Examining the Effect of Environmental Enrichment on the Modulation of Feeding and Aggressive Behaviours in Mice and the Associated Expression of *Bdnf* Transcript Variants

By

Muhammad Shaaban Aldhshan

A Thesis submitted to the Faculty of Graduate Studies of
The University of Manitoba
in partial fulfilment of the requirements of the degree of

MASTER OF SCIENCE

Department of Physiology and Pathophysiology
University of Manitoba
Winnipeg

Copyright © 2021 by Muhammad Shaaban Aldhshan

Abstract

The home environment is a critical modulator of behaviour. Environmental enrichment (EE) is a laboratory housing protocol that employs complex sensory and social stimulations to enrich the cage environment of laboratory rodents. Prior studies suggested that EE elicits improvements in rodents' metabolic profile in addition to profound cognitive and behavioural outcomes, among which is reduced inter-male aggression. However, the underlying mechanism mediating the positive outcomes of EE remains poorly characterised. EE has been associated with altered brain-derived neurotrophic factor (*Bdnf*) gene expression. The *Bdnf* gene consists of multiple transcript variants, all of which encode for an identical protein. Remarkably, different *Bdnf* transcripts expression in different tissues influences different metabolic and behavioural functions. Therefore, we hypothesised that EE modifies the feeding and aggressive behaviours in mice via altering *Bdnf* gene expression in a transcript variant-specific and brain region-specific manner. To test this hypothesis, we employed behavioural and gene expression analyses to examine the influence of EE on feeding and aggressive behaviours and the associated expression of genes encoding neurotrophins in male C57BL/6 mice. In the first study, we showed, for the first time, that EE potentiates the feeding-suppressing effect of glucose in 8-hr fasted mice. Furthermore, intraperitoneal glucose injection promoted hypothalamic activation, as assessed by increased *Fos* expression. Moreover, glucose treatment significantly upregulated hypothalamic glial cell line-derived neurotrophic factor (*Gdnf*) mRNA levels while selectively downregulating *Bdnf* transcript variants levels in the hypothalamus and amygdala of the EE mice. In the second study, we showed that EE significantly reduced inter-male aggression in group-housed mice. This effect was associated with reduced activity of the aggression-linked corticolimbic circuits. Importantly, EE significantly reduced *Bdnf* transcript variant I (*Bdnf I*) expression in the prefrontal cortex, hypothalamus, hippocampus, and parietal cortex, whereas it significantly increased *Bdnf I* mRNA levels in the amygdala. Together, these data show that the effects of EE on feeding and aggressive behaviours are associated with brain region-specific alterations in neurotrophin gene expression. It is highly expected that the novel data reported in this thesis will further the current understanding of the role of the home environment in modulating behaviour at the brain level.

Acknowledgements

I wish to express my deepest gratitude to my supervisor, Dr Tooru M. Mizuno, for his unwavering support, guidance, constructive criticism, tutelage, and wisdom. This work could have never been carried out without his support. I also wish to thank my committee members, Dr Christine Doucette and Dr Mark Fry, for their extremely valuable input, support, and guidance throughout this project.

I similarly wish to thank Dr Kristine Cowley and Dr Katinka Stecina for their moral support and for allowing me to participate in their weekly journal club. I look forward with great excitement to working with Dr Cowley on my PhD.

I have to especially thank Dr Martha Jo (MJ) Willard for her genuine support, friendship, and the many scientific, medical, and ecological discussions.

Thank you to Ms Peisan Lew for her role in getting me settled in the lab. Thank you to my fellow graduate colleagues at the Department of Physiology and Pathophysiology; Geetika for her consistent optimism and support, Mona for all the uplifting Arabic discussions about life, science, and the future, and Urmi for the many "aimless" walks around the campus. Thank you to Gail, Judy, Sharon, and Vanessa for all their administrative assistance. Thank you to Maria, the caretaker on the BMSB fourth floor, for always greeting me with a smile.

Thank you to my mother; you are the strongest and most loving person I know. Thank you to my father; you will always live in my heart. Thank you to my uncle, Mr Muhammad M. Aldhshan, for his genuine interest in my wellbeing. Thank you to the rest of my immediate and extended family. Thank you to Ahmad E. Eyada, Abdelrahman Bakry, Hassan Saleh, and Muhammad Sadek for being there for me when I needed them.

Thank you to the funding co-partners behind this project: Natural Sciences and Engineering Research Council of Canada (NSERC) and the Graduate Enhancement of Tri-Agency Stipends (GETS). Lastly, I wish to thank the Faculty of Graduate Studies (FGS) for their generous Entrance and Research Completion Scholarships.

Dedications

In memory of the many Palestinian children who were killed in the Gaza Strip in the most recent episode of Israel-Gaza aggression and for the thousands and thousands of other children who felt/feel threatened in their own homes.

This thesis is also dedicated to the survivors and victims of the Canadian residential schools. May peace finds them wherever they are.

Table of contents

Abstract i
Acknowledgements ii
Dedications iii
Table of Contents iv
List of Figures viii
List of Tables x
List of Key Abbreviations xi

PART I: Effect of environmental enrichment on feeding behaviour and the expression of *Bdnf* transcript variants 1
1. Introduction 2
1.1. CNS regulation of food intake 3
1.1.1. Hypothalamic circuits regulating food intake 3
1.1.2. Extrahypothalamic circuits regulating food intake 6
1.2. Regulation of caloric intake by peripheral signals 8
1.2.1. Nutritional signals: emphasis on glucose role in feeding modulation 8
1.2.2. Adiposity signals: emphasis on leptin role in feeding modulation 9
1.2.3. GI signals: emphasis on xenin role in feeding modulation 10
1.3. Neurotrophic factors control of energy balance 11
1.3.1. Brain-derived neurotrophic factor (BDNF) 11
1.3.1.1. BDNF role in food intake 12
1.3.1.2. BDNF role in glucose homeostasis 13
1.3.1.3. BDNF role in energy expenditure 14
1.3.1.4. The *Bdnf* genomic structure 14
1.3.1.5. BDNF signalling and energy balance 16
1.3.2. Glial cell line-derived neurotrophic factor (GDNF) role in energy homeostasis 16
1.3.3. Mesencephalic astrocyte-derived neurotrophic factor (MANF) role in energy homeostasis 17
1.4. Social and environmental factors influencing obesity: epidemiological evidence 18
1.4.1. Individual-related factors influencing obesity 18
1.4.2. Environment-related factors influencing obesity 18

1.4.3. Social factors influencing obesity development 20
1.5. Environmental enrichment 21
1.5.1. A tale of two animal cage environments 21
1.5.2. Environmental enrichment beneficial influence on brain, body, and behaviour 22
1.5.3. The metabolic benefits of environmental enrichment 23
1.5.4. The molecular basis of the physiological benefits of environmental enrichment 25
2. Rationale 26
3. Hypotheses 28
4. Objectives 28
5. Experimental design 29
6. Results 37
6.1. Effect of cage environment on body weight and adiposity levels 37
6.2. Effect of cage environment on glucose-induced feeding suppression 39
6.3. Effect of cage environment on high dose (50 μg/g b.w.) xenin-induced feeding suppression 42
6.4. Effect of cage environment on low dose (15 μg/g b.w.) xenin-induced feeding suppression 45
6.5. Effect of cage environment on leptin-induced feeding suppression 48
6.6. Effect of cage environment and glucose treatment on blood glucose, insulin, leptin, and corticosterone levels in mice 51
6.7. Effect of cage environment and glucose treatment on *Fos* mRNA expression 53
6.8. Effect of cage environment and glucose treatment on the glucose transporters 1-5 gene expression in the mouse hypothalamus and amygdala 56
6.9. Effect of cage environment and glucose treatment on the expression of *Bdnf* transcript variants in mouse brain 59
6.10. Effect of cage environment and glucose treatment on the expression of the BDNF receptors genes in the mouse brain 65
6.11. Effect of cage environment and glucose treatment on *Gdnf* and *Manf* mRNA expression in the mouse brain 67
7. Discussion 69
7.1. Summary of the main findings 69
7.2. The metabolic benefits of environmental enrichment 69
7.3. Environmental enrichment potentiates the feeding-suppressing effect of glucose 70

7.4. Effect of EE on *Bdnf* transcript variant-specific and brain region-specific expression 72
7.5. A possible role of GDNF in the mediation of EE-induced feeding suppression 74
7.6. Conclusion 74
7.7. Significance 75
7.8. Limitations 76
7.9. Future directions 78

PART II: Effect of environmental enrichment on aggressive behaviour and the expression of Bdnf transcript variants 80

1. Introduction 81
1.1. Human aggression 81
1.1.1. Nurtured aggression, bullying, and the "social popularity" factor 82
1.1.2. A hefty cost of aggression 83
1.1.3. Aggression during the coronavirus pandemic disease 2019 (Covid-19) 83
1.2. Neuropsychiatry of aggression 84
1.2.1. Intermittent explosive disorder (IED) 84
1.2.2. Schizophrenia 84
1.2.3. Alcoholism and substance abuse 85
1.3. Neuroanatomy of aggression 85
1.3.1. Hypothalamic circuits involved in aggression 86
1.3.2. Limbic structures involved in aggression 86
1.3.2.1. Amygdala 86
1.3.2.2. Prefrontal cortex (PFC) 87
1.3.2.3. Hippocampus 87
1.3.2.4. Lateral septum (LS) 88
1.4. Neurochemistry of aggression 88
1.4.1. Neurotransmitters 88
1.4.2. Hormones 89
1.4.3. BDNF role in aggression 90
1.5. Cage environment and aggressive behaviour in laboratory mice 91
1.6. Environmental enrichment (EE) and inter-male aggression 92
2. Rationale 93

3. Hypotheses 95
4. Objectives 95
5. Experimental design 96
6. Results 99
6.1. Effect of cage environment on inter-male aggressive behaviour in mice 99
6.2. Effect of cage environment on serum corticosterone levels 99
6.3. Effect of cage environment on the expression of immediate early genes 101
6.4. Effect of cage environment on the expression of *Bdnf* transcripts in the brain 103
6.5. Effect of cage environment on the expression of BDNF functional receptors in the mouse brain 103
6.6. Effect of cage environment on GABAergic gene expression in the brain 105
6.7. Effect of cage environment on serotonergic gene expression in the brain 105
7. Discussion 108
7.1. Summary of the main findings 108
7.2. Examining the effect of enriched cage environment on inter-male aggression in group-housed C57BL/6 mice 108
7.3. Examining the brain activation pattern associated with the aggression-lowering effect of enriched environment 109
7.4. Examining the association between the aggression-lowering effect of EE and BDNF 113
7.5. Examining the association between the aggression-lowering effect of EE and *Bdnf* mRNA transcript-variant-specific and brain region-specific expression 113
7.6. Examining the role of GABAergic transmission in mediating the aggression-lowering effect of EE 115
7.7. Examining the role of serotonergic transmission in mediating the aggression-lowering effect of EE 116
7.8. Conclusion 116
7.9. Significance 117
7.10. Limitations 117
7.11. Future directions 120

Overall thesis summary and conclusion remarks..... 121
References 124

List of Figures

Figure 1 Simplified schematic of peripheral and central circuits controlling food intake 5

Figure 2 Schematic representation of the mouse *Bdnf* gene showing alternative splicing and 3'UTR polyadenylation signals 15

Figure 3 Environmental enrichment (EE) cage vs standard environment (SE) cage 24

Figure 4 Effect of environmental enrichment on body weight, food intake, and adiposity in mice 38

Figure 5 Effect of glucose on food intake in mice maintained under standard environment and enriched environment 41

Figure 6 Effect of xenin (50 μg/g body weight) on food intake in mice maintained under standard environment and enriched environment 44

Figure 7 Effect of xenin (15 μg/g body weight) on food intake in mice maintained under standard environment and enriched environment 47

Figure 8 Effect of leptin treatment (2.5 μg/g body weight) on food intake in mice maintained under standard environment and enriched environment..... 50

Figure 9 Effect of environmental enrichment and glucose treatment on *Fos* mRNA expression in the brain 55

Figure 10 Effect of environmental enrichment and glucose treatment on *Slc2a1-5* mRNA expression in the hypothalamus and amygdala 58

Figure 11 Splice variant-specific and brain region-specific *Bdnf* mRNA expression in response to environmental enrichment and glucose 64-65

Figure 12 Expression of the genes encoding BDNF receptors in the brain in response to environmental enrichment and glucose treatment 66

Figure 13 Expression levels of *Gdnf* and *Manf* mRNA in response to environmental enrichment and glucose treatment 68

Figure 14 Assessment of aggressive behaviour in mice 97

Figure 15 Effect of cage environment on aggressive behaviour in group-housed mice 100

Figure 16 Effect of environmental enrichment on immediate early genes expression in the mouse brain 102

Figure 17 Effect of environmental enrichment on *Bdnf* transcripts and BDNF receptors mRNA expression in the mouse brain 104-105

Figure 18 Effect of environmental enrichment on GABAergic gene expression in the brain ... 106

Figure 19 Effect of environmental enrichment on serotonergic gene expression in the brain ... 107

Figure 20 The proposed corticolimbic neural circuitry mediating the aggression-lowering effect of environmental enrichment in mice 112

List of Tables

Table 1 Primer sequences used for real-time PCR 34-35

Table 2 Amplification efficiencies of primers used for real-time PCR 36

Table 3 Results of two-way ANOVA of body weight and food intake data 37

Table 4 (A-B) Three-way and two-way ANOVA of food intake data in response to glucose treatment 40

Table 5 (A-B) Three-way and two-way ANOVA of food intake data in response to xenin treatment at a dose of 50 μg/g body weight 43

Table 6 (A-B) Three-way and two-way ANOVA of food intake data in response to xenin treatment at a dose of 15 μg/g body weight 46

Table 7 (A-B) Three-way and two-way ANOVA of food intake data in response to leptin treatment 49

Table 8 Effect of environmental enrichment and glucose treatment on circulating glucose, insulin, leptin, and corticosterone levels in mice 52

Table 9 Two-way ANOVA of Fos mRNA levels in response to environmental enrichment and glucose treatment 54

Table 10 Two-way ANOVA of Slc2a1-5 mRNA levels in response to environmental enrichment and glucose treatment 57

Table 11 Two-way ANOVA of hypothalamic neurotrophins and receptors gene expression levels in response to environmental enrichment and glucose treatment 61

Table 12 Two-way ANOVA of amygdalar neurotrophins and receptors gene expression levels in response to environmental enrichment and glucose treatment 62

Table 13 Two-way ANOVA of hippocampal neurotrophins and receptors gene expression levels in response to environmental enrichment and glucose treatment 63

Table 14 Two-way ANOVA of cortical neurotrophins and receptors gene expression levels in response to environmental enrichment and glucose treatment 63

List of Key Abbreviations

3' UTR	3' untranslated region
5' UTR	5' untranslated region
5-HT	Serotonin (5-hydroxytryptamine)
AgRP	Agouti-related protein
AMPK	AMP-activated protein kinase
AP	Area postrema
ARC	The arcuate nucleus of the hypothalamus
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor protein
BDNF	Human brain-derived neurotrophic factor gene
Bdnf	Mouse/rat brain-derived neurotrophic factor gene
Bdnf I	*Bdnf* transcript variant *I*
Bdnf II	*Bdnf* transcript variant II
Bdnf IV	*Bdnf* transcript variant IV
Bdnf VI	*Bdnf* transcript variant VI
BLA	Basolateral amygdala
BMI	Body mass index
CART	Cocaine- and amphetamine-regulated transcript
CeA	Centromedial amygdala
CNS	Central nervous system
CSF	Cerebrospinal fluid
DMN	Dorsomedial nucleus of the hypothalamus
DMV	Dorsal motor nucleus of the vagal nerve
DVC	Dorsal vagal complex
EE	Environmental enrichment
EWAT	Epididymal white adipose tissue
FDA	Food and Drug Administration
GABA	Gamma aminobutyric acid
Gad1	Glutamate decarboxylase 1

GDNF	Glial cell line-derived neurotrophic factor protein
Gdnf	Mouse/rat glial cell line-derived neurotrophic factor gene
GFR-α	GDNF family receptor-α
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
GLUTs	Glucose transporters
Htr2a	5-hydroxytryptamine receptor 2A
IED	Intermittent explosive disorder
IGF-1	Insulin-like growth factor 1
ITCs	Intercalated cells of the amygdala
LH	Lateral hypothalamic area
LS	Lateral septum
MANF	Mesencephalic astrocyte-derived neurotrophic factor
Manf	Mouse/rat mesencephalic astrocyte-derived neurotrophic factor gene
Maoa	Monoamine oxidase A
mBDNF	Mature BDNF
MC3R	Melanocortin-3
MC4R	Melanocortin-4
MCH	Melanin-concentrating hormone
ME	Median eminence
MeA	Medial nucleus of the amygdala
mPFC	Medial prefrontal cortex
MWAT	Mesenteric white adipose tissue
NAc	Nucleus accumbens
NDDs	Neuro-developmental disorders
NGF	Nerve growth factor
Ngfr	Nerve growth factor receptor
NPY	Neuropeptide Y
Npy	Neuropeptide Y encoding gene
NTFs	Neurotrophic factors
Ntrk2	Mouse neurotrophic tyrosine kinase receptor 2 encoding gene

NTS	Nucleus tractus solitarii
$P75^{NTR}$	Neurotrophin receptor P75
PFC	Prefrontal cortex
POMC	Proopiomelanocortin
proBDNF	BDNF precursor
Pvalb	Parvalbumin alpha encoding gene
PVN	Paraventricular nucleus
RWAT	Retroperitoneal white adipose tissue
SE	Standard environment
Slc2a1	Solute carrier family 2 member 1 encoding gene
Slc2a2	Solute carrier family 2 member 2 encoding gene
Slc2a3	Solute carrier family 2 member 3 encoding gene
Slc2a4	Solute carrier family 2 member 4 encoding gene
Slc2a5	Solute carrier family 2 member 5 encoding gene
SSRIs	Selective serotonin reuptake inhibitors
Sst	Somatostatin encoding gene
SWAT	Subcutaneous white adipose tissue
T. WAT	Total white adipose tissue
TrkB	Tropomyosin receptor kinase B
VMN	Ventromedial nucleus of the hypothalamus
VMNvl	Ventrolateral subdivision of the hypothalamic ventromedial nucleus
VTA	Ventral tegmental area
WHO	World health organisation
α-MSH	α-melanocyte-stimulating hormone

PART I: Effect of environmental enrichment on feeding behaviour and the expression of *Bdnf* transcript variants

1. Introduction

Obesity is characterised by immoderate accretion of body fats emanating from a chronic imbalance between caloric consumption and caloric expenditure. Obesity is a consequential health problem inextricably linked with reduced life expectancy and deteriorated quality of life (Fontaine et al., 2003; Kitahara et al., 2014; Vallis, 2016; Xu, Cupples, et al., 2018). Additionally, obese individuals have a greater predisposition to develop many fatal conditions, including cardiovascular disease, cancerous tumours, and diabetes (Segula, 2014; Kinlen et al., 2017). The world health organisation (WHO) estimated the global number of individuals who are overweight (body mass index (BMI) $\geq$ 25 kg/m^2)) at 1.9 billion adults in 2016, over one-third of whom were obese (BMI $\geq$ 30 kg/m2) (WHO, 2020). In Canada, 63.1% of the adult population are either overweight (36.3%) or obese (26.8%), according to the 2018 Canadian community health survey (Statistics Canada, 2019). A greater understanding of the genetic, psychological, social, and environmental attributes of obesity, and the interactions thereof, is needed to alleviate, if not fully diminish, many obesity-associated co-morbidities and improve the quality of life of hundreds of millions of overweight and obese individuals.

Presently, the non-invasive approach for the treatment of obesity aims at establishing a state of caloric deficit, forcing the body to mobilise and burn body fats to meet its energy requirements. This state is primarily attained via caloric restriction and further augmented with increased physical activity. However, this approach requires a great deal of self-discipline, which is often hard to accomplish in our increasingly obesogenic and sedentary environments. Thus, increasing attention has been channelled towards inducing the caloric deficit state pharmacologically. Although the option for long-term treatment of obesity is still limited, the number of approved obesity drugs has been increasing. Indeed, while writing this thesis, the United States Food and Drug Administration (FDA) approved Wegovy (semaglutide), an anti-obesity drug that has been referred to in the media as a "game-changer" (FDA Press Release, 2021). Semaglutide is a glucagon-like peptide-1 (GLP-1) receptor agonist that reduces caloric intake via suppressing appetite and reducing the preference for high-fat calorie-dense food (Blundell et al., 2017; Reviewed in Christou et al., 2019).

The central nervous system (CNS) is the master regulator of appetite. It tirelessly surveys the body's energy state via feedback integration of multiple homeostatic, hedonic, and environmental sensory signals to devise a proportionate perception of hunger or satiety, with the

end goal being to maintain energy balance (Berthoud, 2006). In contrast, obesity is associated with a disruption of the CNS regulatory mechanisms of body weight and caloric intake, precipitating a metabolic and hedonic preference for weight stasis, or even weight gain, rather than weight loss (Levin, 2007; Thomas et al., 2013). Therefore, understanding the contributions of different attributes of obesity and the associated CNS mechanisms employed in regulating appetite could hold the key to developing effective and safe drugs to treat obesity.

1.1. CNS regulation of food intake

1.1.1. Hypothalamic circuits regulating food intake

Glucose catabolism is the main route to produce the ATP necessary for brain functions; hence, it is vital to maintain adequate glucose levels in the blood and a constant supply to the brain. To achieve this, the brain, particularly the hypothalamus, evolved to sense the systemic glucose availability and respond by fine-tuning the central and peripheral circuits controlling food intake and metabolism to maintain central and peripheral glucose homeostasis (Lopez-Gambero et al., 2019). In this regard, the hypothalamic arcuate nucleus (ARC) plays a particularly important function in integrating peripheral blood-borne metabolic signals. Such role is thought to be facilitated by its proximity to the median eminence (ME), a circumventricular organ at the base of the hypothalamus (Rodríguez et al., 2010). However, several anatomical studies provide evidence of blood-brain barrier (BBB) integrity in the ARC region, opposing the widespread view that metabolic signals can passively diffuse into the ARC due to a leaky or weak BBB in that area (For review see Fry & Ferguson, 2010). Therefore, discounting facilitated, transporters-mediated, diffusion, the exact mechanism through which lipophobic peripheral signals can access the ARC remains inadequately characterised.

Within the ARC, specialised neurons integrate blood-borne nutritional (e.g., glucose) and hormonal signals (e.g., leptin, insulin, etc.) and modulate the activity of two antagonistic ARC neuronal populations that tightly control food intake (Fig. 1). The first population increases food intake via co-expressing the feeding-inducing neuropeptides agouti-related protein (AgRP) and neuropeptide Y (NPY). In contrast, the other population inhibits food intake via co-expressing the anorectic neuropeptides proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). Both POMC/CART and AgRP/NPY expressing neurons project to second-order neurons in the hypothalamus and extrahypothalamic circuits, leading to a coordinated homeostatic and hedonic feeding response (Timper & Brüning, 2017).

POMC produced in ARC neurons undergoes post-transcriptional cleavage to produce α-melanocyte-stimulating hormone (α-MSH), which suppresses food intake via downstream signalling through the melanocortin-3 and -4 receptors (MC3R and MC4R) on the second-order neurons, especially in the paraventricular nucleus (PVN) of the hypothalamus (Reviewed in Krashes et al., 2016). Indeed, studies have shown that disruption of MC4R expression in the PVN results in hyperphagic obesity in mice (Huszar et al., 1997; Balthasar et al., 2005). Moreover, mutations involving the gene coding for the MC4R are considered the most common cause of monogenic obesity in humans (Reviewed in Tao, 2005). In contrast, AgRP has been shown to antagonise α-MSH anorectic function via signalling through the same receptors, MC3R and MC4R, on second-order neurons (Ollmann et al., 1997). This ongoing competition between POMC and AgRP to bind MC3R and MC4R constitutes the central melanocortin system, an integral component of food intake regulation.

Most of the ARC AgRP/NPY- and POMC-expressing neurons project to second-order neurons in the PVN. Moreover, lesions involving the PVN have been shown to cause hyperphagic obesity in rats (Leibowitz et al., 1981), suggesting that the PVN plays an anorectic role in feeding regulation. Similarly, ARC neurons also project to the ventromedial nucleus (VMN) and the lateral hypothalamic area (LH). Earlier studies showed that destructive lesions involving the VMN cause hyperphagic obesity (Penicaud et al., 1983), while lesions involving the LH were shown to suppress feeding and induce weight loss (Anand & Brobeck, 1951). Therefore, the VMN and LH have long been known as the brain "satiety" and "hunger" centres, respectively (Stellar, 1954).

The LH is generally thought to play a significant role in motivated behaviours, including appetitive behaviour (Petrovich, 2018). LH neurons express the orexigenic neuropeptides orexin and melanin-concentrating hormone (MCH). Orexin produced in the LH neurons increases caloric intake via direct activation of the ARC AgRP/NPY neurons and indirect inhibition of the ARC POMC-expressing neurons (van den Top et al., 2004; Ma et al., 2007). Additionally, MCH overexpression in the LH has been shown to induce a hyperphagic response in mice (Ludwig et al., 2001).

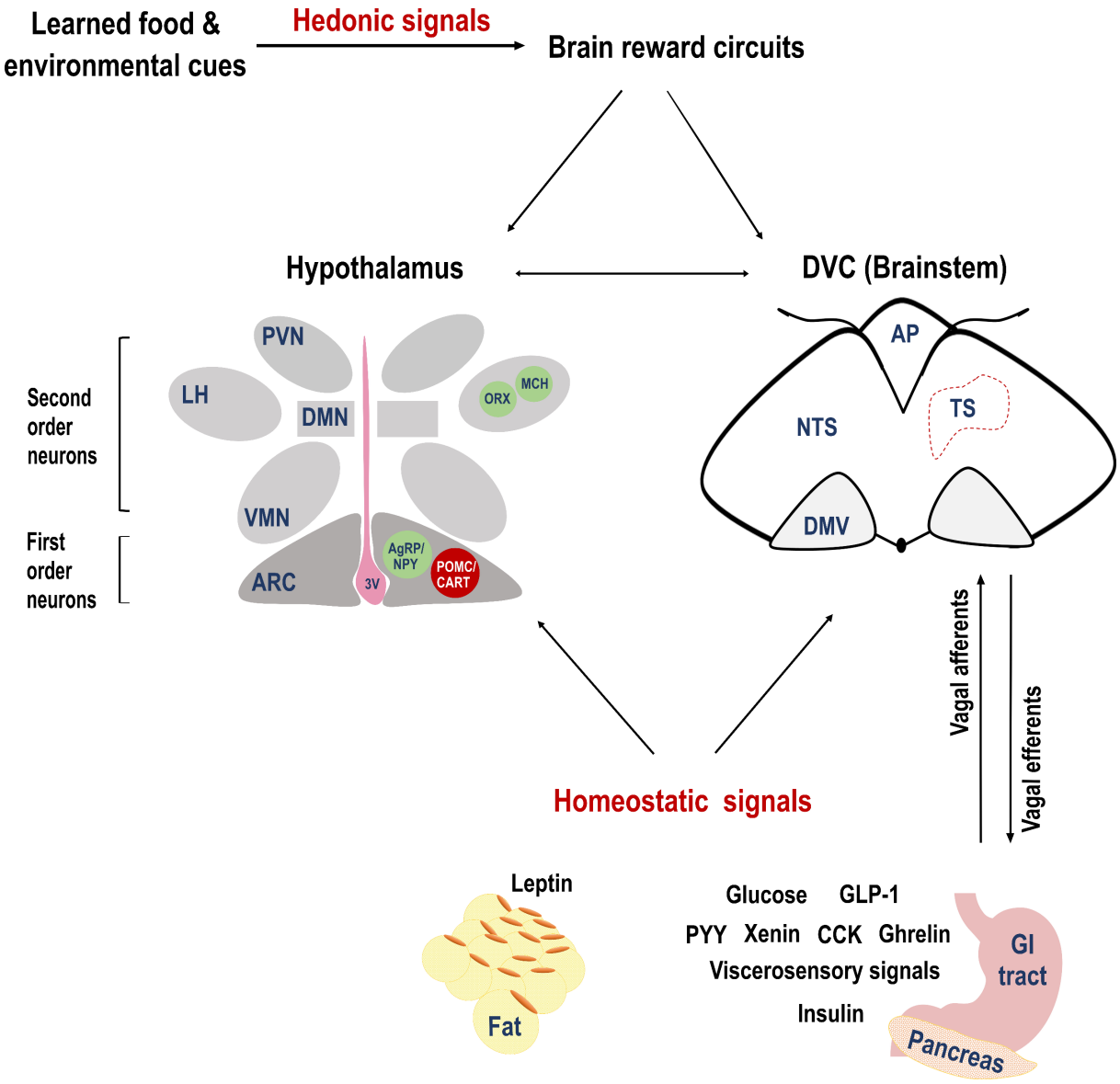


Figure 1 Simplified schematic of peripheral and central circuits controlling food intake. Peripheral homeostatic signals (e.g., leptin, insulin, GLP-1, ghrelin, etc.) are directly or indirectly integrated in the ARC first-order AgRP/NPY or POMC/CART expressing neurons as well as the hindbrain homeostatic circuits (DVC). Both the hypothalamus and the DVC have a reciprocal connection to the corticolimbic reward circuits (i.e., amygdala, VTA, NAc, mPFC), leading to a coordinated hedonic and homeostatic feeding response.

1.1.2. Extrahypothalamic circuits regulating food intake

The CNS control of food intake relies not only on the hypothalamus but also on extrahypothalamic brain regions. The dorsal vagal complex is an important controller of food intake (Fig.1). It comprises three important nuclei located in the caudal brainstem: (1) the nucleus tractus solitarii (NTS), a viscerosensory integration centre that receives multiple inputs from the visceral organs, including signals from the gastrointestinal (GI) tract, via vagal afferents; (2) the area postrema (AP), a circumventricular organ located atop of the NTS and plays a vital role in energy balance; and (3) the dorsal motor nucleus of the vagal nerve (DMV) which controls vagal innervation to many visceral organs and regulates GI secretion and motility. The dorsal vagal complex is reciprocally connected to the hypothalamus, representing an important centre for metabolic and mechanosensory signals integration between peripheral visceral organs and the central circuits controlling food intake (Reviewed in Browning & Carson, 2021). Vagal afferents are thought to initiate satiety perception in response to feeding. Indeed, selective vagal deafferentation has been shown to increase meal size (Schwartz et al., 1999). Also, most GI vagal afferents have been shown to terminate within the NTS, with fewer afferents terminating in the AP and DMV. Of note, the NTS is extensively connected to the PVN, allowing for hypothalamus-brainstem functional coordination in food intake regulation (Geerling et al., 2010). Moreover, some NTS neurons have been shown to express feeding-relevant neuropeptides, including GLP-1, NPY, and POMC (Ahima & Antwi, 2008). In fact, studies have shown that activation of the NTS GLP-1-expressing and POMC-expressing neurons was sufficient to suppress feeding (Reviewed in Trapp & Richards, 2013; Zhan et al., 2013).

Notably, hedonic feeding is under the joint control of the mesolimbic reward circuits, specifically the ventral tegmental area (VTA) neurons and their projections to the nucleus accumbens (NAc), and the LH neurons (Fig. 1). Studies have shown that glucose deprivation increases the activity of the excitatory orexin-expressing LH neurons, which in turn project to and increase the dopaminergic neurotransmission in the VTA neurons (Sheng et al., 2014). Furthermore, optogenetic activation of a subset of the VTA-projecting LH gamma-aminobutyric acid-expressing (GABAergic) neurons increased the consummatory reward-seeking aspect of food intake (Jennings et al., 2015) and compulsive sucrose-seeking behaviour in mice (Nieh et al., 2015). Additionally, consumption of palatable food was shown to increase dopamine transmission

in the NAc and the medial prefrontal cortex (mPFC) in rats (Ghiglieri et al., 1997; Bassareo & Di Chiara, 1999).

The amygdala also participates in the regulation of food intake. It is a deep brain structure composed of a cluster of nuclei bilaterally situated in the medial temporal lobes (LeDoux, 2007). The amygdala consists of three major sub-regions, the basolateral (BLA), centromedial (CeA), and cortical amygdala, as well as the intercalated cell masses (ITCs). Studies have shown that lesions affecting the posterodorsal aspects of the amygdala, spanning all nuclei, increased the preference for carbohydrate-rich food in mice (King et al., 1998; King et al., 2003). Conversely, optogenetic activation of a subset of CeA GABAergic neurons has been shown to induce a robust suppression of food intake in mice (Cai et al., 2014). The amygdala regulates food intake by integrating neural, neuroendocrine, and gastrointestinal signals. For example, the amygdala receives projections of the hypothalamic AgRP-expressing neurons and express MC4R. Administration of an MC4R agonist into the CeA reduced food intake, while MC4R antagonist injection increased food intake (Boghossian et al., 2010). Moreover, the CeA receives indirect neural input from the brainstem, and a subpopulation of the CeA neurons were found to mediate the anorexigenic action of the GI hormone cholecystokinin (CCK) (Wu et al., 2012; Carter et al., 2013; Cai et al., 2014). Additionally, both environmental stressors exposure or intra-BLA infusion of corticotropin-releasing hormone (CRH) were found to reduce food intake significantly (Jochman et al., 2005).

Higher cognitive centres also contribute to the modulation of feeding. Revisiting a hippocampal-dependent memory of the taste or smell of palatable food triggers food-seeking behaviour and influences meal onset (Parent et al., 2014). Therefore, the hippocampus is thought to contribute to food intake regulation. Indeed, studies have shown that functional impairments in hippocampal activity elicit a hyperphagic response in rats (Henderson et al., 2013; Hannapel et al., 2017) and humans (Hebben et al., 1985; Rozin et al., 1998). Accordingly, it has been postulated that overeating might result from an inability of the hippocampus to control the appetitive drive in response to learned food cues (Davidson et al., 2014). Additionally, gastric distention induces an increase in hippocampal activity (Min et al., 2011). Also, the hippocampus is known to express receptors for GI peptides that modulate food intake, including insulin and leptin (Kanoski & Grill, 2017). Moreover, high-sugar diets have been associated with impairments in hippocampus-dependent learning in rats (Hsu et al., 2015; Beilharz et al., 2016) and humans (Wolnerhanssen et al., 2015; Stopyra et al., 2021), suggesting that metabolic cues can alter hippocampal functions.

1.2. Regulation of caloric intake by peripheral signals

Caloric intake is regulated by an array of overlapping, often redundant, pathways. Multiple peripheral signals work in concert to convey the body's energy state to the central neural circuits controlling energy balance.

1.2.1. Nutritional signals: emphasis on glucose role in feeding modulation

Nutritional signals (i.e., glucose, fatty acids) play an essential role in energy homeostasis (Reviewed in Jordan et al., 2010; Tremblay & Bellisle, 2015). Being one of the most important energy sources, glucose is undoubtedly an effective gauge of energy availability and subsequently an effective modulator of food intake. Indeed, numerous studies have shown that peripheral administration of glucose suppresses feeding (Herberg, 1960; Mizuno et al., 2013). Postulated by Mayer and Bates, the glucostatic hypothesis was the first to propose that post-prandial increase in glucose availability elicits changes in the hypothalamic neurons and signals meal termination (Mayer & Bates, 1952; Mayer, 1955; Revisted in Mobbs et al., 2005). This hypothesis was initially met with fierce dismissal due to a lack of empirical evidence directly implicating hypothalamic glucose availability in meal termination. However, subsequent investigations showed that hypothalamic glucose sensing is an integral component in mediating the homeostatic response of glucose, including modulation of food intake (For review see Routh et al., 2014). The hypothalamus, particularly the ARC and VMN, contains glucose-excited and glucose-inhibited neurons whose frequency of action potential firing increases or decreases, respectively, in response to changes in cerebral glucose availability. In connection to this, studies have shown that intra-hypothalamic or intra-ARC glucose infusion reduced food intake and inhibited NPY expression (Kurata et al., 1986; Chang et al., 2005). Furthermore, impairments in hypothalamic glucose sensitivity were found to diminish glucose-induced feeding suppression (Bady et al., 2006; Parton et al., 2007; Stolarczyk et al., 2010; de Andrade et al., 2015). Of note, glucose availability is known to activate AMP-activated protein kinase (AMPK) signalling cascade in the hypothalamic glucose-sensing neurons (Kim et al., 2004; Claret et al., 2007; Murphy et al., 2009).

Glucose-sensing neurons are also found in extrahypothalamic brain regions, including the brainstem (Ritter et al., 1998; Roberts et al., 2017) and amygdala (Nakano et al., 1986; Zhou et al., 2010), and have been shown to contribute to glucose homeostasis and food intake. In addition to glucose-sensing neurons, glial cells expressing glucose transporter 2 (GLUT2), mainly astrocytes and tanycytes, were shown to participate in hypothalamic glucose sensing (García et al., 2003;

Lam et al., 2005; Marty et al., 2005; Stolarczyk et al., 2010). In relation to this, a recent study suggested that glucose can actively diffuse into the cerebrospinal fluid (CSF) of the median eminence via β2 tanycytes expressing GLUT2 and GLUT6, leading to a "fast-track" activation of the glucose-sensing components of the hypothalamus (Martínez et al., 2019).

1.2.2. Adiposity signals: emphasis on leptin role in feeding modulation

During the positive energy balance state, insulin and the adipocyte hormone leptin circulate in the blood and cross the BBB to bind to their respective receptors in the ARC POMC-expressing neurons, causing suppression of feeding (Reviewed in Morton et al., 2014). Moreover, leptin signals through the ARC AgRP/NPY-expressing neurons to reduce its orexigenic tone and consequently reduces food intake (Reviewed in Andermann & Lowell, 2017). In contrast, circulating insulin and leptin levels during the negative energy balance state are reduced, leading to disinhibition of the AgRP/NPY neurons and a subsequent increase in food intake. Thus, the central feeding response of leptin is believed to be mediated through the hypothalamic melanocortin system.

Leptin provides long-term feedback to regulate food intake. Leptin is a multitarget hormone that is secreted from white fat cells in proportion to fat mass, crosses the BBB in proportion to its plasma concentration, and activates the central melanocortin circuits responsible for energy homeostasis (Zhang et al., 2005; Triantafyllou et al., 2016). Studies have shown that intracerebroventricular infusion of leptin reduces food intake (Campfield et al., 1995; Calapai et al., 1998). Moreover, defective central leptin signalling causes hyperphagic obesity (Cohen et al., 2001; de Git et al., 2018). Besides its prominent role in hypothalamic modulation of food intake, leptin also acts on other brain regions where leptin receptors are expressed. For example, leptin reduces hedonic feeding and food-seeking behaviour by acting on the dopaminergic neurons in the VTA (Hommel et al., 2006; Bruijnzeel et al., 2013). Together, these studies present compelling evidence for the role of leptin in reducing food intake.

Leptin resistance is a hallmark of severe obesity. Obese individuals often have high circulating levels of leptin and reduced leptin sensitivity (Izquierdo et al., 2019; Liu et al., 2020). Hypothalamic leptin resistance is believed to result from defective activation of the downstream Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signalling cascades in leptin-sensitive neurons (El-Haschimi et al., 2000; Bates et al., 2003). Of interest, leptin resistance is also thought to result from impairments in leptin transport across the BBB

(Banks et al., 1999). In support of this, intracerebroventricular infusion of leptin potently reduced food intake in leptin-resistant mice, whereas peripheral administration failed to elicit a leptin response (Halaas et al., 1997; Van Heek et al., 1997). Moreover, the ratio of leptin levels in the CSF to leptin levels in the plasma was shown to be significantly lower in obese subjects relative to healthy non-obese control (Caro et al., 1996; Schwartz et al., 1996). These studies support the notion that impaired leptin transport across the BBB is responsible for leptin resistance in obese individuals. Contrary to such notion, a recent study demonstrated intactness of leptin transport across the BBB in obese leptin-resistant mice and also showed a comparable leptin accumulation in metabolically-relevant brain regions in both obese and lean mice (Harrison et al., 2019), reflecting a poorly understood underlying mechanism of leptin resistance in obesity.

Studies have shown that both leptin and glucose converge on the AMPK signalling pathway (Kim et al., 2004; Minokoshi et al., 2004). Moreover, leptin and glucose are thought to influence each other's functions synergistically. For example, leptin has been found to enhance hypothalamic glucose sensitivity (Abraham et al., 2018), and reciprocally glucose was found to enhance leptin signalling (Su et al., 2012). Importantly, glucose-induced feeding suppression is abolished in the leptin-deficient *ob/ob* mice (Poritsanos et al., 2011). Together, these data indicate that leptin and glucose can suppress feeding via a common signalling pathway. However, glucose sensing is also mediated via glia-neuron interaction (Routh et al., 2014; Elizondo-Vega et al., 2015), suggesting an additional leptin-independent pathway for glucose action.

1.2.3. GI signals: emphasis on xenin role in feeding modulation

The GI tract is primarily involved in short-term feeding regulation. It produces hormones that predominantly inhibit food intake (e.g., GLP-1, peptide tyrosine tyrosine [PYY], CCK, xenin; represented in Fig. 1), these anorectic peptides are integrated in the central feeding circuits, eliciting an acute feeding-suppressing response (Leckstrom et al., 2009; Carabotti et al., 2015; Kairupan et al., 2016). Furthermore, the GI tract contains stretch mechanoreceptors that detect food-mediated gastric and intestinal distension and signal to the CNS via vagal afferents, causing inhibition of the orexigenic AgRP neurons in the hypothalamus (Bai et al., 2019). Conversely, ghrelin is the only known feeding-inducing GI peptide that functions via activation of the AgRP/NPY co-expressing neurons in the hypothalamus (Kamegai et al., 2001; Nakazato et al., 2001).

Since this part of the present thesis examined the anorectic response to xenin, it would be of the reader's benefit to expand on xenin's role in regulating feeding behaviour. Xenin is a gut peptide that modulates intestinal motility, exocrine pancreatic secretion and has also been demonstrated to modulate appetite suppression (Reviewed in Craig et al., 2018). The first evidence of the role of xenin in feeding suppression emerged when intracerebroventricular infusion of xenin elicited a dose-dependent anorectic response in fasted rats (Alexiou et al., 1998). Moreover, xenin plasma concentration increased following food intake (Feurle et al., 2003). Subsequent studies demonstrated that both Intracerebroventricular and intraperitoneal administration of xenin suppressed food intake significantly in chicks (Cline et al., 2007) and mice (Cooke et al., 2009; Leckstrom et al., 2009; Taylor et al., 2010). In addition, intraperitoneal administration of xenin was shown to increase hypothalamic activity, as evidenced by an increased Fos-immunoreactivity in chicks (Cline et al., 2007; Nandar et al., 2008) and mice (Leckstrom et al., 2009). Similarly, intraperitoneal xenin treatment increased Fos-immunoreactivity in the NTS (Kim & Mizuno, 2010). Of interest, the anorectic effect of xenin, while not fully characterised, is believed to be independent of the ARC melanocortin signalling pathway (Leckstrom et al., 2009). The same study also showed that xenin treatment suppressed food intake in *ob/ob* mice, suggesting that xenin regulates feeding, at least partly, through a pathway that is independent of leptin and, by proxy, also independent of glucose.

1.3. Neurotrophic factors control of energy balance

Neurotrophic factors (NTFs) are a diverse family of growth factors that are integral to neuronal circuits formation, growth, and survival. NTFs, therefore, support higher cognitive functions, including memory acquisition and retrieval, learning, and behaviour (Reviewed in Platholi & Lee, 2018). NTFs are also known to modulate the central circuits controlling energy homeostasis and has been shown to control energy intake (Rios, 2014).

1.3.1. Brain-derived neurotrophic factor (BDNF)

BDNF is crucial for the normal functioning of brain neuronal circuits and has been shown to support a broad range of cognitive functions (Reviewed in Miranda et al., 2019). Apart from its well-documented role in cognitive development, BDNF has also been found to influence the brain circuits responsible for food intake and energy expenditure, rendering it an important modulator of energy homeostasis (Rios, 2013; Vanevski & Xu, 2013; Xu & Xie, 2016).

1.3.1.1. BDNF role in food intake

The BDNF metabolic role was serendipitously discovered in a study initially aiming to examine the extent of BDNF role in modulating the functions of hippocampal cholinergic neurons in rats (Lapchak & Hefti, 1992). In their study, Lapchak and Hefti found that chronic central administration of recombinant human BDNF (rh-BDNF) protein reduced body weight significantly. Such BDNF-induced reduction in body weight was later attributed to significant suppression of caloric intake, effectively establishing a novel anorectic function of BDNF (Pelleymounter et al., 1995). The abovementioned pharmacological studies were soon corroborated with causal genetic evidence as two independent studies have shown that heterozygous deficiency in the *Bdnf* gene, the mouse homologue of the human *BDNF,* induces hyperphagic obesity in mice (Lyons et al., 1999; Kernie et al., 2000). Also, conditional brain deletion of BDNF postnatally increased food intake in mice (Rios et al., 2001).

BDNF exerts its diverse biological functions via signalling through either its high-affinity receptor tropomyosin receptor kinase B (TrkB) or the non-selective neurotrophin receptor P75 ($P75^{NTR}$), which binds all known members of the neurotrophin family (Sasi et al., 2017). Studies have shown that TrkB-knockdown mice are hyperphagic and obese (Xu et al., 2003). Furthermore, peripheral or hypothalamic injections of neurotrophin-4, a TrkB agonist, were proven sufficient to ameliorate obesity in leptin-deficient mice, as well as in a mouse model of diet-induced obesity (Tsao et al., 2008). While TrkB activation induces feeding suppression and weight loss, $P75^{NTR}$ signalling has been recently shown to promote feeding in response to prolonged fasting (Podyma et al., 2020) and to precipitate obesity in mice fed on a high-fat diet (Baeza-Raja et al., 2016). Thus, it seems that selective BDNF signalling through the TrkB and $P75^{NTR}$ receptors exert antagonistic control of feeding behaviour and body weight, further delineating a complex role of BDNF in energy balance regulation.

The metabolic role of BDNF has also been investigated in humans. A clinical case report showed that an inversion mutation involving the region of the *BDNF* gene on chromosome 11 was associated with hyperphagic obesity and impaired cognitive functions (Gray et al., 2006). Moreover, *BDNF* haploinsufficiency has been shown to cause hyperphagic obesity in patients suffering from WAGR syndrome (Han et al., 2008). Similarly, *BDNF* single-nucleotide polymorphisms (SNPs) have been associated with obesity (Mou et al., 2015; Han, 2016).

Additionally, mutations involving the TrkB-coding gene, albeit rare, have been shown to precipitate obesity in humans (Yeo et al., 2004; Gray et al., 2007; Sonoyama et al., 2020).

Of note, BDNF expression is subject to changes in the nutritional state. Studies have shown that food deprivation causes a drastic reduction in *Bdnf* mRNA levels in the VMN in mice (Xu et al., 2003; Tran et al., 2006; Unger et al., 2007). Interestingly, peripheral administration of glucose was found to increase *Bdnf* mRNA in the VMN (Unger et al., 2007). Similarly, food deprivation reduced BDNF content in the DVC, whereas refeeding or peripheral administration of leptin were sufficient to increase DVC BDNF levels (Bariohay et al., 2005). Collectively, these data show that BDNF is a critical central effector of food intake in response to the body's metabolic state.

1.3.1.2. BDNF role in glucose homeostasis

CNS BDNF has also been implicated in glucoregulation. It has been shown that repetitive peripheral BDNF administration improved impaired glucose tolerance in a mouse model of diet-induced obesity as well as in the leptin resistant lethal yellow *agouti* mice compared with vehicle-treated pair-fed respective controls (Nakagawa et al., 2003). Likewise, intracerebroventricular administration of BDNF reduced serum glucose and increased insulin levels in the pancreas of the leptin receptor-deficient *db/db* mice relative to vehicle-treated pair-fed control (Nonomura et al., 2001). Reciprocally, peripheral glucose administration was found to increase *Bdnf* mRNA expression in the hypothalamus, further arguing for a BDNF protective role in glucose homeostasis (Unger et al., 2007).

Peripheral BDNF signalling also plays a role in glucose homeostasis. A recent study demonstrated that BDNF-TrkB signalling in pancreatic β-cells is vital for glucose-induced insulin secretion (Fulgenzi et al., 2020). Interestingly, the same study showed, for the first time, that BDNF produced in skeletal muscles is necessary for pancreatic TrkB activation and the subsequent improvement in insulin release, providing direct evidence that BDNF can act as a blood-borne hormone in addition to being an autocrine agent. In contrast to TrkB signalling, $P75^{NTR}$ signalling has been suspected in insulin resistance and dysregulation of glucose homeostasis. Indeed, $P75^{NTR}$ knockout mice display improved insulin sensitivity via an extra-pancreatic mechanism involving increased insulin-dependent glucose uptake in skeletal muscles and adipocytes (Baeza-Raja et al., 2012).

1.3.1.3. BDNF role in energy expenditure

Both central and peripheral administration of BDNF were shown to increase the sympathetic outflow and uncoupling protein 1 (UCP1) expression in brown adipose tissue (BAT), leading to improved thermogenesis in obese diabetic mice (Nonomura et al., 2001; Tsuchida et al., 2001). Moreover, BDNF injection into the PVN improved energy expenditure via increasing UCP1-mediated BAT thermogenesis (Wang, Bomberg, Billington, et al., 2007), Whereas BDNF injection into the VMN improved energy expenditure without significantly changing UCP1 expression in BAT (Wang et al., 2010). Also, a recent study has shown that disrupted *Bdnf* expression in the LH led to impairments in BAT thermogenesis, whereas rescue of BDNF-TrkB signalling in the LH restored BAT thermogenesis to normality (You et al., 2020). It has also been shown that $P75^{NTR}$ knockout mice are immune to diet-induced obesity due to an upregulated fat oxidation and an overall increase in energy expenditure (Baeza-Raja et al., 2016). Collectively, these reports, although scarce, suggest that BDNF is also an important modulator of energy expenditure.

1.3.1.4. The *BDNF* genomic structure

The *BDNF* gene has a uniquely complex genomic structure comprised of multiple 5' non-coding exons splicing onto a common 3' single coding-exon and giving rise to multiple *BDNF* mRNA transcripts, remarkably all of which encode an identical BDNF protein. This already complex structure is further complicated by the presence of two alternative poly(A) sites in the coding exon, giving rise to two *BDNF* mRNA species with short and long 3' untranslated regions (3' UTR) copies of each *BDNF* transcript. The human *BDNF* gene is composed of 11 exons and of at least nine promoters driving the expression of at least 18 *BDNF* mRNA transcripts (First published in Pruunsild et al., 2007; Illustrated in Figure 1 of Cattaneo et al., 2016; Reviewed in Notaras & van den Buuse, 2019).

The mouse *Bdnf* gene is an analogue of the human *BDNF* gene but with fewer exons and fewer transcript variants (Fig. 2). The mouse *Bdnf* gene comprises nine exons (I-IX) encoding for 11 *Bdnf* mRNA transcripts (Aid et al., 2007; West et al., 2014). Here, the 5' non-coding exons (exons I-VIII) splice onto a common 3' coding exon (exon IX). Moreover, exon II has three splice donor sites giving rise to three distinct *Bdnf* mRNA transcripts (*IIa*, *IIb*, and *IIc*). Furthermore, exon IX has a longer 5' UTR preceding the coding sequence and has the unique ability to splice onto itself (Fig. 1; Aid et al., 2007; West et al., 2014). The biological need for having multiple

Bdnf mRNA splice variants encoding an identical protein is poorly understood; however, it has been long suspected that *Bdnf* transcription is subject to temporal, spatial, and environmental influences (Greer & Greenberg, 2008; Hong et al., 2008). Moreover, it has recently been shown that different *Bdnf* mRNA transcripts promote different behavioural and metabolic functions (Maynard et al., 2016; McAllan et al., 2018; Ramnauth et al., 2021).

BDNF is first translated in the rough endoplasmic reticulum ribosomes as preproBDNF. The endoplasmic-reticulum-targeting signal peptide (N-terminal pre-domain) in the nascent preproBDNF form is then sequestered in the endoplasmic reticulum and cleaved into a 32 kDa proBDNF which can be packaged and secreted as a functional protein (Lee et al., 2001; Chao & Bothwell, 2002) that binds $P75^{NTR}$ exclusively (Teng et al., 2005a). Alternately, proBDNF can undergo further intracellular cleavage to produce a 17 kDa BDNF pro-peptide and a 14 kDa mature BDNF protein (mBDNF) which binds TrkB preferentially (Reviewed in Sasi et al., 2017).

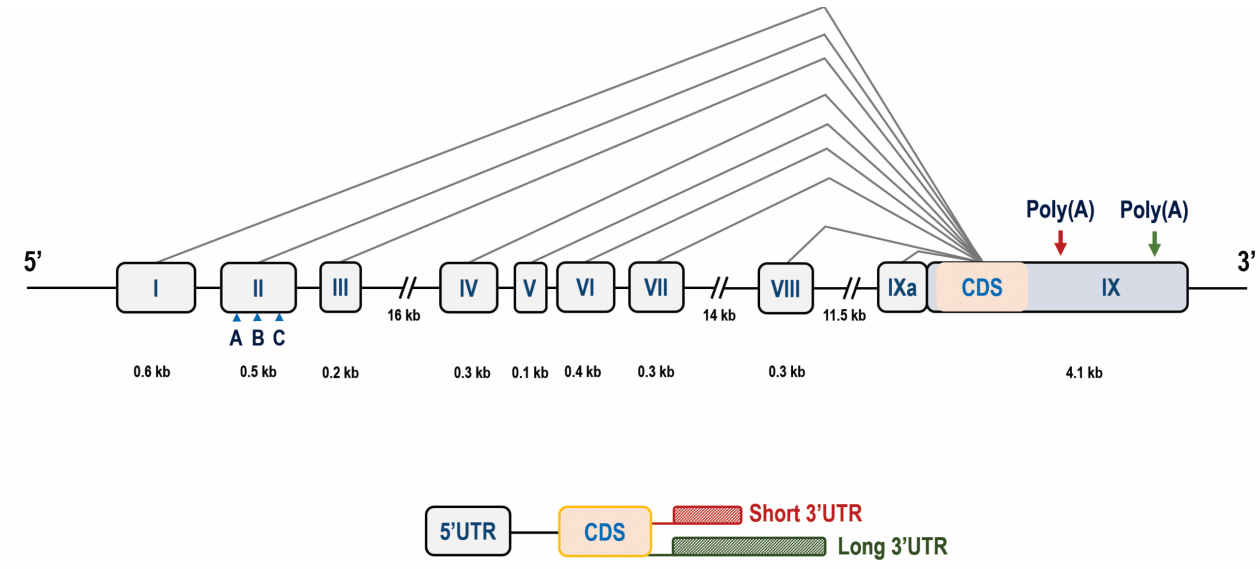


Figure 2 Schematic representation of the mouse *Bdnf* gene showing alternative splicing and 3'UTR polyadenylation signals. Modified from (Aid et al., 2007).

1.3.1.5. BDNF signalling and energy balance

The extent of BDNF functions depends on the type of receptor it binds and its downstream signalling pathways (Yamashita et al., 1999; Huang & Reichardt, 2003; Zampieri & Chao, 2006; Lin et al., 2015). Ergo, BDNF has been shown to support antagonistic receptor-dependent roles on neuronal networks survival and maturation (Harrington et al., 2002; Yoshii & Constantine-Paton, 2010). However, factors influencing BDNF differential signalling through either the TrkB or the $P75^{NTR}$ in the regulation of energy balance remain largely unknown. Although, it has been suggested that the specificity of BDNF signalling could be mediated via the receptor affinity to bind BDNF. Therefore, a cell-type-specific preference to express a specific BDNF receptor under specific metabolic conditions could control BDNF functional metabolic outcomes. Additionally, post-translational modifications of the BDNF protein play a deciding role in BDNF receptor selectivity. For example, the immature precursor of the BDNF protein, proBDNF, is known to bind $P75^{NTR}$ exclusively and antagonise mBDNF-TrkB functions (Teng et al., 2005a).

Previous studies suggested that proBDNF-$P75^{NTR}$ signalling mediates an orexigenic function, which essentially opposes the long-established BDNF-TrkB anorectic role in feeding regulation (Podyma et al., 2020). Therefore, it is likely that proBDNF-$P75^{NTR}$ signalling is active during the state of negative energy balance to promote feeding. Then, when energy balance is restored or during the positive energy balance state, proBDNF is cleaved to produce mBDNF and predominantly signal through TrkB to suppress food intake.

1.3.2. Glial cell line-derived neurotrophic factor (GDNF) role in energy homeostasis

GDNF is primarily involved in the development and maturation of mesencephalic dopamine (DA) neurons and the restoration of damaged adults DA neurons (Ibáñez & Andressoo, 2017). GDNF signals via the GDNF family receptor-α (GFR-α) leading to the recruitment and activation of the rearranged during transfection (RET) receptor tyrosine kinase (Reviewed in Conway et al., 2020). In addition to its trophic functions in dopaminergic neurons, GDNF has been shown to play a role in energy balance and body weight regulation. Studies have shown that intracerebroventricular administration of GDNF reduced body weight significantly in rodents (Lapchak et al., 1997), rhesus monkeys (Su et al., 2009), and humans (Nutt et al., 2003; Whone et al., 2019). Additionally, viral-mediated GDNF overexpression in the hypothalamus reduced body weight in aged rats and hindered body weight gain in young ones (Tümer et al., 2006). The same study concluded that GDNF-mediated reduction in body weight was associated with reduced food

intake and a concurrent increase in energy expenditure, independent of alterations in hypothalamic dopamine levels. Another study has also shown that viral-mediated nigrostriatal GDNF overexpression in obese rats reduced body weight at a more robust rate compared with hypothalamic GDNF overexpression (Manfredsson et al., 2009); such robust reduction in body weight was independent of motor activity. Indeed, it has been shown that a single unilateral nigrostriatal injection of GDNF in adult rats produced a significant anorectic effect that lasted up to seven days post-injection and was also associated with a concurrent increase in motor activity (Hudson et al., 1995). Together, these data show that GDNF plays an important role in energy balance.

1.3.3. Mesencephalic astrocyte-derived neurotrophic factor (MANF) role in energy homeostasis

MANF is a non-canonical neurotrophic factor that does not share any homology of its protein sequence with the neurotrophins family of growth factors; however, its mode of action and signalling pathways are comparatively similar to canonical neurotrophins (Discussed in Yang et al., 2018). The metabolic role of MANF was first described in a clinical exome sequencing study which showed that a *MANF* mutation was responsible for obesity, type 2 diabetes, and intellectual disability in a female patient (Yavarna et al., 2015). A follow-up study in rodents showed that MANF is abundantly expressed in the hypothalamus in response to changes in energy balance and plays an orexigenic role (Yang et al., 2017). Moreover, the same study showed that hypothalamic MANF controls food intake without affecting energy expenditure. Although limited, these studies show that hypothalamic MANF is a novel regulator of food intake.

MANF has also been shown to play a protective role in insulin signalling. MANF-deficient mice develop severe early-onset diabetes precipitated by a post-natal reduction in the proliferations of pancreatic β cells and increased β cells destruction (Lindahl et al., 2014). In contrast, recombinant human MANF reduced stress-induced apoptosis in primary human β cells as well as in the EndoC-βH1 cell line, an experimentally valid model of human β cells (Hakonen et al., 2018). Taken together, MANF plays a role in food intake and glucose homeostasis. Identifying the brain region-specific expression of *Manf* could offer a much needed and novel insight into the brain circuits responsible for mediating its role in food intake.

1.4. Social and environmental factors influencing obesity: epidemiological evidence

The influence of social and environmental factors on the regulation of energy balance is well-documented. Obesity has long been known to be a multifaceted disease process with genetic and environmental attributes (Allison et al., 2008). From an epidemiological perspective, Factors influencing obesity development can be divided into three interconnected categories: (i) individual-related, (ii) environment-related, and (iii) socially-influenced. Individual-related factors are those related to an individual's sex, age, and ethnicity. Environmental-related factors relate to the individual's home and work environments, geographical location, and access to advanced technology. Socially influenced factors relate to the individual's social status and social circles.

1.4.1. Individual-related factors influencing obesity

Obesity prevalence rates exhibit a cross-sectional increase with age. In Canada, obese individuals distributions were 8.6% (18-19 years old), 19.9% (20-34 years old), 30.3% (35-49 years old), 31.3% (50-64 years old), according to the 2018 Canadian community health survey (Statistics Canada, 2019). Moreover, overweight and obesity rates were higher in Canadian males (69.4%) compared to females (56.7%). Additionally, obesity rates were significantly higher in Indigenous individuals living off-reserve compared to the non-Indigenous Canadian population (Gionet & Roshanafshar, 2013). Obesity rates were also significantly lower in landed immigrants and individuals with advanced academic degrees compared to the general Canadian population (Public Health Agency of Canada, 2011).

1.4.2. Environment-related factors influencing obesity

An individual's environment is a potent contributor to the development of obesity and associated co-morbidities (Bray & Champagne, 2005; Eisenmann, 2006; Keith et al., 2006). It is estimated that people spend a quarter of their adult lives in the workforce. Therefore, the work environment is an important determinant of feeding behaviour and physical activity patterns. Indeed, multiple studies showed that desk-bound jobs are associated with higher obesity rates and increased cardiovascular risk (Church et al., 2011; Tigbe et al., 2017). Moreover, a significant positive correlation exists between work-environment-related stresses and BMI (Netterstrøm et al., 1991; Hellerstedt & Jeffery, 1997; Martikainen & Marmot, 1999; Ostry et al., 2006). Also, jobs that require long shifts have been associated with higher BMI and overall increased risk of obesity (van Amelsvoort et al., 1999; Di Lorenzo et al., 2003). On the other side, adult

unemployment has also been associated with increased BMI, especially among females (Laitinen et al., 2002). Together, these studies show that a healthy work environment enriched with positive mental and physical stimulations is necessary for workers' overall physiological and psychological well-being.

An individual's home environment plays a significant role in shaping their metabolic profile, especially during childhood. A recent systematic review highlighted a significant influence of the home media environment on the development of childhood obesity (Kininmonth et al., 2021). This study showed that uncontrolled access to electronic media equipment (e.g., game consoles, handheld devices, etc.) is associated with increased BMI in children aged three to twelve years old. Another systematic review concluded that exposure to air pollutants in the home environment is positively associated with a higher prevalence of obesity (Parasin et al., 2021). Interestingly, accessibility to urban green open spaces and community recreational facilities is positively associated with increased physical activity (Wang, Dai, et al., 2019). However and surprisingly, individuals living in rural environments have been shown to have a significantly higher BMI compared to individuals living in urban environments (N. C. D. Risk Factor Collaboration, 2019).

Neighbourhood socioeconomic and ethnic compositions are also believed to be significant environmental determinants of food choices (Ball et al., 2009; Chen et al., 2016). Moreover, studies have shown that neighbourhood safety (i.e., crime rate, vandalism, general maintenance, and security factor) significantly influences residents' physical activity and obesity prevalence (Mayne et al., 2018; Rees-Punia et al., 2018). Interestingly, a randomised controlled trial showed that a neighbourhood shift from a high-poverty environment to a low-poverty environment was associated with reductions in obesity and diabetes prevalence (Ludwig et al., 2011).

An individual's home proximity to the workplace and other necessary services (e.g., supermarkets, hospitals, schools, etc.) can impact the walkability factor and reduce physical activity. Indeed, it has been shown that a one-hour passive daily commute is associated with a 6% increase in obesity likelihood (Frank et al., 2004). In contrast, a randomised clinical trial showed that active commuting (i.e., walking, running, cycling) was sufficient to reduce fat mass in obese individuals (Quist et al., 2018). Collectively, these reports showcase the heterogeneity and complexity of some environment-related factors influencing obesity prevalence.

1.4.3. Social factors influencing obesity development

Interpersonal relationships have been shown to influence obesity development across the lifespan. Multiple studies have found a positive correlation between social relations and obesity risk. For instance, it has been shown that an individual's likelihood to develop obesity increases by 57% just by having an obese friend; it also increases by 40% if an individual has an obese sibling and by 37% if they have an obese spouse (Christakis & Fowler, 2007; Fowler & Christakis, 2008). Similarly, studies have shown that adolescent friends are likely to have similar feeding behaviour and BMI (Fletcher et al., 2011; Simpkins et al., 2013). Interestingly, social norms and peer-modelling have been shown to influence feeding behaviour and food choices, especially in adolescents (Pachucki et al., 2011; Robinson et al., 2013). Furthermore, familial and social influences have been shown to contribute significantly to physical activity patterns in youth (Macdonald-Wallis et al., 2012; Maturo & Cunningham, 2013).

An interesting study examining the relationship between marital status and obesity showed that married males tend to have a higher body weight compared to males without an active partner, whereas married females were found to have a lower body weight compared to females who were never married (Sobal & Hanson, 2011). Another interesting study showed that individuals perceived as belonging to a lower social class were found to have a higher perception of hunger than individuals belonging to a higher social class (Cheon & Hong, 2017). Notably, multiple studies have shown that social media networks can have a positive influence in reducing obesogenic behaviour and associated health risks, especially in young adults (Centola, 2013; Li et al., 2013; Napolitano et al., 2013).

To reiterate, epidemiological data present clear evidence of the involvement of environmental and social factors in precipitating obesity, and consequently other co-morbidities, across the lifespan. However, the specific physiological and neural mechanisms mediating the social and environmental influences on energy balance remain obscure. Understanding the gene-environment interplay is of paramount importance in understanding and resolving the obesity epidemic.

1.5. Environmental enrichment

Environmental enrichment is an experimental paradigm that modifies the cage environment's physical, cognitive, and social elements, reaping measurable physiological and behavioural benefits across the animal's lifespan. In its essence, the environmental enrichment paradigm attempts to artificially mimic the complex environmental and social experiences that animals would inadvertently encounter in the natural environment but in controlled laboratory settings. The earliest attempt to improve the housing environment of captive lab animals dates back to 1947 when the infamous Canadian psychologist Donald O. Hebb (1904-1985) decided to adopt lab animals in his home and give them free well to interact with his children as part of his scientific quest to understand the influence of experience on cognition. Hebb found that his home-adopted animals were more cognitively advanced and had a higher capacity for learning than their counterparts raised in the standard lab environment (Hebb, 1947; Brown & Milner, 2003). Hebb's findings paved the way for contemporary, now considered classical, experiments examining the influence of environmental enrichment on brain structure and physiology (Diamond et al., 1964; Diamond et al., 1966; Rosenzweig, 1966; Rosenzweig & Bennett, 1969). These earlier attempts substantiated environmental enrichment as a prominent experimental paradigm to examine the influence of the environment on brain development and behaviour. It also added a whole new dimension to the long-contested nature vs nurture debate.

1.5.1. A tale of two animal cage environments

Lab rodents have traditionally been housed in tiny cages supplied with food, water and basic bedding (Fig. 3). Such economically motivated barren conditions deprive the animals of healthy social, sensory, and motor stimulations and prevent them from displaying healthy species-typical behaviours. Indeed, mice housed under the standard cage environment often present abnormal cage-induced stereotypies and increased anxiety levels (Würbel, 2006; Garner et al., 2011; Gross et al., 2012; Novak et al., 2016), impacting their physiological and psychological welfare and reducing the overall applicability of animal-based biomedical research data to humans (Bracken, 2009; Garner et al., 2017).

On the other hand, the environmental enrichment paradigm incorporates complex sensory, motor, and social stimulations in the cage environment (Fig. 3). This is accomplished through group-housing lab rodents in larger-than-standard cages. These enriched cages are supplied with stimulating objects, including running wheels and discs, where animals can exercise, tube mazes,

where they can play and explore, igloos, where they can hide, a house, where they can sleep, and wooden logs to keep them in touch with nature. The primary objective of the environmental enrichment paradigm is to improve the cognitive and physiological welfare of lab animals via increasing the complexity and novelty factors of the cage environment and maximising the display of animals' species-specific typical behaviours.

1.5.2. Environmental enrichment beneficial influence on brain, body, and behaviour

The earliest reported effects of environmental enrichment were anatomical in nature as studies have shown the environmental enrichment elicits an increase in the cortical organisation (Diamond et al., 1964; Diamond et al., 1966; Rosenzweig, 1966; Rosenzweig & Bennett, 1969), dendritic branching (Holloway, 1966; Greenough et al., 1973), and synaptogenesis (Møllgaard et al., 1971; Turner & Greenough, 1985). Other reports showed that environmental enrichment improves hippocampal neurogenesis (Kempermann et al., 1997) and inhibits spontaneous neuronal apoptosis (Young et al., 1999). Importantly, environmental enrichment has also been shown to play a significant neuroprotective function against neurodegenerative disorders (Nithianantharajah & Hannan, 2006; Berardi et al., 2007; Fischer et al., 2007). The same neuroprotective role was also observed in brain lesions (Johansson, 1996; Koopmans et al., 2006; Comeau et al., 2008). Given the abovementioned role of environmental enrichment in improving the brain structural and cellular organisation, it has consequently been shown to influence many behaviours. Indeed, environmental enrichment improves learning and memory and halts the age-associated decline in cognitive functions (Rampon, Tang, et al., 2000; Van Praag et al., 2000; Bennett et al., 2006). Similarly, environmental enrichment has been suggested as a potential therapeutic approach for neuro-developmental disorders (NDDs) in children, including autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD), given its promising results in NDDs animal models (Reviewed in Ball et al., 2019). However, the generalizability of the environmental enrichment therapeutic potential to humans remains challenging to implement.

Fascinatingly, environmental enrichment cognitive benefits have been demonstrated independent of the animal's age (Diamond et al., 1985; Kempermann et al., 2002). Also, multiple studies have presented evidence of transmissibility of enrichment benefits trans-generationally from pregnant mothers to offspring (Arai et al., 2009; Maruoka et al., 2009; Bechard & Lewis, 2016; Yeshurun et al., 2017). These studies showcase the importance of social and environmental stimulations as powerful tools in cognitive development.

Transcending its effect on the brain and behaviour, environmental enrichment has been shown to improve many physiological functions. Multiple studies have shown that environmental enrichment improves immune responses in rodents (Kingston & Hoffman-Goetz, 1996; Marashi et al., 2003a; Benaroya-Milshtein et al., 2004; Arranz et al., 2010) and macaques (Schapiro, 2002). Moreover, different environmental enrichment protocols have been shown to improve cancer remission and reduce tumours growth (Cao et al., 2010; Garofalo et al., 2015; Li et al., 2015; Wu et al., 2016), even the simplest forms of enrichment were proven effective in the activation of anti-tumour immune responses (Takai et al., 2019). Other studies have shown that environmental enrichment improved wound healing and recovery in mice (Vitalo et al., 2009; Vitalo et al., 2012).

1.5.3. The metabolic benefits of environmental enrichment

Of particular relevance to the scope of this study, environmental enrichment has been shown to improve metabolism. Environmental enrichment reduces adiposity, improves glycaemic control, and increases energy expenditure through stimulation of a fat phenotypic transformation from the energy-storing white adipose tissue (WAT) to the thermogenic and energy-expending brown adipose tissue (BAT) via genetic and environmental activation of the hypothalamic-sympathoneural-adipocyte axis (Cao et al., 2011). Another study showed that environmental enrichment improved cognitive impairments and abolished the glucose intolerance associated with chronic high-fat feeding in obese mice (de Souza et al., 2019). Additionally, environmental enrichment was found to reduce adiposity and leptin levels, increase lean mass, and improve glucose tolerance in a mouse model of autism (BTBR mice); this effect, however, was more prominent in males than in females, suggesting a sex-dependent effect of environmental enrichment in metabolism (Queen et al., 2020). Still, much remains to be investigated concerning the role of environmental enrichment on metabolism, particularly its influence on food intake and feeding behaviour.

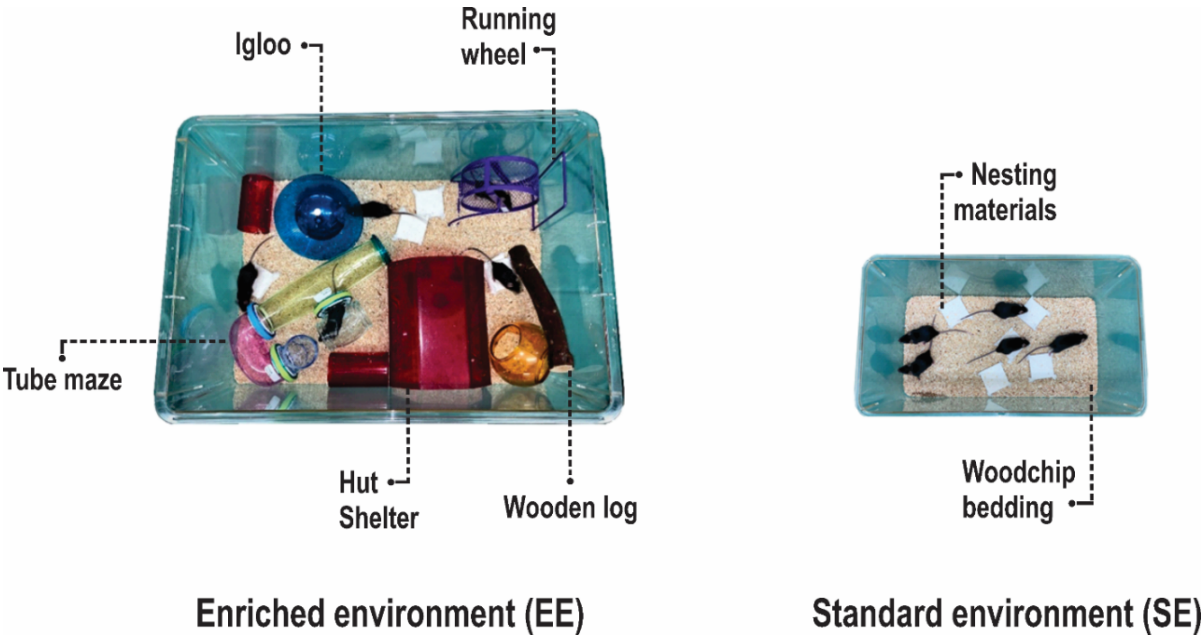


Figure 3 Environmental enrichment (EE) cage vs standard environment (SE) cage. Mice in the EE group were housed in large cages (left) with the following dimensions (58 cm [L] × 48 cm [W] × 30 cm [H]) and supplemented with a hut shelter, a running wheel, a tube maze, an igloo, a hut, a wood gnawing stick, a tube maze, nesting materials, and woodchip bedding. In contrast, mice in the SE group (right cage) were housed in regular cages with the following dimensions (37.5 cm [L] × 19 cm [W] × 12.5 cm [H]) and were only supplemented with nesting materials and woodchip bedding.

1.5.4. The molecular basis of the physiological benefits of environmental enrichment

Earlier studies examining the brain biochemical profile associated with environmental enrichment outlined an improvement in acetylcholinesterase activity, suggesting the involvement of the cholinergic system in mediating the cognitive improvement of the enrichment paradigm (Rosenzweig et al., 1967). Other studies showed that long-term environmental enrichment was associated with upregulated serotonin 1A (5-HT1A) receptor mRNA expression in the hippocampus (Rasmuson et al., 1998; Ragu Varman & Rajan, 2015) and an increase in the noradrenaline content of the brain (Naka et al., 2002), suggesting the involvement of serotonergic and noradrenergic transmission in mediating the response to environmental enrichment.

The environmental enrichment paradigm was also found to influence the expression pattern of multiple genes involved in neuronal signalling and synaptic transmission (Rampon, Jiang, et al., 2000; Keyvani et al., 2004). Some emphasis has also been made on insulin-like growth factor 1 (IGF-1) role as a mediator of the neuroprotective functions of the enrichment paradigm (Carro et al., 2000; Koopmans et al., 2006). Unsurprisingly, a great deal of attention has been given to BDNF, a member of the neurotrophins family of growth factors, in relation to the environmental enrichment role in neuroprotection and hippocampal neurogenesis (Pham et al., 2002; Rossi et al., 2006; Sun et al., 2010; Bekinschtein et al., 2011; Jha et al., 2016; Gualtieri et al., 2017; Dandi et al., 2018). Other neurotrophins, including GDNF and nerve growth factor (NGF), were shown to be upregulated in response to environmental enrichment (Bekinschtein et al., 2011; Williamson et al., 2012). However, as described above, most environmental enrichment protocols include an exercise component represented in running wheels and disks. Physical exercise, on its own, is known to elicit an increase in neurotrophins expression, BDNF in particular, and hippocampal neurogenesis (Vaynman et al., 2004). Therefore, it is difficult to discern with absolute certainty whether the increase in neurotrophins expression associated with environmental enrichment is induced by either the social component of enrichment (Branchi et al., 2006), the motor component, or both.

BDNF has been suggested as a critical mediator of the adiposity-reducing effect of environmental enrichment. Cao and colleagues showed that hypothalamic BNDF signalling activates the hypothalamic-sympathoneural-adipocyte axis, increasing the sympathetic tone to the adipose tissue and fostering the white to brown fats conversion (Cao et al., 2011). Similarly, hypothalamic BDNF signalling has been implicated in cancer remission (Cao et al., 2010). It is

likely that multiple molecular mechanisms, rather than one, mediates the beneficial influence of environmental enrichment on physiological functions. Identification of such molecular mediators, sometimes referred to as “enviromimetics”, could prove fruitful in developing drugs to treat many cognitive, immunologic, and metabolic disorders.

2. Rationale

Energy homeostasis is an intricate multifactorial process that involves maintaining the balance between caloric consumption and caloric expenditure. Obesity, in contrast, emanates from a chronic dysregulation of the caloric consumption/expenditure balance. The central nervous system (CNS), particularly the hypothalamus, controls caloric consumption via integrating multiple peripheral and central homeostatic metabolic signals with individual-specific environmental and hedonic motivating or averting influences, effectively contriving a commensurate perception of hunger or satiety (Berthoud et al., 2017).

Environmental enrichment is an animal housing paradigm that incorporates complex social, motor, and sensory stimulations in the cage environment intending to improve the physiological welfare of laboratory rodents. Indeed, enriched mice have lower adiposity levels, exhibit a lean phenotype, and are resistant to high-fat diet-induced obesity (Cao et al., 2011; Queen et al., 2020). The beneficial effects of environmental enrichment are associated with increased hypothalamic BDNF expression and are recapitulated by experimentally elevating hypothalamic BDNF expression. These studies pointed out a significant role of hypothalamic BDNF signalling in mediating the influence of the enriched cage environment on body weight. BDNF is a neurotrophin that plays an important role in food intake. Studies have shown that perturbations in the expression of BDNF or its cognate receptor TrkB cause severe hyperphagic obesity in mice (Lyons et al., 1999; Rios et al., 2001; Xu et al., 2003) and humans (Yeo et al., 2004; Gray et al., 2006). Interestingly, hypothalamic BDNF expression is influenced by peripherally produced anorectic signals, such as glucose and leptin (Komori et al., 2006; Unger et al., 2007), suggesting a functional integration between peripheral anorectic signals and hypothalamic BDNF in the regulation of food intake. Therefore, it is reasonable to propose that environmental enrichment potentiates the metabolic response, including the anorectic response, to peripherally produced nutrients and hormonal signals via the BDNF-TrkB signalling pathway.

The *BDNF* gene has a unique transcription pattern. The mouse *Bdnf* gene has at least eight promoters driving the expression of 11 known *Bdnf* mRNA transcripts, all of which, remarkably, encode an identical BDNF protein. It has been proposed that different *Bdnf* transcripts exhibit brain region-specific expression patterns in response to glucose availability (Unger et al., 2007; Hossain et al., 2018). Additionally, while the biological drive for having multiple *Bdnf* transcripts encoding an identical protein remains poorly understood, it has been shown that different *Bdnf* transcripts influence different behavioural functions (Maynard et al., 2016; McAllan et al., 2018; Ramnauth et al., 2021) and specific *Bdnf* transcripts have been implicated in food intake regulation (Liao et al., 2012). Therefore, it is equally reasonable to propose that environmental enrichment potentiates the anorectic response of peripherally produced nutrients and hormonal signals via altering *Bdnf* transcript variant-specific and brain region-specific expression patterns.

Like BDNF, GDNF has been shown to reduce food intake and body weight in rodents (Hudson et al., 1995; Martin et al., 1996; Hoane et al., 1999) and humans (Nutt et al., 2003; Whone et al., 2019). Moreover, hypothalamus-specific *Gdnf* overexpression caused reductions in food intake and body weight gain in young rats as well as in a rat model of age-associated obesity (Tümer et al., 2006; Manfredsson et al., 2009). Importantly, studies have shown that environmental enrichment significantly increased *Gdnf* mRNA levels in the hippocampus (Young et al., 1999) and the substantia nigra in the midbrain (Faherty et al., 2005). However, *Gdnf* expression in the hypothalamus has never been examined before in response to environmental enrichment. Therefore, it was relevant to the scope of this study to examine hypothalamic *Gdnf* mRNA expression in response to the enriched cage environment, especially given its well-documented anorectic role in food intake.

3. Hypotheses

a) Environmental enrichment potentiates the anorectic response of peripherally produced nutritional and hormonal anorectic signals.

b) Environmental enrichment alters the *Bdnf* transcript-variant-specific and brain region-specific expression patterns in response to anorectic signals.

4. Objectives

a) To examine the anorectic response of glucose, the GI peptide xenin and the adipocyte hormone leptin in fasted male mice in response to the enriched cage environment.

b) To examine the brain region-specific expression of *Bdnf* transcript variants in response to the enriched cage environment.

c) To examine hypothalamic *Gdnf* expression in response to the enriched cage environment.

5. Experimental design

Animals

Male C57BL/6 mice (3-4-week-old) procured from colonies at the University of Manitoba Central Animal Care Services (CACS) were maintained under a 12-12 light-dark cycle (lights on at 0600 hr). Mice were habituated in the animal holding room for one week before initiating the study. All mice received *ad libitum* standard rodent chow (Prolab RMH 3000, Ralston Purina) and tap water. The animal holding room was maintained at 20 ± 2 °C, 30 to 50% relative humidity. All procedures involving animals were approved by the Animal Protocol Management and Review Committee at the University of Manitoba (Protocol #17-029) and in complete accord with the Guide for the Care and Use of Laboratory Animals published by the Canadian Council on Animal Care.

Environmental enrichment (EE) protocol

Mice were randomly assigned to live in either standard environment (SE) or enriched environment (EE) cages in groups of 5 mice/cage. SE mice were kept in cages (37.5 cm [L] × 19 cm [W] × 12.5 cm [H]) supplemented with nesting materials and woodchip bedding (Fig. 3). EE mice were housed in relatively large cages (58 cm [L] × 48 cm [W] × 30 cm [H]) supplemented with a house, a running wheel, two tunnels, an igloo, a hut, a wooden stick, a crawl ball, a tube maze, nesting materials, and woodchip bedding (Fig. 3). Mice were maintained under both housing conditions for either six or thirteen weeks, depending on the experiment. Cages and beddings were changed, and enrichment objects were cleaned weekly.

Measurement of body weight and food intake

The body weight of each mouse and food intake for each cage were measured at least three times per week throughout the animal study. Individual food intake data were estimated by dividing the food intake per cage over the number of mice in the cage, and averages of daily and weekly intake were calculated in each housing environment

Feeding studies

The present study examined the feeding response to three anorectic agents: glucose, the GI hormone xenin, and the adipocyte hormone leptin. These three agents were selected because each functions through a different brain mechanism to regulate food intake (Ellacott & Cone, 2006;

Leckstrom et al., 2009; Tonon et al., 2013), as described in the introduction section. Five weeks into our housing protocol, mice were fasted for 8 hr during the light phase and received an intraperitoneal injection of glucose (2 mg/g body weight) or saline immediately before the onset of the dark phase. Mice were temporarily subjected to solitary housing 30 minutes before injection, enabling accurate measurements of individual food intake. Mice were given food immediately following intraperitoneal injection, and food intake was recorded up to four hours post-injection at 0.5-1 hr intervals. At the end of the feeding study, mice were returned to their respective group cages and were given a 7-day rest to wash out any possible carry-over effect of the intraperitoneal injection. The same experimental design was repeated at weeks seven and nine into the housing protocol using saline as control or xenin (American Peptide Co., Sunnyvale, CA) at a dose of 50 or 15 μg/g body weight, respectively. The same design was followed with leptin (2.5 μg/g body weight, Sigma–Aldrich, St. Louis, MO) in the 11th week into the housing protocol. Specific dosages of the anorectic agents were decided according to previous reports (Leckstrom et al., 2009; Poritsanos et al., 2011; da Silva et al., 2014).

Tissue dissection

At the end of the experiment, mice were fasted for eight hours during the light phase and received an intraperitoneal injection of glucose (2 mg/g body weight) or saline 30 minutes before euthanasia. Mice were euthanised by isoflurane exposure, followed by decapitation. Brains were then quickly removed, mounted on a mouse brain matrix, and brain blocks containing the hypothalamus (between -0.35 mm and -2.6 mm to Bregma) were prepared according to the mouse brain atlas by Paxinos and Franklin (Paxinos & Franklin, 2001). The hypothalamus was dissected out from the brain block using the following anatomical coordinates: optic chiasm (rostral), mammillary bodies (caudal), optic tract (lateral) and apex of the hypothalamic third ventricle (superior). The amygdala, hippocampus and parietal cortex were also removed from the same brain block. Dissected amygdalar tissue contained central, lateral, basal (basolateral and basomedial) and medial nucleus. Tissues were quickly frozen and stored at -80 °C until RNA extraction. Trunk blood was collected for subsequent blood chemistry analysis, and white adipose tissue (epididymal, retroperitoneal, mesenteric, and subcutaneous WAT) and BAT pads were dissected out and weighed.

Primers design and validation for real-time PCR

All primer pairs used in this study (Table 1) were designed using the Primer Express software (Version 3.0, Applied Biosystems) or the NCBI Primer-Blast tool. Primer pairs were designed by spanning exon-exon junctions except in a small number of genes where it was difficult to design efficient primers by spanning an exon-exon junction. The specificity of each primers pair was confirmed by the presence of a single PCR product of the expected size by gel electrophoresis as well as the presence of a single peak following melt curve analysis. Additionally, the specificity was confirmed by the absence of amplification in negative controls without either reverse transcriptase (RT-) or RNA (RNA-). A standard curve was generated for each primer pair by plotting the x-axis as log nanograms of cDNA (serially diluted cDNA) and the y-axis as threshold cycle number (Ct). The amplification efficiency of each primer pair was determined using the following formula, where S represents the slope of the standard curve:

$$\textbf{Efficiency (\%)} = [10^{-1/S} - 1] \times 100$$

Notably, amplification efficiencies of primers used in this study (Table 2) were between 92.4 and 101.8%, which is well within the accepted efficiency range for real-time PCR primers (Taylor et al., 2019).

Total *Bdnf* mRNA expression was assessed using primer pairs that can detect all *Bdnf* mRNA transcripts with either short or long 3' UTR (*Pan-Bdnf*, Table 1). Moreover, another pair (*L-Bdnf*, Table 1) was also used to specifically detect total *Bdnf* mRNA transcripts alternatively spliced with the long 3' UTR. Transcript variant-specific primer pairs (*I, IIc. IV*, and *VI*) were designed by combining a forward primer within the variant-specific exon and a common reverse primer within the coding region in the common exon IX (Table 1).

RNA analysis using real-time PCR

Total RNA was extracted from individual tissues in TRI reagent (T9424, Sigma-Aldrich, St. Louis, MO), according to the manufacturer instructions. Total RNA (1 μg) was converted to cDNA using iScript™ Reverse Transcription Supermix (Cat#: 170-8840, Bio-Rad, Hercules, CA), and diluted 1:50. Samples prepared without reverse transcriptase or cDNA were used as negative controls. Expression levels of mRNA were measured by real-time PCR using the ABI 7500 Fast thermal cycler (Applied Biosystems, Foster City, CA).

To identify an optimal housekeeping gene for the real-time PCR experiments, three housekeeping genes, *cyclophilin B*, hypoxanthine-guanine phosphoribosyltransferase (*Hprt*), and beta-actin (*Actb*), were simultaneously compared in a pilot study using representative samples from the four experimental groups in the present study. A strong positive correlation was only found between *Hprt* and *cyclophilin B* (r = 0.9634 – 0.7174, $P < 0.0001$, n = 36, 4 independent experiments), while weaker correlations were found between *Actb* and *Hprt* (r = 0.7668 – 0.2976, $P < 0.0001$ – $P = 0.0826$, n = 36) or *cyclophilin B* (r = 0.7720 – 0.2471, $P < 0.0001$ – $P = 0.1323$, n = 36). Moreover, the expression levels of target genes were equivalent whether normalised against either or both of these two genes. Data from this pilot study suggested that *Hprt* and *cyclophilin B* mRNA expression levels were not altered by the experimental conditions in the present study (environmental enrichment and glucose treatment). In contrast, *Actb* presented multiple expression inconsistencies, and therefore it was excluded as a housekeeping gene.

Real-time PCR was performed in a 20-µl reaction containing SYBR green (PowerUp SYBR Green Master Mix, Applied Biosystems), primer mixture (final concentration: 300 nM) and 5 µl of cDNA (5 ng) for 45 cycles at 94°C (denaturing) and 60°C (annealing/extension). To confirm the absence of non-specific amplification, negative controls (RT- and RNA-) were included and melt curve analysis was performed in each PCR run. Relative mRNA levels were determined using the ΔΔCt method by normalising to *Hprt* mRNA levels unless otherwise indicated and are expressed as means (% of the control group) ± standard error of the mean (SEM). All experiments were performed in triplicates, and the coefficient of variation was less than 2% for each triplicate.

Blood chemistry

Animals were euthanised at the end of the experiment, as described above. Trunk blood was collected, and blood glucose levels were measured by a glucometer (ELITE XL, Bayer HealthCare, Mishawaka, IN). Serum was separated and stored at -80 °C. Commercial ELISA kits were subsequently used to determine insulin (EZRMI 13K; Linco, St Charles, MO, USA), leptin (KMC2281, Thermo Fisher Scientific, Waltham, MA), and corticosterone levels (ADI-900-097, Enzo Life Sciences, Farmingdale, NY). Final concentrations were measured using a microplate reader (iMark™ Microplate Absorbance Reader, Bio-Rad Laboratories, Hercules, CA)

Statistical analysis

Food intake data analysis: To determine the interaction between housing condition and treatment (glucose, xenin or leptin intraperitoneal injection), food intake data were initially analysed by three-way ANOVA. Comparison of cumulative food intake between control and experimental treatment groups (glucose, xenin or leptin injection) within each housing condition was performed by two-way ANOVA. Additionally, food intake data at each time point were analysed by two-way ANOVA followed by Tukey-Kramer post-hoc test.

***Gene expression analysis*:** Two complementary statistical approaches were followed in all gene expression data presented in part (I). Firstly, collective gene expression data were analysed by two-way ANOVA (two variables; housing environment and glucose treatment), followed by Tukey-Kramer post-hoc test to detect intergroup variations. Secondly, the per cent change of mRNA expression levels in response to the glucose treatment was calculated against the saline-treated group in each housing environment and compared between the two housing environments using the Student's *t*-test, allowing for maximum interpretation of the data. All data presented are means ± S.E.M. Outliers identified by the Discordance test were omitted from the analysis. In all cases, differences were taken to be significant if *P*-values were below 0.05.

Table 1 Primer sequences used for real-time PCR

Gene	Accession No.	Direction*	Sequences [5′ → 3′]	Exon
Fos	NM_010234	F	GACAGCCTTTCCTACTACCATTCC	1
		R	GGACAGATCTGCGCAAAAGTC	2
Junb	BC092302	F	CACGACGACTCTTACGCAGC	1
		R	GATGCGCCTGTGTCTGATCC	1
Pan-Bdnf	AY057907	F[a]	ATTACCTGGATGCCGCAAAC	9
		R[b]	CAGTTGGCCTTTGGATACCG	9
		R[c]	TGCAACCGAAGTATGAAATAACCA	9
L-Bdnf	AY057907	F	CACTCTTCTGGCATGGGTCA	9
		R	AATGGGAGGAGGGAGGGAAA	9
Bdnf I	NM_007540	F	GCAAAGCCGAACTTCTCACAT	1
Bdnf IIc	NM_001048139	F	TGCAAGTGTTTATCACCAGGATCTA	2c
Bdnf IV	NM_001048141	F	CAGCTGCCTTGATGTTTACTTTGA	4
Bdnf VI	NM_001048142	F	GCTTTGTGTGGACCCTGAGTTC	6-9
Ntrk2	NM_001025074	F[d]	CCACGGATGTTGCTGACCAAAG	11
		R[d]	GCCAAACTTGGAATGTCTCGCC	12
		F[e]	TGAAAAACAGCAACCTGCGG	4
		R[e]	ACAAGTCAAGGTGGCGGAAA	5
Ngfr	NM_033217	F	CTTTGGAGGTGCCAAGGAGA	2
		R	CACACAGGGAGCGGACATAC	3
Gdnf	NM_010275	F	GACTTGGGTTTGGGCTATGAA	3
		R	TGGCCTACTTTGTCACTTGTT	3
Manf	NM_029103	F	GTCACATTTTCACCAGCCAC	2
		R	AGCATCATCTGTGGCTCCAA	3

Slc2a1	NM_011400	F	GGATCACTGCAGTTCGGCTAT	2
		R	CGTAGCGGTGGTTCCATGTT	3
Slc2a2	NM_031197	F	ACCGGGATGATTGGCATGT	9
		R	TGGCAGTCATGCTCACGTAAC	10
Slc2a3	NM_011401	F	CAGCCGCTTCTCATCTCCAT	6
		R	CACCCGCGTCCTTGAAGA	7
Slc2a4	NM_009204	F	CTTGGCTCCCTTCAGTTTGG	2
		R	TGCCTACCCAGCCACGTT	3
Slc2a5	NM_019741	F	AAGCGACGACGTCCAGTATGT	10
		R	GAATCGCCGTCCCCAAAG	11
Maoa	NM_173740	F	ATGCTGAGGAATGGGACAAGA	5
		R	CATACCACAAGAACCACAGGG	6
Htr2a	NM_172812	F	CACCATAGCCGCTTCAACTC	2
		R	CGAATCATCCTGTAGCCCGAA	3
Hprt	NM_013556	F	AGTCCCAGCGTCGTGATTAG	1-2
		R	TGATGGCCTCCCATCTCCTT	3
Cyclophilin	X52803	F	AAGCATACAGGTCCTGGCATCT	4
		R	TGCCATCCAGCCATTCAGT	4-5

*: Forward primer (F) and reverse primer (R).

[a] and [b]: This combination of primers detects all *Bdnf* mRNA variants.

[c]: Common reverse primer for *Bdnf* mRNA variants *I*, *IIc*, *IV* and *IV*.

[d] and [e]: Either of primer pairs ***d*** or ***e*** were used.

Table 2 Amplification efficiencies of primers used for real-time PCR

Gene	Primer efficiency (%)
Fos	94.3
Junb	93.5
Pan-Bdnf	95.3
L-Bdnf	92.4
Bdnf I	94.1
Bdnf IIc	94.3
Bdnf IV	99.9
Bdnf VI	97.5
Ntrk2 (a)	99.5
Ntrk2 (b)	96.3
Ngfr	101.8
Gdnf	97.1
Manf	99.9
Slc2a1	94.1
Slc2a2	100.6
Slc2a3	94.0
Slc2a4	100.8
Slc2a5	94.5
Maoa	92.9
Htr2a	99.3
Hprt	95.4

6. Results

6.1. Effect of cage environment on body weight and adiposity levels

Across three independent animal studies, mice housed in the EE cages consistently showed a significant reduction in body weight compared with the SE mice control ($P = 0.0334$ by two-way ANOVA, Table 3, Fig. 4A). Interestingly, EE mice consumed more food compared with the SE mice control ($P < 0.0001$ by two-way ANOVA, Table 3, Fig. 4B). In addition, the total white adipose tissue (T. WAT) to body weight ratio was significantly reduced in the EE mice compared with the SE control (Fig. 4C). However, such reduction was confined to the epididymal WAT (EWAT) and the retroperitoneal WAT (RWAT), but not to the subcutaneous WAT (SWAT) or the mesenteric WAT (MWAT). Furthermore, the ratio of BAT mass to body weight was not significantly changed in response to the housing environment (Fig. 4C). Collectively, these data show that EE reduced body weight and adiposity levels in mice. It also serves to validate the EE protocol followed in this study, as it replicates previously reported data on the effect of EE on body weight and adiposity (Cao et al., 2011)

Table 3 Results of two-way ANOVA of body weight and food intake data

Two-way ANOVA	Body weight		Food intake	
	F statistics	*P* value	*F* statistics	*P* value
Housing environment	$F(1, 297) = 4.57$	$P = 0.0334$	$F(1, 54) = 200.41$	$P < 0.0001$
Time	$F(8, 297) = 58.86$	$P < 0.0001$	$F(8, 54) = 14.88$	$P < 0.0001$
Environment × Time	$F(8, 297) = 1.29$	$P = 0.2496$	$F(8, 54) = 6.64$	$P < 0.0001$

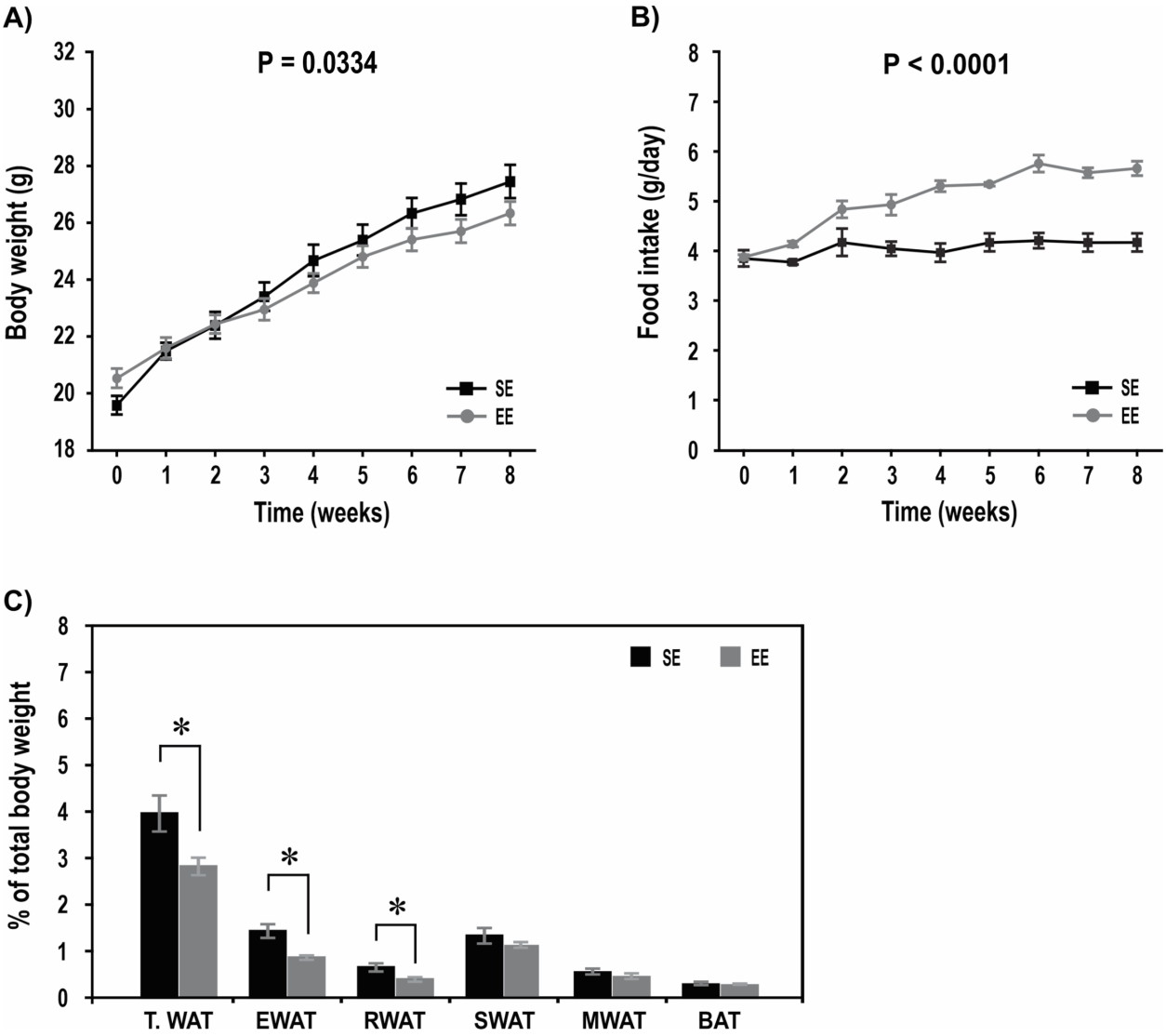


Figure 4 Effect of environmental enrichment on body weight, food intake, and adiposity in mice. Mice were maintained in the standard environment (SE) or enriched environment (EE) in groups of 5 mice per cage for 8 weeks. Body weight (A) and food intake (B) were measured five days a week throughout the experiment. The weight of different fat pads was measured at the end of the experiment, normalised to body weight, and expressed as a percentage (C). Data are means ± S.E.M. (n = 17-18/group). *P* values are for the main effect of the housing condition by two-way ANOVA. *: $P < 0.05$ by Student's *t*-test.

6.2. Effect of cage environment on glucose-induced feeding suppression

Cumulative food intake data in response to intraperitoneal glucose treatment (2 mg/g body weight) were initially analysed by three-way ANOVA, which showed a significant main effect of the glucose treatment ($P < 0.0001$) but not a significant main effect of the housing environment ($P = 0.1667$, Table 4A). This meant that intraperitoneal glucose treatment significantly reduced food intake in both housing environments. Importantly, three-way ANOVA showed a significant interaction between the glucose treatment and the housing environment ($P = 0.0086$, Table 4A), which meant that the housing environment differentially modified the anorectic effect of glucose.

To determine the added anorectic effect of the housing environment on glucose-induced feeding suppression, food intake data in each housing environment were analysed separately by two-way ANOVA. As expected, two-way ANOVA showed a significant main effect of the glucose treatment in both housing environments. However, the main effect of treatment was more significant in the EE cohort compared with the SE cohort ($P < 0.0001$ for the EE cohort and $P = 0.0016$ for the SE cohort, Table 4B, Fig. 5A-B).

To further discern intergroup variations in response to glucose treatment, food intake data were analysed at each time point using two-way ANOVA followed by Tukey-Kramer post-hoc test (Fig. 5C). A significant intergroup variation was only found between the glucose- and saline-treated mice in the enriched cage environment between 1.5 and 2.5 hours post-injection, while no significant change was found between saline- and glucose-treated mice in the standard cage environment over the same period (Fig. 5C). Furthermore, Tukey-Kramer post-hoc test did not pick out any significant intra-environment difference at all other time points (Fig. 5C). Together, these data provide clear evidence that environmental enrichment significantly enhances the feeding-suppressing effect of glucose in a time-dependent manner.

Table 4 Three-way and two-way ANOVA of food intake data in response to glucose treatment

(A)

Three-way ANOVA	**Cumulative food intake in all groups**	
	F statistics	***P*** value
Housing environment	$F(1, 224) = 1.93$	$P = 0.1667$
Glucose treatment	$F(1, 224) = 40.61$	$P < 0.0001$
Time post-treatment	$F(6, 224) = 129.37$	$P < 0.0001$
Housing x Treatment	$F(1, 224) = 17.02$	$P = 0.0086$
Treatment x Time	$F(6, 224) = 0.55$	$P = 0.7674$
Housing x Time	$F(6, 224) = 0.04$	$P = 0.9997$
Housing x Treatment x Time	$F(6, 224) = 0.34$	$P = 0.9135$

(B)

Two-way ANOVA	**Standard cage environment**		**Enriched cage environment**	
	F statistics	***P*** value	***F*** statistics	***P*** value
Glucose treatment	$F(1, 112) = 10.42$	$\mathbf{P = 0.0016}$	$F(1, 112) = 30.48$	$\mathbf{P < 0.0001}$
Time	$F(6, 112) = 99.16$	$P < 0.0001$	$F(6, 112) = 47.54$	$P < 0.0001$
Treatment x Time	$F(6, 112) = 0.16$	$P = 0.9856$	$F(6, 112) = 0.59$	$P = 0.7369$

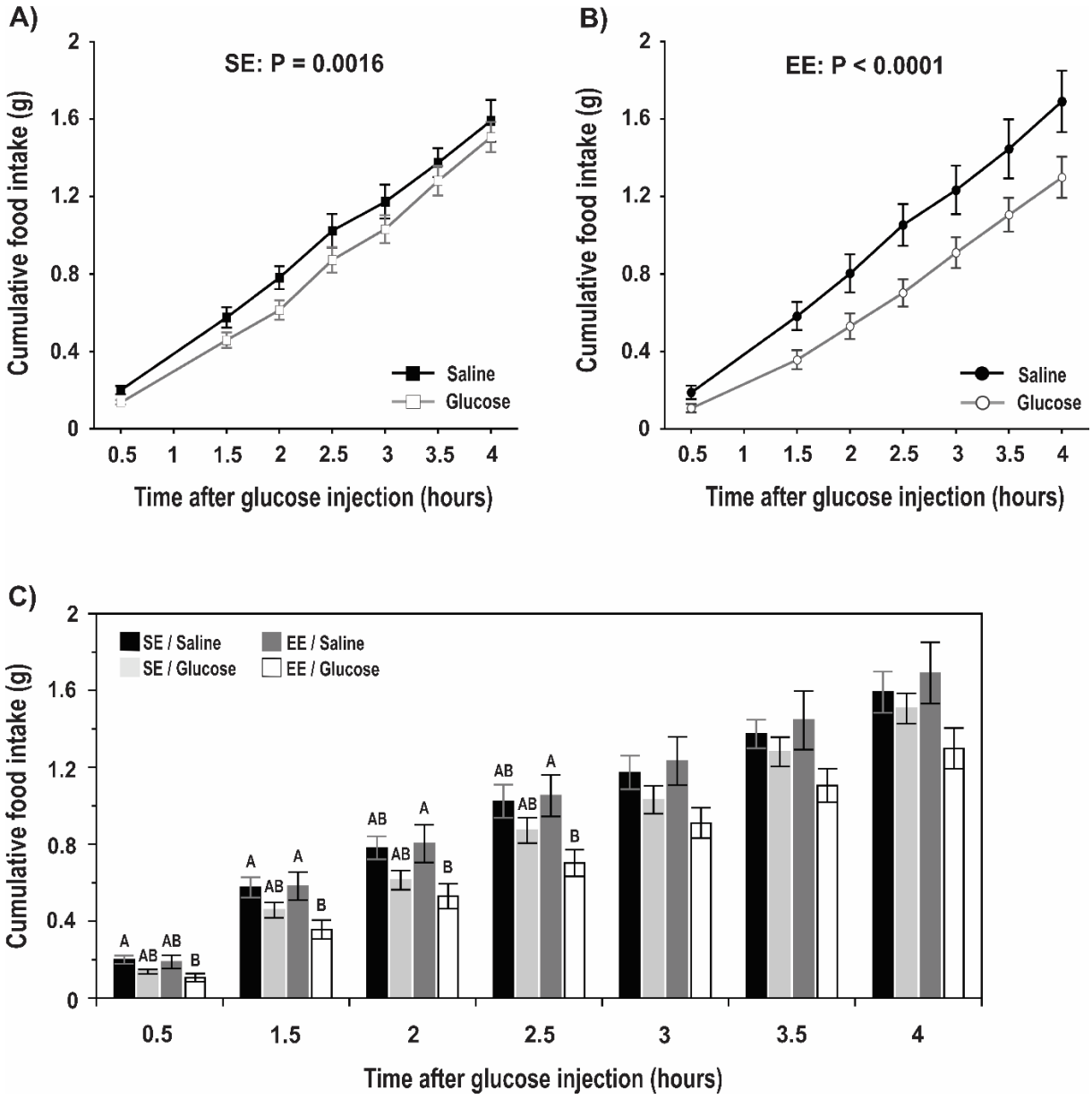


Figure 5 Effect of glucose on food intake in mice maintained under standard environment and enriched environment. Mice were maintained in SE (A) or EE (B) for 5 weeks. Mice were fasted for 8 hr and received an intraperitoneal injection of glucose or saline immediately before the onset of the dark phase. Cumulative food intake was measured for 4 hr post-injection at 0.5-1 hr intervals. Cumulative food intake was also compared between groups at each time point (C). Data are means ± S.E.M. (n = 9/group). *P* values are for the main effect of glucose treatment by two-way ANOVA. Groups with different letters are statistically different ($P < 0.05$ by Tukey-Kramer test).

6.3. Effect of cage environment on high dose (50 µg/g b.w.) xenin-induced feeding suppression

The same experimental design and statistical approach were followed as in the glucose feeding study presented above. The feeding-suppressing effect of xenin was initially examined in the 7th week of the housing protocol at a dose of 50 µg/g of body weight, as this dose consistently reduced food intake in mice (Leckstrom et al., 2009; Kim et al., 2016). Three-way ANOVA (Table 5A) showed a significant main effect of the xenin treatment ($P < 0.0001$), but there was no significant main effect of the housing environment ($P = 0.9239$). Furthermore, the interaction between the housing environment and xenin treatment was not significant ($P = 0.4194$). Taken together, these data meant that xenin treatment (50 µg/g body weight) reduced food intake in both housing groups independent of any added effect of the housing environment.

Food intake data in each housing environment were analysed separately by two-way ANOVA, which showed an equally significant main effect of xenin (50 µg/g body weight) treatment in both housing environments ($P < 0.0001$ for both the SE and the EE group, Table 5B, Fig. 6A-B). Furthermore, this equally significant reduction in food intake was also present at each time point between the xenin- and saline-treated groups in each housing environment, as assessed by Tukey-Kramer post-hoc analysis (Fig. 6C). Collectively, these data show that EE did not enhance the feeding-suppressing effect of xenin at a dose of 50 µg/g of body weight.

Table 5 Three-way and two-way ANOVA of food intake data in response to xenin treatment at a dose of 50 µg/g body weight

(A)

Three-way ANOVA	**Cumulative food intake in all groups**	
	F statistics	***P*** value
Housing environment	$F(1, 160) = 0.01$	$P = 0.9239$
Xenin treatment	$F(1, 160) = 149.78$	$P < 0.0001$
Time post-treatment	$F(4, 160) = 174.77$	$P < 0.0001$
Housing x Treatment	$F(1, 160) = 0.66$	$P = 0.4194$
Treatment x Time	$F(4, 160) = 11.56$	$P < 0.0001$
Housing x Time	$F(4, 160) = 0.49$	$P = 0.9955$
Housing x Treatment x Time	$F(4, 160) = 0.13$	$P = 0.9694$

(B)

Two-way ANOVA	**Standard cage environment**		**Enriched cage environment**	
	F statistics	***P*** value	***F*** statistics	***P*** value
Xenin treatment	$F(1, 80) = 98.87$	$\mathbf{P < 0.0001}$	$F(1, 80) = 63.55$	$\mathbf{P < 0.0001}$
Time	$F(4, 80) = 129.77$	$P < 0.0001$	$F(4, 80) = 66.52$	$P < 0.0001$
Treatment x Time	$F(4, 80) = 7.27$	$P < 0.0001$	$F(4, 80) = 5.15$	$P < 0.0001$

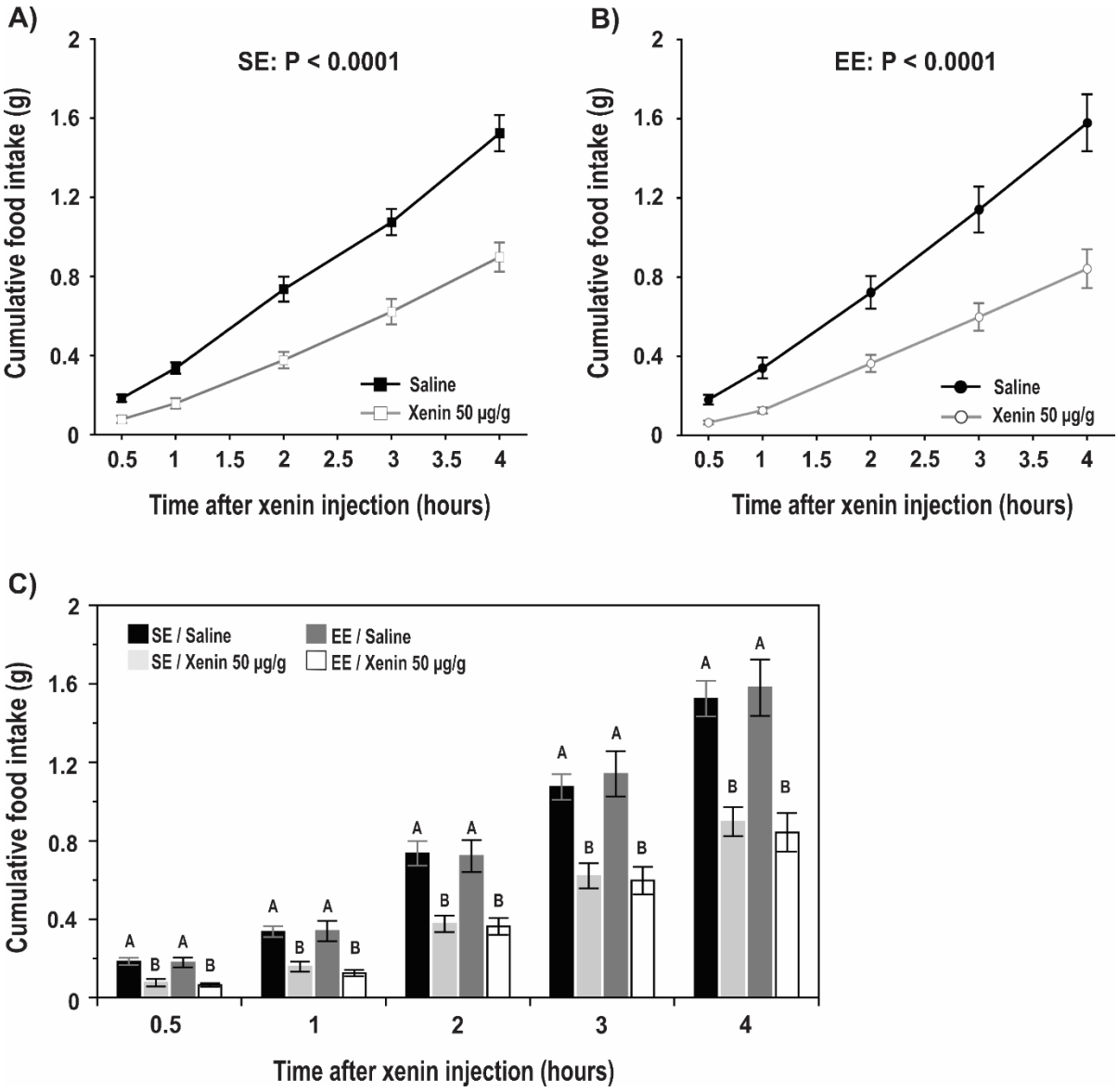


Figure 6 Effect of xenin (50 µg/g body weight) on food intake in mice maintained under standard environment and enriched environment. Mice in the SE (A) or EE (B) housing environments were fasted for 8-hr and received an intraperitoneal injection of xenin (50 µg/g body weight) or saline immediately before the onset of the dark phase. Cumulative food intake was measured for 4-hr post-injection at 0.5-1 hr intervals. Cumulative food intake was also compared between groups at each time point (C). Data are means ± S.E.M. (n = 9/group). *P* values are for the main effect of xenin treatment by two-way ANOVA. Groups with different letters are statistically different ($P < 0.05$ by Tukey-Kramer test).

6.4. Effect of cage environment on low dose (15 µg/g b.w.) xenin-induced feeding suppression

Xenin at a dose of 50 µg/g of body weight might have been strong enough to the point that it exceeded the threshold of showing any differential effect of the housing environment. Therefore, the feeding-suppressing effect of xenin was re-examined in the 9th week of the housing protocol at a lower near-threshold dose of 15 µg/g of body weight; this dose reduced food intake but with a lower magnitude in a previous study (Leckstrom et al., 2009). Three-way ANOVA (Table 6A) still showed a significant main effect of the xenin treatment (P = 0.0001) while continued to show no significant interaction between the xenin treatment and the housing environment (P = 0.3292, Table 6B). However, two-way ANOVA showed a slightly more significant reduction of food intake in mice housed in the enriched cage environment compared with mice housed in the standard cage environment (P = 0.0016 for the EE group and P = 0.0321 for the SE group, Fig. 7A-B). In the meantime, Tukey-Kramer post-hoc analysis failed to detect any significant intergroup variation in each housing condition at any time point (Fig. 7C). Thus, taken together, environmental enrichment showed minimal to no influence on the anorectic effect of xenin at a dose of either 50 or 15 µg/g of body weight.

Table 6 Three-way and two-way ANOVA of food intake data in response to xenin treatment at a dose of 15 µg/g body weight

(A)

Three-way ANOVA	**Cumulative food intake in all groups**	
	F statistics	***P*** value
Housing environment	$F(1, 160) = 1.51$	$P = 0.2202$
Xenin treatment	$F(1, 160) = 15.14$	$P = 0.0001$
Time post-treatment	$F(4, 160) = 139.79$	$P < 0.0001$
Housing x Treatment	$F(1, 160) = 0.96$	$P = 0.3292$
Treatment x Time	$F(4, 160) = 1.94$	$P = 0.1062$
Housing x Time	$F(4, 160) = 0.11$	$P = 0.9133$
Housing x Treatment x Time	$F(4, 160) = 0.22$	$P = 0.9281$

(B)

Two-way ANOVA	**Standard cage environment**		**Enriched cage environment**	
	F statistics	***P*** value	***F*** statistics	***P*** value
Xenin treatment	$F(1, 80) = 4.76$	$\mathbf{P = 0.0321}$	$F(1, 80) = 10.69$	$\mathbf{P = 0.0016}$
Time	$F(4, 80) = 72.86$	$P < 0.0001$	$F(4, 80) = 67.74$	$P < 0.0001$
Treatment x Time	$F(4, 80) = 0.51$	$P = 0.7312$	$F(4, 80) = 1.54$	$P = 0.1987$

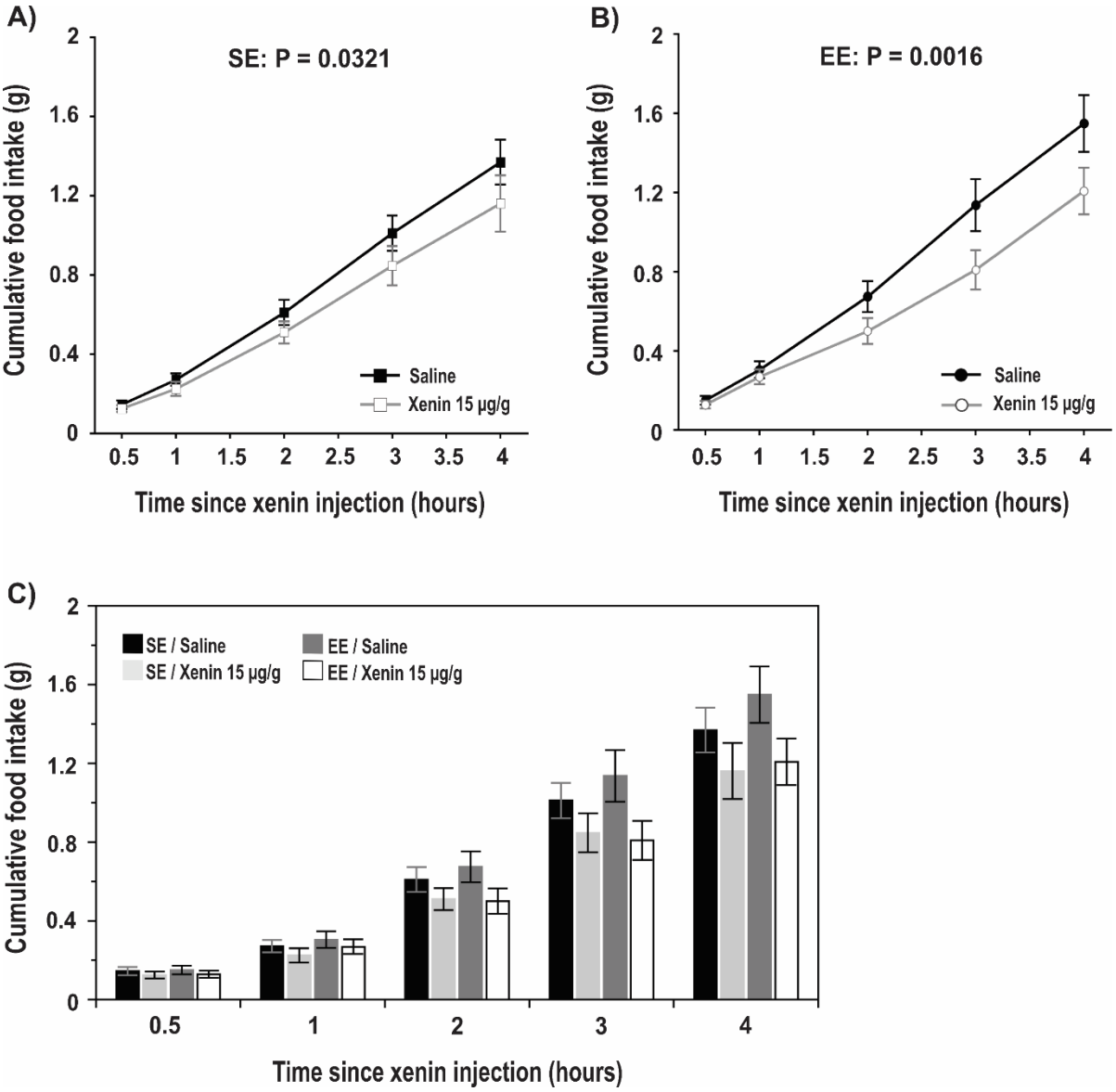


Figure 7 Effect of xenin (15 µg/g body weight) on food intake in mice maintained under standard environment and enriched environment. Mice in the SE (A) or EE (B) housing environments were fasted for 8-hr and received an intraperitoneal injection of xenin (15 µg/g body weight) or saline immediately before the onset of the dark phase. Cumulative food intake was measured for 4-hr post-injection at 0.5-1 hr intervals. Cumulative food intake was also compared between groups at each time point (C). Data are means ± S.E.M. (n = 9/group). *P* values are for the main effect of xenin treatment by two-way ANOVA.

6.5. Effect of cage environment on leptin-induced feeding suppression

The feeding-suppressing effect of the adipocyte hormone leptin was examined in the 11th week of the housing protocol at a dose of 2.5 μg/g of body weight; leptin at this dose has been shown to reduce food intake in mice (da Silva et al., 2014). The same experimental design and statistical analysis in the glucose and xenin feeding studies were followed. Much like xenin, three-way ANOVA (Table 7A) showed a significant main effect of leptin treatment (P = 0.0024) on reducing food intake in both housing conditions, but there was no significant interaction between the housing environment and leptin treatment (P = 0.8537). Additionally, the main effect of treatment was equally significant in both housing environments (P = 0.0217 for the EE group and P = 0.0439 for the SE group by two-way ANOVA, Table 7B, Fig 8A-B). Furthermore, Tukey-Kramer post-hoc analysis did not show any significant intergroup difference in response to leptin treatment in each housing environment at all time points examined (Fig. 8C). Collectively, these data show that EE did not alter the anorectic response to leptin at a dose of 2.5 μg/g of body weight.

Table 7 Three-way and two-way ANOVA of food intake data in response to leptin treatment

(A)

Three-way ANOVA	**Cumulative food intake in all groups**	
	F statistics	***P*** value
Housing environment	$F(1, 150) = 0.81$	$P = 0.3690$
Leptin treatment	$F(1, 150) = 9.56$	$P = 0.0024$
Time post-treatment	$F(4, 150) = 119.69$	$P < 0.0001$
Housing x Treatment	$F(1, 150) = 0.03$	$P = 0.8537$
Treatment x Time	$F(4, 150) = 2.29$	$P = 0.0628$
Housing x Treatment x Time	$F(4, 150) = 0.04$	$P = 0.9971$

(B)

Two-way ANOVA	**Standard cage environment**		**Enriched cage environment**	
	F statistics	***P*** value	***F*** statistics	***P*** value
Leptin treatment	$F(1, 80) = 4.19$	$\mathbf{P = 0.0439}$	$F(1, 70) = 5.52$	$\mathbf{P = 0.0217}$
Time	$F(4, 80) = 58.66$	$P < 0.0001$	$F(4, 70) = 62.42$	$P < 0.0001$
Treatment x Time	$F(4, 80) = 0.91$	$P = 0.4628$	$F(4, 70) = 1.45$	$P = 0.2278$

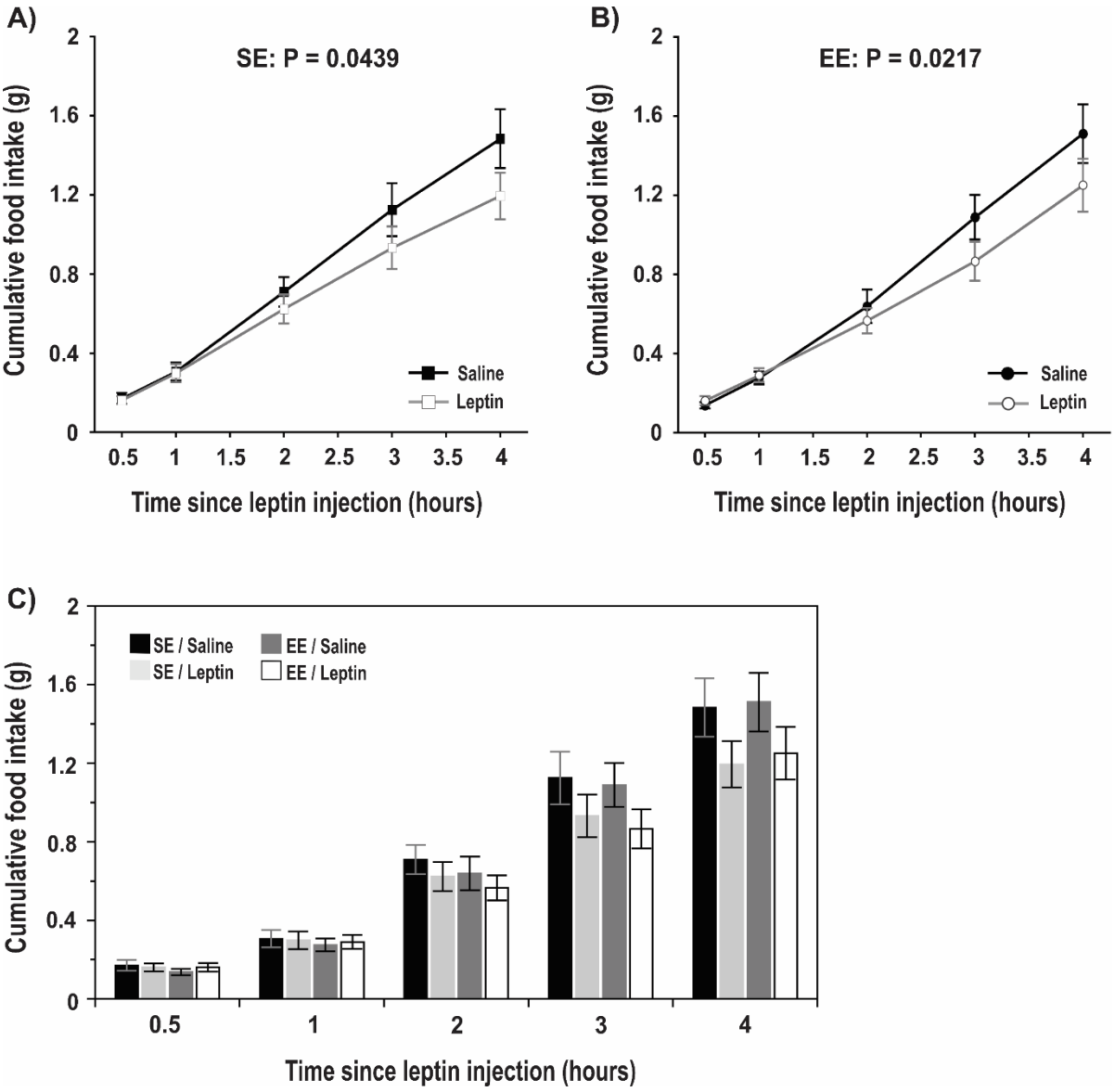


Figure 8 Effect of leptin treatment (2.5 µg/g body weight) on food intake in mice maintained under standard environment and enriched environment. Mice in the SE (A) or EE (B) housing environments were fasted for 8-hr and received an intraperitoneal injection of leptin (2.5 µg/g body weight) or saline immediately before the onset of the dark phase. Cumulative food intake was measured for 4-hr post-injection at 0.5-1 hr intervals. Cumulative food intake was also compared between groups at each time point (C). Data are means ± S.E.M. (n = 8-9/group). *P* values are for the main effect of leptin treatment by two-way ANOVA.

6.6. Effect of cage environment and glucose treatment on blood glucose, insulin, leptin, and corticosterone levels in mice

To examine gene expression patterns associated with the enhanced feeding-suppressing effect of glucose under the enriched cage environment, the experimental conditions of the glucose feeding study were replicated in a separate animal study. Briefly, six weeks into the housing protocol, mice were fasted for eight hours during the light phase and received an intraperitoneal injection of glucose (2 mg/g body weight) or saline as a control. Mice were euthanised 30 minutes later by decapitation following exposure to isoflurane, trunk blood was collected for blood chemistry analysis, and brain tissues were dissected for gene expression analysis as described in the experimental design section.

Glucose treatment caused a significant increase in blood glucose levels in both housing conditions (Table 8). However, plasma insulin levels, although increased, did not differ significantly between the glucose- and saline-treated groups in either housing environment (Table 8). Similarly, intergroup variations in plasma leptin levels were not detected between the glucose- and saline-treated groups in either housing condition despite a significant reduction in baseline plasma leptin levels in saline-treated EE mice relative to saline-treated SE mice ($P < 0.05$ by Tukey-Kramer test, Table 8). Reduced serum leptin levels in EE mice reflect reduced adiposity in these mice. Consistent with this notion, there was a significant positive correlation between total WAT mass and serum leptin levels in all treatment groups ($r = 0.8040$ and $P < 0.0001$). Of note, serum corticosterone levels were indistinguishable between the four treatment groups (Table 8). These data suggest that the enhanced feeding-suppressing effect of glucose under the enriched cage environment conditions may be functionally independent of insulin, leptin, or corticosterone influences.

Table 8 Effect of environmental enrichment and glucose treatment on circulating glucose, insulin, leptin, and corticosterone levels in mice

	Standard cage environment		Enriched cage environment	
	Saline	Glucose	Saline	Glucose
Glucose (mmol/l)	9.79 ± 1.09[a]	14.82 ± 0.54[b]	8.64 ± 0.52[a]	13.64 ± 0.74[b]
Insulin (ng/ml)	0.60 ± 0.14	0.88 ± 0.13	0.94 ± 0.34	1.60 ± 0.56
Leptin (ng/ml)	1.30 ± 0.46[a]	0.55 ± 0.13[ab]	0.32 ± 0.06[b]	0.47 ± 0.08[b]
Corticosterone (ng/ml)	330.3 ± 69.6	287.4 ± 72.5	344.8 ± 35.6	419.7 ± 83.8

Data are mean ± S.E.M. (n = 6 – 9 per group).

Groups with different letters are statistically different ($P < 0.05$ by Tukey-Kramer test).

6.7. Effect of cage environment and glucose treatment on brain *Fos* mRNA expression

The immediate early gene *Fos* is widely accepted as an indirect marker of neuronal activity (Day et al., 2008; VanElzakker et al., 2008). In the present study, *Fos* mRNA levels were measured in the hypothalamus, amygdala, hippocampus, and parietal cortex and were compared between glucose- and saline-treated groups in each housing environment. Two-way ANOVA did not detect a significant main effect of glucose treatment, housing environment, nor a significant interaction thereof on *Fos* mRNA expression levels in the hypothalamus and amygdala (Table 9, Fig. 9A). Two-way ANOVA, however, detected a significant main effect of the housing environment on *Fos* mRNA expression levels in the hippocampus ($P < 0.0001$) and parietal cortex ($P = 0.0125$, Table 9, Fig. 9A). Importantly, Tukey-Kramer post-hoc analysis did not detect any significant difference between glucose- and saline-treated groups in each housing environment in all four tissues examined (Fig. 9A). However, at the baseline levels, hippocampal *Fos* mRNA levels were significantly reduced in the saline-treated enriched mice relative to the saline-treated standard environment control (Fig. 9A). To further determine the effect of glucose on *Fos* mRNA expression, percent change relative to the saline-treated control group was calculated in each housing condition. Glucose treatment elicited a 70.1% increase in hypothalamic *Fos* mRNA expression levels in the enriched cage environment compared with a 6.1% reduction in the standard cage environment ($P < 0.05$ by Student's *t*-test, Fig. 9B). Notably, no significant inter-environment difference was observed in the other tissues (Fig. 9B). These data suggest that environmental enrichment increases hypothalamic activity in response to glucose availability.

Table 9 Two-way ANOVA of *Fos* mRNA levels in response to environmental enrichment and glucose treatment

	Housing environment	Glucose treatment	Environment × Treatment
Hypothalamus	$F(1, 31) = 3.51$, $P = 0.0704$	$F(1, 31) = 1.10$, $P = 0.3016$	$F(1, 31) = 2.21$, $P = 0.1468$
Amygdala	$F(1, 31) = 2.13$, $P = 0.1545$	$F(1, 31) = 0.64$, $P = 0.4298$	$F(1, 31) = 0.31$, $P = 0.5845$
Hippocampus	$F(1, 27) = 24.51$, $\mathbf{P < 0.0001}$	$F(1, 27) = 0.92$, $P = 0.3459$	$F(1, 27) = 0.003$, $P = 0.9530$
Parietal cortex	$F(1, 29) = 7.09$, $\mathbf{P = 0.0125}$	$F(1, 29) = 0.62$, $P = 0.4384$	$F(1, 29) = 0.08$, $P = 0.7729$

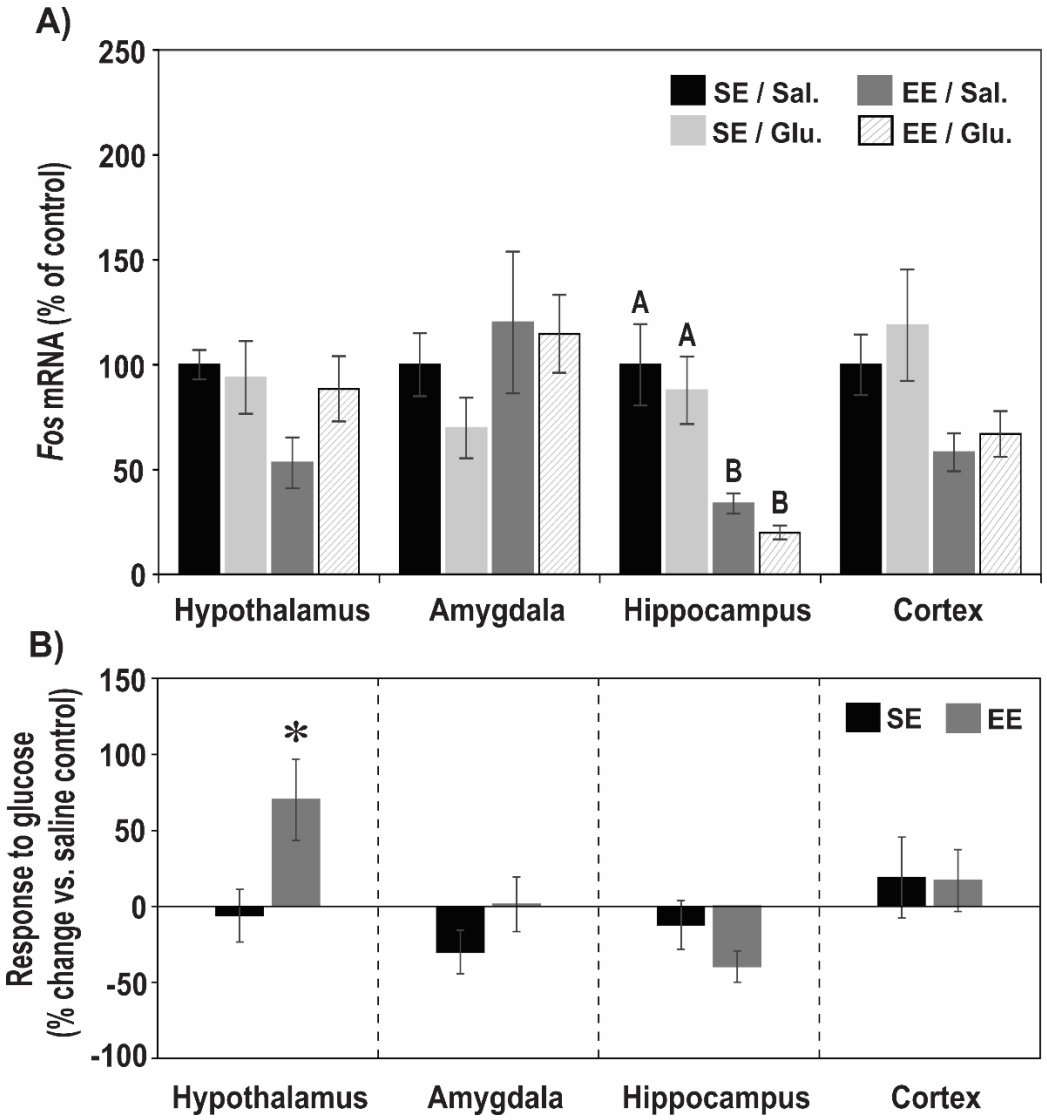


Figure 9 Effect of environmental enrichment and glucose treatment on *Fos* mRNA expression in the brain. Mice were maintained under the SE or EE environments for 8 weeks and received an intraperitoneal injection of saline or glucose. Brain tissues were collected 30 min after the injection. Levels of *Fos* mRNA were measured by real-time PCR. Values in saline-treated SE groups were set to 100% (A). Response to glucose (% of glucose/saline) in each housing condition was compared (B). Data are means ± S.E.M. (n = 8-9/group). Groups that do not share a common letter are statistically different ($P < 0.05$ by Tukey-Kramer test). *: $P < 0.05$ by Student's *t*-test.

6.8. Effect of cage environment and glucose treatment on the glucose transporter 1-5 gene expression in the mouse hypothalamus and amygdala

Glucose transporters (GLUTs) are a diverse group of membrane proteins responsible for the facilitated diffusion of glucose and related hexoses across the plasma membrane (Zhao & Keating, 2007). Brain GLUTs expression exhibits cellular and regional specificity (McEwen & Reagan, 2004); therefore, the present study examined the hypothalamic and amygdalar mRNA expression levels of solute carrier family 2 members 1 through 5 (*Slc2a1*, *Slc2a2, Slc2a3, Slc2a4, and Slc2a5)* genes encoding for the GLUT 1 through 5, respectively. Two-way ANOVA did not show any significant main effect of housing environment, glucose treatment, nor a significant interaction between the housing environment and glucose treatment in all five genes examined in both tissues (Table 10). The only exception is the significant main effect of housing environment on amygdalar *Slc2a1* mRNA levels ($P = 0.0005$, Table 10). Furthermore, Tukey-Kramer post-hoc test did not detect any significant intergroup variation in both tissues except for *Slc2a1* mRNA levels in the amygdala (Fig. 10A and C). Interestingly, the inter-environment per cent change in the GLUT5-encoding *Slc2a5* mRNA expression in response to glucose treatment was significantly increased in the enriched mice group (33.4% increase) compared with the standard environment control (12.4% reduction, $P < 0.05$ by Student's *t*-test, Fig. 10B). No significant inter-environment difference was detected in *Slc2a1-5* mRNA levels in the amygdala (Fig. 10D).

Table 10 Two-way ANOVA of *Slc2a1-5* mRNA levels in response to environmental enrichment and glucose treatment

Gene	**Hypothalamus**		
	Housing environment	**Glucose treatment**	**Environment × Treatment**
Slc2a1	$F(1, 30) = 0.02$, $P = 0.8870$	$F(1, 30) = 0.09$, $P = 0.7658$	$F(1, 30) = 1.14$, $P = 0.2939$
Slc2a2	$F(1, 28) = 3.42$, $P = 0.0749$	$F(1, 28) = 0.66$, $P = 0.4232$	$F(1, 28) = 0.06$, $P = 0.8163$
Slc2a3	$F(1, 29) = 0.52$, $P = 0.4748$	$F(1, 29) = 0.36$, $P = 0.5514$	$F(1, 29) = 1.94$, $P = 0.1738$
Slc2a4	$F(1, 31) = 3.20$, $P = 0.0836$	$F(1, 31) = 3.08$, $P = 0.0891$	$F(1, 31) = 0.85$, $P = 0.3639$
Slc2a5	$F(1, 29) = 0.11$, $P = 0.7408$	$F(1, 29) = 0.05$, $P = 0.8284$	$F(1, 29) = 1.14$, $P = 0.2952$
Gene	**Amygdala**		
	Housing environment	**Glucose treatment**	**Environment × Treatment**
Slc2a1	$F(1, 26) = 15.58$, $\mathbf{P = 0.0005}$	$F(1, 26) = 0.004$, $P = 0.9498$	$F(1, 26) = 1.05$, $P = 0.3151$
Slc2a2	$F(1, 22) = 1.97$, $P = 0.1745$	$F(1, 22) = 1.52$, $P = 0.2307$	$F(1, 22) = 0.08$, $P = 0.7855$
Slc2a3	$F(1, 29) = 1.61$, $P = 0.2146$	$F(1, 29) = 0.17$, $P = 0.6868$	$F(1, 29) = 1.73$, $P = 0.1986$
Slc2a4	$F(1, 27) = 1.54$, $P = 0.2259$	$F(1, 27) = 0.85$, $P = 0.3655$	$F(1, 27) = 0.21$, $P = 0.6520$
Slc2a5	$F(1, 29) = 0.09$, $P = 0.7698$	$F(1, 29) = 0.10$, $P = 0.7550$	$F(1, 29) = 1.65$, $P = 0.2097$

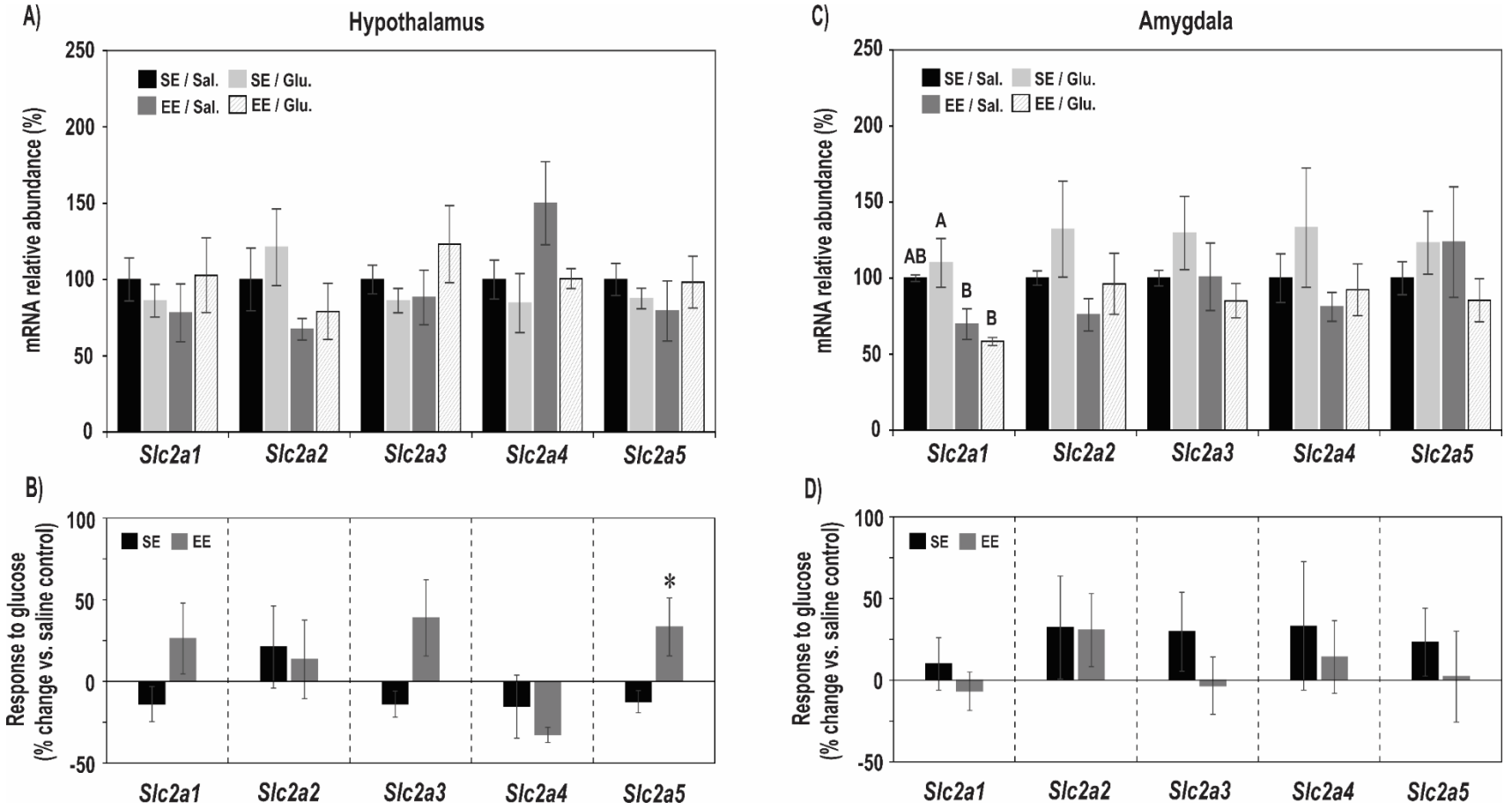


Figure 10 Effect of environmental enrichment and glucose treatment on *Slc2a1-5* mRNA expression in the hypothalamus and amygdala. Levels of *Slc2a1-5* mRNA were measured by real-time PCR in the hypothalamus (A) and amygdala (C). Values in saline-treated SE groups were set to 100%. Response to glucose (% of glucose/saline) in each housing condition was compared in the hypothalamus (B) and amygdala (D). Data are means ± S.E.M. (n = 6-9/group). Groups that do not share a common letter are statistically different ($P < 0.05$ by Tukey-Kramer test). *: $P < 0.05$ by Student's *t*-test.

6.9. Effect of cage environment and glucose treatment on the expression of *Bdnf* transcript variants in mouse brain

The present study examined total *Bdnf* mRNA with either short or long 3' UTR (*Pan-Bdnf*), *Bdnf* mRNA with long 3' UTR (*L-Bdnf*) as well as *Bdnf* transcript variants *I, IIc, IV,* and *VI* in the hypothalamus, amygdala, hippocampus, and parietal cortex of glucose- and saline-treated SE and EE mice. *Bdnf* transcript-variants *I, IIc, IV*, and *VI* were selected in this study because they are associated with a wide array of metabolic and behavioural functions, and therefore they are the most studied *Bdnf* transcripts in the mouse brain (Aid et al., 2007; Pruunsild et al., 2007; Maynard et al., 2016).

In the hypothalamus, two-way ANOVA showed a significant main effect of the housing environment on *Bdnf I* ($P = 0.0005$) and *Bdnf IIc* ($P = 0.0164$) mRNA expression, while it did not detect a significant main effect of housing environment on other *Bdnf* transcript variants (Table 11). Two-way ANOVA, however, did not detect a significant main effect of glucose treatment nor a significant interaction between housing environment and glucose treatment in all of the *Bdnf* transcripts examined. Furthermore, Tukey-Kramer post-hoc test did not detect any meaningful intra- or inter-environment significant difference between the four treatment groups (Fig. 11A). Interestingly, the inter-environment per cent change glucose response analysis showed a significant reduction in *L-Bdnf* and *Bdnf* IV mRNA expression levels in the enriched mice cohort (-6.4% and -17.9%, respectively) compared with the standard environment control (23.2% and 16.5% increase, respectively, $P < 0.05$ by Student's *t*-test, Fig. 11B).

In the amygdala, two-way ANOVA did not show a significant main effect of glucose treatment nor a significant main effect of the housing environment on the expression of all *Bdnf* variants examined (Table 12). Two-way ANOVA, however, only showed a significant interaction between glucose treatment and housing environment on *Bdnf I* expression ($P = 0.0263$, Table 12). Such significant interaction was reflected on the Tukey-Kramer post-hoc analysis, which showed a significant intergroup variation in *Bdnf I* mRNA levels between glucose- and saline-treated groups in the enrichment cohort but not in the standard environment control (Fig. 11C). This significance was also evident in the inter-environment per cent change glucose response analysis which showed a significant reduction in *Bdnf I* mRNA levels in the enriched cohort (-46.9% reduction) compared with the standard environment control (19.5% increase, $P < 0.05$ by Student's *t*-test, Fig. 11D).

In the hippocampus, two-way ANOVA only detected a significant main effect of the housing environment on *Bdnf I* ($P = 0.0023$) and *Bdnf IV* ($P = 0.0480$) transcripts expression but did not detect a significant main effect of treatment nor a significant interaction between glucose treatment and housing environment (Table 13). Tukey-Kramer post-hoc test did not detect any significant intra- or inter-environment difference between the four treatment groups (Fig. 9E). Additionally, the inter-environment per cent change glucose response analysis did not show any significant difference in all of the *Bdnf* transcripts examined (Fig. 9F).

In the parietal cortex, two-way ANOVA showed a significant main effect of the housing environment on the expression of the *Pan-Bdnf* ($P = 0.0005$), *L-Bdnf* ($P = 0.0414$), *Bdnf I* ($P < 0.0001$), and *Bdnf IIc* ($P = 0.0151$) transcripts (Table 14). Concurrently, there was a significant main effect of the glucose treatment on the expression of the *Pan-Bdnf* ($P = 0.0233$) and *L-Bdnf* ($P = 0.0407$) transcripts (Table 14). Nevertheless, two-way ANOVA did not show any significant interaction between the housing environment and glucose treatment in all transcripts examined. Interestingly, Tukey-Kramer post-hoc test detected a significant reduction in baseline *Bdnf I* mRNA levels in the saline-treated enriched mice relative to the saline-treated standard environment mice (Fig. 9G). Of note, the inter-environment per cent change glucose response analysis failed to show any significant difference in all of the *Bdnf* transcripts examined (Fig. 9H). In its entirety, these data suggest that environmental enrichment alters the expression of *Bdnf* mRNA and its response to glucose in a transcript variant-specific and brain region-specific manner.

Table 11 Two-way ANOVA of hypothalamic neurotrophins and receptors gene expression levels in response to environmental enrichment and glucose treatment

Gene	Two-way ANOVA (Hypothalamus)		
	Housing environment	**Glucose treatment**	**Environment × Treatment**
Pan-Bdnf	$F(1, 31) = 3.57$, $P = 0.0682$	$F(1, 31) = 0.14$, $P = 0.7066$	$F(1, 31) = 0.91$, $P = 0.3484$
L-Bdnf	$F(1, 30) = 3.06$, $P = 0.3108$	$F(1, 30) = 0.64$, $P = 0.4287$	$F(1, 30) = 0.69$, $P = 0.4127$
Bdnf I	$F(1, 31) = 14.87$, $\mathbf{P = 0.0005}$	$F(1, 31) = 0.03$, $P = 0.8562$	$F(1, 31) = 0.55$, $P = 0.4632$
Bdnf IIc	$F(1, 31) = 6.44$, $\mathbf{P = 0.0164}$	$F(1, 31) = 0.92$, $P = 0.3451$	$F(1, 31) = 1.67$, $P = 0.2064$
Bdnf IV	$F(1, 31) = 3.10$, $P = 0.0880$	$F(1, 31) = 0.01$, $P = 0.9468$	$F(1, 31) = 2.60$, $P = 0.1171$
Bdnf VI	$F(1, 31) = 0.78$, $P = 0.3838$	$F(1, 31) = 0.53$, $P = 0.4729$	$F(1, 31) = 0.46$, $P = 0.5024$
Ntrk2	$F(1, 30) = 0.75$, $P = 0.3931$	$F(1, 30) = 0.34$, $P = 0.5630$	$F(1, 30) = 2.59$, $P = 0.1180$
Ngfr	$F(1, 30) = 0.10$, $P = 0.7536$	$F(1, 30) = 0.61$, $P = 0.4391$	$F(1, 30) = 0.03$, $P = 0.8670$
Gdnf	$F(1, 28) = 0.06$, $P = 0.8117$	$F(1, 28) = 0.21$, $P = 0.6476$	$F(1, 28) = 1.99$, $P = 0.1697$
Manf	$F(1, 29) = 3.36$, $P = 0.0770$	$F(1, 29) = 3.11$, $P = 0.0882$	$F(1, 29) = 0.19$, $P = 0.6695$

Table 12 Two-way ANOVA of amygdalar neurotrophins and receptors gene expression levels in response to environmental enrichment and glucose treatment

Gene	Two-way ANOVA (Amygdala)		
	Housing environment	**Glucose treatment**	**Environment × Treatment**
Pan-Bdnf	$F(1, 31) = 0.04$, $P = 0.8426$	$F(1, 31) = 2.30$, $P = 0.1398$	$F(1, 31) = 0.40$, $P = 0.5338$
L-Bdnf	$F(1, 30) = 1.31$, $P = 0.2614$	$F(1, 30) = 1.41$, $P = 0.2444$	$F(1, 30) = 1.55$, $P = 0.2228$
Bdnf I	$F(1, 29) = 1.36$, $P = 0.2537$	$F(1, 29) = 2.30$, $P = 0.1410$	$F(1, 29) = 5.48$, $\mathbf{P = 0.0263}$
Bdnf IIc	$F(1, 31) = 0.20$, $P = 0.6597$	$F(1, 31) = 1.69$, $P = 0.2033$	$F(1, 31) = 0.41$, $P = 0.5289$
Bdnf IV	$F(1, 30) = 0.17$, $P = 0.6862$	$F(1, 30) = 2.26$, $P = 0.1432$	$F(1, 30) = 3.76$, $P = 0.0619$
Bdnf VI	$F(1, 30) = 2.22$, $P = 0.1465$	$F(1, 30) = 2.33$, $P = 0.1370$	$F(1, 30) = 1.96$, $P = 0.1720$
Ntrk2	$F(1, 28) = 3.20$, $P = 0.0841$	$F(1, 28) = 0.001$, $P = 0.9757$	$F(1, 28) = 0.41$, $P = 0.5251$
Ngfr	$F(1, 25) = 0.72$, $P = 0.4058$	$F(1, 25) = 0.35$, $P = 0.5567$	$F(1, 25) = 1.95$, $P = 0.1753$
Gdnf	$F(1, 28) = 1.51$, $P = 0.2292$	$F(1, 28) = 1.47$, $P = 0.2351$	$F(1, 28) = 0.002$, $P = 0.9673$
Manf	$F(1, 28) = 7.36$, $\mathbf{P = 0.0113}$	$F(1, 28) = 1.03$, $P = 0.3181$	$F(1, 28) = 0.34$, $P = 0.5646$

Table 13 Two-way ANOVA of hippocampal neurotrophins and receptors gene expression levels in response to environmental enrichment and glucose treatment

Gene	Two-way ANOVA (Hippocampus)		
	Housing environment	**Glucose treatment**	**Environment × Treatment**
Pan-Bdnf	$F(1, 31) = 0.01$, $P = 0.9191$	$F(1, 31) = 0.17$, $P = 0.6805$	$F(1, 31) = 0.05$, $P = 0.8335$
L-Bdnf	$F(1, 31) = 1.15$, $P = 0.2908$	$F(1, 31) = 0.02$, $P = 0.8854$	$F(1, 31) = 0.21$, $P = 0.8854$
Bdnf I	$F(1, 30) = 11.06$, $\mathbf{P = 0.0023}$	$F(1, 30) = 0.001$, $P = 0.9734$	$F(1, 30) = 0.21$, $P = 0.6486$
Bdnf IIc	$F(1, 31) = 0.03$, $P = 0.8567$	$F(1, 31) = 0.45$, $P = 0.5075$	$F(1, 31) = 0.32$, $P = 0.5763$
Bdnf IV	$F(1, 31) = 4.24$, $\mathbf{P = 0.0480}$	$F(1, 31) = 0.001$, $P = 0.9746$	$F(1, 31) = 0.004$, $P = 0.9526$
Bdnf VI	$F(1, 31) = 0.0002$, $P = 0.988$	$F(1, 31) = 0.02$, $P = 0.8852$	$F(1, 31) = 0.14$, $P = 0.7079$
Ntrk2	$F(1, 29) = 21.12$, $\mathbf{P < 0.0001}$	$F(1, 29) = 0.03$, $P = 0.8659$	$F(1, 29) = 0.47$, $P = 0.4972$
Ngfr	$F(1, 28) = 5.11$, $\mathbf{P = 0.0317}$	$F(1, 28) = 0.07$, $P = 0.7931$	$F(1, 28) = 7.01$, $\mathbf{P = 0.0132}$

Table 14 Two-way ANOVA of cortical neurotrophins and receptors gene expression levels in response to environmental enrichment and glucose treatment

Gene	Two-way ANOVA (Parietal cortex)		
	Housing environment	**Glucose treatment**	**Environment × Treatment**
Pan-Bdnf	$F(1, 31) = 15.03$, $\mathbf{P = 0.0005}$	$F(1, 31) = 5.78$, $\mathbf{P = 0.0223}$	$F(1, 31) = 1.52$, $P = 0.2268$
L-Bdnf	$F(1, 30) = 4.54$, $\mathbf{P = 0.0414}$	$F(1, 30) = 4.57$, $\mathbf{P = 0.0407}$	$F(1, 30) = 024$, $P = 0.6259$
Bdnf I	$F(1, 31) = 25.96$, $\mathbf{P < 0.0001}$	$F(1, 31) = 3.43$, $P = 0.0736$	$F(1, 31) = 0.68$, $P = 0.4162$
Bdnf IIc	$F(1, 31) = 6.62$, $\mathbf{P = 0.0151}$	$F(1, 31) = 0.66$, $P = 0.4219$	$F(1, 31) = 0.11$, $P = 0.7462$
Bdnf IV	$F(1, 30) = 3.40$, $P = 0.0750$	$F(1, 30) = 0.91$, $P = 0.3490$	$F(1, 30) = 2.60$, $P = 0.1174$
Bdnf VI	$F(1, 31) = 0.14$, $P = 0.7138$	$F(1, 31) = 2.02$, $P = 0.1657$	$F(1, 31) = 0.85$, $P = 0.3638$
Ntrk2	$F(1, 31) = 0.20$, $P = 0.6542$	$F(1, 31) = 0.20$, $P = 0.6557$	$F(1, 31) = 2.09$, $P = 0.1581$
Ngfr	$F(1, 29) = 0.15$, $P = 0.6979$	$F(1, 29) = 3.48$, $P = 0.0722$	$F(1, 29) = 5.39$, $\mathbf{P = 0.0274}$

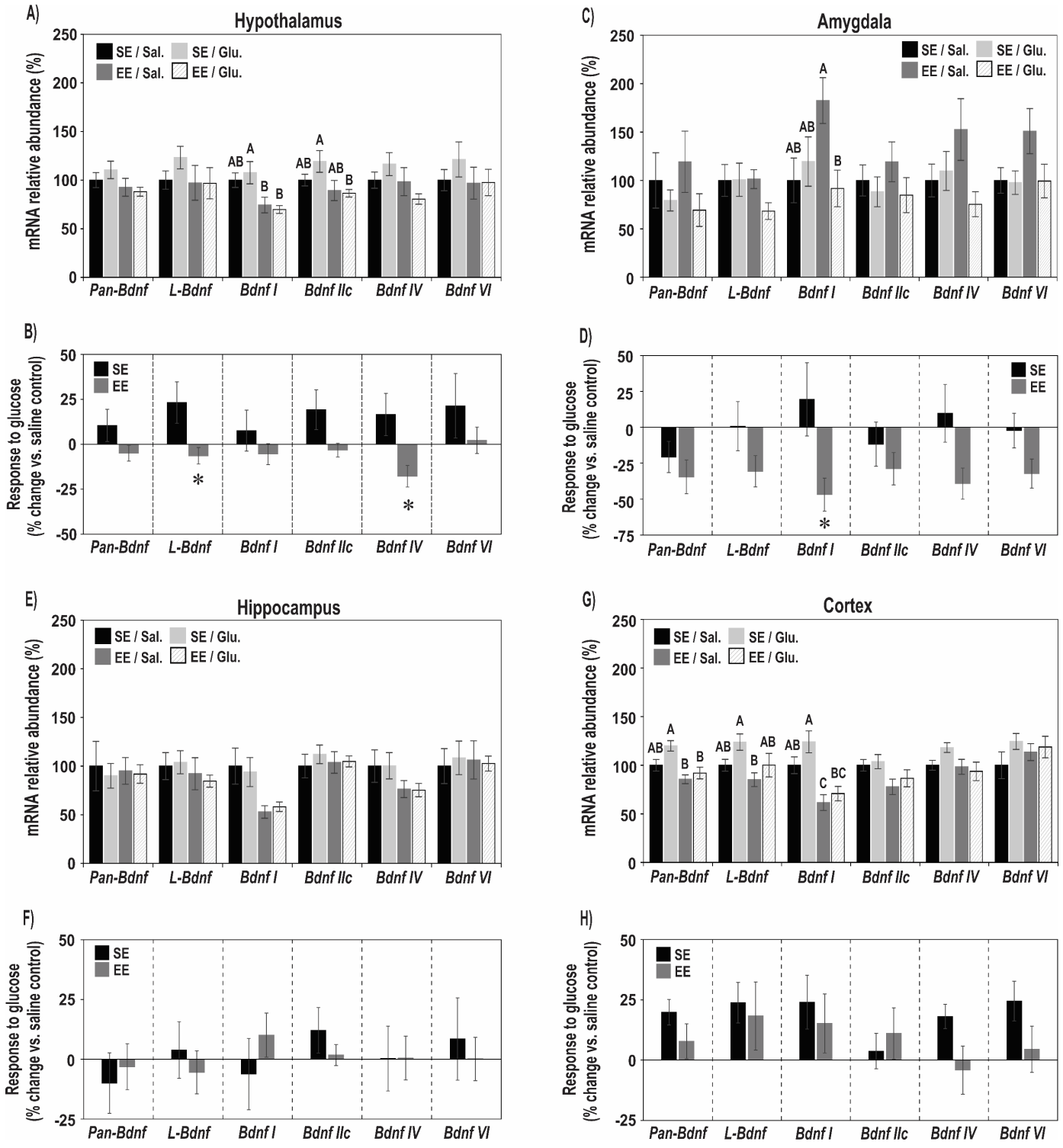

A)
Hypothalamus
mRNA relative abundance (%)
SE / Sal.
SE / Glu.
EE / Sal.
EE / Glu.
Pan-Bdnf
L-Bdnf
Bdnf I
Bdnf IIc
Bdnf IV
Bdnf VI
B)
Response to glucose (% change vs. saline control)
SE
EE
C)
Amygdala
D)
E)
Hippocampus
F)
G)
Cortex
H)

Figure 11 Splice variant-specific and brain region-specific *Bdnf* mRNA expression in response to environmental enrichment and glucose. Effect of environmental enrichment and glucose treatment on *Bdnf* mRNA variants levels in the hypothalamus (A), amygdala (C), hippocampus (E), and parietal cortex (G). Data were initially analysed by two-way ANOVA followed by Tukey-Kramer post-hoc test. Response to glucose (% of glucose/saline) in each housing condition was compared in the hypothalamus (B), amygdala (D), hippocampus (F), and parietal cortex (H). Data are means ± S.E.M. (n = 6-9/group). Groups that do not share a common letter are statistically different ($P < 0.05$ by Tukey-Kramer test). *: $P < 0.05$ by Student's *t*-test

6.10. Effect of cage environment and glucose treatment on the expression of the BDNF receptor genes in mouse brain

The present study examined the expression of the neurotrophic tyrosine kinase receptor 2 (*Ntrk2*) and the nerve growth factor receptor (*Ngfr*) genes that encode for the TrkB and $P75^{NTR}$ receptors, respectively. Two-way ANOVA only showed a significant main effect of the housing environment on hippocampal *Ntrk2* mRNA expression ($P < 0.0001$, Table 13). Consistently, Tukey-Kramer post-hoc test showed a significant reduction in baseline hippocampal *Ntrk2* mRNA levels in the saline-treated EE mice compared with the saline-treated SE control (Fig. 12A). Interestingly, the inter-environment per cent change glucose response analysis showed a significant increase in hypothalamic *Ntrk2* mRNA levels in the EE cohort (35% increase) compared with the SE control (-13.8% reduction, $P < 0.05$ by Student's *t*-test, Fig. 12B).

Two-way ANOVA showed a significant main effect of the housing environment on hippocampal *Ngfr* mRNA expression ($P = 0.0317$, Table 13). Furthermore, two-way ANOVA showed a significant interaction between the housing environment and glucose treatment in hippocampal and cortical *Ngfr* mRNA expression ($P < 0.05$, Tables 13 and 14). In agreement with these data, Tukey-Kramer post-hoc test showed a significant reduction in baseline hippocampal *Ngfr* mRNA levels in the saline-treated EE mice compared with the saline-treated SE control (Fig. 12C). Moreover, glucose treatment significantly increased cortical *Ngfr* mRNA expression in the EE cohort but not in the SE control (Fig. 12C). Additionally, the inter-environment per cent change glucose response analysis showed a significant increase in cortical *Ngfr* mRNA levels in the EE cohort (160% increase) compared with the SE cohort (-8.1% reduction, $P < 0.05$ by Student's *t*-test, Fig. 12D).

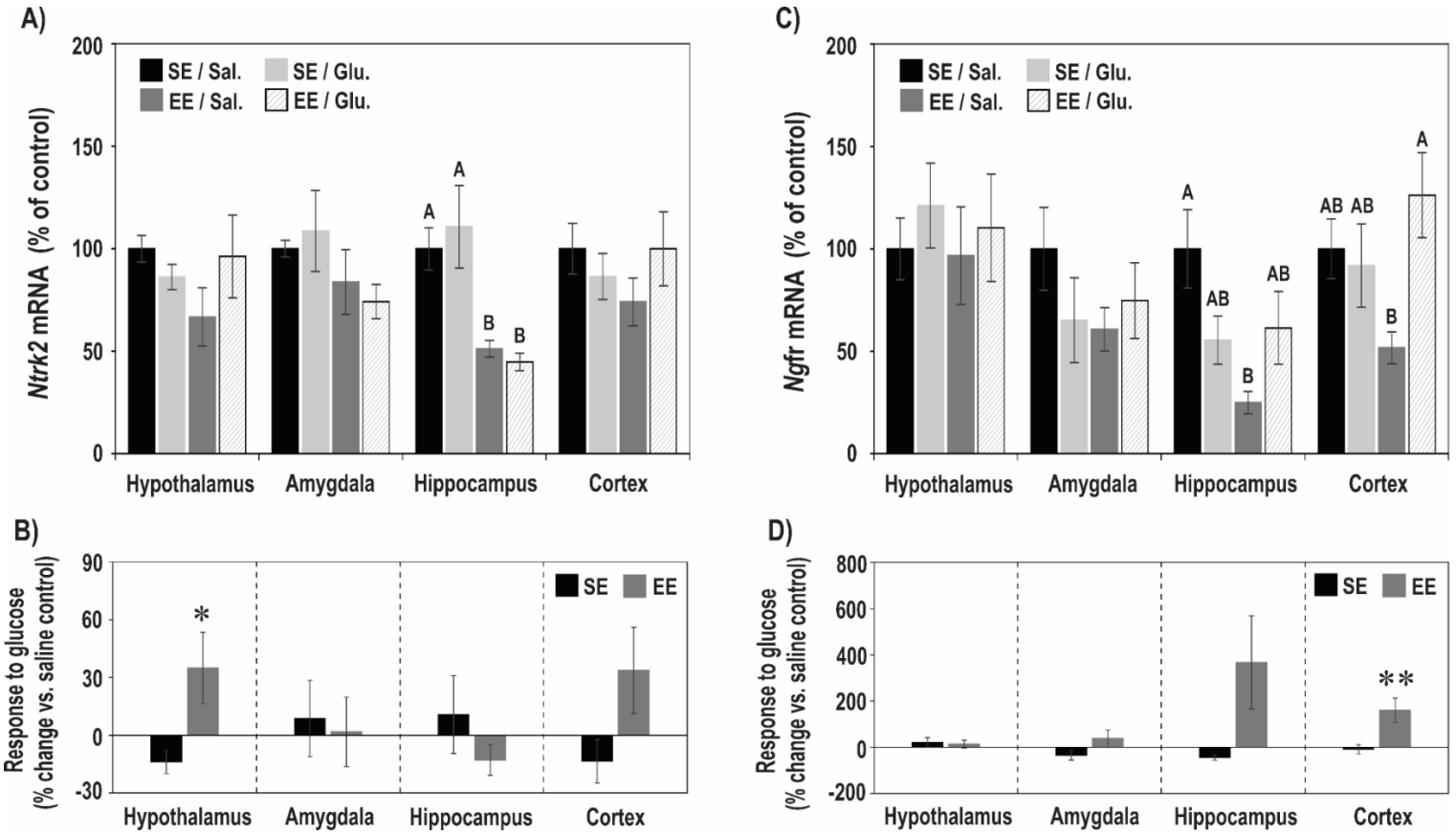


Figure 12 Expression of the genes encoding BDNF receptors in the brain in response to environmental enrichment and glucose treatment. Levels of *Ntrk2* (A) and *Ngfr* (C) mRNA were measured by real-time PCR in the hypothalamus, amygdala, hippocampus, and parietal cortex. Values in saline-treated SE groups were set to 100%. *Ntrk2* (B) and *Ngfr* (D) expression levels in response to glucose (% of glucose/saline) were compared between housing environments. Data are means ± S.E.M. (n = 6-9/group). Groups that do not share a common letter are statistically different ($P < 0.05$ by Tukey-Kramer test). *: $P < 0.05$, **: $P < 0.01$ by Student's *t*-test.

6.11. Effect of cage environment and glucose treatment on *Gdnf* and *Manf* mRNA expression in the mouse brain

GDNF and MANF have been shown to regulate energy balance (Mwangi et al., 2014; Yang et al., 2017). Therefore, the present study examined the mRNA expression of the *Gdnf* and *Manf* genes in the hypothalamus and amygdala. Two-way ANOVA did not show a significant main effect of housing environment, glucose treatment, nor a significant interaction between the housing environment and glucose treatment in the expression of both genes in both tissues (Tables 11 and 12) except for a significant main effect of the housing environment on amygdalar *Manf* expression levels ($P < 0.05$, Table 12). Tukey-Kramer post-hoc test did not detect any intra- or inter-environment significant difference between the treatment groups (Fig. 13A and 13C). Notably, the inter-environment per cent change glucose response analysis showed a significant increase in hypothalamic, but not amygdalar, *Gdnf* mRNA levels in the enriched cohort (24.7% increase) compared with the standard environment cohort (-10.5% reduction, $P < 0.05$ by Student's *t*-test, Fig. 13B and 13D). The same analysis did not detect a significant difference in *Manf* mRNA response to glucose between the two housing conditions (Fig. 13B and 13D). These data suggest that environmental enrichment increases hypothalamic *Gdnf* expression in response to glucose treatment.

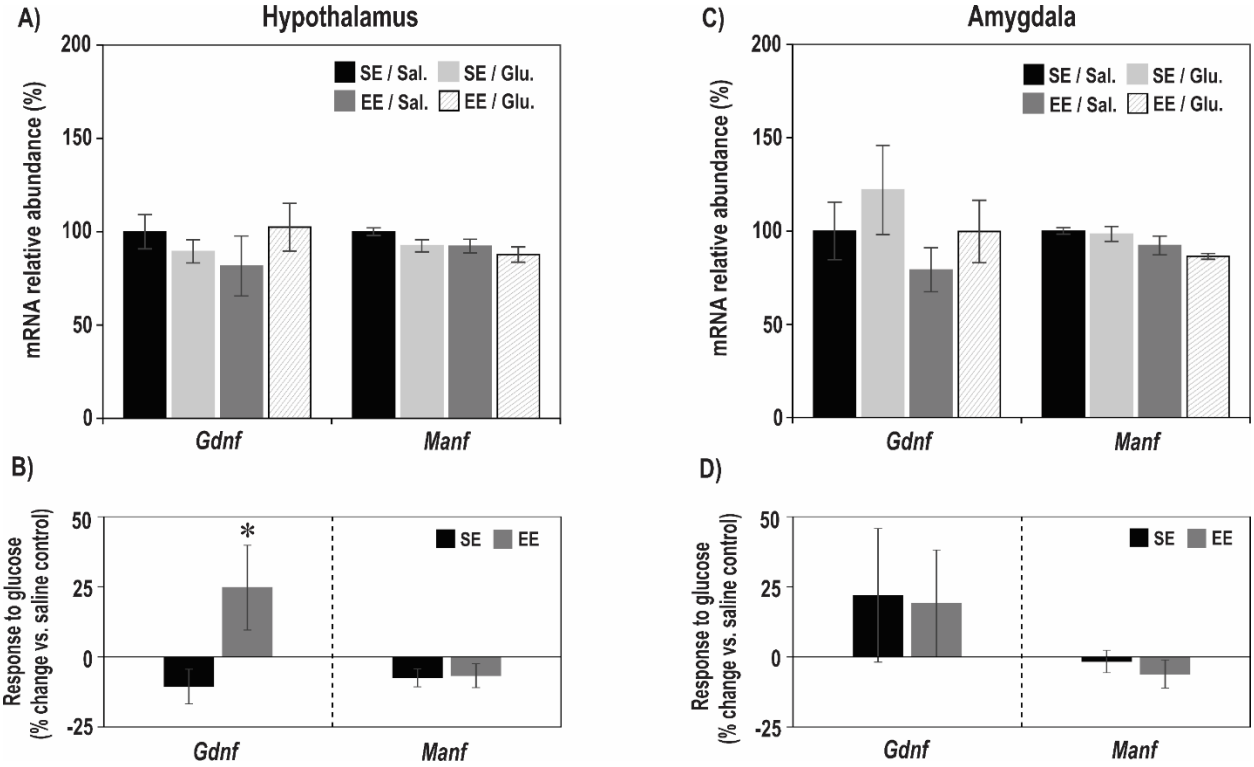


Figure 13 Expression levels of *Gdnf* and *Manf* mRNA in response to environmental enrichment and glucose treatment. *Gdnf* and *Manf* mRNA Levels were measured by real-time PCR in the hypothalamus (A) and the amygdala (C). Values in saline-treated SE groups were set to 100%, and data were analysed by two-way ANOVA followed by Tukey-Kramer post hoc test. Additionally, *Gdnf* and *Manf* mRNA expression levels in response to glucose (% of glucose/saline) were compared between housing environments in the hypothalamus (B) and the amygdala (D). Data are means ± S.E.M. (n = 6-9/group). *: $P < 0.05$ by Student's *t*-test.

7. Discussion

7.1. Summary of the main findings

The present study demonstrated, for the first time, that the environmental enrichment (EE) paradigm potentiates the anorectic response to glucose but not the gastrointestinal hormone xenin or the adipocyte hormone leptin. It also showed that hypothalamic *Fos* and *Slc2a5* mRNA expression levels were upregulated in response to glucose treatment under the enriched environment conditions. Furthermore, the present study showed selective glucose-induced down-regulation of *Bdnf* mRNA transcript variants in the hypothalamus and amygdala of the enriched mice relative to the standard environment (SE) control mice. In contrast, hypothalamic *Ntrk2* mRNA expression levels were increased in the enriched mice compared with the SE control. Moreover, there was a significant increase in glucose-induced hypothalamic *Gdnf* mRNA expression in the enriched mice compared with the SE control. Collectively, these findings suggest that EE potentiates the anorectic response to glucose by altering the pattern of hypothalamic neurotrophins gene expression.

7.2. The metabolic benefits of environmental enrichment

EE is a laboratory group housing protocol that combines inanimate and social stimulations to elicit BDNF-mediated physical and mental health benefits in laboratory animals (Vitalo et al., 2009; Hüttenrauch et al., 2016; Song et al., 2017; Takai et al., 2019). Despite a lack of consensus among researchers on a universal EE protocol, a consistent finding in EE research, including the present study, is that EE mice always display a lean phenotype characterised by reduced adiposity and body weight compared to the non-enriched SE mice (Cao et al., 2010; Mainardi et al., 2010; Cao et al., 2011; McQuaid et al., 2018). The EE-associated lean phenotype is independent of increased physical activity or reduced food intake and is mediated by activation of the hypothalamic-sympathoneural-adipocyte axis (Cao et al., 2011). While the previously reported EE-mediated increase in hypothalamic BDNF expression should theoretically reduce food intake, this study, and others, showed that EE mice consumed more food compared to the SE mice control (Cao et al., 2010; Mainardi et al., 2010; Cao et al., 2011; Queen et al., 2020). Such an increase in food intake may be a compensatory response to the EE-associated fat mass reduction and the low circulating levels of serum leptin observed in the present study. However, the precise mechanism controlling feeding under EE remains largely unknown.

7.3. Environmental enrichment potentiates the feeding-suppressing effect of glucose

It was previously reported that EE improves glucose tolerance, suggesting that EE enhances glucose sensitivity (Mainardi et al., 2010). The hypothalamus is the point of convergence between the neuroendocrine and autonomic pathways regulating glucose homeostasis. Therefore, hypothalamic glucose sensing represents a vital mechanism through which the brain can efficiently optimise energy balance to meet its energy demands without compromising the body's energy needs. Not surprisingly, multiple studies have demonstrated that impaired hypothalamic glucose sensing results in defective glucose-induced feeding suppression in rodents (Bady et al., 2006; Parton et al., 2007; Stolarczyk et al., 2010; de Andrade et al., 2015). The present study demonstrated that EE enhanced the feeding-suppressing effect of glucose while it had a negligible effect on xenin- or leptin-induced feeding suppression. It similarly showed that hypothalamic activity, as assessed by *Fos* mRNA expression, was increased in the hypothalamus in response to intraperitoneal glucose injection in the EE mice relative to the SE control, whereas no change was seen in the amygdala, hippocampus, or parietal cortex. Moreover, EE also enhanced glucose-induced expression of the GLUT5-encoding *Slc2a5* mRNA in the hypothalamus but not in the other tissues. Together, these data suggest that EE enhances glucose-induced satiation partly by increasing hypothalamic glucose sensitivity.

The present study also showed that the mice housed in the EE cages consumed significantly more food than the mice housed in the SE cages, across three independent animal studies. Therefore, the present finding that EE enhanced the anorectic response to glucose seems contradictory to the overall 24-hr food intake data. One may rightfully ask; if total 24-hr intake is increased, does the enhanced anorectic response to glucose contribute to the metabolic benefits of EE? The answer to this question might have to do with the feeding rhythm (i.e., temporal spreading of food intake) rather than the total 24-hr intake. Indeed, it has been shown that increased caloric intake per meal rather than per 24-hr precipitated high-fat-diet-induced obesity in rodents, suggesting that meal size, rather than the 24-hr overnutrition, is a more significant determinant of obesity development (Furnes et al., 2009). Moreover, a report involving 3,610 randomly selected participants showed that obesity development was associated with increased self-reported portion sizes of the main meals (Berg et al., 2009). Importantly, the same study reported no significant relationship between total 24-hr energy intake and obesity prevalence in the study participants. Additionally, another study reported that mice subjected to time-restricted feeding of high-fat diet

are protected against the deleterious metabolic syndrome consequences associated with high-fat-diet-induced obesity despite consuming an equal number of calories as in mice with *ad libitum* access to high-fat diet (Hatori et al., 2012). The same study by Hatori and colleagues also showed that time-restricted feeding improved the circadian oscillator proteins expression, leading to improved liver functions and increased thermogenesis and motor functions, further supporting the notion that alterations in diurnal feeding patterns affect metabolism. Based on the three studies mentioned above, the present study proposes that the EE-mediated glucose-induced feeding suppression could induce an early termination of meals, restricting the number of calories consumed per meal (i.e., reduced meal size), without reducing total daily energy intake and therefore could contribute to the overall reduced adiposity levels and increased thermogenesis in EE mice. Further investigation is necessary to determine whether EE causes a reduction in meal size and an increase in meal frequency by examining the diurnal feeding rhythm.

The present study showed an increase in hypothalamic *Fos* mRNA levels, suggesting that hypothalamic activity is increased in response to glucose treatment under the EE housing conditions. Although glucose-sensing neurons are also present in extrahypothalamic tissues, the reported increase in hypothalamic *Fos* mRNA expression, but not in the other tissues examined, in response to glucose treatment suggests that the hypothalamus is the primary site mediating the EE-induced potentiation of glucose effect. Hypothalamic glucose-sensing represents a vital component of energy homeostasis, and hypothalamic neurons can be classified into glucose-excited or glucose-inhibited neurons (Reviewed in Yoon & Diano, 2021). In addition to hypothalamic glucose-sensing neurons, glial cells have also been shown to contribute to glucose sensing and glucose homeostasis. The hypothalamic astrocytes are known to express glucose transporters (i.e., GLUT2 and GLUT1). Of note, glucose uptake in the astrocytes can either be stored as glycogen or converted to lactate and shuttled to glucose-excited neurons where it can be further converted to pyruvate and metabolised to increase intracellular ATP levels, resulting in membrane depolarisation (Reviewed in Bélanger et al., 2011; Yoon & Diano, 2021). There is also enough evidence to suggest a role of hypothalamic microglia in glucose sensing. Indeed, hypothalamic microglia are known to express many glucose transporters, including GLUT1 (Wang, Pavlou, et al., 2019) and GLUT5 (Payne et al., 1997). Moreover, acute glucose fluctuations have been shown to induce stress-mediated alterations in microglial activity (Hsieh et al., 2019). The present study showed that glucose treatment elicited an increase in the microglia-specific

GLUT5-encoding *Slc2a5* mRNA, suggesting a possible involvement of microglia in mediating the improved response to glucose under EE. Although GLUT5 is mainly associated with fructose transport, a recent study showed that peripherally-administered glucose could be transferred to the brain and converted to fructose (Hwang et al., 2017). Therefore, it is possible that intraperitoneal glucose treatment increased the activation of hypothalamic microglia in EE mice, as manifested by increased hypothalamic *Fos* and *Slc2a5* mRNA levels. However, further investigations are needed to examine this hypothesis.

7.4. Effect of EE on *Bdnf* transcript variant-specific and brain region-specific expression

In addition to its neurotrophic actions, BDNF and its receptor TrkB play a critical role in energy homeostasis. Administration of BDNF into the hypothalamic VMN or PVN reduced food intake in rats (Wang, Bomberg, Billington, et al., 2007; Wang, Bomberg, Levine, et al., 2007). Furthermore, mice display hyperphagia and abnormally increased body weight in the absence of BDNF in the hypothalamus (Rios et al., 2001; Unger et al., 2007; An et al., 2015). On the other hand, rescuing hypothalamic *Bdnf* expression reversed hyperphagia and obesity in *Bdnf*-deficient mice (Liao et al., 2012). Interestingly, previous reports have shown that EE increased *Bdnf* expression in the brain, specifically in the hypothalamus (Cao et al., 2011; Hu et al., 2013; Mansour et al., 2021). These findings suggest that changes in hypothalamic *Bdnf* expression mediate the effect of EE on energy homeostasis. However, the current study showed that EE did not significantly alter baseline *Bdnf* mRNA expression in the hypothalamus in 8-hr fasted mice. The lack of EE-induced *Bdnf* expression may be attributed to food deprivation as previous studies have shown that 24-48 hr fast significantly reduced *Bdnf* mRNA levels in the VMN (Xu et al., 2003; Liu et al., 2014). It may also be explained by the EE-associated reduction in serum leptin levels, as it has been previously reported that leptin increases *Bdnf* mRNA expression in the VMN (Komori et al., 2006).

It has been reported that glucose treatment increased *Bdnf* mRNA (*pan-Bdnf* and *Bdnf I* mRNA) in the VMN but not in the dorsomedial nucleus (DMN), hippocampus, and cortex (Unger et al., 2007). Conversely, inhibition of glucose metabolism by 2-deoxy-D-glucose lowers *Bdnf* mRNA levels in the rat VMN as well as in hypothalamic neuronal primary culture (Maekawa et al., 2013). Moreover, glucose treatment promoted *Bdnf* transcription from promoter *I* and *IV* in neuronal cell lines (Hossain et al., 2018). Additionally, mice with truncated long 3′ UTR *Bdnf* mRNA develop hyperphagic obesity and impaired glucose homeostasis; such features were

attenuated upon viral-mediated expression of long 3′ UTR *Bdnf* mRNA in the hypothalamus (Liao et al., 2012). Together, these findings suggest that increased glucose availability in the hypothalamus stimulates the expression of specific *Bdnf* transcript variants, leading to the hypothesis that EE augments glucose-induced *Bdnf* mRNA expression. Contrary to this hypothesis, the present study showed that levels of *Bdnf* mRNA transcript variants in EE mice were not increased in response to glucose treatment in the hypothalamus. If anything, *Bdnf* mRNA transcripts were selectively downregulated in response to glucose treatment under EE. Although these conflicting data are inexplicable, the lack of glucose-induced *Bdnf* mRNA expression in the hypothalamus of EE mice may contribute to the increased food intake in these mice. As stated above, this is consistent with the possibility that EE mice show a compensatory increase in food intake in response to reduced adiposity and serum leptin levels that may reduce hypothalamic *Bdnf* mRNA expression in EE mice.

Hypothalamic VMN and PVN neurons not only produce BDNF but also are targets of BDNF action, as they express TrkB receptors. Intriguingly, a relatively recent report demonstrated that lower glucose concentration in the brain alters BDNF responsiveness in a subset of hypothalamic PVN neurons, resulting in hyperpolarisation of these neurons (McIsaac & Ferguson, 2017). This study raises the possibility that increased hypothalamic glucose concentration could potentiate endogenous BDNF responsiveness in the PVN, leading to enhanced BDNF-mediated anorectic response, possibly without changes in BDNF protein or *Bdnf* mRNA levels. The current finding that hypothalamic *Ntrk2* mRNA levels were increased in the EE mice compared to the SE control in response to glucose treatment further supports this possibility.

BDNF plays a differential role in the regulation of metabolism in a brain region-specific manner. Contrary to hypothalamic *Bdnf* functions, a recent study has revealed that deletion of *Bdnf* in the amygdala results in a lean phenotype in mice. This lean phenotype persisted even when mice were fed a high-fat diet (Xie et al., 2019). Moreover, the same study showed that rescue of amygdalar *Bdnf* expression precipitated obesity in response to high-fat diet. Although glucose-sensing neurons are present in the amygdala, the precise role of the amygdala in glucose-sensing remains unclear (Nakano et al., 1986; Zhou et al., 2010). Our finding that glucose treatment reduced *Bdnf I* mRNA expression in the amygdala supports a role of amygdalar *Bdnf I* in promoting the transition from negative to positive energy balance. However, empirical investigations need to be carried out to examine such possibility.

Together, these studies indicate that BDNF plays a multidimensional role in regulating food intake and body weight under the enriched environment.

7.5. A possible role of GDNF in the mediation of EE-induced feeding suppression

Glial cell line-derived neurotrophic factor (GDNF) plays a role in energy homeostasis. Despite its name, GDNF is mainly expressed in neurons and functions to promote the growth and survival of dopaminergic and motor neurons (Kopra et al., 2017; Cintrón-Colón et al., 2020). Metabolically speaking, enhanced *Gdnf* expression reduced food intake and prevented high-fat-diet-induced obesity in mice (Mwangi et al., 2014). Moreover, hypothalamus-specific enhancement of *Gdnf* expression caused reductions in food intake and body weight gain in young rats as well as in a rat model of age-associated obesity (Tümer et al., 2006; Manfredsson et al., 2009). Previous studies reported conflicting data on the effect of EE on *Gdnf* mRNA expression. EE significantly increased *Gdnf* mRNA levels in the hippocampus (Young et al., 1999) and the substantia nigra in the midbrain (Faherty et al., 2005), while other studies reported no change in *Gdnf* mRNA expression in the hippocampus (Williamson et al., 2012). No previous study, according to the available published knowledge, has examined *Gdnf* mRNA expression in the hypothalamus or amygdala in response to EE or glucose treatment. Here, the current study reports for the first time that EE did not alter *Gdnf* mRNA expression in the hypothalamus or the amygdala in mice. However, glucose treatment significantly increased *Gdnf* mRNA expression in the hypothalamus of EE mice relative to the SE mice. Of note, our laboratory has recently found that intracerebroventricular glucose treatment led to an increase in hypothalamic *Gdnf* mRNA expression in mice (Mizuno & Poritsanos, unpublished data). The present study, therefore, supports the notion that hypothalamic *Gdnf* mRNA expression is, at least in part, regulated by glucose. It also proposes that EE amplifies glucose-induced *Gdnf* expression in the hypothalamus, which may contribute to the observed enhanced glucose-induced feeding suppression in EE mice.

7.6. Conclusion

Environmental enrichment potentiates glucose-induced feeding-suppressing and alters hypothalamic activity and neurotrophic factors gene expression in response to glucose availability. Specifically, increased hypothalamic *Gdnf* expression may constitute an additional mechanism through which enriched environment augments hypothalamic glucose sensing and induces its reported metabolic benefits.

7.7. Significance

Obesity prevalence has increased unabated in recent years with a concurrent increase in the obesity-associated co-morbidities and mortality rates. Obesity is a product of dysregulation in energy homeostasis, which is typically maintained by an intricate interplay between genetic and environmental factors. Such factors converge in a complex way to influence the feeding behaviour and the pattern of physical activity. While most obesity research is focused on delineating the genetic component of obesity, less attention has historically been paid to the environmental factors. Similarly, the interconnectedness between genetic and environmental influences in multifactorial obesity remains poorly understood. The present study attempted to examine the influence of the housing environment on feeding behaviour. More specifically, data from the present study showed, for the first time, that EE potentiates the feeding suppressing effect of glucose, leading to a reduction in food intake in 8-hr fasted mice. Building on the results from the present study, it is expected that subsequent investigations could outline a role of EE in regulating the feeding pattern rather than total food intake, which would partly explain the reduced adiposity levels and the lean phenotype associated with EE.

It has been generally accepted that exercise improves metabolic functions (Reviewed in Ibeas et al., 2021; Sgrò et al., 2021). However, the adiposity-reducing effect of EE is thought to be independent of physical activity (Cao et al., 2011). Thus, the present study provided a unique opportunity to uncover potential agents/mechanisms that promote the beneficial metabolic effect of the enriched environment beyond physical activity. Indeed, the present study showed that EE altered neurotrophins expression in response to glucose availability. More importantly, the present study showed that hypothalamic *Gdnf* mRNA levels are increased in response to glucose availability under the EE paradigm, suggesting, for the first time, that GDNF could be an important metabolic enviromimetic and therefore a potential candidate for the pharmacological treatment of obesity. It is reasonably expected that the novel data published in this study will further the current understanding of the gene-environment interplay in the regulation of feeding behaviour.

7.8. Limitations

Sex difference in response to EE

The present study only used male mice to examine the anorectic response to peripheral metabolic signals. It is generally accepted that metabolic differences exist between males and females (Reviewed in Brown & Clegg, 2010). For instance, body fats exhibit sex-dependent distribution in humans and rodents. Whereas females have more subcutaneous fats, males tend to have more visceral fats (Wajchenberg, 2000). Notably, sex differences have also been reported in the metabolic response to hormones such as insulin and leptin (Clegg et al., 2003). In addition, it has also been reported that long-term social isolation increased locomotor activity in male mice compared with females (Guo et al., 2004). Furthermore, environmental enrichment improved the cognitive functions in females but failed to do so in males (Girbovan & Plamondon, 2013). Therefore, it is unclear whether the findings reported in this study can be applicable to females. Future studies should include and compare the effect of EE in both males and females.

Timing of the feeding studies

The present study examined the anorectic response to three different metabolic signals but at different time points (different ages of the animals) and consequently at different lengths of exposure to the EE protocol. For instance, the glucose feeding-suppressing effect was examined in the 5th week into the housing protocol (mice were 9-week-old), whereas the leptin feeding-suppressing effect was measured in the 11th week into the housing protocol (mice were 15-week-old). It is unclear whether the age of the animals or the length of EE exposure influenced the outcome of the four feeding studies described in the present study. Therefore, future studies should consider age and EE exposure uniformity in testing the metabolic response of anorectic agents. For example, future investigations should examine all treatment agents or the same agent with different treatment doses simultaneously to avoid age or EE exposure variability in the experimental design.

Tissue dissection protocol

As discussed in the introduction section, different hypothalamic and amygdalar nuclei support distinct metabolic and behavioural functions. The dissection protocol followed in the present study is limiting as it only allows whole tissue dissection rather than a specific nucleus or subregion in the tissue. Therefore, the present study could not examine *Bdnf* mRNA transcript

variants expression in the hypothalamic nuclei where it is most abundant such as the VMN and PVN. Future studies should use techniques such as laser-capture microdissection (LCM) to procure specific hypothalamic nuclei or cellular subpopulations for gene and protein expression analysis (Espina et al., 2006). In addition to LCM, *in situ* hybridisation (ISH) can also be used to visualise RNA expression in distinct tissues or cellular populations (Jensen, 2014).

Correlation between mRNA and protein expression

The present study examined gene expression exclusively without examining the correlation between gene and protein expression. It is not known, at this point, whether mRNA levels showed in this study correlate to functional protein levels in the tissues examined. In particular, the interpretations of *Bdnf* mRNA levels presented in this study should be taken with caution given the transcriptional complexity as well as the level of temporal and spatial epigenetic and post-translational modifications of the BDNF precursor and mature proteins (Mowla et al., 2001; Martínez-Levy & Cruz-Fuentes, 2014). Moreover, previous studies showed that hypothalamic BDNF levels increase in response to EE housing (Cao et al., 2011; Hu et al., 2013; Mansour et al., 2021), whereas the present study showed that *Bdnf* transcript variants mRNA levels, which all encode the same BDNF protein, were not changed significantly in response to EE housing. Therefore, future studies should examine the effect of EE on the tissue-specific BDNF expression (both precursor and mature proteins) by employing techniques such as immunoblotting and immunohistochemistry.

Insulin ELISA

The present study measured blood glucose levels and insulin concentration in response to intraperitoneal glucose treatment at the time of sacrifice (30 minutes post-injection). Analysis of blood glucose levels showed an expected significant increase in blood glucose in response to treatment. However, Tukey-Kramer post-hoc test failed to detect any significant difference in insulin levels between saline- and glucose-treated groups in both housing conditions. While these data can be interpreted as a lack of an insulin-dependent mechanism in influencing the EE-mediated enhanced feeding-suppression in response to glucose treatment, caution should be exercised as insulin levels were only measured at a single time point, 30 minutes post-injection, and it might not have been enough to potentiate glucose-stimulated insulin secretion (GSIS) in the pancreas. Moreover, the present study showed that EE potentiated the anorectic response of

glucose between 1.5 to 2.5 hours post glucose injection, and it is unknown whether insulin contributed to feeding suppression at this time frame. Therefore, future studies should utilise a time-course analysis of insulin levels, rather than a single point analysis, to conclusively discern the contribution of insulin to the enhanced glucose-induced anorexia under EE.

7.9. Future directions

As an extension of the present study, future investigations should aim to determine the cell-type-specific activation of CNS cells that are both responsive to environmental enrichment and glucose availability using immunohistochemistry/immunofluorescence (IHC/IF). For example, double staining IHC/IF should be performed for c-Fos, as a marker of cell activation, combined with either neuronal nuclei (NeuN), glial fibrillary acidic protein (GFAP) or ionized calcium-binding adaptor molecule 1 (Iba1) as markers of neurons, astrocytes, or microglia, respectively. Previous studies have also shown that BDNF mediates the metabolic effects of EE. Therefore, it would be of value to characterise the cell type-specific expression of BDNF in response to EE using double staining IHC/IF for BDNF combined with the markers mentioned above. It is equally valuable to determine the neurochemical characteristics of BDNF and GDNF expressing neurons in response to glucose treatment under EE.

The present study proposed that feeding rhythm (temporal pattern) rather than the 24-hr food intake contribute to the metabolic phenotype associated with EE. It is plausible that the EE-potentiated glucose-induced feeding suppression could induce an early termination of meals, restricting the number of calories consumed per meal without affecting total daily energy intake and therefore could contribute to the overall reduced adiposity levels in EE mice. However, the present study did not examine the temporal pattern of food intake under normal conditions. Future studies should utilise advanced pellet dispensers with time-stamp and quantification analyses of the dispensed chow pellets to quantify caloric intake with maximum temporal precision (Nguyen et al., 2017; Oh et al., 2017). However, special considerations should be made to measure caloric intake without disturbing the social component of the EE paradigm. This objective could be accomplished by combining pellet dispensers with video surveillance to assess each mouse's temporal pattern of food intake in the EE cage (Concept reviewed in Ali & Kravitz, 2018).

The present study proposes that hypothalamic GDNF contributes to the enhanced glucose-induced feeding suppression under EE. However, it falls short in providing clear causal evidence linking GDNF to glucose-induced anorexia under EE. Therefore, future investigations can

establish such causal evidence using multiple approaches. For instance, replicating the glucose feeding study in response to EE, as described here, but in *Gdnf* floxed mice in the C57BL/6 background (Described in Kopra et al., 2015) or in mice with conditional *Gdnf* deletion in the hypothalamus (Similar to what has been described in Harasztosi et al., 2020). Alternatively, the glucose-induced feeding suppression can be examined in mice overexpressing GDNF in the hypothalamus (Tümer et al., 2006) independent of EE. Moreover, it would be of value to perform a comparative feeding study examining cumulative food intake in response to intracerebroventricular treatment of GDNF alone, or intracerebroventricular glucose treatment alone, or under a combined intracerebroventricular injection of GDNF and glucose in wild-type C57BL/6 mice under the SE and EE conditions.

Recent studies have shown an important metabolic role of a protein called growth and differentiation factor 15 (GDF15), a member of the transforming growth factor-β (TGF-β) superfamily of proteins. GDF15 overexpression decreased food intake, body weight and improved glucose metabolism in mice (Macia et al., 2012). More importantly, it has been recently shown that GDF15 produces these beneficial metabolic effects via signalling through a novel receptor called GDNF receptor-α-like (GFRAL) in the brainstem (Emmerson et al., 2017; Hsu et al., 2017; Mullican et al., 2017; Yang et al., 2017). Interestingly, GFRAL does not bind GDNF, but it has been demonstrated that GDF15-GFRAL signalling activates RET receptor tyrosine kinase signalling cascade in an identical fashion to GDNF-GFR-α signalling (Hsu et al., 2017; Mullican et al., 2017; Yang et al., 2017). Intriguingly, GDF15 is as potent as GDNF in promoting the growth and survival of dopaminergic neurons (Strelau et al., 2000). Collectively, these reports reflect a strong need to understand the cross-talk between GDNF and GDF15 in the regulation of energy balance and the brain circuits mediating their potent metabolic effect, particularly in the hypothalamus. The present study showed that EE increased hypothalamic *Gdnf* mRNA expression in response to glucose treatment. Given the functional similarity of GDNF and GDF15, it would be of value to examine GDF15 protein and mRNA expression in response to EE and glucose treatment.

PART II: Effect of environmental enrichment on aggressive behaviour and the expression of *Bdnf* transcript variants

1. Introduction

1.1. Human aggression

Human aggression is defined as any hostile social interaction that involves inflicting physical or psychological harm to another unwilling individual with the immediate intent to hurt them (Anderson & Bushman, 2002). From a strictly functional perspective, aggression can be viewed as a primal instinct that ensures self-preservation in the face of increased resource competition (Archer, 2009b; Lindenfors & S.Tullberg, 2011). Conspecific competitions for food, mate or land represent the most significant driving forces for aggression in social species (Georgiev et al., 2013). Given their favourable sexual dimorphism traits, males tend to have a higher propensity for violence than females. This naturally driven male aggressiveness can be explained in light of the sexual selection theory as aggressive males have a higher chance of obtaining mates, procuring food, and defending their territorial dominance than weaker acquiescent males (Archer, 2009a; Puts, 2010). Therefore, male aggression is a hard-wired behaviour fuelling survival and genetic continuity from a Darwinian evolutionary viewpoint.

Not surprisingly, aggressive tendencies are considered a universal behaviour in early childhood, reaching its peak at four years of age followed by a gradual socially driven decline, supported by parental guidance and gentle behavioural modifications to meet social standards (Tremblay, 2010). As the child's brain matures, the child social skills and emotional processing improve, leading to a substantial control of the innate aggression tendencies typically seen in primary school age (Côté et al., 2007). Failure to conform to modern-day socially acceptable behaviours, especially uncontrolled aggressive behaviour, is often associated with a pathopsychological or a neurological disease process that requires professional interventions. Furthermore, the interactions of the hard-wired innate aggressive tendencies with social and environmental influences could accurately predict an individual's capacity for aggression and other disruptive social behaviours (Lanza, 1983). Indeed, in many cases, aggressive interactions could be explained as an attempt of an individual to achieve self-mastery over undesired social or environmental agents threatening the individual's core needs or the integrity of the individual's self-system (Boettcher, 1983). Ergo, aggression is an innate behaviour modified by social and environmental influences to achieve self-preservation.

1.1.1. Nurtured aggression, bullying, and the "social popularity" factor

In many instances, a child's home environment, including family, friends, and exposure, encourages aggression to achieve self-assertiveness and dominance. The social learning theory proposes that children exposed to domestic interpersonal aggressive altercations are likely to become aggressive adults (Bandura, 1977). Direct evidence of the home environment influence on aggression development in children came from studies that showed that male children who witnessed physical marital abuse directed towards their mothers are three times more prone to physically abuse their partners as adults (Johnson, 1996; Abrahams & Jewkes, 2005). On the other hand, female children who witnessed marital abuse directed towards their mothers tend to have low self-esteem and therefore are more likely to be subjects of abuse in their adult relationships (Silvern et al., 1995; Dauvergne et al., 2001). Aggression is also influenced by different cultural and social norms, especially in less developed countries, that encourage corporal punishment as a time-tested parenting method to modify any unwanted behaviour in children (Silvern et al., 1995). Ironically, it is generally believed that childhood exposure to violence could in itself help create social norms that effectuate violence as a potent negative reinforcement tool (Lilleston et al., 2017). However, it is also believed that targeted interventions of the social norms that perpetrate children-directed violence could help reduce adulthood aggression (WHO, 2009)

An incredibly prevalent example of nurtured aggression is peer victimisation among young adolescents or what is more commonly known as bullying. Peer victimisation comprises any premeditated peer-based aggressive interaction involving a power differential (Smith et al., 1999). Peer victimisation may either be direct, encompassing verbal or physical abuse, or indirect, encompassing psychological torment and intentional damage of the victim's character (van der Wal et al., 2003). It has been estimated that up to 38% of all adult Canadian males and up to 30% of adult Canadian females have been exposed to isolated or frequent incidents of peer victimisation at some point in their school years (Kim & Leventhal, 2008). In agreement with this study, one in three Canadian adolescent students reported being bullied in school between the years 1993/94 to 2005/06 (Molcho et al., 2009). Peer pressure plays a significant role in effectuating bullying behaviour as children who have violent friends tend to be violent and engage in bullying as an attempt to fit in a friendship group (Espelage et al., 2003) and to achieve a desired sense of affiliation (Brown et al., 1986). A disconcerting association exists between social prominence in young adolescents, those perceived as being "cool and popular", and peer victimisation (Adler &

Adler, 1998; LaFontana & Cillessen, 2002). In an effort to seek and maintain dominance or popularity, young adolescents in middle schools were found to use a somewhat effective combination of physical aggression and psychological intimidation (Juvonen et al., 2013). It is even more disconcerting to see this pattern of social aggression being accepted as a "rite of passage" and indirectly reinforced in popular culture and media (Martins & Wilson, 2012).

In conclusion, peer victimisation represents a prevalent social problem with intersecting familial, cultural, social, and environmental influences impacting the normal emotional and social development of young adolescents and community well-being at large.

1.1.2. A hefty cost of aggression

More than 1.6 million people worldwide lose their lives to interpersonal or collective acts of violence every year, with the number of injured individuals in aggressive altercations reaching as many as 40 times the number of reported deaths (Krug et al., 2002). A recent estimation of the economic impact of aggression reported the global cost of interpersonal and collective aggression at 14.8 trillion USD, accounting for 12.4% of the global gross domestic product (GDP) in 2017 (Iqbal et al., 2019). In Canada, the economic cost of intimate partner violence alone amounted to an astonishing 7.4 billion dollars in 2009 (Zhang, 2013). In 2019, there were up to 400,000 police-reported severe incidents of violence in Canada (Conroy, 2021).

1.1.3. Aggression during the coronavirus pandemic disease 2019 (Covid-19)

While it is still early days to gauge the effect of Covid-19 on aggression accurately, a body of research has recently emerged suggesting that Covid-19 increased aggressive behaviour on a global scale. Due to Covid-19 imposed restrictions, many people felt deprived of personal freedom and a persistent traumatic situation further complicated by undeniable economic disruption. The Covid-19 restrictions and the associated social and economic limitations represent a fertile environment for aggression. Not surprisingly, intimate partner violence has seen a global increase of 20% during periods of mandatory lockdown (Mahase, 2020). Moreover, the number of women seeking psychological support or sheltering from intimate partner violence increased by 60% on an annual basis in Europe (Mahase, 2020). In Canada, the number of calls received by domestic violence support lines surged in many provinces during the first few months into the pandemic (Bradley et al., 2020). Also, police-reported incidents of intimate partner violence increased exponentially in many countries, including Canada (Telles et al., 2020).

In summary, aggression represents a grave problem with social, cultural, and environmental attributes. The hefty interpersonal and economic cost of aggression reflects a pressing need to empirically investigate the instigating factors and the physiological mechanisms contributing to aggression development.

1.2. Neuropsychiatry of aggression

Uncontrolled aggressive behaviour is inextricably linked to many psychiatric disorders. While out of the patients' control, pathological aggression is a highly stigmatised disease presentation often associated with discriminatory consequences against afflicted individuals and their families. Pathological aggression also poses persistent physical and emotional challenges for mental health workers. Therefore, understanding the underlying neurological or psychological disorders instigating aggressive behaviour is necessary to reduce, if not fully treat, aggression.

1.2.1. Intermittent explosive disorder (IED)

Intermittent explosive disorder (IED) involves discrete and episodic acute bouts of aggressive behaviour resulting from failure to resist aggressive impulses, causing the afflicted individual to engage in serious acts of physical violence (American Psychiatric Association, 2013). IED is diagnosed after a careful examination of the nature of aggression and exclusion of other psychiatric or medical conditions with a similar presentation of acute aggression like substance abuse or head trauma. IED is treated using a psychopharmacological approach involving a combination of cognitive behavioural therapy and drugs like selective serotonin reuptake inhibitors (SSRIs) and mood stabilisers (McCloskey et al., 2012).

1.2.2. Schizophrenia

Schizophrenia is a severe mental illness involving a distorted perception of reality typically associated with psychotic hallucinations, delusions, disorganised speech, and disturbed emotions. Schizophrenia is a multifactorial disease with an early onset and a chronic course that seems to be precipitated by genetic disposition and environmental influences (Moran et al., 2016). Patients who have paranoid schizophrenia display aggressive behaviour of heterogenous nature, meaning that it could simply be an impulsive act out of the patient's control or could also be a calculated response to manipulate and intimidate others (Volavka & Citrome, 2011).

1.2.3. Alcoholism and substance abuse

Alcohol abuse and dependence are more closely associated with aggressive behaviour than any other psychotropic agent (*Who expert committee on problems related to alcohol consumption.*, 2007). Alcohol abuse has been shown to significantly influence sexual aggression (Testa, 2002) and other violent crimes (Darke, 2010). A recent study showed a causal link between alcohol abuse and disruptive behaviours such as vandalism and fighting in young adults (Evans et al., 2021). Additionally, males with chronic alcohol dependency are more likely to engage in intimate partner violence and sexual aggression compared with males who are not alcohol dependent (O'Farrell et al., 2003). Studies have also shown that alcohol-dependent individuals are more likely to engage in deliberate self-harm and ponder suicidal thoughts (Riedi et al., 2012; Darvishi et al., 2015). It is believed that alcohol-related aggression results from alcohol-induced impairments of the inhibitory centres in the brain (Gan et al., 2014), followed by subsequent exposure to psychosocial or environmental instigating influences that trigger aggression (Heinz et al., 2011). In addition to alcohol, other psychotropic substances have also been linked to aggression. A recently published meta-analysis of 18 studies conducted between the years 1990 and 2019 concluded that individuals with diagnosed single or polydrug substance use disorder involving opioids, cannabis, stimulants, or hallucinogens have a higher risk of committing violent crimes or engaging in aggressive behaviours compared with the general population (Zhong et al., 2020).

1.3. Neuroanatomy of aggression

Aggressive behaviour emanates in the brain through coordination between the subcortical hypothalamic and limbic structures and the prefrontal centres where emotions are born and matured (Cases et al., 1995; Siever, 2008). Aggression is believed to be under the control of the prefrontal cortex, which exerts inhibitory control over the subcortical structures, including the hypothalamus and amygdala. Aberrations in the inhibitory tone of the prefrontal cortex result in disinhibition of subcortical structures and unhinged expression of aggression (Siegel & Victoroff, 2009; Siegel & Douard, 2011).

1.3.1. Hypothalamic circuits involved in aggression

External stimuli (e.g., environmental and social influences) and internal homeostatic signals converge at the hypothalamus, making it an ideal regulatory centre for various physiologic and behavioural functions (Kruk et al., 1998). The hypothalamus role in aggression is well documented, dating back as far as the 1920s when W. R. Hess found that electrical stimulations of a specific region in the hypothalamus evoked aggressive responses in cats (Hess, 1928). Following Hess's discovery, subsequent studies (Kruk et al., 1979; Kruk et al., 1983; Lammers et al., 1988) were able to precisely map the area of the hypothalamus responsible for aggression which is often called the "hypothalamic attack area" and comprises the intermediate hypothalamic area and the ventrolateral subdivision of the hypothalamic ventromedial nucleus (VMNvl).

VMNvl plays an integral role in initiating and expressing aggressive behaviour. Optogenetic stimulation of VMNvl neurons elicited rapid and indiscriminate aggressive attacks against intruders, including inanimate objects in the resident-intruder assay, while inhibition of the VMNvl neurons suppressed aggression (Lin et al., 2011). Furthermore, a recent study showed increased activation of the VMNvl neurons during aggression-seeking behaviour, demonstrating a complex role of the VMNvl in aggression not only during the acute phase of the attack but also during the aggression-seeking phase preceding the attack (Falkner et al., 2016).

1.3.2. Limbic structures involved in aggression

1.3.2.1. Amygdala

The basolateral amygdala (BLA) serves as the primary locus of sensory input to the amygdala, whereas the centromedial amygdala (CeA) is the main output component to other brain regions. The amygdala plays a significant role in processing emotions and analysing fearful or threatening stimuli allowing for a maximum adaptation to social and environmental influences (Baxter & Croxson, 2012). Interestingly, neuroimaging studies have found a significant negative correlation between amygdalar volume and aggression in females, where healthy non-clinical subjects with reduced amygdalar volume displayed higher levels of trait aggression (Matthies et al., 2012). A similar negative correlation was found in men with a previous history of aggression, where adult subjects with reduced amygdalar volume showed higher levels of violent behaviour and increased psychopathic features at an early age (Pardini et al., 2014).

The medial nucleus of the amygdala (MeA) is part of the CeA that plays a significant role in aggression and other innate social behaviours (Newman, 1999). The MeA consists of a

heterogeneous mixture of glutamatergic and GABAergic neurons, and both neuronal populations were found to play a direct role in controlling aggressive behaviour. Indeed, optogenetic activation of the MeA GABAergic neurons evoked intense aggressive attacks against male and female intruders, whereas optogenetic activation of the neighbouring MeA glutamatergic neurons inhibited aggressive attacks in the resident-intruder assay (Hong et al., 2014).

1.3.2.2. Prefrontal cortex (PFC)

The prefrontal cortex (PFC) has long been known to play an inhibitory role in aggression across many species (Kolb & Nonneman, 1974; Siegel et al., 1977). A meta-analysis of 43 neuroimaging studies showed the aggressive antisocial behaviour seen in patients afflicted with many neuropsychiatric disorders to be associated with structural and functional deficits in the PFC (Yang & Raine, 2009). Furthermore, optogenetic activation of glutamatergic neurons in the medial PFC (mPFC) suppressed inter-male aggressive behaviour in mice, while inhibition of these excitatory neurons was sufficient to elicit aggressive behaviour (Takahashi et al., 2014). Moreover, the PFC sends glutamatergic projections to other aggression-linked brain structures, including the amygdala and hypothalamus (Vertes, 2004; Gabbott et al., 2005). Interestingly, stimulation of glutamatergic neurons in the PFC was found to increase the activity of GABAergic interneurons in the BLA, leading to a reduction in the activity of BLA output neurons (Rosenkranz & Grace, 2002).

1.3.2.3. Hippocampus

The hippocampus supports a wide range of behavioural functions. While the dorsal aspect of the hippocampus is primarily responsible for spatial memory, the ventral hippocampus is mainly involved in the regulation of emotions and defensive behaviour (Maggio & Segal, 2012). The ventral hippocampus is functionally connected with many aggression-linked brain regions, including the MeA, PFC, and VMN (Sahay & Hen, 2007). A recent study showed that activation of VMNvl-projecting hippocampal neurons elicited stress-provoked impulsive attacks in a post-weaning mouse model of social isolation (Chang & Gean, 2019). Interestingly, repeated inter-male fighting incidents in which there were positive outcomes increased hippocampal neurogenesis and hippocampal activity in the winner mice (Smagin et al., 2015).

1.3.2.4. Lateral septum (LS)

The lateral septum (LS) is a relay centre interconnected with many aggression-linked brain regions, including the hypothalamus, hippocampus, and MeA (Risold & Swanson, 1997). The LS has been implicated in aggression as human patients with lesions involving the LS suffer from increased aggressiveness and irritability (Zeman & King, 1958). The LS is primarily composed of interconnected GABAergic neurons, and it has been shown that activation of the $GABA_A$ receptors in the LS neurons increased aggressive behaviour in hamsters (McDonald et al., 2012). Like other aggression-linked structures, LS neurons have been shown to project to the VMNvl (Risold & Swanson, 1997). Interestingly, optogenetic activation of the GABAergic VMNvl-projecting LS neurons reduced aggressiveness in mice (Wong et al., 2016). A recent study showed that hippocampal CA2 pyramidal neurons project to the LS, and optogenetic activation of the LS-projecting CA2 neurons was sufficient to elicit aggressive attacks in mice (Leroy et al., 2018). Intriguingly, the same study showed that activation of the LS-projecting CA2 neurons promoted aggression via inhibition of the aforementioned VMNvl-projecting LS neurons which is known to suppress aggression in mice. Collectively, the LS plays a vital gatekeeping role in controlling aggression upstream of the VMNvl.

1.4. Neurochemistry of aggression

The above listed corticolimbic neuronal circuits work in concert with central and peripheral neuromodulators to regulate aggressive behaviour. At a brain level, neurotransmitters including serotonin (5-HT), dopamine, and GABA have been shown to control aggression (Faccidomo et al., 2006). At a peripheral level, hormones including androgens and glucocorticoids have long been implicated in physical aggression (Ramirez, 2003).

1.4.1. Neurotransmitters

Serotonin (5-hydroxytryptamine [5-HT]) is a monoamine neurotransmitter that plays an extremely complex receptor-specific role in the modulation of inter-male aggression. High 5-HT levels are positively associated with adaptive aggressive behaviour in mice, which is meant to establish territorial or social dominance (Kulikov et al., 2012). In contrast, pathological forms of aggression are thought to be inversely associated with 5-HT activity (De Boer et al., 2009). Generally speaking, low 5-HT levels are associated with increased aggression. Moreover, pharmacological manipulations that lower serotonin signalling also cause increased aggression

(Chiavegatto et al., 2001). On the other hand, elevated 5-HT levels or increased 5-HT activity have been shown to suppress aggression in rodents (Miczek et al., 2001; Nelson & Trainor, 2007).

Dopamine plays a significant role in decision making and reward; therefore, it has been postulated to contribute to aggression (Schultz, 2002). Indeed, studies in mice and rats showed an association between dopaminergic signalling and aggressive behaviour/interactions (Couppis & Kennedy, 2008; Anstrom et al., 2009; Montoya et al., 2012). A relatively recent study showed a negative correlation between dopamine synthesis and storage in the midbrain and the frequency of aggressive reactions in response to social and environmental provocation in healthy male subjects (Schlüter et al., 2013). These data, although limited, suggest that dopamine availability may be protective against aggressive responses to environmental provocations.

GABA is a highly conserved inhibitory neurotransmitter found in many life forms, including plants, fungi, and animals. GABA functions in the brain through binding to three receptors, $GABA_A$, $GABA_B$, and $GABA_C$, among which $GABA_A$ is the most abundant (Sankar, 2012). Previous reports linking GABA to aggressive behaviour mostly showed an inhibitory effect (Miczek et al., 2003). Allosteric modulations of the $GABA_A$ receptor using alcohol, barbiturates, or benzodiazepines have been associated with increased aggressiveness in a dose-dependent manner (de Almeida et al., 2005). Moreover, activation of $GABA_A$ receptors in the dorsal raphe nucleus has been linked to alcohol-heightened aggression (Takahashi et al., 2010).

1.4.2. Hormones

Androgens, most prominently testosterone, have long been associated with male aggression. It has been postulated that testosterone-mediated aggression increase during puberty in response to sexual arousal and social competition (Archer, 2006). Studies in non-human animals showed a strong positive correlation between circulating testosterone levels and aggressive behaviour (Rose et al., 1971; Rada et al., 1976; vom Saal, 1983). In humans, however, the relationship is more discrepant. While a positive correlation was reported in many studies, some studies still showed a negative correlation or no relation between testosterone levels and aggression (Archer, 1991; Turner, 1994; Book et al., 2001). Such discrepancies might be attributed to experimental design differences that did not consider the circadian nature of testosterone secretion or the influence of the immediate environmental and social cues at the time of measurement.

Disruptive behaviours in adolescent males highlighted a positive correlation between low cortisol levels and enhanced aggression (Dabbs et al., 1991; McBurnett et al., 2000). Intriguingly, low serum cortisol was found to be causal in mediating the effect of serum testosterone on aggressive behaviour (Popma et al., 2007). Therefore, it has been suggested that the testosterone to cortisol ratio might be an effective serological marker of male propensity for aggression (Terburg et al., 2009; van Honk et al., 2010).

Given the state of physical and emotional arousal achieved in anticipation of and following aggressive encounters, noradrenaline is generally believed to play a role in aggression. In support of this, post-synaptic β-receptors blockers (e.g., metoprolol and propranolol) have been used effectively to calm and suppress pathological aggression in psychiatric patients (Elliott, 1977; Yudofsky et al., 1981). Moreover, escalated aggression has been associated with a disrupted enzymatic activity of catechol-O-methyltransferase (COMT), an enzyme responsible for catecholamines degradation (Gogos et al., 1998).

The abovementioned neuromodulators (neurotransmitters and hormones) can either act alone or interdependently to control aggression. For instance, corticosterone and testosterone have been shown to increase serotonin turnover in the hippocampus and MeA (Summers et al., 2000). Moreover, glucocorticoids, including cortisol, have been shown to alter serotonergic transmission (Pompili et al., 2010). It is justifiably expected that understanding the functional interactions between different neuromodulators and the associated anatomical significance would undoubtedly contribute to pharmacological treatment of aggression.

1.4.3. BDNF role in aggression

BDNF has been shown to play a role in the modulation of aggressive behaviour. However, the role of BDNF in aggression is poorly understood due to a scarce and contradictory nature of previous reports. Studies in rodents showed that heterozygous *Bdnf* $^{+/-}$ mice have significantly higher dispositions for inter-male aggression (Lyons et al., 1999). Moreover, brain-specific deletion of *Bdnf* was shown to increase anxiety and aggressive behaviour in mice (Chan et al., 2006; Ito et al., 2011; Ernst et al., 2012). Interestingly, a recent study showed that ablation of BDNF/TrkB signalling in corticolimbic GABAergic interneurons increased inter-male aggression to establish territorial dominance (Tan et al., 2018). In agreement with studies in rodents, clinical studies in humans showed that a *BDNF* single-nucleotide polymorphism causing valine-to-methionine substitution (Val66Met) increased aggressiveness (Spalletta et al., 2010) and violent

behaviour in response to environmental provocations (Kretschmer et al., 2014; Musci et al., 2014). Collectively, these studies indicate that BDNF attenuates aggressive behaviour.

In contrast, it has also been reported that central administration of BDNF elicited aggressive asocial behaviour in mice (Naumenko et al., 2014). Additionally, a significantly higher concentration of BDNF protein was reported in the striatum, hippocampus, and frontal cortex of the highly aggressive AB-Halle mice compared with the genetically related but nonaggressive AB-Gatersleben mice (Lang et al., 2009). Moreover, *Bdnf* mRNA levels were found to be elevated in the frontal cortex, hypothalamus, and amygdala of a rat model of severe fear-induced aggression relative to nonaggressive rats (Ilchibaeva et al., 2018). The same study showed an increase in mBDNF protein levels in the hippocampus, striatum, and amygdala of aggressive rats relative to the nonaggressive rat control. Collectively, these studies indicate that BDNF is an inducer of aggressive behaviour.

1.5. Cage environment and aggressive behaviour in laboratory mice

Mice are a highly social species with a complex social system structured around territorial dominance. Wild mice generally live in colonies; each colony inhabits a self-identified territory and represents a family unit, typically composed of a dominant adult male, several females, and their offspring (Hurst et al., 1993). Such social organisation is maintained via a highly stringent dominance hierarchy imposed by the dominant male over every other male in the colony, ensuring the elimination of within-colony competition over resources however limited these resources might be (Anholt & Mackay, 2012). As could be expected, wild male mice depend on physical aggression to defend their territorial and social dominance (Bisazza, 1981).

Laboratory mice are often kept in tiny enclosures supplied with essential tools to sustain their lives and ensure maximum animal experimentations' reproducibility. Despite the availability of within-cage resources, laboratory mice still seek a hierarchical social structure and often use aggression to establish within-cage dominance (Williamson et al., 2016). Depriving laboratory mice of a social structure is often associated with heightened anxiety, increased aggressiveness, and display of captivity-associated stereotypies (Würbel, 2001). Therefore, social housing is necessary for the general welfare of laboratory rodents. Indeed, male mice in a laboratory setting have been shown to choose the company of other males, even an aggressive male, over individual housing (Van Loo, Van Zutphen, et al., 2003).

However, group housing male mice is often hard to accomplish in a laboratory environment without substantial incidents of inter-male fighting. Intra-cage aggression is inevitable as the confines of the small cage make it hard, if not impossible, to avoid inter-male confrontation to establish territorial dominance. Furthermore, the persistent need to defend territorial dominance in the challenging cage environment often leads to a persistent and unavoidable display of aggression from the dominant male against his subordinate cage mates who can neither escape the confines of the small cage nor overpower the dominant male (Gaskill et al., 2017). Importantly, the pain and physical injuries associated with inter-male fighting pose a constant source of experimental variability, jeopardising the validity of scientific data (Sherwin, 2004).

Multiple factors are thought to contribute to the inter-male aggression phenotype associated with social housing in the laboratory environment. These factors are related to cage size and group size (Van Loo et al., 2001), mouse strain (Van Loo, Van der Meer, et al., 2003), presence of cage enrichment (Lockworth et al., 2015), weaning age (Gaskill et al., 2017), and frequency of mice handling by human carers (Jirkof et al., 2020). However, attempts to alter the impacts of the above-mentioned factors have been made with conflicting results. For example, while some researchers claim that weaning mice into experimental groups before puberty reduced inter-male aggression (Annas et al., 2013), other researchers reported the exact opposite (Bartolomucci et al., 2004). Similarly, while some researchers showed that keeping mice in small groups (i.e., three mice/group) reduced aggression (Van Loo et al., 2001), other researchers showed that small groups were associated with increased aggressiveness (Poole & Morgan, 1973). Based on these conflicting reports, it is highly plausible that simultaneous modification of multiple factors, rather than a single factor, could be more beneficial in reducing inter-male aggression.

1.6. Environmental enrichment (EE) and inter-male aggression

Conflicting data have been reported regarding the effect of EE on aggressive behaviour. Multiple studies have shown positive effects of EE on reducing inter-male aggressive behaviour in laboratory mice (Vestal & Schnell, 1986; Chamove, 1989; Armstrong et al., 1998; Ambrose & Morton, 2000; Van Loo et al., 2002; Pietropaolo et al., 2004). On the other hand, multiple studies showed that EE increased aggression in mice (Henderson, 1976; Haemisch & Gärtner, 1994; Bergmann et al., 1995; Barnard et al., 1996; Haemisch & Gärtner, 1997; Marashi et al., 2003b; Würbel & Garner, 2007; McQuaid et al., 2018; Mertens et al., 2020). Such variability in reporting the effect of EE on aggression could be attributed to differences in experimental design including,

mouse strain and age, group size, colour and type of enrichment devices used, duration of the enrichment protocol, and the criteria used for assessing aggressive behaviour. For instance, a study showed that incorporating nesting materials increased inter-male aggression, whereas the incorporation of shelters was sufficient to reduce inter-male fighting in NIH/S mice (Kaliste et al., 2006). In contrast, another study showed that the incorporation of shelters increased aggression in BALB/c mice (Van Loo et al., 2002). While contradictory, the abovementioned data reflect a need to study the strain-dependent effect of different enrichment items on inter-male aggression. It similarly reflects a need for a standardised enrichment protocol. Identification of a strain-specific EE protocol that maximises the expression of species-typical behaviours while minimising inter-male aggression would likely create better animal research models and undoubtedly increase the external validity of animal studies.

2. Rationale

Aggression is a ubiquitous multifaceted social behaviour with innumerous psychological, social, and economic consequences. Aggression is among the leading causes of death in individuals aged 15 to 44 years, with an estimated 1.6 million people losing their lives to violence annually (Krug et al., 2002). Furthermore, uncontrolled aggressive behaviour is often considered a manifestation of many serious mental disorders, representing a persistent public health concern and a taxing clinical challenge for mental health professionals (Rueve & Welton, 2008). Understanding the physiological and environmental attributes of aggression could uncover new therapeutic tools to control violence and improve the quality of life for afflicted individuals.

Studies in humans have shown that the Val66Met mutation in the *BDNF* gene is associated with diminished activity-dependent BDNF secretion and increased aggressive tendencies (Egan et al., 2003; Spalletta et al., 2010). In agreement with these findings in humans, studies in rodents have shown that genetic mutations in the *Bdnf* gene, the mouse homologue of human *BDNF,* were associated with hyperactivity and hyperaggressive behaviour (Lyons et al., 1999; Chan et al., 2006). Interestingly, targeted deletion of *Bdnf* in the cortex or the hippocampus precipitated aggressive behaviour in mice while deleting *Bdnf* in other areas did not modify aggression (Gorski et al., 2003; Unger et al., 2007; Ito et al., 2011). A unique feature in the mouse *Bdnf* gene is the presence of nine individual promoters driving the transcription of multiple *Bdnf* variants, all of which encode an identical BDNF protein. It has been shown that transgenic mice with disrupted *Bdnf* production from promoters *I* or *II* were highly aggressive, while loss of *Bdnf* production from

promoters *IV* or *VI* did not influence aggression (Maynard et al., 2016; Hobbs et al., 2017). Collectively, these findings show that BDNF modulates aggression in a transcript-variant-specific and brain region-specific manner.

Social and environmental factors influence behaviour. Environmental enrichment (EE) is an animal housing protocol in which mice interact with complex social, sensory, and motor stimulations designed to help them partly experience and express the behavioural repertoire of their wild counterparts in a controlled and reproducible setting. EE has been shown to promote animals welfare and improve neurogenesis, metabolism, and immunity in rodents via improved production and signalling of BDNF in the brain (Vitalo et al., 2009; Beauquis et al., 2010; Cao et al., 2010; Cao et al., 2011; Foglesong et al., 2019; Kempermann, 2019; Takai et al., 2019; Queen et al., 2020). Importantly, EE has been reported to reduce inter-male aggression in mice (Chamove, 1989; Armstrong et al., 1998; Ambrose & Morton, 2000; Van Loo et al., 2002; Pietropaolo et al., 2004). However, the exact mechanism modulating the EE-induced reduction in aggression is yet to be elucidated. Previous studies showed that EE alters *Bdnf* mRNA expression in the mouse brain, including some brain structures implicated in aggression, such as the hypothalamus, the hippocampus, and the PFC (Wolf et al., 2006; Cao et al., 2010; Chourbaji et al., 2012; Jurgens & Johnson, 2012; Foglesong et al., 2016; McQuaid et al., 2018). However, the BDNF role in mediating the EE-induced reduction in aggression, albeit plausible, has not been examined.

The EE paradigm represents a unique approach to studying the influence of social and environmental factors on the expression of aggression and generally outlines the BDNF role in social aggression. Overall, it is reasonable to address the hypothesis that EE suppresses aggression by modulating the activity of aggression-related corticolimbic circuitry that involves BDNF. It is similarly reasonable to hypothesise that the EE-induced reduction in inter-male aggression is associated with altered *Bdnf* gene expression in a transcript variant-specific and brain region-specific manner. It is expected that the outcome of this study will provide valuable data on the brain circuitry involved in mediating the effect of the housing environment on aggression modulation and the *Bdnf* transcripts involved, putting us a step closer to creating effective therapeutic modalities that target the root causes of aggression.

3. Hypotheses

a) EE suppresses inter-male aggression by modulating the activity of aggression-related corticolimbic circuitry.

b) The EE-induced reduction in inter-male aggression is associated with the *Bdnf* gene expression in a transcript variant-specific and brain region-specific manner.

4. Objectives

a) To determine the effect of EE on the incidence and frequency of inter-male aggression in group-housed male C57BL/6 mice.

b) To determine the corticolimbic activity pattern associated with the effect of EE on inter-male aggression.

c) To study the transcript-variant-specific and brain region-specific expression of *Bdnf* in response to EE.

5. Experimental design

The same animal strain (male C57BL/6), EE and RNA analysis protocols from part (I) were employed in part (II). Kindly refer to the experimental design section on page 28 for the detailed design.

Assessment of aggressive behaviour

Aggressive interactions were evaluated by direct visual observations for one hour during the light phase (1400-1500 h) five days a week throughout the experiment. Biting, chasing, and fighting were considered aggressive behaviours. Fighting was characterised by any combination of wrestling, kicking, mounting, and rolling (Fig. 14) (Giles et al., 2018). The number of aggressive mice was counted and compared between the two types of housing conditions. The days with inter-male fighting were recorded for each cage. The overall frequency of aggressive interaction was calculated for each cage as follows, frequency of fighting (%) = (number of days with aggressive interaction)/(total number of days during the experimental period) x 100. The visual assessment of aggression could not be performed in a blinded fashion due to the apparent difference in cage setup between the groups.

Corticosterone assay

Animals were euthanised at the end of the experiment, as described in the experimental design section of part (I). Trunk blood was collected, and serum was separated and stored at -80 °C. Corticosterone levels were assessed using a commercial ELISA kit (ADI-900-097, Enzo Life Sciences, Farmingdale, NY), and final concentrations were measured using a microplate reader (iMark™ Microplate Absorbance Reader, Bio-Rad Laboratories, Hercules, CA)

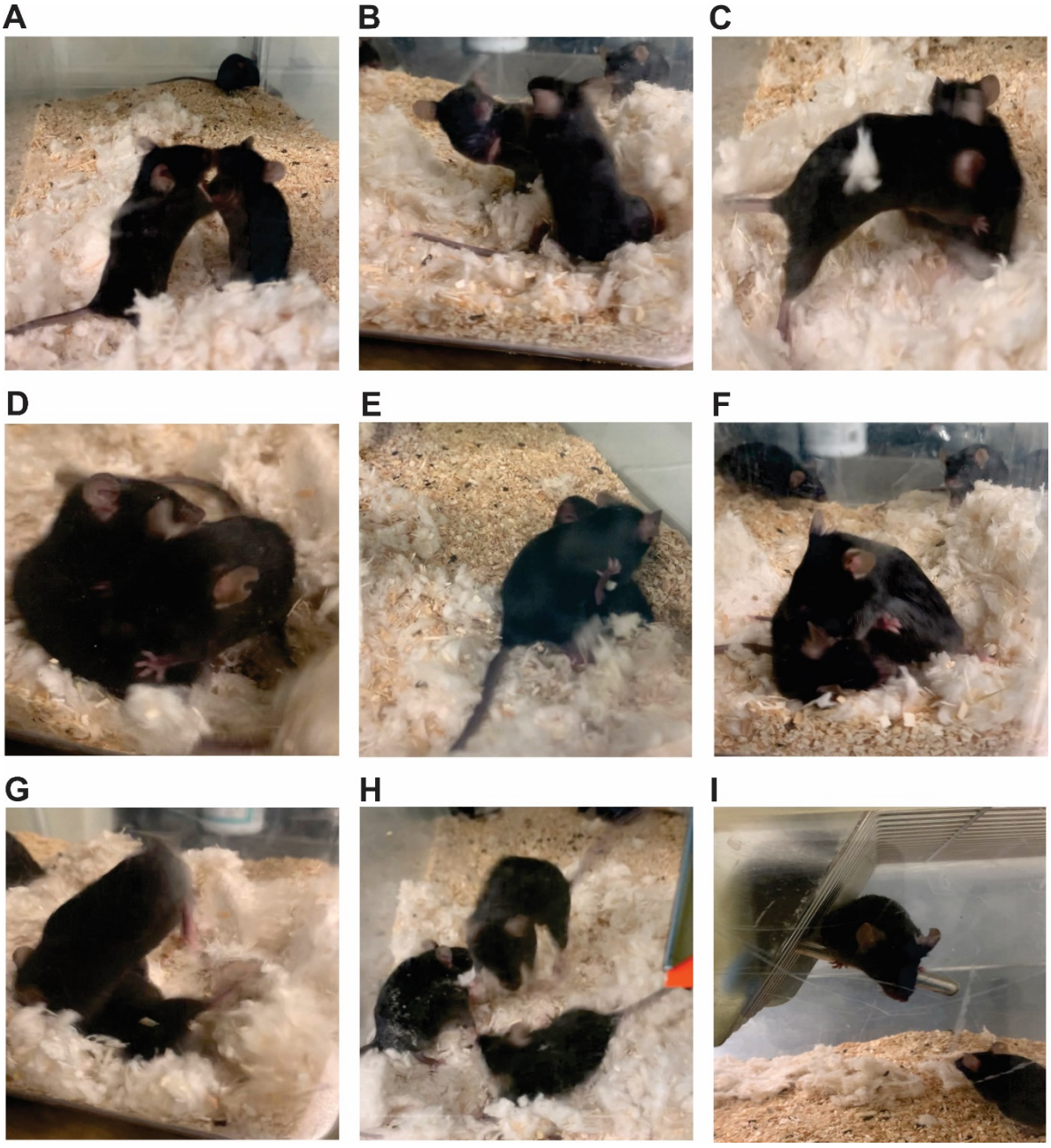


Figure 14. Assessment of aggressive behaviour in mice. Inter-male aggressive interactions were evaluated by direct visual observations for one hour during the light phase (1400-1500 h) five days a week throughout the experiment. Pictures in the figure represent; “upright posture” confrontation associated with attack initiation (A and B), ano-genital sniffing and “lateral threat” attacks (C and D), “keep down” and “clinch attacks” (E and F), severe attacks involving aerial acrobatics (G and H), and evasion tactic to avoid aggressive attacks (I).

Tissue dissection

Mice were euthanised by exposing them to isoflurane, followed by decapitation at the end of the experiment. To minimise individual variations in the influence of nutritional state and stress on gene expression, mice were fasted for 8-hr during the light phase and received an intraperitoneal injection of saline 30 minutes before euthanasia. Brains were quickly removed and cut into three coronal blocks (i.e., anterior, middle, and posterior blocks) at -0.35 mm and -2.6 mm to Bregma using a brain matrix. Specific brain regions were identified and dissected according to the mouse brain atlas by Paxinos and Franklin (Paxinos & Franklin, 2001). The PFC was removed from the anterior brain block by inserting a razor blade at a 60° angle from both sides to the midline 2.0 mm deep from the top of the brain. Our dissection protocol collected parts of the prelimbic cortex (PrL), medial orbital cortex (MO), infralimbic cortex (IL), cingulate cortex area 1 and 2 (Cg1 and Cg2), as well as a part of the frontal association cortex (FrA) and secondary motor cortex (M2). The parietal cortex was removed from the middle brain block and contained medial parietal association cortex (MPtA), lateral parietal association cortex (LPtA), mediomedial secondary visual cortex (V2MM), mediolateral secondary visual cortex (V2ML) as well as a part of primary and motor cortex (M1), M2, retrosplenial granular cortex (RSG) and retrosplenial agranular cortex (RSA). The hypothalamus, hippocampus, amygdala, and parietal cortex were collected as described in the experimental design section of part (I).

Statistical analysis

Data are presented as means ± S.E.M. Statistical comparison of the incidence of aggressive behaviour (number of mice with aggressive behaviour) between housing environments was performed by chi-squared test. For other variables, comparisons between the two housing environments were performed by Student's *t*-test in parametric data and by Wilcoxon test when the data were not normally distributed. In all cases, differences were taken to be significant if *P*-values were below 0.05.

6. Results

6.1. Effect of cage environment on inter-male aggressive behaviour in group-housed mice

Intra-cage aggressive interactions were identified directly through timed (1 h) and repeated (5 times per week) visual observation during the light phase throughout the study (Fig. 14). Inter-male aggressive interactions were found to be significantly lower in mice housed under EE (17.1%, 6 out of 35 mice) compared with mice housed under standard environmental conditions (51.4%, 18 out of 35 mice, $P < 0.005$ by the chi-squared test of independence, Fig. 15A and B). The frequency of aggressive interactions was significantly lower in the SE mice compared with the EE mice (5.9 ± 1.4% vs 31.7 ± 6.8%, $P < 0.005$ by Wilcoxon test, Fig. 15A and C). Latency to the first aggressive interaction was not significantly different between the two housing environments (SE [n = 7 cages]: 11.3 ± 4.9 days vs. EE [n = 7 cages]: 22.1 ± 4.9 days, $P = 0.15$ by Student's *t*-test, Fig. 3D). However, the first attack in the SE cages was observed in the first two weeks of the study except in a single cage (cage 1, Fig 15A), where it was observed in the final week. Upon exclusion of this cage from the statistical analysis, first attack latency was found to be significantly longer in the EE mice (22.1 ± 4.9 days, n = 7 cages) compared with the SE mice (6.7 ± 2.1 days, n = 6 cages, $P < 0.05$ by Student's *t*-test).

6.2. Effect of cage environment on serum corticosterone levels

Serum corticosterone levels were not significantly different between the EE mice (344.8 ± 35.6 ng/ml) and the SE control (330.3 ± 69.6 ng/ml, $P = 0.99$ by Student's *t*-test). Thus, while serum corticosterone levels were elevated in both housing environments, the lack of a statistical inter-group difference suggests that the EE-induced aggression lowering effect is independent of the stress response.

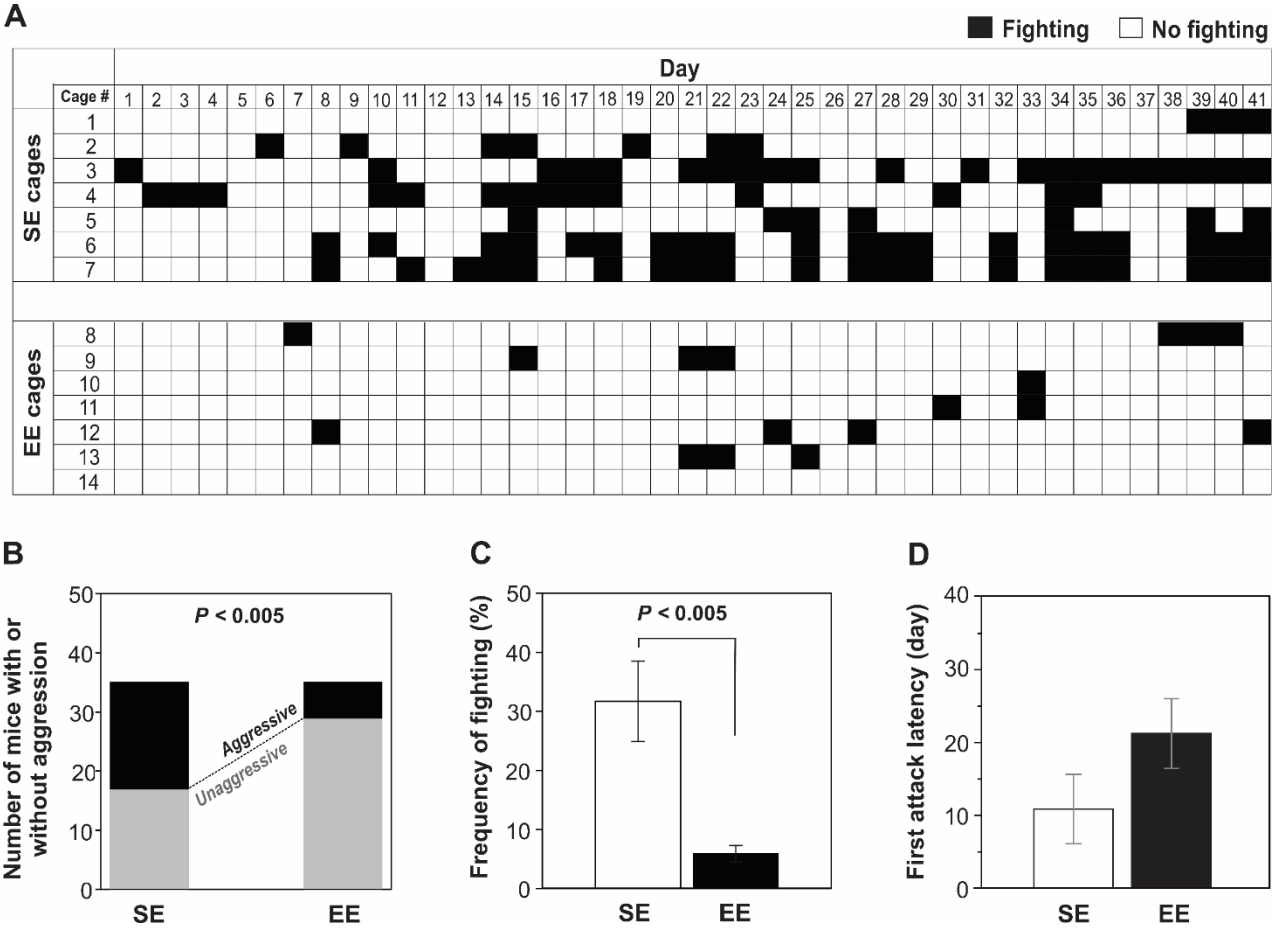


Figure 15. Effect of cage environment on aggressive behaviour in group-housed male mice. (A): Binary heatmap of presence (black) or absence (white) of aggressive interaction within each cage during the 6-week experimental period. (B): Number of mice that displayed aggressive behaviour was reduced by environmental enrichment (EE). (C): Frequency of fighting, [the number of days with fighting]/[the number of experimental days] x 100 (%) was reduced by EE. (D). Latency to the first attack. Data are means ± S.E.M. (n = 7-9/group). *P* values were obtained by chi-squared test (B) or Wilcoxon test (C-D).

6.3. Effect of cage environment on the expression of immediate early genes

Expression levels of the immediate early genes *Fos* and *Junb* were assessed to determine brain activation patterns associated with each housing environment. Levels of *Fos* mRNA were significantly reduced in the PFC (45.2% reduction, $P < 0.05$, Fig. 16A), hypothalamus (50.5% reduction, $P < 0.05$, Fig. 16A), hippocampus (70.7% reduction, $P < 0.005$, Fig. 16A), and parietal cortex (35.9% reduction, $P < 0.05$, Fig. 16A) in EE mice relative to the SE control. *Fos* mRNA levels in the amygdala did not differ significantly between the two housing environments (Fig. 16A). *Junb* mRNA levels were significantly reduced in the hippocampus of EE mice (40.0% reduction, $P < 0.05$, Fig. 16B) relative to the SE control. *Junb* mRNA levels were not significantly different in the other brain regions.

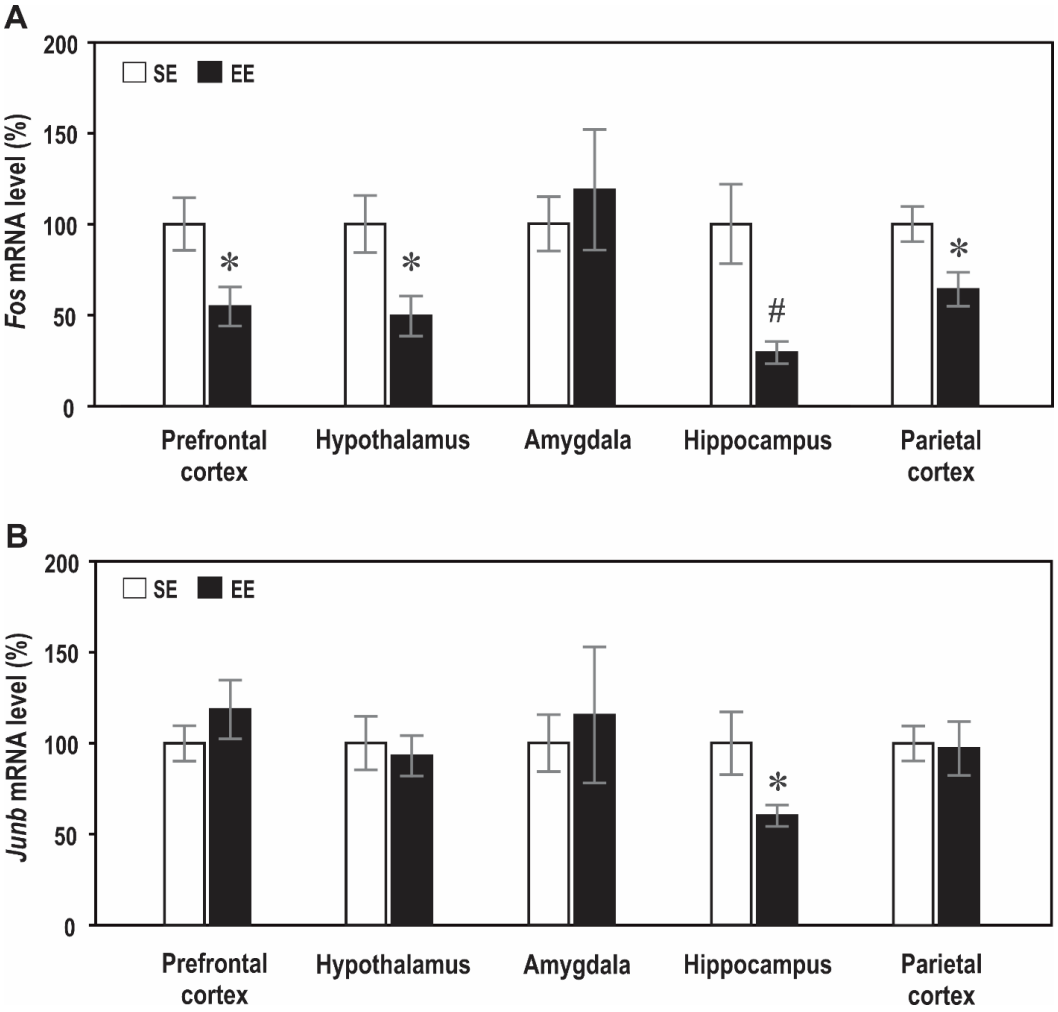


Figure 16. Effect of environmental enrichment on immediate early genes expression in the brain. Levels of *Fos* (A) and *Junb (B)* mRNA in the prefrontal cortex, hypothalamus, amygdala hippocampus, and parietal cortex were measured by real-time PCR. Values in the standard environment (SE) group were set to 100%. Data are means ± S.E.M. (n = 7-9/group). *: $P < 0.05$, #: $P < 0.005$ by Student's *t*-test.

6.4. Effect of cage environment on the expression of *Bdnf* transcripts in the brain

Total *Bdnf* mRNA expression was assessed using a primer pair that can detect all *Bdnf* mRNA variants with either short or long 3' UTR (*Pan-Bdnf*, Table 1). Another set of primers (*L-Bdnf*, Table 1) was also used to detect all long 3' UTR *Bdnf* mRNA variants. The expression levels of *Bdnf* transcripts *I, IIc, IV*, and *VI* were also assessed given their documented role in modulating behaviour (Aid et al., 2007; Pruunsild et al., 2007; Maynard et al., 2016). Total *Bdnf* mRNA levels, as detected by the *Pan-Bdnf* primers, were significantly reduced in the PFC of EE mice compared with the SE control (Fig. 17A). Total *Bdnf* mRNA levels were not significantly changed in the other brain regions examined (Fig. 17B-E). Interestingly, *Bdnf I* mRNA levels were significantly reduced in the PFC (55.8% reduction, $P < 0.05$, Fig. 17A), hypothalamus (25.8% reduction, $P < 0.05$, Fig. 17B), hippocampus (47.1% reduction, $P < 0.05$, Fig. 17D), and parietal cortex (37.5% reduction, $P < 0.05$, Fig. 17E) in the EE mice relative to the SE control. In contrast, *Bdnf I* mRNA levels were significantly increased in the amygdala of the EE mice relative to the SE control (78.7% increase, $P < 0.05$, Fig. 17C). Levels of *Bdnf* transcripts *IIc*, *IV*, and *VI* did not differ significantly between the two housing environments in all brain regions examined (Fig. 17).

6.5. Effect of cage environment on the expression of BFNF receptor genes in the brain

BDNF exerts its diverse biological functions via signalling through either the TrkB or the p75 neurotrophin receptor ($p75^{NTR}$) (Huang & Reichardt, 2001; Chao, 2003). The TrkB protein is encoded by the neurotrophic tyrosine kinase receptor 2 (*Ntrk2*) gene, whereas the $p75^{NTR}$ protein is encoded by the nerve growth factor receptor (*Ngfr*) gene. In this study, *Ntrk2* mRNA levels were significantly reduced in the hypothalamus (40.6% reduction, $P < 0.05$, Fig. 17B) and the hippocampus (50.7% reduction, $P < 0.001$, Fig. 17D) of the EE mice relative to the SE control. *Ngfr* mRNA levels were significantly reduced in the hippocampus (79.0% reduction, $P < 0.01$, Fig. 17D) and the parietal cortex (48.5% reduction, $P < 0.05$, Fig. 17E) of the EE mice relative to the SE control. *Ntrk2* and *Ngfr* mRNA levels did not differ significantly between the two housing environments in the other brain region examined (Fig. 17).

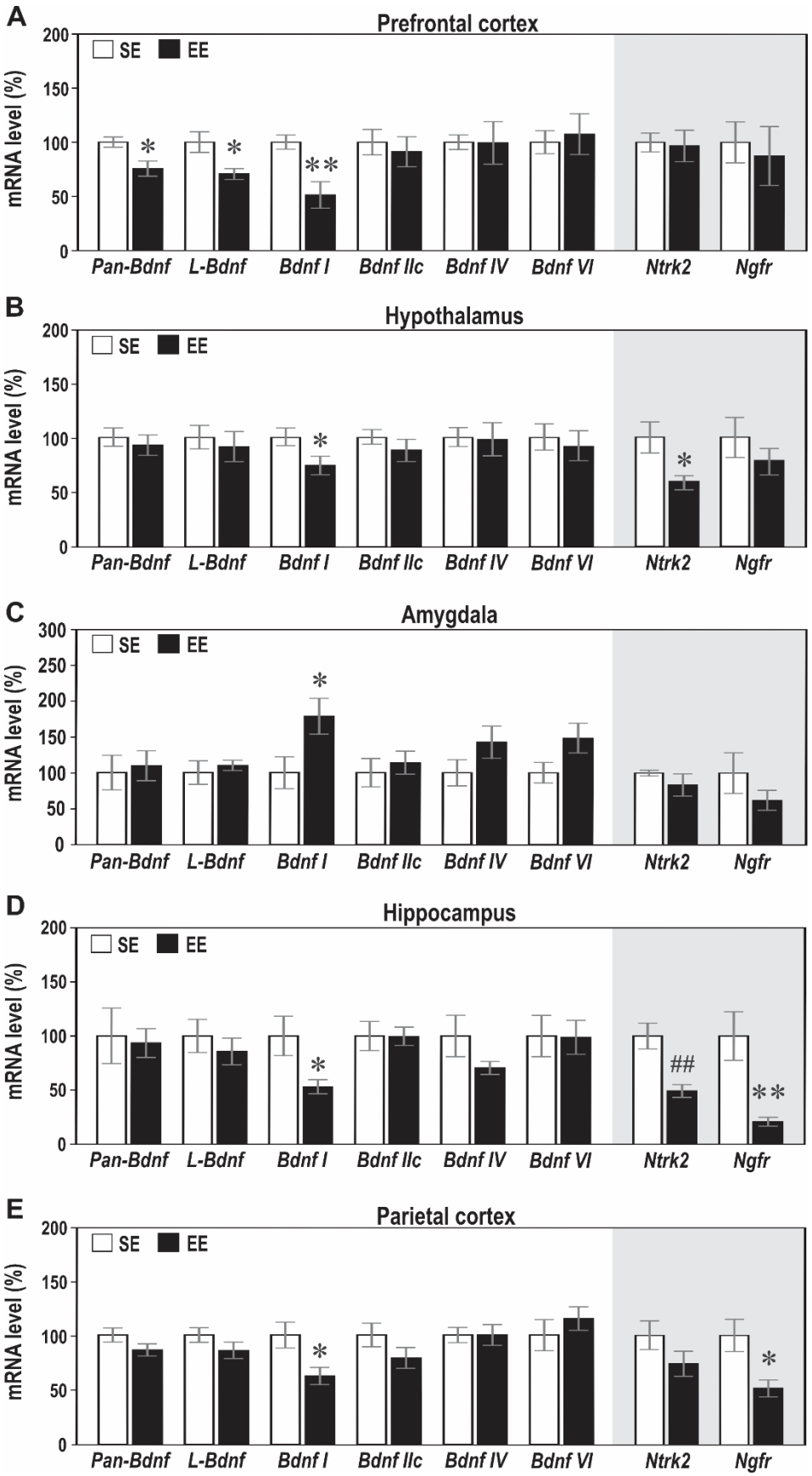

A
Prefrontal cortex
SE
EE
mRNA level (%)
200
150
100
50
0
*
*
**
Pan-Bdnf
L-Bdnf
Bdnf I
Bdnf IIc
Bdnf IV
Bdnf VI
Ntrk2
Ngfr
B
Hypothalamus
SE
EE
mRNA level (%)
200
150
100
50
0
*
*
Pan-Bdnf
L-Bdnf
Bdnf I
Bdnf IIc
Bdnf IV
Bdnf VI
Ntrk2
Ngfr
C
Amygdala
SE
EE
mRNA level (%)
300
250
200
150
100
50
0
*
Pan-Bdnf
L-Bdnf
Bdnf I
Bdnf IIc
Bdnf IV
Bdnf VI
Ntrk2
Ngfr
D
Hippocampus
SE
EE
mRNA level (%)
200
150
100
50
0
*
##
**
Pan-Bdnf
L-Bdnf
Bdnf I
Bdnf IIc
Bdnf IV
Bdnf VI
Ntrk2
Ngfr
E
Parietal cortex
SE
EE
mRNA level (%)
200
150
100
50
0
*
*
Pan-Bdnf
L-Bdnf
Bdnf I
Bdnf IIc
Bdnf IV
Bdnf VI
Ntrk2
Ngfr

Figure 17. Effect of environmental enrichment on *Bdnf* transcripts and BDNF receptor mRNA expression in the mouse brain. Levels of *Bdnf* mRNA variants and TrkB- and $p75^{NTR}$-encoding mRNA in the prefrontal cortex (A), hypothalamus (B), amygdala (C), hippocampus (D), and parietal cortex (E) were measured by real-time PCR. Values in the standard environment (SE) group were set to 100%. Data are means ± S.E.M. (n = 6-9/group). *: $P < 0.05$, **: $P < 0.01$, ##: $P < 0.001$ by Student's *t*-test. *L-Bdnf*: long-form *Bdnf* mRNA.

6.6. Effect of cage environment on GABAergic gene expression in the brain

Glutamate decarboxylase 1 (*Gad1*), parvalbumin alpha (*Pvalb*), somatostatin (*Sst*) and neuropeptide Y (*Npy*) mRNA levels were assessed in the SE and EE mice. A significant inter-group change was only seen in the levels of *Gad1* mRNA in the hippocampus (47.1% reduction, $P < 0.05$, Fig. 18D) and *Sst* mRNA in the hypothalamus (33.9% reduction, $P < 0.005$, Fig. 18B) of the EE mice relative to the SE control. Other genes in the other brain regions examined were not changed significantly (Fig.18).

6.7. Effect of cage environment on serotonergic gene expression in the brain

Monoamine oxidase A (*Maoa*) and 5-hydroxytryptamine receptor 2A (*Htr2a*) mRNA levels were significantly reduced in the hippocampus (33.3% and 38.8%, respectively, $P < 0.05$, Fig. 19A-B) of the EE mice group relative to the SE control. No significant inter-group difference was seen in the other brain regions examined.

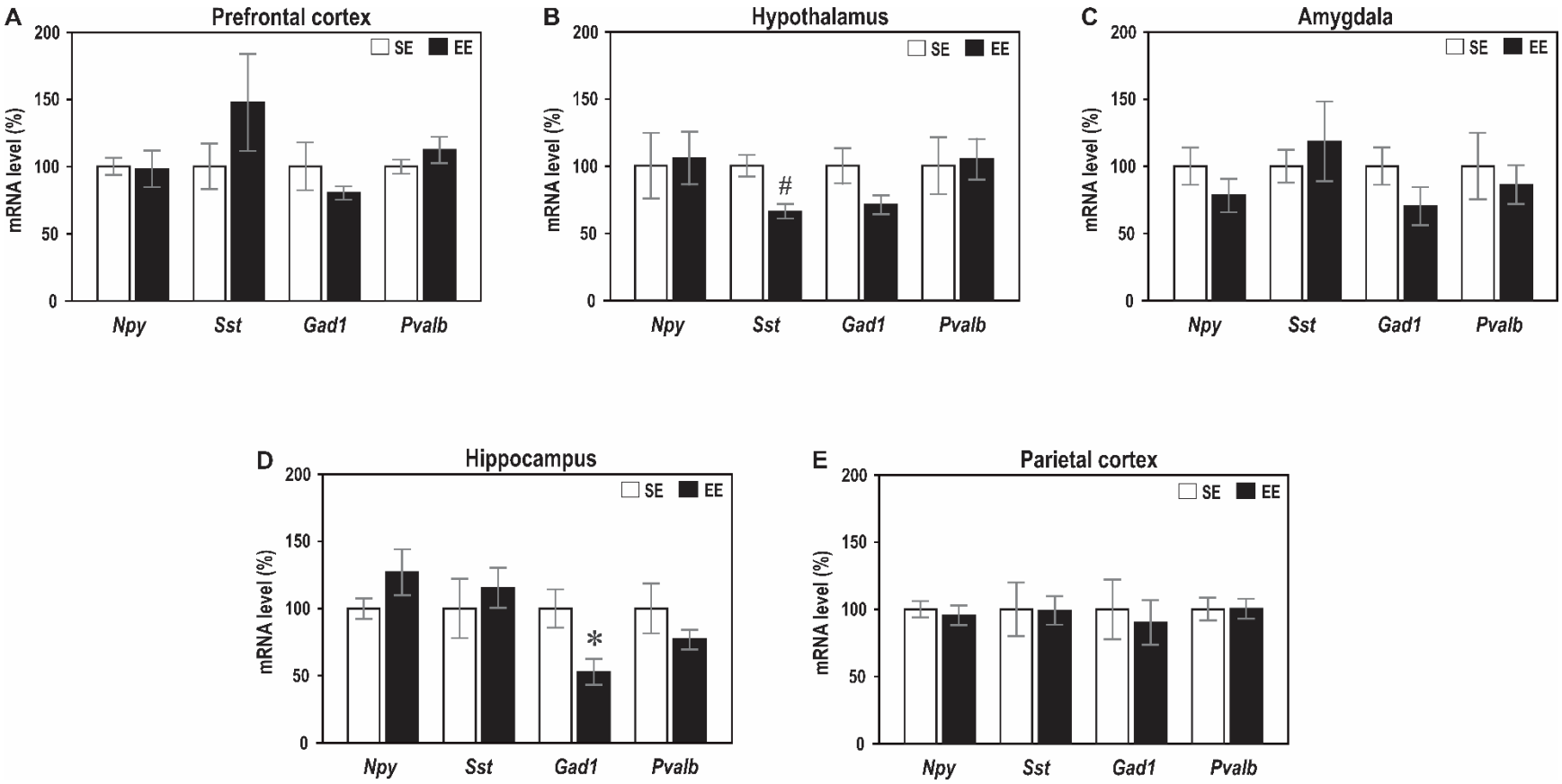


Figure 18. Effect of environmental enrichment on GABAergic gene expression in the brain. mRNA levels of *Npy, Sst, Gad1*, and *Pvalb* in the prefrontal cortex (A), hypothalamus (B), amygdala (C), hippocampus (D), and parietal cortex (E) were measured by real-time PCR. Values in the standard environment (SE) group were set to 100%. Data are means ± S.E.M. (n = 6-9/group). *: $P < 0.05$, #: $P < 0.05$ by Student's *t*-test.

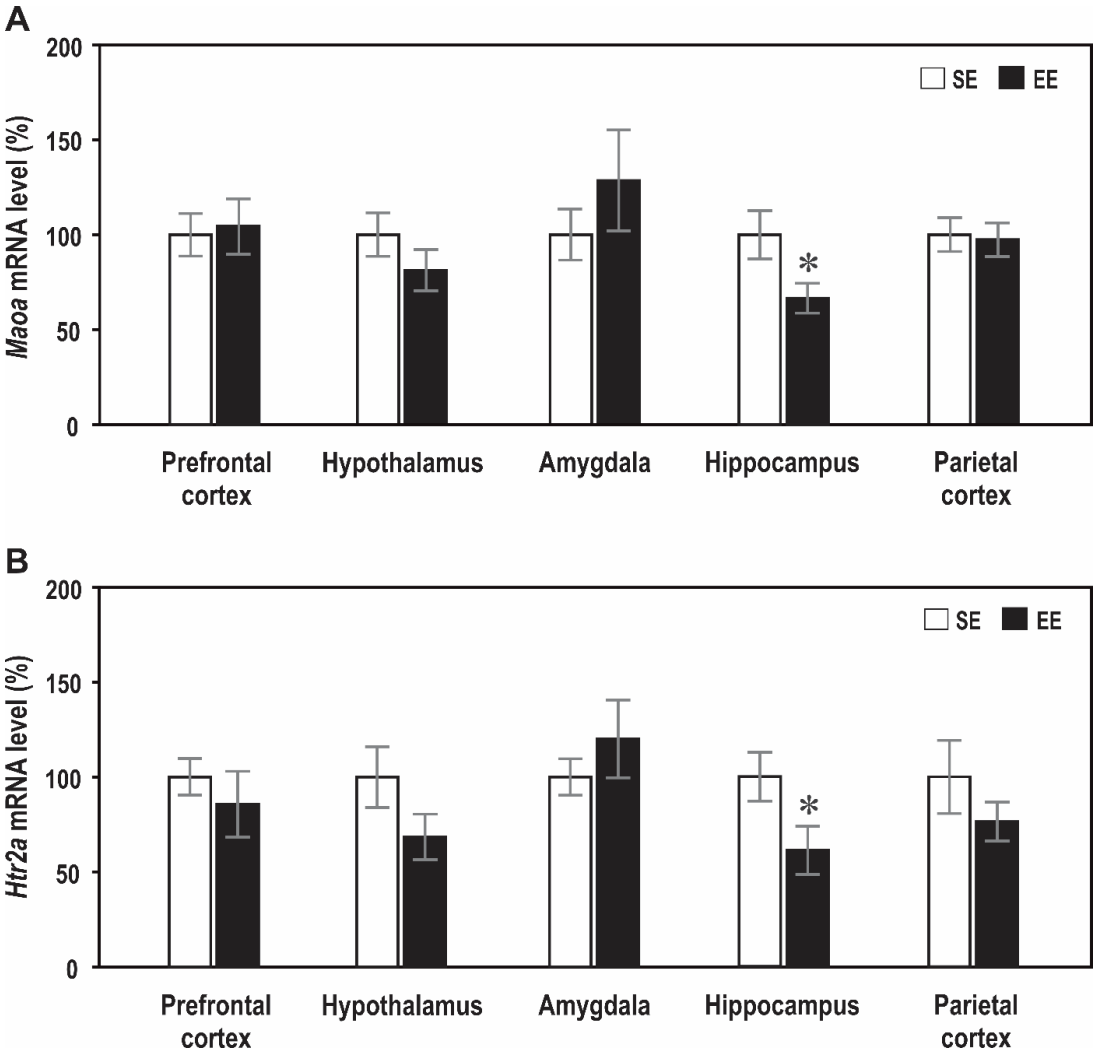


Figure 19. Effect of environmental enrichment on serotonergic gene expression in the brain. Levels of *Maoa* (A) and *Htr2a* (B) mRNA expression in the prefrontal cortex, hypothalamus, amygdala, hippocampus, and parietal cortex were measured by real-time PCR. Values in the standard environment (SE) group were set to 100%. Data are means ± S.E.M. (n = 7-9/group). *: $P < 0.05$ by Student's *t*-test.

7. Discussion

7.1. Summary of the main findings

The present study showed that environmental enrichment is effective in reducing inter-male aggression in group-housed C57BL/6 mice. The aggression-lowering effect of the enriched cage environment was associated with upregulated *Bdnf I* mRNA transcript expression in the amygdala and significant reduction of the same transcript in the hippocampus, hypothalamus, prefrontal cortex, and parietal cortex of environmental enrichment (EE) mice relative to mice housed under standard environment (SE). Total *Bdnf* mRNA levels were significantly reduced in the prefrontal cortex but not in the other tissues examined. Moreover, mRNA levels of *Bdnf* transcripts *IIc, IV*, and *VI* were not significantly altered in response to the enriched environment in any of the brain regions examined. The present study also examined expression levels of immediate early genes *Fos* and *Junb* to assess the pattern of brain activation associated with EE. The aggression-lowering effect of EE was associated with a significant reduction in the activity of aggression-related brain regions, namely the hippocampus, hypothalamus, prefrontal cortex, and parietal cortex, but not in the amygdala.

7.2. Examining the effect of enriched cage environment on inter-male aggression in group-housed C57BL/6 mice.

Previous studies reported contradictory results on the effect of environmental enrichment on reducing inter-male aggression in group-housed mice. Such inconsistency is likely attributed to design differences pertaining to mouse strain and age, group size, cage size, colour and type of enrichment devices used, arrangement of enrichment items in the cage, duration of the enrichment protocol, and the methodology used for assessing aggressive behaviour. For example, previous studies examined the effect of different types of enrichment on inter-male aggression in C57BL/6 mice at different ages and reported inconsistent results (Eskola & Kaliste-Korhonen, 1999; Gaskill et al., 2017; Mertens et al., 2020). Interestingly, there appear to be strain differences in aggressiveness in male mice (Lidster et al., 2019). EE successfully reduced inter-male aggression in docile BALB/c mice, while failed to achieve the same effect in high-aggression strains such as CD-1 mice (Haemisch & Gärtner, 1994; Haemisch et al., 1994; Armstrong et al., 1998; Ambrose & Morton, 2000; Van Loo et al., 2002; Pietropaolo et al., 2004; Howerton et al., 2008; Swetter et al., 2011; McQuaid et al., 2018). Moreover, EE suppressed inter-male aggression in low-aggression sub-strain BALB/cByJ, while failed to produce the same effect in high-aggression sub-

strain BALB/cJ (Giles et al., 2018). In the present study, enrichment of the cage environment, as described in the experimental design section, suppressed the incidence and frequency of inter-male aggression in the low-aggression strain C57BL/6 mice. These findings corroborate previous reports and suggest that the response to EE depends on aggression tendencies that may be innate or learned through experience. More specifically, the present study provides further evidence that low-aggression mouse strains are more amenable to the aggression-lowering effect of EE compared to high-aggression strains.

7.3. Examining the brain activation pattern associated with the aggression-lowering effect of enriched environment

The hypothalamus plays a critical role in the regulation of aggression. In particular, the ventrolateral subdivision of the hypothalamic ventromedial nucleus (VMNvl) is a critical brain region for controlling aggressive behaviour. Other brain regions modulate aggression by projecting to the VMNvl either directly or indirectly. Optogenetic stimulation of VMNvl neurons elicited attack by a resident male mouse towards intruder male mice, whilst inhibition of VMNvl neurons suppressed aggression (Lin et al., 2011; Yang et al., 2013; Lee et al., 2014). Moreover, neuronal activation marker c-Fos was upregulated in VMNvl neurons following aggressive attacks (Lee et al., 2014). In agreement with these data, the present study showed a significant reduction in hypothalamic *Fos* mRNA levels in EE mice, suggesting that reduced activity of hypothalamic cell populations, including VMNvl neurons, may mediate the observed aggression-lowering effect of EE.

The ventromedial nucleus (VMN) of the hypothalamus receives inputs from various brain regions, such as the hippocampus. Activation of hippocampal glutamatergic neurons projecting to the VMN promoted aggressive behaviour, whereas inhibition of these neurons blocked stress-induced aggression (Chang & Gean, 2019). In addition to monosynaptic hippocampus-hypothalamus projections, the hippocampal formation utilises a multisynaptic projection to the hypothalamus via the lateral septum (LS) (Risold & Swanson, 1997; Strange et al., 2014). The activity of LS-projecting hippocampal neurons is increased during aggression. Stimulation of these neurons leads to activation of neurons in the dorsal lateral septum, while silencing of this neural connection inhibits aggression, suggesting that hippocampal neurons promote aggression at least partly through their projections to the LS (Leroy et al., 2018). The dorsal LS sends inhibitory projections to the ventral LS, which in turn sends GABAergic inputs to VMNvl glutamatergic

neurons (Toth et al., 2010; Wong et al., 2016). Collectively, activation of hippocampal neurons activates inhibitory dorsal lateral septum neurons, leading to disinhibition of VMNvl-projecting ventral LS neurons, triggering aggression. In the present study, the hippocampal activity was significantly reduced in EE mice, as indicated by reduced *Fos* and *Junb* mRNA expression. Since the hypothalamic activity was also reduced in EE mice, it is plausible that the aggression-lowering effect of EE is partly mediated by reduced activity of the hippocampus-VMNvl and hippocampus-LS-VMNvl neural circuitries (Fig. 20).

The amygdala functions as a relay station of emotion and behaviour. The basolateral amygdala (BLA) serves as the primary locus of sensory input to the amygdala, whereas the centromedial amygdala is the main output component to other brain regions. The medial nucleus of the amygdala (MeA) contains heterogeneous populations of neurons, including glutamatergic and GABAergic neurons that project to the VMNvl. GABAergic neurons are predominantly (>90%) activated following aggressive attacks, while only a small fraction of glutamatergic neurons (<10%) are activated in response to aggressive attacks. Stimulation of the MeA GABAergic neurons elicits aggressive behaviour while silencing these neurons suppresses aggressive behaviour (Hong et al., 2014; Unger et al., 2015). Activation of vesicular glutamate transporter 2 (Vglut2)-expressing glutamatergic neurons in the MeA interrupts ongoing attack (Hong et al., 2014). In contrast, stimulation of VMNvl-projecting Vglut1-expressing glutamatergic neurons in the posterior amygdala promotes aggression (Zha et al., 2020). The complex amygdala-VMNvl neural circuitry-mediated regulation of aggression can be explained by the following mechanisms: **(1)** MeA GABAergic neurons may induce aggression by disinhibiting VMNvl output neurons, possibly via GABAergic interneurons in the VMNvl. **(2)** In contrast, Vglut2-expressing glutamatergic neurons in the MeA may activate VMNvl GABAergic interneurons and reduce VMNvl glutamatergic output, leading to suppression of aggression. **(3)** Vglut1-expressing glutamatergic neurons may synapse onto VMNvl output neurons and promote aggressive behaviour by directly stimulating these neurons. The present finding that EE did not alter *Fos* mRNA in the amygdala but reduced *Fos* mRNA levels in the hypothalamus argues that MeA-to-VMNvl glutamatergic (Vglut2) transmission overwhelms GABAergic and glutamatergic (Vglut1) inputs, leading to an inhibition of VMNvl output neurons and a reduction of aggression under EE housing conditions.

Cortical inputs to the amygdala influence amygdala-mediated behaviour, including aggressive behaviour. PFC glutamatergic neurons project to the BLA as well as the ITCs and regulate amygdalar activity. Activation of excitatory neurons in the PFC inhibits aggression in mice, while inhibition of these neurons increases aggressiveness (Takahashi et al., 2014). Stimulation of PFC glutamatergic neurons suppresses the activity of BLA output neurons by stimulating GABAergic interneurons in the BLA (Rosenkranz & Grace, 2002). ITCs are densely packed with GABAergic interneurons that project to the centromedial amygdala, where output neurons (both excitatory and inhibitor) project to the VMNvl. In the present study, EE reduced *Fos* mRNA levels in the PFC. This finding is consistent with the concept that reduced glutamatergic inputs from the PFC can inhibit BLA and/or ITC GABAergic interneurons, resulting in the increased firing of output neurons from the amygdala. Thus, the present *Fos* mRNA data suggest that inhibition of PFC-BLA circuitry mediates EE-induced suppression of aggression. BLA and ITC GABAergic interneurons may synapse onto both glutamatergic and GABAergic neurons that project to the VMNvl. Reduced activity of GABAergic interneurons may stimulate GABAergic output neurons that synapse onto VMNvl glutamatergic neurons, resulting in a suppression of aggression. Another possibility would be that reduced activity of GABAergic interneurons may stimulate VMNvl-projecting glutamatergic neurons followed by stimulation of VMNvl GABAergic interneurons, leading to an inhibition of VMNvl output glutamatergic neurons and suppressing aggressive behaviour (Fig. 20). Taken together, analysis of *Fos* mRNA expression suggests that the aggression-lowering effect of EE results from modulating the activity of the aggression-linked corticolimbic circuitry. It is important to note that the abovementioned interpretation of the *Fos* mRNA data is oversimplified. The function of the abovementioned neural circuitry is likely to be more complex in regulating aggression in response to environmental provocations.

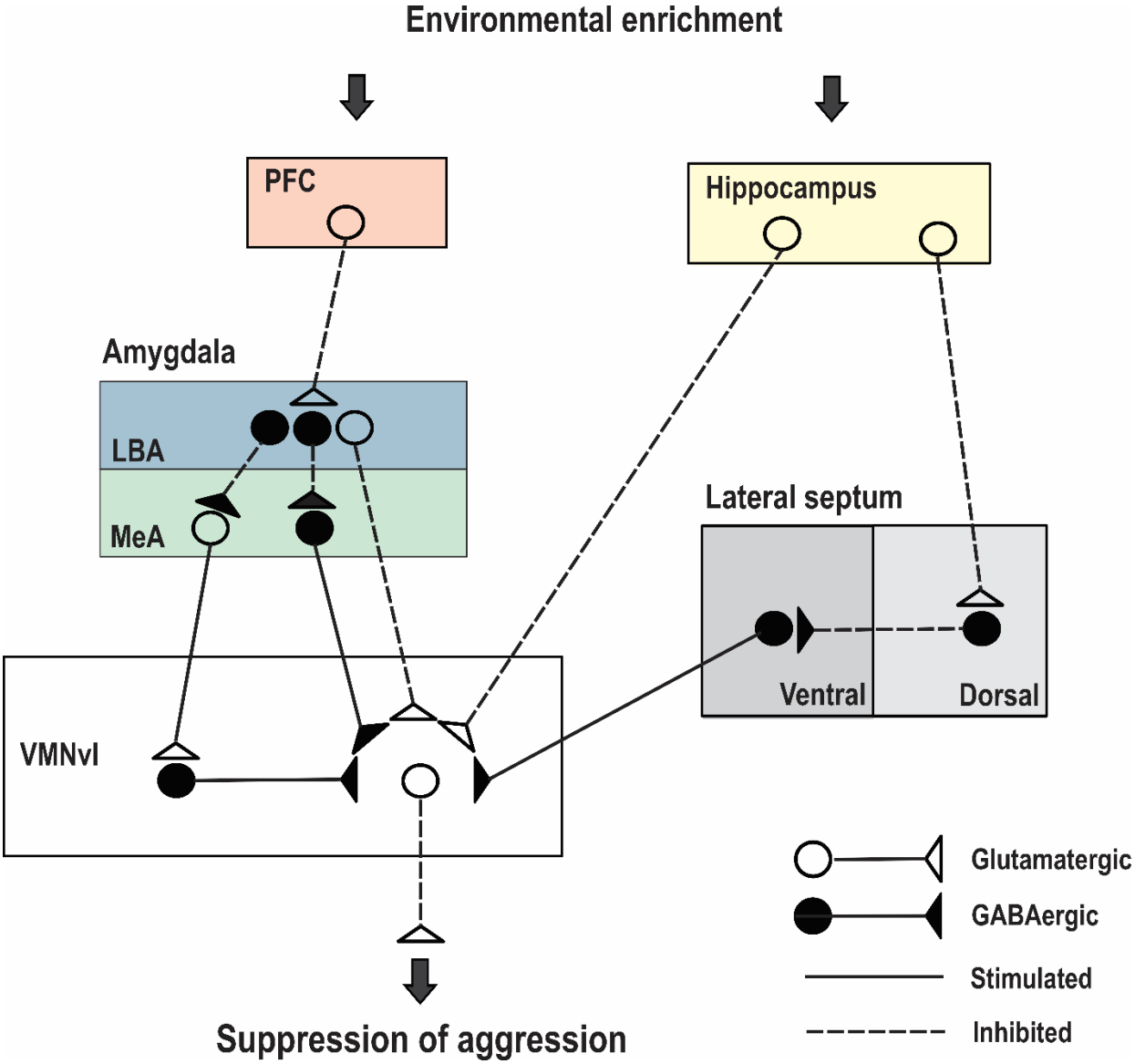


Figure 20. The proposed corticolimbic neural circuitry mediating the aggression-lowering effect of environmental enrichment in mice. Glutamatergic neurons and GABAergic neurons are shown with empty or filled cell body and nerve terminal, respectively. Neurons stimulated by environmental enrichment are shown as a solid line, while neurons inhibited are shown as a dotted line. PFC: prefrontal cortex, BLA: basolateral amygdala, MeA: medial nucleus of the amygdala, VMNvl: ventrolateral subdivision of the ventromedial hypothalamus. Some PFC, MeA, hippocampal and VMNvl glutamatergic neurons may be BDNF-expressing neurons.

7.4. Examining the association between the EE aggression-lowering effect and BDNF

BDNF signalling via TrkB has been implicated in the modulation of aggressive behaviour. Reduced BDNF-TrkB signalling causes increased aggressive behaviour in mice (Lyons et al., 1999; Gorski et al., 2003; Bremond-Gignac et al., 2005; Chan et al., 2006; Unger et al., 2007; Ito et al., 2011; Ernst et al., 2012). Furthermore, enriching the cage environment has been associated with increased *Bdnf* mRNA levels in brain tissues associated with the initiation and expression of aggressive behaviour in mice (Wolf et al., 2006; Cao et al., 2010; Chourbaji et al., 2012; Jurgens & Johnson, 2012; Foglesong et al., 2016; McQuaid et al., 2018). Together, these findings gave ground to hypothesise that the observed aggression-lowering effect of EE is mediated via increased expression and signalling of BDNF in the aggression-associated corticolimbic circuits. This hypothesis, however, was short-lived as total *Bdnf* mRNA (*Pan-Bdnf* mRNA) levels were not altered in response to EE in this study; if anything, total *Bdnf* mRNA levels were significantly reduced in the PFC. Moreover, the TrkB-encoding *Ntrk2* mRNA levels were significantly reduced in the hypothalamus of enriched mice, suggesting that BDNF-TrkB signalling is also reduced in the hypothalamus.

A change in perspective had to take place in order to explain the nature of the BDNF role in the aggression-lowering effect of EE. As mentioned in the introduction, the BDNF role in aggression is contradictory. Some recent studies showed that *Bdnf* mRNA levels are upregulated in the PFC, amygdala, and hypothalamus of aggressive rats (Ilchibaeva et al., 2015; Ilchibaeva et al., 2018). Furthermore, central administration of BDNF induces acute aggressive behaviour in mice characterised by increased attack time and reduced first attack latency (Naumenko et al., 2014). These data suggest that BDNF is an inducer of aggression and conveniently explain the reduced *Bdnf* mRNA level associated with the aggression-lowering effect of EE reported in this study.

7.5. Examining the association between the aggression-lowering effect of EE and *Bdnf* mRNA transcript variant-specific and brain region-specific expression.

Bdnf transcription is under the control of nine promoters driving the expression of multiple splice variants, all of which encode an identical BDNF protein. Selective cell type-specific or brain region-specific expression of *Bdnf* transcripts has been associated with a variety of stimuli (Metsis et al., 1993; Nanda & Mack, 1998; Tao et al., 1998; Pattabiraman et al., 2005; Tsankova et al., 2006; Unger et al., 2007; Karpova et al., 2011; Baj et al., 2012). Furthermore, the subcellular

localisation of different *Bdnf* transcripts influences neuronal functions (Pattabiraman et al., 2005; An et al., 2008; Baj et al., 2011; Liao et al., 2012). In the present study, *L-Bdnf* mRNA levels were significantly reduced in the PFC of EE mice relative to control mice in the SE cages but were not significantly changed in the other tissues examined. Additionally, *Bdnf I* mRNA levels were significantly reduced in the PFC, hippocampus, hypothalamus, and parietal cortex in response to EE, whereas *Bdnf I* mRNA levels were significantly increased in the amygdala. Moreover, *Bdnf* variants *IIc, IV,* and *VI* were not significantly altered in response to EE. These data argue for a variant-specific and brain region-specific role of *Bdnf* in mediating the observed aggression-lowering effect of EE. Interestingly, *Bdnf I* has been linked to aggressive behaviour in previous studies. Mice with disrupted *Bdnf* transcripts *I* or *II* are hyperaggressive, whilst mice with disrupted *Bdnf* transcripts *IV* or *VI* do not display heightened aggressive behaviour (Maynard et al., 2016; Hobbs et al., 2017). These data further suggest that the aggression-lowering effect of EE seen in this study is likely mediated via upregulated expression of *Bdnf I* mRNA in the amygdala.

The relative contribution of each transcript to the total BDNF protein is complex. There are multiple biologically active BDNF proteins and receptors that may exert opposing effects. For example, mature BDNF (mBDNF) promotes neuronal survival via TrkB signalling, whereas BDNF precursor (proBDNF) functions as a pro-apoptotic factor via $p75^{NTR}$ signalling (Teng et al., 2005b). Glutamate release is stimulated by mBDNF via TrkB but is inhibited by proBDNF via $p75^{NTR}$ (Gibon et al., 2016). Studies have also suggested that the ratio of mBDNF/proBDNF is associated with aggressiveness (Ilchibaeva et al., 2015; Ilchibaeva et al., 2018). It is probable that different *Bdnf* transcript variants are differentially processed to BDNF proteins via altered translation efficiency and post-translational modification or cleavage. Therefore, future studies should investigate *Bdnf* transcript variant-specific and brain region-specific processing of BDNF proteins and its relationship to the aggression-lowering effect of EE.

Although *Bdnf* mRNA is expressed widely in the brain, there is a lack of information on the neural circuit and the neuronal populations expressing BDNF. It is possible that subpopulations of PFC, amygdala and VMNvl output excitatory neurons are BDNF-expressing neurons. Thus, EE may inhibit PFC BDNF-expressing glutamatergic neurons projecting to the BLA. This may cause an inhibition of BLA GABAergic interneurons followed by stimulation of BDNF–expressing glutamatergic neurons projecting to the VMNvl. Subsequently, it may stimulate VMNvl GABAergic interneurons, leading to an inhibition of aggression-promoting VMNvl BDNF-

expressing glutamatergic neurons under EE conditions. Alternatively or concurrently, stimulation of amygdala GABAergic neurons projecting to the VMNvl inhibits BDNF-expressing glutamatergic neurons in the VMNvl directly.

Additionally, levels of *Ntrk2* mRNA were reduced in the hypothalamus of EE mice in the present study, suggesting that BDNF-TrkB signalling is reduced in the hypothalamus of EE mice. This finding conjectures the possibility that VMNvl BDNF-expressing glutamatergic neurons may be inhibited by reduced input from amygdala BDNF-expressing glutamatergic neurons via reduced availability of BDNF receptor in the VMNvl. This sequence of events is consistent with the neural circuitry proposed as a possible pathway mediating the aggression-lowering effect of EE according to *Fos* mRNA data as discussed above (Fig. 20). Thus, data from the present study cautiously propose that BDNF-expressing neurons within the PFC-amygdala-VMNvl neural circuitry mediate EE's aggression-lowering effect. A theory worthy of empirical investigation in future studies.

7.6. Examining the role of GABAergic transmission in mediating the aggression-lowering effect of EE

Regulation of aggressive behaviour involves a complex interplay between different neurotransmitter systems. Neurons in the hypothalamic attack area, including VMNvl, are predominantly glutamatergic, whose activity may be inhibited by local GABAergic interneurons (Gregg & Siegel, 2001; Hrabovszky et al., 2005). Microinjection of $GABA_A$ antagonists into the HAA elicited aggressive behaviour in rats (Adams et al., 1993; Roeling et al., 1993). These findings led to the assumption that increased hypothalamic GABAergic tone contributes to the aggression-lowering effect of EE. To investigate this hypothesis, the present study examined the mRNA expression of GABAergic marker genes *Gad1, Pvalb, Sst,* and *Npy.*

Contrary to the initial assumption, EE reduced *Sst* mRNA levels in the hypothalamus and *Gad1* mRNA levels in the hippocampus of EE mice relative to control mice in the SE cages. Other genes were not significantly altered in other brain regions. These changes in GABAergic marker genes may be due to reduced *Bdnf I* expression because BDNF-TrkB signalling positively influences *Gad1* and *Gad2* mRNA and protein expression (Matsumoto et al., 2006; Sanchez-Huertas & Rico, 2011). For the time being, it remains highly questionable whether reduced GABAergic signalling, as represented by reduced GABAergic marker gene expression, in the hypothalamus and the hippocampus contributes to the observed aggression-lowering effect of EE

aggressive; however, these findings suggest that EE may reduce aggression partly by altering GABAergic transmission in these brain regions.

7.7. Examining the role of serotonergic transmission in mediating the aggression-lowering effect of EE

5-HT plays a complex role in the modulation of aggressive behaviour. Selective serotonin reuptake inhibitors (SSRIs) are known to suppress impulsive aggression in a range of neuropsychiatric disorders (Meyer et al., 2016). Furthermore, neuroimaging studies showed a positive association between impulsive aggression and increased 5-HT_{2A} receptor concentration in the PFC (Rylands et al., 2012). Consistent with these findings in humans, 5-HT_{2A} receptor agonists increase aggression, while 5-HT_{2A} receptor antagonists reduce aggressive behaviour in mice (White et al., 1991; Rodriguez-Arias et al., 1998; Sakaue et al., 2002). 5-HT and BDNF are functionally co-dependent, 5-HT induces BDNF expression and BDNF, in turn, fosters the maturation and survival of serotonergic neurons (Mattson et al., 2004). For instance, 5-HT_{2A} knock-out mice show a reduction in *Bdnf* mRNA expression, while SSRIs, like fluoxetine, were found to increase *Bdnf I* mRNA levels in the hippocampus and BLA, suggesting that improved serotonergic transmission increases *Bdnf I* expression (Karpova et al., 2011; Jaggar et al., 2019). In the present study, expression levels of the serotonergic markers *Htr2a, which* encodes 5-HT_{2A}, and *Maoa* were significantly reduced in the hippocampus. Moreover, *Bdnf I*, *Ntrk2*, and *Ngfr* mRNA levels were also significantly reduced in the hippocampus. Collectively, these data strongly suggest that a BDNF-mediated reduction in 5-HT-5-HT_{2A} signalling in the hippocampus may modulate the aggression-lowering effect of EE.

7.8. Conclusion

Environmental enrichment diminishes inter-male aggression by reducing the activity of the corticolimbic circuits involved in aggression. Additionally, the aggression-lowering effect of EE was associated with altered CNS *Bdnf I* expression. Specifically, the present study suggests that environmental enrichment reduces aggression via increasing *Bdnf I* mRNA expression in the amygdala.

7.9. Significance

Aggression is a multifaceted ubiquitous behaviour that is poorly understood. Aggressive behaviour is a serious disease presentation in many psychiatric disorders and represents a resident threat to mental health workers, families of patients, and the community at large. Treatment of aggression is very complicated and usually involves a combination of cognitive behavioural therapy and drugs that mostly sedate the patient without treating the underlying predisposing factors. Social and environmental factors are thought to influence the initiation and expression of aggressive behaviour via modulating the corticolimbic circuits controlling aggression; however, the underlying brain mechanisms are severely understudied. The present study showed that environmental enrichment effectively reduces inter-male aggressive behaviour in group-housed mice with concomitant changes in the expression of specific *Bdnf* transcripts in a brain region-specific manner. The current study proposed a preliminary model of the corticolimbic circuitry and the neuronal populations that mediate the effect of social and environmental factors on aggression development. This study paves the way for subsequent studies that would further explore the effects of social and environmental influences on the brain and potentially identify effective therapeutic modalities for impulsive aggression, especially in the face of environmental provocations. Above all, the present study serves as a reminder of the importance of enriching the home, social and work environments as an effective means of reducing human aggression.

7.10. Limitations

Assessment of aggressive behaviour in mice

The present study investigated inter-male aggression using timed (1 h) and repeated (5 days/week) direct visual observations carried out by the same investigator according to pre-set criteria. Visual observations were carried out during the light phase (1400 h) and might reflect or do not reflect the nature of inter-male fighting during the active phase. However, physical evaluation of mice in both cage environments validated the inter-male fighting data, as mice in the standard cages were associated with a much higher rate of fighting-induced injuries like biting, scratches, and ano-genital wounds compared with mice in the enriched cages, in which these signs were almost absent. The objective of this study was to examine the intra-cage aggressive behaviour in group-housed mice, therefore using standardised tests (e.g., the resident-intruder paradigm or the tube dominance test) that depend on external provocations is irrelevant in the present study.

Mice strain, group size and cage size

The present study examined the aggression-lowering effect of EE in group-housed male C57BL/6 mice. C57BL/6 is a low aggression mouse strain extensively used in biomedical research (Lidster et al., 2019). Mice in this study were not selectively bred for aggression and were randomly assigned to standard or enriched cage environments. The present study did not investigate the strain-specific response to the aggression-lowering effect of EE. It is, however, expected that different mouse strains would likely respond differently to the aggression-lowering effect of the EE protocol used in the present study. Nevertheless, it would be valuable to use multiple strains simultaneously and examine the inter-strain response to EE-mediated behavioural and physiological effects.

In the present study, mice were housed in groups of 5 mice/cage. As already discussed, previous reports showed a highly contradictory role of group size in influencing inter-male aggressive behaviour (Van Loo et al., 2001). At this point, it is unknown if the group size in the standard cage environment helped increase inter-male aggression. Similarly, it is also unknown if the group size influenced the relatively peaceful behaviour in the enriched cage environment. Therefore, it would be valuable to explore the influence of group size in the observed aggression-lowering effect of EE and the associated gene expression pattern in future studies.

Similarly, in the present study, the EE mice had larger cages (58 cm [L] × 48 cm [W] × 30 cm [H]) compared to the SE mice control (37.5 cm [L] × 19 cm [W] × 12.5 cm [H]). It is possible that the reduction in the display of aggression seen in EE mice could be attributed to the larger cage space and not necessarily a direct effect of the enrichment items included in the EE protocol. In support of this possibility, a previous study has reported that cage size, in itself, contributes significantly to the development of intra-cage aggression (Van Loo et al., 2001). In their study, Van Loo and colleagues found a significant main effect of the cage size and a significant interaction between cage and group sizes. Still, they also showed that cage size did not significantly influence aggression in mice aged 7-8 weeks or 11-12 weeks when they were housed in groups of 5 mice/cage, which is very similar to the conditions employed in the present study. Notwithstanding, it remains probable that cage size could have influenced the aggression-lowering effect seen in EE mice independent of the presence of the enrichment items.

Sex differences

Males are more socially and genetically prone to physical aggression compared with females (Björkqvist, 2018). Group-housed female mice show little to no intra-cage aggression compared with group-housed male mice. For these reasons, the present study focused on inter-male aggression. That being said, it would be interesting to study the effect of EE on maternal aggression (Bosch, 2013).

Tissue dissection protocol

Much like in part (I), the dissection protocol, described in the experimental design section, allows for gross dissection of tissues of interest but does not allow for a targeted dissection of a specific nucleus within the tissue. For instance, different subdivisions of the amygdala (e.g., BLA and MeA) have distinct neuronal populations and therefore play different roles in controlling aggression. Similarly, the VMN is more relevant to aggression than other hypothalamic nuclei. To circumvent this limitation, future studies should use techniques such as immunohistochemistry, *in situ* hybridisation, and laser capture microdissection to examine the role of different neuronal populations in each tissue in relation to the aggression-lowering effect of EE.

Causality

The present study provides preliminary evidence that the aggression-lowering effect of EE is mediated via increased *Bdnf I* transcript expression in the amygdala. However, this study does not provide causal evidence conclusively linking amygdalar *Bdnf I* to the aggression-lowering outcome of EE.

7.11. Future directions

To better understand cage's social dynamics throughout the day and avoid any undesired human influences, it would be best to use video surveillance with night vision capabilities. Video surveillance of mice during the active phase will provide quantifiable and accurate information of the social and hierarchical structure in each cage. It also offers a chance to **(a)** identify the pattern of aggressive behaviour, including injuries caused by inter-male fighting; **(b)** measure the duration and number of aggressive attacks per cage; **(c)** calculate the area of territorial dominance of the dominant mouse in each cage; and **(d)** categorise the behaviour of mice in each cage into one of three categories: aggressive dominant, subordinate, or neutral. Such categorisation would help examine the gene and protein expression pattern associated with each social category and allow for inter-category behavioural and biological comparisons.

Future studies should use cages of the same size to exclude any possible stand-alone effect of the cage size in both experimental environments. Also, a third experimental SE group of an equal cage size to that of the EE group could be added to the present design to conclusively discern the contribution of the cage size in the aggression-lowering effect of EE and the associated gene expression changes, if any.

To investigate and establish a causal link between amygdalar *Bdnf I* overexpression and the observed reduction in inter-male aggression, future studies should examine the effect of overexpressing *Bdnf I* in the amygdala in relation to inter-male aggression under the standard cage environment. Simultaneously, the elimination of the *Bdnf I* transcript in the amygdala should be examined in relation to inter-male aggression under EE. Additionally, it would be valuable to examine the effect of switching the cage environment on *Bdnf I* expression in the brain. For instance, mice housed in the enriched cage environment for six weeks, the experimental period in this study, can be deprived of the enrichment items and moved to standard cages for another six weeks through which the aggressive behaviour can be evaluated as described and gene expression analysis can be carried out at the end of the study using 6-week enriched mice and 12-week standard cages mice as controls. Reciprocally, standard environment mice can be moved to enriched cages, and a similar approach could be followed.

Overall thesis summery and conclusion remarks

Aggression and feeding are evolutionarily linked behaviours in many species. Animals often display aggressive behaviour to protect their food from conspecific competition; hence, the term "food aggression" was coined to refer to such behaviour, especially in canines (Beaver, 1993; Marder et al., 2013). Not surprisingly, multiple brain pathways were found to be common in the regulation of both feeding and aggressive behaviours, including the NPY-5HT pathway (Karl et al., 2004; Reviewed in Emeson & Morabito, 2005) and the BDNF-TrkB signalling pathway, which is discussed extensively in this thesis. From a metabolic perspective, aggressive behaviour is associated with a significant metabolic cost. Indeed, studies have shown that aggressive behaviour is associated with a significant increase in metabolic rate and mobilisation of energy reserves (Briffa & Elwood, 2004; Ros et al., 2006; Briffa & Sneddon, 2007; Seebacher et al., 2013). Moreover, it has been shown that repeated exposure to aggressive encounters resulted in a significant reduction in fat mass in resident striped hamsters in the resident-intruder paradigm due to increased fat mobilisation (Xu, Chi, et al., 2018). Together, these studies serve to establish a strong link between aggression and feeding behaviours.

Since the environment is a strong determinant of behaviour, the present thesis examined the effect of enriching the cage environment of captive laboratory mice on modulating feeding and aggressive behaviours. We also examined mRNA expression of neurotrophins, and other genes, implicated in the regulation of both behaviours at the level of the brain. In the first part of the thesis, we showed that enriching the cage environment improved the feeding suppressing effect of glucose in group-housed male mice. However, environmental enrichment failed to produce a similar feeding suppressing effect with the gastrointestinal hormone xenin or the adipokine leptin. Also, hypothalamic and amygdalar *Bdnf* mRNA transcripts levels were altered in response to glucose treatment under the enriched environment relative to the standard environment control. Hypothalamic *Fos* mRNA levels were significantly increased in the enriched environment mice relative to the standard environment control, suggesting that environmental enrichment increased hypothalamic activity in response to glucose treatment. Interestingly, hypothalamic *Gdnf* mRNA levels were significantly increased in response to glucose treatment in the enriched environment mice compared with the standard environment control, suggesting for the first time that glucose may regulate hypothalamic *Gdnf* expression and that GDNF may play a role in enhancing the anorectic response to glucose under the enriched environment.

In the second part of this thesis, we showed that environmental enrichment diminishes inter-male aggression in group-housed mice, as indicated by reduced intra-cage fighting incidence and frequency compared with the standard environment control group. We also showed that environmental enrichment significantly reduced *Fos* mRNA levels in the prefrontal cortex, hypothalamus, hippocampus, and parietal cortex, suggesting that environmental enrichment reduced the activity of the corticolimbic circuits controlling aggressive behaviour. Importantly, *Bdnf I* transcript levels were significantly reduced in the prefrontal cortex, hypothalamus, hippocampus, and parietal cortex, whereas *Bdnf I* transcript levels were significantly increased in the amygdala. Together, our data suggest that improved amygdalar *Bdnf I* expression is, at least partly, responsible for the aggression-lowering effect of environmental enrichment.

Animals exposed to frequent aggressive encounters have higher energy demands and display a lean phenotype due to persistent fat mobilisation (Xu, Chi, et al., 2018). Therefore, we initially expected standard environment mice to have a leaner phenotype compared with environmental enrichment mice. Contrary to our assumption, we found that environmental enrichment mice showed a significant reduction in fat mass relative to standard environment mice. Such effect might be attributed to the following possibilities: **(1)** the metabolic effect of environmental enrichment, manifested as fat mass reduction, is very profound to the degree that it exceeded any expected aggression-associated reduction in fat mass; or **(2)** the mechanism controlling the aggression-lowering effect of environmental enrichment is independent of the mechanism modulating the metabolic effect of environmental enrichment. Both possibilities should be thoroughly investigated in future studies.

The novel data uncovered in this thesis further our knowledge of how the CNS participates in modulating behaviour and examined an understudied aspect of the gene-environment interplay and the associated behavioural phenotypes. It is justifiably expected that the data presented in this thesis would pave the way for subsequent detailed and specific studies aiming at deciphering the neuronal circuits responsible for the modulation of aggressive and feeding behaviours in response to environmental and social stimuli, which would play a crucial part in developing effective drugs (enviromimetics) to treat pathological aggression and obesity. Additionally, this thesis substantiates environmental enrichment as a powerful tool for non-pharmacological behavioural modifications and psychological adjustment. We, therefore, wholeheartedly urge the readers to enrich their home and social environments by introducing more positive stimulations, including

exercise, travel, nature walks, changing home décor, learning new language, listening to different music genres, exposure to different visual art forms, and re-establish a healthy balance in their social relationships.

References

Abraham, M. A., et al. (2018). Leptin enhances hypothalamic lactate dehydrogenase a (ldha)-dependent glucose sensing to lower glucose production in high-fat-fed rats. *J Biol Chem, 293*(11), 4159-4166. doi:10.1074/jbc.RA117.000838

Abrahams, N.& Jewkes, R. (2005). Effects of south african men's having witnessed abuse of their mothers during childhood on their levels of violence in adulthood. *Am J Public Health, 95*(10), 1811-1816. doi:10.2105/ajph.2003.035006

Adams, D. B., et al. (1993). Offense produced by chemical stimulation of the anterior hypothalamus of the rat. *Physiol Behav, 53*(6), 1127-1132. doi:10.1016/0031-9384(93)90369-q

Adler, P. A.& Adler, P. (1998). *Peer power: Preadolescent culture and identity*. Piscataway, NJ, US: Rutgers University Press.

Ahima, R. S.& Antwi, D. A. (2008). Brain regulation of appetite and satiety. *Endocrinology and metabolism clinics of North America, 37*(4), 811-823. doi:10.1016/j.ecl.2008.08.005

Aid, T., et al. (2007). Mouse and rat bdnf gene structure and expression revisited. *Journal of Neuroscience Research, 85*(3), 525-535. doi:https://doi.org/10.1002/jnr.21139

Alexiou, C., et al. (1998). Xenin--a novel suppressor of food intake in rats. *Brain research, 800*(2), 294-299. doi:10.1016/s0006-8993(98)00535-6

Ali, M. A.& Kravitz, A. V. (2018). Challenges in quantifying food intake in rodents. *Brain research, 1693*(Pt B), 188-191. doi:10.1016/j.brainres.2018.02.040

Allison, D. B., et al. (2008). Obesity as a disease: A white paper on evidence and arguments commissioned by the council of the obesity society. *Obesity (Silver Spring), 16*(6), 1161-1177. doi:10.1038/oby.2008.231

Ambrose, N.& Morton, D. B. (2000). The use of cage enrichment to reduce male mouse aggression. *Journal of Applied Animal Welfare Science, 3*(2), 117-125.

American Psychiatric Association, D.-. (2013). *Diagnostic and statistical manual of mental disorders : Dsm-5*. Arlington, VA: American Psychiatric Association.

An, J. J., et al. (2008). Distinct role of long 3' utr bdnf mrna in spine morphology and synaptic plasticity in hippocampal neurons. *Cell, 134*(1), 175-187. doi:10.1016/j.cell.2008.05.045

An, J. J., et al. (2015). Discrete bdnf neurons in the paraventricular hypothalamus control feeding and energy expenditure. *Cell Metab, 22*(1), 175-188. doi:10.1016/j.cmet.2015.05.008

Anand, B. K.& Brobeck, J. R. (1951). Localization of a "feeding center" in the hypothalamus of the rat. *Proc Soc Exp Biol Med, 77*(2), 323-324. doi:10.3181/00379727-77-18766

Andermann, M. L.& Lowell, B. B. (2017). Toward a wiring diagram understanding of appetite control. *Neuron, 95*(4), 757-778. doi:https://doi.org/10.1016/j.neuron.2017.06.014

Anderson, C. A.& Bushman, B. J. (2002). Human aggression. *Annual Review of Psychology, 53*(1), 27-51. doi:10.1146/annurev.psych.53.100901.135231

Anholt, R. R. H.& Mackay, T. F. C. (2012). Genetics of aggression. *Annual Review of Genetics, 46*(1), 145-164. doi:10.1146/annurev-genet-110711-155514

Annas, A., et al. (2013). Group housing of male cd1 mice: Reflections from toxicity studies. *Lab Anim, 47*(2), 127-129. doi:10.1177/0023677213476278

Anstrom, K. K., et al. (2009). Increased phasic dopamine signaling in the mesolimbic pathway during social defeat in rats. *Neuroscience, 161*(1), 3-12. doi:10.1016/j.neuroscience.2009.03.023

Arai, J. A., et al. (2009). Transgenerational rescue of a genetic defect in long-term potentiation and memory formation by juvenile enrichment. *J Neurosci, 29*(5), 1496-1502. doi:10.1523/jneurosci.5057-08.2009

Archer, J. (1991). The influence of testosterone on human aggression. *British journal of psychology, 82*(1), 1-28.

Archer, J. (2006). Testosterone and human aggression: An evaluation of the challenge hypothesis. *Neurosci Biobehav Rev, 30*(3), 319-345. doi:10.1016/j.neubiorev.2004.12.007

Archer, J. (2009a). Does sexual selection explain human sex differences in aggression? *Behav Brain Sci, 32*(3-4), 249-266; discussion 266-311. doi:10.1017/s0140525x09990951

Archer, J. (2009b). The nature of human aggression. *International Journal of Law and Psychiatry, 32*(4), 202-208. doi:https://doi.org/10.1016/j.ijlp.2009.04.001

Armstrong, K. R., et al. (1998). Use of corn-husk nesting material to reduce aggression in caged mice. *Contemp Top Lab Anim Sci, 37*(4), 64-66.

Arranz, L., et al. (2010). Environmental enrichment improves age-related immune system impairment: Long-term exposure since adulthood increases life span in mice. *Rejuvenation research, 13*(4), 415-428.

Bady, I., et al. (2006). Evidence from glut2-null mice that glucose is a critical physiological regulator of feeding. *Diabetes, 55*(4), 988-995. doi:10.2337/diabetes.55.04.06.db05-1386

Baeza-Raja, B., et al. (2012). P75 neurotrophin receptor regulates glucose homeostasis and insulin sensitivity. *Proceedings of the National Academy of Sciences, 109*(15), 5838. doi:10.1073/pnas.1103638109

Baeza-Raja, B., et al. (2016). P75 neurotrophin receptor regulates energy balance in obesity. *Cell Reports, 14*(2), 255-268. doi:https://doi.org/10.1016/j.celrep.2015.12.028

Bai, L., et al. (2019). Genetic identification of vagal sensory neurons that control feeding. *Cell, 179*(5), 1129-1143.e1123. doi:10.1016/j.cell.2019.10.031

Baj, G., et al. (2012). Physical exercise and antidepressants enhance bdnf targeting in hippocampal ca3 dendrites: Further evidence of a spatial code for bdnf splice variants. *Neuropsychopharmacology, 37*(7), 1600-1611. doi:10.1038/npp.2012.5

Baj, G., et al. (2011). Spatial segregation of bdnf transcripts enables bdnf to differentially shape distinct dendritic compartments. *Proc Natl Acad Sci U S A, 108*(40), 16813-16818. doi:10.1073/pnas.1014168108

Ball, K., et al. (2009). Neighbourhood socioeconomic inequalities in food access and affordability. *Health & Place, 15*(2), 578-585. doi:https://doi.org/10.1016/j.healthplace.2008.09.010

Ball, N. J., et al. (2019). Enriched environments as a potential treatment for developmental disorders: A critical assessment. *Frontiers in Psychology, 10*(466). doi:10.3389/fpsyg.2019.00466

Balthasar, N., et al. (2005). Divergence of melanocortin pathways in the control of food intake and energy expenditure. *Cell, 123*(3), 493-505. doi:10.1016/j.cell.2005.08.035

Bandura, A. (1977). Self-efficacy: Toward a unifying theory of behavioral change. *Psychological review, 84 2*, 191-215.

Banks, W. A., et al. (1999). Impaired transport of leptin across the blood-brain barrier in obesity. *Peptides, 20*(11), 1341-1345. doi:10.1016/s0196-9781(99)00139-4

Bariohay, B., et al. (2005). Brain-derived neurotrophic factor plays a role as an anorexigenic factor in the dorsal vagal complex. *Endocrinology, 146*(12), 5612-5620. doi:10.1210/en.2005-0419

Barnard, C. J., et al. (1996). Environmental enrichment, immunocompetence, and resistance to babesia microti in male mice. *Physiol Behav, 60*(5), 1223-1231. doi:10.1016/s0031-9384(96)00174-6

Bartolomucci, A., et al. (2004). Age at group formation alters behavior and physiology in male but not female cd-1 mice. *Physiol Behav, 82*(2-3), 425-434. doi:10.1016/j.physbeh.2004.04.011

Bassareo, V.& Di Chiara, G. (1999). Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. *Eur J Neurosci, 11*(12), 4389-4397. doi:10.1046/j.1460-9568.1999.00843.x

Bates, S. H., et al. (2003). Stat3 signalling is required for leptin regulation of energy balance but not reproduction. *Nature, 421*(6925), 856-859. doi:10.1038/nature01388

Baxter, M. G.& Croxson, P. L. (2012). Facing the role of the amygdala in emotional information processing. *Proceedings of the National Academy of Sciences, 109*(52), 21180. doi:10.1073/pnas.1219167110

Beauquis, J., et al. (2010). Short-term environmental enrichment enhances adult neurogenesis, vascular network and dendritic complexity in the hippocampus of type 1 diabetic mice. *PLoS One, 5*(11), e13993. doi:10.1371/journal.pone.0013993

Beaver, B. (1993). Profiles of dogs presented for aggression. *Journal of the American Animal Hospital Asscociation (USA)*.

Bechard, A. R.& Lewis, M. H. (2016). Transgenerational effects of environmental enrichment on repetitive motor behavior development. *Behav Brain Res, 307*, 145-149. doi:10.1016/j.bbr.2016.04.005

Beilharz, J. E., et al. (2016). Short-term exposure to a diet high in fat and sugar, or liquid sugar, selectively impairs hippocampal-dependent memory, with differential impacts on inflammation. *Behavioural Brain Research, 306*, 1-7. doi:10.1016/j.bbr.2016.03.018

Bekinschtein, P., et al. (2011). Effects of environmental enrichment and voluntary exercise on neurogenesis, learning and memory, and pattern separation: Bdnf as a critical variable? *Seminars in Cell & Developmental Biology, 22*(5), 536-542. doi:https://doi.org/10.1016/j.semcdb.2011.07.002

Bélanger, M., et al. (2011). Brain energy metabolism: Focus on astrocyte-neuron metabolic cooperation. *Cell Metabolism, 14*(6), 724-738. doi:https://doi.org/10.1016/j.cmet.2011.08.016

Benaroya-Milshtein, N., et al. (2004). Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. *European Journal of Neuroscience, 20*(5), 1341-1347.

Bennett, J. C., et al. (2006). Long-term continuous, but not daily, environmental enrichment reduces spatial memory decline in aged male mice. *Neurobiology of Learning and Memory, 85*(2), 139-152. doi:https://doi.org/10.1016/j.nlm.2005.09.003

Berardi, N., et al. (2007). Environmental enrichment delays the onset of memory deficits and reduces neuropathological hallmarks in a mouse model of alzheimer-like neurodegeneration. *Journal of Alzheimer's disease, 11*(3), 359-370.

Berg, C., et al. (2009). Eating patterns and portion size associated with obesity in a swedish population. *Appetite, 52*(1), 21-26. doi:https://doi.org/10.1016/j.appet.2008.07.008

Bergmann, P., et al. (1995). Environmental enrichment and aggressive behaviour: Influence on body weight and body fat in male inbred hlg mice. *Journal of Experimental Animal Science (Germany)*.

Berthoud, H. R. (2006). Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance. *Obesity (Silver Spring), 14 Suppl 5*, 197s-200s. doi:10.1038/oby.2006.308

Berthoud, H. R., et al. (2017). Blaming the brain for obesity: Integration of hedonic and homeostatic mechanisms. *Gastroenterology, 152*(7), 1728-1738. doi:10.1053/j.gastro.2016.12.050

Bisazza, A. (1981). Social organization and territorial behaviour in three strains of mice. *Bollettino di zoologia, 48*(2), 157-167. doi:10.1080/11250008109439329

Björkqvist, K. (2018). Gender differences in aggression. *Current Opinion in Psychology, 19*, 39-42. doi:https://doi.org/10.1016/j.copsyc.2017.03.030

Blundell, J., et al. (2017). Effects of once-weekly semaglutide on appetite, energy intake, control of eating, food preference and body weight in subjects with obesity. *Diabetes Obes Metab, 19*(9), 1242-1251. doi:10.1111/dom.12932

Boettcher, E. G. (1983). Preventing violent behavior. An integrated theoretical model for nursing. *Perspect Psychiatr Care, 21*(2), 54-58.

Boghossian, S., et al. (2010). Melanocortin activity in the amygdala controls appetite for dietary fat. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 298*(2), R385-R393. doi:10.1152/ajpregu.00591.2009

Book, A. S., et al. (2001). The relationship between testosterone and aggression: A meta-analysis. *Aggression and Violent Behavior, 6*(6), 579-599. doi:https://doi.org/10.1016/S1359-1789(00)00032-X

Bosch, O. J. (2013). Maternal aggression in rodents: Brain oxytocin and vasopressin mediate pup defence. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences, 368*(1631), 20130085-20130085. doi:10.1098/rstb.2013.0085

Bracken, M. B. (2009). Why animal studies are often poor predictors of human reactions to exposure. *Journal of the Royal Society of Medicine, 102*(3), 120-122. doi:10.1258/jrsm.2008.08k033

Bradley, N. L., et al. (2020). Health care practitioners' responsibility to address intimate partner violence related to the covid-19 pandemic. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne, 192*(22), E609-E610. doi:10.1503/cmaj.200634

Branchi, I., et al. (2006). Early social enrichment augments adult hippocampal bdnf levels and survival of brdu-positive cells while increasing anxiety- and "depression"-like behavior. *Journal of Neuroscience Research, 83*(6), 965-973. doi:https://doi.org/10.1002/jnr.20789

Bray, G. A.& Champagne, C. M. (2005). Beyond energy balance: There is more to obesity than kilocalories. *J Am Diet Assoc, 105*(5 Suppl 1), S17-23. doi:10.1016/j.jada.2005.02.018

Bremond-Gignac, D., et al. (2005). Combination of wagr and potocki-shaffer contiguous deletion syndromes in a patient with an 11p11.2-p14 deletion. *Eur J Hum Genet, 13*(4), 409-413. doi:10.1038/sj.ejhg.5201358

Briffa, M.& Elwood, R. W. (2004). Use of energy reserves in fighting hermit crabs. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 271*(1537), 373-379.

Briffa, M.& Sneddon, L. U. (2007). Physiological constraints on contest behaviour. *Functional Ecology, 21*(4), 627-637. doi:https://doi.org/10.1111/j.1365-2435.2006.01188.x

Brown, B. B., et al. (1986). The importance of peer group ("crowd") affiliation in adolescence. *J Adolesc, 9*(1), 73-96. doi:10.1016/s0140-1971(86)80029-x

Brown, L. M.& Clegg, D. J. (2010). Central effects of estradiol in the regulation of food intake, body weight, and adiposity. *J Steroid Biochem Mol Biol, 122*(1-3), 65-73. doi:10.1016/j.jsbmb.2009.12.005

Brown, R. E.& Milner, P. M. (2003). The legacy of donald o. Hebb: More than the hebb synapse. *Nature Reviews Neuroscience, 4*(12), 1013-1019. doi:10.1038/nrn1257

Browning, K. N.& Carson, K. E. (2021). Central neurocircuits regulating food intake in response to gut inputs-preclinical evidence. *Nutrients, 13*(3), 908. doi:10.3390/nu13030908

Bruijnzeel, A. W., et al. (2013). Anorexic effects of intra-vta leptin are similar in low-fat and high-fat-fed rats but attenuated in a subgroup of high-fat-fed obese rats. *Pharmacol Biochem Behav, 103*(3), 573-581. doi:10.1016/j.pbb.2012.10.012

Cai, H., et al. (2014). Central amygdala pkc-δ(+) neurons mediate the influence of multiple anorexigenic signals. *Nature Neuroscience, 17*(9), 1240-1248. doi:10.1038/nn.3767

Calapai, G., et al. (1998). Effects of intracerebroventricular leptin administration on food intake, body weight gain and diencephalic nitric oxide synthase activity in the mouse. *Br J Pharmacol, 125*(4), 798-802. doi:10.1038/sj.bjp.0702121

Campfield, L. A., et al. (1995). Recombinant mouse ob protein: Evidence for a peripheral signal linking adiposity and central neural networks. *Science, 269*(5223), 546-549. doi:10.1126/science.7624778

Cao, L., et al. (2011). White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. *Cell Metabolism, 14*(3), 324-338. doi:https://doi.org/10.1016/j.cmet.2011.06.020

Cao, L., et al. (2010). Environmental and genetic activation of a brain-adipocyte bdnf/leptin axis causes cancer remission and inhibition. *Cell, 142*(1), 52-64.

Carabotti, M., et al. (2015). The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. *Annals of gastroenterology, 28*(2), 203-209. Retrieved from https://pubmed.ncbi.nlm.nih.gov/25830558

Caro, J. F., et al. (1996). Decreased cerebrospinal-fluid/serum leptin ratio in obesity: A possible mechanism for leptin resistance. *Lancet, 348*(9021), 159-161. doi:10.1016/s0140-6736(96)03173-x

Carro, E., et al. (2000). Circulating insulin-like growth factor i mediates effects of exercise on the brain. *Journal of Neuroscience, 20*(8), 2926-2933.

Carter, M. E., et al. (2013). Genetic identification of a neural circuit that suppresses appetite. *Nature, 503*(7474), 111-114. doi:10.1038/nature12596

Cases, O., et al. (1995). Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking maoa. *Science, 268*(5218), 1763-1766. doi:10.1126/science.7792602

Cattaneo, A., et al. (2016). The human bdnf gene: Peripheral gene expression and protein levels as biomarkers for psychiatric disorders. *Translational Psychiatry, 6*(11), e958-e958. doi:10.1038/tp.2016.214

Centola, D. (2013). Social media and the science of health behavior. *Circulation, 127*(21), 2135-2144. doi:10.1161/circulationaha.112.101816

Chamove, A. S. (1989). Cage design reduces emotionality in mice. *Lab Anim, 23*(3), 215-219. doi:10.1258/002367789780810608

Chan, J. P., et al. (2006). Examination of behavioral deficits triggered by targeting bdnf in fetal or postnatal brains of mice. *Neuroscience, 142*(1), 49-58. doi:10.1016/j.neuroscience.2006.06.002

Chang, C.-H.& Gean, P.-W. (2019). The ventral hippocampus controls stress-provoked impulsive aggression through the ventromedial hypothalamus in post-weaning social isolation mice. *Cell Reports, 28*(5), 1195-1205.e1193. doi:https://doi.org/10.1016/j.celrep.2019.07.005

Chang, G.-Q., et al. (2005). Glucose injection reduces neuropeptide y and agouti-related protein expression in the arcuate nucleus: A possible physiological role in eating behavior. *Molecular Brain Research, 135*(1), 69-80. doi:https://doi.org/10.1016/j.molbrainres.2004.12.017

Chao, M. V. (2003). Neurotrophins and their receptors: A convergence point for many signalling pathways. *Nat Rev Neurosci, 4*(4), 299-309. doi:10.1038/nrn1078

Chao, M. V.& Bothwell, M. (2002). Neurotrophins: To cleave or not to cleave. *Neuron, 33*(1), 9-12. doi:10.1016/s0896-6273(01)00573-6

Chen, D., et al. (2016). Food environments and obesity: Household diet expenditure versus food deserts. *Am J Public Health, 106*(5), 881-888. doi:10.2105/ajph.2016.303048

Cheon, B. K.& Hong, Y. Y. (2017). Mere experience of low subjective socioeconomic status stimulates appetite and food intake. *Proc Natl Acad Sci U S A, 114*(1), 72-77. doi:10.1073/pnas.1607330114

Chiavegatto, S., et al. (2001). Brain serotonin dysfunction accounts for aggression in male mice lacking neuronal nitric oxide synthase. *Proc Natl Acad Sci U S A, 98*(3), 1277-1281. doi:10.1073/pnas.031487198

Chourbaji, S., et al. (2012). The impact of environmental enrichment on sex-specific neurochemical circuitries - effects on brain-derived neurotrophic factor and the serotonergic system. *Neuroscience, 220*, 267-276. doi:10.1016/j.neuroscience.2012.06.016

Christakis, N. A.& Fowler, J. H. (2007). The spread of obesity in a large social network over 32 years. *N Engl J Med, 357*(4), 370-379. doi:10.1056/NEJMsa066082

Christou, G. A., et al. (2019). Semaglutide as a promising antiobesity drug. *Obesity Reviews, 20*(6), 805-815. doi:https://doi.org/10.1111/obr.12839

Church, T. S., et al. (2011). Trends over 5 decades in u.S. Occupation-related physical activity and their associations with obesity. *PLoS One, 6*(5), e19657. doi:10.1371/journal.pone.0019657

Cintrón-Colón, A. F., et al. (2020). Gdnf synthesis, signaling, and retrograde transport in motor neurons. *Cell and Tissue Research, 382*(1), 47-56. doi:10.1007/s00441-020-03287-6

Claret, M., et al. (2007). Ampk is essential for energy homeostasis regulation and glucose sensing by pomc and agrp neurons. *J Clin Invest, 117*(8), 2325-2336. doi:10.1172/jci31516

Clegg, D. J., et al. (2003). Differential sensitivity to central leptin and insulin in male and female rats. *Diabetes, 52*(3), 682-687. doi:10.2337/diabetes.52.3.682

Cline, M. A., et al. (2007). Xenin reduces feed intake by activating the ventromedial hypothalamus and influences gastrointestinal transit rate in chicks. *Behav Brain Res, 179*(1), 28-32. doi:10.1016/j.bbr.2007.01.008

Cohen, P., et al. (2001). Selective deletion of leptin receptor in neurons leads to obesity. *The Journal of Clinical Investigation, 108*(8), 1113-1121. doi:10.1172/JCI13914

Comeau, W., et al. (2008). Therapeutic effects of complex rearing or bfgf after perinatal frontal lesions. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology, 50*(2), 134-146.

Conroy, S. (2021). *Family violence in canada: A statistical profile, 2019*. Retrieved from https://www150.statcan.gc.ca/n1/pub/85-002-x/2021001/article/00001-eng.htm

Conway, J. A., et al. (2020). Gdnf/ret signaling in dopamine neurons in vivo. *Cell and Tissue Research, 382*(1), 135-146. doi:10.1007/s00441-020-03268-9

Cooke, J. H., et al. (2009). Peripheral and central administration of xenin and neurotensin suppress food intake in rodents. *Obesity (Silver Spring), 17*(6), 1135-1143. doi:10.1038/oby.2008.652

Côté, S. M., et al. (2007). The joint development of physical and indirect aggression: Predictors of continuity and change during childhood. *Dev Psychopathol, 19*(1), 37-55. doi:10.1017/s0954579407070034

Couppis, M. H.& Kennedy, C. H. (2008). The rewarding effect of aggression is reduced by nucleus accumbens dopamine receptor antagonism in mice. *Psychopharmacology (Berl), 197*(3), 449-456. doi:10.1007/s00213-007-1054-y

Craig, S. L., et al. (2018). Emerging therapeutic potential for xenin and related peptides in obesity and diabetes. *Diabetes/Metabolism Research and Reviews, 34*(6), e3006. doi:https://doi.org/10.1002/dmrr.3006

da Silva, R. P., et al. (2014). Leptin resistance is not the primary cause of weight gain associated with reduced sex hormone levels in female mice. *Endocrinology, 155*(11), 4226-4236. doi:10.1210/en.2014-1276

Dabbs, J. M., Jr., et al. (1991). Salivary testosterone and cortisol among late adolescent male offenders. *J Abnorm Child Psychol, 19*(4), 469-478. doi:10.1007/bf00919089

Dandi, E., et al. (2018). Beneficial effects of environmental enrichment on behavior, stress reactivity and synaptophysin/bdnf expression in hippocampus following early life stress. *International Journal of Developmental Neuroscience, 67*, 19-32. doi:https://doi.org/10.1016/j.ijdevneu.2018.03.003

Darke, S. (2010). The toxicology of homicide offenders and victims: A review. *Drug Alcohol Rev, 29*(2), 202-215. doi:10.1111/j.1465-3362.2009.00099.x

Darvishi, N., et al. (2015). Alcohol-related risk of suicidal ideation, suicide attempt, and completed suicide: A meta-analysis. *PLoS One, 10*(5), e0126870. doi:10.1371/journal.pone.0126870

Dauvergne, M., et al. (2001). *Children witnessing family violence*: Canadian Centre for Justice Statistics.

Davidson, T. L., et al. (2014). A view of obesity as a learning and memory disorder. *J Exp Psychol Anim Learn Cogn, 40*(3), 261-279. doi:10.1037/xan0000029

Day, H. E. W., et al. (2008). Conditioned fear inhibits c-fos mrna expression in the central extended amygdala. *Brain research, 1229*, 137-146. doi:10.1016/j.brainres.2008.06.085

de Almeida, R. M. M., et al. (2005). Escalated aggressive behavior: Dopamine, serotonin and gaba. *European Journal of Pharmacology, 526*(1), 51-64. doi:https://doi.org/10.1016/j.ejphar.2005.10.004

de Andrade, I. S., et al. (2015). Diet-induced obesity impairs hypothalamic glucose sensing but not glucose hypothalamic extracellular levels, as measured by microdialysis. *Nutrition & Diabetes, 5*(6), e162-e162. doi:10.1038/nutd.2015.12

De Boer, S., et al. (2009). The vicious cycle towards violence: Focus on the negative feedback mechanisms of brain serotonin neurotransmission. *Frontiers in Behavioral Neuroscience, 3*(52). doi:10.3389/neuro.08.052.2009

de Git, K. C. G., et al. (2018). Is leptin resistance the cause or the consequence of diet-induced obesity? *International Journal of Obesity, 42*(8), 1445-1457. doi:10.1038/s41366-018-0111-4

de Souza, R. M., et al. (2019). Behavioural, metabolic and neurochemical effects of environmental enrichment in high-fat cholesterol-enriched diet-fed mice. *Behavioural Brain Research, 359*, 648-656. doi:https://doi.org/10.1016/j.bbr.2018.09.022

Di Lorenzo, L., et al. (2003). Effect of shift work on body mass index: Results of a study performed in 319 glucose-tolerant men working in a southern italian industry. *Int J Obes Relat Metab Disord, 27*(11), 1353-1358. doi:10.1038/sj.ijo.0802419

Diamond, M. C., et al. (1985). Plasticity in the 904-day-old male rat cerebral cortex. *Exp Neurol, 87*(2), 309-317. doi:10.1016/0014-4886(85)90221-3

Diamond, M. C., et al. (1964). The effects of an enriched environment on the histology of the rat cerebral cortex. *J Comp Neurol, 123*, 111-120. doi:10.1002/cne.901230110

Diamond, M. C., et al. (1966). Increases in cortical depth and glia numbers in rats subjected to enriched environment. *J Comp Neurol, 128*(1), 117-126. doi:10.1002/cne.901280110

Egan, M. F., et al. (2003). The bdnf val66met polymorphism affects activity-dependent secretion of bdnf and human memory and hippocampal function. *Cell, 112*(2), 257-269. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12553913

Eisenmann, J. C. (2006). Insight into the causes of the recent secular trend in pediatric obesity: Common sense does not always prevail for complex, multi-factorial phenotypes. *Prev Med, 42*(5), 329-335. doi:10.1016/j.ypmed.2006.02.002

El-Haschimi, K., et al. (2000). Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *The Journal of Clinical Investigation, 105*(12), 1827-1832. doi:10.1172/JCI9842

Elizondo-Vega, R., et al. (2015). The role of tanycytes in hypothalamic glucosensing. *Journal of cellular and molecular medicine, 19*(7), 1471-1482. doi:10.1111/jcmm.12590

Ellacott, K. L.& Cone, R. D. (2006). The role of the central melanocortin system in the regulation of food intake and energy homeostasis: Lessons from mouse models. *Philos Trans R Soc Lond B Biol Sci, 361*(1471), 1265-1274. doi:10.1098/rstb.2006.1861

Elliott, F. A. (1977). Propranolol for the control of belligerent behavior following acute brain damage. *Ann Neurol, 1*(5), 489-491. doi:10.1002/ana.410010516

Emeson, R. B.& Morabito, M. V. (2005). Food fight: The npy-serotonin link between aggression and feeding behavior. *Sci STKE, 2005*(277), pe12. doi:10.1126/stke.2772005pe12

Emmerson, P. J., et al. (2017). The metabolic effects of gdf15 are mediated by the orphan receptor gfral. *Nature Medicine, 23*(10), 1215-1219. doi:10.1038/nm.4393

Ernst, C., et al. (2012). Highly penetrant alterations of a critical region including bdnf in human psychopathology and obesity. *Archives of General Psychiatry, 69*(12), 1238-1246. doi:10.1001/archgenpsychiatry.2012.660

Eskola, S.& Kaliste-Korhonen, E. (1999). Aspen wood-wool is preferred as a resting place, but does not affect intracage fighting of male balb/c and c57bl/6j mice. *Lab Anim, 33*(2), 108-121. doi:10.1258/002367799780578273

Espelage, D. L., et al. (2003). Examination of peer-group contextual effects on aggression during early adolescence. *Child Dev, 74*(1), 205-220. doi:10.1111/1467-8624.00531

Espina, V., et al. (2006). Laser-capture microdissection. *Nature Protocols, 1*(2), 586-603. doi:10.1038/nprot.2006.85

Evans, I., et al. (2021). The influence of alcohol consumption on fighting, shoplifting and vandalism in young adults. *International journal of environmental research and public health, 18*(7), 3509. doi:10.3390/ijerph18073509

Faccidomo, S., et al. (2006). Neurochemistry and molecular neurobiology of aggressive behavior. In (pp. 285-336).

Faherty, C. J., et al. (2005). Environmental enrichment in adulthood eliminates neuronal death in experimental parkinsonism. *Molecular Brain Research, 134*(1), 170-179. doi:https://doi.org/10.1016/j.molbrainres.2004.08.008

Falkner, A. L., et al. (2016). Hypothalamic control of male aggression-seeking behavior. *Nature Neuroscience, 19*(4), 596-604. doi:10.1038/nn.4264

FDA Press Release. (2021). Fda approves new drug treatment for chronic weight management, first since 2014. *Press Announcements* Retrieved from https://www.fda.gov/news-events/press-announcements/fda-approves-new-drug-treatment-chronic-weight-management-first-2014

Feurle, G. E., et al. (2003). Xenin plasma concentrations during modified sham feeding and during meals of different composition demonstrated by radioimmunoassay and chromatography. *Regul Pept, 111*(1-3), 153-159. doi:10.1016/s0167-0115(02)00281-1

Fischer, A., et al. (2007). Recovery of learning and memory is associated with chromatin remodelling. *Nature, 447*(7141), 178-182.

Fletcher, A., et al. (2011). You are what your friends eat: Systematic review of social network analyses of young people's eating behaviours and bodyweight. *J Epidemiol Community Health, 65*(6), 548-555. doi:10.1136/jech.2010.113936

Foglesong, G. D., et al. (2016). Role of hypothalamic vgf in energy balance and metabolic adaption to environmental enrichment in mice. *Endocrinology, 157*(3), 983-996. doi:10.1210/en.2015-1627

Foglesong, G. D., et al. (2019). Enriched environment inhibits breast cancer progression in obese models with intact leptin signaling. *Endocr Relat Cancer, 26*(5), 483-495. doi:10.1530/ERC-19-0075

Fontaine, K. R., et al. (2003). Years of life lost due to obesity. *Jama, 289*(2), 187-193. doi:10.1001/jama.289.2.187

Fowler, J. H.& Christakis, N. A. (2008). Estimating peer effects on health in social networks: A response to cohen-cole and fletcher; and trogdon, nonnemaker, and pais. *J Health Econ, 27*(5), 1400-1405. doi:10.1016/j.jhealeco.2008.07.001

Frank, L. D., et al. (2004). Obesity relationships with community design, physical activity, and time spent in cars. *Am J Prev Med, 27*(2), 87-96. doi:10.1016/j.amepre.2004.04.011

Fry, M.& Ferguson, A. V. (2010). Ghrelin: Central nervous system sites of action in regulation of energy balance. *Int J Pept, 2010*. doi:10.1155/2010/616757

Fulgenzi, G., et al. (2020). Novel metabolic role for bdnf in pancreatic β-cell insulin secretion. *Nature Communications, 11*(1), 1950. doi:10.1038/s41467-020-15833-5

Furnes, M. W., et al. (2009). Development of obesity is associated with increased calories per meal rather than per day. A study of high-fat diet-induced obesity in young rats. *Obesity Surgery, 19*(10), 1430-1438. doi:10.1007/s11695-009-9863-1

Gabbott, P. L., et al. (2005). Prefrontal cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. *J Comp Neurol, 492*(2), 145-177. doi:10.1002/cne.20738

Gan, G., et al. (2014). Alcohol-induced impairment of inhibitory control is linked to attenuated brain responses in right fronto-temporal cortex. *Biological psychiatry, 76*(9), 698-707. doi:10.1016/j.biopsych.2013.12.017

García, M., et al. (2003). Hypothalamic ependymal-glial cells express the glucose transporter glut2, a protein involved in glucose sensing. *J Neurochem, 86*(3), 709-724. doi:10.1046/j.1471-4159.2003.01892.x

Garner, J. P., et al. (2017). Introducing therioepistemology: The study of how knowledge is gained from animal research. *Lab Animal, 46*(4), 103-113. doi:10.1038/laban.1224

Garner, J. P., et al. (2011). Reverse-translational biomarker validation of abnormal repetitive behaviors in mice: An illustration of the 4p's modeling approach. *Behavioural Brain Research, 219*(2), 189-196.

Garofalo, S., et al. (2015). Enriched environment reduces glioma growth through immune and non-immune mechanisms in mice. *Nature Communications, 6*(1), 1-13.

Gaskill, B. N., et al. (2017). The effect of early life experience, environment, and genetic factors on spontaneous home-cage aggression-related wounding in male c57bl/6 mice. *Lab Anim (NY), 46*(4), 176-184. doi:10.1038/laban.1225

Geerling, J. C., et al. (2010). Paraventricular hypothalamic nucleus: Axonal projections to the brainstem. *J Comp Neurol, 518*(9), 1460-1499. doi:10.1002/cne.22283

Georgiev, A. V., et al. (2013). When violence pays: A cost-benefit analysis of aggressive behavior in animals and humans. *Evolutionary psychology : an international journal of evolutionary approaches to psychology and behavior, 11*(3), 678-699.

Ghiglieri, O., et al. (1997). Palatable food induces an appetitive behaviour in satiated rats which can be inhibited by chronic stress. *Behav Pharmacol, 8*(6-7), 619-628. doi:10.1097/00008877-199711000-00018

Gibon, J., et al. (2016). Opposing presynaptic roles of bdnf and probdnf in the regulation of persistent activity in the entorhinal cortex. *Mol Brain, 9*, 23. doi:10.1186/s13041-016-0203-9

Giles, J. M., et al. (2018). Effect of environmental enrichment on aggression in balb/cj and balb/cbyj mice monitored by using an automated system. *J Am Assoc Lab Anim Sci*. doi:10.30802/AALAS-JAALAS-17-000122

Gionet, L.& Roshanafshar, S. (2013). *Select health indicators of first nations people living off reserve, métis and inuit.* . Retrieved from https://www150.statcan.gc.ca/n1/pub/82-624-x/2013001/article/11763-eng.htm

Girbovan, C.& Plamondon, H. (2013). Environmental enrichment in female rodents: Considerations in the effects on behavior and biochemical markers. *Behavioural Brain Research, 253*, 178-190. doi:https://doi.org/10.1016/j.bbr.2013.07.018

Gogos, J. A., et al. (1998). Catechol-o-methyltransferase-deficient mice exhibit sexually dimorphic changes in catecholamine levels and behavior. *Proc Natl Acad Sci U S A, 95*(17), 9991-9996. doi:10.1073/pnas.95.17.9991

Gorski, J. A., et al. (2003). Learning deficits in forebrain-restricted brain-derived neurotrophic factor mutant mice. *Neuroscience, 121*(2), 341-354. doi:10.1016/s0306-4522(03)00426-3

Gray, J., et al. (2007). Functional characterization of human ntrk2 mutations identified in patients with severe early-onset obesity. *International Journal of Obesity, 31*(2), 359-364. doi:10.1038/sj.ijo.0803390

Gray, J., et al. (2006). Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (bdnf) gene. *Diabetes, 55*(12), 3366-3371. doi:10.2337/db06-0550

Greenough, W. T., et al. (1973). Effects of rearing complexity on dendritic branching in frontolateral and temporal cortex of the rat. *Experimental Neurology, 41*(2), 371-378. doi:https://doi.org/10.1016/0014-4886(73)90278-1

Greer, P. L.& Greenberg, M. E. (2008). From synapse to nucleus: Calcium-dependent gene transcription in the control of synapse development and function. *Neuron, 59*(6), 846-860. doi:10.1016/j.neuron.2008.09.002

Gregg, T. R.& Siegel, A. (2001). Brain structures and neurotransmitters regulating aggression in cats: Implications for human aggression. *Prog Neuropsychopharmacol Biol Psychiatry, 25*(1), 91-140. doi:10.1016/s0278-5846(00)00150-0

Gross, A. N., et al. (2012). Cage-induced stereotypies, perseveration and the effects of environmental enrichment in laboratory mice. *Behavioural Brain Research, 234*(1), 61-68.

Gualtieri, F., et al. (2017). Effects of environmental enrichment on doublecortin and bdnf expression along the dorso-ventral axis of the dentate gyrus. *Frontiers in neuroscience, 11*(488). doi:10.3389/fnins.2017.00488

Guo, M., et al. (2004). Sex difference in psychological behavior changes induced by long-term social isolation in mice. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 28*(1), 115-121. doi:https://doi.org/10.1016/j.pnpbp.2003.09.027

Haemisch, A.& Gärtner, K. (1994). The cage design affects intermale aggression in small groups of male laboratory mice: Strain specific consequences on social organization, and endocrine activations in two inbred strains (dba/2j and cba/j). *J Exp Anim Sci, 36*(4-5), 101-116.

Haemisch, A.& Gärtner, K. (1997). Effects of cage enrichment on territorial aggression and stress physiology in male laboratory mice. *Acta Physiol Scand Suppl, 640*, 73-76.

Haemisch, A., et al. (1994). Effects of environmental enrichment on aggressive behavior, dominance hierarchies, and endocrine states in male dba/2j mice. *Physiol Behav, 56*(5), 1041-1048. doi:10.1016/0031-9384(94)90341-7

Hakonen, E., et al. (2018). Manf protects human pancreatic beta cells against stress-induced cell death. *Diabetologia, 61*(10), 2202-2214. doi:10.1007/s00125-018-4687-y

Halaas, J. L., et al. (1997). Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proceedings of the National Academy of Sciences of the United States of America, 94*(16), 8878-8883. doi:10.1073/pnas.94.16.8878

Han, J. C. (2016). Chapter three - rare syndromes and common variants of the brain-derived neurotrophic factor gene in human obesity. In Y.-X. Tao (Ed.), *Progress in molecular biology and translational science* (Vol. 140, pp. 75-95): Academic Press.

Han, J. C., et al. (2008). Brain-derived neurotrophic factor and obesity in the wagr syndrome. *New England Journal of Medicine, 359*(9), 918-927. doi:10.1056/NEJMoa0801119

Hannapel, R. C., et al. (2017). Ventral hippocampal neurons inhibit postprandial energy intake. *Hippocampus, 27*(3), 274-284. doi:https://doi.org/10.1002/hipo.22692

Harasztosi, C., et al. (2020). Differential deletion of gdnf in the auditory system leads to altered sound responsiveness. *Journal of Neuroscience Research, 98*(9), 1764-1779. doi:https://doi.org/10.1002/jnr.24544

Harrington, A. W., et al. (2002). Activation of rac gtpase by p75 is necessary for c-jun n-terminal kinase-mediated apoptosis. *The Journal of Neuroscience, 22*(1), 156. doi:10.1523/JNEUROSCI.22-01-00156.2002

Harrison, L., et al. (2019). Fluorescent blood-brain barrier tracing shows intact leptin transport in obese mice. *Int J Obes (Lond), 43*(6), 1305-1318. doi:10.1038/s41366-018-0221-z

Hatori, M., et al. (2012). Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metabolism, 15*(6), 848-860. doi:https://doi.org/10.1016/j.cmet.2012.04.019

Hebb, D. O. (1947). The effects of early experience on problem-solving at maturity. *American Psychologist, 2*, 306-307. Retrieved from https://ci.nii.ac.jp/naid/10024426978/en/

Hebben, N., et al. (1985). Diminished ability to interpret and report internal states after bilateral medial temporal resection: Case h.M. *Behav Neurosci, 99*(6), 1031-1039. doi:10.1037//0735-7044.99.6.1031

Heinz, A. J., et al. (2011). Cognitive and neurobiological mechanisms of alcohol-related aggression. *Nat Rev Neurosci, 12*(7), 400-413. doi:10.1038/nrn3042

Hellerstedt, W. L.& Jeffery, R. W. (1997). The association of job strain and health behaviours in men and women. *Int J Epidemiol, 26*(3), 575-583. doi:10.1093/ije/26.3.575

Henderson, N. D. (1976). Short exposures to enriched environments can increase genetic variability of behavior in mice. *Dev Psychobiol, 9*(6), 549-553. doi:10.1002/dev.420090608

Henderson, Y. O., et al. (2013). Hippocampal neurons inhibit meal onset. *Hippocampus, 23*(1), 100-107. doi:https://doi.org/10.1002/hipo.22062

Herberg, L. J. (1960). Hunger reduction produced by injecting glucose into the lateral ventricle of the rat. *Nature, 187*(4733), 245-246. doi:10.1038/187245b0

Hess, W. R. (1928). Stammganglien-reizversuche. Berichte der gesamten. *Physiologie, 42*, 554-555.

Hoane, M. R., et al. (1999). Differential in vivo effects of neurturin and glial cell-line-derived neurotrophic factor. *Exp Neurol, 160*(1), 235-243. doi:10.1006/exnr.1999.7175

Hobbs, J., et al. (2017). Disruption of brain-derived neurotrophic factor production from promoter i causes social behavioral deficits. *Biological Psychiatry, 81*(10 Suppl), S201-202.

Holloway, R. L. (1966). Dendritic branching: Some preliminary results of training and complexity in rat visual cortex. *Brain research, 2*(4), 393-396. doi:https://doi.org/10.1016/0006-8993(66)90009-6

Hommel, J. D., et al. (2006). Leptin receptor signaling in midbrain dopamine neurons regulates feeding. *Neuron, 51*(6), 801-810. doi:10.1016/j.neuron.2006.08.023

Hong, E. J., et al. (2008). A biological function for the neuronal activity-dependent component of bdnf transcription in the development of cortical inhibition. *Neuron, 60*(4), 610-624. doi:10.1016/j.neuron.2008.09.024

Hong, W., et al. (2014). Antagonistic control of social versus repetitive self-grooming behaviors by separable amygdala neuronal subsets. *Cell, 158*(6), 1348-1361. doi:https://doi.org/10.1016/j.cell.2014.07.049

Hossain, M. S., et al. (2018). Glucose can epigenetically alter the gene expression of neurotrophic factors in the murine brain cells. *Mol Neurobiol, 55*(4), 3408-3425. doi:10.1007/s12035-017-0578-3

Howerton, C. L., et al. (2008). Effects of a running wheel-igloo enrichment on aggression, hierarchy linearity, and stereotypy in group-housed male cd-1 (icr) mice. *Applied Animal Behaviour Science, 115*(1-2), 90-103. doi:10.1016/j.applanim.2008.05.004

Hrabovszky, E., et al. (2005). Neurochemical characterization of hypothalamic neurons involved in attack behavior: Glutamatergic dominance and co-expression of thyrotropin-releasing hormone in a subset of glutamatergic neurons. *Neuroscience, 133*(3), 657-666. doi:10.1016/j.neuroscience.2005.03.042

Hsieh, C.-F., et al. (2019). Acute glucose fluctuation impacts microglial activity, leading to inflammatory activation or self-degradation. *Scientific Reports, 9*(1), 840. doi:10.1038/s41598-018-37215-0

Hsu, J.-Y., et al. (2017). Non-homeostatic body weight regulation through a brainstem-restricted receptor for gdf15. *Nature, 550*(7675), 255-259. doi:10.1038/nature24042

Hsu, T. M., et al. (2015). Effects of sucrose and high fructose corn syrup consumption on spatial memory function and hippocampal neuroinflammation in adolescent rats. *Hippocampus, 25*(2), 227-239. doi:10.1002/hipo.22368

Hu, Y. S., et al. (2013). Molecular mechanisms of environmental enrichment: Impairments in akt/gsk3β, neurotrophin-3 and creb signaling. *PLoS One, 8*(5), e64460. doi:10.1371/journal.pone.0064460

Huang, E. J.& Reichardt, L. F. (2001). Neurotrophins: Roles in neuronal development and function. *Annu Rev Neurosci, 24*, 677-736. doi:10.1146/annurev.neuro.24.1.677

Huang, E. J.& Reichardt, L. F. (2003). Trk receptors: Roles in neuronal signal transduction. *Annual Review of Biochemistry, 72*(1), 609-642. doi:10.1146/annurev.biochem.72.121801.161629

Hudson, J., et al. (1995). Glial cell line-derived neurotrophic factor augments midbrain dopaminergic circuits in vivo. *Brain Research Bulletin, 36*(5), 425-432. doi:https://doi.org/10.1016/0361-9230(94)00224-O

Hurst, J. L., et al. (1993). The role of substrate odours in maintaining social tolerance between male house mice, mus musculus domesticus. *Animal Behaviour, 45*(5), 997-1006. doi:https://doi.org/10.1006/anbe.1993.1117

Huszar, D., et al. (1997). Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell, 88*(1), 131-141. doi:10.1016/s0092-8674(00)81865-6

Hüttenrauch, M., et al. (2016). Effects of long-term environmental enrichment on anxiety, memory, hippocampal plasticity and overall brain gene expression in c57bl6 mice. *Frontiers in Molecular Neuroscience, 9*(62). doi:10.3389/fnmol.2016.00062

Hwang, J. J., et al. (2017). The human brain produces fructose from glucose. *JCI Insight, 2*(4), e90508-e90508. doi:10.1172/jci.insight.90508

Ibáñez, C. F.& Andressoo, J. O. (2017). Biology of gdnf and its receptors - relevance for disorders of the central nervous system. *Neurobiol Dis, 97*(Pt B), 80-89. doi:10.1016/j.nbd.2016.01.021

Ibeas, K., et al. (2021). Hypothalamus-skeletal muscle crosstalk during exercise and its role in metabolism modulation. *Biochem Pharmacol, 190*, 114640. doi:10.1016/j.bcp.2021.114640

Ilchibaeva, T. V., et al. (2015). Brain-derived neurotrophic factor (bdnf) and its precursor (probdnf) in genetically defined fear-induced aggression. *Behav Brain Res, 290*, 45-50. doi:10.1016/j.bbr.2015.04.041

Ilchibaeva, T. V., et al. (2018). Genetically defined fear-induced aggression: Focus on bdnf and its receptors. *Behav Brain Res, 343*, 102-110. doi:10.1016/j.bbr.2018.01.034

Iqbal, M., et al. (2019). Estimating the global economic cost of violence: Methodology improvement and estimate updates. *Defence and Peace Economics*, 1-24. doi:10.1080/10242694.2019.1689485

Ito, W., et al. (2011). Bdnf-restricted knockout mice as an animal model for aggression. *Genes Brain Behav, 10*(3), 365-374. doi:10.1111/j.1601-183X.2010.00676.x

Izquierdo, A. G., et al. (2019). Leptin, obesity, and leptin resistance: Where are we 25 years later? *Nutrients, 11*(11). doi:10.3390/nu11112704

Jaggar, M., et al. (2019). 5-ht2a receptor loss does not alter acute fluoxetine-induced anxiety and exhibit sex-dependent regulation of cortical immediate early gene expression. *Neuronal Signal, 3*(1), NS20180205. doi:10.1042/NS20180205

Jennings, J. H., et al. (2015). Visualizing hypothalamic network dynamics for appetitive and consummatory behaviors. *Cell, 160*(3), 516-527. doi:10.1016/j.cell.2014.12.026

Jensen, E. (2014). Technical review: In situ hybridization. *The Anatomical Record, 297*(8), 1349-1353. doi:https://doi.org/10.1002/ar.22944

Jha, S., et al. (2016). Antidepressive and bdnf effects of enriched environment treatment across ages in mice lacking bdnf expression through promoter iv. *Translational Psychiatry, 6*(9), e896-e896. doi:10.1038/tp.2016.160

Jirkof, P., et al. (2020). The effect of group size, age and handling frequency on inter-male aggression in cd 1 mice. *Scientific Reports, 10*(1), 2253-2253. doi:10.1038/s41598-020-59012-4

Jochman, K. A., et al. (2005). Corticotropin-releasing factor-1 receptors in the basolateral amygdala mediate stress-induced anorexia. *Behavioral Neuroscience, 119*(6), 1448-1458. doi:10.1037/0735-7044.119.6.1448

Johansson, B. B. (1996). Functional outcome in rats transferred to an enriched environment 15 days after focal brain ischemia. *Stroke, 27*(2), 324-326.

Johnson, H. (1996). *Dangerous domains : Violence against women in canada / holly johnson*. Toronto ; Albany: Nelson Canada.

Jordan, S. D., et al. (2010). Sensing the fuels: Glucose and lipid signaling in the cns controlling energy homeostasis. *Cellular and Molecular Life Sciences, 67*(19), 3255-3273. doi:10.1007/s00018-010-0414-7

Jurgens, H. A.& Johnson, R. W. (2012). Environmental enrichment attenuates hippocampal neuroinflammation and improves cognitive function during influenza infection. *Brain Behav Immun, 26*(6), 1006-1016. doi:10.1016/j.bbi.2012.05.015

Juvonen, J., et al. (2013). Physical aggression, spreading of rumors, and social prominence in early adolescence: Reciprocal effects supporting gender similarities? *Journal of Youth and Adolescence, 42*(12), 1801-1810. doi:10.1007/s10964-012-9894-0

Kairupan, T. S., et al. (2016). Role of gastrointestinal hormones in feeding behavior and obesity treatment. *J Gastroenterol, 51*(2), 93-103. doi:10.1007/s00535-015-1118-4

Kaliste, E. K., et al. (2006). Environmental modification and agonistic behavior in nih/s male mice: Nesting material enhances fighting but shelters prevent it. *Comp Med, 56*(3), 202-208.

Kamegai, J., et al. (2001). Chronic central infusion of ghrelin increases hypothalamic neuropeptide y and agouti-related protein mrna levels and body weight in rats. *Diabetes, 50*(11), 2438. doi:10.2337/diabetes.50.11.2438

Kanoski, S. E.& Grill, H. J. (2017). Hippocampus contributions to food intake control: Mnemonic, neuroanatomical, and endocrine mechanisms. *Biological psychiatry, 81*(9), 748-756. doi:10.1016/j.biopsych.2015.09.011

Karl, T., et al. (2004). Y1 receptors regulate aggressive behavior by modulating serotonin pathways. *Proceedings of the National Academy of Sciences of the United States of America, 101*(34), 12742. doi:10.1073/pnas.0404085101

Karpova, N. N., et al. (2011). Fear erasure in mice requires synergy between antidepressant drugs and extinction training. *Science, 334*(6063), 1731-1734. doi:10.1126/science.1214592

Keith, S. W., et al. (2006). Putative contributors to the secular increase in obesity: Exploring the roads less traveled. *Int J Obes (Lond), 30*(11), 1585-1594. doi:10.1038/sj.ijo.0803326

Kempermann, G. (2019). Environmental enrichment, new neurons and the neurobiology of individuality. *Nature Reviews Neuroscience, 20*(4), 235-245. doi:10.1038/s41583-019-0120-x

Kempermann, G., et al. (2002). Neuroplasticity in old age: Sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. *Ann Neurol, 52*(2), 135-143. doi:10.1002/ana.10262

Kempermann, G., et al. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature, 386*(6624), 493-495. doi:10.1038/386493a0

Kernie, S. G., et al. (2000). Bdnf regulates eating behavior and locomotor activity in mice. *The EMBO journal, 19*(6), 1290-1300. doi:https://doi.org/10.1093/emboj/19.6.1290

Keyvani, K., et al. (2004). Gene expression profiling in the intact and injured brain following environmental enrichment. *Journal of Neuropathology & Experimental Neurology, 63*(6), 598-609.

Kim, E. R., et al. (2016). Xenin-induced feeding suppression is not mediated through the activation of central extracellular signal-regulated kinase signaling in mice. *Behav Brain Res, 312*, 118-126. doi:10.1016/j.bbr.2016.06.026

Kim, E. R.& Mizuno, T. M. (2010). Xenin delays gastric emptying rate and activates the brainstem in mice. *Neurosci Lett, 481*(1), 59-63. doi:10.1016/j.neulet.2010.06.055

Kim, M. S., et al. (2004). Anti-obesity effects of alpha-lipoic acid mediated by suppression of hypothalamic amp-activated protein kinase. *Nat Med, 10*(7), 727-733. doi:10.1038/nm1061

Kim, Y. S.& Leventhal, B. (2008). Bullying and suicide. A review. *Int J Adolesc Med Health, 20*(2), 133-154. doi:10.1515/ijamh.2008.20.2.133

King, B. M., et al. (2003). Obesity-inducing amygdala lesions: Examination of anterograde degeneration and retrograde transport. *Am J Physiol Regul Integr Comp Physiol, 284*(4), R965-982. doi:10.1152/ajpregu.00249.2002

King, B. M., et al. (1998). Amygdaloid-lesion hyperphagia: Impaired response to caloric challenges and altered macronutrient selection. *Am J Physiol, 275*(2), R485-493. doi:10.1152/ajpregu.1998.275.2.R485

Kingston, S.& Hoffman-Goetz, L. (1996). Effect of environmental enrichment and housing density on immune system reactivity to acute exercise stress. *Physiology & Behavior, 60*(1), 145-150.

Kininmonth, A. R., et al. (2021). The relationship between the home environment and child adiposity: A systematic review. *International Journal of Behavioral Nutrition and Physical Activity, 18*(1), 4. doi:10.1186/s12966-020-01073-9

Kinlen, D., et al. (2017). Complications of obesity. *QJM: An International Journal of Medicine, 111*(7), 437-443. doi:10.1093/qjmed/hcx152

Kitahara, C. M., et al. (2014). Association between class iii obesity (bmi of 40-59 kg/m2) and mortality: A pooled analysis of 20 prospective studies. *PLoS Med, 11*(7), e1001673. doi:10.1371/journal.pmed.1001673

Kolb, B.& Nonneman, A. J. (1974). Frontolimbic lesions and social behavior in the rat. *Physiol Behav, 13*(5), 637-643. doi:10.1016/0031-9384(74)90234-0

Komori, T., et al. (2006). Induction of brain-derived neurotrophic factor by leptin in the ventromedial hypothalamus. *Neuroscience, 139*(3), 1107-1115. doi:10.1016/j.neuroscience.2005.12.066

Koopmans, G. C., et al. (2006). Circulating insulin-like growth factor i and functional recovery from spinal cord injury under enriched housing conditions. *European Journal of Neuroscience, 23*(4), 1035-1046.

Kopra, J., et al. (2015). Gdnf is not required for catecholaminergic neuron survival in vivo. *Nat Neurosci, 18*(3), 319-322. doi:10.1038/nn.3941

Kopra, J. J., et al. (2017). Dampened amphetamine-stimulated behavior and altered dopamine transporter function in the absence of brain gdnf. *J Neurosci, 37*(6), 1581-1590. doi:10.1523/jneurosci.1673-16.2016

Krashes, M. J., et al. (2016). Melanocortin-4 receptor-regulated energy homeostasis. *Nat Neurosci, 19*(2), 206-219. doi:10.1038/nn.4202

Kretschmer, T., et al. (2014). The association between peer and own aggression is moderated by the bdnf val-met polymorphism. *J Res Adolesc, 24*(1), 177-185. doi:10.1111/jora.12050

Krug, E. G., et al. (2002). The world report on violence and health. *Lancet, 360*(9339), 1083-1088. doi:10.1016/s0140-6736(02)11133-0

Kruk, M. R., et al. (1979). The induction of aggressive behaviour by electrical stimulation in the hypothalamus of male rats. *Behaviour, 70*(3-4), 292-322. doi:10.1163/156853979x00106

Kruk, M. R., et al. (1983). Discriminant analysis of the localization of aggression-inducing electrode placements in the hypothalamus of male rats. *Brain research, 260*(1), 61-79. doi:https://doi.org/10.1016/0006-8993(83)90764-3

Kruk, M. R., et al. (1998). The hypothalamus: Cross-roads of endocrine and behavioural regulation in grooming and aggression. *Neuroscience & Biobehavioral Reviews, 23*(2), 163-177. doi:https://doi.org/10.1016/S0149-7634(98)00018-9

Kulikov, A. V., et al. (2012). A pharmacological evidence of positive association between mouse intermale aggression and brain serotonin metabolism. *Behavioural Brain Research, 233*(1), 113-119. doi:https://doi.org/10.1016/j.bbr.2012.04.031

Kurata, K., et al. (1986). D-glucose suppression of eating after intra-third ventricle infusion in rat. *Physiology & Behavior, 37*(4), 615-620. doi:https://doi.org/10.1016/0031-9384(86)90295-7

LaFontana, K. M.& Cillessen, A. H. (2002). Children's perceptions of popular and unpopular peers: A multimethod assessment. *Dev Psychol, 38*(5), 635-647. doi:10.1037//0012-1649.38.5.635

Laitinen, J., et al. (2002). Unemployment and obesity among young adults in a northern finland 1966 birth cohort. *Int J Obes Relat Metab Disord, 26*(10), 1329-1338. doi:10.1038/sj.ijo.0802134

Lam, T. K., et al. (2005). Regulation of blood glucose by hypothalamic pyruvate metabolism. *Science, 309*(5736), 943-947. doi:10.1126/science.1112085

Lammers, J. H. C. M., et al. (1988). Hypothalamic substrates for brain stimulation-induced attack, teeth-chattering and social grooming in the rat. *Brain research, 449*(1), 311-327. doi:https://doi.org/10.1016/0006-8993(88)91046-3

Lang, U. E., et al. (2009). Higher bdnf concentrations in the hippocampus and cortex of an aggressive mouse strain. *Behavioural Brain Research, 197*(1), 246-249. doi:https://doi.org/10.1016/j.bbr.2008.08.025

Lanza, M. L. (1983). Origins of aggression. *Journal of Psychosocial Nursing and Mental Health Services, 21*(6), 11-16. doi:doi:10.3928/0279-3695-19830601-03

Lapchak, P. A., et al. (1997). Adenoviral vector-mediated gdnf gene therapy in a rodent lesion model of late stage parkinson's disease. *Brain research, 777*(1), 153-160. doi:https://doi.org/10.1016/S0006-8993(97)01100-1

Lapchak, P. A.& Hefti, F. (1992). Bdnf and ngf treatment in lesioned rats: Effects on cholinergic function and weight gain. *Neuroreport, 3*(5), 405-408. doi:10.1097/00001756-199205000-00007

Leckstrom, A., et al. (2009). Xenin, a gastrointestinal peptide, regulates feeding independent of the melanocortin signaling pathway. *Diabetes, 58*(1), 87-94. doi:10.2337/db08-0260

LeDoux, J. (2007). The amygdala. *Curr Biol, 17*(20), R868-874. doi:10.1016/j.cub.2007.08.005

Lee, H., et al. (2014). Scalable control of mounting and attack by esr1+ neurons in the ventromedial hypothalamus. *Nature, 509*(7502), 627-632. doi:10.1038/nature13169

Lee, R., et al. (2001). Regulation of cell survival by secreted proneurotrophins. *Science, 294*(5548), 1945. doi:10.1126/science.1065057

Leibowitz, S. F., et al. (1981). Hypothalamic paraventricular nucleus lesions produce overeating and obesity in the rat. *Physiology & Behavior, 27*(6), 1031-1040. doi:https://doi.org/10.1016/0031-9384(81)90366-8

Leroy, F., et al. (2018). A circuit from hippocampal ca2 to lateral septum disinhibits social aggression. *Nature, 564*(7735), 213-218. doi:10.1038/s41586-018-0772-0

Levin, B. E. (2007). Why some of us get fat and what we can do about it. *The Journal of physiology, 583*(Pt 2), 425-430. doi:10.1113/jphysiol.2007.135434

Li, G., et al. (2015). Enriched environment inhibits mouse pancreatic cancer growth and down-regulates the expression of mitochondria-related genes in cancer cells. *Scientific Reports, 5*(1), 1-9.

Li, J. S., et al. (2013). Approaches to the prevention and management of childhood obesity: The role of social networks and the use of social media and related electronic technologies: A scientific statement from the american heart association. *Circulation, 127*(2), 260-267. doi:10.1161/CIR.0b013e3182756d8e

Liao, G. Y., et al. (2012). Dendritically targeted bdnf mrna is essential for energy balance and response to leptin. *Nat Med, 18*(4), 564-571. doi:10.1038/nm.2687

Lidster, K., et al. (2019). Cage aggression in group-housed laboratory male mice: An international data crowdsourcing project. *Scientific Reports, 9*(1), 15211. doi:10.1038/s41598-019-51674-z

Lilleston, P. S., et al. (2017). Understanding social norms and violence in childhood: Theoretical underpinnings and strategies for intervention. *Psychology, Health & Medicine, 22*(sup1), 122-134. doi:10.1080/13548506.2016.1271954

Lin, D., et al. (2011). Functional identification of an aggression locus in the mouse hypothalamus. *Nature, 470*(7333), 221-226. doi:10.1038/nature09736

Lin, Z., et al. (2015). Structural basis of death domain signaling in the p75 neurotrophin receptor. *eLife, 4*, e11692. doi:10.7554/eLife.11692

Lindahl, M., et al. (2014). Manf is indispensable for the proliferation and survival of pancreatic β cells. *Cell Reports, 7*(2), 366-375. doi:10.1016/j.celrep.2014.03.023

Lindenfors, P.& S.Tullberg, B. (2011). 2 - evolutionary aspects of aggression: The importance of sexual selection. In R. Huber, D. L. Bannasch, & P. Brennan (Eds.), *Advances in genetics* (Vol. 75, pp. 7-22): Academic Press.

Liu, W., et al. (2020). Serum leptin, resistin, and adiponectin levels in obese and non-obese patients with newly diagnosed type 2 diabetes mellitus: A population-based study. *Medicine, 99*(6), e19052-e19052. doi:10.1097/MD.0000000000019052

Liu, X., et al. (2014). Effects of energy status and diet on bdnf expression in the ventromedial hypothalamus of male and female rats. *Physiol Behav, 130*, 99-107. doi:10.1016/j.physbeh.2014.03.028

Lockworth, C. R., et al. (2015). Effect of enrichment devices on aggression in manipulated nude mice. *J Am Assoc Lab Anim Sci, 54*(6), 731-736.

Lopez-Gambero, A. J., et al. (2019). Brain glucose-sensing mechanism and energy homeostasis. *Mol Neurobiol, 56*(2), 769-796. doi:10.1007/s12035-018-1099-4

Ludwig, D. S., et al. (2001). Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance. *J Clin Invest, 107*(3), 379-386. doi:10.1172/jci10660

Ludwig, J., et al. (2011). Neighborhoods, obesity, and diabetes — a randomized social experiment. *New England Journal of Medicine, 365*(16), 1509-1519. doi:10.1056/NEJMsa1103216

Lyons, W. E., et al. (1999). Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proceedings of the National Academy of Sciences, 96*(26), 15239. doi:10.1073/pnas.96.26.15239

Ma, X., et al. (2007). Electrical inhibition of identified anorexigenic pomc neurons by orexin/hypocretin. *J Neurosci, 27*(7), 1529-1533. doi:10.1523/jneurosci.3583-06.2007

Macdonald-Wallis, K., et al. (2012). Social network analysis of childhood and youth physical activity: A systematic review. *Am J Prev Med, 43*(6), 636-642. doi:10.1016/j.amepre.2012.08.021

Macia, L., et al. (2012). Macrophage inhibitory cytokine 1 (mic-1/gdf15) decreases food intake, body weight and improves glucose tolerance in mice on normal & obesogenic diets. *PLoS One, 7*(4), e34868. doi:10.1371/journal.pone.0034868

Maekawa, F., et al. (2013). Brain-derived neurotrophic factor in vmh as the causal factor for and therapeutic tool to treat visceral adiposity and hyperleptinemia in type 2 diabetic goto-kakizaki rats. *Front Synaptic Neurosci, 5*, 7. doi:10.3389/fnsyn.2013.00007

Maggio, N.& Segal, M. (2012). Steroid modulation of hippocampal plasticity: Switching between cognitive and emotional memories. *Frontiers in Cellular Neuroscience, 6*, 12-12. doi:10.3389/fncel.2012.00012

Mahase, E. (2020). Covid-19: Eu states report 60% rise in emergency calls about domestic violence. *Bmj, 369*, m1872. doi:10.1136/bmj.m1872

Mainardi, M., et al. (2010). A sensitive period for environmental regulation of eating behavior and leptin sensitivity. *Proceedings of the National Academy of Sciences, 107*(38), 16673. doi:10.1073/pnas.0911832107

Manfredsson, F. P., et al. (2009). Nigrostriatal raav-mediated gdnf overexpression induces robust weight loss in a rat model of age-related obesity. *Molecular Therapy, 17*(6), 980-991. doi:https://doi.org/10.1038/mt.2009.45

Mansour, A. G., et al. (2021). Enriched environment enhances nk cell maturation through hypothalamic bdnf in male mice. *European Journal of Immunology, 51*(3), 557-566. doi:https://doi.org/10.1002/eji.201948358

Marashi, V., et al. (2003a). Effects of different forms of environmental enrichment on behavioral, endocrinological, and immunological parameters in male mice. *Hormones and Behavior, 43*(2), 281-292.

Marashi, V., et al. (2003b). Effects of different forms of environmental enrichment on behavioral, endocrinological, and immunological parameters in male mice. *Horm Behav, 43*(2), 281-292. doi:10.1016/s0018-506x(03)00002-3

Marder, A. R., et al. (2013). Food-related aggression in shelter dogs: A comparison of behavior identified by a behavior evaluation in the shelter and owner reports after adoption. *Applied Animal Behaviour Science, 148*(1), 150-156. doi:https://doi.org/10.1016/j.applanim.2013.07.007

Martikainen, P. T.& Marmot, M. G. (1999). Socioeconomic differences in weight gain and determinants and consequences of coronary risk factors. *Am J Clin Nutr, 69*(4), 719-726. doi:10.1093/ajcn/69.4.719

Martin, D., et al. (1996). Glial cell line-derived neurotrophic factor: The lateral cerebral ventricle as a site of administration for stimulation of the substantia nigra dopamine system in rats. *European Journal of Neuroscience, 8*(6), 1249-1255. doi:https://doi.org/10.1111/j.1460-9568.1996.tb01293.x

Martínez-Levy, G. A.& Cruz-Fuentes, C. S. (2014). Genetic and epigenetic regulation of the brain-derived neurotrophic factor in the central nervous system. *The Yale journal of biology and medicine, 87*(2), 173-186. Retrieved from https://pubmed.ncbi.nlm.nih.gov/24910563

Martínez, F., et al. (2019). The median eminence as the hypothalamic area involved in rapid transfer of glucose to the brain: Functional and cellular mechanisms. *Journal of Molecular Medicine, 97*(8), 1085-1097. doi:10.1007/s00109-019-01799-5

Martins, N.& Wilson, B. J. (2012). Mean on the screen: Social aggression in programs popular with children. *Journal of Communication, 62*(6), 991-1009. doi:https://doi.org/10.1111/j.1460-2466.2011.01599.x

Marty, N., et al. (2005). Regulation of glucagon secretion by glucose transporter type 2 (glut2) and astrocyte-dependent glucose sensors. *J Clin Invest, 115*(12), 3545-3553. doi:10.1172/jci26309

Maruoka, T., et al. (2009). Maternal enrichment affects prenatal hippocampal proliferation and open-field behaviors in female offspring mice. *Neurosci Lett, 454*(1), 28-32. doi:10.1016/j.neulet.2009.02.052

Matsumoto, T., et al. (2006). Brain-derived neurotrophic factor-induced potentiation of glutamate and gaba release: Different dependency on signaling pathways and neuronal activity. *Mol Cell Neurosci, 31*(1), 70-84. doi:10.1016/j.mcn.2005.09.002

Matthies, S., et al. (2012). Small amygdala-high aggression? The role of the amygdala in modulating aggression in healthy subjects. *World J Biol Psychiatry, 13*(1), 75-81. doi:10.3109/15622975.2010.541282

Mattson, M. P., et al. (2004). Bdnf and 5-ht: A dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends in Neurosciences, 27*(10), 589-594. doi:10.1016/j.tins.2004.08.001

Maturo, C. C.& Cunningham, S. A. (2013). Influence of friends on children's physical activity: A review. *Am J Public Health, 103*(7), e23-38. doi:10.2105/ajph.2013.301366

Mayer, J. (1955). Regulation of energy intake and the body weight: The glucostatic theory and the lipostatic hypothesis. *Ann N Y Acad Sci, 63*(1), 15-43. doi:10.1111/j.1749-6632.1955.tb36543.x

Mayer, J.& Bates, M. W. (1952). Blood glucose and food intake in normal and hypophysectomized, alloxan-treated rats. *American Journal of Physiology-Legacy Content, 168*(3), 812-819.

Maynard, K. R., et al. (2016). Functional role of bdnf production from unique promoters in aggression and serotonin signaling. *Neuropsychopharmacology, 41*(8), 1943-1955. doi:10.1038/npp.2015.349

Mayne, S. L., et al. (2018). Neighborhood disorder and obesity-related outcomes among women in chicago. *International journal of environmental research and public health, 15*(7). doi:10.3390/ijerph15071395

McAllan, L., et al. (2018). Disruption of brain-derived neurotrophic factor production from individual promoters generates distinct body composition phenotypes in mice. *American Journal of Physiology-Endocrinology and Metabolism, 315*(6), E1168-E1184. doi:10.1152/ajpendo.00205.2018

McBurnett, K., et al. (2000). Low salivary cortisol and persistent aggression in boys referred for disruptive behavior. *Arch Gen Psychiatry, 57*(1), 38-43. doi:10.1001/archpsyc.57.1.38

McCloskey, M., et al. (2012). Assessment and treatment of intermittent explosive disorder. *The Oxford Handbook of Impulse Control Disorders*. doi:10.1093/oxfordhb/9780195389715.013.0099

McDonald, M. M., et al. (2012). Gabaa receptor activation in the lateral septum reduces the expression of conditioned defeat and increases aggression in syrian hamsters. *Brain research, 1439*, 27-33. doi:10.1016/j.brainres.2011.12.042

McEwen, B. S.& Reagan, L. P. (2004). Glucose transporter expression in the central nervous system: Relationship to synaptic function. *Eur J Pharmacol, 490*(1-3), 13-24. doi:10.1016/j.ejphar.2004.02.041

McIsaac, W.& Ferguson, A. V. (2017). Glucose concentrations modulate brain-derived neurotrophic factor responsiveness of neurones in the paraventricular nucleus of the hypothalamus. *J Neuroendocrinol, 29*(4). doi:10.1111/jne.12464

McQuaid, R. J., et al. (2018). Post-weaning environmental enrichment in male cd-1 mice: Impact on social behaviors, corticosterone levels and prefrontal cytokine expression in adulthood. *Frontiers in Behavioral Neuroscience, 12*, 145-145. doi:10.3389/fnbeh.2018.00145

Mertens, S., et al. (2020). Effect of a partial cage dividing enrichment on aggression-associated parameters in group-housed male c57bl/6ncrl mice. *Applied Animal Behaviour Science, 224*, 104939. doi:https://doi.org/10.1016/j.applanim.2020.104939

Metsis, M., et al. (1993). Differential usage of multiple brain-derived neurotrophic factor promoters in the rat brain following neuronal activation. *Proc Natl Acad Sci U S A, 90*(19), 8802-8806. doi:10.1073/pnas.90.19.8802

Meyer, J. M., et al. (2016). Psychopharmacology of persistent violence and aggression. *Psychiatr Clin North Am, 39*(4), 541-556. doi:10.1016/j.psc.2016.07.012

Miczek, K. A., et al. (2003). Neurosteroids, gabaa receptors, and escalated aggressive behavior. *Hormones and Behavior, 44*(3), 242-257. doi:https://doi.org/10.1016/j.yhbeh.2003.04.002

Miczek, K. A., et al. (2001). Aggressive behavioral phenotypes in mice. *Behavioural Brain Research, 125*(1), 167-181. doi:https://doi.org/10.1016/S0166-4328(01)00298-4

Min, D. K., et al. (2011). Gastric distention induced functional magnetic resonance signal changes in the rodent brain. *Neuroscience, 179*, 151-158. doi:https://doi.org/10.1016/j.neuroscience.2011.01.051

Minokoshi, Y., et al. (2004). Amp-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature, 428*(6982), 569-574. doi:10.1038/nature02440

Miranda, M., et al. (2019). Brain-derived neurotrophic factor: A key molecule for memory in the healthy and the pathological brain. *Frontiers in Cellular Neuroscience, 13*(363). doi:10.3389/fncel.2019.00363

Mizuno, T. M., et al. (2013). Mediation of glucose-induced anorexia by central nervous system interleukin 1 signaling. *Behav Brain Res, 256*, 512-519. doi:10.1016/j.bbr.2013.08.050

Mobbs, C. V., et al. (2005). Impaired glucose signaling as a cause of obesity and the metabolic syndrome: The glucoadipostatic hypothesis. *Physiology & Behavior, 85*(1), 3-23. doi:https://doi.org/10.1016/j.physbeh.2005.04.005

Molcho, M., et al. (2009). Cross-national time trends in bullying behaviour 1994-2006: Findings from europe and north america. *Int J Public Health, 54 Suppl 2*, 225-234. doi:10.1007/s00038-009-5414-8

Møllgaard, K., et al. (1971). Quantitative synaptic changes with differential experience in rat brain. *International Journal of Neuroscience, 2*(3), 113-127. doi:10.3109/00207457109148764

Montoya, E. R., et al. (2012). Testosterone, cortisol, and serotonin as key regulators of social aggression: A review and theoretical perspective. *Motivation and emotion, 36*(1), 65-73.

Moran, P., et al. (2016). Gene × environment interactions in schizophrenia: Evidence from genetic mouse models. *Neural plasticity, 2016*, 2173748-2173748. doi:10.1155/2016/2173748

Morton, G. J., et al. (2014). Neurobiology of food intake in health and disease. *Nature Reviews Neuroscience, 15*(6), 367-378. doi:10.1038/nrn3745

Mou, Z., et al. (2015). Human obesity associated with an intronic snp in the brain-derived neurotrophic factor locus. *Cell Reports, 13*(6), 1073-1080. doi:https://doi.org/10.1016/j.celrep.2015.09.065

Mowla, S. J., et al. (2001). Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor *. *Journal of Biological Chemistry, 276*(16), 12660-12666. doi:10.1074/jbc.M008104200

Mullican, S. E., et al. (2017). Gfral is the receptor for gdf15 and the ligand promotes weight loss in mice and nonhuman primates. *Nature Medicine, 23*(10), 1150-1157. doi:10.1038/nm.4392

Murphy, B. A., et al. (2009). Amp-activated protein kinase and nitric oxide regulate the glucose sensitivity of ventromedial hypothalamic glucose-inhibited neurons. *Am J Physiol Cell Physiol, 297*(3), C750-758. doi:10.1152/ajpcell.00127.2009

Musci, R. J., et al. (2014). Reducing aggression and impulsivity through school-based prevention programs: A gene by intervention interaction. *Prev Sci, 15*(6), 831-840. doi:10.1007/s11121-013-0441-3

Mwangi, S. M., et al. (2014). Glial cell line-derived neurotrophic factor protects against high-fat diet-induced obesity. *Am J Physiol Gastrointest Liver Physiol, 306*(6), G515-525. doi:10.1152/ajpgi.00364.2013

N. C. D. Risk Factor Collaboration. (2019). Rising rural body-mass index is the main driver of the global obesity epidemic in adults. *Nature, 569*(7755), 260-264. doi:10.1038/s41586-019-1171-x

Naka, F., et al. (2002). An enriched environment increases noradrenaline concentration in the mouse brain. *Brain research, 924*(1), 124-126. doi:https://doi.org/10.1016/S0006-8993(01)03257-7

Nakagawa, T., et al. (2003). Antiobesity and antidiabetic effects of brain-derived neurotrophic factor in rodent models of leptin resistance. *International Journal of Obesity, 27*(5), 557-565. doi:10.1038/sj.ijo.0802265

Nakano, Y., et al. (1986). Feeding-related activity of glucose-and morphine-sensitive neurons in the monkey amygdala. *Brain research, 399*(1), 167-172. doi:https://doi.org/10.1016/0006-8993(86)90613-X

Nakazato, M., et al. (2001). A role for ghrelin in the central regulation of feeding. *Nature, 409*(6817), 194-198. doi:10.1038/35051587

Nanda, S.& Mack, K. J. (1998). Multiple promoters direct stimulus and temporal specific expression of brain-derived neurotrophic factor in the somatosensory cortex. *Brain Res Mol Brain Res, 62*(2), 216-219. doi:10.1016/s0169-328x(98)00242-3

Nandar, W., et al. (2008). Mechanisms of xenin-induced anorectic response in chicks (gallus gallus). *Gen Comp Endocrinol, 157*(1), 58-62. doi:10.1016/j.ygcen.2008.03.012

Napolitano, M. A., et al. (2013). Using facebook and text messaging to deliver a weight loss program to college students. *Obesity (Silver Spring), 21*(1), 25-31. doi:10.1002/oby.20232

Naumenko, V. S., et al. (2014). On the role of 5-ht(1a) receptor gene in behavioral effect of brain-derived neurotrophic factor. *J Neurosci Res, 92*(8), 1035-1043. doi:10.1002/jnr.23381

Nelson, R. J.& Trainor, B. C. (2007). Neural mechanisms of aggression. *Nature Reviews Neuroscience, 8*(7), 536-546. doi:10.1038/nrn2174

Netterstrøm, B., et al. (1991). Job strain and cardiovascular risk factors: A cross sectional study of employed danish men and women. *Br J Ind Med, 48*(10), 684-689. doi:10.1136/oem.48.10.684

Newman, S. W. (1999). The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network. *Ann N Y Acad Sci, 877*, 242-257. doi:10.1111/j.1749-6632.1999.tb09271.x

Nguyen, K. P., et al. (2017). Feeding experimentation device (fed): Construction and validation of an open-source device for measuring food intake in rodents. *J Vis Exp*(120). doi:10.3791/55098

Nieh, E. H., et al. (2015). Decoding neural circuits that control compulsive sucrose seeking. *Cell, 160*(3), 528-541. doi:10.1016/j.cell.2015.01.003

Nithianantharajah, J.& Hannan, A. J. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nature Reviews Neuroscience, 7*(9), 697-709.

Nonomura, T., et al. (2001). Brain-derived neurotrophic factor regulates energy expenditure through the central nervous system in obese diabetic mice. *Int J Exp Diabetes Res, 2*(3), 201-209. doi:10.1155/edr.2001.201

Notaras, M.& van den Buuse, M. (2019). Brain-derived neurotrophic factor (bdnf): Novel insights into regulation and genetic variation. *The Neuroscientist, 25*(5), 434-454. doi:10.1177/1073858418810142

Novak, J., et al. (2016). Effect of cage-induced stereotypies on measures of affective state and recurrent perseveration in cd-1 and c57bl/6 mice. *PLoS One, 11*(5), e0153203. doi:10.1371/journal.pone.0153203

Nutt, J. G., et al. (2003). Randomized, double-blind trial of glial cell line-derived neurotrophic factor (gdnf) in pd. *Neurology, 60*(1), 69-73. doi:10.1212/wnl.60.1.69

O'Farrell, T. J., et al. (2003). Partner violence before and after individually based alcoholism treatment for male alcoholic patients. *J Consult Clin Psychol, 71*(1), 92-102. doi:10.1037//0022-006x.71.1.92

Oh, J., et al. (2017). An open source automatic feeder for animal experiments. *HardwareX, 1*, 13-21.

Ollmann, M. M., et al. (1997). Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science, 278*(5335), 135-138. doi:10.1126/science.278.5335.135

Ostry, A. S., et al. (2006). Psychosocial and other working conditions in relation to body mass index in a representative sample of australian workers. *BMC Public Health, 6*, 53. doi:10.1186/1471-2458-6-53

Pachucki, M. A., et al. (2011). Social network concordance in food choice among spouses, friends, and siblings. *Am J Public Health, 101*(11), 2170-2177. doi:10.2105/ajph.2011.300282

Parasin, N., et al. (2021). Effect of air pollution on obesity in children: A systematic review and meta-analysis. *Children, 8*(5), 327. Retrieved from https://www.mdpi.com/2227-9067/8/5/327

Pardini, D. A., et al. (2014). Lower amygdala volume in men is associated with childhood aggression, early psychopathic traits, and future violence. *Biological psychiatry, 75*(1), 73-80. doi:10.1016/j.biopsych.2013.04.003

Parent, M. B., et al. (2014). Remembering to eat: Hippocampal regulation of meal onset. *American journal of physiology. Regulatory, integrative and comparative physiology, 306*(10), R701-R713. doi:10.1152/ajpregu.00496.2013

Parton, L. E., et al. (2007). Glucose sensing by pomc neurons regulates glucose homeostasis and is impaired in obesity. *Nature, 449*(7159), 228-232. doi:10.1038/nature06098

Pattabiraman, P. P., et al. (2005). Neuronal activity regulates the developmental expression and subcellular localization of cortical bdnf mrna isoforms in vivo. *Mol Cell Neurosci, 28*(3), 556-570. doi:10.1016/j.mcn.2004.11.010

Paxinos, G.& Franklin, K. B. J. (2001). *The mouse brain in stereotaxic coordinates*: Academic Press.

Payne, J., et al. (1997). Glucose transporter glut 5 expression in microglial cells. *Glia, 21*(3), 327-331. doi:10.1002/(sici)1098-1136(199711)21:3<327::aid-glia7>3.0.co;2-1

Pelleymounter, M. A., et al. (1995). Characteristics of bdnf-induced weight loss. *Experimental Neurology, 131*(2), 229-238. doi:https://doi.org/10.1016/0014-4886(95)90045-4

Penicaud, L., et al. (1983). Endocrine basis for weight gain after fasting or vmh lesion in rats. *Am J Physiol, 245*(3), E246-252. doi:10.1152/ajpendo.1983.245.3.E246

Petrovich, G. D. (2018). Lateral hypothalamus as a motivation-cognition interface in the control of feeding behavior. *Frontiers in Systems Neuroscience, 12*(14). doi:10.3389/fnsys.2018.00014

Pham, T. M., et al. (2002). Environmental influences on brain neurotrophins in rats. *Pharmacology Biochemistry and Behavior, 73*(1), 167-175.

Pietropaolo, S., et al. (2004). Long-term effects of the periadolescent environment on exploratory activity and aggressive behaviour in mice: Social versus physical enrichment. *Physiol Behav, 81*(3), 443-453. doi:10.1016/j.physbeh.2004.02.022

Platholi, J.& Lee, F. S. (2018). Chapter 5 - neurotrophic factors. In W. Slikker, M. G. Paule, & C. Wang (Eds.), *Handbook of developmental neurotoxicology (second edition)* (pp. 55-64): Academic Press.

Podyma, B., et al. (2020). The p75 neurotrophin receptor in agrp neurons is necessary for homeostatic feeding and food anticipation. *eLife, 9*, e52623. doi:10.7554/eLife.52623

Pompili, M., et al. (2010). The hypothalamic-pituitary-adrenal axis and serotonin abnormalities: A selective overview for the implications of suicide prevention. *European Archives of Psychiatry and Clinical Neuroscience, 260*(8), 583-600. doi:10.1007/s00406-010-0108-z

Poole, T. B.& Morgan, H. D. R. (1973). Differences in aggressive behaviour between male mice (mus musculus l.) in colonies of different sizes. *Animal Behaviour, 21*(4), 788-795. doi:https://doi.org/10.1016/S0003-3472(73)80105-8

Popma, A., et al. (2007). Cortisol moderates the relationship between testosterone and aggression in delinquent male adolescents. *Biological psychiatry, 61*(3), 405-411. doi:10.1016/j.biopsych.2006.06.006

Poritsanos, N. J., et al. (2011). Impaired hypothalamic fto expression in response to fasting and glucose in obese mice. *Nutr Diabetes, 1*(10), e19. doi:10.1038/nutd.2011.15

Pruunsild, P., et al. (2007). Dissecting the human bdnf locus: Bidirectional transcription, complex splicing, and multiple promoters. *Genomics, 90*(3), 397-406. doi:10.1016/j.ygeno.2007.05.004

Public Health Agency of Canada. (2011). *Obesity in canada: A joint report from the public health agency of canada and the canadian institute for health information*. Retrieved from https://www.canada.ca/en/public-health/services/health-promotion/healthy-living/obesity-canada.html

Puts, D. A. (2010). Beauty and the beast: Mechanisms of sexual selection in humans. *Evolution and Human Behavior, 31*(3), 157-175. doi:https://doi.org/10.1016/j.evolhumbehav.2010.02.005

Queen, N. J., et al. (2020). Environmental enrichment improves metabolic and behavioral health in the btbr mouse model of autism. *Psychoneuroendocrinology, 111*, 104476-104476. doi:10.1016/j.psyneuen.2019.104476

Quist, J. S., et al. (2018). Effects of active commuting and leisure-time exercise on fat loss in women and men with overweight and obesity: A randomized controlled trial. *International Journal of Obesity, 42*(3), 469-478. doi:10.1038/ijo.2017.253

Rada, R. T., et al. (1976). Plasma testosterone and aggressive behavior. *Psychosomatics, 17*(3), 138-142. doi:https://doi.org/10.1016/S0033-3182(76)71132-0

Ragu Varman, D.& Rajan, K. E. (2015). Environmental enrichment reduces anxiety by differentially activating serotonergic and neuropeptide y (npy)-ergic system in indian field mouse (mus booduga): An animal model of post-traumatic stress disorder. *PLoS One, 10*(5), e0127945. doi:10.1371/journal.pone.0127945

Ramirez, J. M. (2003). Hormones and aggression in childhood and adolescence. *Aggression and Violent Behavior, 8*(6), 621-644. doi:https://doi.org/10.1016/S1359-1789(02)00102-7

Ramnauth, A. D., et al. (2021). Induction of bdnf from promoter i following electroconvulsive seizures contributes to structural plasticity in neurons of the piriform cortex. *bioRxiv*, 2021.2003.2004.433962. doi:10.1101/2021.03.04.433962

Rampon, C., et al. (2000). Effects of environmental enrichment on gene expression in the brain. *Proceedings of the National Academy of Sciences, 97*(23), 12880-12884.

Rampon, C., et al. (2000). Enrichment induces structural changes and recovery from nonspatial memory deficits in ca1 nmdar1-knockout mice. *Nature Neuroscience, 3*(3), 238-244.

Rasmuson, S., et al. (1998). Environmental enrichment selectively increases 5-ht1a receptor mrna expression and binding in the rat hippocampus. *Molecular Brain Research, 53*(1), 285-290. doi:https://doi.org/10.1016/S0169-328X(97)00317-3

Rees-Punia, E., et al. (2018). Crime, perceived safety, and physical activity: A meta-analysis. *Prev Med, 111*, 307-313. doi:10.1016/j.ypmed.2017.11.017

Riedi, G., et al. (2012). Alcohol and repeated deliberate self-harm: Preliminary results of the french cohort study of risk for repeated incomplete suicides. *Crisis, 33*(6), 358-363. doi:10.1027/0227-5910/a000148

Rios, M. (2013). Bdnf and the central control of feeding: Accidental bystander or essential player? *Trends in Neurosciences, 36*(2), 83-90. doi:https://doi.org/10.1016/j.tins.2012.12.009

Rios, M. (2014). Neurotrophins and the regulation of energy balance and body weight. In G. R. Lewin & B. D. Carter (Eds.), *Neurotrophic factors* (pp. 283-307). Berlin, Heidelberg: Springer Berlin Heidelberg.

Rios, M., et al. (2001). Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Mol Endocrinol, 15*(10), 1748-1757. doi:10.1210/mend.15.10.0706

Risold, P. Y.& Swanson, L. W. (1997). Connections of the rat lateral septal complex1published on the world wide web on 2 june 1997.1. *Brain Research Reviews, 24*(2), 115-195. doi:https://doi.org/10.1016/S0165-0173(97)00009-X

Ritter, S., et al. (1998). Subgroups of hindbrain catecholamine neurons are selectively activated by 2-deoxy-d-glucose induced metabolic challenge. *Brain research, 805*(1-2), 41-54. doi:10.1016/s0006-8993(98)00655-6

Roberts, B. L., et al. (2017). High glucose increases action potential firing of catecholamine neurons in the nucleus of the solitary tract by increasing spontaneous glutamate inputs. *Am J Physiol Regul Integr Comp Physiol, 313*(3), R229-r239. doi:10.1152/ajpregu.00413.2016

Robinson, E., et al. (2013). Social influences on eating: Implications for nutritional interventions. *Nutr Res Rev, 26*(2), 166-176. doi:10.1017/s0954422413000127

Rodriguez-Arias, M., et al. (1998). Effects of risperidone and sch 23390 on isolation-induced aggression in male mice. *Eur Neuropsychopharmacol, 8*(2), 95-103. doi:10.1016/s0924-977x(97)00051-5

Rodríguez, E. M., et al. (2010). The design of barriers in the hypothalamus allows the median eminence and the arcuate nucleus to enjoy private milieus: The former opens to the portal blood and the latter to the cerebrospinal fluid. *Peptides, 31*(4), 757-776. doi:10.1016/j.peptides.2010.01.003

Roeling, T. A., et al. (1993). Behavioural responses of bicucculline methiodide injections into the ventral hypothalamus of freely moving, socially interacting rats. *Brain Res, 615*(1), 121-127. doi:10.1016/0006-8993(93)91122-9

Ros, A. F., et al. (2006). Aggressive behaviour and energy metabolism in a cichlid fish, oreochromis mossambicus. *Physiology & Behavior, 89*(2), 164-170.

Rose, R. M., et al. (1971). Plasma testosterone, dominance rank and aggressive behaviour in male rhesus monkeys. *Nature*.

Rosenkranz, J. A.& Grace, A. A. (2002). Cellular mechanisms of infralimbic and prelimbic prefrontal cortical inhibition and dopaminergic modulation of basolateral amygdala neurons in vivo. *J Neurosci, 22*(1), 324-337. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11756516

Rosenzweig, M. R. (1966). Environmental complexity, cerebral change, and behavior. *American Psychologist, 21*(4), 321.

Rosenzweig, M. R.& Bennett, E. L. (1969). Effects of differential environments on brain weights and enzyme activities in gerbils, rats, and mice. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology, 2*(2), 87-95.

Rosenzweig, M. R., et al. (1967). *Effects of differential environments on brain anatomy and brain chemistry.* Paper presented at the Proceedings of the annual meeting of the American Psychopathological Association.

Rossi, C., et al. (2006). Brain-derived neurotrophic factor (bdnf) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. *European Journal of Neuroscience, 24*(7), 1850-1856.

Routh, V. H., et al. (2014). Hypothalamic glucose sensing: Making ends meet. *Frontiers in Systems Neuroscience, 8*, 236-236. doi:10.3389/fnsys.2014.00236

Rozin, P., et al. (1998). What causes humans to begin and end a meal? A role for memory for what has been eaten, as evidenced by a study of multiple meal eating in amnesic patients. *Psychological Science, 9*(5), 392-396. doi:10.1111/1467-9280.00073

Rueve, M. E.& Welton, R. S. (2008). Violence and mental illness. *Psychiatry (Edgmont), 5*(5), 34-48.

Rylands, A. J., et al. (2012). Pre- and postsynaptic serotonergic differences in males with extreme levels of impulsive aggression without callous unemotional traits: A positron emission tomography study using (11)c-dasb and (11)c-mdl100907. *Biological psychiatry, 72*(12), 1004-1011. doi:10.1016/j.biopsych.2012.06.024

Sahay, A.& Hen, R. (2007). Adult hippocampal neurogenesis in depression. *Nature Neuroscience, 10*(9), 1110-1115. doi:10.1038/nn1969

Sakaue, M., et al. (2002). Modulation by 5-ht2a receptors of aggressive behavior in isolated mice. *Jpn J Pharmacol, 89*(1), 89-92. doi:10.1254/jjp.89.89

Sanchez-Huertas, C.& Rico, B. (2011). Creb-dependent regulation of gad65 transcription by bdnf/trkb in cortical interneurons. *Cereb Cortex, 21*(4), 777-788. doi:10.1093/cercor/bhq150

Sankar, R. (2012). Gaba a receptor physiology and its relationship to the mechanism of action of the 1, 5-benzodiazepine clobazam. *CNS drugs, 26*(3), 229-244.

Sasi, M., et al. (2017). Neurobiology of local and intercellular bdnf signaling. *Pflügers Archiv - European Journal of Physiology, 469*(5), 593-610. doi:10.1007/s00424-017-1964-4

Schapiro, S. J. (2002). Effects of social manipulations and environmental enrichment on behavior and cell-mediated immune responses in rhesus macaques. *Pharmacology Biochemistry and Behavior, 73*(1), 271-278.

Schlüter, T., et al. (2013). The impact of dopamine on aggression: An [18f]-fdopa pet study in healthy males. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 33*(43), 16889-16896. doi:10.1523/JNEUROSCI.1398-13.2013

Schultz, W. (2002). Getting formal with dopamine and reward. *Neuron, 36*(2), 241-263. doi:10.1016/s0896-6273(02)00967-4

Schwartz, G. J., et al. (1999). Gut vagal afferent lesions increase meal size but do not block gastric preload-induced feeding suppression. *Am J Physiol, 276*(6), R1623-1629. doi:10.1152/ajpregu.1999.276.6.R1623

Schwartz, M. W., et al. (1996). Cerebrospinal fluid leptin levels: Relationship to plasma levels and to adiposity in humans. *Nat Med, 2*(5), 589-593. doi:10.1038/nm0596-589

Seebacher, F., et al. (2013). Increased aggression during pregnancy comes at a higher metabolic cost. *Journal of Experimental Biology, 216*(5), 771-776.

Segula, D. (2014). Complications of obesity in adults: A short review of the literature. *Malawi medical journal : the journal of Medical Association of Malawi, 26*(1), 20-24. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4062780/

Sgrò, P., et al. (2021). Exercise as a drug for glucose management and prevention in type 2 diabetes mellitus. *Current opinion in pharmacology, 59*, 95-102. doi:10.1016/j.coph.2021.05.006

Sheng, Z., et al. (2014). Metabolic regulation of lateral hypothalamic glucose-inhibited orexin neurons may influence midbrain reward neurocircuitry. *Mol Cell Neurosci, 62*, 30-41. doi:10.1016/j.mcn.2014.08.001

Sherwin, C. M. (2004). The influences of standard laboratory cages on rodents and the validity of research data. *Animal Welfare, 13*, 9-15.

Siegel, A.& Douard, J. (2011). Who's flying the plane: Serotonin levels, aggression and free will. *International Journal of Law and Psychiatry, 34*(1), 20-29. doi:10.1016/j.ijlp.2010.11.004

Siegel, A., et al. (1977). Suppression of attack behavior in the cat by the prefrontal cortex: Role of the mediodorsal thalamic nucleus. *Brain research, 127*(1), 185-190. doi:10.1016/0006-8993(77)90392-4

Siegel, A.& Victoroff, J. (2009). Understanding human aggression: New insights from neuroscience. *Int J Law Psychiatry, 32*(4), 209-215. doi:10.1016/j.ijlp.2009.06.001

Siever, L. J. (2008). Neurobiology of aggression and violence. *The American journal of psychiatry, 165*(4), 429-442. doi:10.1176/appi.ajp.2008.07111774

Silvern, L., et al. (1995). Retrospective reports of parental partner abuse: Relationships to depression, trauma symptoms and self-esteem among college students. *Journal Family Violence, 10*, 177-202. doi:10.1007/BF02110599

Simpkins, S. D., et al. (2013). Adolescent friendships, bmi, and physical activity: Untangling selection and influence through longitudinal social network analysis. *J Res Adolesc, 23*(3). doi:10.1111/j.1532-7795.2012.00836.x

Smagin, D. A., et al. (2015). Altered hippocampal neurogenesis and amygdalar neuronal activity in adult mice with repeated experience of aggression. *Frontiers in neuroscience, 9*, 443-443. doi:10.3389/fnins.2015.00443

Smith, P. K., et al. (1999). *The nature of school bullying: A cross-national perspective*: Routledge.

Sobal, J.& Hanson, K. L. (2011). Marital status, marital history, body weight, and obesity. *Marriage & Family Review, 47*(7), 474-504. doi:10.1080/01494929.2011.620934

Song, Y., et al. (2017). Enriching the housing environment for mice enhances their nk cell antitumor immunity via sympathetic nerve–dependent regulation of nkg2d and ccr5. *Cancer Research, 77*(7), 1611. doi:10.1158/0008-5472.CAN-16-2143

Sonoyama, T., et al. (2020). Human bdnf/trkb variants impair hippocampal synaptogenesis and associate with neurobehavioural abnormalities. *Scientific Reports, 10*(1), 9028. doi:10.1038/s41598-020-65531-x

Spalletta, G., et al. (2010). Bdnf val66met polymorphism is associated with aggressive behavior in schizophrenia. *Eur Psychiatry, 25*(6), 311-313. doi:10.1016/j.eurpsy.2009.10.008

Statistics Canada. (2019). *Overweight and obese adults, 2018*. Retrieved from https://www150.statcan.gc.ca/n1/en/pub/82-625-x/2019001/article/00005-eng.pdf?st=IEsMRYO_

Stellar, E. (1954). The physiology of motivation. *Psychol Rev, 61*(1), 5-22. doi:10.1037/h0060347

Stolarczyk, E., et al. (2010). Detection of extracellular glucose by glut2 contributes to hypothalamic control of food intake. *Am J Physiol Endocrinol Metab, 298*(5), E1078-1087. doi:10.1152/ajpendo.00737.2009

Stopyra, M. A., et al. (2021). The effect of intestinal glucose load on neural regulation of food craving. *Nutritional Neuroscience, 24*(2), 109-118. doi:10.1080/1028415X.2019.1600275

Strange, B. A., et al. (2014). Functional organization of the hippocampal longitudinal axis. *Nat Rev Neurosci, 15*(10), 655-669. doi:10.1038/nrn3785

Strelau, J., et al. (2000). Growth/differentiation factor-15/macrophage inhibitory cytokine-1 is a novel trophic factor for midbrain dopaminergic neurons <em>in vivo</em&gt. *The Journal of Neuroscience, 20*(23), 8597. doi:10.1523/JNEUROSCI.20-23-08597.2000

Su, H., et al. (2012). Glucose enhances leptin signaling through modulation of ampk activity. *PLoS One, 7*(2), e31636. doi:10.1371/journal.pone.0031636

Su, X., et al. (2009). Safety evaluation of aav2-gdnf gene transfer into the dopaminergic nigrostriatal pathway in aged and parkinsonian rhesus monkeys. *Hum Gene Ther, 20*(12), 1627-1640. doi:10.1089/hum.2009.103

Summers, C. H., et al. (2000). Serotonergic responses to corticosterone and testosterone in the limbic system. *Gen Comp Endocrinol, 117*(1), 151-159. doi:10.1006/gcen.1999.7408

Sun, H., et al. (2010). Environmental enrichment influences bdnf and nr1 levels in the hippocampus and restores cognitive impairment in chronic cerebral hypoperfused rats. *Curr Neurovasc Res, 7*(4), 268-280. doi:10.2174/156720210793180819

Swetter, B. J., et al. (2011). Separating the effects of shelter from additional cage enhancements for group-housed balb/cj mice. *Neurosci Lett, 495*(3), 205-209. doi:10.1016/j.neulet.2011.03.067

Takahashi, A., et al. (2010). Gabaa receptors in the dorsal raphé nucleus of mice: Escalation of aggression after alcohol consumption. *Psychopharmacology, 211*(4), 467-477. doi:10.1007/s00213-010-1920-x

Takahashi, A., et al. (2014). Control of intermale aggression by medial prefrontal cortex activation in the mouse. *PLoS One, 9*(4), e94657. doi:10.1371/journal.pone.0094657

Takai, D., et al. (2019). Minimum environmental enrichment is effective in activating antitumor immunity to transplanted tumor cells in mice. *Experimental animals, 68*(4), 569-576. doi:10.1538/expanim.19-0055

Tan, S., et al. (2018). Postnatal trkb ablation in corticolimbic interneurons induces social dominance in male mice. *Proceedings of the National Academy of Sciences of the United States of America, 115*(42), E9909-E9915. doi:10.1073/pnas.1812083115

Tao, X., et al. (1998). Ca2+ influx regulates bdnf transcription by a creb family transcription factor-dependent mechanism. *Neuron, 20*(4), 709-726. doi:10.1016/s0896-6273(00)81010-7

Tao, Y. X. (2005). Molecular mechanisms of the neural melanocortin receptor dysfunction in severe early onset obesity. *Mol Cell Endocrinol, 239*(1-2), 1-14. doi:10.1016/j.mce.2005.04.012

Taylor, A. I., et al. (2010). Evaluation of the degradation and metabolic effects of the gut peptide xenin on insulin secretion, glycaemic control and satiety. *J Endocrinol, 207*(1), 87-93. doi:10.1677/joe-10-0085

Taylor, S. C., et al. (2019). The ultimate qpcr experiment: Producing publication quality, reproducible data the first time. *Trends in Biotechnology, 37*(7), 761-774. doi:10.1016/j.tibtech.2018.12.002

Telles, L. E. B., et al. (2020). Domestic violence in the covid-19 pandemic: A forensic psychiatric perspective. *Braz J Psychiatry*. doi:10.1590/1516-4446-2020-1060

Teng, H. K., et al. (2005a). Probdnf induces neuronal apoptosis via activation of a receptor complex of p75ntr and sortilin. *The Journal of Neuroscience, 25*(22), 5455. doi:10.1523/JNEUROSCI.5123-04.2005

Teng, H. K., et al. (2005b). Probdnf induces neuronal apoptosis via activation of a receptor complex of p75ntr and sortilin. *J Neurosci, 25*(22), 5455-5463. doi:10.1523/JNEUROSCI.5123-04.2005

Terburg, D., et al. (2009). The testosterone-cortisol ratio: A hormonal marker for proneness to social aggression. *Int J Law Psychiatry, 32*(4), 216-223. doi:10.1016/j.ijlp.2009.04.008

Testa, M. (2002). The impact of men's alcohol consumption on perpetration of sexual aggression. *Clin Psychol Rev, 22*(8), 1239-1263. doi:10.1016/s0272-7358(02)00204-0

Thomas, E. A., et al. (2013). Eating-related behaviors and appetite during energy imbalance in obese-prone and obese-resistant individuals. *Appetite, 65*, 96-102. doi:10.1016/j.appet.2013.01.015

Tigbe, W. W., et al. (2017). Time spent in sedentary posture is associated with waist circumference and cardiovascular risk. *Int J Obes (Lond), 41*(5), 689-696. doi:10.1038/ijo.2017.30

Timper, K.& Brüning, J. C. (2017). Hypothalamic circuits regulating appetite and energy homeostasis: Pathways to obesity. *Disease models & mechanisms, 10*(6), 679-689. doi:10.1242/dmm.026609

Tonon, M. C., et al. (2013). Hypothalamic glucose-sensing: Role of glia-to-neuron signaling. *Horm Metab Res, 45*(13), 955-959. doi:10.1055/s-0033-1355357

Toth, M., et al. (2010). Neural inputs of the hypothalamic "aggression area" in the rat. *Behav Brain Res, 215*(1), 7-20. doi:10.1016/j.bbr.2010.05.050

Tran, P. V., et al. (2006). Diminished hypothalamic bdnf expression and impaired vmh function are associated with reduced sf-1 gene dosage. *Journal of Comparative Neurology, 498*(5), 637-648. doi:https://doi.org/10.1002/cne.21070

Trapp, S.& Richards, J. E. (2013). The gut hormone glucagon-like peptide-1 produced in brain: Is this physiologically relevant? *Current opinion in pharmacology, 13*(6), 964-969. doi:10.1016/j.coph.2013.09.006

Tremblay, A.& Bellisle, F. (2015). Nutrients, satiety, and control of energy intake. *Appl Physiol Nutr Metab, 40*(10), 971-979. doi:10.1139/apnm-2014-0549

Tremblay, R. E. (2010). Developmental origins of disruptive behaviour problems: The 'original sin' hypothesis, epigenetics and their consequences for prevention. *Journal of Child Psychology and Psychiatry, 51*(4), 341-367. doi:https://doi.org/10.1111/j.1469-7610.2010.02211.x

Triantafyllou, G. A., et al. (2016). Leptin and hormones: Energy homeostasis. *Endocrinol Metab Clin North Am, 45*(3), 633-645. doi:10.1016/j.ecl.2016.04.012

Tsankova, N. M., et al. (2006). Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat Neurosci, 9*(4), 519-525. doi:10.1038/nn1659

Tsao, D., et al. (2008). Trkb agonists ameliorate obesity and associated metabolic conditions in mice. *Endocrinology, 149*(3), 1038-1048. doi:10.1210/en.2007-1166

Tsuchida, A., et al. (2001). The effects of brain-derived neurotrophic factor on insulin signal transduction in the liver of diabetic mice. *Diabetologia, 44*(5), 555-566. doi:10.1007/s001250051661

Tümer, N., et al. (2006). Hypothalamic raav-mediated gdnf gene delivery ameliorates age-related obesity. *Neurobiology of Aging, 27*(3), 459-470. doi:https://doi.org/10.1016/j.neurobiolaging.2005.03.018

Turner, A. K. (1994). Genetic and hormonal influences on male violence. *Male violence. New York: Routledge*, 233-252.

Turner, A. M.& Greenough, W. T. (1985). Differential rearing effects on rat visual cortex synapses. I. Synaptic and neuronal density and synapses per neuron. *Brain research, 329*(1), 195-203. doi:https://doi.org/10.1016/0006-8993(85)90525-6

Unger, E. K., et al. (2015). Medial amygdalar aromatase neurons regulate aggression in both sexes. *Cell Rep, 10*(4), 453-462. doi:10.1016/j.celrep.2014.12.040

Unger, T. J., et al. (2007). Selective deletion of bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. *J Neurosci, 27*(52), 14265-14274. doi:10.1523/JNEUROSCI.3308-07.2007

Vallis, M. (2016). Quality of life and psychological well-being in obesity management: Improving the odds of success by managing distress. *International Journal of Clinical Practice, 70*(3), 196-205. doi:https://doi.org/10.1111/ijcp.12765

van Amelsvoort, L. G., et al. (1999). Duration of shiftwork related to body mass index and waist to hip ratio. *Int J Obes Relat Metab Disord, 23*(9), 973-978. doi:10.1038/sj.ijo.0801028

van den Top, M., et al. (2004). Orexigen-sensitive npy/agrp pacemaker neurons in the hypothalamic arcuate nucleus. *Nat Neurosci, 7*(5), 493-494. doi:10.1038/nn1226

van der Wal, M. F., et al. (2003). Psychosocial health among young victims and offenders of direct and indirect bullying. *Pediatrics, 111*(6 Pt 1), 1312-1317. doi:10.1542/peds.111.6.1312

Van Heek, M., et al. (1997). Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *J Clin Invest, 99*(3), 385-390. doi:10.1172/jci119171

van Honk, J., et al. (2010). Socially explosive minds: The triple imbalance hypothesis of reactive aggression. *J Pers, 78*(1), 67-94. doi:10.1111/j.1467-6494.2009.00609.x

Van Loo, P. L. P., et al. (2002). Influence of cage enrichment on aggressive behaviour and physiological parameters in male mice. *Applied Animal Behaviour Science, 76*(1), 65-81. doi:https://doi.org/10.1016/S0168-1591(01)00200-3

Van Loo, P. L. P., et al. (2001). Modulation of aggression in male mice: Influence of group size and cage size. *Physiology & Behavior, 72*(5), 675-683. doi:https://doi.org/10.1016/S0031-9384(01)00425-5

Van Loo, P. L. P., et al. (2003). Strain-specific aggressive behavior of male mice submitted to different husbandry procedures. *Aggressive Behavior, 29*(1), 69-80. doi:https://doi.org/10.1002/ab.10035

Van Loo, P. L. P., et al. (2003). Male management: Coping with aggression problems in male laboratory mice. *Lab Anim, 37*(4), 300-313. doi:10.1258/002367703322389870

Van Praag, H., et al. (2000). Neural consequences of enviromental enrichment. *Nature Reviews Neuroscience, 1*(3), 191-198.

VanElzakker, M., et al. (2008). Environmental novelty is associated with a selective increase in fos expression in the output elements of the hippocampal formation and the perirhinal cortex. *Learning & memory (Cold Spring Harbor, N.Y.), 15*(12), 899-908. doi:10.1101/lm.1196508

Vanevski, F.& Xu, B. (2013). Molecular and neural bases underlying roles of bdnf in the control of body weight. *Frontiers in neuroscience, 7*(37). doi:10.3389/fnins.2013.00037

Vaynman, S., et al. (2004). Hippocampal bdnf mediates the efficacy of exercise on synaptic plasticity and cognition. *Eur J Neurosci, 20*(10), 2580-2590. doi:10.1111/j.1460-9568.2004.03720.x

Vertes, R. P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse, 51*(1), 32-58. doi:10.1002/syn.10279

Vestal, B. M.& Schnell, G. D. (1986). Influence of environmental complexity and space on social interactions of mice (mus musculus and peromyscus leucopus). *Journal of Comparative Psychology, 100*(2), 143-154. doi:10.1037/0735-7036.100.2.143

Vitalo, A., et al. (2009). Nest making and oxytocin comparably promote wound healing in isolation reared rats. *PLoS One, 4*(5), e5523. doi:10.1371/journal.pone.0005523

Vitalo, A. G., et al. (2012). Environmental enrichment with nesting material accelerates wound healing in isolation-reared rats. *Behavioural Brain Research, 226*(2), 606-612. doi:https://doi.org/10.1016/j.bbr.2011.09.038

Volavka, J.& Citrome, L. (2011). Pathways to aggression in schizophrenia affect results of treatment. *Schizophr Bull, 37*(5), 921-929. doi:10.1093/schbul/sbr041

vom Saal, F. S. (1983). Models of early hormonal effects on intrasex aggression in mice. In *Hormones and aggressive behavior* (pp. 197-222): Springer.

Wajchenberg, B. L. (2000). Subcutaneous and visceral adipose tissue: Their relation to the metabolic syndrome. *Endocr Rev, 21*(6), 697-738. doi:10.1210/edrv.21.6.0415

Wang, C., et al. (2007). Brain-derived neurotrophic factor in the hypothalamic paraventricular nucleus increases energy expenditure by elevating metabolic rate. *Am J Physiol Regul Integr Comp Physiol, 293*(3), R992-1002. doi:10.1152/ajpregu.00516.2006

Wang, C., et al. (2010). Brain-derived neurotrophic factor (bdnf) in the hypothalamic ventromedial nucleus increases energy expenditure. *Brain research, 1336*, 66-77. doi:10.1016/j.brainres.2010.04.013

Wang, C., et al. (2007). Brain-derived neurotrophic factor in the ventromedial nucleus of the hypothalamus reduces energy intake. *Am J Physiol Regul Integr Comp Physiol, 293*(3), R1037-1045. doi:10.1152/ajpregu.00125.2007

Wang, H., et al. (2019). Influence of urban green open space on residents' physical activity in china. *BMC Public Health, 19*(1), 1093. doi:10.1186/s12889-019-7416-7

Wang, L., et al. (2019). Glucose transporter 1 critically controls microglial activation through facilitating glycolysis. *Mol Neurodegener, 14*(1), 2. doi:10.1186/s13024-019-0305-9

West, A. E., et al. (2014). Neurotrophins: Transcription and translation. In G. R. Lewin & B. D. Carter (Eds.), *Neurotrophic factors* (pp. 67-100). Berlin, Heidelberg: Springer Berlin Heidelberg.

White, S. M., et al. (1991). Effects of serotonergic agents on isolation-induced aggression. *Pharmacol Biochem Behav, 39*(3), 729-736. doi:10.1016/0091-3057(91)90155-u

WHO. (2009). Changing cultural and social norms that support violence. In. Geneva: World Health Organization.

WHO. (2020). Obesity and overweight. *WHO fact sheets.* 1 April 2020. Retrieved from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight#:~:text=%2Fm2).-,Adults,than%20or%20equal%20to%2030.

Who expert committee on problems related to alcohol consumption. (0512-3054 (Print)

0512-3054). (2007). Retrieved from https://www.who.int/news-room/events/detail/2006/10/10/default-calendar/who-expert-committee-on-problems-related-to-alcohol-consumption

Whone, A. L., et al. (2019). Extended treatment with glial cell line-derived neurotrophic factor in parkinson's disease. *Journal of Parkinson's Disease, 9*, 301-313. doi:10.3233/JPD-191576

Williamson, C. M., et al. (2016). Temporal dynamics of social hierarchy formation and maintenance in male mice. *Animal Behaviour, 115*, 259-272. doi:https://doi.org/10.1016/j.anbehav.2016.03.004

Williamson, L. L., et al. (2012). Environmental enrichment alters glial antigen expression and neuroimmune function in the adult rat hippocampus. *Brain Behav Immun, 26*(3), 500-510. doi:10.1016/j.bbi.2012.01.003

Wolf, S. A., et al. (2006). Cognitive and physical activity differently modulate disease progression in the amyloid precursor protein (app)-23 model of alzheimer's disease. *Biol Psychiatry, 60*(12), 1314-1323. doi:10.1016/j.biopsych.2006.04.004

Wolnerhanssen, B. K., et al. (2015). Dissociable behavioral, physiological and neural effects of acute glucose and fructose ingestion: A pilot study. *PLoS One, 10*(6), 15. doi:10.1371/journal.pone.0130280

Wong, L. C., et al. (2016). Effective modulation of male aggression through lateral septum to medial hypothalamus projection. *Current biology : CB, 26*(5), 593-604. doi:10.1016/j.cub.2015.12.065

Wu, Q., et al. (2012). Deciphering a neuronal circuit that mediates appetite. *Nature, 483*(7391), 594-597. doi:10.1038/nature10899

Wu, Y., et al. (2016). Enriched environment housing enhances the sensitivity of mouse pancreatic cancer to chemotherapeutic agents. *Biochemical and biophysical research communications, 473*(2), 593-599.

Würbel, H. (2001). Ideal homes? Housing effects on rodent brain and behaviour. *Trends Neurosci, 24*(4), 207-211. doi:10.1016/s0166-2236(00)01718-5

Würbel, H. (2006). The motivational basis of caged rodents' stereotypies. *Stereotypic Animal Behaviour: Fundamentals and Applications to Welfare*, 86-120. doi:10.1079/9780851990040.0086

Würbel, H.& Garner, J. P. (2007). Refinement of rodent research through environmental enrichment and systematic randomization. *NC3Rs, 9*, 1-9.

Xie, X., et al. (2019). Activation of anxiogenic circuits instigates resistance to diet-induced obesity via increased energy expenditure. *Cell Metab, 29*(4), 917-931 e914. doi:10.1016/j.cmet.2018.12.018

Xu, B., et al. (2003). Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. *Nat Neurosci, 6*(7), 736-742. doi:10.1038/nn1073

Xu, B.& Xie, X. (2016). Neurotrophic factor control of satiety and body weight. *Nature Reviews Neuroscience, 17*(5), 282-292. doi:10.1038/nrn.2016.24

Xu, H., et al. (2018). Association of obesity with mortality over 24 years of weight history: Findings from the framingham heart study. *JAMA network open, 1*(7), e184587-e184587. doi:10.1001/jamanetworkopen.2018.4587

Xu, X.-M., et al. (2018). The effect of aggression i: The increases of metabolic cost and mobilization of fat reserves in male striped hamsters. *Hormones and Behavior, 98*, 55-62. doi:https://doi.org/10.1016/j.yhbeh.2017.12.015

Yamashita, T., et al. (1999). Neurotrophin binding to the p75 receptor modulates rho activity and axonal outgrowth. *Neuron, 24*(3), 585-593. doi:https://doi.org/10.1016/S0896-6273(00)81114-9

Yang, C. F., et al. (2013). Sexually dimorphic neurons in the ventromedial hypothalamus govern mating in both sexes and aggression in males. *Cell, 153*(4), 896-909. doi:10.1016/j.cell.2013.04.017

Yang, S., et al. (2018). Manf: A new player in the control of energy homeostasis, and beyond. *Frontiers in Physiology, 9*, 1725-1725. doi:10.3389/fphys.2018.01725

Yang, S., et al. (2017). Manf regulates hypothalamic control of food intake and body weight. *Nature Communications, 8*(1), 579. doi:10.1038/s41467-017-00750-x

Yang, Y.& Raine, A. (2009). Prefrontal structural and functional brain imaging findings in antisocial, violent, and psychopathic individuals: A meta-analysis. *Psychiatry research, 174*(2), 81-88. doi:10.1016/j.pscychresns.2009.03.012

Yavarna, T., et al. (2015). High diagnostic yield of clinical exome sequencing in middle eastern patients with mendelian disorders. *Hum Genet, 134*(9), 967-980. doi:10.1007/s00439-015-1575-0

Yeo, G. S., et al. (2004). A de novo mutation affecting human trkb associated with severe obesity and developmental delay. *Nat Neurosci, 7*(11), 1187-1189. doi:10.1038/nn1336

Yeshurun, S., et al. (2017). Paternal environmental enrichment transgenerationally alters affective behavioral and neuroendocrine phenotypes. *Psychoneuroendocrinology, 77*, 225-235. doi:10.1016/j.psyneuen.2016.11.013

Yoon, N. A.& Diano, S. (2021). Hypothalamic glucose-sensing mechanisms. *Diabetologia, 64*(5), 985-993. doi:10.1007/s00125-021-05395-6

Yoshii, A.& Constantine-Paton, M. (2010). Postsynaptic bdnf-trkb signaling in synapse maturation, plasticity, and disease. *Developmental Neurobiology, 70*(5), 304-322. doi:https://doi.org/10.1002/dneu.20765

You, H., et al. (2020). A subpopulation of bdnf-e1–expressing glutamatergic neurons in the lateral hypothalamus critical for thermogenesis control. *Molecular Metabolism, 31*, 109-123. doi:https://doi.org/10.1016/j.molmet.2019.11.013

Young, D., et al. (1999). Environmental enrichment inhibits spontaneous apoptosis, prevents seizures and is neuroprotective. *Nature Medicine, 5*(4), 448-453. doi:10.1038/7449

Yudofsky, S., et al. (1981). Propranolol in the treatment of rage and violent behavior in patients with chronic brain syndromes. *The American journal of psychiatry, 138*(2), 218-220. doi:10.1176/ajp.138.2.218

Zampieri, N.& Chao, M. V. (2006). Mechanisms of neurotrophin receptor signalling. *Biochemical Society Transactions, 34*(4), 607-611. doi:10.1042/bst0340607

Zeman, W.& King, F. A. (1958). Tumors of the septum pellucidum and adjacent structures with abnormal affective behavior: An anterior midline structure syndrome. *J Nerv Ment Dis, 127*(6), 490-502. doi:10.1097/00005053-195812000-00002

Zha, X., et al. (2020). Vmhvl-projecting vglut1+ neurons in the posterior amygdala gate territorial aggression. *Cell Reports, 31*(3), 107517. doi:https://doi.org/10.1016/j.celrep.2020.03.081

Zhan, C., et al. (2013). Acute and long-term suppression of feeding behavior by pomc neurons in the brainstem and hypothalamus, respectively. *The Journal of Neuroscience, 33*(8), 3624. doi:10.1523/JNEUROSCI.2742-12.2013

Zhang, F., et al. (2005). Leptin: Structure, function and biology. *Vitam Horm, 71*, 345-372. doi:10.1016/S0083-6729(05)71012-8

Zhang, T. (2013). An estimation of the economic impact of spousal violence in canada, 2009. Retrieved from https://www.deslibris.ca/ID/237372

Zhao, F. Q.& Keating, A. F. (2007). Functional properties and genomics of glucose transporters. *Curr Genomics, 8*(2), 113-128. doi:10.2174/138920207780368187

Zhong, S., et al. (2020). Drug use disorders and violence: Associations with individual drug categories. *Epidemiologic Reviews, 42*(1), 103-116. doi:10.1093/epirev/mxaa006

Zhou, L., et al. (2010). The medial amygdalar nucleus: A novel glucose-sensing region that modulates the counterregulatory response to hypoglycemia. *Diabetes, 59*(10), 2646-2652. doi:10.2337/db09-0995