. New-York: Nova Science Publishers: 75-114.
Tomiyama, A. J., M. F. Dallman and E. S. Epel (2011). "Comfort food is comforting to those most stressed: evidence of the chronic stress response network in high stress women." Psychoneuroendocrinology **36**(10): 1513-1519.
Ulrich-Lai, Y. M. and J. P. Herman (2009). "Neural regulation of endocrine and autonomic stress responses." Nature Reviews Neuroscience **10**(6): 397.
Uylings, H., C. Van Eden, J. De Bruin, M. Feenstra and C. Pennartz (2000). "The integration of stress by the hypothalamus, amygdala and prefrontal cortex: balance between the autonomic nervous system and the neuroendocrine system." Prog Brain Res **126**: 117-132.
Vajnerova, O., E. Bielavska, P. Jiruska and G. Brozek (2003). "Level of vigilance influences licking frequency in rats." Physiological research **52**(2): 243-250.
van de Giessen, E., F. Celik, D. H. Schweitzer, W. van den Brink and J. Booij (2014). "Dopamine D2/3 receptor availability and amphetamine-induced dopamine release in obesity." Journal of Psychopharmacology **28**(9): 866-873.
van der Kooy, D., L. Y. Koda, J. F. McGinty, C. R. Gerfen and F. E. Bloom (1984). "The organization of projections from the cortes, amygdala, and hypothalamus to the nucleus of the solitary tract in rat." Journal of Comparative Neurology **224**(1): 1-24.
Vanhoutte, P., J.-V. Barnier, B. Guibert, C. Pagès, M.-J. Besson, R. A. Hipskind and J. Caboche (1999). "Glutamate induces phosphorylation of Elk-1 and CREB, along with c-fos activation, via an extracellular signal-regulated kinase-dependent pathway in brain slices." Molecular and cellular biology **19**(1): 136-146.
Verbalis, J. G. and J. Dohanics (1991). "Vasopressin and oxytocin secretion in chronically hyposmolar rats." American Journal of Physiology-Regulatory, Integrative and Comparative Physiology **261**(4): R1028-R1038.
Vocks, S., B. Tuschen‐Caffier, R. Pietrowsky, S. J. Rustenbach, A. Kersting and S. Herpertz (2010). "Meta‐analysis of the effectiveness of psychological and pharmacological treatments for binge eating disorder." International Journal of Eating Disorders **43**(3): 205-217.
Volkow, N. D., G.-J. Wang and R. D. Baler (2011). "Reward, dopamine and the control of food intake: implications for obesity." Trends in Cognitive Sciences **15**(1): 37-46.
Voon, V. (2015). "Cognitive biases in binge eating disorder: the hijacking of decision making." CNS spectrums **20**(6): 566-573.

Voon, V., L. S. Morris, M. A. Irvine, C. Ruck, Y. Worbe, K. Derbyshire, V. Rankov, L. R. Schreiber, B. L. Odlaug and N. A. Harrison (2015). "Risk-taking in disorders of natural and drug rewards: neural correlates and effects of probability, valence, and magnitude." Neuropsychopharmacology **40**(4): 804.
Wallis, J. D. (2011). "Cross-species studies of orbitofrontal cortex and value-based decision-making." Nature Neuroscience **15**: 13.
Wanat, M., F. Hopf, G. Stuber, P. Phillips and A. Bonci (2008). "Corticotropin‐releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C‐dependent enhancement of Ih." The Journal of physiology **586**(8): 2157-2170.
Wang, G.-J., N. D. Volkow, P. K. Thanos and J. S. Fowler (2004). "Similarity between obesity and drug addiction as assessed by neurofunctional imaging: a concept review." Journal of addictive diseases **23**(3): 39-53.
Watabe-Uchida, M., N. Eshel and N. Uchida (2017). "Neural circuitry of reward prediction error." Annual review of neuroscience **40**: 373-394.
Waterson, Michael J. and Tamas L. Horvath (2015). "Neuronal Regulation of Energy Homeostasis: Beyond the Hypothalamus and Feeding." Cell Metabolism **22**(6): 962-970.
Wayman, G. A., Y.-S. Lee, H. Tokumitsu, A. Silva and T. R. Soderling (2008). "Calmodulin-kinases: modulators of neuronal development and plasticity." Neuron **59**(6): 914-931.
Weidner, G., C.-W. Kohlmann, E. Dotzauer and L. R. Burns (1996). "The effects of academic stress on health behaviors in young adults." Anxiety, stress, and coping **9**(2): 123-133.
Weiss, M. S. and P. M. Di Lorenzo (2012). "Not so fast: taste stimulus coding time in the rat revisited." Frontiers in integrative neuroscience **6**: 27.
Welsh, J. P., E. J. Lang, I. Suglhara and R. Llinás (1995). "Dynamic organization of motor control within the olivocerebellar system." Nature **374**(6521): 453.
West, A. E., W. G. Chen, M. B. Dalva, R. E. Dolmetsch, J. M. Kornhauser, A. J. Shaywitz, M. A. Takasu, X. Tao and M. E. Greenberg (2001). "Calcium regulation of neuronal gene expression." Proceedings of the National Academy of Sciences **98**(20): 11024-11031.
West, A. E. and M. E. Greenberg (2011). "Neuronal activity‐regulated gene transcription in synapse development and cognitive function." Cold Spring Harbor perspectives in biology **3**(6): a005744.
Westenbroek, R. E., M. K. Ahlijanian and W. A. Catterall (1990). "Clustering of L-type Ca2+ channels at the base of major dendrites in hippocampal pyramidal neurons." Nature **347**(6290): 281.
Wolfe, B. E., C. W. Baker, A. T. Smith and S. Kelly‐Weeder (2009). "Validity and utility of the current definition of binge eating." international Journal of Eating disorders **42**(8): 674-686.
Wolkowitz, O. M., E. S. Epel and V. I. Reus (2001). "Stress hormone-related psychopathology: pathophysiological and treatment implications." The World Journal of Biological Psychiatry **2**(3): 115-143.
Woolley, J., B. Lee and H. Fields (2006). "Nucleus accumbens opioids regulate flavor-based preferences in food consumption." Neuroscience **143**(1): 309-317.
Wren, A., L. Seal, M. Cohen, A. Brynes, G. Frost, K. Murphy, W. Dhillo, M. Ghatei and S. Bloom (2001). "Ghrelin enhances appetite and increases food intake in humans."
Wu, Q., M. S. Clark and R. D. Palmiter (2012). "Deciphering a neuronal circuit that mediates appetite." Nature **483**(7391): 594.
Xing, J., D. D. Ginty and M. E. Greenberg (1996). "Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase." Science **273**(5277): 959-963.
Yager, L. M., A. F. Garcia, A. M. Wunsch and S. M. Ferguson (2015). "The ins and outs of the striatum: role in drug addiction." Neuroscience **301**: 529-541.
Yamanaka, A., K. Kunii, T. Nambu, N. Tsujino, A. Sakai, I. Matsuzaki, Y. Miwa, K. Goto and T. Sakurai (2000). "Orexin-induced food intake involves neuropeptide Y pathway." Brain research **859**(2): 404-409.
Zeidan, M. A., S. A. Igoe, C. Linnman, A. Vitalo, J. B. Levine, A. Klibanski, J. M. Goldstein and M. R. Milad (2011). "Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats." Biological psychiatry **70**(10): 920-927.
Zellner, D. A., S. Loaiza, Z. Gonzalez, J. Pita, J. Morales, D. Pecora and A. Wolf (2006). "Food selection changes under stress." Physiology & behavior **87**(4): 789-793.
Zheng, J., A. Dobner, R. Babygirija, K. Ludwig and T. Takahashi (2009). "Effects of repeated restraint stress on gastric motility in rats." American Journal of Physiology-Regulatory, Integrative and Comparative Physiology **296**(5): R1358-R1365.

Zigman, J. M., J. E. Jones, C. E. Lee, C. B. Saper and J. K. Elmquist (2006). "Expression of ghrelin receptor mRNA in the rat and the mouse brain." Journal of Comparative Neurology **494**(3): 528-548.

Annex A

Supplementary Data

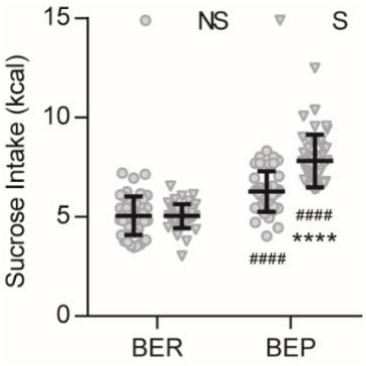

Figure SD 1. Sucrose intake in non-stressful condition and after foot-shock stress of the BERs and BEPs.

####, significantly ($p<0.0001$) different from the BERs within the same condition. ****, significantly ($p<0.0001$) different from non-stressed rats within the same phenotype. Error bars, SEM. NS - No stress, S - Stress

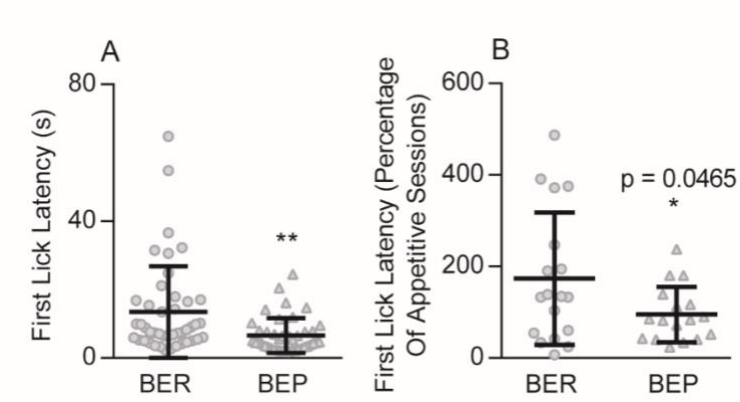

Figure SD 2. First lick latency.

A, First lick latency during *Appetitive* sessions. B, First lick latency in the *Test* session normalized to the *Appetitive* session