Impaired GABA Plasticity at ovBNST Synapses Predicts Compulsive Drinking in Schedule-Induced Polydipsia.

By

Staci Angelis

A thesis submitted to the graduate program in Neuroscience in conformity with the requirements for the degree of Masters of Science.

Queen’s University

Kingston, Ontario, Canada

[September, 2016]

Copyright © (Staci Angelis, 2016)
Abstract

Schedule-Induced Polydipsia (SIP) is an animal model of adjunctive drinking induced when a hungry rat receives food on a fixed interval of time. This model has been implemented as a model of compulsive behaviour and may represent a powerful tool to understand the neural mechanisms of compulsion. The bed nucleus of the stria terminalis (BNST) is thought to translate challenges to energy homeostasis into consummatory behaviours, and is therefore likely to contribute to drinking behaviours displayed by food restricted rats in the SIP paradigm. Furthermore, the BNST seems implicated in various compulsive behaviors, including compulsive water drinking in rats. Therefore, the goal of this project was to determine whether compulsive drinking in the SIP paradigm was associated with alterations in transmission at oval BNST (ovBNST) synapses. Rats undergoing the SIP procedure had restricted food access (1-hours/day) for a total of 29 days. After 7 days of food restriction and for the next 21 consecutive days, the rats had daily 2-hour access to operant conditioning chambers where they were presented with a 45-mg food pellet every minute. Water consumed during these 2-hour sessions was measured and the rats that drank 15 ml or more water for a minimum of 3 consecutive days were considered High Drinkers (HD; n=17) or otherwise, Low Drinkers (LD; n=13). Brain slices whole-cell patch clamp recordings conducted 18-hours after the last SIP training showed that chronic food restriction changed low frequency stimulation (LFS) - induced long-term potentiation of ovBNST inhibitory synaptic transmission (iLTP) into LFS - induced long-term depression (iLTD) in a majority of neurons, regardless of drinking behaviours. However, ad libitum access to food between the last day of SIP training and brain slice recordings (18-hour refeed) rescued LFS-induced iLTP in LD but not in HD, suggesting that impaired bi-directional plasticity of ovBNST synapses may contribute to compulsive drinking in the SIP paradigm.
Co-Authorship

Research for this thesis was conducted by Staci Angelis under the supervision of Dr. Eric Dumont. All behavioural training and data analysis was conducted by Staci Angelis. Whole-cell patch clamping for this thesis was done by Staci Angelis, James Gardner Gregory, Catherine Normandeau and Michael Naughton. Dr. Emily Hawken provided background knowledge of the SIP protocol and trained Staci Angelis.
Acknowledgements

I am grateful first and foremost to my fiancé, James Gardner Gregory, for his help collecting data and his support throughout my degree. I could not have completed my MSc. without his wise words, patience and love. Second, I would like to thank Dr. Eric Dumont for pushing me to strive for excellence. I am incredibly grateful for his countless hours editing and helping me with my thesis. Most importantly, for believing in my potential as a researcher. Additionally, I would like to thank Catherine Normandeau, Michael Naughton and Dr. Emily Hawken for their help collecting data and training me on whole-cell patch clamp and schedule-induced polydipsia. I would like to thank my parents, Mary Arhontas and Peter Angelis, for instilling in me a good work ethic. I am the person I am today because of all of their unconditional love and support. Thank you for always pushing me towards becoming the best version of myself. To my siblings, Nicholas and Diana Angelis, thank you for your encouragement and for always knowing how to make me laugh. Finally, I would like to thank my committee members for taking the time to read my thesis.
# Table of Contents

Abstract .................................................................................................................................................. i  
Co-Authorship ...................................................................................................................................... ii  
Acknowledgements ................................................................................................................................ iii  
List of Figures ....................................................................................................................................... v  
List of Abbreviations ............................................................................................................................. vii  
Chapter 1: Introduction ......................................................................................................................... 1  
Chapter 2: Method ............................................................................................................................... 11  
Chapter 3: Results .............................................................................................................................. 19  
Chapter 4: Discussion .......................................................................................................................... 31  
Chapter 5: Reference List .................................................................................................................... 38
List of Figures

Figure 1. Schematic depicting the differences in drinking patterns between High Drinkers and Low Drinkers. *Adapted from Gardner Gregory et al., (2015), SFN*………………………………………2

Figure 2. Schematic demonstrating the relative position of the ovBNST within the BNST. *Image adapted from Hammack, Mania & Rainie (2007)*……………………………………………………………..6

Figure 3. Representative traces of the three patterns of GABAA-IPSC amplitude changes (LTP, LTD, NC) following the LFS protocol…………………………………………………………………………………………18

Figure 4. Water consumption (ml) as a function of time (days) for both HD and LD during the 2h SIP training………………………………………………………………………………………………………………………………………………22

Figure 5. Mean water consumption (ml) as a function of time (days) during the 22h period in the home cage environment for both HD and LD…………………………………………………………………………………………………………………………23

Figure 6. Mean total water consumption (ml) as a function of time (days) during the 24h period of SIP training and home cage environment for both HD and LD…………………………………………………………………………………………………………………………24

Figure 7. GABAA-IPSC amplitude percent change in the naïve control condition…………….25

Figure 8. GABAA-IPSC amplitude percent change in the cFDR condition…………….25

Figure 9. GABAA-IPSC amplitude percent change in the cFDR-refeed condition…………….26

Figure 10. Percentage of cells that either demonstrated a LTP, LTD or NC following LFS-induced GABAA-IPSC for the following conditions: naïve control, cFDR and cFDR-refeed….26

Figure 11. GABAA-IPSC amplitude percent change in the HD condition…………………………………27

Figure 12. GABAA-IPSC amplitude percent change in the LD condition…………………………………27

Figure 13. GABAA-IPSC amplitude percent change in the HD-refeed condition………………………….28
Figure 14. GABA$_A$-IPSC amplitude percent change in the LD-refeed condition ....................28

Figure 15. Percentage of cells that either demonstrated a LTP, LTD or NC following LFS-induced GABA$_A$-IPSC for the following conditions: HD, LD, HD-refeed and LD-refeed ..............29

Figure 16. Correlation between GABA$_A$-IPSC amplitude percentage at 20 minutes post-LFS and water consumed on the last day of SIP training in refeed rats ..................................................29

Figure 17. Summary of the effect of SIP on the ratio of AMPA to NMDA receptor for the following conditions: HD, LD, HD-refeed and LD-refeed .................................................30

Figure 18. Mechanism underlying LFS-induced GABA$_A$-IPSC amplitude ......................37
List of Abbreviations

AMPA: amino-3-hydrox-5-methyl-4-isoxazolepropionic acid

ANOVA: analysis of variance

ATP: adenosine triphosphate

BNST: bed nucleus of the stria terminalis

cFDR: chronic food restriction

cFDR-refeed: chronic food restriction followed by an 18-hour refeed

D-AP5: D-amino-phosphonopentanoid acid

DMSO: dimethyl sulfoxide

DNQX: 6,7-dinitroquinoxaline-2,3-dione

DSM-V: Diagnostic and Statistical Manual of Mental Disorder, fifth edition

E2: 17β estradiol

eCB: endocannabinoid

EPSC: excitatory post-synaptic current

ERα: estrogen receptor alpha

fMRI: functional magnetic resonance imaging

GABA: gamma-aminobutyric acid

GTP: guanosine triphosphate
HD: High Drinkers

HD-refeed: High Drinkers with an 18-hour refeed

iLTD: long term depression of inhibitory synaptic transmission

iLTP: long term potentiation of inhibitory synaptic transmission

IPSC: inhibitory post-synaptic current

LD: Low Drinkers

LD-refeed: Low Drinkers with an 18-hour refeed

LH: lateral hypothalamus

LFS: low frequency stimulation

LTD: long term depression

LTP: long term potentiation

NC: no change

NMDA: $N$-methyl-$D$-aspartate

OCD: Obsessive-Compulsive Disorder

ovBNST: oval bed nucleus of the stria terminalis

PVT: posterior paraventricular thalamus

SIP: Schedule-Induced Polydipsia
Chapter 1: Introduction

Compulsions are defined as repetitive actions, usually aimed at reducing anxiety. These actions are usually time-consuming and cause distress and impairment over an individual’s quality of life (DSM-V). Investigating the neuronal basis of compulsivity is a step forwards for identifying risk factors and preventative strategies. Therefore, understanding compromised neuronal synaptic transmission is key for determining individual susceptibility for developing compulsive behaviours. Schedule-induced polydipsia (SIP), is an animal model and useful tool to further understand the neuronal mechanisms underlying compulsive behaviours.

1.1 Schedule-induced polydipsia, stress and individual differences

SIP is a model of adjunctive drinking arising in food-restricted rats when presented with food according to a fixed time schedule (Falk, 1966; Platt, Beyer, Schechter & Rosenzweig-Lipson, 2008). This model was developed by John L. Falk and first published in 1961 intending to investigate renal functioning. This model grew in relevance as multiple human case studies describing this excess water drinking, which was later coined as psychogenic polydipsia and compulsive polydipsia, came to light (Goodner, Arnas, Andros & Waterhouse, 1971; Rendell, McGrane & Cuesta, 1978). Prior to the characterisation of polydipsia, cases of excess water drinking were predominantly associated with diabetes insipidus. Psychogenic polydipsia became a medical concern as more case studies warned of the intoxication risks and increases in related fatalities (Bewley, 1964; Raskind, 1974; Rendell, McGrane & Cuesta, 1978). At the time, medical doctors were unsure what the leading causes of polydipsia were (Leiken & Caplan, 1967; Goodner, Arnas, Andros & Waterhouse, 1971). However, as more case studies were published, it was hard to ignore a common link between patients: a history of mental illness (Mendelson & Deza, 1976, Raskind, 1974; Goodner, Arnas, Andros & Waterhouse, 1971; Rendell, McGrane &
Cuesta, 1978). Specifically, patients usually had a history of psychomotor seizures, schizophrenia, depression, suicidal ideation, violent behaviour, drug addiction and alcoholism (Goodner, Arnas, Andros & Waterhouse, 1971; Mendelson & Deza, 1976; Rendell, McGrane & Cuesta, 1978; Bewley, 1964). Thus, polydipsia, like that observed in the SIP model, is often found to be co-morbid with patients diagnosed with schizophrenia (Hawken & Beninger, 2014; Mercier-Guidez & Loas, 2000; de Leon, Tracy, McCann, & McGrory, 2002). Two risk factors were identified for developing polydipsia. First, this behaviour may have resulted from the strict hospital schedule implemented for the daily functioning of patients; by analogy to the fixed time schedule of food presentation in SIP. Second, there was a high association between smoking and polydipsia (Mercier-Guidez & Loas, 2000; de Leon, Tracy, McCann, & McGrory, 2002). Current research determined that enhanced dopaminergic sensitivity, a phenomenon observed in schizophrenia, increased the susceptibility of SIP in rats (Hawken & Beninger, 2014). Moreover, only a proportion, 6 to 20%, of individuals diagnosed with schizophrenia developed the comorbidity with polydipsia (Evenson, Jos, & Mallya, 1987). This suggests that there may be an underlying vulnerability driving this behaviour. Thus, understanding the underlying mechanisms of vulnerability to SIP may provide insight into understanding the co-morbidity into polydipsia with other psychological disorders.

SIP is unique in that the entire population is not vulnerable in developing the compulsive drinking behaviour (Falk, 1966; Gardner Gregory et al., 2015). Thus, two groups are ultimately formed: High Drinkers (HD) and Low Drinkers (LD) representing compulsive and resistant rats, respectively (Figure 1). These organically

Figure 1. Schematic depicting the difference in drinking patterns between High Drinkers (HD; black) and Low Drinkers (LD; grey). Adapted from Gardner Gregory et al., 2015.
formed groups make this model ideal for investigating the vulnerability for developing compulsive behaviours. Additionally, other adjunctive behaviours that have been observed in animals exposed to food presented in a fixed time schedule include: air licking, wheel running, ingestion of wood shavings, and pecking behaviours in birds (Wallace & Singer, 1976). As a result, this model has recently generated interest as a model for investigating the vulnerability of developing compulsive behaviours in psychological disorders such as in Obsessive-Compulsive Disorder (OCD; Moreno & Flores, 2012). In fact, SIP causes alterations to areas of the brain highly associated with the development and maintenance of OCD and habitual behaviours such as the prefrontal cortex regions (Gardner Gregory et al., 2015). Furthermore, research investigating its face validity and comparability with human cases of OCD has determined that drugs prescribed to reduce OCD symptoms such as fluoxetine, fluvoxamine and clomipramine, decreased the amount of water consumed by rats exposed to SIP training (Woods et al., 1993). Additionally, drugs that have no effects on OCD symptoms similarly had no effects on the water intake of rats exposed to SIP training (Woods et al., 1993). Taken together, SIP research has multiple implications affecting various aspects of mental illness.

As stated in the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V), the compulsions observed in clinical cases of OCD are postulated to manifest as a result of stress with the intent of alleviating it. Stress is a construct used to describe a physiological response to a homeostatic challenge. This challenge, or stressor, causes an activation of the sympathetic nervous system and of the hypothalamo-pituitary-adrenal axis. Thus, resulting in an increase in cortisol or corticosterone levels for humans and rodents respectively (Fink, 2009). Usually, the parasympathetic and sympathetic nervous systems work together so that homeostasis can be restored following a stressor. However, in pathological
disorders, this balance might be compromised. For HD to develop, rats need be in a stressful state, accomplished through chronic food restriction (cFDR) in conjunction with the scheduled-delivery of food (Lopez-Grancha et al., 2006; Falk, 1961). This is further observed with adrenalectomy; removing the body’s primary source of corticosterone attenuated drinking. This was reversed with corticosterone replacement, supporting that corticosterone elevation is necessary for compulsive drinking to occur (Levine & Levine, 1989). Interestingly, having access to a drinking spout during SIP decreases plasma corticosterone levels (Mittleman, Jones & Robbins, 1988; Tazi, Dantzer, Mormede & Le Moal, 1986; Brett & Levine, 1979; Brett & Levine, 1981). Furthermore, an inverse relationship exists between plasma corticosterone levels and volume of water consumed during SIP (Mittleman, Jones & Robbins, 1988). Thus, at a glance it appears that drinking water is an adaptive response to being in this stressful state.

Contrarily, arguments have been made that water drinking in SIP is maladaptive due to the risks of hyponatremia (Hawken et al., 2013). Thus, examining the differences between HD and LD is crucial for understanding why certain rats are more vulnerable in developing maladaptive behaviours. It is theorized that HD might have a heightened sensitivity to stress compared to LD resulting in an increase and overacting drive to restore homeostasis (Danzter, 1991). Furthermore, differences in stress sensitivity might account for the variability in prevalence rates observed in OCD (Kim et al., 2008; Kircanski, Wu, & Piacentini, 2014).

Ultimately, SIP has been used to investigate multiple aspects of mental illness. The categorization of HD and LD makes it ideal for investigating differences of susceptibility and sensitivity between rats in developing compulsive water drinking when presented with stressful state (i.e. a shift in their homeostasis following food restriction). Therefore, this model is a useful tool in the investigation of neurobiological differences between HD and LD.
1.2 Chronic food restriction: a necessary condition for SIP

Being in a food deprived state increases the likelihood of developing compulsive behaviors (Shalev, 2012). Therefore, it is critical for the compulsive drinking to occur during SIP. This is related to the shift in homeostasis previously discussed, resulting in elevated corticosterone levels. The rats require this stressor when presented with the fixed schedule delivery of food during SIP training (Falk, 1966). Furthermore, drinking water during the training session reduces the elevated corticosterone levels (Mittleman, Jones & Robbins, 1988; Tazi, Dantzer, Mormede & Le Moal, 1986; Brett & Levine, 1979; Brett & Levine, 1981). It can be suggested that HD have a predisposition sensitivity to such a homeostatic challenge that results in an overcompensation of behavior. This sensitivity has never been explored at the neurophysiological level. Furthermore, it may account for the difference in behaviour (HD versus LD). Ultimately, exploring the sensitivity towards hunger might account for variations in drinking observed in SIP.

1.3 The bed nucleus stria terminalis: critically involved in homeostasis and compulsivity

The bed nucleus of the stria terminalis (BNST) is a critical area when investigating compulsive behaviours. This is a result of its crucial role in maintaining physiological homeostasis (Dumont, 2009). Traditionally, the BNST is divided into two sections, the posterior and anterior, each being comprised of multiple nuclei. One such nucleus is the oval nucleus of the BNST (ovBNST), found in the anterior BNST (Figure 2). The posterior BNST is implicated in reproduction and defensive behaviours while the anterior is involved in energy balance and homeostasis (Dumont, 2009; Dong & Swanson, 2006; Dong & Swanson 2004; Dong & Swanson 2004; Dong & Swanson 2006; Ju & Swanson, 1989; Ju, Swanson, & Simerly, 1989). The BNST receives and integrates multiple inputs before sending the subsequent projections to other brain
structures. Therefore, it is involved in numerous mechanisms ranging from physiological drives and pathophysiological states such as food intake, anxiety, fear, social interaction, addiction, and compulsive behaviours (Ciccocioppo et al., 2003; Crowne, King, Meagher, & Grau, 2000; Deyama et al., 2007; Dumont, Mark, Mader & Williams, 2005; Dunn & Williams, 1995; Fendt, Endres, & Apfelbach, 2003; Jasnow, Davis & Huhman, 2004; Liu, Chen & Liang, 2009; Sajdyk, Johnson, Fitz & Schekhar, 2008). Due to its position in addiction pathways, the ovBNST has been a target area for investigating compulsive drug seeking (deBacker et al., 2015). Overall, the BNST presents as a promising target area for understanding what drives the difference in compulsive tendencies between HD and LD.

As previously discussed, a homeostatic shift is a critical component driving SIP and may contribute to the vulnerability creating the two groups (HD and LD). Therefore, investigating the BNST regulation of homeostasis may provide important insights for maladaptive drinking in HD. This is easily observed through an anecdote depicting specific phobias. Spider phobias have a rational basis in that there are venomous spiders, so being afraid and removing oneself is adaptive. However, being crippled by fear by a spider can have a devastating and distressful impact on an individual’s daily functioning. Research investigating this in human functional magnetic resonance imaging (fMRI) studies demonstrates an increase activation of the BNST in individuals with a spider phobia when they were presented when a picture of a spider. This activation was not observed in the amygdala, an area critically involved in stress and emotion. However, when the actual threat was physically presented, both areas are activated. This research suggests that the BNST is involved in assessing future threats (Straube, Mentzel & Miltner, 2007; Lebow & Chen, 2016). Moreover, when comparing unpredictable and predictable
stressors, the BNST demonstrates greatest activation with an unpredictable and unavoidable stressor in conjunction with an increased sense of loss of control (Lebow & Chen, 2016; Alvarez et al., 2011; Somerville, Whalenm & Kelly, 2010). Thus, the BNST is involved in mediating anticipatory threats, especially when in a state of decreased control to prevent such threat from occurring (Lebow & Chen, 2016). Recently, it has been reported that the BNST assigns salience to external stimuli (Lebow & Chen, 2016; Daniel & Rannie, 2015). In SIP, the homeostatic challenge of food restriction is a threat to survival. These findings support that a potential dysregulation within the BNST might account for the shift from adaptive to maladaptive behaviour, and specifically how rats cope when presented with this challenge. Thus, this imbalanced system may be involved in the misattribution of stress coping behaviours, such as the compulsive behaviours observed in OCD or drinking in SIP. Hence, the BNST remains an interesting and promising area of investigating this pathological behaviour.

These findings have been corroborated with animal research examining the BNST and compulsive behaviours. Using SIP, scientists observe an increase in firing rate of single-cell neurons, in vivo, of the medial BNST in HD when compared to the LD (Welkenhysen et al., 2013). Additionally, a decrease in bursting behaviour of these neurons is observed in HD when compared to LD (Welkenhysen et al., 2013). This pattern of neuronal firing suggests an increase vulnerability in the development of compulsive behaviour, in this case, compulsive water consummation (Welkenhysen et al., 2013). Furthermore, water consumption in compulsive rats decreased following high frequency stimulation of BNST neurons (van Kuyck et al., 2008). These findings were the first to characterize differences between HD and LD at the level of neuronal activity. However, this provides a limited scope of the neurophysiological trace as this technique is unable to distinguish between excitatory and inhibitory transmission within a living
organism. Therefore, exploring the synaptic drives may further explain potential neuronal dysregulation between HD and LD. Furthermore, exploring the synaptic inputs into the BNST may explain how synaptic transmission is altered in HD.

1.4. Neuronal transmission at BNST synapses: excitatory versus inhibitory transmission

Contrary to in vivo electrophysiology that records cell activity directly from living organisms, in vitro whole-cell patch clamp electrophysiology records from a brain slice preparation. A small glass pipette “patched” onto a neuron enables live measures of electrical activity. The benefit of slice electrophysiology is that it allows the researcher to control whether they are investigating inhibitory or excitatory transmission. Thus, this technique allows us to investigate synaptic plasticity in a given brain area (Accardi et al., 2016).

1.4.1 The role of glutamate and excitatory transmission in compulsive behaviours

There are two primary excitatory transmission receptors: N-methyl-D-aspartate (NMDA) and amino-3-hydrox-5-methyl-4-isoxazolepropionic acid (AMPA) (Iijima Kurosu & Chaki, 2010; Egashira et al., 2008). The current ratio of AMPA and NMDA receptors is used as a tool for investigating changes in synaptic plasticity. An increase in AMPA peak current in relation to NMDA peak current demonstrates increases synaptic strength. Importantly, this occurs in the ovBNST following compulsive drug administration, but may be generalized to other compulsive behaviours like those observed in HD in SIP (Dumont et al., 2005).

1.4.2 The role of GABA and inhibitory transmission in compulsive behaviors.

The main neurotransmitter for inhibitory transmission is gamma-aminobutyric acid (GABA). The $\Gamma\text{GABA}_A$ receptor is a ligand-gated ionotropic receptor in which the major inhibitory neurotransmitter GABA binds to. This causes an influx of chloride anions (Schartz,
In addition to endogenous GABA, this protein complex is the target site for benzodiazepines, drugs with anxiolytic effects (Sigel & Luscher, 2011). Previous research investigating the functioning in SIP explored the effects of benzodiazepines on GABA\textsubscript{A} receptors (Altamura et al., 2013). The effects of benzodiazepines on the behavioural outputs of SIP demonstrates that a GABA\textsubscript{A} positive allosteric modulator decreases polydipsic behaviour in a dose-dependent fashion (Mittleman, Jones & Robbins 1988; Pellon, Ruiz, Lamas & Rodriguez 2007). Furthermore, when a selective benzodiazepine antagonist is administered with an agonists, the drinking behaviour is restored (Mittleman, Jones & Robbins 1988). These experiments are of the first to demonstrate that SIP behaviour can be modulated through manipulating the GABA\textsubscript{A} receptor. This dysregulation is additionally observed in the BNST. Decreased GABA synthesis in the BNST elicits anxiety-like behaviour in rats (Sajdyk, Johnson, Fitz & Schekhar 2008). Chao and colleagues (2010) revealed that decreased GABA produces stereotyped, repetitive, behaviours such as compulsive grooming. Hence, characterizing GABA transmission in SIP in the ovBNST may elucidate underling mechanism of the dysregulated system driving compulsive drinking.

Furthermore, interference in GABAergic transmission is associated with feeding behaviour. BNST GABA projections suppresses excitatory glutamatergic neurons to the lateral hypothalamus (LH) resulting in an increases food consumption (Jennings 2013). Taken together, not only is GABAergic transmission critically involved in compulsive behaviour, it has a crucial role in feeding. Therefore, understanding the neuronal inhibitory inputs to the ovBNST is critical to further discover upstream dysregulation that may account for the compulsive drinking observed by HD.
Overall, Welkenhysen and colleagues (2013) determined that there is an increase in neuronal activity in the BNST in HD when compared to LD. As previously, discussed it is unsure whether this occurs from a decrease in glutamatergic activity or through a dysregulation of GABAergic projections. Therefore, exploring inhibitory transmission is necessary to understand what is driving the differences observed between HD and LD.

1.5 Aim and Hypothesis

Identifying a neurophysiological trace in SIP (i.e. maladaptive compulsive drinking) in the ovBNST will lead to a better understanding to the development of maladaptive compulsive behaviours. Both excitatory and inhibitory synaptic transmission contribute in compulsive behaviours (deBacker et al., 2015; Krawczyk et al., 2013). However, there has yet been a study exploring both aspects of transmission on the synaptic plasticity underlying the compulsive drinking observed in SIP. Specifically, it is critical to investigate the factors that contribute to the susceptibility in developing such a compulsive behaviour so that preventative manipulations can be implemented. Due to its implications in anxiety and compulsive drug seeking, the ovBNST is an interesting area to explore these findings. Additionally, previous researching investigating SIP demonstrates alterations in synaptic transmission in the BNST (Welkenhysen et al., 2013; van Kuyck et al, 2008). Therefore, I hypotheses that an alteration in both GABAergic and glutamatergic synaptic transmission will be observed in the HD when compared to the LD group.

The aim of the current research is to explore and identify the neuronal transmission of the ovBNST synapses in rats that developed compulsive water drinking and those that were resistant (HD and LD respectively). This will be conducted through the investigation of the effects of chronic food restriction (cFDR) and subsequently an 18-hour refeed period, generating a sated
state. Finally, the association between volume of water consumed and impaired synaptic plasticity will be investigated.

Chapter 2: Method

2.1 Animals.

Fifty-one Long Evan male rats weighing 250-275 g (Charles River, QC) were pair-housed in clear Plexiglas shoe-box style cages (45 x 23 x 20 cm deep) lined with bedding (Beta Chip, NEPCO, Warrenburg, NY). These cages were located in a climate-controlled colony room (21±1°C; humidity 40-70%) on a reversed light/dark cycle (8:00 A.M. lights off – 8:00 P.M. lights on). The rats had seven days of acclimatization upon their arrival where they were provided ad libitum food (rat chow; LabDiet rodent feed #5001, PMI Nutrition International, Brentwood, MO) and water; following this period they were individually housed. All the experiments were conducted in accordance with the Canadian Council on Animal Care guidelines for use of animals in experiments and approved by the Queen’s University Animal Care Committee (protocol # 2014-1537).

2.2. Groups

The rats were randomly assigned to five groups: chronic food restriction (cFDR), chronic food restriction followed by refeed (cFDR-refeed), schedule-induced polydipsia (HD, LD), schedule induced polydipsia followed by refeed (HD-refeed, LD-refeed), and naïve control. During all conditions the rats had ad libitum access to water and were handled daily. Variations in feeding time occurred to ensure that all rats that received food manipulations were within 85-90% of their starting weight.

2.2.1 Chronic Food Restriction.
Seven rats were individually housed for 29 days where they only received *ad libitum* rat chow for a 2-hour period daily (12:00–2:00 PM). At day 28 point they were sacrificed for electrophysiology recordings at the start of the dark cycle (see 2.4.1 Slices preparation and electrophysiology).

2.2.2 Chronic Food Restriction – Refeed.

Nine rats were individually housed for 29 days where they only received *ad libitum* rat chow for a 2-hour period daily (12:00 – 2:00 PM). At day 28, the rats were given *ad libitum* access to food overnight (18-hours). At day 29 point they were scarified for electrophysiology recordings at the start of the dark cycle (see 2.4.1 Slices preparation and electrophysiology).

2.2.3 Schedule-Induced Polydipsia.

Twelve rats were individually housed for 29 days. For the first seven days, the rats only received *ad libitum* rat chow for a 2-hour period (12:00 – 2:00 PM). From days 8-28, the rats began their behavioural training in the operant conditioning chambers (see 2.3 Apparatus and Training) and their water consumption was recorded. Immediately following their training they received *ad libitum* access to food for a one-hour period. On day 29, they were sacrificed for electrophysiology recordings at the start of the dark cycle (see 2.4.1 Slices preparation and electrophysiology).

2.2.3.1 High Drinkers and Low Drinkers.

Rats that consume at least 15 ml of water for a minimum of 3 consecutive days are considered HD. Rats that do not meet this criterion are considered LD (Gardner-Gregory et al., 2015; Hawken & Beninger, 2014). Thus, HD are considered to be engaging in compulsive drinking.
2.2.4 Schedule-Induced Polydipsia – Refeed.

Seventeen rats were individually housed for 29 days and underwent the SIP protocol (see 2.2.3 Schedule-Induced Polydipsia). On day 28, the rats were given *ad libitum* access to food overnight (18-hours). On day 29 point they were sacrificed for electrophysiology recordings at the start of the dark cycle (see 2.4.1 Slices preparation and electrophysiology) and were divided into their respective groups (see 2.2.3.1 High Drinkers and Low Drinkers).

2.2.5 Naïve Control.

Six rats were individually housed for 29 days where they had *ad libitum* access to food. At this point they were sacrificed for electrophysiology recordings at the start of the dark cycle (see 2.4.1 Slices preparation and electrophysiology).

2.3 Apparatus and Training.

2.3.1 Apparatus.

Experiments were done using six commercial (Med Associates, ST-Albans, VT) operant conditioning chambers inside sound-attenuating cabinets and equipped with fan for ventilation. These chambers were composed of a metal grid floor, two metal side panels and three Plexiglas panels. A red house light remained on throughout the training sessions. Additionally, there was a food dispenser tray (5 x 8 x 4 cm) and a metal, ball-bearing, drinking spout. The operant conditioning chambers were controlled by a computer running MED-PC-IV (Med Associates Inc., St. Albans, VT).

2.3.2 Training.

Rats undergoing SIP training were placed in the operant conditioning chambers for 2-hours, daily for 21 days. In these chambers they received a 45 mg dustless precision
food pellet (Bio-Serv, Frenchtown, NJ) on a fixed-time 60 sec for 120 mins, released in a recessed dispenser tray (5 x 8 x 4 cm). On the opposite side of the chamber there was a ball-bearing drinking spout filled with water. The water was weighed before and after each session and the volume drunk was recorded for each rat.

2.4 Brain Slice Preparation and Electrophysiology.

2.4.1 Brain Slice Preparation.

The rats were deeply anesthetized with isoflurane (5% at 5 ml/min) and their brains rapidly removed and kept in an iced-cold physiological solution containing (in mM): 126 NaCl, 2.5 KCl, 1.2 MgCl₂, 6 CaCl₂, 1.2 NaH₂PO₄, 25 NaHCO₃ and 12.5 D-glucose equilibrated with 95% O₂/5% CO₂. The brains were cut in coronal slices (250 μm) with a vibrating-blade microtome (Leica VT-1000) in the physiological solution and maintained at 2°C throughout the slicing procedure. Slices containing the BNST were incubated at 34°C for 60 minutes and then transferred to a chamber that was constantly perfused (3 ml/min) with the physiological solution maintained at 34°C.

2.4.2 Measures of Inhibitory synaptic transmission.

Whole-cell voltage-clamp recordings were made using glass microelectrodes (3.5 MOhm) filled with a solution containing (in mM): 70 Cs⁺MeSO₃⁻, 58 KCl, 0.5EGTA, 7.5 HEPES, 1.2 MgCl₂, 12 NaCl, 1 MgATO, 0.3 Guanosine triphosphate (GTP), 1 Adenosine triphosphate (ATP) and 1 phosphocreatine. The E_cl in these conditions was approximately -32 mV such that evoked GABA_A postsynaptic responses were inward currents when neurons were voltage-clamped at -60 mV. Postsynaptic GABA_A currents were evoked by local fiber stimulations with tungsten electrodes (FHC, Bowdoin, ME, USA) using a bipolar stimulus isolator (World Precision Instruments, Sarasota, FL, USA).
in the presence of the AMPA antagonist DNQX (50\(\mu\)M). Electrodes were placed in the ovBNST, 100-500 \(\mu\)m dorsal from the recorded neurons, and an electrical stimuli (10-100\(\mu\)A, 0.1 ms duration) was evoked at 0.1Hz. Following 5 minutes of stable and steady baseline recording, neurons were subjected to a synaptic plasticity-inducing stimulation protocol (1Hz, 5 mins) followed by a minimum of 30 minutes post-manipulation period (Gardner Gregory et al., 2015, SFN). Recordings were made using a Multiclamp 700B amplifier and a Digidata 1440A (Molecular Devices Scientific, Sunnyvale, CA, USA). Data was acquired and analyzed with Axograph running on Apple and PC computers respectively.

2.4.3 Measures of Excitatory synaptic transmission.

Whole-cell voltage-clamp recordings were made using glass microelectrodes (3.5MOhm) filled with a solution containing (in mM): 140 Cs\(^{+}\)MeSO\(_3\), 1 EGTA, 10 HEPES, 1 MgCl\(_2\), 12 NaCl, 0.3 Guanosine triphosphate (GTP), 2 Adenosine triphosphate (ATP), 1 phosphocreatine, and picrotoxin. Neurons were voltage-clamped at -60mV until stable. The voltage was then increased to +40 mV. Electrodes were placed in the ovBNST 100-500 \(\mu\)m dorsal from the recorded neurons, and repeated electrical stimulation (0.1 ms duration, 3000 ms interval) was evoked at 0.1Hz. The amplitude of the evoke EPSC was recorded. Following five minutes of stable and steady baseline recording, the NMDA receptor antagonist D-AP5 was bath applied for 5 minutes to isolate AMPA currents. Recordings were made using a Multiclamp 700B amplifier and a Digidata 1440A (Molecular Devices Scientific, Sunnyvale, CA, USA) until the effect of D-AP5 completely washed. Data was acquired and analyzed with Axograph running on Apple and PC computers respectively.
2.5 Drugs

Stock solution of 6,7-dinitroquinoxaline-2,3-dione (DNQX; 100mM; R & D Systems, Minneapolis, MN, USA) was prepared in dimethyl sulfoxide (DMSO; 100%; Fisher Canada) then further dissolved in the physiological solutions at the desired concentration such that the final DMSO concentration never exceeded 0.1%. Picrotoxin (R & D Systems, Minneapolis, MN, USA) was added to the physiological solution made daily yielding a concentration of 100µM. D-amino-phosphonopentanoid acid (D-AP5) was diluted in stock solution of DMSO (0.1%).

2.6 Statistical Analysis

2.6.1 Behavioural Analysis.

Volume of water consumed across time between HD and LD is measured using a two-way mixed analysis of variance (ANOVA). The between-groups variable is condition (HD, LD) and the within-groups variable is time (days). A statistical significance of less that \( p = 0.05 \) is considered statistically significant. To control of type I error rates across multiple comparisons, a Bonferroni correction was applied to all \( p \) values.

2.6.2 GABA\(_A\)-inhibitory postsynaptic current.

In order to investigate changes in inhibitory post-synaptic currents (IPSC) following LFS, the baseline amplitude is compared to the post-synaptic current amplitude (((Peak amplitude post - Peak amplitude baseline)/Peak amplitude baseline)*100) and the data were reported as mean ± S.E.M. Measurements of the peak amplitudes was completed using Axograph X. In graphs denoting electrophysiology time-course, each data point represents the average of 1-minute bins (6 evoked GABA\(_A\)-IPSCs) across
recorded neurons. Changes in amplitude greater than 20% are considered long term potentiation (LTP), a decrease of 20% or more is considered long term depression (LTD), and changes between +20% to -20% are considered no change (NC) (Figure 3). Once the data points have been identified, the number of cells in each group (LTP, LTD and NC) were compared between conditions using chi-squared test for independence across all groups. Finally, the relation between water consumed on last day of behaviour and GABA<sub>A</sub>-IPSCs at 20 minutes post the low frequency stimulation manipulation is analyzed using a person correlation. The time point of 20 minutes is used as it is the shorted recording that was collected and analyzed. A statistical significance of less that \( p = 0.05 \) was considered statistically significant.

2.6.3 AMPA/NMDA Ratio.

Measurements of the peak amplitude of evoked excitatory post-synaptic currents (EPSC) were completed using Axograph X and peak amplitudes for each minute of recording was evaluated as a percent change from baseline. A one-way ANOVA was used to examine differences in AMPA/NMDA receptor ratio in the ovBNST between varying conditions: HD, LD, HD-refeed and LD-refeed. Statistical significance of less that \( p = 0.05 \) was considered statistically significant.
Figure 3. Representative traces of GABA<sub>A</sub>-IPSC amplitude changes (LTP, LTD, NC) following the LFS protocol.
Chapter 3: Results

3.1 High Drinkers consumed more water than Low Drinkers during schedule-induced polydipsia training.

Following SIP training, rats were categorized into two groups – HD or LD – according to a drinking criterion. Rats that consumed a minimum of 15 ml of water in the 2-hour session for a minimum of three consecutive days were placed in the HD group whereas rats that did not meet this criterion were placed in the LD group. This criterion has been previously established as a statistically significant means of separating the groups (Gardner Gregory et al., 2015; Hawken & Beninger, 2014) (Figure 4). There is a statistically significant main effect of group (HD, LD; $F(1, 28) = 22.27, p< 0.001$; two-way mixed ANOVA, between variable) confirming that HD did indeed consume more water during the session. Furthermore, HD continuously drank increasing amounts of water over the course of the experiment (main effect of time (days); $F(20, 560)= 17.66, p<0.001$; two-way mixed ANOVA, within variable). Comparatively, LD continue to consume a consistent volume over time (interaction between group and time; $F(20, 560)=8.08, p<0.001$; two-way mixed ANOVA). These results indicate that there are in fact individual differences within the rats that contribute to whether they become HD or LD.

On the contrary, there is no difference in the amount of water consumed in the home cage between HD and LD ($F(1,28)=3.42, p=0.08$; two-way mixed ANOVA, between variable) (Figure 5). The rats did drink less over time (main effects of time (days); $F(26,728) = 22.21, p<0.001$; two-way mixed ANOVA; within variable), but, it was not a result of their drinking during the SIP training (interaction between time (days) and group (HD, LD); $F(26,728)= 0.77, p=0.79$; two-way mixed ANOVA). Overall, in a 24-hour period, HD drank significantly more than LD ($F(1, 28) = 8.64, p< 0.006$; two-way mixed ANOVA; between variable) over the course
of the experiment (days; \( F(26, 728) = 2.20, p<0.001 \), two-way mixed ANOVA, within variable). Additionally, there was a statistically significant interaction between group and time (\( F(26, 728) = 3.56, p<0.001 \), two-way mixed ANOVA). Therefore, HD consumed more water than LD across time (Figure 6).

3.2 Food restriction reveals bidirectional plasticity at ovBNST GABA synapses.

Oval BNST GABA synapses display satiety state-dependent bi-directional change in strength when submitted to a plasticity-inducing stimulation protocol (LFS, 1 Hz, 5 mins; Gardner Gregory et al., 2015 SFN) in brain slices. LFS produced robust LTP\(^{GABA}\) in all neurons recorded from slices prepared from naïve control rats (\( n_{\text{LTP}} = 11, n_{\text{LTD}} = 0, n_{\text{NC}} = 0 \); Figure 7). However, LFS produced LTP\(^{GABA}\) in only a small proportion (25%) of ovBNST neurons (\( n_{\text{LTP}} = 4, n_{\text{LTD}} = 7, n_{\text{NC}} = 5 \); Figure 8) following cFDR.

To determine whether refeeding would rescue LTP\(^{GABA}\) after chronic food restriction, rats were given \textit{ad libitum} access to food for 18 hours before brain slice preparation and recordings. This only partially recovered LFS-induced LTP\(^{GABA}\) (\( n_{\text{LTP}} = 7, n_{\text{LTD}} = 5, n_{\text{NC}} = 5 \); Figure 9). Thus, there was no statistically significant difference between the cFDR and cFDR-refeed conditions (\( X^2(2) = 1.12, p = 0.57 \)) and there continued to be a statistically significant difference between the naïve control and cFDR-refeed condition (\( X^2(2) = 10.07, p = 0.006 \); Figure 10).

3.3 Impaired bidirectional GABA plasticity at ovBNST synapses in high drinkers.

We next determined whether the SIP paradigm would affect bidirectional GABA plasticity beyond the effect of cFDR. Similar to cFDR, LFS resulted in LTD\(^{GABA}\) or no change in a majority of ovBNST rats that underwent SIP training. There is no differences in proportion of LFS-induced LTP\(^{GABA}\) between HD (\( n_{\text{LTP}} = 1, n_{\text{LTD}} = 7, n_{\text{NC}} = 6 \); Figure 11) and LD (\( n_{\text{LTP}} = 2, n_{\text{LTD}} = 5, n_{\text{NC}} = 7 \); Figure 12; \( X^2(2) = 0.74, p = 0.69 \); Figure 15). Thus, are no differences between
cFDR, HD and LD ($X^2(4) = 2.52, p = 0.64$). This suggests, at first glance, that plasticity of ovBNST GABA synapses is tightly linked to the chronic metabolic challenge imposed by food restriction but not further related to the SIP training.

When investigating the partial refeed-induced recovery of LTP in cFDR rats, we hypothesized that the bidirectional plasticity might be impaired in HD only. Impaired recovery of $LTP^{GABA}$ in SIP trained rats would be consistent with a reduction of inhibition in the ovBNST and enhanced neuronal excitability measured in the BNST of SIP trained rats. As predicted, bidirectional plasticity was impaired in the HD-refeed group ($n_{LTP} = 2, n_{LTD} = 7, n_{NC} = 8$; Figure 13) as it did not recover the lost $LTP^{GABA}$ observed in the HD group ($X^2(2) = 9.02, p = 0.01$; Figure 15). Interestingly, an 18-hour refeed rescued the $LTP^{GABA}$ in the LD group ($n_{LTP} = 9, n_{LTD} = 1, n_{NC} = 7$; Figure 14). This effect is statistically significantly different from the original LD group ($X^2(2) = 6.89, p = 0.03$) and from the HD-refeed group ($X^2(2) = 9.02, p = 0.01$; Figure 15).

Moreover, the volume of water consumed by HD-refeed rats and LD-refeed rats negatively correlated with GABA$_A$-IPSC percentages 20 minutes post LFS-induction, $r(16) = -0.57, p = 0.008$ (Figure 16). Thus, indicating that rat displayed more compulsive behaviours – drinking more water – had neurons that demonstrated a greater decrease in LFS-induced GABA$_A$-IPSC amplitude, while rats that consumed less water had neurons that demonstrated a recovered $LTP^{GABA}$ at ovBNST synapses.

3.4 Changes in AMPA to NMDA ratio is unrelated to compulsive drinking in SIP trained rats.

Excitatory glutamatergic transmission was investigated through the analysis of AMPA/NMDA receptor ratio in the ovBNST. Increases in AMPA receptors is a marker of learning and synaptic plasticity (Henley & Wilkinson, 2013). There were no statistically
significant differences between HD, LD, HD-refeed and LD-refeed conditions ($F(3,21) = 0.59, p = 0.63$; one-way ANOVA; Figure 17).

*Figure 4.* Water consumption (ml) as a function of time (days) for both HD and LD during 2h SIP training. The grey and black shades areas represent the range of water consumption for HD and LD respectively. The blue and green lines represent the mean water consumption for HD and LD respectively. *Denotes a statistically significant difference of $p<0.05$. 
Figure 5. Mean water consumption (ml) as a function of time (days) during the 22 h period in the home cage environment for both HD and LD.
Figure 6. Mean total water consumption (ml) as a function of time (days) during the 24 h period of SIP training and home cage environments for both HD and LD. *Denotes a statistically significant difference of $p<0.05$. 
Figure 7. [A] GABA_A-IPSC percent change as a function of time (minutes) with each data point representing an average of six events for rats in the naïve control condition (n_cells = 11; n_rats = 6). [B] Traces are of the dominant GABA_A-IPSC amplitudes (LTD, LTD, NC) and scale bar is to 0.1μA and 10ms. Each trace is the average of 5 events immediately before LFS and 20 minutes after LFS, respectively.

Figure 8. [A] GABA_A-IPSC percent change as a function of time (minutes) with each data point representing an average of six events for rats in the cFDR condition (n_cells = 16; n_rats = 7). [B] Traces are of the dominant GABA_A-IPSC amplitudes (LTD, LTD, NC) and scale bar is to 0.1μA and 10ms. Each trace is the average of 5 events immediately before LFS and 20 minutes after LFS, respectively.
Figure 9. [A] GABA$_A$-IPSC percent change as a function of time (minutes) with each data point representing an average of six events for rats in the cFDR-18 h refeed condition ($n_{\text{cells}}=17$; $n_{\text{rats}}=9$). [B] Traces are of the dominant GABA$_A$-IPSC amplitudes (LTD, LTD, NC) and scale bar is to 0.1 nA and 10 ms. Each trace is the average of 5 events immediately before LFS and 20 minutes after LFS, respectively.

Figure 10. Percentage of cells that either demonstrated an LTP, LTD or NC following LFS-induced GABA$_A$-IPSC for naive control ($n_{\text{LTP}}=11$, $n_{\text{LTD}}=0$, $n_{\text{NC}}=0$), cFDR ($n_{\text{LTP}}=4$, $n_{\text{LTD}}=7$, $n_{\text{NC}}=5$) and cFDR followed by an 18 h refeed ($n_{\text{LTP}}=7$, $n_{\text{LTD}}=5$, $n_{\text{NC}}=5$). *Denotes a statistically significant difference of $p<0.05$. 
Figure 11. [A] GABA<sub>A</sub>-IPSC percent change as a function of time (minutes) with each data point representing an average of six events for rats in the HD condition (n<sub>cells</sub>= 14; n<sub>rats</sub>=7). [B]Traces are of the dominant GABA<sub>A</sub>-IPSC amplitudes (LTD, LTD, NC) and scale bar is to 0.1µA and 10ms. Each trace is the average of 5 events immediately before LFS and 20 minutes after LFS, respectively.

Figure 12. [B] GABA<sub>A</sub>-IPSC percent change as a function of time (minutes) with each data point representing an average of six events for rats in the LD condition (n<sub>cells</sub>= 17; n<sub>rats</sub>=7). [B]Traces are of the dominant GABA<sub>A</sub>-IPSC amplitudes (LTD, LTD, NC) and scale bar is to 0.1µA and 10ms. Each trace is the average of 5 events immediately before LFS and 20 minutes after LFS, respectively.
Figure 13. [A] GABA$_A$-IPSC percent change as a function of time (minutes) with each data point representing an average of six events for rats in the HD followed by an 18 h refeed condition ($n_{\text{cells}}=17$; $n_{\text{rats}}=10$). [B] Traces are of the dominant GABA$_A$-IPSC amplitudes (LTD, LTD, NC) and scale bar is to 0.1$\mu$A and 10ms. Each trace is the average of 5 events immediately before LFS and 20 minutes after LFS, respectively.

Figure 14. [A] GABA$_A$-IPSC percent change as a function of time (minutes) with each data point representing an average of six events for rats in the LD followed by an 18 h refeed condition ($n_{\text{cells}}=17$; $n_{\text{rats}}=7$). [B] Traces are of the dominant GABA$_A$-IPSC amplitudes (LTD, LTD, NC) and scale bar is to 0.1$\mu$A and 10ms. Each trace is the average of 5 events immediately before LFS and 20 minutes after LFS, respectively.
Figure 15. Percentage of cells that either demonstrated an LTP, LTD or NC following LFS-induced GABA_A-IPSC for HD (n_{LTP} = 1, n_{LTD} = 7, n_{NC} = 6), HD followed by an 18 h refeed (n_{LTP} = 2, n_{LTD} = 7, n_{NC} = 8), LD (n_{LTP} = 2, n_{LTD} = 5, n_{NC} = 7), and LD followed by an 18 h refeed (n_{LTP} = 9, n_{LTD} = 1, n_{NC} = 7). *Denotes a statistically significant difference of $p<0.05$.

Figure 16. Correlation between GABA_A-IPSC percent change at 20 minutes post low frequency stimulation protocol and water consumed on last day of behaviour in refeed rats ($r = -0.57$, $p = 0.008$). Each data point represents the average GABA_A-IPSC percent change for each cell per rat and that rat’s specific water consumptions on the last day of behavioural training.
Figure 17. Summary of the effects of SIP on the ratio of AMPA to NMDA for the following conditions: HD (n_{cells} = 9; n_{rats} = 4), LD (n_{cells} = 10; n_{rats} = 4), HD followed by an 18 h refeed period (n_{cells} = 10; n_{rats} = 5) and LD followed by an 18 h refeed period (n_{cells} = 7; n_{rats} = 3).
Chapter 4: Discussion

The goal of this project was to determine whether compulsive drinking in the SIP paradigm was associated with alterations in synaptic transmission at ovBNST synapses. I hypothesized that there are differences in both inhibitory and excitatory synaptic transmission in the ovBNST between HD and LD. Furthermore, that these differences are associated with the degree of compulsivity as defined by volume of water consumed. Refeed revealed an impairment in bidirectional plasticity of ovBNST GABA synapses in animals displaying high drinking behaviour in the SIP paradigm. This GABA plasticity was biased towards depression in HD, suggesting a reduction in synaptic inhibition which might explain enhanced neuronal activity previously observed in HD rats (Welkenhysen et al., 2013). Thus, this may serve as a potential neuromarker for compulsive behaviours.

4.1 Schedule-induced polydipsia and drinking behaviours

Rats that met the HD criteria (15mL of water for 3 consecutive days) did in fact drink statistically significantly more water than LD as replicated from previous experiments (Figure 4; Gardner Gregory et al., 2015; Hawken & Beninger, 2014); however there is no difference in their home cage drinking patterns (Figure 5). This demonstrates that the HD were drinking beyond their physiological need. When investigating their water consumption during a 24-hour period, there was a statistically significant difference coinciding with the difference observed in the behavioural sessions (Figure 6).

4.2 Recovered GABA$_A$-IPSC amplitudes in LD but not in HD

Chronic FDR changed LFS-induced iLTP into iLTD in a majority of neurons in the ovBNST (Figure 8). This shift was only partially recovered following an 18-hour refeed (Figure
The same pattern of LFS-induced shift towards iLTD in GABA<sub>A</sub>-IPSC amplitude was observed in both HD and LD groups (Figure 11, 12). Therefore, we believe that the LFS-induced iLTD was a manifestation of the state of cFDR. However, an 18-hour refeed rescued the iLTP in LD, but not in HD, exposing a potential neural mechanism underlying compulsive drinking in the SIP paradigm (Figure 13, 14). This alteration in GABA<sub>A</sub>-IPSC amplitude in the ovBNST supports the hypotheses that both cFDR and SIP (HD and LD groups) demonstrate alterations in GABAergic synaptic transmission. Furthermore, our hypothesis was correct in that the impaired GABAergic plasticity was specific to HD following a refeed period. This disinhibition in the ovBNST of HD corroborates research demonstrating that a decrease in GABA synthesis elicits stereotyped compulsive behaviours (Sajdyk, Johnson, Fitz & Schekhar 2008; Choa et al., 2010). It can be interpreted that refeed did not satiate the HD as it pertains to their GABAergic synaptic transmission. Thus, a potential prolonged disinhibition of GABA neurons in the ovBNST inhibit glutamatergic neurons in the LH. Interestingly, a significant negative correlation between GABA<sub>A</sub>-IPSC percent change and volume of water consumed on last day of behaviour in refeed rats was observed (Figure 16). This suggests that greater LFS-induced iLTD was observed in HD. Taken together, these results suggest that there is a dysfunctional GABAergic transmission that is strongly associated with compulsive water drinking, such that the greater the dysfunction the more severe the compulsive behaviours are.

The iLTD observed in cFDR, HD and LD compliments the circuitry presented by Jennings and colleagues (2013) such that the disinhibition of neurons in the ovBNST presently observed may be involved in promoting feeding through greater inhibition of the LH glutamatergic neurons. Moreover, the ovBNST receives excitatory inputs from the posterior paraventricular thalamus (PVT) (Li & Kirouac, 2008). The PVT is involved in homeostatic
regulation and importantly, in food anticipation (Bhatnagar & Dallman, 1999; Nakaharam Fukui, & Murakami, 2004). Thus, this pathway regulating hunger from anticipation of food to consummation of food is critical in understanding the neuromechanism underlying the shift to LFS-induced iLTD of GABA_A IPSCs.

The mechanism underlying the effects of LFS-induced GABA_A-IPSCs acts through endocannabinoid (eCB) inhibition of pre-synaptic GABA release through its binding of CB1 endocannabinoid receptors (Figure 18). In naïve conditions, LFS reduces the CB1 inhibition of GABA release, resulting in an iLTP of GABA_A IPSCs. Additionally, LFS may reduce CB1 inhibition through an endogenous estrogenic mechanism. Hypothetically, LFS promotes the aromatisation of testosterone into estrogen, causing binding to estrogen receptor (ERα) alpha reducing eCB functioning and consequently, causing LTP of GABA_A - IPSCs. Disruption of the production of estrogen or in its ability to bind to ERα yields an effect comparable to cFDR (Gardner Gregory et al., 2015 SFN). Therefore, it is likely that both compulsive drinking behaviour from the SIP paradigm and cFDR is altering endocannabinoid function within the ovBNST. In order to confirm this hypothesis future studies may want to examine if altering either endocannabinoid or estrogen function in the ovBNST with cannulations will change SIP behaviour.

4.3 SIP did not produce detectable change in strength at excitatory synapses

Increased firing rate, as well as decreased bursting behaviour is demonstrated in BNST neurons of HD compared to the LD group (Welkenhysen et al., 2013). However, these experiments were unable to discriminate whether inhibitory or excitatory synaptic transmission in the BNST was regulating compulsive drinking induced by SIP. We predicted that alterations in AMPA/NMDA ratios would be present between HD and LD, similar to previous findings of
cocaine administration (Dumont et al., 2005). Contrary to our hypothesis, none of the tested conditions affected the strength of ovBNST excitatory synapses as measured by differences in AMPA/NMDA receptor ratios in the ovBNST (Figure 17). Thus with regards to the susceptibility between HD and LD in SIP, glutamatergic transmission in the ovBNST does not appear to be a critical contributor. Thus, these results demonstrate that the underlying mechanisms driving the susceptibility for developing compulsive water drinking is a consequence to a dysregulated GABAergic synaptic transmission at ovBNST synapses.

4.4 Compulsivity: predisposition vulnerability

A hunger state is suggested to exacerbate a pre-existing or predisposition vulnerability towards developing compulsive tendencies (O’Brien & Vincent, 2003). Therefore, the iLTD of GABA\textsubscript{A}-IPSC in the ovBNST generated through cFDR may be necessary for the compulsive behaviours to manifest. Interestingly, refeed only recovered the LFS-induced iLTP lost in LD. An 18-hour refeed had no effect on HD, thus demonstrating an impaired plasticity of inhibitory transmission. As cFDR induces homeostatic shift in the organisms, LD displayed the ability to return to a pre-challenged state once their hunger was satisfied by the refeed. However, compulsive rats did not demonstrate this ability. Hence, alterations of LFS-induced GABA\textsubscript{A}-IPSC amplitudes following an 18-hour refeed may serve as a neuromarker for compulsive tendencies. It would be of interest for future research to investigate the permanence of the impaired GABA\textsubscript{A}-IPSC in compulsive rats by increasing the refeed period. Furthermore, understanding other factors that might contribute and promote individual susceptibility for developing compulsive behaviours are key in understanding the division of behaviour observed in SIP.

4.6 Future Directions
Future research should aim at investigating whether the changes in LFS-induced GABA$_A$-IPSC amplitude in the ovBNST in LD and HD are replicated in other compulsive behaviours such as wheel-running. If the dysfunctional GABAergic synaptic transmission in the ovBNST continues to be observed in other behaviours, then it can be stated that this is a neuromarker for compulsive behaviours. In addition, it can be stated that water loading is not contributing to this effect. Moreover, it would be of interest to increase the refeed time to evaluate the permanence of the iLTD observed following LFS-induced GABA$_A$-IPSC in compulsive rats.

Additionally, future research should aim to confirm whether the underlying mechanism regulating the changes in LFS-induced GABA$_A$-IPSC amplitudes in HD and LD is indeed the eCB and E2 mechanism previously described. First, in vivo manipulations, such as bilateral cannulations to the ovBNST, can be implemented to abolish one of the two conditions, thus, creating either all HD or LD phenotypes. It is hypothesized that to foster conditions which attenuate compulsive drinking, rats should be administered E2, hence, increasing binding to ER$_\alpha$ receptors, or through blocking CB1 receptors with AM251. These manipulations should promote increased LFS-induced GABA$_A$–IPSC amplitudes. It is hypothesized that to increase compulsive drinking, there must be decreases in LFS-induced GABA$_A$ –IPSC amplitude. Therefore, preventing the conversion from estrogen to testosterone via aromatase with the compound letrozole will promote this LTD. Additionally, ICI and DOG will have similar actions on LFS-induced GABA$_A$–IPSC amplitude. Finally, the same manipulations can be explored through in vitro whole cell patch-clamp electrophysiology. This permits us to observed LFS-induced GABA$_A$ –IPSC amplitude changes from each manipulation. Hence, understanding how SIP is involved with this established mechanism can further understanding of compulsive behaviours.
and provide potential therapeutic targets for disorders involving a compulsive component such as OCD.

4.7 Concluding Remarks

A GABAergic dysfunction in the ovBNST might underlie a predisposed vulnerability to SIP that may explain why some rats are HD, while others are LD. An 18-hour refeed was unable to recover an impaired GABAergic synaptic transmission in HD, while LD demonstrate a normally functioning plasticity of GABAergic neurons in the ovBNST. Moreover, there is a negative correlation associating severity of compulsive drinking with this impaired GABAergic synaptic transmission in the HD-refeed group. Thus, HD may have a predisposition vulnerability for developing compulsive drinking. Knowing the biological susceptibility to develop compulsive behaviours can lead to preventative screening measures in both healthy individuals and newly diagnosed psychiatric patients.
**Figure 18.** Mechanism underlying LFS-induced GABA_A–IPSC amplitudes (Gardner Gregory et al., 2015 SFN). LFS acts on aromatase (P450), converting testosterone (T) to 17β-estradiol (E2). E2 binds to ERα which then acts on DGK that inhibits release of eCB. Decrease CB1 binding ultimately results in an iLTP GABA_A–IPSC. However, manipulations in yellow promote increase eCB (through the inhibition of P450, ERα or DGK) or decrease it’s binding to CB1, resulting in iLTD of GABA_A–IPSC. Manipulations in purple, either further inhibit eCB production (increase ERα binding) or decrease its binding to CB1 receptors (blocking CB1 receptors), thus promoting an iLTP GABA_A–IPSC.
Chapter 5: Reference List


