Chemically Guided Electrical Stimulation as a Cell Type-Specific Treatment for Depression in Rodents

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Abstract

Major depressive disorder is a mental health condition that severely impacts the lives of those affected. Although many treatments have been developed to alleviate symptoms, their efficiency is limited by low response rates and unwanted side effects. Advancements in the field of neurostimulation provide a promising new avenue for treatment; however, research is still ongoing to determine the optimal parameters and underlying mechanisms of the procedure. In this thesis, I examine the efficacy of a novel combination therapy for depression that applies transcranial direct current stimulation (tDCS) to neurons that have been chemically primed for excitability using the small-conductance, calcium-activated potassium channel antagonist, NS8593, in order to selectively activate a specific subset of neurons in the prefrontal cortex. First, we identified a subthreshold dose for both treatments individually that failed to elicit any behavioural or neurochemical changes in adult rats. These electrical and chemical doses (0.05mA and 1.0mg/kg, respectively) were then combined into one treatment known as EC stimulation and examined across a battery of behavioural tests in the olfactory bulbectomy (OBX) model of rodent depression. We found that EC stimulation reversed OBX-induced hyperlocomotion in the open field test, while also blocking anxiety-related behaviours in the open field and novelty suppressed feeding tests that manifested following tDCS application. These effects were associated with increased serotonin (5-HT) turnover in the left prefrontal cortex and hippocampus, as indicated by a reduction in overall 5-HT and corresponding increase in its metabolite, 5-HIAA. Combination treatment also blocked the increase in hippocampal dopamine that was observed following administration of tDCS or NS8593 alone. However, these effects were observed solely in male rats, as females failed to exhibit a depressive-like phenotype following OBX. The societal burden imposed by increasing depression rates makes the

development of novel interventions for the disorder a priority for public health. Taken together, these results support further preclinical development of EC stimulation as a safe and effective novel treatment for depression.

General Summary

Depression is a serious mental illness that greatly reduces the quality of life of those affected by it. Even though many drugs and therapies exist that can treat depressive symptoms, nearly half of those who start treatment either see no improvements or experience unwanted side effects such as weight gain, dizziness, or anxiety. This has led scientists to develop new techniques to combat depression, such as the use of non-invasive electrical stimulation to directly influence brain activity.

We examined a technique known as transcranial direct current stimulation (tDCS), which sends weak electrical current directly through the skull to increase the activity of brain cells. However, because this current affects all cells underneath the stimulating electrode indiscriminately, we combined it with a drug (NS8593) that predisposes certain cell types to react to tDCS. This method allowed us to use a dose for each treatment that alone would have no effect, but together would activate these specific cell types to reduce depressive symptoms. This combination treatment that electrically and chemically influences cells is known as EC stimulation.

To test EC stimulation as a potential treatment, we used a rat model of depression known as olfactory bulbectomy (OBX), which involves surgical removal of the olfactory bulbs. We found that OBX rats were hyperactive when exposed to a large open arena, but this behavior was reversed when the rats received EC stimulation beforehand. Furthermore, tDCS alone also increased anxiety-like behaviours, such as avoiding the center of the arena and increasing the time it took for rats to start eating food, an effect that was blocked when using the combined EC stimulation technique. Using brain chemical analysis techniques, we found that these effects

were associated with increased serotonin consumption in key depression-related brain areas such as the prefrontal cortex and hippocampus, as well as decreased levels of dopamine.

In conclusion, we found that EC stimulation has potential as a safe and effective treatment for depression that may alleviate symptoms without producing unwanted side effects. We hope this work guides future research towards the implementation of the technique in clinical settings.

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List of Abbreviations

5-HIAA	5-hydroxyindoleacetic acid
5-HT	Serotonin
ACC	Anterior Cingulate Cortex
ACTH	Adrenocorticotropic Hormone
BDNF	Brain Derived Neurotrophic Factor
CAMKII	Ca2+/Calmodulin-Dependent Protein Kinase II
CBT	Cognitive Behavioural Therapy
CCK	Cholecystokinin
CREB	cAMP Response Element-Binding Protein
CUMS	Chronic Unpredictable Mild Stress
DBS	Deep Brain Stimulation
DMSO	Dimethylsulfoxide
dlPFC	Dorsolateral Prefrontal Cortex
DA	Dopamine
DR	Dorsal Raphe
EEG	Electroencephalogram
ERK	Extracellular Signal-Regulated Kinase
FST	Forced Swim Test
GAD	Glutamic Acid Decarboxylase
HPA	Hypothalamic–Pituitary–Adrenal
HPLC	High-Pressure Liquid Chromatography
LC	Locus Coeruleus
LH	Learned Helplessness
LTP	Long Term Potentiation
MDD	Major Depressive Disorder
mPFC	Medial Prefrontal Cortex
MS	Maternal Separation
NE	Noradrenaline

NS8593	(*R*)-*N*-(benzimidazole-2-yl)-1,2,3,4-tetrahydro-1-naphtylamine
NMDA	N-Methyl-D-aspartate
NSFT	Novelty Suppressed Feeding Test
OBX	Olfactory Bulbectomy
OFT	Open Field Test
PFC	Prefrontal Cortex
PTZ	Pentylenetetrazole
rTMS	Repetitive Transcranial Magnetic Stimulation
SD	Social Defeat
SERT	Serotonin Transporter
sgACC	Subgenual Anterior Cingulate Cortex
SK	Small-Conductance, Calcium-Activated Potassium Channels
SPT	Sucrose Preference Test
SSRI	Selective Serotonin Reuptake Inhibitor
tACS	Transcranial Alternating Current Stimulation
tDCS	Transcranial Direct Current Stimulation
TMS	Transcranial Magnetic Stimulation
TRP	Tryptophan
vmPFC	Ventromedial Prefrontal Cortex

Co-Authorship Statement

I, Shannon C. Waye, in collaboration with my supervisor, Francis Bambico, designed, performed, and analyzed all experiments and wrote all portions of this thesis unless specified below. Essential contributions provided by collaborators are also listed below.

In Chapter 2, Rachel Noel and Nageeb Hasan performed the behavioural tests to examine the dose-response to NS8593. Rachel Noel, Chandani Dinesh, and Gerryt Bruijns assisted in performing RNAscope and counting cells following brain extraction. Tadhg Strand and Gavin Afonso assisted in performing behavioural tests for tDCS response, while Courtney Clarke assisted in preparing the biosensor setup.

In Chapter 3, Stefano Comai at the University of Padova performed the HPLC procedure and consolidated the data.

Chapter 1: Introduction

Major Depressive Disorder (MDD) is a debilitating condition characterized by a variety of symptoms such as chronic depressed mood, anhedonia, fatigue, suicidal thoughts and behaviours, and feelings of worthlessness or guilt (American Psychiatric Association, 2013). These symptoms have major repercussions in the lives of those affected by impacting their personal relationships, school achievement, and work performance. As one of the most common mental disorders, these impairments have led researchers to designate MDD as a leading cause of burden worldwide (Ferrari et al., 2013). It has a lifetime prevalence rate of 11.2% in Canada, and studies suggest that this rate is increasing, especially among young adults (Knoll & MacLennan, 2017; Mojtabai et al., 2016). Furthermore, MDD is highly comorbid with other psychiatric disorders, including substance abuse, anxiety, and personality disorders (Hasin et al., 2018), and may also serve as a risk factor for future impairments, such as Alzheimer disease and type 2 diabetes (Knol et al., 2006; Ownby et al., 2006).

Because of its devastating and widespread impact, many interventions have been utilized to treat MDD, such as cognitive behavioural therapy (CBT) and various types of antidepressant drugs. However, these treatments often have limited success in alleviating depressive symptoms and are often associated with undesirable side effects (Baldwin, 2006; Sugarman et al., 2014). CBT is a common type of psychotherapy that guides patients to re-evaluate their perceptions of reality and eliminate distorted patterns of thought, and although effective when combined with other strategies (Cuijpers et al., 2013), it often produces little benefit over active controls when used as a standalone treatment (Oar et al., 2017). Alternatively, antidepressant drugs are families of substances, such as selective serotonin reuptake inhibitors or tricyclic antidepressants, that are administered to reduce depressive symptoms. They have been shown to be mildly successful at

alleviating depressive symptoms as a standalone treatment (Rush et al., 2006; Sugarman et al., 2014) and moderately successful when used as part of a treatment battery (Trivedi et al., 2006). However, they are also associated with a variety of adverse side effects, including nausea, headache, weight gain, and sexual dysfunction (Baldwin, 2006).

The issues with these conventional treatments have guided researchers towards the development of alternative strategies to combat MDD. Of particular interest is the rapidly advancing field of neuromodulation, which includes techniques such as deep brain stimulation (DBS), transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), and transcranial alternating current stimulation (tACS). While the specific procedures and parameters utilized by these techniques differ, they all share a common mechanism of action by altering intrinsic electrical activity within the brain, leading to normalized cell activity within brain regions and circuits that exhibit depression-related abnormalities. This dissertation will begin by examining the capacity of these neuromodulation strategies to treat depressive symptoms in both human and rodent models, by first describing the neural substrates of depression and the rodent models used to mimic this human condition, then directly addressing the mechanisms, strengths, and limitations of each technique in turn.

1.1. Neurobiology of Depression

Depression is a complex psychiatric disorder that is associated with abnormalities in the structure, function, and connectivity of various regions throughout the brain, including frontal, limbic, and brainstem structures (Mayberg, 1997; Singh & Gotlib, 2014). A simplified overview of the brain circuitry implicated in MDD is presented in Figure 1.

The Monoamine Hypothesis of Depression

Before reviewing the various brain areas often implicated in depression, it is important to understand the chemical underpinnings of the disorder. The currently prevailing hypothesis is that a depletion of monoamines, including serotonin, norepinephrine, and dopamine, underlies the pathophysiology of depression (Delgato, 2000). The deficiency of these monoamines could be due to numerous factors, including increased monoamine oxidase enzyme activity, abnormalities in channel proteins and receptors, or dysregulation of other systems such as glutamate and GABA (for review, see Jesulola et al., 2018). The monoamine hypothesis is based on the observation that depressed patients exhibit lower levels of these neurotransmitters throughout the brain, which is corroborated by the discovery that drugs which increase their activity are effective at reversing symptoms (Ressler & Nemeroff, 2000). These findings have led to the development of selective serotonin reuptake inhibitors as the first line treatment for depression, which exert their effects upon the serotonin transporter to block reuptake, leading to increased serotonergic activity resulting from elevated levels of serotonin in the synaptic cleft. The role of serotonin in depression will be further examined in the following sections.

Prefrontal Cortex

The prefrontal cortex (PFC) is a common area of focus in depression research because of its role in the cognitive regulation of emotion (Kerestes et al., 2014). Indeed, Dunlop et al. (2017a) reported that larger resting-state functional connectivity scores between prefrontal regions was associated with an increased effectiveness of CBT, but not antidepressant drug treatment. As CBT focuses on changing a patient's perceptions about their own emotions, this finding provides support for the role of the PFC in the cognitive aspect of depression.

To further characterize the region's involvement in depression, Drevets et al. (1997) used positron emission tomography to measure brain activity as a function of cerebral blood flow and glucose metabolism. They found abnormal reductions of activity in the lateral and dorsolateral PFC of depressed patients in comparison to healthy controls, a finding that has been replicated across numerous other studies (for review, see Rigucci et al., 2010). These abnormalities are reversed during symptom remission and with chronic antidepressant drug treatment, bringing activity of the region back to control levels (Bench et al., 1995; Drevets et al., 1997; Kennedy et al., 2001). Furthermore, morphological changes have also been observed in prefrontal areas, with studies reporting decreases in pyramidal neuronal size and reductions of gray matter volume in depressed patients (Bora et al., 2012; Kempton et al., 2011; Manji et al., 2001).

Cellular alterations in the PFC have been found to be associated with cognitive dysfunction and clinical symptoms of depression (Drevets et al., 1997; Manji et al., 2001). Quan et al. (2011) found that rats exposed to the chronic unpredictable mild stress paradigm, a rodent model of depression, showed lower field excitatory postsynaptic potential amplitudes in the medial PFC (mPFC), which was associated with depressive-like symptoms such as decreased sucrose preference, reduced weight gain, and memory impairment. This effect may be mediated by alterations in GABAergic and glutamatergic transmission within the PFC, as the dysfunction of these systems have been implicated in the pathophysiology of depression (Krystal et al., 2002; Moriguchi et al., 2019). Reductions in frontal GABA concentration has been observed in models of depression, which are reversed following typical antidepressant treatment (Kim et al., 2014; Sanacora et al., 2002). Beneyto and Meador-Woodruff (2008) found lowered expression of N-Methyl-D-aspartate (NMDA) receptor subunits (NR1 and NR2A) in the PFC of MDD patients without alterations in receptor binding or number, suggesting abnormal receptor stoichiometry

and altered response to glutamate. Further evidence for abnormal glutamate signalling in frontal areas comes from studies investigating the use of ketamine as an antidepressant. Ketamine is a NMDA receptor antagonist that increases the amount of glutamate within the synaptic cleft, leading to a cascade of events that results in synaptogenesis within the prefrontal cortex (Abdallah et al., 2016). This leads to a remission of symptoms within depressive patients, as suggested by a randomized, double blind clinical trial conducted by Zarate et al. (2006). They found that a single intravenous injection of ketamine produced rapid antidepressant effects in patients with treatment-resistant depression, occurring two hours post-infusion and lasting up to one week. Furthermore, ketamine-induced NMDA receptor blockage results in increased activity in the PFC, an effect that is also observed following administration of serotonin-enhancing drugs (Kennedy et al., 2001; Vollenweider et al., 1997). Together, these results highlight the role of frontal glutamatergic signalling in the pathology of depression.

Anterior Cingulate Cortex

The anterior cingulate cortex (ACC) is comprised of two portions that have differential function and connectivity. Of particular interest in the pathology of depression is the ventral portion, known as the subgenual cingulate (sgACC), which has bilateral connections with limbic and frontal regions (for review, see Pandya et al., 2012). Therefore, it acts as a link between the limbic areas that generate emotion and the frontal areas that regulate these emotions. The sgACC also has extensive reciprocal connections with other regions implicated in the pathophysiology of depression, including the hypothalamus, nucleus accumbens, ventral tegmental area, raphe nucleus, and locus coeruleus (Drevets, 2000).

Abnormalities in function and structure of the sgACC have been reported in patients suffering from depression. Cerebral blood flow has been found to be increased in patients with

MDD, which correlates with the severity of depressive symptoms (Mayberg et al., 2005; Osuch et al., 2000). Studies have also shown remission-associated decreases in the activity of this region during antidepressant drug treatment (Drevets et al., 1997), electroconvulsive therapy administration (Nobler et al., 2001), and deep brain stimulation (Mayberg et al., 2005). Furthermore, mean gray matter volume of the sgACC is abnormally reduced in subjects with MDD (Drevets et al., 1997; Drevets et al., 2008), and animal studies have reported that lesions of sgACC homologue areas in rats contribute to altered corticosterone secretion in response to restraint stress (Drevets, 2000; Sullivan & Gratton, 1999). Considering its influence on dopaminergic neurons in the striatum and ventral tegmental area, sgACC dysfunction may contribute to the anhedonia and lack of motivated behavior observed in depression patients (Drevets, 1999).

Striatum

The striatum is divided into a dorsal portion, containing the caudate and putamen, and a ventral portion, containing the nucleus accumbens, and has extensive connections with the prefrontal cortex, anterior cingulate cortex, and amygdala (Price et al., 1996). Studies have found that cerebral blood flow and metabolism are abnormally decreased in the striatum of depressed patients, and that volumes of the caudate head and ventral striatum are significantly reduced in postmortem studies (Baumann et al., 1999; Baxter et al., 1985; Manji et al., 2001).

Similar to the PFC, studies have uncovered dysregulation of glutamate at the cellular level within the striatum. Kristiansen and Meador-Woodruff (2005) found decreased expression of SAP-102, a scaffolding protein for the NMDA receptor, in the striatum in patients with depression. These proteins are known to be essential for NMDA receptor function, so decreased concentrations may result in failure to insert glutamate receptor complexes into the post synaptic

membrane of the dendritic spine (Proctor et al., 2010). Furthermore, because the striatum receives extensive glutamatergic innervation from the frontal cortex, this may also suggest a dysregulation of frontostriatal circuitry in depression (Kristiansen & Meador-Woodruff, 2005).

Amygdala and Hippocampus

Limbic areas, such as the amygdala and hippocampus, are often examined in depression research because of their known contributions to emotion and negative affect (for review, see Rolls, 2015). In humans, electrically stimulating the amygdala produces anxiety, fear, dysphoria, recollection of emotional events, and increases secretion of the stress hormone cortisol (Drevets, 1999). Increased amygdalar activity correlates positively with depression severity and chronic antidepressant drug treatment has been found to normalize this hyperactivity, supporting the amygdala's role in the emotional component of depression (Abercrombie et al., 1996; Abercrombie et al., 1998; Drevets, 2001; Drevets et al., 2008). This overactivity may also contribute to excessive cortisol release via intrinsic corticotropin-releasing hormone neurons and hypothalamic projections, which has been hypothesized to underlie anxiety-like symptoms observed in rodent models of depression, such as appetite suppression, decreased sexual behavior, and sleep disturbance (Musselman & Nemeroff, 1993). Similarly, studies have also reported increased blood flow in the hippocampus in patients with depression, which positively correlates with scores on the Hamilton Depression Rating Scale and decreases following antidepressant drug treatment (Kennedy et al., 2001; Videbech et al., 2002).

Structural abnormalities have also been observed in limbic areas of depressed subjects, such as abnormal reductions in glial cell count and neuron size in the amygdala and decreased volume of both the amygdala and hippocampus (Bremner et al., 2000; Kronenberg et al., 2009; Manji et al., 2001; Stockmeier et al., 2004). These abnormalities are thought to be caused by

glucocorticoid-induced neurotoxicity via glutamatergic connections between the amygdala and hippocampus, and are reversed following electroconvulsive therapy (Nordanskog et al., 2010; Sheline et al., 1998).

Abnormalities within the limbic system have also been found at the cellular level in MDD patients. Karolewicz et al. (2009) reported a large increase in the expression of PSD-95 and GluN2A in the lateral amygdala of depressed subjects, suggesting disruption of glutamate signaling at the NMDA receptor. Other studies have also found evidence of abnormal glutamate signaling, as well as lowered gene transcription of synaptic proteins and brain-derived neurotrophic factor (BDNF), within the hippocampus of depressed patients (Duric et al., 2013; Law & Deakin, 2001). BDNF, which is critical for neuronal survival and growth, has also been found to be decreased at both the RNA and protein levels in the amygdala in MDD subjects (Guilloux et al., 2012), suggesting its role in the volumetric reductions observed in both of these structures.

Dorsal Raphe Nucleus

The dorsal raphe nucleus (DRN) is a brainstem structure that provides extensive serotonergic input to many parts of the brain. Serotonin (5-HT) is a major neurotransmitter in the brain and has a key role in the pathophysiology of depression, with many studies reporting decreased levels of serotonin throughout the brain in depressed subjects (Ressler & Nemeroff, 2000). Further demonstrating its importance, the most commonly prescribed drugs for treating depression are selective serotonin reuptake inhibitors (SSRIs), which inhibit the reuptake of serotonin from the synaptic cleft to increase its concentration within the brain (Petersen et al., 2002).

Serotonergic neurons in the DRN form extensive reciprocal connections with forebrain structures, including the amygdala and PFC, forming feedback loops that regulate emotions and anxiety states (Lowry et al., 2008). Furthermore, the ventrolateral portion of the DRN also receives direct visual input from the retina and innervates the hypothalamus, medulla, and periaqueductal gray, suggesting a role in the modulation of physiological and behavioral responses to severe stressors (for review, see Jasinska et al., 2012; Michelsen et al., 2007). This hypothesis is supported by a study from Nishitani et al. (2019), who demonstrated that optogenetic activation of serotonergic neurons in the DRN increased active coping in the forced swim test in rats, while inhibition of these neurons increased anxiety-like behaviors in the elevated plus maze test.

Considering the widespread influence of serotonin in the brain, it is unsurprising that the function of the DRN is altered in MDD. Gos et al. (2008) found that suicidal patients with depression had significantly less activity in DRN neurons, while Lira et al. (2003) reported that serotonin transporter knockout mice had a 50% reduction of serotonergic neurons and a fourfold decrease in firing in the DRN, which was associated with depressive-like behaviours in the novelty suppressed feeding test, forced swim test, and shock escape paradigm. Studies have also found depression-related abnormalities in the structure of the DRN. Baumann et al. (2002) reported that the number of neurons in the DRN was reduced by 31% in patients with mood disorders compared with non-psychiatric control subjects. Similarly, Matthews and Harrison (2012) reported that the cross-sectional area of the DRN was decreased in depressed patients without changes in cell size or density, which was suggested to be the result of a reduction in neuropil, which contains glia, dendrites, and axons.

1.2. Animal Models of Depression

Clearly, depression is a complex and heterogeneous disorder that involves numerous intertwining systems and complex signalling cascades. As a result, animal models are critical for examining the underlying neurological substrates of the disorder and the mechanisms of its treatment. Many animal models have been developed to simulate the disease profile of depression, and are evaluated based on their reliability, reproducibility, and their ability to predict outcomes in humans (Duman, 2010).

Chronic Unpredictable Mild Stress

The chronic unpredictable mild stress (CUMS) paradigm is a frequently used animal model of depression that mimics the instigation and progression of human depression (Willner, 2005). It has been found to be a valid, reliable, and sensitive method for studying depressive disorders in rodents (Willner, 1997). It involves the exposure of rodents to a series of mild and unpredictable stressors for two to three weeks, such as isolation, restraint, food or water deprivation, disruption of the light-dark cycle, tilting of home cages, and soiled bedding (Deussing, 2006). These stressors, administered daily in a random order, are designed to mirror unpredictable life stressors in humans and focus on the predominant role of stress in the etiology of depression (Willner, 2005).

Studies suggest that CUMS may induce behavioural and physiological changes that resemble symptoms of human depression (Willner, 2005). It has been found to interfere with intrinsic reward and hedonic value of appetitive stimuli, such as decreasing sucrose preference and intracranial self-stimulation, which reflects anhedonia-like behaviour that is reversed by chronic antidepressant treatment (Monleon et al., 1995). CUMS also consistently produces symptoms of

behavioural despair in rodents, evidenced by increased immobility in the forced swim test, which is thought to resemble hopelessness (Filho et al., 2015; Xin-hua et al., 2017).

In tandem with these behavioural changes, CUMS has also been found to alter neurochemistry and structure. In addition to the typical anhedonic and despairing behaviour, Xin-hua et al. (2017) found that rats exposed to three weeks of chronic stress had significantly higher concentrations of plasma corticosterone and increased corticotrophin-releasing hormone mRNA expression in the hypothalamus, an effect that was successfully reversed following treatment with the SSRI fluoxetine. Furthermore, repeated stress results in dendritic atrophy and reduction of glial cells in rodents, mirroring the loss of gray matter in depressed humans in regions such as the PFC and hippocampus (for review, see Drevets et al., 2008). It has also been associated with dendritic retraction in the CA3 region of the rat hippocampus, as well as with inhibition of neurogenesis in dentate gyrus granule neurons (Conrad, 2006; Jayatissa et al., 2008). Notably, these dendritic abnormalities have been reversed by treatment with lithium, which resembles the effects of lithium on the gray matter reductions observed in bipolar disorder (McEwen & Magarinos, 2001). A study by Yang et al. (2009) investigated cytoskeletal alterations in the hippocampus of rats exposed and then re-exposed to CUMS to mimic the reoccurring nature of depression in humans. They found that one series of CUMS resulted in impairment of microtubule dynamics and decreased levels of phosphor-MAP-2, while re-exposure to CUMS produced even more dramatic effects. These effects were associated with significant reductions in sucrose preference, and treatment with fluoxetine was able to reverse both the physiological and behavioural alterations. These data, supported by the finding that BDNF is reduced in the hippocampus after exposure to CUMS, suggest that changes in microtubule dynamics may be responsible for the abnormalities in neuronal plasticity observed

in the hippocampus after chronic stress (Filho et al., 2015; Xin-hua et al., 2017; Yang et al., 2009).

Although CUMS has proven to be a reliable method of emulating depression in rodents (Willner, 1997), its practicality is limited by some procedural disadvantages. Applying the model requires extensive labour over an extended period of time, as stressors must be changed daily for two to three weeks for a large number of subjects. Furthermore, because individuals are separated and bedding is often changed, it is demanding of both space and resources. Studies have also reported that up to a third of subjects are resistant to the CUMS paradigm, which increases the need for larger sample sizes (Bergström et al., 2007; Strekalova et al., 2006). However, despite these issues, CUMS is still one of the most commonly used models because its non-invasive application of chronic stress mirrors the development of depression in humans (Willner, 2005).

Olfactory Bulbectomy

Another commonly used model of depression in rodents is olfactory bulbectomy (OBX), a surgical procedure involving bilateral ablation of the olfactory bulbs that results in behavioural and physiological changes that are reversed by antidepressant treatment (for review, see Kelly et al., 1997).

The most notable behavioural change following OBX is an increase in locomotor activity in a novel, brightly lit environment (Klein & Brown, 1969). This change is most likely the result of some neurological substrate rather than the loss of olfaction, as this effect on locomotor activity is not observed in anosmia induced by other methods (Sieck & Baumbach, 1973). This hyperlocomotion is suggested to be related to agitated hyposerotonergic depression, which is a

major risk factor for suicide in human patients (Klein & Brown, 1969; Lumia et al., 1992; Rihmer, 2007). Other behavioural changes that often manifest following the OBX procedure include increases in immobility in the forced swim test, latency to feed in the novelty-suppressed feeding test, and time spent in closed arms in the elevated plus maze, as well as a decrease in sucrose preference (Islam et al., 2014; Morales-Medina et al., 2013; Padilla et al., 2018; Pudell et al., 2014). These changes mirror symptoms commonly observed in depressed patients, such as hopelessness, lethargy, anxiety, and anhedonia, and are reversed following administration of SSRIs (Mar et al., 2002).

The OBX procedure results in a multitude of neurological changes in structure and function, many of which are also observed in humans with MDD (Rajkumar & Dave, 2018). Of particular interest are changes in limbic structures, including the amygdala and hippocampus, which receive projections from the olfactory bulb and play important roles in controlling emotional and cognitive functioning (Shepherd, 1972). Neural degeneration, including reductions of synapses and dendritic spines, within the limbic system has been observed in both OBX animals and depressed humans (Drevets et al., 2008; Ramaker & Dulawa, 2017). Furthermore, Wrynn et al. (2000) used magnetic resonance imaging and found pronounced enlargement of the lateral and 3rd ventricles following OBX, as well as decreased signal intensity in the PFC and striatum, similar to the patterns of activity observed in depressed humans (Baxter et al., 1995; Rigucci et al., 2010).

Unsurprisingly, the OBX procedure also results in dysfunction of the serotonergic system. Many studies have reported reductions in extracellular serotonin levels following OBX in key structures, including the frontal cortex, amygdala, and hippocampus, which has been associated with depressive-like behaviours such as hyperlocomotion (Song & Leonard, 2005; van der Stelt

et al., 2005). These changes in serotonin have been found to be reversed by antidepressant treatment with SSRIs (van der Stelt et al., 2005; Wang et al., 2017). Abnormal regulation of other neurotransmitters and peptides have also been observed following OBX, including neuropeptide Y, acetylcholine, noradrenaline, and glutamate (for review, see Wang et al., 2017). Ho et al. (2000) found that OBX resulted in increased vulnerability to stressors, indicated by increased glutamate release in the striatum following novelty stress. Finally, OBX has also been found to decrease levels of BDNF in the serum and hippocampus in rats, an effect commonly observed in depressed humans (Kucera et al., 2019; Molendijk et al., 2011; Pudell et al., 2014).

The OBX model uses a surgical procedure to model depression, and as such has been criticized for its invasive nature. Furthermore, researchers are required to obtain training to perform the procedure and must possess adequate surgical skills to ensure animal health and lesion specificity. Despite these drawbacks, OBX is still considered a well-validated depression model because of its high sensitivity, specificity, and reliability for assessing the efficacy of antidepressant treatment (Ramaker & Dulawa, 2017).

Learned Helplessness

The learned helplessness (LH) model is based on the cognitive view of depression, in which events that are perceived as negative and uncontrollable lead to feelings of anxiety and helplessness (Wang et al., 2017). It initially exposes rodents to inescapable stress, such as an unavoidable foot shock. When given the opportunity to escape the stressful stimuli in future sessions, many subjects will show reduced ability to acquire escape responses and often make no effort to do so (Duman, 2010; Yang et al., 2015). This uncontrollable stressful event leads to depression-like behaviours in rodents, which models the onset of clinical depression in some humans after experiencing stressful life experiences (Lloyd, 1980). Furthermore, animals that are

susceptible to learned helplessness also exhibit several features that are observed in depressed humans, including decreased motor activity, weight loss, altered sleep, decreased motivation, and increased stress hormones, all of which have been found to be reversed by antidepressant treatment (Duman, 2010). Attesting to the specificity of the LH model, Sherman et al. (1982) found that chronic administration of tricyclic antidepressants, atypical antidepressants, monoamine oxidase inhibitors, and electroconvulsive shock were effective in reversing LH, while treatment with anxiolytics, neuroleptics, stimulants, and depressants were not.

Despite these findings, the LH model is not as commonly used by researchers as other methods to study depression. It has been criticized because of its sensitivity to sub-chronic treatment with classical antidepressants and non-antidepressant agents, which limits its usefulness for examining antidepressant onset (Ramaker & Dulawa, 2017). Furthermore, nearly half of the animals exposed to LH do not develop the behavioural deficits characteristic of the model, limiting its reliability and practicality (Muneoka et al., 2013; Ramaker & Dulawa, 2017). However, this disadvantage may be beneficial to some researchers who seek to examine tolerance to stressors and resilience to depressive-like tendencies.

Early Life Stress

Early life stress models, including prenatal stress, early postnatal handling, and maternal separation, are widely used in rodents to study how early-life adversities contribute to the future development of psychopathologies such as anxiety, depression, or addiction (Daskalakis et al., 2013). These models have been shown to produce long-lasting behavioural and neurological effects that are reversed by antidepressant treatment (Duman, 2010; Lippmann et al., 2007). The most widely used model of early life stress is the maternal separation (MS) paradigm, which separates pups from the dam for a set period of time per day (ranging from 1-24 hours,

depending on the study) during the first two postnatal weeks (Deussing, 2006). This neglect-like environment leads to long-term depressive-like behaviours and alteration of homeostatic mechanisms (Pryce et al., 2005).

Maternal separation results in many behavioural abnormalities that resemble symptoms observed in human depression, including increased anxiety, reduced social motivation, appetite disturbances, and anhedonia, which are also associated with increased HPA axis response and are counteracted by antidepressant therapies (Duman, 2010; de Kloet, 2005). Many researchers have reported increased immobility in the forced swim test, reduced entries into open arms of the elevated plus maze, and increased defecation in rats exposed to the MS paradigm, supporting the development of a depressive-like phenotype in these animals (Daniels et al., 2004; Lee et al., 2007). Physiological changes include dysregulation of the HPA axis, reductions in serotonergic activity in the hippocampus and dorsal raphe, and impairments in frontal BDNF expression (Lee et al., 2007; Lippman et al., 2007; Plotsky et al., 2005; Roth et al., 2009).

Criticism for the MS model of depression mainly focuses on the inconsistency of separation procedures, which could vary significantly between studies. It has been suggested that this lack of standardization could produce inconsistent effects by generating different degrees of stress exposure in pups (Tractenberg et al., 2016). Despite this, the MS paradigm is still widely used because of its stability of symptoms and face validity for disrupted parenting in humans, which provides a useful model for examining the effects of childhood neglect on vulnerability to developing depression in adulthood (Duman, 2010).

Social Defeat

The social defeat (SD) paradigm is a rodent model of depression that uses social conflict as a stressor to inflict a persistent state of tension, anxiety, and fear (Siegfried et al., 1984). To achieve this, a male rodent is introduced into the home cage of an older, aggressive male. After being attacked, the animals are separated and the test rodent is exposed to different aggressors before undergoing testing (for review, see Wang et al., 2017). Exposure to this social stress leads to many behavioural and physiological changes that resemble the development of depression and other psychopathologies in humans (Agid et al., 2000). These changes include behavioural deficits such as anhedonia, anxiety, decreased locomotion and sexual behavior, changes in circadian rhythm and sleep, alterations in feeding and body weight, and impaired immune function, as well as neuroendocrine alterations such as overactivity of the HPA axis (Duman, 2010). As with other stress models, these abnormalities have been found to be reversed by long-term antidepressant treatment (Deussing, 2006; Duman, 2010).

Despite these robust effects, the social defeat model is not as widely used as other depression models because of its many disadvantages, most notably its inability to be studied in non-aggressive populations such as female and adolescent subjects (Wang et al., 2017).

1.3. Neuromodulation Strategies

Interest in the field of neuromodulation has steadily grown since the first treatment with electroconvulsive therapy (ECT) in 1938. ECT passes weak electric currents through the brain to induce brief seizures and has proven to be effective in treating severe treatment-resistant depression (Kellner et al., 2012; Pagnin et al., 2004). However, misconceptions about its use have resulted in poor public perception of ECT, leading researchers to develop alternative

methods of brain stimulation (Payne & Prudic, 2009). These techniques, which include deep brain stimulation, transcranial magnetic stimulation, transcranial direct current stimulation, and transcranial alternating current stimulation, have been shown to normalize aberrant brain function in clinical settings and have provided a useful tool to attribute causal relationships between brain activity and behaviour (Drobisz & Damborská, 2019; Holtzheimer et al., 2010; Thut et al., 2011).

Deep Brain Stimulation

Deep brain stimulation (DBS) is a technique that applies chronic electric current to alter neural function by surgically implanting a pair of neural electrodes that are connected to a pulse generator in the chest (Holtzheimer & Mayberg, 2010). The surgery is completed under local anesthesia and the final location of electrodes is confirmed by postoperative MRI and electrophysiological exploration of the target area (Mayberg et al., 2005). Once the surgery is complete, stimulation can be programmed and monitored remotely from a handheld device, which allows treatment parameters to be adjusted in response to evolving patient needs (Benabid, 2003). Because of this utility, DBS has received extensive study for treatment of motor complications in Parkinson disease as well as other neuropathologies such as depression and obsessive-compulsive disorder (Drobisz & Damborská, 2019; Greenberg et al., 2010; Ramirez-Zamora & Ostrem, 2018).

The effects of DBS have been found to be immediate, long-lasting, and reversible, with low risk of causing any permanent damage to neural tissue (Jakobs et al., 2019). Furthermore, stimulation results in network and biochemical effects that extend beyond both the time and location of stimulation (McIntyre & Hahn, 2010). However, these effects are dependent on the parameters of stimulation, including voltage, polarity, frequency, and pulse width, as well as the

nature of the tissue at the target site, which can result in varying electrophysiological, cellular, and molecular outcomes (Jakobs et al., 2019; Kuncel & Grill, 2004; Milev et al., 2016).

Although the mechanisms of DBS have yet to be fully uncovered, researchers agree that stimulation produces effects at both local and remote regions. At the point of stimulation, a change in transmembrane voltage occurs that can open voltage-gated sodium channels on the axon which can generate an action potential that propagates both antidromically and orthodromically (McIntyre & Anderson, 2016). This effect can be used to normalize aberrant neurotransmission by increasing or decreasing neural communication. Lower frequencies of stimulation (30-60Hz) have excitatory effects, while higher frequencies (100Hz) have inhibitory effects due to stimulation-induced action potentials travelling antidromically to block intrinsically generated action potentials, which results in signal nullification and generates a reversible informational lesion (Benabid, 2003; McIntyre & Anderson, 2016). While this immediate effect alters the firing pattern of neural circuits, prolonged stimulation results in long term changes, such as altered neurotransmitter dynamics and protein expression, which affects the behaviour of networks as a whole (Mayberg et al., 2005; McIntyre & Hahn, 2010).

While DBS has been found to produce effective symptom remission in many disorders, it remains a controversial technique because of its highly invasive nature, which introduces many risks that can outweigh the potential therapeutic benefits, including the possibility of intracranial hemorrhaging, infection, and death (Holtzheimer & Mayberg, 2010). In addition to these physiological complications, cognitive and psychosocial side effects have also been observed, including speech disturbances, dementia, altered self-perception, and familial problems (Clausen, 2010). Furthermore, the process of optimizing stimulation parameters for individual patients must be performed by trained professionals, which can be time consuming and requires extensive

long-term follow-up care (Greenberg et al., 2010). Despite these issues, DBS is still widely used because of its robust and immediate effects. The flexible and reversible neuromodulation provided by DBS allows physicians to explore potential brain targets, optimize treatment for individual patients, and cease treatment if unacceptable side effects arise (Benabid, 2003; Greenberg et al., 2010). Therefore, weighing the risks and benefits is essential when considering treatment with DBS.

Transcranial Magnetic Stimulation

Criticism of the invasive nature of DBS has guided researchers to begin investigating non-invasive brain stimulation techniques, such as transcranial magnetic stimulation (TMS). This technique circulates a strong electric current within a coil resting on the scalp, which generates a brief and intense magnetic field that non-invasively affects current flow within the brain that can depolarize neural tissue (Hallett, 2000; Rossini & Rossi, 2007). By using different sizes and shapes of coils in varying orientations, a precise region of the brain can be mapped on the scalp and selectively stimulated. Many studies suggest that TMS can be useful in treating many neurological disorders, including Parkinson disease, Alzheimer disease, and depression (Arias et al., 2010; Holtzheimer et al., 2010; Nardone at al., 2014). Therapeutic TMS is usually delivered under the supervision of a trained professional; however, unlike DBS, no anesthesia is required for application (Milev et al., 2016).

Delivering single pulses of TMS to the brain has been shown to be safe, however it does not appear to have lasting effects (Hallett, 2000). Instead, devices deliver repetitive TMS (rTMS), which can produce powerful effects that outlast the period of stimulation by activating axons to produce new action potentials, which induces plastic changes in the brain (Paulus, 2011). Lower frequencies of stimulation (1Hz) cause a decrease in brain excitability, while higher frequencies

(5Hz) result in increased excitability (Hallett, 2007). Therefore, like DBS, TMS can transiently activate or disrupt activity in discrete brain regions, allowing researchers to localize brain function and examine the relationship between brain activity and behavioural processes (Hallett, 2000).

In a clinical guideline for therapeutic neurostimulation, Milev et al. (2016) state that standard protocols administer rTMS daily for five days per week with variable stimulation parameters, which include intensity, frequency, pattern, and site of application. These parameters are tweaked to each patient's individual needs by identifying their resting motor threshold to determine stimulus intensity, and then choosing the correct coil configuration and placement on the scalp to stimulate the targeted brain region. Conventionally, stimulation is delivered in trains lasting 2-10 seconds at 10-60 second intervals, with sessions lasting 15-45 minutes in total (Milev et al., 2016).

The most common adverse effects associated with rTMS are scalp pain and headache following stimulation; however, both of these effects diminish steadily over treatment, are remedied by over-the-counter medications, and rarely result in discontinuation of treatment (Borckardt et al., 2013). In rare cases, high intensity rTMS may induce epileptic seizures, however this risk can be minimized by carefully selecting subjects and strictly following safety guidelines (Rossini & Rossi, 2007). Furthermore, the long-term treatment schedule, which consists of daily administration over several weeks, also limits the feasibility of rTMS for patients who work full time, have busy schedules, or have limited access to transportation (Sonmez et al., 2019). This issue has led to the development of refined TMS techniques, such as theta burst stimulation, which mimics endogenous hippocampal theta patterns, and accelerated

TMS, which combines TMS protocols, to consolidate treatment and increase the utility of TMS (Hallett, 2007; Sonmez et al., 2019).

Transcranial Direct Current Stimulation

Another widely used non-invasive brain stimulation technique is transcranial direct current stimulation (tDCS), which sends weak electrical current through the scalp using a pair of surface sponge electrodes connected to a constant current generator (Liebetanz et al., 2002; Nitsche & Paulus, 2001). The current flows in a single direction, from anode to cathode, rather than oscillating in both directions (Arul-Anandam & Loo, 2009). Many studies have supported tDCS as a safe and effective method of neurostimulation, reporting benefits in cognitive performance and reductions in psychiatric symptoms following its application (Alonzo et al., 2013; Boggio et al., 2008; Gillick et al., 2015; Mondino et al., 2014). The effects of tDCS depend on whether the anode or cathode is chosen as the stimulating electrode, as well as other stimulation parameters such as current intensity, duration of treatment, and the size and location of electrodes (Arul-Anandam & Loo, 2009; Kuo et al., 2016).

Unlike the previously described methods of neurostimulation, tDCS is unable to directly generate action potentials. Instead, it modulates spontaneous firing rates and alters resting membrane potentials, with anodal stimulation enhancing excitability through neuronal depolarization and cathodal stimulation reducing excitability through neuronal hyperpolarization (Kuo et al., 2016; Paulus, 2011). Nitsche and Paulus (2001) reported that these neuromodulatory effects persist following the cessation of stimulation after observing that neural excitability in the stimulated area increased approximately 150% above baseline for up to 90 minutes following stimulation. The effects of tDCS are also reliant on current intensity, as a recent study reported

that 100μA or 200μA of repetitive anodal tDCS resulted in behavioural and neurochemical changes in a rat model of Alzheimer disease, while 20μA or 60μA did not (Yu et al., 2015).

In addition to altering resting membrane potentials, the effects of tDCS can also be attributed to alterations in brain plasticity and to modified transcription of certain genes (Nitsche et al., 2012). Anodal tDCS can increase cerebral concentrations of BDNF, a protein that is involved in long term potentiation (LTP) and memory in addition to neuronal plasticity (Fritsch et al., 2010; Podda et al., 2016). In a rat model, Kim et al. (2017) found that repetitive anodal tDCS over the right sensorimotor area increased the transcription of plasticity-related genes ipsilaterally in the stimulated area, including BDNF, cAMP response element-binding protein (CREB), synapsin I, and Ca2+/calmodulin-dependent protein kinase II (CaMKII). Similarly, Yoon et al. (2012) reported that five days of tDCS increased the expression of plasticity-related genes in a rat model of cerebral ischemia. These epigenetic changes have been suggested to be the reason why the repetitive application of tDCS has long-lasting effects (Podda et al., 2016). Furthermore, studies have suggested that these neuroplastic effects of repetitive tDCS administration are mediated by NMDA receptor-dependent mechanisms, as antagonistic blocking of these receptors has been found to prevent the long-lasting after-effects of tDCS (Liebetanz et al., 2002; Nitsche et al., 2003; Stagg & Nitsche, 2011).

Although tDCS is generally regarded as a safe procedure, some studies have reported mild side effects following its application, including burn-like lesions and contact dermatitis (Matsumoto & Ugawa, 2017). Furthermore, while the current supplied by the generator during stimulation can be standardized between subjects, the final current that reaches the tissue of interest may vary slightly between subjects due to differences in skin, skull, and tissue resistance (Brunoni et al., 2012). While these drawbacks should be acknowledged by researchers and

clinicians, they fail to outweigh the advantages provided by tDCS, which include feasibility of use, cost efficiency, portability, and ability for use in combination with other treatments (Milev et al., 2016).

Transcranial Alternating Current Stimulation

Rather than using a constant direct current, transcranial alternating current stimulation (tACS) applies an alternating electrical current with a sinusoidal waveform that interacts with and manipulates natural brain oscillations (Paulus, 2011). Similar to tDCS, this current is generated between two or more scalp electrodes to alter brain activity non-invasively; however, unlike tDCS, the current flow alternates between the anode and cathode (Reato et al., 2013). A recent meta-analysis reported that despite small-to-moderate effect sizes, tACS is able to reliably improve perceptual and cognitive performance (Schutter & Wischnewski, 2016). It also shows potential as a treatment for many pathological conditions, including depression, schizophrenia, and attention deficit hyperactivity disorder, by correcting the abnormal brain rhythms associated with these disorders (Alexander et al., 2019; Farokhzadi et al., 2020; Kallel et al., 2016). In addition to correcting pathological activity, tACS provides another use by allowing researchers to desynchronize normal rhythms to investigate causal relationships between brain oscillations and cognitive functions (Thut et al., 2011).

Like other methods of neurostimulation, the outcomes of tACS treatment are reliant on the intensity, frequency, location, and phase of the stimulation (Antal & Paulus, 2013). While the intensity of stimulation is often consistent (1-2mA), the other parameters can vary wildly between studies, which can result in variable effects on the brain and behavioural processes (Schutter & Wischnewski, 2016). Researchers suggest that tACS modulates oscillatory rhythms

in the brain in a frequency-dependent manner by synchronizing or desynchronizing neuronal networks (Reato et al., 2013). When applied at conventional electroencephalographic (EEG) frequencies (0.1–80 Hz), tACS may entrain with or synchronize neural networks, while higher frequencies in the kHz range may instead alter membrane excitability through accumulation of calcium, leading to changes in synaptic plasticity (Antal & Paulus, 2013; Citri & Malenka, 2008). However, Vossen et al. (2015) reported that stimulation at alpha (α) frequency (8–12 Hz) enhanced EEG α-oscillation long after cessation of tACS, which was suggested to be caused by both tACS-induced entrainment of cortical oscillations and plasticity-related changes in oscillatory circuits.

At the present, there are no reports of persistent adverse events associated with the application of tACS. However, because of the technique's novelty, there are considerably fewer reports available of its safety than other methods of neurostimulation (Matsumoto & Ugawa, 2017). It is logical to assume that, like tDCS, current flow may be shunted by scalp, skull, and tissue resistance before reaching the region of interest (Brunoni et al., 2012). Nonetheless, tACS provides many advantages over the neurostimulation techniques previously described. It is relatively cheap to implement, the equipment is compact and portable, and it produces no indicators of active treatment, including noise or sensations, which makes it more suitable for double-blind, sham-controlled studies (Antal & Paulus, 2013).

1.4. Neuromodulation to Treat Depression

Antidepressant drugs such as SSRIs are often the first choice of physicians to treat depression; however, many patients are resistant to their effects or are unable to tolerate their side effects (Rush et al., 2006; Sugarman et al., 2014). It has been suggested that this treatment-resistant depression is the result of serotonergic abnormalities at pre- and post-synaptic sites, as

well as environmental and genetic factors such as early life stress and possession of the short arm of the serotonin transporter gene (Coplan et al., 2014). With the failings of conventional antidepressants in this subpopulation, researchers have turned to alternative methods of treatment such as neurostimulation. The studies examined in this section are summarized in Table 1.

Deep Brain Stimulation

Despite being one of the oldest and most heavily studied methods of neurostimulation, DBS is reserved as a last-resort technique for severe, treatment-resistant depression because of its invasive nature. Mayberg et al. (2005) applied high-frequency DBS to white matter tracts adjacent to the subgenual cingulate gyrus (sgACC), a region found to exhibit hyperactivity in depressed patients (Osuch et al., 2000). They found that chronic stimulation reduced cerebral blood flow in this region and that this change was associated with the reversal of depressive symptoms, an effect that has similarly been achieved by SSRI treatment (Mayberg et al., 2000). Although the sample size was small, four of the six patients achieved sustained clinical response or remission after 6 months without any changes in medication (Mayberg et al., 2005). The pervasive antidepressive effects of DBS were further supported by Kennedy et al. (2011), who conducted a long-term follow-up study of 20 patients who received DBS to the sgACC for treatment-resistant depression. They reported that the average rate of response 1, 2, and 3 years after implantation of DBS electrodes was 63%, 46%, and 75% respectively. Furthermore, physical and social functioning progressively improved at each follow-up, likely due to normalization of activity in the sgACC, which has bilateral connections to both limbic and frontal regions that regulate emotion and motivation (Kennedy et al., 2011; Pandya et al., 2012). Although these studies focused solely on stimulation of the sgACC, DBS of numerous other brain regions that show depression-related abnormalities, including the nucleus accumbens,

ventral striatum, and medial forebrain bundle, has also been found to alleviate depression symptoms, further supporting the efficacy of DBS as a treatment for depression (for review, see Drobisz & Damborská, 2019).

As an augment to clinical studies, rodent models provide an excellent opportunity to study the underlying neurochemical changes and corresponding behavioural effects resulting from DBS. A study by Hamani et al. (2012) found that chronically stressed rats that received long-term DBS to the ventromedial prefrontal cortex (vmPFC), considered the rodent homologue of the human sgACC (Gabbott et al., 2003), had a reversal of anhedonic-like behavior in the sucrose preference test. This effect became apparent after only 1 week of treatment, and by the second week the chronically stressed rats had a rate of sucrose intake similar to that of non-stressed control subjects. The authors also reported that chronic DBS had no effect on sucrose preference in these control subjects, ruling out the possibility that the stimulation itself caused this effect. Furthermore, this anti-anhedonic effect in stressed rats was associated with partial reversal of stress-induced reduction in hippocampal BDNF levels (Hamani et al., 2012).

Another study found that DBS of the vmPFC induced a fourfold increase of hippocampal 5-HT release, which was associated with antidepressant-like behaviours in the forced swim test (Hamani et al., 2010). However, these effects were completely abolished by neurotoxic 5-HT depleting lesions in the raphe, highlighting the importance of the serotonergic system in the mechanism of action of DBS. Similar results were reported by Bregman et al. (2018), who found that DBS of the vmPFC increased serotonin levels by 33-55% and induced antidepressant-like behaviour in the forced swim test. This effect was observed in both wild-type and serotonin transporter (SERT) knockout mice, suggesting that while serotonergic activity may be necessary for DBS-related effects to occur, the serotonin transporter is not. Because SSRIs work to increase

extracellular serotonin by inhibiting SERT, and lower SERT transcription alleles are correlated with treatment-resistant depression, this suggests that DBS may be an effective alternative treatment for patients with abnormal SERT expression (Bonvicini et al., 2010; Bregman et al., 2018).

The antidepressant effects of DBS have also been examined in the OBX model of rodent depression. Jiménez-Sánchez et al. (2016) found that 1 hour of DBS of the infralimbic cortex diminished the hyperlocomotion, anhedonia, and social deficits characteristic of OBX rats, while also increasing prefrontal efflux of glutamate and serotonin in both OBX and sham-operated rats. Furthermore, DBS also increased the synthesis of BDNF and the GluA1 AMPA receptor subunit, both of which are implicated in the mechanism of chronic antidepressant drug treatment (Chourbaji et al., 2008; Jiménez-Sánchez et al., 2016; Martínez-Turrillas et al., 2005).

Transcranial Magnetic Stimulation

Studies have demonstrated that rTMS of the left dorsolateral PFC (dlPFC) reduces symptoms in treatment-resistant depression, making rTMS a first-line treatment for patients who have failed to achieve remission with antidepressant drug treatment (Holtzheimer et al., 2010; Milev et al., 2016). In one of the earliest studies, Pascual-Leone et al. (1996) applied 5 days of daily rTMS over the dlPFC of patients with treatment-resistant depression. Most patients reported feeling an improvement of symptoms after left, but not right, dlPFC stimulation, which was reflected by significantly decreased scores on the Hamilton depression rating scale and Beck questionnaire following left dlPFC stimulation. However, TMS in this study was applied at a frequency of 10Hz, which has been shown to be excitatory (Hallett, 2007). In depression, a lateralization of activity has been observed within the PFC, with the left exhibiting hypoactivity and the right exhibiting hyperactivity (Bench et al., 1992; Rotenberg, 2004). Therefore, it is of little surprise

that excitatory 10Hz rTMS failed to decrease depressive symptoms following stimulation of the right dlPFC. Indeed, a study by Kito et al. (2011) applied low frequency rTMS to the right PFC in patients with treatment-resistant depression. After 12 treatment sessions at 1Hz over 3 weeks, they found significant decreases in depression scores on the Hamilton depression rating scale, which was correlated with decreases of cerebral blood flow in the right PFC, sgACC, putamen, and insula.

These studies, in addition to others, suggest that both high frequency rTMS of the left dlPFC and low frequency rTMS of the right dlPFC are capable of producing robust antidepressant effects in patients with treatment-resistant depression (Berlim et al., 2014; Hovington et al., 2013; Kito et al., 2011; Pascual-Leone et al. 1996). Some researchers have suggested that the combination of these two strategies, known as bilateral stimulation, may produce stronger antidepressant effects; however, meta-analyses have shown that bilateral rTMS is not more efficacious for the treatment of depression than unilateral rTMS (Chen et al., 2014; Fitzgerald et al., 2006).

The efficacy of rTMS for treating depression has also been examined by rodent studies. Feng et al. (2012) applied 3 weeks of daily bilateral high frequency rTMS to rats exposed to the CUMS paradigm. They reported that long term chronic rTMS significantly reversed anhedonic- and despair-like behaviours in the sucrose preference and forced swim tests, decreased plasma levels of ACTH and CORT, as well as increased hippocampal cell proliferation and BDNF protein level. They also reported increased phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, proteins that play important roles in synaptic plasticity and gene transcription, an outcome that is also observed following treatment with antidepressant drugs (Feng et al., 2012; Gourley et al., 2008). Similar results were obtained in a study by Kim et al. (2014), who

also applied chronic rTMS in the CUMS rat model of depression. They reported that exposure to CUMS significantly impaired sucrose preference and reduced the concentration of GABA in prefrontal and hippocampal areas. However, these effects were completely reversed following 2 weeks of daily rTMS at 10Hz, which is also observed after chronic treatment with SSRIs (Sanacora et al., 2002).

An experiment by Müller et al. (2000) examined the effects of rTMS on the expression of mRNA for various neuropeptides and proteins in rats. They reported that long term rTMS at a frequency of 20Hz resulted in significant increases in BDNF mRNA in the hippocampus, supporting the results obtained from Feng et al. (2012). Furthermore, there was also a significant increase in hippocampal mRNA for cholecystokinin (CCK), an abundant peptide in the mammalian brain that mediates depression-like behaviour (Becker et al., 2008). These increases in BDNF and CCK expression mirror results produced from antidepressant drug and ECT treatment, suggesting a common mechanism underlying these depression therapies (Lindefors et al., 1991; Molteni et al., 2006; Müller et al., 2000).

Transcranial Direct Current Stimulation

Similar to TMS, studies investigating tDCS as a treatment for depression often target the left dlPFC because of its predictable hypoactivity in depressed patients, and that normalization of this activity is characteristic of successful antidepressant drugs (Drevets et al., 1997; Kennedy et al., 2001).

A randomized, double-blind clinical trial conducted by Boggio et al. (2008) applied 2mA of anodal tDCS over the left dlPFC or occipital cortex in patients with MDD. After ten 20-minute sessions, patients in the dlPFC group had a significant reduction in depression scores as compared to the occipital or sham stimulation groups, with the beneficial effects of tDCS

persisting one month after treatment. While supporting the antidepressant properties of tDCS, these results also highlight the region specificity of the technique. Furthermore, another study by Ferrucci et al. (2009) also found that anodal tDCS applied to the left dlPFC in patients with treatment-resistant depression resulted in a significant decrease in depression scores that persisted for one month after treatment. Results from meta-analyses have further supported the use of tDCS as a treatment for MDD, as well as for other subtypes including bipolar and pregnancy-related depression (Berlim et al., 2013; Dondé et al., 2017; Vigod et al., 2019).

Although many studies support the use of tDCS as a treatment for depression, others have failed to produce positive results. Palm et al. (2012) found no difference between active and sham tDCS after two weeks of treatment, although both reduced scores on the Hamilton depression rating scale as compared to baseline. However, there was considerable variability among the medication status of patients, which may have interacted with stimulation effects to mask treatment results (Brunoni et al., 2012). Loo et al. (2010) also reported no significant benefit of active tDCS as compared to sham tDCS, despite using stimulation parameters similar to previously successful studies. However, this outcome may be a result of stimulation being administered on alternate days, as well as the use of concurrent drug therapy in only a subset of the participants. Together, these mixed results suggest that the antidepressant properties of tDCS may only manifest under certain sets of conditions and parameters. Therefore, a multitude of factors must be considered before using tDCS to treat depression, including the unique circumstances of the patient as well as the location, duration, and intensity of stimulation.

Rodent studies have provided valuable information in the determination of optimal stimulation parameters and corresponding neurochemical changes that occur following tDCS. Higher intensities of anodal tDCS produce corresponding increases in cerebral blood flow while

higher intensities of cathodal tDCS produce corresponding decreases, which supports the feasibility of tDCS to correct the abnormal activity patterns observed in the PFC in depressed humans (Bench et al., 1992; Rotenberg, 2004; Wachter et al., 2011). Furthermore, a study by Peanlikhit et al. (2017) reported reduced immobility in the forced swim test following a single 200μA tDCS session in mice, but not from 25μA or 100μA. This effect was associated with an increase in c-fos expression in the PFC, hippocampus, and ventral tegmental area, indicating activation of these areas following frontal stimulation. This enhanced resilience in the forced swim test persisted for up to 60 days in mice receiving 10 stimulations; however, at this timepoint there was no observable difference in c-fos expression. This suggests that transient activation of frontal and midbrain regions facilitate the short-term effects of tDCS, while repetition of stimulation is necessary to induce synaptic plasticity and produce long-term antidepressant effects (Nitsche & Paulus, 2001; Peanlikhit et al., 2017; Yoon et al., 2012).

Long-term tDCS application results in neurochemical changes that are also observed following chronic antidepressant treatment. Kim et al. (2017) reported that 7 days of unilateral anodal tDCS in rats significantly increased transcription of plasticity-related genes in the region of stimulation, including BDNF, cAMP response element-binding protein (CREB), and Ca2+/calmodulin-dependent protein kinase II (CaMKII). Chronic administration of SSRIs has been found to upregulate BDNF and CREB mRNA (Molteni et al., 2006; Tiraboschi et al., 2004), while treatment with tricyclic antidepressants increases the activity of CaMKII in the hippocampus and PFC (Consogno et al., 2001). Additionally, Podda et al. (2016) also observed that anodal tDCS increases expression of BDNF in mice. Reductions in BDNF have been observed in both rodent models of depression and human MDD; therefore, the long-term antidepressant effect of tDCS may in part be due to the normalization of this deficit (Duric et al.,

2013; Kucera et al., 2019; Molendijk et al., 2011). Taken together, these findings suggest a common mechanism of action underlying the antidepressant effects of tDCS and drug treatment.

Transcranial Alternating Current Stimulation

As a relatively new technique, there are few studies that examine the efficacy of tACS as a treatment for depression. A recent case series by Haller et al. (2020) applied tACS in gamma frequency (40Hz) over the PFC in patients with treatment-resistant depression. They reported that chronic stimulation significantly reduced depressive symptoms as assessed by the Hamilton depression rating scale and Beck depression inventory, while also improving cognitive functions in the word fluency and n-back tests. Although the underlying mechanisms are still unclear, the authors hypothesize that gamma tACS produced this effect by normalizing disturbed frequency bands within the PFC (Haller et al., 2020). Another study also found that tACS at 30Hz produced improvement in prefrontal-executive functions at 4 weeks following stimulation (Del Felice et al., 2019). This long-term effect is most likely attributable to stimulation-induced changes in neuronal plasticity, similar to how antidepressant drugs reverse impaired cognitive function and increase expression of plasticity-related genes in patients with depression (Martinowich & Lu, 2008; Prado et al., 2018; Vossen et al., 2015).

Another small pilot study by Alexander et al. (2019) investigated the efficacy of tACS in alpha frequency (10Hz) over the left dlPFC. Patients were assigned to a 10Hz, 40Hz, or sham stimulation condition and received daily 40-minute sessions for 5 consecutive days. At a follow-up session 2 weeks after completion, the authors reported that the 10Hz tACS group had significantly lower scores on both the Montgomery–Åsberg and Hamilton depression rating scales, as well as a reduction in alpha oscillatory activity over the left dlPFC. Increased alpha oscillation is associated with hypoactivity in depression and contributes to abnormal emotional

processing, which suggests that modulating these oscillations with tACS may be useful to decrease depressive symptoms (Alexander et al., 2019; Galynker et al., 1998). The use of alpha tACS is further supported by a case study that applied weekly bifrontal 10Hz tACS for 12 weeks on a single patient (Riddle et al., 2020a). This protocol resulted in effective remission of depressive symptoms as measured by the Montgomery–Åsberg depression rating scale for up to 6 months. Although this result was obtained from only a single patient, it presents initial evidence that weekly tACS treatment could replace daily administration and provides further support for the use of alpha tACS to treat depression.

Similar to other methods of neurostimulation, the long-term antidepressant effects of tACS are thought to be the result of BDNF-dependent plasticity. A naturally occurring polymorphism at codon 66 (Val66Met) in the gene that codes for BDNF results in deficits in BDNF secretion and neuroplasticity, as well as reduced effectiveness of non-invasive brain stimulation and increased susceptibility to developing depression (Chaieb et al., 2014; Fritsch et al., 2010; Kleim et al., 2006; Youssef et al., 2018). The effect of this polymorphism on tACS response was examined in a study by Riddle et al. (2020b). They found that Val66Val carriers had larger alpha amplitude increases in the motor cortex from alpha tACS as compared to Val66Met carriers, suggesting a role of BDNF in the mechanism of tACS. However, there are currently no published studies that directly examine changes in BDNF expression in response to tACS.

1.5. Research Objectives and Hypotheses

The current project aimed to develop a novel treatment for depression by manipulating the activity of specific neuron subtypes using a combination of tDCS and drug therapy. Electrical brain stimulation often results in indiscriminate cell activation at the site of application, which could lead to the jamming of synaptic signals and limit the efficacy of therapeutic stimulation

(Breit et al., 2004; Klooster et al., 2016). To address this problem, we attempted to chemically prime a subset of cell types within the rodent mPFC, considered the functional homologue of the dlPFC in humans (Farovik et al., 2008), to respond to a subthreshold dose of tDCS, modifying their membrane potential to render the cells more sensitive to input. This was accomplished by targeting small-conductance, calcium-activated potassium (SK) channels, which control neural excitability and brain plasticity by allowing potassium ions to exit the cell (Faber & Sah, 2007; Imbrici et al., 2013; Sah & Faber, 2002). Previous studies have found that blocking these channels, and therefore increasing the cell's likelihood of firing, is associated with antidepressant-like responses (Stocker et al., 2004; van der Staay et al., 1999). Combining tDCS with SK-selective drugs will exclusively modulate the activity of these SK channel-containing cells, constraining signal propagation within well-defined pathways (Faber & Sah, 2007; Imbrici et al., 2013; Sah & Faber, 2002). This process of electrically guiding cells that have been chemically primed for excitability is known as EC stimulation.

To test the hypothesis that EC stimulation can be effectively utilized as a novel approach to treat depression, this research was conducted over two experiments. Experiment 1 determined the optimal subthreshold dose of the SK3 antagonist NS8593 and tDCS current intensity that produced no discernable neurochemical or behavioural changes in rats. After determination of these optimal treatment parameters, Experiment 2 evaluated EC stimulation in terms of behaviourally and neurochemically relevant changes in adult rats that had undergone OBX surgery to induce a depressive-like phenotype (Kelly et al., 1997). The OBX model of depression was chosen for its highly specific surgical approach, which more reliably produces the desired phenotypic changes compared to other models (Ramaker & Dulawa, 2017).

It was hypothesized that EC stimulation, but not a subthreshold dose of NS8593 or tDCS alone, would result in normalization of the depressive-like behaviours induced by the OBX procedure. These behaviours would in turn be associated with increased concentrations of depression-related neurotransmitters across frontal and limbic areas of the brain. The confirmation of these hypotheses would support the development of EC stimulation as an effective method for achieving safe and highly selective neurostimulation, while also providing a novel treatment alternative for difficult to treat disorders such as depression.

Chapter 2: Experiment 1. Determination of Optimal Dosage for EC Stimulation

2.1. Introduction

Depression is associated with a myriad of complex signaling cascades that involve numerous types of cells and ion channels. Of particular significance are small-conductance, calcium-activated potassium (SK) channels, which play a role in the regulation of firing patterns and dendritic excitability by mediating the medium afterhyperpolarization following action potentials in neuronal cells (Faber & Sah, 2007). Three subtypes of SK channels have been identified (SK1, SK2, & SK3), each with highly similar structural properties but distinct localizations within the central nervous system.

SK channels consist of multiple subunits assembled as a tetramer, with each subunit containing six transmembrane spanning regions with a calmodulin-binding domain in the C terminus that responds to intracellular changes in calcium concentration (Faber & Sah, 2007; Xia et al., 1998). Because of their similar structural features, the three subtypes of SK channels are distinguished by their sensitivity to the bee venom apamin, with SK2 exhibiting the strongest affinity and SK1 exhibiting the least (Köhler et al., 1996). They also differ based on their location within the brain. While SK1 and SK2 channels share some colocalization in the neocortex and hippocampus, each subtype is generally concentrated in different brain tissue, with SK1 being expressed within the olfactory bulb, SK2 within the amygdala, and SK3 within the basal ganglia, thalamus, and brainstem nuclei (Sailer et al., 2004; Stocker & Pedarzani, 2000). Most importantly, however, the SK3 subtype is also located on the cell bodies of GABAergic interneurons in the mPFC, a key regulator in the pathology of depression (Markram et al., 2004; Pandya et al., 2012).

Numerous studies have implicated SK3 channels in the development and prognosis of depression, as well as various other neurological disorders such as schizophrenia and Alzheimer disease (Martin et al., 2017; Strøbæk et al., 2006; Tomita et al., 2003). A study by Nashed et al. (2022) utilizing the CUMS model of depression in rats observed SK3 overexpression in the prelimbic cortex, which was associated with region hypoactivity as assessed by a decrease in mRNA for the activity marker, *zif268*. Furthermore, genetic SK3 deletion reduced immobility time in the forced swim test in homozygotes as compared to their wild-type counterparts. Similar work by Bambico et al. (2020) found that infusions of the SK3 antagonist apamin resulted in a rapid antidepressant response, while infusions of the SK3 agonist 1-EBIO blocked scopolamine-induced antidepressant activity, which has been observed in numerous other studies (Faber & Sah, 2007; Galeotti et al., 1999; Wang et al., 2023). Together, these results suggest that the overexpression or hyperactivity of SK3 channels play a key role in the mechanism of depression, and that therapies targeting these channels may prove useful in mitigating its symptoms.

This experiment aims to determine the optimal current intensity of tDCS and dosage of the SK3-selective antagonist, (*R*)-*N*-(benzimidazole-2-yl)-1,2,3,4-tetrahydro-1-naphtylamine (NS8593). Rather than blocking the channel's pore like other SK antagonists, this compound acts as an inhibitory gating modifier by decreasing the calcium sensitivity of the channel, resulting in reduced potassium efflux and increased rate of action potentials and burst firing (Strøbæk et al., 2006). Similarly, stimulation of neurons with tDCS alters resting membrane potential to make cell firing more likely to occur (Kuo et al., 2016; Paulus, 2011). Therefore, we anticipate that by combining a subthreshold dose of both NS8593 and tDCS, we can selectively activate SK3-containing interneurons within the mPFC to achieve a more robust antidepressant effect than with either treatment alone. The mPFC is a major source of glutamate in the raphe and the

extensive reciprocal connections between the two regions have been implicated in emotional regulation (Amat et al., 2005; Bambico et al., 2007; Hajós et al., 1998). It is known that glutamate-mediated activation of raphe interneurons inhibits serotonergic neuron activity; therefore, reducing glutamatergic input by increasing GABAergic activity within the mPFC may remediate depressive symptoms (Amat et al., 2005).

2.2. Method

2.2.1. Animals

Adult male Sprague-Dawley rats (Charles-River Saint-Constant, Quebec, Canada), weighing between 500 and 600 g at the start of the experiment, were pair-housed under standard conditions upon arrival. All animals were given *ad libitum* access to food and water and were kept on a 12-hr light-dark cycle under standard laboratory conditions (lights on at 0700).

All procedures and protocols for experiments and animal housing followed the guidelines of the Canadian Council on Animal Care and Memorial University of Newfoundland's Animal Care Committee.

2.2.2. Phase One: Determination of NS8593 dose

Thirty animals were divided into five groups (n = 6) to receive a varying intraperitoneal dose of the SK3 antagonist, NS8593. The drug was prepared using 90% saline (0.9% NaCl) and 10% DMSO. Four doses of NS8593 were assessed (0.1mg/kg, 0.5mg/kg, 1mg/kg, and 3mg/kg) as well as a control group that received an injection of vehicle at a similar volume. Animals were then tested on the Open Field Test (OFT), euthanized, and had their brains collected for RNAscope analysis.

Based on these results, a second set of twelve animals were split into two groups (n = 6), receiving an intraperitoneal injection of either 1mg/kg of NS8593 or vehicle before being subjected to a Novelty Suppressed Feeding Test (NSFT) and Sucrose Preference Test (SPT).

Open Field Test. The open field test (OFT) was conducted to assess the locomotor effects of the varying doses of NS8593, with hyperlocomotion being characteristic of rodent depression. Twenty minutes post-injection, rats were placed facing the corner in an open field apparatus (60 cm x 60 cm x 60 cm) and were allowed to freely explore the chamber during their 10-min trial. Trials were counterbalanced based on group, and the chamber was cleaned with 70% ethanol in between trials. Each trial was videotaped and distance travelled was analyzed offline using Ethovision XT14 software.

Novelty Suppressed Feeding Test. The novelty suppressed feeding test (NSFT) was conducted to measure anxiety by examining the latency for a rat to eat in a novel environment (Britton & Britton, 1981). Animals were food deprived for 24 hours before given an injection of 1mg/kg NS8593. After 20 minutes they were placed facing the corner of a large arena (60 cm x 60 cm x 60 cm) with 9 food pellets placed in the center. The latency for each rat to reach the center of the arena and to commence eating was recorded. Once the animal started eating, or after 10 min had passed without eating, rats were promptly removed from the maze and placed back in their home cage, along with some food pellets. Upon return to the colony room, rats were once again allowed *ad libitum* access to food.

Sucrose Preference Test. The sucrose preference test (SPT) was conducted as an indicator of anhedonia (Papp et al., 1991). Five hours post NSFT, a two-bottle, overnight sucrose preference test was given to all animals, with one bottle containing a 1% sucrose solution and one bottle containing water. Animals had free access to both bottles for 12 hours. Sucrose and water levels were measured before and after the test and sucrose preference was determined by dividing the amount of sucrose consumed by the total amount of liquid consumed.

Sample Preparation. After testing, animals were euthanized by CO_2 and brains were flash frozen in 2-methylbutane before being stored at -80 °C. Brains were then sliced using a cryostat (Leica Microsystems, Germany) into 20-µm thick coronal sections collected on Superfrost Plus slides (Fisher Scientific, USA) and stored at −80 °C for RNAscope™ in-situ hybridization.

RNAscope™ Fluorescent In-situ Hybridization. The RNAscope assay is a novel in-situ hybridization technique designed to have less background interference and increased selectivity over traditional in situ hybridization techniques (Wang et al. 2012). To test the degree at which varying doses of NS8593 activate GABAergic interneurons, RNAscope was used to target *zif268* and *GAD* mRNA in the rat mPFC. Zif268 is a marker for cellular activity, while GAD is an enzyme used to localize GABAergic interneurons. The prepared slides were processed according to the RNAscope Multiplex Fluorescent Reagents Kit V2 Assay User Manual for fresh frozen tissue, provided by Advanced Cell Diagnostics (Newark, CA).

First, the sections were fixed using 10% neutral buffered formalin (NBF) and successively dehydrated in 50%, 70%, and 100% ethanol. Hydrophobic barriers were drawn

around each section using the ImmEdge Hydrophobic Barrier Pen (ACD, cat# 310018) before adding RNAscope hydrogen peroxide and Protease IV, each with their own incubation and washing steps. Next, the probes were hybridized by dropping probe solution on each section and incubating at 40°C for two hours. After double rinsing in wash buffer, the sections were treated with AMP 1 (30 minutes, 40°C), AMP 2 (30 minutes, 40°C), and AMP 3 (15 minutes, 40°C), with double rinsing in wash buffer also occurring between each amplification step. Next, horseradish peroxidase signals were developed by treating the sections with HRP-C1 (15 minutes, 40°C), TSA Vivid Dye 520 (1:1500, green) (30 minutes, 40°C), HRP blocker (15 minutes, 40°C), HRP-C2 (15 minutes, 40°C), TSA Vivid Dye 650 (1:1500, far-red) (30 minutes, 40°C), and HRP blocker (15 minutes, 40°C), with double rinsing in wash buffer occurring between each incubation step.

Finally, sections were counterstained by dipping slides in hematoxylin for 30 seconds, followed by two quick rinses in tap water, five dips in 0.02% ammonia water, and three rinses in tap water before being incubated for 15 minutes at 40°C. After cooling, glass coverslips were applied and the slides were stored overnight in complete darkness. The slides were then imaged using an Olympus BX51 confocal microscope (Richmond Hill, ON) at 20x magnification. Images underwent colour inversion and were analyzed in ImageJ (National Institutes of Health, USA) by four individuals blinded to treatment groups. The number of cells in the left mPFC expressing mRNA for *zif268, GAD*, both *zif268* and *GAD*, or neither, were counted and expressed as a percentage of the total cell count for each image.

2.2.3. Phase Two: Determination of tDCS current intensity

To determine the intensity-related behavioural effects of tDCS, 21 animals underwent a series of behavioural tests, including the OFT, NSFT, and forced swim test (FST), following a 20-minute treatment session with 0.05mA (n = 7), 0.10mA (n = 7), or sham (n = 7) stimulation. These parameters were determined based on previous experiments in our lab and others (Boggio et al., 2008; Peanlikhit et al., 2017; Waye et al., 2021).

Then, to assess tDCS-induced changes in raphe glutamate concentration, 21 additional animals had a glutamate biosensor surgically implanted in the dorsal raphe and were divided into four groups to receive a varying intensity of tDCS over the left mPFC. The four intensities tested were 0.05mA (n = 6), 0.1mA (n = 6), 0.2mA (n = 6), as well as a sham group that received no stimulation (n = 3). After a two-hour baseline reading of raphe glutamate, animals received three 20-minute sessions of tDCS, with 20 minutes of rest in between each session. The percent change in raphe glutamate compared to baseline was assessed for the first five minutes, as well as for the full 20 minutes of stimulation. The rest periods in between sessions were also included in the analysis in the no stimulation condition.

A second set of nine animals receiving 0.2mA of stimulation were further split into two groups receiving either a 25mg/kg dose of pentylenetetrazol (PTZ) (n = 3) or vehicle (n = 6) and were compared to the sham group receiving no stimulation (n = 3). PTZ is a $GABA_A$ receptor antagonist and was administered intraperitoneally at a dose that would not induce seizures (Corda et al., 1990; Samokhina & Samokhin, 2018). Assessment of glutamate in the dorsal raphe was conducted as described above.

Surgery and tDCS Treatment for Behavioural Examination. Animals were anesthetized using isoflurane (induction levels at 2.5%, maintenance at 2%) and mounted on a standard stereotaxic apparatus in the skull-flat position. Four jeweler screws were tightly twisted into the skull to act as anchors for the dental acrylic resin. A male JST connector was installed on the skull, connecting a conducting metal plate sized 2.5±0.25 x 1.5±0.25 mm at the anodal end positioned over the mPFC (AP: +2.2 mm to +4.7 mm). The cathodal end was linked to a screw behind the cerebellum serving as the ground electrode. Dental acrylic resin was applied to stabilize the assembly during treatment. After post-surgical recovery, the electrodes were connected to the tDCS system, with a current generator developed at the Center for Addiction and Mental Health (Toronto, ON, Canada) that delivered currents of 0.05 mA or 0.10 mA. The anodal current was delivered for 20 min for three sessions: 24, 5, and 0.5 hours before behavioral testing, as determined by pilot experiments in our lab. The animals were gently held in place while being connected to the generator, then were allowed to freely roam their cages throughout the treatment under close observation. A multimeter was attached to the generator to ensure that each animal received the full extent of the stimulation. Control rats receiving sham stimulation were handled in the same way but did not undergo electrical stimulation.

Behavioural Tests. The OFT and NSFT were conducted as described in section 2.2.2. The FST measured passive immobility in an inescapable cylindrical water pool (20 cm diameter, 50 cm high; 30 cm water depth, 25–27°C water temperature). Animals were placed in the pool for a videotaped 10-minute trial 24 hours following a swim pre-exposure to induce learned passive coping. For behavioural quantification, we utilized a pixel motion-based algorithm to differentiate between immobility, swimming, and climbing actions. Immobility was defined as

minimal movements to keep the head above water, swimming as moderate horizontal movements for navigation, and climbing as vigorous movements along the cylinder walls.

Surgery for Glutamate Examination. Animals were anesthetized with an intraperitoneal injection of urethane at a dose of 1.5g/kg and mounted on a standard stereotaxic apparatus in the skull-flat position. A cranial window was drilled over the dorsal raphe and a glutamate biosensor cannula (Pinnacle Technology, Inc., USA) was slowly lowered into place (AP: -7.8 mm, ML: 0.0 mm, DV: -5.5 mm) based on coordinates obtained from the stereotaxic atlas by Paxinos and Watson (2007). Immediately after lowering the cannula into place, a polypropylene epicranial electrode holder (Soterix Medical, USA) was mounted on the skull above the left medial prefrontal cortex (AP: +3.0 mm, ML: +1.0 mm). Dental acrylic resin (Jet Set-4™ Denture Repair Powder & Jet™ Liquid, Lang Dental, USA) was applied to keep both assemblies in place on the skull during stimulation and recording. Following surgery, animals were left undisturbed for one hour to allow for the resin to harden.

Biosensor Recording and tDCS Treatment. Animals were mounted in a secure chamber and a biosensor probe was lowered into the implanted cannula to measure glutamate concentration in real-time within the dorsal raphe. The probe was connected to a pre-amplifier and amplifier, with glutamate activity being displayed on screen and saved for later offline analyses using data acquisition software (Sirenia Acquisition, Pinnacle Technology, Inc., USA). The electrode holder was filled with conductive electrogel, and an epicranial stimulation electrode connected to a current generator (Soterix Medical, USA) was fastened into place. A reference electrode was placed inside a moistened sponge and kept tightly in place on the right

thorax using a restraint jacket. After a two-hour baseline recording, the current generator was switched on and rats received 20 minutes of active or sham stimulation at a given intensity. This was repeated 3 times for each animal, with 20 minutes of rest in between sessions. Immediately following the final session, the probe was removed and the animals were euthanized by CO_2.

PTZ Administration. To test if the effects of tDCS on raphe glutamate were mediated by $GABA_A$ receptors, a second set of animals underwent the same procedures outlined above with the addition of an intraperitoneal injection of either PTZ (25mg/kg) or vehicle 30 minutes before the start of stimulation, as determined based on previous studies (Corda et al., 1990; Samokhina & Samokhin, 2018). To prepare the drug, 500 mg of PTZ was dissolved in 20 ml of physiological saline (0.9% NaCl) to obtain an injection volume of 1ml/kg. Control animals were injected with saline at the same volume of 1ml/kg.

Statistical Analyses. Datasets were analyzed using Jamovi 2.2.5. One-way ANOVAs were predominately used to assess group differences, with Tukey's post hoc comparisons following up on pairs of group means when appropriate. Independent samples t-tests were used to assess the behavioural effects of 1.0mg/kg NS8593 in comparison to vehicle. Effect size was measured using Cohen's *f* for one-way ANOVAs, with a value of 0.4 indicating a large effect. For independent samples *t*-tests or pairwise comparisons, effect size was measured using Cohen's *d*, with a value of 0.8 being considered a large effect. Non-parametric analyses were used when assumptions of normality and homogeneity of variance were not met, with Mann-Whitney's *r* being used as a measure of effect size. For all analyses, a value of $p < .05$ was considered to be significant.

2.3. Results

To determine the optimal dosage of NS8593 and current intensity of tDCS, we compared the effects of varying doses and intensities across numerous behavioural and neurochemical outcomes.

2.3.1. Dose Determination for NS8593

Four doses of the SK3 antagonist NS8593 (0.1mg/kg, 0.5mg/kg, 1.0mg/kg, and 3.0mg/kg), as well as a control group receiving an injection of vehicle at a similar volume, were assessed using the open field test and RNAscope in situ hybridization, with further assessment of the 1.0mg/kg dose in the novelty suppressed feeding test and sucrose preference test.

2.3.1.1. Open Field Test

The open field test examined agitated depression- and anxiety-like behaviour by measuring hyperlocomotion within a novel environment. A one-way ANOVA revealed no significant differences in distance traveled between the groups (0mg/kg: M = 2061.396, SD = 742.468, n = 6; 0.1mg/kg: M = 2288.357, SD = 643.843, n = 6; 0.5mg/kg: M = 2274.851, SD = 772.655, n = 5; 1.0mg/kg: M = 1838.505, SD = 446.359, n = 6; 3.0mg/kg: M = 1286.394, SD = 538.655, n = 5), however a downward trend was observed as dosage increased, $F(4,11.112)$ = 2.161, p = .140, Cohen's f = .616 (Figure 2). This f value corresponds to a large effect, so Tukey's post hoc tests were used to compare each dosage to the vehicle condition. As the 3.0mg/kg dose was closest to achieving significance when compared to vehicle injection, $t(23)$ = 2.005, p = .295, Cohen's d = 1.214, it was determined that 1.0mg/kg would be a more appropriate choice as a sub-threshold dose, $t(23)$ = .605, p = .973, Cohen's d = 0.349.

2.3.1.2. RNAscope In Situ Hybridization

RNAscope in situ hybridization examined the co-localization of *zif268* mRNA within interneurons in the prefrontal cortex as a measure of neural activation in response to NS8593 injection. A one-way ANOVA revealed that the percentage of colocalized *zif268* in *GAD*-positive cells between treatment groups (0mg/kg: $M = 14.662$, $SD = 6.136$, $n = 4$; 0.1mg/kg: $M = 16.338$, $SD = 5.679$, $n = 3$; 0.5mg/kg: $M = 10.706$, $SD = 7.473$, $n = 3$; 1.0mg/kg: $M = 15.505$, $SD = 8.416$, $n = 3$; 3.0mg/kg: $M = 28.975$, $SD = 12.453$, $n = 3$) was not significant, $F(4, 11) = 2.148$, $p = .143$, Cohen's $f = .885$ (Figure 3). However, this large effect size suggested an undetected difference between groups. Upon analysis using Tukey's post hoc comparisons, the 3.0mg/kg group had a noticeably larger amount of co-expression when compared to vehicle, $t(11) = 2.283$, $p = .221$, Cohen's $d = 1.744$, while the 1.0mg/kg group was nearly indistinguishable, $t(11) = .135$, $p = .999$, Cohen's $d = .103$. When combined with the results from the open field test, this suggests that 1.0mg/kg may be the more appropriate dose to examine the sub-threshold effects of NS8593.

2.3.1.3. Novelty Suppressed Feeding Test

To further verify the behaviourally inert consequences of a 1.0mg/kg NS8593 injection, a novelty suppressed feeding test was conducted to examine anxiety by measuring latency to feed in an open arena. An independent samples t-test revealed that the latency to reach the food pellets in the center of the arena was not significantly different between the two groups (Drug: $M = 114.190$, $SD = 215.331$, $n = 7$; VEH: $M = 140.167$, $SD = 230.893$, $n = 6$), $U = 19.500$, $p = .886$, Mann-Whitney's $r = .071$(Figure 4). Additionally, the latency to start eating the food pellets was not significantly different between the two groups (Drug: $M = 454.286$, $SD = 151.176$, $n = 7$; VEH: $M = 566.667$, $SD = 81.65$, $n = 6$), $U = 11.500$, $p = .143$, Mann-Whitney's $r = .452$, nor was

the latency to begin eating when placed back in the home cage (Drug: $M = 73.714$, $SD = 19.973$, $n = 7$; VEH: $M = 138.500$, $SD = 72.729$, $n = 6$), $U = 12.000$, $p = .223$, Mann-Whitney's $r = .429$. These results suggest that an injection of 1.0mg/kg of NS8593 does not result in any behavioural changes as measured by the NSFT.

2.3.1.4. Sucrose Preference Test

The sucrose preference test measured anhedonia by assessing the drinking preferences of rats when provided with bottles containing a palatable sucrose solution or regular tap water. An independent samples t-test revealed that rats receiving a 1.0mg/kg injection of NS8593 ($M = 89.571$, $SD = 9.769$, $n = 7$) did not consume a significantly different amount of sucrose when compared to vehicle-injected rats ($M = 94.633$, $SD = 3.789$, $n = 6$), $U = 15.000$, $p = .445$, Mann-Whitney's $r = .286$ (Figure 5). This suggests that a 1.0mg/kg dose of NS8593 does not produce an anhedonia-like response as assessed by the SPT.

2.3.2. Current Intensity Determination for tDCS

Three current intensities (0mA, 0.05mA, and 0.10mA) were used to examine the antidepressant-like properties of tDCS in the OFT, NSFT, and FST. Then, the effects of four intensities (0mA, 0.05mA, 0.1mA, 0.2mA) on glutamate release within the dorsal raphe were examined using an indwelling biosensor probe, with a follow-up experiment examining the changes in raphe glutamate after 0.2mA of tDCS both with and without a concurrent injection of the $GABA_A$ receptor antagonist, PTZ.

2.3.2.1. Open Field Test

Our results suggest that neither 0.05mA nor 0.10mA affect overall locomotion in the OFT. A one-way ANOVA revealed no significant differences in distance traveled between the

groups (0mA: M = 6456.411, SD = 3745.325, n = 7; 0.05mA: M = 6725.257, SD = 3314.813, n = 7; 0.10mA: M = 7493.197, SD = 2475.035, n = 7), $F(2,11.615) = .221$, $p = .805$, Cohen's $f = .146$ (Figure 6).

2.3.2.2. Novelty Suppressed Feeding Test

Analysis of the results obtained from the NSFT suggest a mild anxiogenic effect of tDCS at the 0.10mA dose. A one-way ANOVA revealed no significant differences in the time taken to start eating within the testing chamber between the groups (0mA: M = 145.833, SD = 61.756, n = 6; 0.05mA: M = 195.714, SD = 90.517, n = 7; 0.10mA: M = 235.143, SD = 61.110, n = 7), $F(2,11.084) = 3.218$, $p = .079$, Cohen's $f = .533$ (Figure 7A). However, the test closely approached significance, with an increase in current intensity being associated with an increase in latency to feed. Follow up tests using Tukey's post hoc comparisons showed that the latency to begin feeding for rats receiving 0.10mA of tDCS approached significance when compared to sham animals, $t(17) = -2.199$, $p = .100$, Cohen's $d = 1.223$ whereas rats receiving 0.05mA were nearly indistinguishable, $t(17) = -1.228$, $p = .454$, Cohen's $d = .683$. This effect was abolished once returned to the home cage, as an additional one-way ANOVA revealed no significant differences in the latency to feed between groups in this environment (0mA: M = 13.000, SD = 11.983, n = 6; 0.05mA: M = 18.286, SD = 13.124, n = 7; 0.10mA: M = 20.857, SD = 10.189, n = 7), $F(2,10.976) = .753$, $p = .494$, Cohen's $f = .293$ (Figure 7B).

2.3.2.3. Forced Swim Test

Our results suggest that 0.10mA, but not 0.05mA, reduces passivity and promotes active coping in rats as measured by the FST. A one-way ANOVA indicated a significant difference in immobility time between the groups (0mA: M = 136.614, SD = 59.573, n = 7; 0.05mA: M =

91.714, *SD* = 46.500, *n* = 7; 0.10mA: *M* = 57.971, *SD* = 29.115, *n* = 7), *F*(2,10.995) = 5.042, *p* = .028, Cohen's *f* = .743 (Figure 8A). Rats receiving 0.10mA of tDCS spent significantly less time immobile than sham rats, *t*(18) = 3.147, *p* = .015, while those receiving 0.05mA did not, *t*(18) = 1.796, *p* = .199.

An additional one-way ANOVA also revealed a significant difference in swimming time between the groups (0mA: *M* = 117.571, *SD* = 29.529, *n* = 7; 0.05mA: *M* = 133.471, *SD* = 37.507, *n* = 7; 0.10mA: *M* = 179.700, *SD* = 18.042, *n* = 7), *F*(2,10.934) = 12.361, *p* = .002, Cohen's *f* = .967 (Figure 8B). Rats receiving 0.10mA of stimulation spent significantly more time swimming than both sham rats, *t*(18) = 3.945, *p* = .003, and rats receiving 0.05mA, *t*(18) = 2.935, *p* = .023, while those receiving the lower intensity of tDCS were nearly indistinguishable from shams, *t*(18) = 1.010, *p* = .580.

A final one-way ANOVA indicated no significant differences in climbing behaviour between the groups (0mA: *M* = 45.843, *SD* = 36.123, *n* = 7; 0.05mA: *M* = 74.814, *SD* = 59.881, *n* = 7; 0.10mA: *M* = 62.371, *SD* = 22.925, *n* = 7), *F*(2,10.716) = .738, *p* = .501, Cohen's *f* = .301 (Figure 8C).

2.3.2.4. Glutamate Biosensor

Biosensor analysis examined the effect of tDCS on glutamate release within the dorsal raphe. A one-way ANOVA revealed a significant effect of current intensity on the change in raphe glutamate concentration compared to baseline (0mA: *M* = -.291, *SD* = 2.311, *n* = 29; 0.05mA: *M* = -1.603, *SD* = 1.705, *n* = 18; 0.1mA: *M* = -1.894, *SD* = 1.205, *n* = 18; 0.2mA: *M* = -2.543, *SD* = 2.074, *n* = 17) after 5 minutes of stimulation, *F*(3,40.590) = 4.481, *p* = .008, Cohen's *f* = .464 (Figure 9). Tukey's follow-up tests revealed that the 0.2mA group had

significantly reduced glutamate concentration as compared to the sham group, $t(78) = 3.808$, $p = .002$, Cohen's $d = 1.163$, and that the 0.1mA group also showed inhibited glutamate release when compared to sham, $t(78) = 2.758$, $p = .036$, Cohen's $d = .828$. No other comparisons were significant.

Similarly, an additional one-way ANOVA examining the full 20 minute stimulation period also revealed a significant effect of current intensity on the change in raphe glutamate concentration compared to baseline (0mA: $M = -.291$, $SD = 2.638$, $n = 29$; 0.05mA: $M = -2.473$, $SD = 3.902$, $n = 18$; 0.1mA: $M = -2.831$, $SD = 3.510$, $n = 18$; 0.2mA: $M = -2.543$, $SD = 3.285$, $n = 17$), $F(3,37.374) = 3.702$, $p = .020$, Cohen's $f = .350$ (Figure 9). Tukey's follow-up tests revealed no significant comparisons; however, all forms of current showed reduced glutamate when compared to sham, with the 0.1mA group closely approaching significance, $t(78) = 2.586$, $p = .055$, Cohen's $d = .776$. Taken together, these results suggest that 0.05mA is the ideal choice for a subthreshold current intensity.

2.3.2.5. Glutamate Biosensor following PTZ

The obtained results suggest that the glutamate suppressive activity of tDCS is not mediated by the $GABA_A$ receptor. A one-way ANOVA revealed a significant effect of injection on the change in raphe glutamate concentration compared to baseline (Control: $M = .268$, $SD = 1.855$, $n = 9$; Vehicle: $M = -2.543$, $SD = 2.074$, $n = 17$; PTZ: $M = -2.154$, $SD = 1.097$, $n = 9$) after 5 minutes of 0.2mA tDCS stimulation, $F(2,18.729) = 6.907$, $p = .006$, Cohen's $f = .678$ (Figure 10). Tukey's follow-up tests revealed that 0.2mA of tDCS resulted in decreased concentrations of raphe glutamate regardless of whether the rat received an injection of PTZ, $t(32) = 2.823$, $p = .022$, Cohen's $d = 1.331$, or an injection of vehicle, $t(32) = 3.747$, $p = .002$, Cohen's $d = 1.545$.

An additional one-way ANOVA examining the full 20 minute stimulation period also revealed a significant effect of injection on the change in raphe glutamate concentration compared to baseline (Control: $M = .267$, $SD = 3.061$, $n = 9$; Vehicle: $M = -2.543$, $SD = 3.285$, $n = 17$; PTZ: $M = -2.712$, $SD = 1.457$, $n = 9$), $F(2,18.270) = 3.526$, $p = .050$, Cohen's $f = .459$ (Figure 10). Tukey's follow-up tests revealed no significant comparisons; however, both stimulation groups showed trending reductions in glutamate when compared to the control group, with significance being approached by both the PTZ injection group, $t(32) = 2.207$, $p = .085$, Cohen's $d = 1.041$, and the vehicle injection group, $t(32) = 2.381$, $p = .059$, Cohen's $d = .981$.

2.4. Discussion

The present study highlighted the dose-dependent behavioural and neurochemical effects of both NS8593 and tDCS administration, allowing us to pinpoint a duo of synergistic sub-threshold doses for both treatments. Our results suggest that 1.0mg/kg is optimal for NS8593, as it is shown to be behaviourally and neurochemically inert. Meanwhile, a dose of 3.0mg/kg showed markedly decreased locomotion in the OFT and increased cell activity in the PFC. These effects are likely the result of preferential binding to SK3 channels located on GABAergic interneurons within the PFC, where these channels are more heavily distributed (Sailer et al., 2004; Stocker & Pedarzani, 2000). This hypothesis is supported by results obtained by Nashed et al. (2021), who subjected rats to a CUMS model of depression and reported that apamin binding was increased in the prelimbic area of the PFC, but not in the dorsal raphe. Furthermore, inhibition of the SK3 channel increased resilience in the forced swim test, suggesting that the antidepressant activity of SK3 antagonists occur primarily within the frontal cortex, with cascading effects on downstream targets through various signalling pathways.

The most prominent of these pathways is the PFC-raphe-hippocampal circuit, which is often implicated in the pathology of depression and targeted for treatment of symptoms (Drevets et al., 2008). SK3 inhibition within infralimbic GABAergic interneurons enhances GABA release in the PFC, which inhibits the overactive pyramidal neurons (Drevets et al., 1997; Fuchikami et al., 2015). This decreased glutamatergic input from the infralimbic cortex to the GABAergic interneurons within the dorsal raphe disinhibits and stimulates serotonergic neurons, normalizing aberrant serotonergic activity and reversing depressive symptoms (Nashed et al., 2021).

Similarly, stimulation of the PFC with tDCS is thought to activate this pathway in an identical manner. This idea is supported both by our observation of reduced glutamatergic activity in the raphe in response to frontal stimulation as well as by the findings of other researchers that highlight altered activity- and plasticity-related genes in limbic regions (Kim et al., 2014; Peanlikhit et al., 2017). Increasing the intensity of stimulation results in stronger effects; however, this also increases its effective range by extending stimulation to surrounding tissue, which may lead to competing responses from opposing neural groups and pathways. With this caveat in mind, we determined that an intensity of 0.05mA is ideal for tDCS. Our behavioural results suggest that 0.1mA of stimulation produced antidepressant-like effects across numerous tests, while 0.05mA was only marginally effective. Previous work in our lab uncovered identical results, where adolescent rats treated with 0.05mA of tDCS for either 2 or 14 consecutive days did not exhibit any behavioural changes in the OFT, NSFT, FST, or SPT (Waye, 2019). These findings are corroborated by our biosensor analyses, which showed significantly reduced glutamatergic activity within the raphe nucleus in response to 0.1mA and

0.2mA stimulation, but not 0.05mA. Taken together, these results support the use of 0.05mA as a subthreshold dose of tDCS.

An additional finding from our biosensor analysis suggests that concurrent administration of the $GABA_A$ receptor antagonist PTZ was incapable of blocking tDCS's glutamatergic inhibition in the raphe nucleus, suggesting that this effect occurs independently of the $GABA_A$ receptor. However, numerous studies have shown altered concentrations of GABA in stimulated and associated areas following tDCS (Bachtiar et al., 2015; Bachtiar et al., 2018; Bunai et al., 2021). While it is possible that tDCS instead acts upon the slower metabotropic $GABA_B$ receptor, the rapid action of the stimulation technique makes the ionotropic $GABA_A$ receptor a more likely target, suggesting a methodological issue in our procedure. We administered PTZ as a single dose of 25mg/kg to avoid the development of seizures; however, many epileptic studies employ a kindling model that involves administration of higher doses (up to 40mg/kg) that are repeated over several days (Bascuñana et al., 2016; Corda et al., 1990). Therefore, our choice to utilize a lower dose may have led to incomplete antagonism of the $GABA_A$ receptor, allowing tDCS to exert its downstream effects relatively unimpeded. Future studies are warranted to determine the exact methods of action and signaling cascades involved.

Chapter 3: Experiment 2. Behavioural and Neurochemical Outcomes of EC Stimulation

3.1. Introduction

Both SK channel antagonism and non-invasive electrical stimulation of neurons have been shown to produce antidepressant effects (Bambico et al., 2020; Boggio et al., 2008; Galeotti et al., 1999; Mondino et al., 2014; Nashed et al., 2022). However, these methods are not without limitations. Apamin is a neurotoxic compound with ubiquitous SK channel targeting that may result in the development of seizures, while newly developed SK antagonists still have questionable safety and efficacy profiles in a clinical setting (Strøbæk et al., 2006; van der Staay et al., 1999). Meanwhile, neurostimulation techniques such as tDCS produce conflicting results in clinical trials, possibly due to the indiscriminate targeting of all cell types within the stimulation region (Klooster et al., 2016; Loo et al., 2010; Palm et al., 2012). However, the synergistic and subthreshold combination of these treatments may prove advantageous by focusing therapeutic intervention along the intended mPFC-raphe pathway while also reducing the chance of any adverse side effects.

To test this hypothesis, this experiment was split into four subcomponents. First, we aimed to validate the OBX procedure as a model of depression using a battery of behaviourally relevant tests. Second, we evaluated the efficacy of EC stimulation as a treatment for depression. NS8593 was injected intraperitoneally, as the compound carries no charge and can readily bypass the blood brain barrier (Strøbæk et al., 2006). Concurrently, stimulation was administered over the left mPFC, a region whose dorsal and ventral portions are considered to be the rodent analogues of the human dlPFC and sgACC respectively, which are both often targeted in clinical settings (Drevets, 2000; Farovik et al., 2008; Gabbott et al., 2003; Mayberg at al., 2005). Third, we compared the behavioural effects of stimulating different sides of the mPFC to confirm that

left hemispheric stimulation produces more robust antidepressant effects. Lateralization of activity within the PFC is characteristic of depression, with the left exhibiting hypoactivity and the right exhibiting hyperactivity (Bench et al., 1992; Rotenberg, 2004). And finally, we investigated the effects of the OBX model and EC stimulation in females. Depression etiology, manifestation, and treatment efficacy is known to vary based on sex in both human and rodent populations (Kessler, 2003; Kim et al., 2018; McCallum et al., 2018; Ruiz et al., 2018), therefore we sought to also quantify the effectiveness of EC stimulation as an antidepressant treatment in female subjects.

3.2. Method

Animals. Adult male or female Sprague-Dawley rats (Charles-River Saint-Constant, Quebec, Canada) were pair-housed under standard conditions upon arrival. Males weighed between 500 and 600 g at the start of the experiment, while females weighed between 250 and 300 g. All animals were given *ad libitum* access to food and water and were kept on a 12-hr light-dark cycle under standard laboratory conditions (lights on at 0700).

All procedures and protocols for experiments and animal housing followed the guidelines of the Canadian Council on Animal Care and Memorial University of Newfoundland's Animal Care Committee.

OBX Surgery. One week after arrival, animals were subjected to olfactory bulbectomy surgery. All animals were anesthetized with isoflurane (induction at 2.5%, maintenance at 2%) and mounted on a standard stereotaxic apparatus in the skull-flat position. We used a modified version of the OBX procedure described elsewhere (Kelly et al., 1997). A cranial window was drilled over the olfactory bulbs (AP: +6.0 to +9.0 mm, ML: ±2.0 mm) based on coordinates

obtained from the stereotaxic atlas by Paxinos and Watson (2007). A sterilized 23G needle/syringe was then used to bilaterally aspirate the visible olfactory bulbs, with sterile hemostatic sponge being placed in the empty area on both sides to prevent regrowth. The incision was then closed using sutures. Post-mortem visual analysis was performed to confirm the complete ablation of the olfactory bulbs. Control animals underwent a similar procedure, with the dura being punctured but bulbs left intact. Following surgery, animals were allowed to recover for 14 days before the commencement of treatment in order to allow for the development of OBX-induced neuroplastic changes (for review see Song & Leonard, 2005).

Electrode Placement. One week following OBX surgery, animals were anesthetized with isoflurane (induction at 2.5%, maintenance at 2%) and mounted on a standard stereotaxic apparatus in the skull-flat position. A polypropylene epicranial electrode holder (Soterix Medical, USA) was mounted on the skull above either the left, center, or right medial prefrontal cortex (AP: +3.0 mm, ML: +1.0, 0.0, or -1.0 mm). Dental acrylic resin (Jet Set-4™ Denture Repair Powder & Jet™ Liquid, Lang Dental, USA) was applied to keep the assembly in place on the skull during treatment. Animals were left to recover for 5-7 days before the beginning of treatment. A diagram outlining electrode placement, stimulation protocol, and current flow is provided in Figure 11A.

EC Stimulation. Antidepressant treatment consisted of an injection of the SK3 antagonist NS8593 immediately followed by administration of transcranial direct current stimulation. To test for acute effects, animals received a single treatment of EC stimulation before a battery of behavioural tests on week 1, including the OFT, FST, and SPT. To test for chronic effects,

animals received this treatment again on week 2, with an additional session being administered before the FST. The schedule of treatment and behavioural testing is shown in Figure 11B.

Animals were given an intraperitoneal injection of NS8593 at a dose of 1mg/kg, created by dissolving 10 mg of NS8593 in 18 ml of vehicle (16.2 ml 0.9% NaCl, 0.9 ml tween 80, 0.9 ml polyethylene glycol). Control animals were injected with vehicle at the same volume.

Following the injection, rats were subjected to 20 minutes of 0.05mA anodal tDCS delivered from an external current generator (Soterix Medical, USA). Each animal was outfitted with a restraint jacket to keep a cathodal reference electrode in contact with the right thorax. Each animal was lightly restrained while being connected to the generator but was promptly placed back in its home cage for the duration of the stimulation. Stimulation intensity was monitored using a current meter built into the generator to ensure that each animal received the full extent of the stimulation. Rats receiving sham stimulation were handled in a similar manner, but no current was delivered.

Behavioural Tests

Immediately after stimulation, a battery of tests assessing depressive-like behaviours were conducted to assess the effects of EC stimulation.

Open Field Test. The open field test (OFT) was conducted using hyperlocomotion as a measure of agitated depression. Rats were placed facing the corner in an open field apparatus (60 cm x 60 cm x 60 cm) with aluminum foil covering the walls and three 60-watt lights placed overhead for illumination (1000 lux at the center of the arena), as described in previous experiments (Kelly et al., 1997). Animals were allowed to freely explore the chamber during their 10-min trial. Trials were counterbalanced based on group, and the chamber was cleaned

with 70% ethanol between trials. Each trial was videotaped and distance travelled was analyzed offline using Ethovision XT14 software. During analysis, the chamber was divided into a 4x4 grid, resulting in 16 squares of equal size, with the inner 4 squares being marked as the center of the chamber. Time spent in the center was also analyzed using Ethovision XT14 software.

Forced Swim Test. The forced swim test (FST) was conducted using immobility as a measure of passivity, an indication of a depressive-like state in rodents (Porsolt, 1979). Twenty-four hours before the first treatment, rats were given a 15 min pre-exposure to the FST. For both the pre-exposure and the trial, rats were placed in a transparent, inescapable cylindrical bin (38.0 cm height x 27.0 cm diameter) filled with water (27 ± 1 ℃) such that the animal could not touch the bottom or top of the bin. Each trial lasted 10 min and was videotaped for analysis of swimming behaviour on Ethovision XT14 software.

Sucrose Preference Test. The sucrose preference test (SPT) was conducted as described in section 2.2.2.

Novelty Suppressed Feeding Test. The novelty suppressed feeding test (NSFT) was conducted in place of the OFT on week 2. Animals were food deprived for 16 hours and then placed facing the corner of a large arena (60 cm x 60 cm x 60 cm) with 6 food pellets placed in the center. To create a novel testing environment compared to the previous week's OFT, the aluminum foil was removed from the walls of the arena and the overhead lights were removed. The latency for each rat to reach the center of the arena and to commence eating was recorded. Once the animal started eating, or after 10 min had passed without eating, rats were promptly removed from the maze and placed back in their home cage along with some food pellets. The time spent to begin feeding in the home cage was recorded, at which point the rats were once again allowed *ad libitum* access to food.

Brain Extraction. Immediately following the sucrose preference test on week 2, rats were euthanized using CO_2. Their brains were flash frozen in 2-methylbutane and stored at -80°C until preparation for analysis.

High-Pressure Liquid Chromatography. The quantification of dopamine (DA), as well as serotonin (5-HT), its precursor tryptophan (TRP), and its metabolite 5-hydroxyindoleacetic acid (5-HIAA), was achieved using high-pressure liquid chromatography (HPLC). To prepare samples for analysis, brains were warmed to -20°C and hemisected to separate the left and right hemispheres. Tissue was collected using a clean razor blade in four target regions: the mPFC, caudate putamen, dorsal hippocampus, and midbrain. Samples were placed in separate microcentrifuge tubes and weighed prior to preparation. Brain areas were then homogenized and sonicated for 2 min in 200 µl of a 0.2 M perchloric acid solution before being centrifuged for 6 min at 13,000 g at 4°C. The separation and quantification of DA, 5-HT, TRP, and 5-HIAA was conducted with a Shimadzu LC-10AD HPLC system equipped with a Shimadzu RF-10AXL fluorometric detector set at excitation and emission wavelengths of 279 and 320 nm, respectively. The chromatographic separation was performed on an Apollo C18 (5 µm 250 mm x 4.6 mm) column (Sepachrom Mega Srl, Milan, Italy) using a mobile phase at a flow rate of 1 ml/min composed by milliQ water/acetonitrile (5% water, 95% acetonitrile) and milliQ water/methanol (90% water, 10% methanol) in a ratio of 5:95 v/v, respectively, that were acidified with orthophosphoric acid to a pH of 3.5.

Statistical Analyses. Datasets were analyzed using Jamovi 2.2.5. ANOVAs were predominately used to assess group differences, with Tukey's post hoc comparisons following up on pairs of group means when appropriate. Independent samples *t*-tests were used to assess the differences between OBX and sham animals. Due to mechanical issues with the swimming apparatus, some animals were excluded from the FST analysis in section 3.3.1.3. (OBX: $n = 10$; sham: $n = 12$). For all analyses, a value of $p < .05$ was considered to be significant.

3.3. Results

To examine the efficacy of EC stimulation as a treatment for depression, this experiment was split into four subcomponents, including the validation of the OBX model in males (3.3.1), assessment of EC stimulation in males (3.3.2), analysis of hemisphere-specific stimulation (3.3.3), and the assessment of OBX and EC stimulation in females (3.3.4).

3.3.1. Validation of OBX

First, we established OBX as a valid model of depression by comparing male OBX and sham animals that had received no treatments across multiple behavioural tests.

3.3.1.1. Open Field Test

Our results suggest that OBX successfully produced the hyperlocomotion characteristic of the procedure, but did not alter anxiety-like states as assessed by the time spent in the center of the arena. An independent samples *t*-test revealed that the distance traveled by OBX animals ($M = 4093.280$, $SD = 1631.210$, $n = 15$) was significantly greater than the distance traveled by sham animals ($M = 3126.384$, $SD = 682.177$, $n = 19$), $t(17.868) = 2.152$, $p = .045$, Cohen's $d = .773$ (Figure 12A). Furthermore, they also entered significantly more quadrants in the maze

(OBX: M = 248.600, SD = 117.040, n = 15; Sham: M = 175.684, SD = 54.142, n = 19), t(18.714) = 2.232, p = .038, Cohen's d = .800 (Figure 12B).

However, the amount of time spent in the center of the chamber was not significantly different between the two groups (OBX: M = 14.327, SD = 10.754, n = 15; Sham: M = 12.679, SD = 19.112, n = 19), t(32) = .298, p = .768, Cohen's d = .103 (Figure 12C), nor was the number of entries into the center (OBX: M = 13.467, SD = 9.768, n = 15; Sham: M = 7.789, SD = 9.704, n = 19), t(32) = .298, p = .101, Cohen's d = .583 (Figure 12D).

3.3.1.2. Novelty Suppressed Feeding Test

The results from this test suggest that OBX did not affect anxiety-like behaviours as assessed by the NSFT. An independent samples t-test revealed that the latency to reach the food pellets in the center of the arena was not significantly different between the two groups (OBX: M = 101.600, SD = 96.324, n = 15; Sham: M = 74.059, SD = 82.597, n = 17), t(30) = .871, p = .391, Cohen's d = .309, nor was the latency to begin eating in the arena (OBX: M = 218.462, SD = 111.288, n = 13; Sham: M = 289.667, SD = 105.582, n = 9), t(20) = -1.506, p = .148, Cohen's d = -.653, or in their home cage (OBX: M = 63.000, SD = 53.968, n = 9; Sham: M = 103.133, SD = 30.680, n = 15), t(11.163) = -2.042, p = .066, Cohen's d = -.914 (Figure 13).

3.3.1.3. Forced Swim Test

Our results suggest that no differences in passivity, as measured by the FST, resulted from the OBX procedure. At the acute time point, an independent samples t-test revealed that OBX animals (M = 162.840, SD = 36.263, n = 5) did not spend more time immobile than sham animals (M = 151.000, SD = 52.614, n = 7), t(10) = .432, p = .675, Cohen's d = .253. There was also no difference between groups in swimming behaviour (OBX: M = 98.86, SD = 24.464, n =

5; Sham: M = 106.857, SD = 39.160, n = 7), $t(10)$ = -.401, p = .697, Cohen's d = -.235, or climbing behaviour (OBX: M = 38.280, SD = 20.949, n = 5; Sham: M = 42.186, SD = 18.733, n = 7), $t(10)$ = -.339, p = .741, Cohen's d = -.199 (Figure 14A).

Similar results were obtained at the chronic time point on Week 2. An independent samples t-test revealed that OBX animals (M = 152.200, SD = 50.340, n = 5) did not exhibit more immobility than sham animals (M = 180.214, SD = 52.692, n = 7), $t(10)$ = -.924, p = .377, Cohen's d = -.541. There were also no differences between groups in swimming behaviour (OBX: M = 103.700, SD = 17.798, n = 5; Sham: M = 92.557, SD = 29.007, n = 7), $t(10)$ = .757, p = .466, Cohen's d = .443, or climbing behaviour (OBX: M = 44.120, SD = 37.479, n = 5; Sham: M = 27.257, SD = 25.264, n = 7), $t(10)$ = .937, p = .371, Cohen's d = .549 (Figure 14B).

3.3.1.4. Sucrose Preference Test

The results from this test suggest that the OBX procedure did not affect anhedonia as measured by the SPT, although the data trended towards a reduction in sucrose consumed following OBX. At the acute time point, an independent samples t-test revealed that OBX animals (M = 87.471, SD = 10.234, n = 14) did not consume significantly less sucrose than sham animals (M = 92.247, SD = 6.643, n = 17), $t(29)$ = -1.567, p = .128, Cohen's d = -.566 (Figure 15A).

Similarly, at the chronic time point, an independent samples t-test revealed that OBX animals (M = 89.079, SD = 11.173, n = 14) did not consume significantly less sucrose than sham animals (M = 92.194, SD = 6.270, n = 18), $t(30)$ = -1.001, p = .325, Cohen's d = -.357 (Figure 15B).

3.3.1.5. HPLC

The obtained results suggest that some alterations in DA and 5-HT signaling occur in frontal regions following OBX, but not in the hippocampus or midbrain.

Dopamine. Analysis of DA concentrations across the four brain regions revealed mild alterations following OBX. In the PFC, an ANOVA indicated no main effect of surgery on DA concentration (OBX groups: $M = 130.953$, $SD = 19.068$, $n = 12$; sham groups: $M = 106.967$, $SD = 35.311$, $n = 12$), $F(1,20) = 3.779$, $p = .066$, $\eta_p^2 = .159$, although this value closely approached significance. However, there was clearly no main effect of hemisphere (left groups: $M = 117.280$, $SD = 18.749$, $n = 12$; right groups: $M = 120.640$, $SD = 35.630$, $n = 12$), $F(1,20) = .074$, $p = .788$, $\eta_p^2 = .004$, as well as no interaction, $F(1,20) = .916$, $p = .350$, $\eta_p^2 = .044$ (Figure 16).

In the CPu, an ANOVA indicated no main effect of surgery on DA concentration (OBX groups: $M = 231.748$, $SD = 69.132$, $n = 12$; sham groups: $M = 219.550$, $SD = 48.394$, $n = 12$), $F(1,20) = .251$, $p = .622$, $\eta_p^2 = .012$, as well as no main effect of hemisphere (left groups: $M = 200.280$, $SD = 59.091$, $n = 12$; right groups: $M = 251.018$, $SD = 58.435$, $n = 12$), although this comparison verged on significance, $F(1,20) = 4.337$, $p = .050$, $\eta_p^2 = .178$. This finding is unsurprising, as asymmetry in nigrostriatal DA levels between the hemispheres has been previously documented (Glick et al., 1977). There was also no interaction between the two variables, $F(1,20) = .025$, $p = .877$, $\eta_p^2 = .001$ (Figure 16).

In the hippocampus, an ANOVA revealed no main effect of OBX on DA concentration (OBX groups: $M = 131.842$, $SD = 57.524$, $n = 11$; sham groups: $M = 109.764$, $SD = 26.824$, $n = 12$), $F(1,19) = 1.430$, $p = .247$, $\eta_p^2 = .070$, as well as no main effect of hemisphere (left groups: M

= 120.266, *SD* = 49.777, *n* = 11; right groups: *M* = 121.340, *SD* = 34.571, *n* = 12), *F*(1,19) = .003, *p* = .954, η_p^2 = .000, and no interaction, *F*(1,19) = .286, *p* = .015, η_p^2 = .015 (Figure 16).

Finally, an ANOVA indicated no main effect of OBX on midbrain DA concentration (OBX groups: *M* = 124.077, *SD* = 43.643, *n* = 12; sham groups: *M* = 115.486, *SD* = 57.316, *n* = 12), *F*(1,20) = .170, *p* = .684, η_p^2 = .008, no main effect of hemisphere (left groups: *M* = 122.154, *SD* = 50.213, *n* = 12; right groups: *M* = 117.409, *SD* = 50.745, *n* = 12), *F*(1,20) = .052, *p* = .822, η_p^2 = .003, as well as no interaction, *F*(1,20) = .001, *p* = .973, η_p^2 = .000 (Figure 16).

Serotonin. Similar to DA, analysis of 5-HT levels found moderate differences across the four regions in response to OBX surgery. In the PFC, an ANOVA indicated no main effect of surgery on 5-HT concentration (OBX groups: *M* = 15.466, *SD* = 16.537, *n* = 12; sham groups: *M* = 23.743, *SD* = 26.562, *n* = 12), *F*(1,20) = .458, *p* = .506, η_p^2 = .022. However, there was a main effect of hemisphere (left groups: *M* = 36.960, *SD* = 41.094, *n* = 12; right groups: *M* = 2.249, *SD* = 2.004, *n* = 12), *F*(1,20) = 8.050, *p* = .010, η_p^2 = .287, but no observed interaction, *F*(1,20) = .496, *p* = .489, η_p^2 = .024 (Figure 17).

Within the CPu, an ANOVA revealed no main effect of OBX on 5-HT concentration (OBX groups: *M* = 11.675, *SD* = 6.869, *n* = 12; sham groups: *M* = 20.221, *SD* = 11.820, *n* = 12), *F*(1,20) = 3.890, *p* = .063, η_p^2 = .163. However, there was a significant main effect of hemisphere (left groups: *M* = 11.223, *SD* = 6.514, *n* = 12; right groups: *M* = 20.673, *SD* = 12.175, *n* = 12), *F*(1,20) = 4.756, *p* = .041, η_p^2 = .192, as well as an interaction between the two variables, *F*(1,20) = 5.399, *p* = .031, η_p^2 = .213 (Figure 17). Tukey's follow up tests revealed that 5-HT levels in the right hemisphere of sham rats were significantly higher than those in the right hemisphere of OBX rats, *t*(20) = 3.038, *p* = .030, Cohen's *d* = 1.754, as well as those in the left hemispheres of

both OBX, $t(20) = 2.937$, $p = .038$, Cohen's $d = 1.695$, and sham rats, $t(20) = 3.185$, $p = .022$, Cohen's $d = 1.839$.

An additional ANOVA also revealed no main effect of OBX on hippocampal 5-HT concentration (OBX groups: $M = 3.733$, $SD = 3.491$, $n = 11$; sham groups: $M = 4.106$, $SD = 3.393$, $n = 12$), $F(1,19) = .066$, $p = .800$, $\eta_p^2 = .003$, no main effect of hemisphere (left groups: $M = 4.365$, $SD = 4.092$, $n = 11$; right groups: $M = 3.475$, $SD = 2.792$, $n = 12$), $F(1,19) = .377$, $p = .547$, $\eta_p^2 = .019$, as well as no interaction, $F(1,19) = .049$, $p = .827$, $\eta_p^2 = .003$ (Figure 17).

Finally, in the midbrain, an ANOVA indicated no main effect of surgery on 5-HT concentration (OBX groups: $M = 3.894$, $SD = 2.982$, $n = 12$; sham groups: $M = 5.108$, $SD = 3.515$, $n = 12$), $F(1,20) = .790$, $p = .385$, $\eta_p^2 = .038$, no main effect of hemisphere (left groups: $M = 4.046$, $SD = 3.704$, $n = 12$; right groups: $M = 4.956$, $SD = 2.793$, $n = 12$), $F(1,20) = .443$, $p = .513$, $\eta_p^2 = .022$, as well as no interaction, $F(1,20) = 1.154$, $p = .295$, $\eta_p^2 = .055$ (Figure 17).

Tryptophan. Unlike the previous two metabolites, no differences emerged in TRP concentrations across the four regions in response to OBX. In the PFC, an ANOVA indicated no main effect of surgery on TRP concentration (OBX groups: $M = 8321.910$, $SD = 3298.310$, $n = 12$; sham groups: $M = 8073.610$, $SD = 2955.960$, $n = 12$), $F(1,20) = .037$, $p = .850$, $\eta_p^2 = .002$, no main effect of hemisphere (left groups: $M = 8958.280$, $SD = 3185.660$, $n = 12$; right groups: $M = 7437.240$, $SD = 3068.620$, $n = 12$), $F(1,20) = 1.373$, $p = .255$, $\eta_p^2 = .064$, as well as no interaction, $F(1,20) = .565$, $p = .461$, $\eta_p^2 = .027$ (Figure 18).

Likewise, an ANOVA indicated no main effect of OBX on TRP levels within the CPu (OBX groups: $M = 13123.400$, $SD = 2754.060$, $n = 12$; sham groups: $M = 14198.400$, $SD = 3451.740$, $n = 12$), $F(1,20) = .666$, $p = .424$, $\eta_p^2 = .032$, no main effect of hemisphere (left

groups: M = 13438.000, SD = 3517.190, n = 12; right groups: M = 13883.800, SD = 2688.620, n = 12), $F(1,20) = .115$, $p = .739$, $\eta_p^2 = .006$, as well as no interaction, $F(1,20) = .173$, $p = .682$, $\eta_p^2 = .009$ (Figure 18).

In the hippocampus, an ANOVA revealed no main effect of surgery on TRP concentration (OBX groups: M = 12169.000, SD = 1707.040, n = 11; sham groups: M = 14922.200, SD = 5103.630, n = 12), $F(1,19) = 2.836$, $p = .109$, $\eta_p^2 = .130$, no main effect of hemisphere (left groups: M = 12957.400, SD = 3382.200, n = 11; right groups: M = 14133.800, SD = 3428.470, n = 12), $F(1,19) = .518$, $p = .481$, $\eta_p^2 = .027$, as well as no interaction, $F(1,19) = .059$, $p = .811$, $\eta_p^2 = .003$ (Figure 18).

Finally, an ANOVA indicated no main effect of OBX on TRP concentration within the midbrain (OBX groups: M = 29304.000, SD = 6826.170, n = 12; sham groups: M = 26745.100, SD = 5726.470, n = 12), $F(1,20) = .910$, $p = .352$, $\eta_p^2 = .044$, no main effect of hemisphere (left groups: M = 29947.500, SD = 6990.250, n = 12; right groups: M = 26101.600, SD = 5562.390, n = 12), $F(1,20) = 2.055$, $p = .167$, $\eta_p^2 = .093$, as well as no interaction, $F(1,20) = .383$, $p = .543$, $\eta_p^2 = .019$ (Figure 18).

5-hydroxyindoleacetic acid. Similar to TRP, no differences were observed in 5-HIAA concentrations across the four target regions following OBX. In the PFC, an ANOVA indicated no main effect of surgery on 5-HIAA concentration (OBX groups: M = 255.578, SD = 109.093, n = 12; sham groups: M = 252.333, SD = 124.558, n = 12), $F(1,20) = .005$, $p = .947$, $\eta_p^2 = .000$. There was also no main effect of hemisphere (left groups: M = 233.888, SD = 109.216, n = 12; right groups: M = 274.023, SD = 124.435, n = 12), $F(1,20) = .692$, $p = .415$, $\eta_p^2 = .033$, as well as no interaction, $F(1,20) = .088$, $p = .770$, $\eta_p^2 = .004$ (Figure 19).

In the CPu, an ANOVA also indicated no main effect of OBX on 5-HIAA concentration (OBX groups: $M = 254.467$, $SD = 79.98$, $n = 12$; sham groups: $M = 237.803$, $SD = 162.307$, $n = 11$), $F(1,19) = .095$, $p = .761$, $\eta_p^2 = .005$, as well as no main effect of hemisphere (left groups: $M = 252.431$, $SD = 107.175$, $n = 11$; right groups: $M = 239.839$, $SD = 135.112$, $n = 12$), $F(1,19) = .055$, $p = .818$, $\eta_p^2 = .003$, and no interaction, $F(1,19) = .383$, $p = .543$, $\eta_p^2 = .020$ (Figure 19).

Likewise, an ANOVA indicated no main effect of surgery on hippocampal 5-HIAA levels (OBX groups: $M = 145.648$, $SD = 43.471$, $n = 11$; sham groups: $M = 176.602$, $SD = 68.396$, $n = 12$), $F(1,19) = 1.570$, $p = .225$, $\eta_p^2 = .076$, no main effect of hemisphere (left groups: $M = 143.557$, $SD = 50.854$, $n = 11$; right groups: $M = 178.693$, $SD = 61.013$, $n = 12$), $F(1,19) = 2.023$, $p = .171$, $\eta_p^2 = .096$, and no interaction, $F(1,19) = .000$, $p = .989$, $\eta_p^2 = .000$ (Figure 19).

Finally, in the midbrain, an ANOVA revealed no main effect of surgery on 5-HIAA concentration (OBX groups: $M = 165.992$, $SD = 77.970$, $n = 12$; sham groups: $M = 136.605$, $SD = 38.527$, $n = 12$), $F(1,20) = 1.288$, $p = .270$, $\eta_p^2 = .061$, as well as no main effect of hemisphere (left groups: $M = 173.026$, $SD = 44.989$, $n = 12$; right groups: $M = 129.572$, $SD = 71.508$, $n = 12$), $F(1,20) = 2.816$, $p = .109$, $\eta_p^2 = .123$, and no interaction between the two, $F(1,20) = .415$, $p = .527$, $\eta_p^2 = .020$ (Figure 19).

3.3.2. Evaluation of EC Stimulation

Second, we evaluated EC stimulation in terms of reversing the depressive-like behaviours and neurochemical changes following OBX surgery in male rats. All results for this section are reported in Table 2.

3.3.2.1. Open Field Test

Our results suggest that tDCS, but not NS8593, is effective in reversing the hyperlocomotion characteristic of the OBX procedure. An ANOVA indicated a main effect of stimulation on distance traveled (tDCS groups: $M = 2509.098$, $SD = 1242.989$, $n = 25$; sham groups: $M = 3393.028$, $SD = 1553.688$, $n = 32$), $F(1,53) = 5.374$, $p = .024$, $\eta_p^2 = .092$. However, there was no main effect of drug (NS8593 groups: $M = 2572.960$, $SD = 1276.970$, $n = 30$; vehicle groups: $M = 3329.170$, $SD = 1519.700$, $n = 27$), $F(1,53) = 3.933$, $p = .053$, $\eta_p^2 = .069$, as well as no interaction, $F(1,53) = 2.855$, $p = .097$, $\eta_p^2 = .051$, although both analyses trended closely towards significance (Figure 20A). Similar results were obtained when comparing the number of quadrant entries to control for small movements. An ANOVA indicated a main effect of stimulation on quadrant entries (tDCS groups: $M = 128.584$, $SD = 85.252$, $n = 25$; sham groups: $M = 208.947$, $SD = 113.618$, $n = 32$), $F(1,53) = 8.611$, $p = .005$, $\eta_p^2 = .140$, but no main effect of drug (NS8593 groups: $M = 144.147$, $SD = 89.656$, $n = 30$; vehicle groups: $M = 193.384$, $SD = 109.213$, $n = 27$), $F(1,53) = 3.232$, $p = .078$, $\eta_p^2 = .057$, as well as no interaction, $F(1,53) = 1.206$, $p = .277$, $\eta_p^2 = .022$ (Figure 20B).

The results also suggest that tDCS, but not NS8593, increases anxiety-like behaviour in the OFT by reducing both the amount of time spent in and the number of entries into the center of the chamber. An ANOVA comparing the amount of time spent in the center of the maze found a main effect of stimulation (tDCS groups: $M = 6.786$, $SD = 10.773$, $n = 25$; sham groups: $M = 14.228$, $SD = 11.681$, $n = 32$), $F(1,53) = 5.874$, $p = .019$, $\eta_p^2 = .100$. However, there was no main effect of drug (NS8593 groups: $M = 9.938$, $SD = 12.812$, $n = 30$; vehicle groups: $M = 11.076$, $SD = 9.642$, $n = 27$), $F(1,53) = .137$, $p = .712$, $\eta_p^2 = .003$, as well as no interaction, $F(1,53) = .094$, $p = .761$, $\eta_p^2 = .002$ (Figure 21A). Similarly, an ANOVA comparing the number of entries into the

center of the maze revealed a main effect of stimulation (tDCS groups: $M = 4.686$, $SD = 5.338$, $n = 25$; sham groups: $M = 11.175$, $SD = 8.454$, $n = 32$), $F(1,53) = 11.115$, $p = .002$, $\eta_p^2 = .173$, but no main effect of drug (NS8593 groups: $M = 6.210$, $SD = 6.351$, $n = 30$; vehicle groups: $M = 9.650$, $SD = 7.441$, $n = 27$), $F(1,53) = 3.123$, $p = .083$, $\eta_p^2 = .056$, as well as no interaction, $F(1,53) = .346$, $p = .559$, $\eta_p^2 = .006$ (Figure 21B).

3.3.2.2. Novelty Suppressed Feeding Test

The results from this test suggest that tDCS, but not NS8593, increases anxiety as measured by the NSFT. An ANOVA comparing the latency to reach the food in the center of the arena revealed no main effects of stimulation (tDCS groups: $M = 73.955$, $SD = 56.441$, $n = 22$; sham groups: $M = 88.536$, $SD = 78.873$, $n = 32$), $F(1,50) = .539$, $p = .466$, $\eta_p^2 = .011$, or drug (NS8593 groups: $M = 77.190$, $SD = 53.324$, $n = 28$; vehicle groups: $M = 85.300$, $SD = 81.989$, $n = 26$), $F(1,50) = .167$, $p = .685$, $\eta_p^2 = .003$, as well as no interaction, $F(1,50) = .824$, $p = .368$, $\eta_p^2 = .016$ (Figure 22).

There was also no main effect of stimulation on the latency to begin eating (tDCS groups: $M = 278.500$, $SD = 135.293$, $n = 18$; sham groups: $M = 205.654$, $SD = 108.502$, $n = 26$), $F(1,40) = 3.743$, $p = .060$, $\eta_p^2 = .086$, however it did closely approach significance. There was also no main effect of drug (NS8593 groups: $M = 254.173$, $SD = 132.872$, $n = 23$; vehicle groups: $M = 229.981$, $SD = 110.923$, $n = 21$), $F(1,40) = .413$, $p = .524$, $\eta_p^2 = .010$, as well as no interaction, $F(1,40) = 1.750$, $p = .193$, $\eta_p^2 = .042$ (Figure 22).

Finally, an ANOVA revealed a main effect of stimulation on the latency to eat in the home cage (tDCS groups: $M = 132.857$, $SD = 59.535$, $n = 12$; sham groups: $M = 86.167$, $SD = 54.606$, $n = 18$), $F(1,26) = 4.216$, $p = .050$, $\eta_p^2 = .140$, but not for drug (NS8593 groups: $M =$

116.667, *SD* = 45.018, *n* = 14; vehicle groups: *M* = 102.357, *SD* = 69.123, *n* = 16), *F*(1,26) = .396, *p* = .535, η_p^2 = .015. There was also no interaction, *F*(1,26) = 1.983, *p* = .171, η_p^2 = .071 (Figure 22).

3.3.2.3. Forced Swim Test

Our results suggest that tDCS does not affect passivity in the FST, but NS8593 reduces swimming behaviour after acute treatment only. At the acute time point, an ANOVA indicated that climbing behaviour was not influenced by stimulation (tDCS groups: *M* = 40.897, *SD* = 23.679, *n* = 25; sham groups: *M* = 40.112, *SD* = 19.256, *n* = 22), *F*(1,43) = .015, *p* = .904, η_p^2 = .000, or drug (NS8593 groups: *M* = 42.184, *SD* = 23.698, *n* = 25; vehicle groups: *M* = 38.824, *SD* = 19.238, *n* = 22), *F*(1,43) = .271, *p* = .606, η_p^2 = .006, and there was also no interaction between the two, *F*(1,43) = .076, *p* = .784, η_p^2 = .002. There was also no main effect for stimulation on swimming behaviour (tDCS groups: *M* = 125.035, *SD* = 35.320, *n* = 25; sham groups: *M* = 120.230, *SD* = 34.68, *n* = 22), *F*(1,43) = .218, *p* = .643, η_p^2 = .005; However, a main effect of drug was observed (NS8593 groups: *M* = 110.710, *SD* = 35.062, *n* = 25; vehicle groups: *M* = 134.555, *SD* = 34.933, *n* = 22), *F*(1,43) = 5.380, *p* = .025, η_p^2 = .111, with no interaction between the two variables, *F*(1,43) = 2.762, *p* = .104, η_p^2 = .060. Despite this, an ANOVA comparing time spent immobile did not reveal any significant effects of stimulation (tDCS groups: *M* = 134.085, *SD* = 46.834, *n* = 25; sham groups: *M* = 139.684, *SD* = 38.497, *n* = 22), *F*(1,43) = .194, *p* = .662, η_p^2 = .004, or drug (NS8593 groups: *M* = 147.135, *SD* = 41.577, *n* = 25; vehicle groups: *M* = 126.634, *SD* = 43.753, *n* = 22), *F*(1,43) = 2.603, *p* = .114, η_p^2 = .057, and there was also no interaction between the two, *F*(1,43) = 2.201, *p* = .145, η_p^2 = .049 (Figure 23A).

At the chronic time point, an ANOVA revealed that there was no main effect of stimulation on time spent climbing (tDCS groups: $M = 41.892$, $SD = 23.052$, $n = 25$; sham groups: $M = 43.879$, $SD = 27.024$, $n = 22$), $F(1,43) = .072$, $p = .790$, $\eta_p^2 = .002$. There was also no main effect of drug (NS8593 groups: $M = 39.905$, $SD = 18.925$, $n = 25$; vehicle groups: $M = 45.866$, $SD = 31.151$, $n = 22$), $F(1,43) = .645$, $p = .426$, $\eta_p^2 = .015$, as well as no interaction, $F(1,43) = .056$, $p = .814$, $\eta_p^2 = .001$. Likewise, no significant comparisons were observed in swimming behaviour for stimulation (tDCS groups: $M = 132.728$, $SD = 36.604$, $n = 25$; sham groups: $M = 123.532$, $SD = 33.080$, $n = 22$), $F(1,43) = .785$, $p = .381$, $\eta_p^2 = .018$, drug (NS8593 groups: $M = 121.057$, $SD = 38.287$, $n = 25$; vehicle groups: $M = 135.203$, $SD = 31.397$, $n = 22$), $F(1,43) = 1.856$, $p = .180$, $\eta_p^2 = .041$, or their interaction, $F(1,43) = 2.101$, $p = .155$, $\eta_p^2 = .047$. Finally, ANOVA analysis of time spent immobile also revealed no main effects for stimulation (tDCS groups: $M = 125.425$, $SD = 51.821$, $n = 25$; sham groups: $M = 132.630$, $SD = 47.788$, $n = 22$), $F(1,43) = .241$, $p = .626$, $\eta_p^2 = .006$, or drug (NS8593 groups: $M = 139.079$, $SD = 48.010$, $n = 25$; vehicle groups: $M = 118.976$, $SD = 51.599$, $n = 22$), $F(1,43) = 1.880$, $p = .178$, $\eta_p^2 = .042$, as well as no interaction between the two, $F(1,43) = .818$, $p = .371$, $\eta_p^2 = .019$ (Figure 23B).

3.3.2.4. Sucrose Preference Test

Neither the application of tDCS nor treatment with NS8593 resulted in altered sucrose intake. At the acute time point, an ANOVA indicated that there were no main effects of stimulation (tDCS groups: $M = 88.503$, $SD = 13.717$, $n = 25$; sham groups: $M = 82.204$, $SD = 20.475$, $n = 32$), $F(1,53) = 1.728$, $p = .194$, $\eta_p^2 = .032$, or drug (NS8593 groups: $M = 86.793$, $SD = 14.211$, $n = 30$; vehicle groups: $M = 83.914$, $SD = 19.981$, $n = 27$), $F(1,53) = .361$, $p = .551$, $\eta_p^2 = .007$, nor was there an interaction between the two, $F(1,53) = .293$, $p = .591$, $\eta_p^2 = .005$ (Figure 24A). Likewise, at the chronic time point, there were no differences in sucrose preference

resulting from stimulation (tDCS groups: $M = 82.629$, $SD = 20.046$, $n = 25$; sham groups: $M = 82.183$, $SD = 23.215$, $n = 32$), $F(1,53) = .005$, $p = .942$, $\eta_p^2 = .000$, or drug (NS8593 groups: $M = 79.161$, $SD = 24.969$, $n = 30$; vehicle groups: $M = 85.651$, $SD = 18.292$, $n = 27$), $F(1,53) = 1.150$, $p = .288$, $\eta_p^2 = .021$, and no interaction was also observed, $F(1,53) = .467$, $p = .497$, $\eta_p^2 = .009$ (Figure 24B).

3.3.2.5. HPLC

The obtained results highlight a variety of neurochemical alterations across the four target areas in response to both EC stimulation and its constituent parts. Due to the large volume of data, only significant results will be described in this section. All results, including insignificant comparisons, are reported in Table 3.

Dopamine. Analysis of DA concentrations revealed alterations in the hippocampus following EC stimulation, but not in the PFC, CPu, or midbrain. In the hippocampus, an ANOVA indicated a significant interaction between NS8593 and tDCS on DA concentration, $F(1,39) = 9.480$, $p = .004$, $\eta_p^2 = .196$ (Figure 25). Although Tukey's follow up tests showed no significant comparisons, rats receiving EC stimulation had a lower concentration of DA ($M = 120.209$, $SD = 46.098$) than rats receiving stimulation only ($M = 191.088$, $SD = 80.605$) that closely approached significance, $t(39) = 2.526$, $p = .071$, Cohen's $d = 1.031$.

Serotonin. Analysis of 5-HT concentrations following EC stimulation revealed numerous alterations within the PFC, CPu, and hippocampus, but not in the midbrain. In the PFC, an ANOVA indicated a main effect of drug (NS8593 groups: $M = 3.823$, $SD = 4.512$, $n = 24$; VEH groups: $M = 12.863$, $SD = 14.223$, $n = 24$) on 5-HT concentration, $F(1,40) = 5.857$, $p = .020$, $\eta_p^2 = .128$, as well as a nearly-significant effect of hemisphere (Left groups: $M = 11.943$, $SD =$

13.502, n = 24; Right groups: M = 4.743, SD = 5.234, n = 24), $F(1,40) = 3.716$, $p = .061$, $\eta_p^2 = .085$ (Figure 26). The interactions between drug and hemisphere, $F(1,40) = 3.861$, $p = .056$, $\eta_p^2 = .088$, as well as stimulation and hemisphere, $F(1,40) = 3.148$, $p = .084$, $\eta_p^2 = .073$, also closely approached significance, indicating that in the absence of treatment, 5-HT levels are elevated in the left hemisphere when compared to the right.

In the CPu, an ANOVA indicated a main effect of stimulation (tDCS groups: M = 30.993, SD = 12.735, n = 24; SHAM groups: M = 17.559, SD = 7.101, n = 24) on 5-HT concentration, $F(1,40) = 19.596$, $p < .001$, $\eta_p^2 = .329$, as well as a nearly-significant effect of drug (NS8593 groups: M = 27.195, SD = 9.093, n = 24; VEH groups: M = 21.357, SD = 10.744, n = 24), $F(1,40) = 3.702$, $p = .061$, $\eta_p^2 = .085$ (Figure 26). The interaction between stimulation and drug closely approached significance, $F(1,40) = 3.817$, $p = .058$, $\eta_p^2 = .087$, indicating that rats receiving no treatment had significantly lower concentrations of 5-HT than rats receiving stimulation, drug, or a combination of the two.

Finally, in the hippocampus, an ANOVA indicated a main effect of hemisphere (Left groups: M = 8.586, SD = 8.776, n = 23; Right groups: M = 2.646, SD = 1.712, n = 24) on 5-HT concentration, $F(1,39) = 8.681$, $p = .005$, $\eta_p^2 = .182$ (Figure 26). However, there was also a significant three-way interaction between stimulation, drug, and hemisphere, $F(1,39) = 4.400$, $p = .042$, $\eta_p^2 = .101$. Tukey's follow up tests revealed no significant comparisons; however, examination of the data indicates that either treatment alone increases 5-HT concentration within the left hippocampus, but this effect is blocked with combination treatment. Large effect sizes were observed when comparing sham-treatment animals to animals receiving stimulation, $t(39)$ = -1.944, p = .531, Cohen's d = -1.177, or NS8593, $t(39)$ = -2.216, p = .365, Cohen's d = -1.342,

but not when comparing them to animals receiving combination treatment, $t(39) = -.214$, $p = 1.000$, Cohen's $d = -.130$.

Typtophan. Examination of TRP concentrations following EC stimulation revealed alterations within the hippocampus, but not within the PFC, CPu, or midbrain. An ANOVA indicated a main effect of stimulation (tDCS groups: $M = 17116.400$, $SD = 2603.390$, $n = 24$; SHAM groups: $M = 13520.500$, $SD = 2653.360$, $n = 23$) on TRP concentration in the hippocampus, $F(1,39) = 16.458$, $p < .001$, $\eta_p^2 = .297$ (Figure 27). Therefore, with the exception of stimulation within the hippocampus, cerebral TRP levels were largely unaffected by our treatments.

5-hydroxyindoleacetic acid. Analysis of 5-HIAA concentrations in response to EC stimulation revealed alterations within the PFC and hippocampus, but not in the CPu or midbrain. In the PFC, an ANOVA indicated a main effect of stimulation (tDCS groups: $M = 365.750$, $SD = 106.665$, $n = 24$; SHAM groups: $M = 263.358$, $SD = 102.889$, $n = 24$) on 5-HIAA concentration, $F(1,40) = 9.102$, $p = .004$, $\eta_p^2 = .185$ (Figure 28).

In the hippocampus, a significant interaction was observed between drug and hemisphere, $F(1,39) = 7.242$, $p = .010$, $\eta_p^2 = .157$, with Tukey's follow up tests suggesting that NS8593 administration results in elevated 5-HIAA in the left hemisphere when compared to the right hemisphere, $t(39) = 2.468$, $p = .081$, Cohen's $d = 1.007$, or in the left hemisphere of vehicle-treated rats, $t(39) = 2.517$, $p = .073$, Cohen's $d = 1.053$. There was also a significant interaction between stimulation and drug, $F(1,39) = 6.215$, $p = .017$, $\eta_p^2 = .137$ (Figure 28). Although Tukey's follow up tests revealed no significant comparisons, both stimulation and NS8593, as well as their combination, appeared to increase 5-HIAA concentration, especially within the left hippocampus.

3.3.3. Evaluation of Stimulation Site

Third, we assessed whether lateralization of stimulation within the medial prefrontal cortex resulted in differential outcomes across numerous behavioural tests.

3.3.3.1. Open Field Test

Our results suggest that stimulation of the right or center mPFC is incapable of reversing OBX-induced hyperactivity in the OFT. An ANOVA revealed a significant main effect of stimulation on distance traveled (tDCS groups: $M = 3472.280$, $SD = 917.799$, $n = 46$; sham groups: $M = 3393.030$, $SD = 1553.690$, $n = 32$), $F(3,70) = 4.886$, $p = .004$, $\eta_p^2 = .173$. However, there was no main effect of drug (NS8593 groups: $M = 3347.740$, $SD = 986.663$, $n = 41$; vehicle groups: $M = 3557.190$, $SD = 1166.880$, $n = 37$), $F(1,70) = .396$, $p = .531$, $\eta_p^2 = .006$, and no interaction between the two, $F(3,70) = 2.192$, $p = .097$, $\eta_p^2 = .086$ (Figure 29A), although the data trended towards significance. Tukey's follow up tests on stimulation showed that rats in the left condition ($M = 2509.098$, $SD = 1242.989$, $n = 25$) traveled significantly less distance than those in the right ($M = 3965.800$, $SD = 908.375$, $n = 10$), $t(70) = -2.999$, $p = .019$, Cohen's $d = -1.122$, and center ($M = 3941.930$, $SD = 602.035$, $n = 11$) conditions, $t(70) = -3.042$, $p = .017$, Cohen's $d = -1.104$, with no other comparisons reaching significance.

Similarly, an ANOVA comparing the number of quadrant entries indicated a main effect of stimulation (tDCS groups: $M = 199.367$, $SD = 64.229$, $n = 46$; sham groups: $M = 208.947$, $SD = 113.618$, $n = 32$), $F(3,70) = 5.656$, $p = .002$, $\eta_p^2 = .195$, but no main effect of drug (NS8593 groups: $M = 196.182$, $SD = 69.626$, $n = 41$; vehicle groups: $M = 207.342$, $SD = 83.526$, $n = 37$), $F(1,70) = .218$, $p = .642$, $\eta_p^2 = .003$, and no interaction between the two, $F(3,70) = 1.420$, $p = .244$, $\eta_p^2 = .057$ (Figure 29B). Tukey's follow up tests on stimulation showed that rats in the left

condition (M = 128.584, SD = 85.252, n = 25) entered significantly fewer quadrants than those in the sham (M = 208.947, SD = 113.618, n = 32), $t(70)$ = -3.224, p = .010, Cohen's d = -.862, right (M = 233.800, SD = 62.880, n = 10), $t(70)$ = -3.015, p = .018, Cohen's d = -1.128, and center conditions (M = 235.717, SD = 44.555, n = 11), $t(70)$ = -3.165, p = .012, Cohen's d = -1.149, with no other comparisons reaching significance.

The results also suggest that right and center stimulation do not elicit the increased anxiety response observed with left stimulation in the OFT. An ANOVA indicated a significant main effect of stimulation on the number of entries into the center of the chamber (tDCS groups: M = 9.106, SD = 6.104, n = 46; sham groups: M = 11.175, SD = 8.454, n = 32), $F(3,70)$ = 4.717, p = .005, η_p^2 = .168, but no main effect of drug (NS8593 groups: M = 10.072, SD = 7.266, n = 41; vehicle groups: M = 9.175, SD = 6.118, n = 37), $F(1,70)$ = .232, p = .632, η_p^2 = .003, and no interaction between the two, $F(3,70)$ = 2.132, p = .104, η_p^2 = .084 (Figure 30B). Tukey's follow up tests on stimulation showed that rats in the left condition (M = 4.686, SD = 5.338, n = 25) entered the center significantly less often than rats in the sham (M = 11.175, SD = 8.454, n = 32), $t(70)$ = -3.343, p = .007, Cohen's d = -.893, and right conditions (M = 12.400, SD = 8.600, n = 10), $t(70)$ = -2.838, p = .030, Cohen's d = -1.062, with no other comparisons reaching significance.

An additional ANOVA comparing the amount of time spent in the center of the arena found no main effect of stimulation (tDCS groups: M = 12.134, SD = 10.919, n = 46; sham groups: M = 14.228, SD = 11.681, n = 32), $F(3,70)$ = 2.465, p = .069, η_p^2 = .096, however the data trended closely towards significance. There was also no main effect of drug (NS8593 groups: M = 15.127, SD = 14.988, n = 41; vehicle groups: M = 10.188, SD = 7.231, n = 37),

$F(1,70) = 2.450$, $p = .122$, $\eta_p^2 = .034$, and no interaction observed, $F(3,70) = 1.872$, $p = .142$, $\eta_p^2 = .074$ (Figure 30A).

3.3.3.2. Novelty Suppressed Feeding Test

Our results suggest that right mPFC stimulation, but not center or left stimulation, increases anxiety-like behaviour as assessed by the NSFT, an effect that is blocked by concurrent administration of NS8593. An ANOVA indicated that stimulation did not affect the latency to approach food placed in the center of the arena (tDCS groups: $M = 134.932$, $SD = 136.976$, $n = 43$; sham groups: $M = 88.536$, $SD = 78.873$, $n = 32$), $F(3,64) = 2.342$, $p = .081$, $\eta_p^2 = .099$, but drug did (NS8593 groups: $M = 93.329$, $SD = 93.828$, $n = 41$; vehicle groups: $M = 153.338$, $SD = 151.072$, $n = 34$), $F(1,64) = 8.569$, $p = .005$, $\eta_p^2 = .118$ (Figure 31A). However, a significant interaction was observed, $F(3,64) = 3.688$, $p = .016$, $\eta_p^2 = .147$. Tukey's follow up tests revealed that rats receiving stimulation over the right mPFC in the absence of NS8593 had significantly higher latency to approach the food when compared to every other group, suggesting an anxiogenic effect of right stimulation that is blocked by SK3 antagonism.

An additional ANOVA investigating the latency to begin feeding within the chamber found no main effect of stimulation (tDCS groups: $M = 302.679$, $SD = 157.963$, $n = 43$; sham groups: $M = 278.990$, $SD = 184.708$, $n = 32$), $F(3,52) = 1.708$, $p = .177$, $\eta_p^2 = .090$, drug (NS8593 groups: $M = 286.325$, $SD = 153.974$, $n = 41$; vehicle groups: $M = 307.188$, $SD = 175.325$, $n = 34$), $F(1,52) = .145$, $p = .705$, $\eta_p^2 = .003$, and no interaction between the two, $F(3,52) = 2.378$, $p = .080$, $\eta_p^2 = .121$ (Figure 31B)

Similarly, an ANOVA comparing the latency to begin feeding in the home cage also found no main effect of stimulation (tDCS groups: $M = 140.344$, $SD = 84.253$, $n = 39$; sham

groups: $M = 166.622$, $SD = 115.270$, $n = 29$), $F(3,38) = 1.310$, $p = .285$, $\eta_p^2 = .094$, drug (NS8593 groups: $M = 170.408$, $SD = 100.561$, $n = 37$; vehicle groups: $M = 123.419$, $SD = 83.454$, $n = 31$), $F(1,38) = .979$, $p = .329$, $\eta_p^2 = .025$, and no interaction between the two, $F(3,38) = .539$, $p = .658$, $\eta_p^2 = .041$ (Figure 31C)

3.3.3.3. Sucrose Preference Test

The obtained results indicate that the site of stimulation has no effect on anhedonia as measured by the SPT. At the acute time point, an ANOVA revealed no significant main effect of stimulation (tDCS groups: $M = 86.489$, $SD = 11.701$, $n = 46$; sham groups: $M = 82.204$, $SD = 20.475$, $n = 32$), $F(3,70) = .705$, $p = .552$, $\eta_p^2 = .029$, drug (NS8593 groups: $M = 86.489$, $SD = 11.610$, $n = 41$; vehicle groups: $M = 85.407$, $SD = 16.179$, $n = 37$), $F(1,70) = .064$, $p = .802$, $\eta_p^2 = .001$, and no significant interaction, $F(3,70) = .456$, $p = .714$, $\eta_p^2 = .019$ (Figure 32A). Similarly, at the chronic time point, an ANOVA also revealed no significant main effect of stimulation (tDCS groups: $M = 82.822$, $SD = 16.495$, $n = 46$; sham groups: $M = 82.183$, $SD = 23.215$, $n = 32$), $F(3,70) = 1.116$, $p = .348$, $\eta_p^2 = .046$, drug (NS8593 groups: $M = 82.255$, $SD = 16.487$, $n = 41$; vehicle groups: $M = 83.071$, $SD = 19.863$, $n = 37$), $F(1,70) = .022$, $p = .884$, $\eta_p^2 = .000$, and no significant interaction between the two, $F(3,70) = .543$, $p = .654$, $\eta_p^2 = .023$ (Figure 32B).

3.3.4. Evaluation of Sex

Finally, we examined the effects of the OBX procedure and EC stimulation in female rats across a number of behavioural tests.

3.3.4.1. Validation of Female OBX

First, we examined whether OBX continues to be a valid model of depression in female rats by comparing OBX and sham animals that had received no treatments across the same behavioural tests outlined in 3.3.1.

3.3.4.1.1. Open Field Test

Our results suggest that OBX did not produce the characteristic hyperlocomotion associated with the procedure and did not alter anxiety-like states as assessed by the time spent in the center of the arena. An independent samples t-test revealed that the distance traveled by OBX animals (M = 3178.443, SD = 1855.644, n = 7) was not significantly greater than the distance traveled by sham animals (M = 3105.943, SD = 1064.898, n = 7), $t(12) = 0.090$, $p = .930$, Cohen's $d = .048$ (Figure 33A). They also did not enter more quadrants in the maze (OBX: M = 173.286, SD = 140.315, n = 7; Sham: M = 156.286, SD = 69.267, n = 7), $t(12) = .287$, $p = .779$, Cohen's $d = .154$ (Figure 33B).

Furthermore, the amount of time spent in the center of the chamber was not significantly different between the two groups (OBX: M = 13.543, SD = 25.280, n = 7; Sham: M = 5.243, SD = 8.177, n = 7), $t(12) = .827$, $p = .425$, Cohen's $d = .442$ (Figure 33C), nor was the number of entries into the center (OBX: M = 6.429, SD = 9.589, n = 7; Sham: M = 3.571, SD = 6.079, n = 7), $t(12) = .666$, $p = .518$, Cohen's $d = .356$ (Figure 33D).

3.3.4.1.2. Novelty Suppressed Feeding Test

The results from this test suggest that OBX did not affect anxiety-like behaviours in female rats as assessed by the NSFT. An independent samples t-test revealed that the latency to reach the food pellets in the center of the arena was not significantly different between the two

groups (OBX: M = 101.200, SD = 68.653, n = 5; Sham: M = 90.857, SD = 62.464, n = 7), $t(10)$ = .272, p = .791, Cohen's d = .159, nor was the latency to begin eating in the arena (OBX: M = 227.000, SD = 192.333, n = 2; Sham: M = 376.500, SD = 23.335, n = 2), $t(1.029)$ = -1.091, p = .468, Cohen's d = -1.091, although most rats failed to eat within the 10 minute time limit. There was also no difference in feeding latency in their home cage (OBX: M = 86.167, SD = 22.833, n = 6; Sham: M = 91.200, SD = 35.365, n = 5), $t(9)$ = -.286, p = .782, Cohen's d = -.173 (Figure 34).

3.3.4.1.3. Forced Swim Test

Our results suggest that no differences in passivity, as measured by the FST, resulted from the OBX procedure in female rats. At the acute time point, an independent samples t-test revealed that OBX animals (M = 121.514, SD = 64.421, n = 7) did not spend more time immobile than sham animals (M = 175.771, SD = 63.427, n = 7), $t(12)$ = -1.588, p = .138, Cohen's d = -.849. There was also no difference between groups in swimming behaviour (OBX: M = 169.829, SD = 61.473, n = 7; Sham: M = 114.829, SD = 61.821, n = 7), $t(12)$ = 1.669, p = .121, Cohen's d = .829, or climbing behaviour (OBX: M = 8.671, SD = 4.779, n = 7; Sham: M = 9.400, SD = 4.860, n = 7), $t(12)$ = -.283, p = .782, Cohen's d = -.151 (Figure 35A).

Similar results were obtained at the chronic time point on Week 2. An independent samples t-test revealed that OBX animals (M = 132.614, SD = 71.129, n = 7) did not exhibit more immobility than sham animals (M = 193.271, SD = 51.963, n = 7), $t(12)$ = -1.822, p = .094, Cohen's d = -.974. There were also no differences between groups in swimming behaviour (OBX: M = 156.143, SD = 66.320, n = 7; Sham: M = 103.114, SD = 51.905, n = 7), $t(12)$ = 1.666, p = .122, Cohen's d = .890, or climbing behaviour (OBX: M = 8.371, SD = 8.101, n = 7; Sham: M = 3.629, SD = 2.369, n = 7), $t(12)$ = 1.487, p = .163, Cohen's d = .795 (Figure 35B).

3.3.4.1.4. Sucrose Preference Test

The obtained results suggest that the OBX procedure did not affect anhedonia in female rats as measured by the SPT. At the acute time point, an independent samples t-test revealed that the amount of sucrose consumed by OBX animals (M = 96.671, SD = 3.432, n = 7) did not differ from the amount consumed by sham animals (M = 89.300, SD = 8.551, n = 7), although the data approached significance, $t(7.884) = 2.117$, $p = .068$, Cohen's $d = 1.131$ (Figure 36A).

Similarly, at the chronic time point, an independent samples t-test revealed that OBX animals (M = 93.940, SD = 3.437, n = 5) did not consume a different amount of sucrose compared to sham animals (M = 89.750, SD = 4.187, n = 4), $t(7) = 1.654$, $p = .142$, Cohen's d = 1.109 (Figure 36B).

3.3.4.2. Evaluation of EC Stimulation

Although the OBX procedure failed to produce a depressive-like phenotype in female rats, we still investigated the effects of EC stimulation on depression- and anxiety-like responses in this population.

3.3.4.2.1. Open Field Test

Our results suggest that neither tDCS nor NS8593 alters the locomotor activity of female rats in the OFT. An ANOVA indicated no main effect of stimulation on distance traveled (tDCS groups: M = 4386.480, SD = 1178.672, n = 14; sham groups: M = 3417.179, SD = 1783.897, n = 14), $F(1,24) = 2.871$, $p = .103$, $\eta_p^2 = .107$, no main effect of drug (NS8593 groups: M = 4146.557, SD = 1480.446, n = 14; vehicle groups: M = 3657.100, SD = 1482.123, n = 14), $F(1,24) = .732$, $p = .401$, $\eta_p^2 = .030$, as well as no interaction, $F(1,24) = .000$, $p = .984$, $\eta_p^2 = .000$ (Figure 37A). Similar results were obtained when comparing the number of quadrant entries to

control for small movements. An ANOVA indicated no main effect of stimulation on quadrant entries (tDCS groups: $M = 242.714$, $SD = 74.618$, $n = 14$; sham groups: $M = 189.929$, $SD = 131.904$, $n = 14$), $F(1,24) = 1.693$, $p = .206$, $\eta_p^2 = .066$, no main effect of drug (NS8593 groups: $M = 230.643$, $SD = 99.747$, $n = 14$; vehicle groups: $M = 202.000$, $SD = 106.775$, $n = 14$), $F(1,24) = .499$, $p = .487$, $\eta_p^2 = .020$, as well as no interaction, $F(1,24) = .013$, $p = .910$, $\eta_p^2 = .001$ (Figure 37B).

The results also suggest that neither tDCS nor NS8593 affect anxiety-like behaviours in female rats in the OFT. An ANOVA comparing the amount of time spent in the center of the maze found no main effect of stimulation (tDCS groups: $M = 7.793$, $SD = 3.223$, $n = 14$; sham groups: $M = 15.950$, $SD = 21.277$, $n = 14$), $F(1,24) = 1.944$, $p = .176$, $\eta_p^2 = .075$, no main effect of drug (NS8593 groups: $M = 12.522$, $SD = 10.096$, $n = 14$; vehicle groups: $M = 11.222$, $SD = 14.404$, $n = 14$), $F(1,24) = .049$, $p = .826$, $\eta_p^2 = .002$, as well as no interaction, $F(1,24) = .361$, $p = .554$, $\eta_p^2 = .015$ (Figure 38A). Similarly, an ANOVA comparing the number of entries into the center of the maze revealed no main effect of stimulation (tDCS groups: $M = 8.215$, $SD = 4.251$, $n = 14$; sham groups: $M = 10.143$, $SD = 10.113$, $n = 14$), $F(1,24) = .431$, $p = .518$, $\eta_p^2 = .018$, no main effect of drug (NS8593 groups: $M = 11.000$, $SD = 7.618$, $n = 14$; vehicle groups: $M = 7.358$, $SD = 6.747$, $n = 14$), $F(1,24) = 1.539$, $p = .227$, $\eta_p^2 = .060$, as well as no interaction, $F(1,24) = 1.662$, $p = .210$, $\eta_p^2 = .065$ (Figure 38B).

3.3.4.2.2. Novelty Suppressed Feeding Test

The results from this test suggest that neither tDCS nor NS8593 affect anxiety in female rats as measured by the NSFT. An ANOVA comparing the latency to reach the food in the center of the arena revealed no main effects of stimulation (tDCS groups: $M = 140.750$, $SD = 82.837$, n

= 12; sham groups: M = 91.000, SD = 53.557, n = 10), $F(1,18) = 2.346$, $p = .143$, $\eta_p^2 = .115$, or drug (NS8593 groups: M = 122.150, SD = 76.621, n = 11; vehicle groups: M = 109.600, SD = 59.773, n = 11), $F(1,18) = .149$, $p = .704$, $\eta_p^2 = .008$, as well as no interaction, $F(1,18) = 1.029$, $p = .324$, $\eta_p^2 = .054$ (Figure 39A).

There was also no main effect of stimulation on the latency to begin eating (tDCS groups: M = 393.375, SD = 135.730, n = 8; sham groups: M = 248.167, SD = 184.129, n = 5), $F(1,9) = 2.552$, $p = .145$, $\eta_p^2 = .221$, as well as no main effect of drug (NS8593 groups: M = 357.042, SD = 132.010, n = 7; vehicle groups: M = 284.500, SD = 187.848, n = 6), $F(1,9) = .637$, $p = .445$, $\eta_p^2 = .066$, as well as no interaction, $F(1,9) = .110$, $p = .747$, $\eta_p^2 = .012$ (Figure 39B).

Finally, an ANOVA revealed no main effect of stimulation on the latency to eat in the home cage (tDCS groups: M = 100.000, SD = 45.317, n = 9; sham groups: M = 82.000, SD = 35.395, n = 12), $F(1,17) = .876$, $p = .362$, $\eta_p^2 = .049$, no main effect of drug (NS8593 groups: M = 94.917, SD = 49.351, n = 12; vehicle groups: M = 87.084, SD = 31.360, n = 9), $F(1,17) = .166$, $p = .689$, $\eta_p^2 = .010$, and no interaction, $F(1,17) = .707$, $p = .412$, $\eta_p^2 = .040$ (Figure 39C).

3.3.4.2.3. Forced Swim Test

Our results suggest that neither treatment affects passivity in female rats as assessed by the FST. At the acute time point, an ANOVA indicated that climbing behaviour was not influenced by stimulation (tDCS groups: M = 15.150, SD = 10.563, n = 14; sham groups: M = 9.621, SD = 8.045, n = 14), $F(1,24) = 2.175$, $p = .153$, $\eta_p^2 = .083$, or drug (NS8593 groups: M = 13.971, SD = 12.499, n = 14; vehicle groups: M = 10.800, SD = 6.110, n = 14), $F(1,24) = .716$, $p = .406$, $\eta_p^2 = .029$, and there was also no interaction between the two, $F(1,24) = .115$, $p = .737$, $\eta_p^2 = .005$ (Figure 40A). There was also no main effect of stimulation on swimming behaviour

(tDCS groups: $M = 166.393$, $SD = 46.839$, $n = 14$; sham groups: $M = 180.808$, $SD = 50.562$, $n = 14$), $F(1,24) = .595$, $p = .448$, $\eta_p^2 = .024$, no main effect of drug (NS8593 groups: $M = 171.915$, $SD = 40.953$, $n = 14$; vehicle groups: $M = 175.286$, $SD = 56.449$, $n = 14$), $F(1,24) = .033$, $p = .858$, $\eta_p^2 = .001$, and no interaction between the two, $F(1,24) = 1.837$, $p = .188$, $\eta_p^2 = .071$ (Figure 40B). Finally, an ANOVA comparing time spent immobile did not reveal any significant effects of stimulation (tDCS groups: $M = 118.465$, $SD = 51.329$, $n = 14$; sham groups: $M = 109.464$, $SD = 52.435$, $n = 14$), $F(1,24) = .205$, $p = .655$, $\eta_p^2 = .008$, or drug (NS8593 groups: $M = 114.000$, $SD = 43.941$, $n = 14$; vehicle groups: $M = 113.929$, $SD = 59.824$, $n = 14$), $F(1,24) = 1.476$, $p = .236$, $\eta_p^2 = .058$, and there was also no interaction between the two, $F(1,24) = 1.476$, $p = .236$, $\eta_p^2 = .058$ (Figure 40C).

Similar results were obtained at the chronic timepoint. An ANOVA revealed that there was no main effect of stimulation on time spent climbing (tDCS groups: $M = 15.365$, $SD = 11.658$, $n = 14$; sham groups: $M = 12.364$, $SD = 14.447$, $n = 14$), $F(1,24) = .326$, $p = .573$, $\eta_p^2 = .013$. There was also no main effect of drug (NS8593 groups: $M = 16.422$, $SD = 16.896$, $n = 14$; vehicle groups: $M = 11.307$, $SD = 9.209$, $n = 14$), $F(1,24) = .947$, $p = .340$, $\eta_p^2 = .038$, as well as no interaction, $F(1,24) = .299$, $p = .590$, $\eta_p^2 = .012$ (Figure 41A). Likewise, no significant comparisons were observed in swimming behaviour for stimulation (tDCS groups: $M = 190.186$, $SD = 44.859$, $n = 14$; sham groups: $M = 173.365$, $SD = 71.015$, $n = 14$), $F(1,24) = .556$, $p = .463$, $\eta_p^2 = .023$, drug (NS8593 groups: $M = 183.029$, $SD = 63.615$, $n = 14$; vehicle groups: $M = 180.522$, $SD = 52.260$, $n = 14$), $F(1,24) = .012$, $p = .912$, $\eta_p^2 = .001$, or their interaction, $F(1,24) = 2.005$, $p = .170$, $\eta_p^2 = .077$ (Figure 41B). Finally, ANOVA analysis of time spent immobile also revealed no main effects for stimulation (tDCS groups: $M = 94.465$, $SD = 43.455$, $n = 14$; sham groups: $M = 112.843$, $SD = 74.967$, $n = 14$), $F(1,24) = .628$, $p = .436$, $\eta_p^2 = .026$, or drug

(NS8593 groups: $M = 100.579$, $SD = 62.265$, $n = 14$; vehicle groups: $M = 106.729$, $SD = 56.157$, $n = 14$), $F(1,24) = .070$, $p = .793$, $\eta_p^2 = .003$, as well as no interaction between the two, $F(1,24) = 2.074$, $p = .163$, $\eta_p^2 = .080$ (Figure 41C).

3.3.4.2.4. Sucrose Preference Test

Neither the application of tDCS nor treatment with NS8593 resulted in altered sucrose intake in female rats. At the acute time point, an ANOVA indicated that there were no main effects of stimulation (tDCS groups: $M = 95.965$, $SD = 5.986$, $n = 14$; sham groups: $M = 95.214$, $SD = 3.848$, $n = 14$), $F(1,24) = .125$, $p = .727$, $\eta_p^2 = .005$, or drug (NS8593 groups: $M = 94.122$, $SD = 6.872$, $n = 14$; vehicle groups: $M = 97.057$, $SD = 2.962$, $n = 14$), $F(1,24) = 1.915$, $p = .179$, $\eta_p^2 = .074$, nor was there an interaction between the two, $F(1,24) = .000$, $p = .992$, $\eta_p^2 = .000$ (Figure 42A). Likewise, at the chronic time point, there were no differences in sucrose preference resulting from stimulation (tDCS groups: $M = 96.198$, $SD = 2.974$, $n = 9$; sham groups: $M = 92.680$, $SD = 4.942$, $n = 10$), $F(1,15) = 3.034$, $p = .102$, $\eta_p^2 = .168$, or drug (NS8593 groups: $M = 93.370$, $SD = 5.199$, $n = 10$; vehicle groups: $M = 95.508$, $SD = 2.717$, $n = 9$), $F(1,15) = 1.120$, $p = .307$, $\eta_p^2 = .070$, and no interaction was also observed, $F(1,15) = .036$, $p = .852$, $\eta_p^2 = .002$ (Figure 42B).

3.4. Discussion

Taken together, the obtained results support the use of EC stimulation as a promising new therapy for alleviating depressive symptoms. In the first sub-experiment, we verified our OBX procedure as a valid instigator of rodent depression. While the data obtained from our novelty-suppressed feeding and forced swim tests were inconclusive, we uncovered a significant hyperlocomotive effect in response to the procedure, which is the main hallmark of OBX (Klein

& Brown, 1969). This hyperlocomotion may have potentially masked the behavioural despair that is often observed in the FST following depression-inducing paradigms by manifesting as increased swimming behaviour within the testing chamber. This idea is supported by research conducted by Vieyra-Reyes et al. (2008), who found that OBX increased locomotor activity in the OFT, while time spent immobile in the FST was unaffected. We also failed to observe a significant decrease in sucrose preference; however, there was a clear trend towards an OBX-induced decrease at both the acute and chronic timepoints. With anhedonia being a commonly observed effect following OBX (Jiménez-Sánchez et al., 2016; Padilla et al., 2018), this result was likely influenced by methodological issues. For example, rats were not acclimatized to the sucrose solution before testing, which may have induced neophobia in some subjects. Many animals exhibit hesitancy to consume newly introduced foods as a defense against the ingestion of toxic elements, which may have reduced the amount of sucrose consumed in more cautious rats (Corey, 1978).

In addition to the hyperlocomotive behavioural effect, we found moderate alterations to neurochemical balance within frontal regions of the brain in response to OBX. A reduction in 5-HT was observed within the right CPu, which may underscore the increased motor activity observed following OBX as reductions of 5-HT within the striatum have been associated with hyperlocomotion (Spanos & Yamamoto, 1989). An increase in DA within the PFC was also observed, which may signal enhanced hedonic response and underlie the commonly reported comorbidity between addiction and emotional depressive symptoms. The PFC plays a key role in addiction circuitry as part of the mesocorticolimbic pathway, where rises in DA within the PFC increase addiction-related behaviours through its glutamatergic projections to the nucleus accumbens (Yap & Miczek, 2008). Similar alterations within the PFC, as well as other frontal

and limbic structures, are also observed in both MDD and substance use disorders (Dunlop et al., 2017b). Taken together, these behavioural and neurochemical outcomes support our use of the OBX model to evaluate EC stimulation in the following sub-experiments.

Both the use of tDCS and the administration of NS8593 were successful in reversing OBX-induced hyperlocomotion, with the combination treatment appearing to have marginally increased effectiveness. However, only tDCS was effective when comparing the number of quadrant entries, which controls for smaller movements by only counting the number of times a rat travels between quadrants within the maze. While these results seem to suggest that EC stimulation's combination approach provides no additional benefit, we also observed that tDCS increases anxiety-like behaviours in the OFT and NSFT, an effect that was blocked with concurrent NS8593 treatment. This anxiogenic effect of stimulation has been observed across numerous other studies in both rodent and human populations (Clarke et al., 2020; Garcia et al., 2020; Waye, 2019). Therefore, EC stimulation posits itself as a procedure that is more efficacious than drug therapy while also encompassing fewer side effects than stimulation treatment. Previous work in our lab uncovered similar results when combining tDCS with the SSRI paroxetine in adolescent rats (Waye et al., 2021). This outcome may be the result of stimulation-induced activation of the prefrontal-amygdala pathway. tDCS, with its non-discriminative field of activation, may activate excitatory glutamatergic pyramidal neurons within the PFC that synapse onto GABAergic interneurons within the amygdala, which in turn inhibit local endocannabinoid CB1R signaling to increase anxiety-like behaviours (Katona et al., 2001; Lutz et al., 2015). This effect may then be attenuated by NS8593 administration, by either activating the opposing GABAergic pathways from the PFC or by binding to the SK3 channels present within the amygdala to antagonize these interneurons directly (Sailer et al., 2004).

The relative advantages of using EC stimulation rather than tDCS or NS8593 alone may be explained by the treatment-specific effects we observed in cerebral 5-HT and DA concentrations. All three treatments led to a reduction in 5-HT within the left mPFC, which was also associated with an increase in 5-HIAA. This suggests a significant increase in 5-HT turnover, as a larger ratio of 5-HIAA to 5-HT indicates that more 5-HT is being metabolized within the area of interest. This increased metabolic activity is associated with remission of depressive symptoms and is also observed following administration of other antidepressant therapies (Mann, 1999; Oh et al., 2018; Wang et al., 2009). However, within the hippocampus, this increased turnover effect was observed following combination treatment only. Stimulation and drug administration increased 5-HT concentration, while all three forms of treatment were associated with an increase in TRP, the precursor for 5-HT. This suggests that while each technique increased the amount of extracellular 5-HT, only combination treatment resulted in increased metabolism of 5-HT within the hippocampus, an effect that is associated with remission of depressive symptoms (Campbell & MacQueen, 2004; Hershey et al., 2021). Furthermore, a similar pattern of effect was observed in regional DA concentrations. The use of tDCS or NS8593 increased the availability of DA within the hippocampus, an effect that was blocked through the use of EC stimulation. An increase in dopaminergic activity within the hippocampus could produce undesirable effects in patients with MDD, as elevated hippocampal DA has been shown to increase depression and addiction-related behaviours (Tang & Dani, 2009). Taken together, these results suggest that EC stimulation may provide safer, more efficacious treatment for MDD.

To confirm the ideal location of electrode placement for EC stimulation, sub-experiment three was designed to examine the effects of stimulation lateralization within the mPFC. We

found that stimulation applied above the center of the mPFC was ineffective at reversing OBX-induced behavioural impairments, while stimulation of the right mPFC appeared to exacerbate depressive-like symptoms. Many studies report that depression is associated with hypoactivity within the left and hyperactivity within the right of frontal brain regions (Bench et al., 1992; Rotenberg, 2004). As such, excitatory anodal stimulation of the left results in remission of depressive symptoms, while inhibitory cathodal stimulation of the right achieves similar results (Bench et al., 1992; Rotenberg, 2004; Wachter et al., 2011). Therefore, central stimulation of the mPFC likely caused competing responses between the left and right hemispheres, normalizing the aberrant activity within the left while exacerbating it within the right, which neutralized the effectiveness of the technique. Similarly, sole stimulation of the right mPFC increased the hyperactivity within this region, leading to the worsening of depressive-like symptoms observed in the OFT and NSFT. Thus, the findings of the present study suggest that stimulating the left, but not the right, mPFC is effective in reversing depressive-like phenotypes.

Our results suggest that the OBX procedure did not elicit a depressive-like phenotype in female rats. Although this finding was unexpected, other studies have reported differential outcomes between sexes following OBX. Ruda-Kucerova et al. (2018) showed that male and female Listar-hooded rats exhibit different responses to natural rewards depending on the amount of effort required to obtain them, likely due to sex-dependent changes in dopaminergic signaling following OBX. Furthermore, an experiment by Stepanichev et al. (2016) found that female Wistar rats did not exhibit differences in sucrose preference, exploratory behaviour in the OFT, or behavioural despair in the FST following OBX, as well as no differences in cholinergic transmission or neuron density, whereas all of these outcomes have been observed in males (Hozumi et al., 2003; Islam et al., 2014; Morales-Medina et al., 2013; Padilla et al., 2018; Pudell

et al., 2014). It has also been reported that female rats have greater frontal and limbic levels of 5-HT than males, which could mask the behavioural effects of OBX in this population (Roca et al., 1999). Therefore, the apparent ineffectiveness of OBX in female rats is likely the result of their resilience to OBX-induced changes in neurotransmitter systems, including serotonergic, dopaminergic, cholinergic, and noradrenergic signaling pathways. This resilience may be due to the protective effect of the sex hormone estradiol, which has been found to prevent neuronal cell death, suppress pro-inflammatory mechanisms, and enhance neurotrophic support of neurons (Brown et al., 2009). With a defunct depression model, neither tDCS, NS8593, nor their combination elicited changes in our behavioural measures, which mirrors previous null results obtained in our lab using a combination stimulation-drug treatment in healthy male rats (Waye, 2019). However, this in itself is a useful finding, as it suggests that these treatments will not worsen symptoms in patients who were misdiagnosed with depression.

Chapter 4: General Discussion

4.1. Summary of Findings and Implications

Overall, this study showed for the first time that the combination of electrical and chemical (EC) stimulation results in rapid antidepressant action in a rodent model of depression, providing a promising first step towards the validation of EC stimulation as a new treatment for depression. In Experiment 1, we identified subthreshold doses for both tDCS and NS8593 that resulted in no discernable behavioural or neurochemical changes in rats. For tDCS, a current intensity of 0.05mA produced no behavioural alterations in the OFT, NSFT, and FST, and also had no effect on glutamate release within the dorsal raphe during biosensor recording. Likewise, an injection of 1.0mg/kg NS8593 produced no detectable changes in behaviour in the OFT, NSFT, and SPT. Furthermore, this dosage failed to increase expression of the cell activity marker *zif268* within SK3-containing interneurons in the PFC. An important aspect of medical intervention is identifying the minimum effective dose (MED) of a treatment, which is the lowest dose that, on average, produces a therapeutic effect in most patients (Filloon, 1995). In this study, we went a step further by identifying doses for both tDCS and NS8593 that were below this MED, with the hypothesis that the combination of these separate subthreshold treatments would produce a synergistic effect that achieves desired treatment outcomes while further minimizing potential side effects. This is of particular clinical importance, as non-invasive stimulation methods are an emerging discipline whose long term effects have yet to be elucidated, and SK channel antagonists have yet to be administered to humans so their safety profile is currently unknown.

To test our hypothesis, Experiment 2 evaluated the ability of EC stimulation to reverse the behavioural and neurochemical impairments induced by the OBX model of rodent

depression. We found that both EC stimulation and singular treatment with either tDCS or NS8593 reversed depressive-like symptoms in the OFT; however, EC stimulation also blocked the manifestation of anxiety-like behaviours in the OFT and NSFT. These findings support our hypothesis by indicating that EC stimulation is an effective intervention for depression that also protects against unwanted side effects. To further examine this effect, we measured changes in neurotransmitter concentrations across the brain in response to treatment. Most notably, we discovered that while tDCS, NS8593 and EC stimulation all increased 5-HT metabolism in the PFC, only EC stimulation increased 5-HT metabolism in the hippocampus. Elevated serotonergic turnover in frontal regions of the brain is associated with remission of depressive symptoms (Bregman et al. 2018; Ressler & Nemeroff, 2000), providing a neurophysiological explanation for the observed antidepressant properties of both EC stimulation and its constituent parts. However, the unique effects of EC stimulation on 5-HT activity in the hippocampus may provide an explanation for its anxiety-blocking effect. The hippocampus contains extensive reciprocal connections with the amygdala and the two regions exhibit strong positive resting state connectivity with each other, linking them both structurally and functionally (Petrovich et al., 2001; Roy et al., 2008). Increased serotonergic metabolism in the hippocampus inhibits local pyramidal cells to reduce the activity of the region, which in turn inhibits the activity of the amygdala, leading to a lessened anxiety response (Bocchio et al., 2016; Dale et al., 2015).

As part of Experiment 2, we also investigated whether the lateralization of the tDCS component of EC stimulation affects treatment efficacy. In line with our hypothesis, we confirmed that EC stimulation's antidepressant effects are left hemisphere-dependent, as bilateral stimulation of the mPFC failed to reverse OBX-induced hyperlocomotion in the OFT, while stimulation of the right mPFC exacerbated depressive-like symptoms by increasing locomotor

activity and latency to feed in the NSFT. Previous research has found that excitatory anodal stimulation of the left PFC, but inhibitory cathodal stimulation of the right, reverses depressive-like symptoms in both humans and rodents (van Dam & Chrysikou, 2021; Wachter et al., 2011). Our results suggest that these hemisphere-specific effects of tDCS continue to manifest when the technique is applied in tandem with the priming chemical agent NS8593, confirming that stimulation of the left hemisphere should be used in any further preclinical or clinical applications of the EC stimulation technique.

Finally, we aimed to examine the antidepressant-like effects of EC stimulation in females, as treatment efficacy for both stimulation and drug interventions have been shown to vary depending on sex (Hanlon & McCalley, 2022; Kim et al., 2018; McCallum et al., 2018; Ruiz et al., 2018). However, the OBX model failed to produce a depressive-like phenotype in this population, as females that underwent the procedure showed no differences in behaviour when compared with sham animals. Unsurprisingly, we also found no differences across any behavioural test in response to EC stimulation. This does not, however, suggest that the technique is ineffective in female rats. With an unsuccessful depression model, our results are generated from a healthy, non-depressive like population. Many studies show that both stimulation and drug therapies are effective at reversing depressive symptoms in females (Hanlon & McCalley, 2022; Sramek et al., 2016), suggesting that further study is warranted using a different depression paradigm, which will be discussed further in the following section.

4.2. Limitations and Future Directions

While this study provides an initial foothold for the use of EC stimulation to treat depression, it is not without limitations. First, and most importantly, rodent studies do not directly translate to humans. They provide a necessary initial proof of concept that inform future

translational studies; however, more preclinical research should be conducted before applying results in a clinical setting.

Assessing the methodology directly, our SK3 antagonist NS8593 is structurally similar to the SK1-3 antagonist apamin, which has been shown to elicit its antidepressant-like effects by modulating the activity of monoaminergic neurons, which all express the SK3 subtype in differing concentrations (Galeotti et al., 1999; Strøbæk et al., 2006). By utilizing a systemic injection rather than a direct intracerebral injection into the target region, NS8593 may have exerted effects on dopaminergic or noradrenergic neurons throughout the brain, leading to competing neural responses that could have masked the effects of treatment. SK3 channels are also present on glial cells, which aid neural functioning through neurotransmitter reuptake and maintenance of homeostasis, potentially counteracting the intended excitatory effects of NS8593 (Armstrong et al., 2005). Furthermore, this systemic injection may have altered SK3 activity in the periphery, as expression of these channels has been reported in peripheral tissues such as the heart, kidneys, and skeletal muscle (Rimini et al., 2000). Future experiments should use a direct intracerebral injection of NS8593 into the medial prefrontal cortex, as well as the inclusion of electrophysiological and histological verification of its SK3-specific action in this region. Additionally, some of our analyses had low sample sizes due to mechanical issues and the corruption of video files, which may have reduced the power of our study to uncover potential effects of treatment. Therefore, future experiments should increase sample sizes to more clearly examine the antidepressant properties of EC stimulation.

In Experiment 1, we identified subthreshold doses for both tDCS and NS8593 using healthy animals. However, it is possible that these doses, while inert in unoperated animals, may exert antidepressant effects when administered to subjects that have experienced OBX surgery.

This may provide an explanation as to why both tDCS and NS8593 alone were successful in reversing some of the behavioural and neurochemical changes associated with the OBX depression model. An alternative explanation is that the selection of these doses did not undergo sufficient physiological validation. We utilized just one measure each to examine both tDCS and NS8593, however these doses may have yielded neurophysiological alterations that were not detected by RNAscope or biosensor analysis. Future studies should further investigate these subthreshold doses by including additional measures of neural activity, such as calcium imaging, electrophysiology, and EEG recordings. Furthermore, the outcomes of neurostimulation treatment are also highly sensitive to minute changes in stimulation protocol (Arul-Anandam & Loo, 2009; Kuo et al., 2016). While we directly examined the effects of multiple current intensities to determine the optimal dose of tDCS, other varying factors that could impact the therapeutic efficacy of the technique were left unexplored, such as the duration of stimulation and the size of the electrodes. A study by Pavlova et al. (2018) found that a stimulation duration of 30 minutes resulted in a significantly greater improvement of depressive symptoms than 20 minutes, although both were effective when compared to sham. We also only examined EC stimulation as an acute treatment for depression; however, other methods of neurostimulation often employ repetitive treatment batteries as part of their clinical protocols to strengthen and prolong the effects of treatment. Thus, it is important for future studies to determine more specific parameters of stimulation to maximize treatment efficiency.

Finally, we were unable to assess the efficacy of EC stimulation as a treatment for depression in females, as the OBX model proved to be ineffective in this population. Therefore, the positive results we obtained are applicable only to male subjects. This presents an issue in translatability, as women are nearly twice as likely than men to be diagnosed with depression

(Albert, 2005). Future studies will need to examine EC stimulation in female rodents that have undergone a valid model of depression, such as the chronic unpredictable mild stress paradigm (Deussing, 2006; Willner, 2005). Alternatively, the OBX procedure may be modified to create a more representative model of depression. A study by Stock et al. (2000) found that female rats were more receptive to OBX-induced behavioural impairments in a gonadectomized model, suggesting that their gonadal hormones may provide a protective effect against the changes normally induced by the OBX procedure. Ideally, the effectiveness of EC stimulation should be assessed using multiple depression models to fully encapsulate its effects and increase translatability.

4.3. Conclusion

The individual and societal burden imposed by depression has resulted in decades of dedicated research. Preclinical models of depression have provided an invaluable resource by allowing examination of potential treatments, which then are tested in clinical settings for feasibility and effectiveness. The synergistic contributions of these fields have allowed researchers to examine the underlying mechanisms of depression and create a variety of strategies for combating symptoms, culminating with the investigation of non-invasive neurostimulation techniques. As a rapidly growing field, research is quickly amassing to suggest that these stimulation techniques, in combination with drug therapy, have the potential to become first line treatment options for people suffering from depression. The primary focus of this study was to assess EC stimulation as a novel therapeutic approach to depression. Although the initial results are promising, future studies must determine the precise parameters and conditions that maximize the effectiveness of this multifaceted approach.

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Table 1

Summary of Neurostimulation Studies in Clinical and Preclinical Models

Author (year)	Subjects	Stimulation Parameters	Target	Results
Mayberg et al. (2005)	6 patients with TRD (3 male)	DBS: 4.0 Volts, 60 μs pulsewidth, 130 Hz, 6 months	sgACC	CBF decreased in sgACC, OFC, and hypothalamus, increased in dlPFC. Decreased HDRS scores was maintained in four subjects
Kennedy et al. (2011)	20 patients with TRD (9 male)	DBS: 4.3 Volts, 70.6 μs pulsewidth, 124.7 Hz, 3 years	sgACC	Response rates after 1, 2, and 3 years were 63%, 46%, and 75% respectively. More than 1/3 of patients achieved remission by year 3
Hamani et al. (2012)	45 male Wistar rats (CUMS)	DBS: 200 μA, 130 Hz, 90 μs pulsewidth, 8 hours/day for 2 weeks	vmPFC	Reversed stress-induced decrease in sucrose preference and partially increased BDNF in hippocampus
Hamani et al. (2010)	30 male Sprague-Dawley rats	DBS: 100 μA, 130 Hz, 90 μs pulsewidth, 2 days (4h day 1; 2h day 2)	vmPFC	45% decrease in immobility in FST, abolished by 5-HT depleting lesions. 1 hour of DBS induced a fourfold increase in 5-HT release in hippocampus
Bregman et al. (2018)	18 male mice (SERT KO)	DBS: 100 μA, 130 Hz, 90 μs pulsewidth, one 4h session	vmPFC	Significant reductions in immobility in FST in both KO and WT mice, as well as a 33-55% increase in 5-HT levels in hippocampus
Jiménez-Sánchez et al. (2016)	81 male Wistar rats (OBX)	DBS: 200 μA, 130 Hz, 90 μs pulsewidth, one 1h session	IL	Stimulation reversed OBX-induced abnormalities in locomotion, emotionality, social interaction, and sucrose preference. Increased release of 5-HT and glutamate in mPFC

Table 1 (continued).

Pascual-Leone et al. (1996)	17 patients with TRD (6 male)	rTMS: 20 trains of 10 s duration separated by 1 min pauses, 10 Hz, intensity of 90% of the patient's MTI, 5 daily sessions	dlPFC	Significant decrease in scores on the HDRS and Beck questionnaire following left dlPFC stimulation
Kito et al. (2011)	26 patients with TRD (14 male)	rTMS: 5 trains of 60s duration separated by 1 min pauses, 1 Hz, intensity of 100% of the patient's MTI, 12 sessions over 3 weeks	Right dlPFC	HDRS scores decreased, which was correlated with decreases of CBF in the right PFC, sgACC, putamen, and insula
Feng et al. (2012)	84 male Sprague-Dawley rats (CUMS)	rTMS: 15s trains of 60 pulses separated by 15s pauses, 15 Hz, intensity at 100% of the device's maximum power, 21 consecutive daily sessions	Vertex of skull	Stimulation increased sucrose preference and decreased the immobility in the FST. Reversed stress-induced increases in ACTH and cortisol as well as stress-induced decreases in BDNF, ERK1/2, and BrdU-positive cells in the hippocampus
Kim et al. (2014)	20 male Sprague-Dawley rats (CUMS)	rTMS: 20 trains of 50 pulses separated by 25s pauses, 10 Hz, intensity of 1.4 T at the surface of the coil, 14 consecutive sessions lasting 10 min each	Frontal cortex	Stimulation reversed stress-induced disruption of sucrose preference and GABAergic neurotransmission in the hippocampus and prefrontal cortex
Müller et al. (2000)	Male Wistar rats	rTMS: 3 trains of 2.5s duration separated by 2 min pauses, 20 Hz, 120 A/μs (130% of rats' MTI), 5 weekly sessions for 11 weeks	Left frontal cortex	Stimulation increased BDNF and CCK mRNA in the hippocampus, as well as BDNF-like immunoreactivity in the parietal and piriform cortex

Table 1 (continued).

Boggio et al. (2008)	40 patients with MDD (12 male)	tDCS: anodal, 2mA, 20 min, 10 sessions over 2 weeks	dlPFC or occipital cortex	Stimulation of dlPFC reduced HDRS scores, occipital and sham stimulation produced no effect
Ferrucci et al. (2009)	14 patients with severe MDD (1 male)	tDCS: anodal, 2mA, 20 min, twice a day for 5 consecutive days	Left dlPFC	HDRS and BDI scores decreased following treatment and this effect lasted up to 1 month
Palm et al. (2012)	22 patients with MDD (8 male)	tDCS: anodal, 1mA or 2mA, 20 min, 20 sessions (10 active, 10 sham) over 4 weeks	Left dlPFC	No significant difference in HDRS or BDI scores between active and sham tDCS at the primary endpoint. Active tDCS was slightly superior to sham during the first study phase
Loo et al. (2010)	40 patients with MDD (18 male)	tDCS: anodal, 1mA, 20 min, 10 sessions occurring 3 times per week. Patients either received 10 active or 5 sham/5 active sessions.	Left dlPFC	Depression scores, as measured by HDRS, BDI, and MADRS, improved significantly over ten treatments, but there was no difference between active and sham tDCS
Peanlikhit et al. (2017)	211 female Swiss mice (naïve) 36 male C57Bl/6 mice (Corticosterone exposure)	tDCS: For naïve mice, a series of variable parameters were used: anodal or cathodal, 200, 100, or 25 μA, 20, 10, or 3 min, 1 or 10 stimulations over 5 consecutive days For corticosterone exposure mice: anodal, 200 μA, 20 min, 15 consecutive daily sessions	Left frontal cortex	A single 200 μA session of tDCS reduces immobility in the FST for up to 21 days, while 10 sessions decrease immobility up to 60 days. Lower intensities, 10 min duration, and cathodal stimulation did not reduce immobility. Stimulation also reverses the corticosterone-induced increase in immobility. A single session increased c-fos expression in the IL, PrL, hippocampus, NAcc, thalamus, and VTA.

Table 1 (continued).

Kim et al. (2017)	19 male Sprague-Dawley rats	tDCS: anodal, 250 μA, 20 mins, 7 consecutive daily sessions	Somato-sensory cortex	Stimulation increased mRNA expression of plasticity-associated genes, including BDNF, CREB, CaMKII, and synapsin I in the ipsilateral sensorimotor cortex
Podda et al. (2016)	Male C57bl/6 mice	tDCS: anodal, 350 μA, 20 min, single session	Left hippo-campus	Anodal tDCS increased LTP in slices compared to controls, while cathodal tDCS decreased LTP. Anodal tDCS also increased spatial learning and memory in the MWM and NOR tests, and these effects persisted 1 week after stimulation. Hippocampal *Bdnf* levels were significantly higher in tDCS-mice than in controls both 24h and 1 week after stimulation.
Haller et al. (2020)	6 patients with MDD (5 male)	tACS: 2 mA (amplitude −1 mA to +1 mA), 40 Hz, 10 min twice a day or 20 min once a day, 10 sessions over 2 weeks	PFC	Stimulation significantly reduced HDRS and BDI scores, while also improving cognitive functions in the word fluency and n-back tests.
Del Felice et al. (2019)	15 patients with Parkinson Disease (9 male)	tACS: 1 to 2 mA, 4 or 30 Hz, 30 min, 10 sessions over 2 weeks	Left or right mastoid	Stimulation improved prefrontal-executive functions at 4 weeks following stimulation
Alexander et al. (2019)	32 patients with MDD (5 male)	tACS: 2mA, 10 or 40 Hz, 40 min, 5 consecutive sessions	Frontal cortex	10Hz-tACS resulted in lower MADRS and HDRS scores than 40 Hz and sham stimulation and reduced alpha oscillatory activity over the left dlPFC

Table 1 (continued).

Riddle et al. (2020a)	1 female patient with MDD	tACS: 10 Hz, 40 min, 5 consecutive sessions, then once weekly for 12 consecutive weeks	Not reported	12 weeks of stimulation caused remission of depressive symptoms as measured by MADRS, improvements lasted up to 6 months after treatment
Riddle et al. (2020b)	Not reported	tACS: 10 Hz, other parameters not reported	Frontal cortex	Alpha-tACS increased alpha oscillations in Val66Val carriers more than Val66Met carriers

5-HT, serotonin; *ACTH*, adrenocorticotropic hormone; *BDI*, Beck Depression Inventory; *BDNF*, brain-derived neurotrophic factor; *CBF*, cerebral blood flow; *CCK*, cholecystokinin; *CUMS*, chronic unpredictable mild stress; *DBS*, deep brain stimulation; *dlPFC*, dorsolateral profrontal cortex; *FST*, forced swim test; *HDRS*, Hamilton depression rating scale; *IL*, infralimbic cortex; *KO*, knockout; *LTP*, long term potentiation; *MADRS*, Montgomery–Asberg depression rating scale, *MDD*, major depressive disorder; *MTI*, motor threshold intensity; *MWM*, morris water maze; *NAcc*, nucleus accumbens; *NOR*, novel object recognition; *OBX*, olfactory bulbectomy; *OFC*, orbital frontal cortex; *PrL*, prelimbic cortex; *rTMS*, repetitive transcranial magnetic stimulation; *SERT*, serotonin transporter; *sgACC*, subgenual anterior cingulate cortex; *tACS*, transcranial alternating current stimulation; *tDCS*, transcranial direct current stimulation; *TRD*, treatment-resistant depression; *vmPFC*, ventromedial prefrontal cortex; *VTA*, ventral tegmental area; *WT*, wild-type

Table 2

ANOVA Table for Behavioural Outcomes in Response to EC Stimulation

Test	Measure	Predictor	Mean (SD)	*n*	*F*	*p*	η_p^2
OFT	Distance Traveled	Stim	*STIM:* 2509.098 (1242.989) *SHAM:* 3393.028 (1553.688)	25 32	5.374	.024	.092
		Drug	*NS8593:* 2572.960 (1276.970) *VEH:* 3329.170 (1519.700)	30 27	3.933	.053	.069
		Stim * Drug			2.855	.097	.051
	Quadrant Entries	Stim	*STIM:* 128.584 (85.252) *SHAM:* 208.947 (113.618)	25 32	8.611	.005	.140
		Drug	*NS8593:* 144.147 (89.656) *VEH:* 193.384 (109.213)	30 27	3.232	.078	.057
		Stim * Drug			1.206	.277	.022
	Time in Center	Stim	*STIM:* 6.786 (10.773) *SHAM:* 14.228 (11.681)	25 32	5.874	.019	.100
		Drug	*NS8593:* 9.938 (12.812) *VEH:* 11.076 (9.642)	30 27	.137	.712	.003
		Stim * Drug			.094	.761	.002
	Center Entries	Stim	*STIM:* 4.686 (5.338) *SHAM:* 11.175 (8.454)	25 32	11.115	.002	.173
		Drug	*NS8593:* 6.210 (6.351) *VEH:* 9.650 (7.441)	30 27	3.123	.083	.056
		Stim * Drug			.346	.559	.006
NSFT	Time to Reach Food	Stim	*STIM:* 73.955 (56.441) *SHAM:* 88.536 (78.873)	22 32	.539	.466	.011
		Drug	*NS8593:* 77.190 (53.324) *VEH:* 85.300 (81.989)	28 26	.167	.685	.003

Table 2 (continued).

		Stim * Drug			.824	.368	.016
	Time to Start Eating	Stim	*STIM:* 278.500 (135.293) *SHAM:* 205.654 (108.502)	18 26	3.743	.060	.086
		Drug	*NS8593:* 254.173 (132.872) *VEH:* 229.981 (110.923)	23 21	.413	.524	.010
		Stim * Drug			1.750	.193	.042
	Time to Start Eating in Home Cage	Stim	*STIM:* 132.857 (59.535) *SHAM:* 86.167 (54.606)	12 18	4.216	.050	.140
		Drug	*NS8593:* 116.667 (45.018) *VEH:* 102.357 (69.123)	14 16	.396	.535	.015
		Stim * Drug			1.983	.171	.071
FST (Acute)	Climbing Time	Stim	*STIM:* 40.897 (23.679) *SHAM:* 40.112 (19.256)	25 22	.015	.904	.000
		Drug	*NS8593:* 42.184 (23.698) *VEH:* 38.824 (19.238)	25 22	.271	.606	.006
		Stim * Drug			.076	.784	.002
	Swimming Time	Stim	*STIM:* 125.035 (35.320) *SHAM:* 120.230 (34.680)	25 22	.218	.643	.005
		Drug	*NS8593:* 110.710 (35.062) *VEH:* 134.555 (34.933)	25 22	5.380	.025	.111
		Stim * Drug			2.762	.104	.060
	Immobility Time	Stim	*STIM:* 134.085 (46.834) *SHAM:* 139.684 (38.497)	25 22	.194	.662	.004
		Drug	*NS8593:* 147.135 (41.577) *VEH:* 126.634 (43.753)	25 22	2.603	.114	.057
		Stim * Drug			2.201	.145	.049
FST (Chronic)	Climbing Time	Stim	*STIM:* 41.892 (23.052) *SHAM:* 43.879 (27.024)	25 22	.072	.790	.002
		Drug	*NS8593:* 39.905 (18.925)	25	.645	.426	.015

Table 2 (continued).

			VEH: 45.866 (31.151)	22			
		Stim * Drug			.056	.814	.001
	Swimming Time	Stim	*STIM:* 132.728 (36.604) *SHAM:* 123.532 (33.080)	25 22	.785	.381	.018
		Drug	*NS8593:* 121.057 (38.287) *VEH:* 135.203 (31.397)	25 22	1.856	.180	.041
		Stim * Drug			2.101	.155	.047
	Immobility Time	Stim	*STIM:* 125.425 (51.821) *SHAM:* 132.630 (47.788)	25 22	.241	.626	.006
		Drug	*NS8593:* 139.079 (48.010) *VEH:* 118.976 (51.599)	25 22	1.880	.178	.042
		Stim * Drug			.818	.371	.019
SPT (Acute)	Sucrose Preference	Stim	*STIM:* 88.503 (13.717) *SHAM:* 82.204 (20.475)	25 32	1.728	.194	.032
		Drug	*NS8593:* 86.793 (14.211) *VEH:* 83.914 (19.981)	30 27	.361	.551	.007
		Stim * Drug			.293	.591	.005
SPT (Chronic)	Sucrose Preference	Stim	*STIM:* 82.629 (20.046) *SHAM:* 82.183 (23.215)	25 32	.005	.942	.000
		Drug	*NS8593:* 79.161 (24.969) *VEH:* 85.651 (18.292)	30 27	1.150	.288	.021
		Stim * Drug			.467	.497	.009

Table 3

ANOVA Table for Neurochemical Response to EC Stimulation Across Four Brain Regions

NT	Region	Predictor	Mean (SD)	*n*	*F*	*p*	η_p^2
DA	PFC	Stim	*STIM:* 171.767 (80.195) *SHAM:* 139.227 (35.708)	24 24	3.128	.085	.073
		Drug	*NS8593:* 157.190 (64.891) *VEH:* 153.805 (51.013)	24 24	.034	.855	.001
		Hemisphere	*LEFT:* 159.661 (56.329) *RIGHT:* 151.333 (59.575)	24 24	.205	.653	.005
		Stim * Drug			.512	.478	.013
		Stim * Hemisphere			1.367	.249	.033
		Drug * Hemisphere			.446	.508	.011
		Stim * Drug * Hemisphere			.258	.614	.006
	CPu	Stim	*STIM:* 266.640 (104.412) *SHAM:* 235.277 (57.774)	24 24	1.586	.215	.038
		Drug	*NS8593:* 241.948 (66.986) *VEH:* 259.969 (95.200)	24 24	.524	.474	.013
		Hemisphere	*LEFT:* 232.334 (79.211) *RIGHT:* 269.582 (82.975)	24 24	2.237	.143	.053
		Stim * Drug			1.014	.320	.025
		Stim * Hemisphere			.138	.713	.003
		Drug * Hemisphere			.005	.942	.000

Table 3 (continued).

	Stim * Drug * Hemisphere			.158	.694	.004
Hipp	Stim	*STIM:* 155.648 (63.353) *SHAM:* 158.243 (67.237)	24 23	.017	.898	.000
	Drug	*NS8593:* 152.426 (61.524) *VEH:* 161.465 (69.066)	24 23	.203	.655	.005
	Hemisphere	*LEFT:* 163.240 (59.553) *RIGHT:* 150.651 (71.037)	23 24	.393	.535	.010
	Stim * Drug			9.480	.004	.196
	Stim * Hemisphere			.087	.770	.002
	Drug * Hemisphere			1.402	.244	.035
	Stim * Drug * Hemisphere			.094	.761	.002
MB	Stim	*STIM:* 129.967 (72.005) *SHAM:* 133.637 (49.562)	24 24	.036	.850	.001
	Drug	*NS8593:* 125.873 (47.642) *VEH:* 137.731 (73.924)	24 24	.381	.541	.009
	Hemisphere	*LEFT:* 125.365 (55.206) *RIGHT:* 138.240 (66.360)	24 24	.449	.507	.011
	Stim * Drug			2.600	.115	.061
	Stim * Hemisphere			.040	.843	.001
	Drug * Hemisphere			.017	.897	.000
	Stim * Drug * Hemisphere			.303	.585	.008

Table 3 (continued).

5-HT	PFC	Stim	*STIM:* 7.396 (8.460) *SHAM:* 9.290 (10.276)	24 24	.257	.615	.006
		Drug	*NS8593:* 3.823 (4.512) *VEH:* 12.863 (14.223)	24 24	5.857	.020	.128
		Hemisphere	*LEFT:* 11.943 (13.502) *RIGHT:* 4.743 (5.234)	24 24	3.716	.061	.085
		Stim * Drug			.786	.381	.019
		Stim * Hemisphere			3.148	.084	.073
		Drug * Hemisphere			3.861	.056	.088
		Stim * Drug * Hemisphere			1.735	.195	.042
	CPu	Stim	*STIM:* 30.993 (12.735) *SHAM:* 17.559 (7.101)	24 24	19.596	<.0001	.329
		Drug	*NS8593:* 27.195 (9.092) *VEH:* 211.357 (10.744)	24 24	3.702	.062	.085
		Hemisphere	*LEFT:* 24.055 (9.435) *RIGHT:* 24.497 (10.401)	24 24	.021	.885	.001
		Stim * Drug			3.817	.058	.087
		Stim * Hemisphere			.065	.800	.002
		Drug * Hemisphere			.320	.575	.008
		Stim * Drug * Hemisphere			.002	.968	.000
	Hipp	Stim	*STIM:* 5.500 (5.738) *SHAM:* 5.731 (4.749)	24 23	.013	.909	.000

Table 3 (continued).

		Drug	*NS8593:* 5.608 (4.799) *VEH:* 5.624 (5.688)	24 23	.000	.994	.000
		Hemisphere	*LEFT:* 8.586 (8.776) *RIGHT:* 2.646 (1.712)	23 24	8.681	.0054	.182
		Stim * Drug			3.961	.054	.092
		Stim * Hemisphere			.003	.957	.000
		Drug * Hemisphere			.262	.612	.007
		Stim * Drug * Hemisphere			4.400	.043	.101
	MB	Stim	*STIM:* 5.201 (3.524) *SHAM:* 3.727 (2.459)	24 24	2.136	.152	.051
		Drug	*NS8593:* 5.045 (3.441) *VEH:* 3.883 (2.542)	24 24	1.327	.256	.032
		Hemisphere	*LEFT:* 5.205 (4.048) *RIGHT:* 3.723 (1.935)	24 24	2.154	.150	.051
		Stim * Drug			2.197	.146	.052
		Stim * Hemisphere			.026	.873	.001
		Drug * Hemisphere			.328	.570	.008
		Stim * Drug * Hemisphere			.033	.857	.001
TRP	PFC	Stim	*STIM:* 8633.110 (3987.430) *SHAM:* 8844.780 (3679.670)	24 24	.035	.852	.001
		Drug	*NS8593:* 9071.040 (3904.600) *VEH:* 8406.850 (3762.500)	24 24	.346	.560	.009

Table 3 (continued).

	Hemisphere	*LEFT:* 9443.470 (3736.890) *RIGHT:* 8034.420 (3930.210)	24 24	1.558	.219	.037
	Stim * Drug			.114	.737	.003
	Stim * Hemisphere			.479	.493	.012
	Drug * Hemisphere			.395	.533	.010
	Stim * Drug * Hemisphere			.128	.723	.003
CPu	Stim	*STIM:* 14018.200 (3311.770) *SHAM:* 13108.600 (2454.050)	24 24	1.005	.322	.025
	Drug	*NS8593:* 14250.600 (3308.100) *VEH:* 12876.200 (2457.720)	24 24	2.294	.138	.054
	Hemisphere	*LEFT:* 13460.300 (3671.900) *RIGHT:* 13666.500 (2093.930)	24 24	.052	.821	.001
	Stim * Drug			2.394	.130	.056
	Stim * Hemisphere			.341	.562	.008
	Drug * Hemisphere			2.191	.147	.052
	Stim * Drug * Hemisphere			2.975	.092	.069
Hipp	Stim	*STIM:* 17116.400 (2603.390) *SHAM:* 13520.500 (2653.360)	24 23	16.458	<.001	.297
	Drug	*NS8593:* 15956.700 (3732.990) *VEH:* 14680.200 (1523.760)	24 23	2.074	.158	.050
	Hemisphere	*LEFT:* 15522.400 (2252.510) *RIGHT:* 15114.500 (3004.240)	23 24	.212	.648	.005

Table 3 (continued).

		Stim * Drug			2.590	.116	.062
		Stim * Hemisphere			.001	.971	.000
		Drug * Hemisphere			.400	.531	.010
		Stim * Drug * Hemisphere			.556	.460	.014
	MB	Stim	*STIM:* 28120.500 (4506.700) *SHAM:* 28640.900 (6186.340)	24 24	.101	.752	.003
		Drug	*NS8593:* 28384.100 (5496.780) *VEH:* 28377.200 (5196.260)	24 24	.000	.997	.000
		Hemisphere	*LEFT:* 29005.900 (5671.530) *RIGHT:* 27755.500 (5021.510)	24 24	.586	.449	.014
		Stim * Drug			.666	.419	.016
		Stim * Hemisphere			.724	.400	.018
		Drug * Hemisphere			.891	.351	.022
		Stim * Drug * Hemisphere			.656	.423	.016
5-HIAA	PFC	Stim	*STIM:* 365.750 (106.665) *SHAM:* 263.358 (102.889)	24 24	9.102	.004	.185
		Drug	*NS8593:* 309.620 (94.692) *VEH:* 319.488 (114.862)	24 24	.085	.773	.002
		Hemisphere	*LEFT:* 288.409 (82.033) *RIGHT:* 340.699 (127.521)	24 24	2.374	.131	.056
		Stim * Drug			.561	.458	.014
		Stim * Hemisphere			.138	.713	.003

Table 3 (continued).

	Drug * Hemisphere			.047	.830	.001
	Stim * Drug * Hemisphere			.009	.927	.000
CPu	Stim	*STIM:* 302.959 (108.221) *SHAM:* 262.679 (72.035)	24 23	2.111	.154	.051
	Drug	*NS8593:* 272.507 (78.929) *VEH:* 293.131 (101.327)	23 24	.553	.461	.014
	Hemisphere	*LEFT:* 271.999 (81.622) *RIGHT:* 293.639 (98.634)	23 24	.609	.440	.015
	Stim * Drug			1.786	.189	.044
	Stim * Hemisphere			2.052	.160	.050
	Drug * Hemisphere			.198	.659	.005
	Stim * Drug * Hemisphere			.314	.578	.008
Hipp	Stim	*STIM:* 188.241 (87.684) *SHAM:* 185.498 (60.904)	24 23	.014	.907	.000
	Drug	*NS8593:* 197.545 (75.260) *VEH:* 176.195 (73.328)	24 23	.832	.367	.021
	Hemisphere	*LEFT:* 195.715 (83.956) *RIGHT:* 178.025 (64.632)	23 24	.571	.454	.014
	Stim * Drug			6.215	.017	.137
	Stim * Hemisphere			.269	.607	.007
	Drug * Hemisphere			7.242	.010	.157

Table 3 (continued).

	Stim * Drug * Hemisphere			.881	.354	.022
MB	Stim	*STIM:* 131.963 (83.450) *SHAM:* 152.506 (76.650)	24 24	.758	.389	.019
	Drug	*NS8593:* 133.052 (85.705) *VEH:* 151.417 (74.395)	24 24	.606	.441	.015
	Hemisphere	*LEFT:* 143.264 (67.900) *RIGHT:* 141.205 (92.200)	24 24	.008	.931	.000
	Stim * Drug			.133	.717	.003
	Stim * Hemisphere			.276	.602	.007
	Drug * Hemisphere			.043	.837	.001
	Stim * Drug * Hemisphere			.531	.471	.013

5-HIAA, 5-hydroxyindoleacetic acid; *5-HT*, serotonin; *CPu*, caudate putamen; *DA*, dopamine; *Hipp*, hippocampus; *MB*, midbrain; *Stim*, stimulation; *TRP*, tryptophan

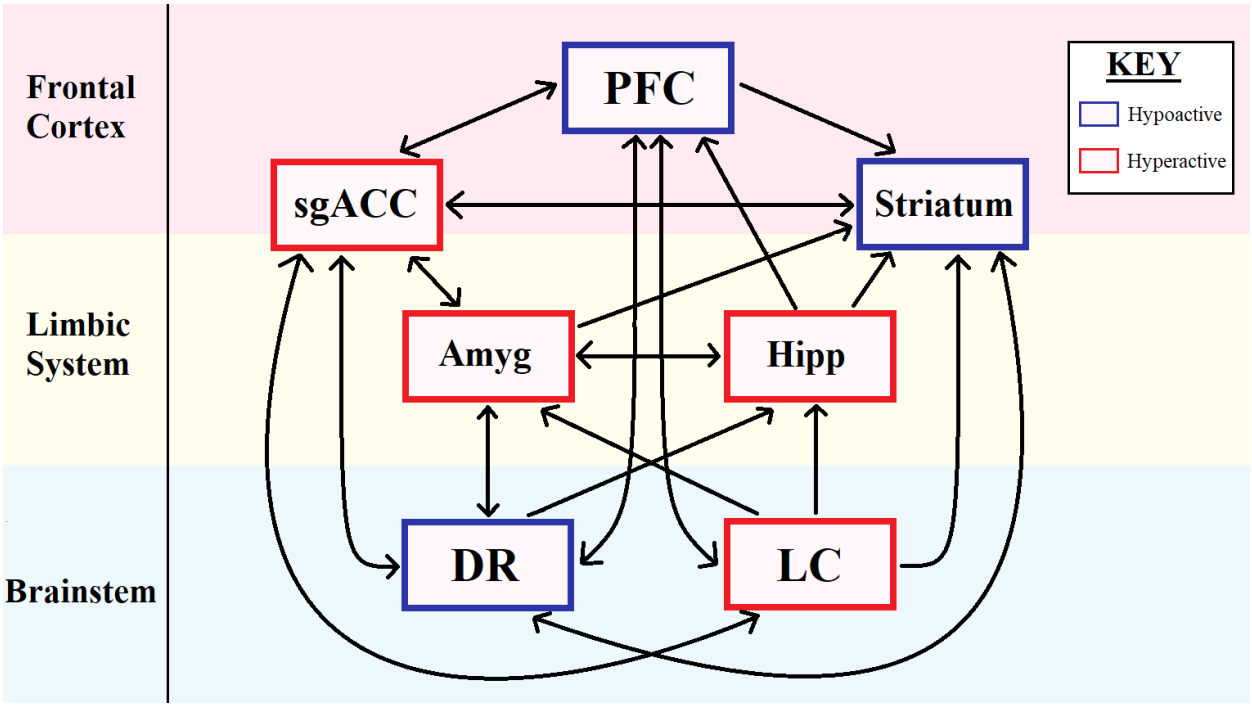


Figure 1: Simplified outline of brain circuitry implicated in MDD. Abbreviations: *Amyg*, amygdala; *DR*, dorsal raphe; *Hipp*, hippocampus; *LC*, locus coereleus; *PFC*, prefrontal cortex; *sgACC*, subgenual anterior cingulate cortex.

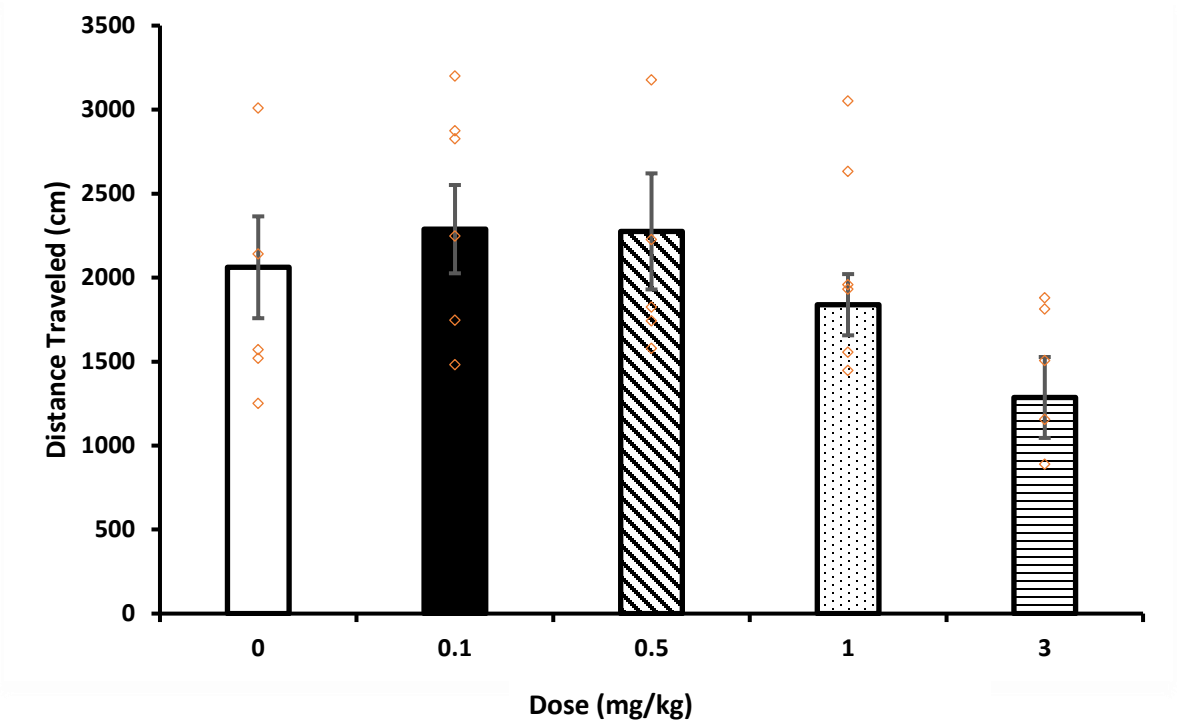


Figure 2: Average (±SEM) distance traveled in the OFT after varying doses of NS8593. No differences were found between groups on this measure, *n* = 5-6/group.

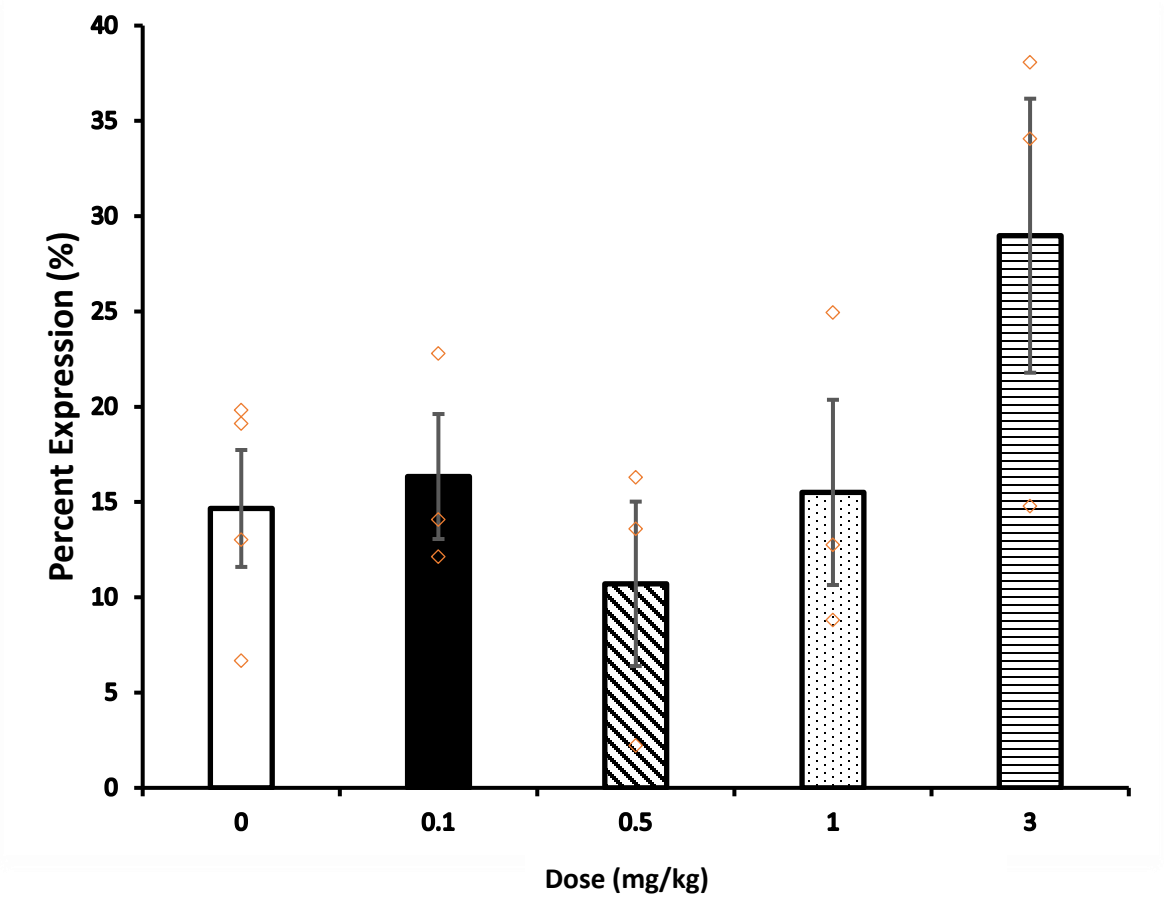


Figure 3: Average (±SEM) co-expression of *zif268* and *GAD* mRNA after varying doses of NS8593. Co-expression was quantified by first counting the number of cells containing both *zif268* and *GAD* staining and then calculating it as a percentage of the total cell count for each image. No differences were found between groups on this measure, n = 3-4/group.

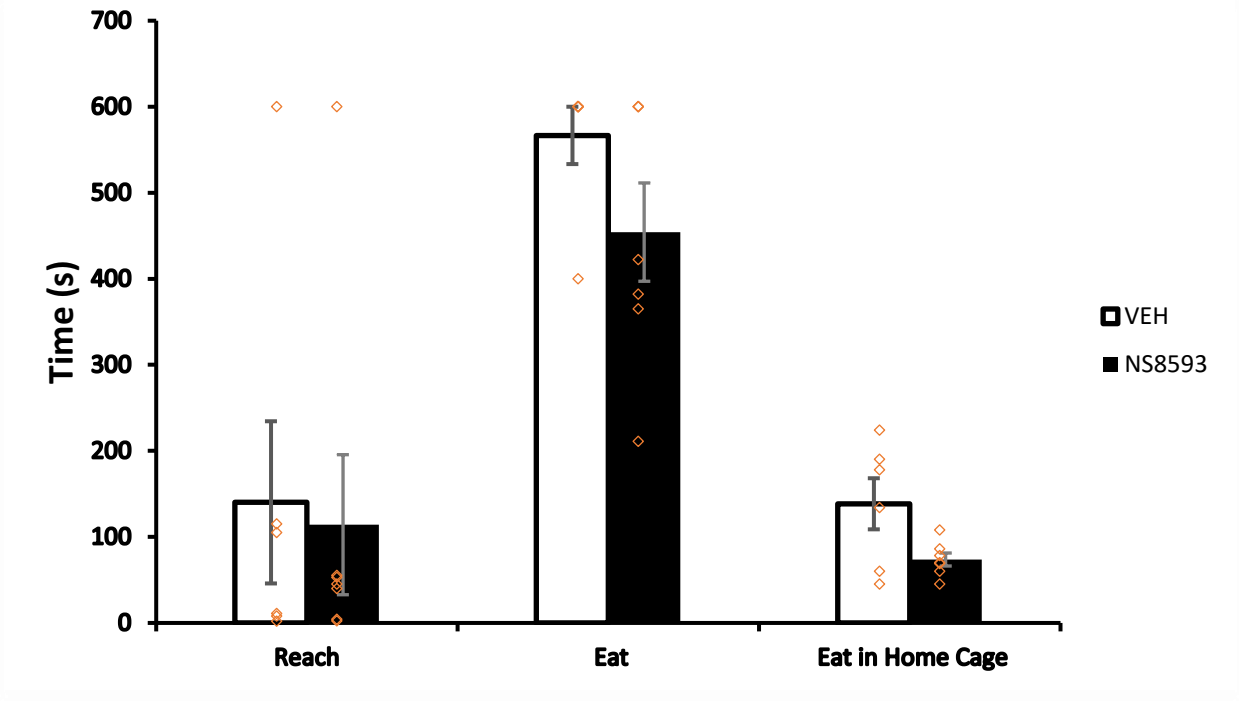


Figure 4: Average (±SEM) latency to reach food, eat within the chamber, and eat within the home cage in the NSFT following administration of 1.0mg/kg NS8593. No differences were found between groups on these measures, *n* = 6-7/group.

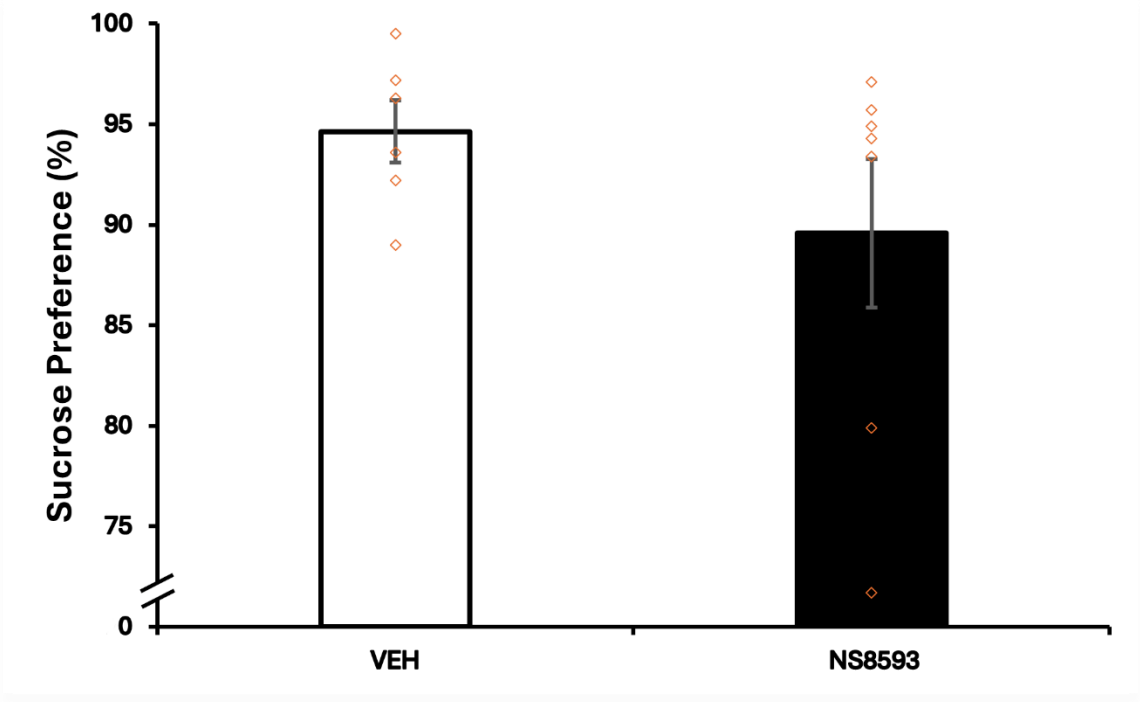


Figure 5: Average (±SEM) sucrose preference in the SPT following administration of 1.0mg/kg NS8593. No differences were found between groups on this measure, *n* = 6-7/group.

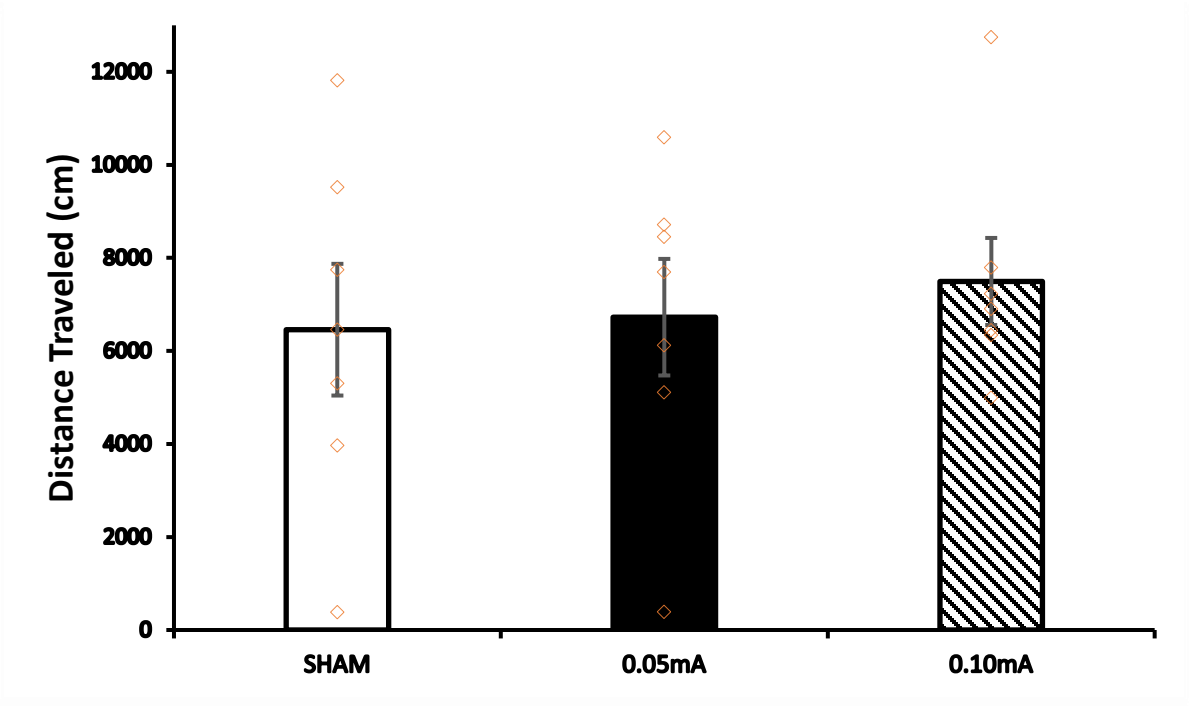


Figure 6: Average (±SEM) distance traveled in the OFT following treatment with varying intensities of tDCS current. No differences were found between groups on this measure, *n* = 7/group.

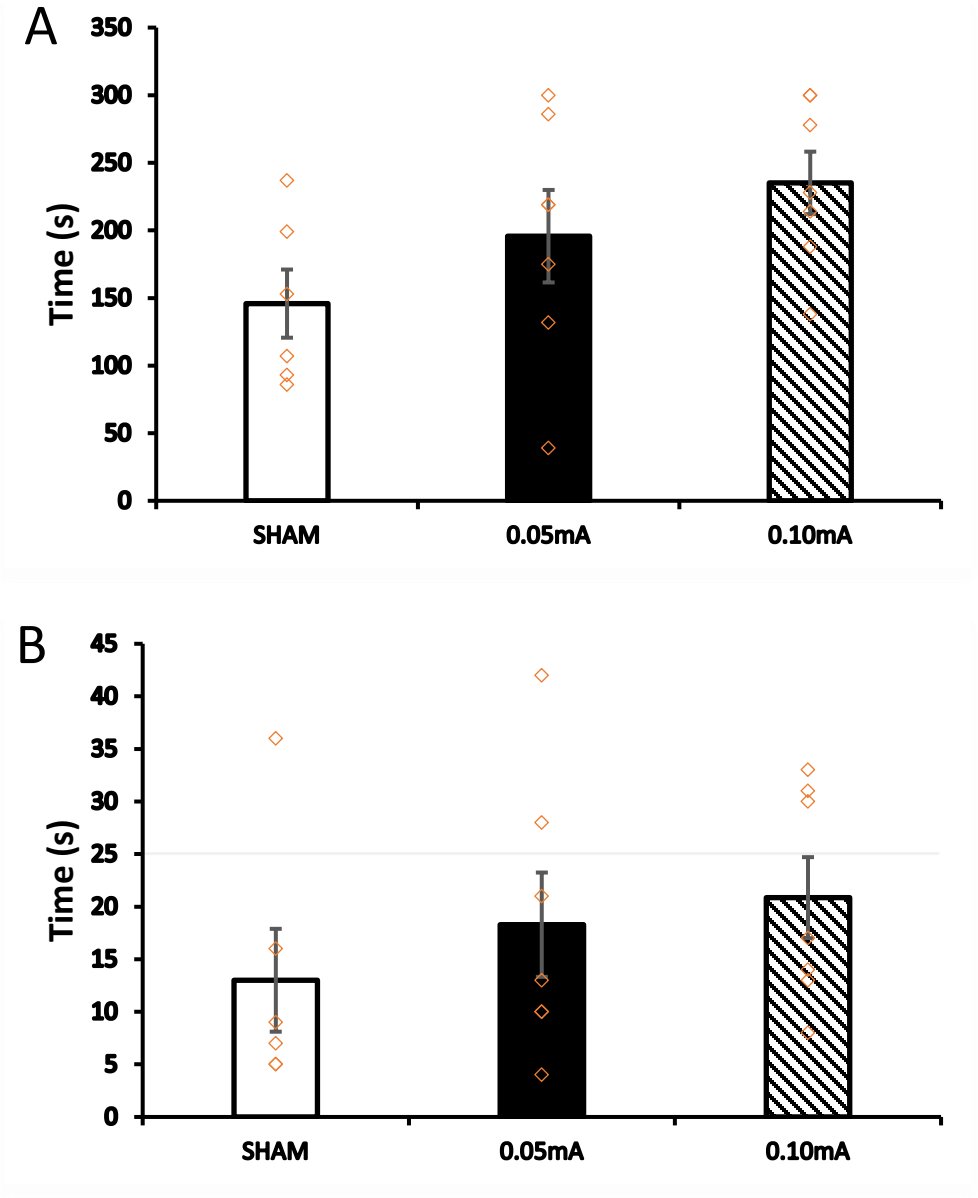


Figure 7: Average (±SEM) latency to eat within the chamber (A) and the home cage (B) in the NSFT following treatment with varying intensities of tDCS current. No differences were found between groups on this measure, *n* = 6-7/group.

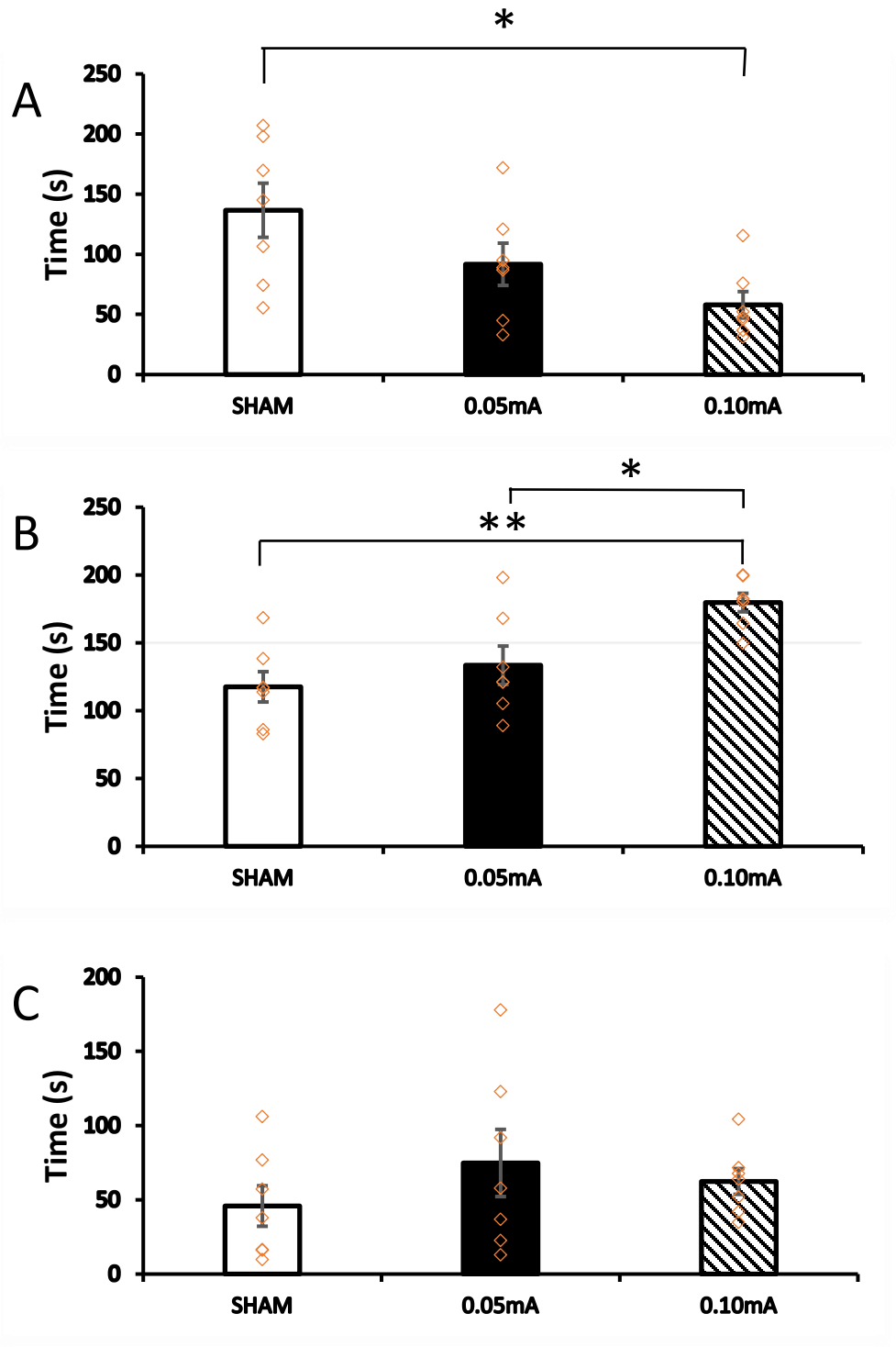


Figure 8: Average (±SEM) time spent immobile (A), swimming (B), and climbing (C) in the FST following treatment with varying intensities of tDCS current. The results suggest that 0.10mA, but not 0.05mA, reduces passivity and promotes active coping in the FST, n = 7/group, *$p < .05$, **$p < .01$

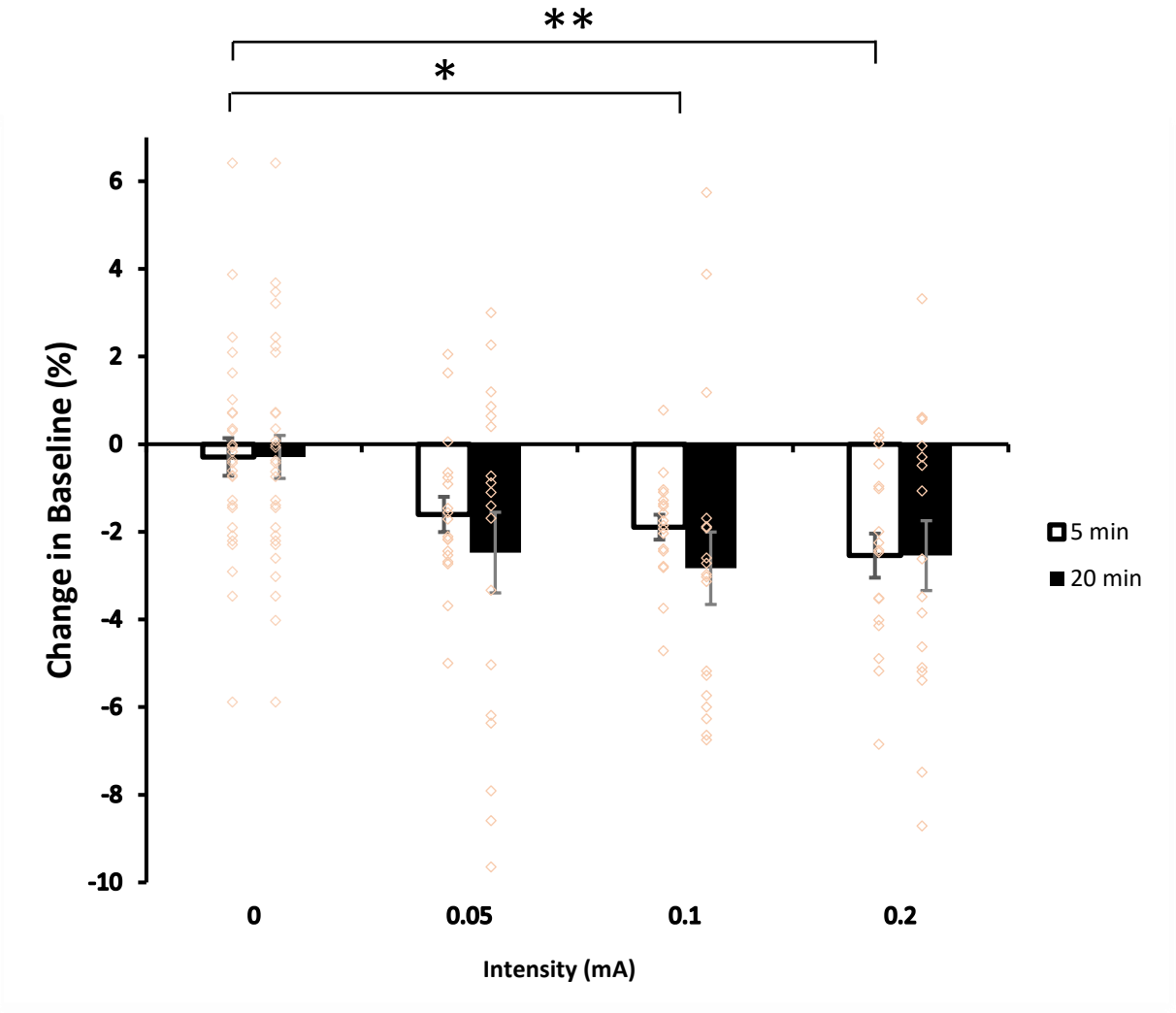


Figure 9: Average (±SEM) change in raphe glutamate concentration compared to baseline in response to varying intensities of tDCS during biosensor recording. The results indicate that both 0.10mA and 0.20mA of tDCS reduce glutamate concentration within the dorsal raphe, n = 17-29/group, *$p < .05$, **$p < .01$

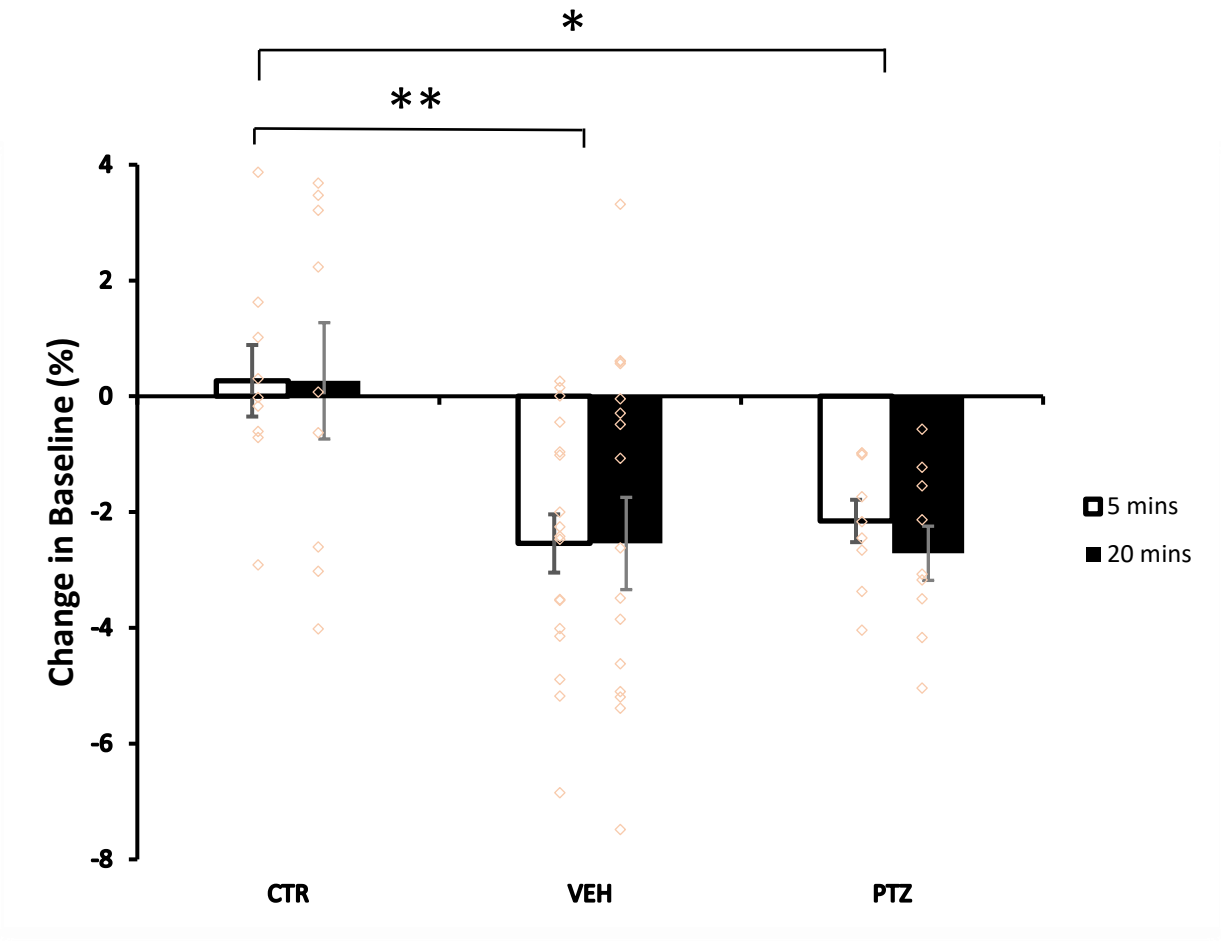


Figure 10: Average (±SEM) change in raphe glutamate concentration compared to baseline in response to PTZ and 0.2mA of tDCS during biosensor recording. The results indicate that 0.20mA of tDCS reduces glutamate concentration within the dorsal raphe; however, this effect is unaffected by PTZ administration, n = 9-17/group, *$p < .05$, **$p < .01$

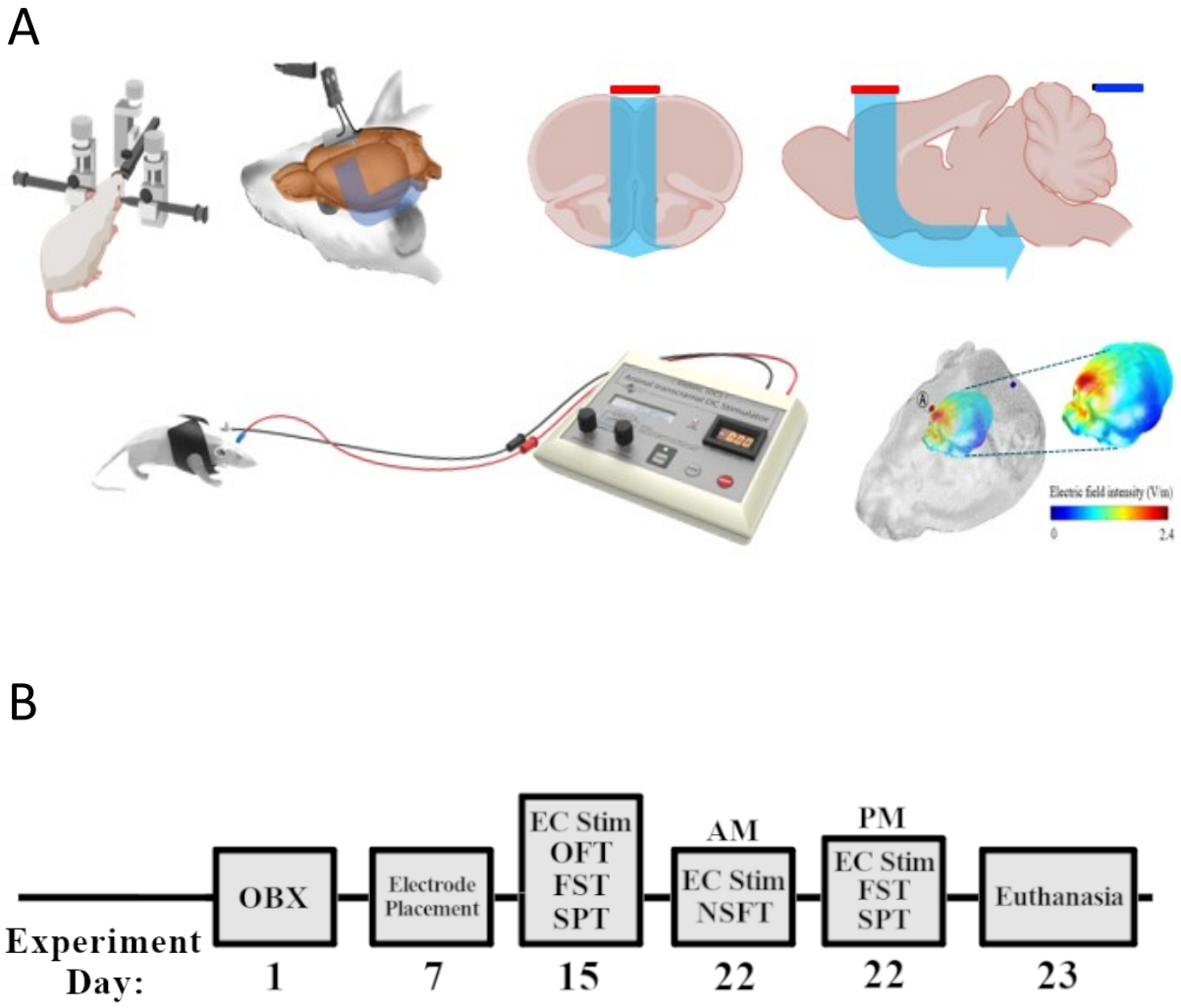


Figure 11: (A) Diagram of Electrode Placement and tDCS Protocol. Animals were mounted in a stereotaxic frame and an electrode base was affixed to the skull above the mPFC (AP: +3.0mm). Anodal stimulation passed a current through the underlying tissue to create a localized electric field. **(B) Timeline of Experiment 2.** Abbreviations: *FST*, forced swim test; *NSFT*, novelty suppressed feeding test; *OBX*, olfactory bulbectomy; *OFT*, open field test; *SPT*, sucrose preference test.

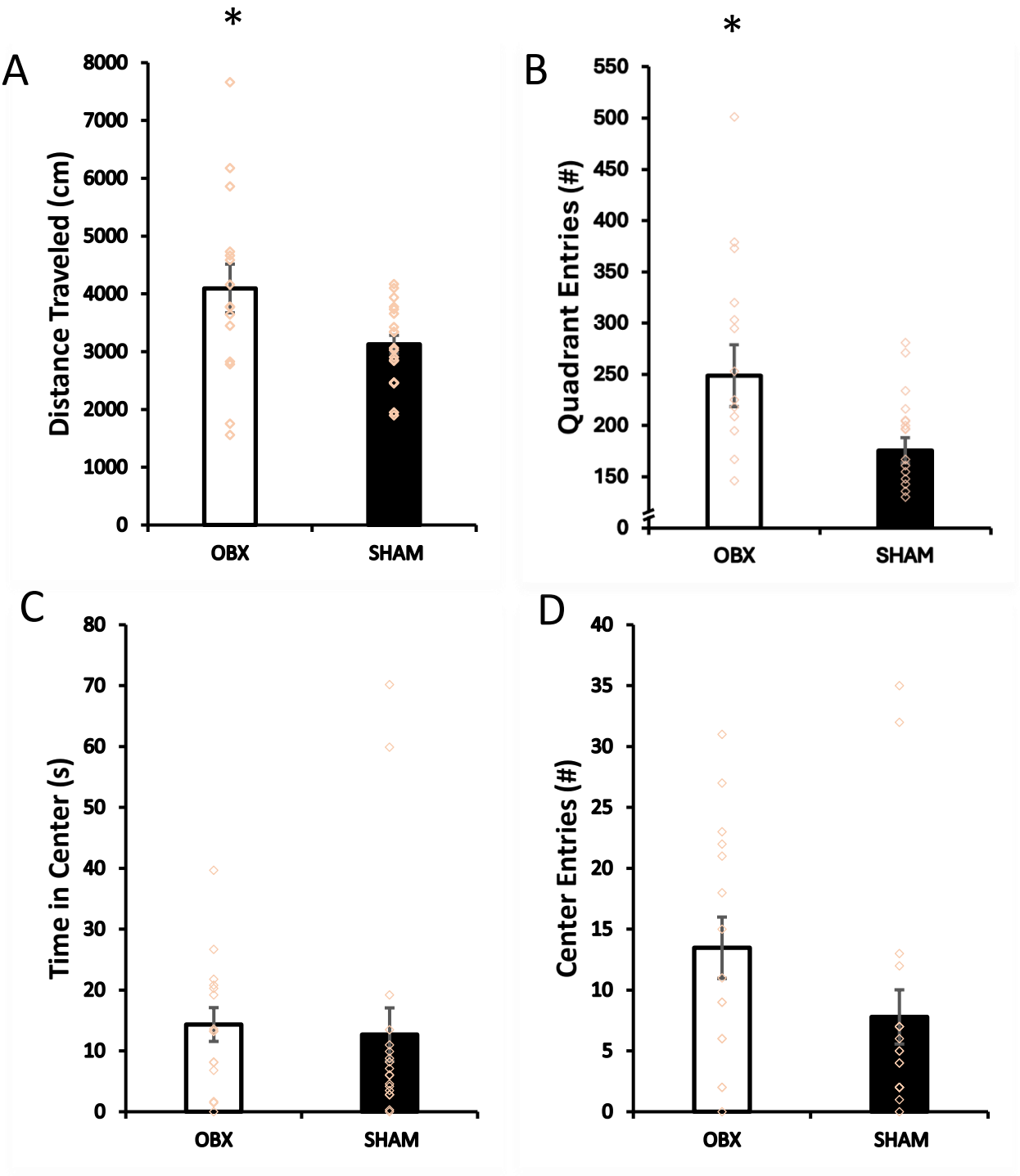


Figure 12: Average (±SEM) distance traveled (A), number of quadrants visited (B), time spent in the center of the arena (C), and number of center entries (D) in the OFT following OBX. The results suggest that OBX animals traveled more distance and entered more quadrants than sham animals but did not spend more time entering or standing in the center of the arena, indicating a hyperactive, but not anxiety-like, state, n = 15-19/group, *$p < .05$

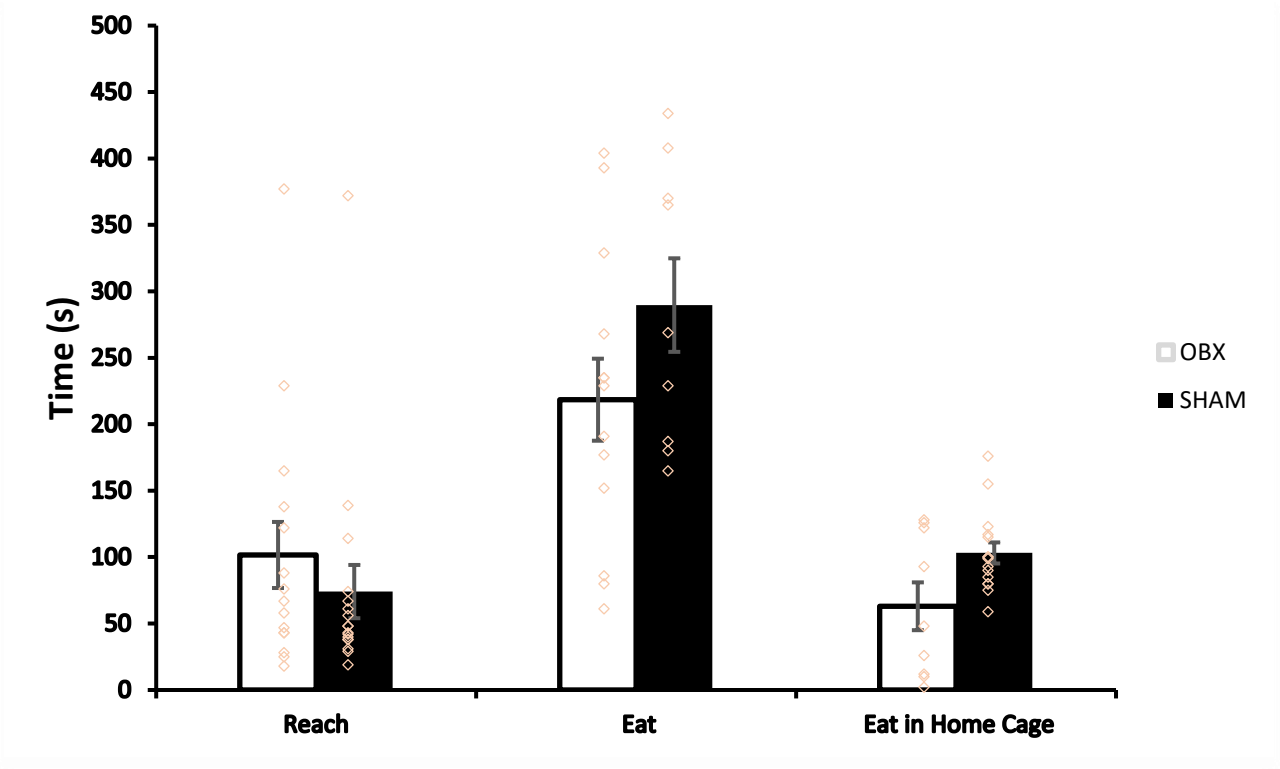


Figure 13: Average (±SEM) latency to reach food, eat within the chamber, and eat within the home cage in the NSFT following OBX. No differences were found between groups on these measures, *n* = 9-17/group.

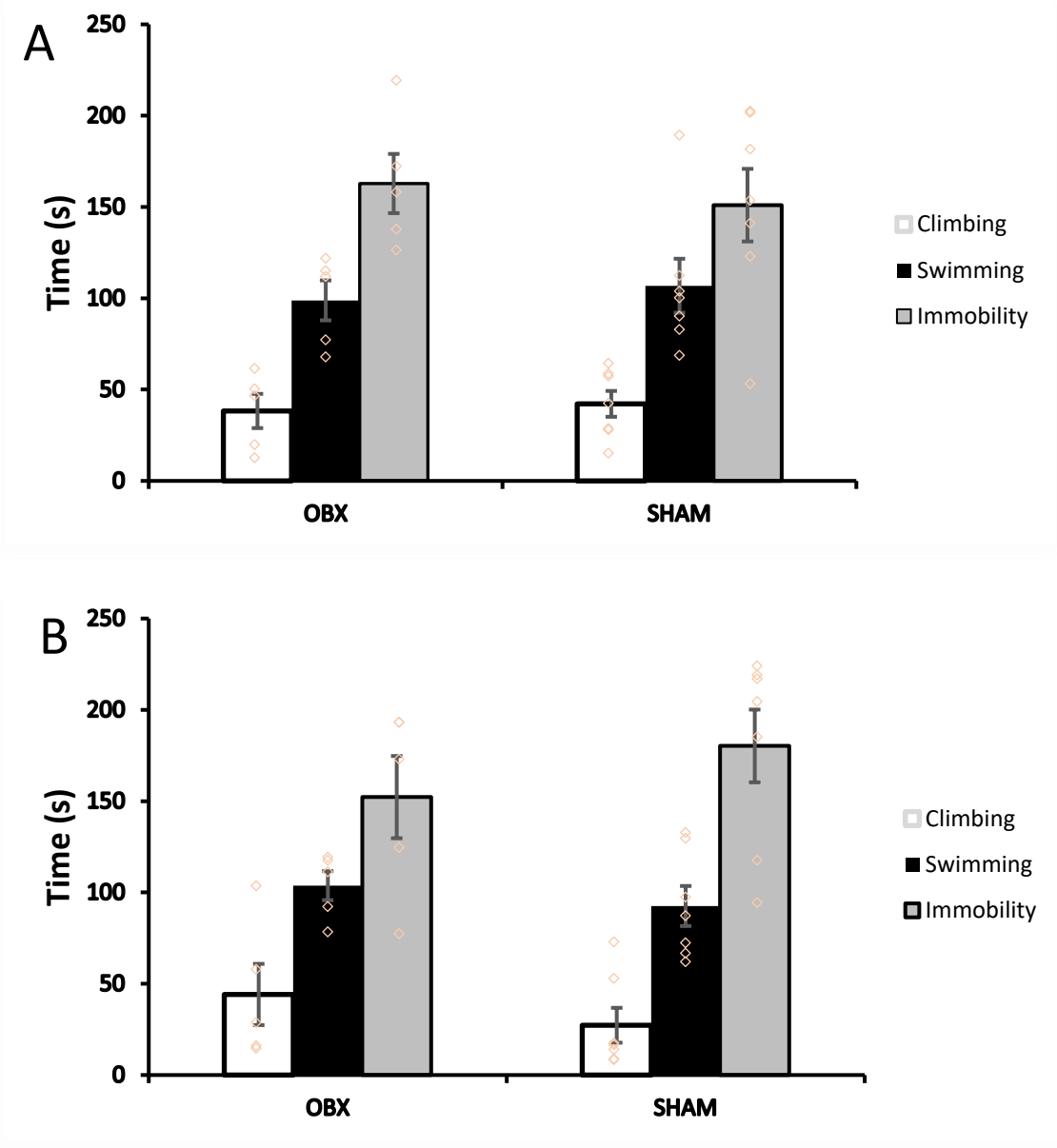


Figure 14: Average (±SEM) climbing, swimming, and immobility time in the FST following OBX on week 1 (A) and week 2 (B) of testing. No differences were found between groups on these measures at either time point, *n* = 5-7/group.

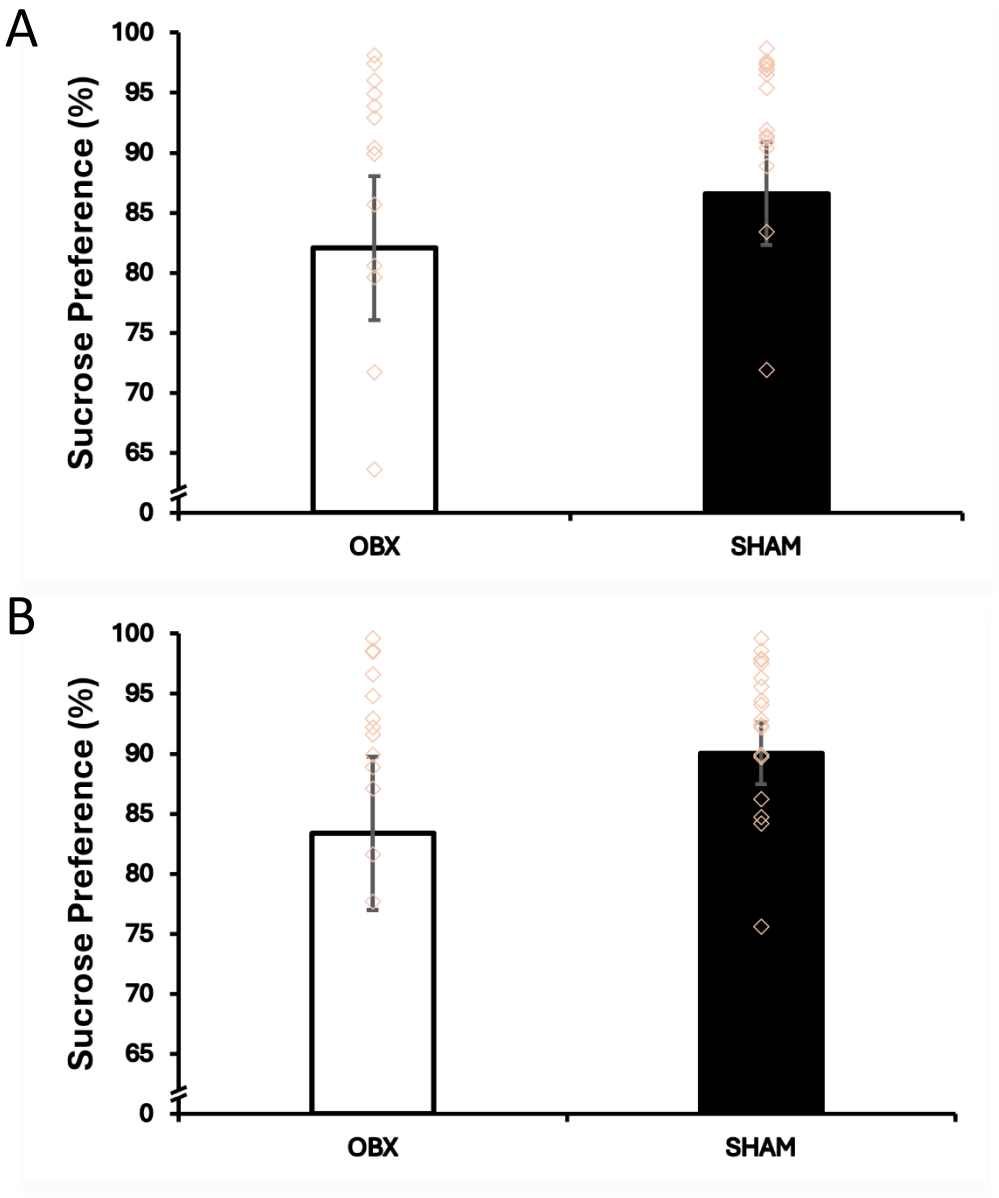


Figure 15: Average (±SEM) sucrose preference in the SPT following OBX on week 1 (A) and week 2 (B) of testing. No differences were found between groups on this measure at either time point, *n* = 14-18/group.

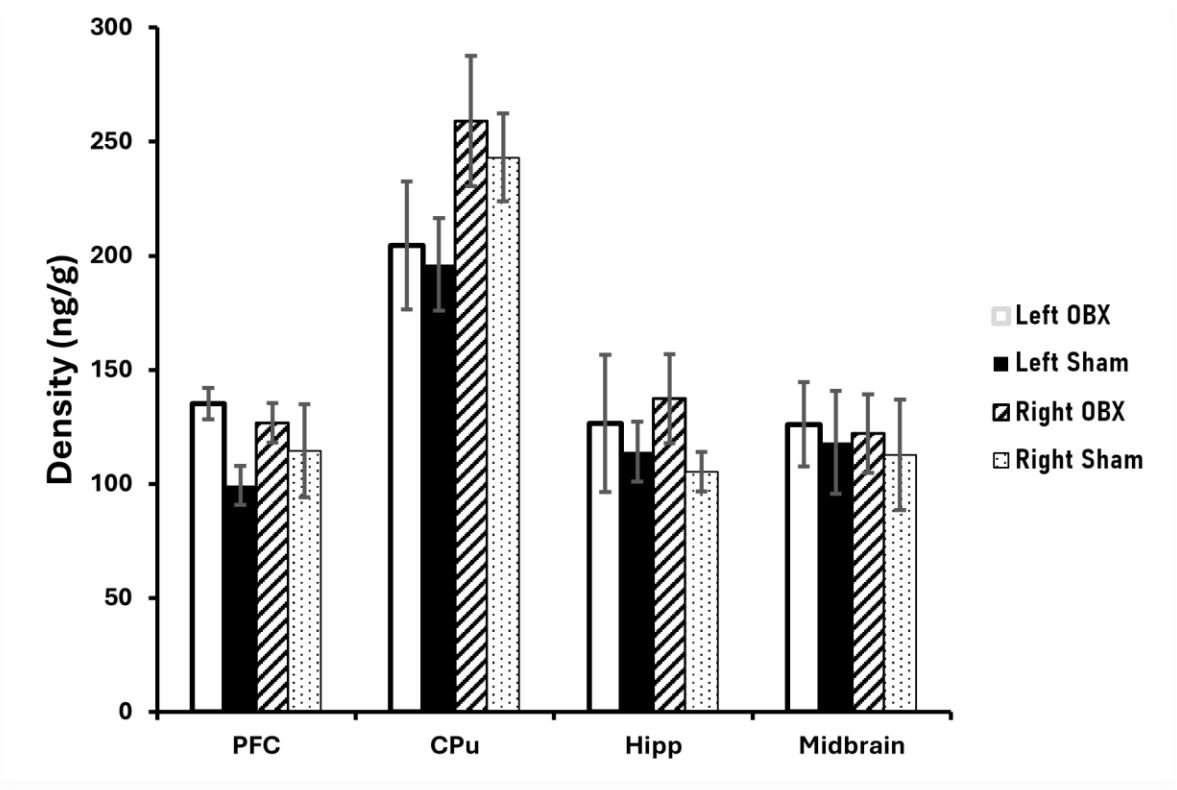


Figure 16: Average (±SEM) density of Dopamine in the left and right hemispheres across each brain region following OBX. No significant differences were found between groups on this measure, *n* = 11-12/group.

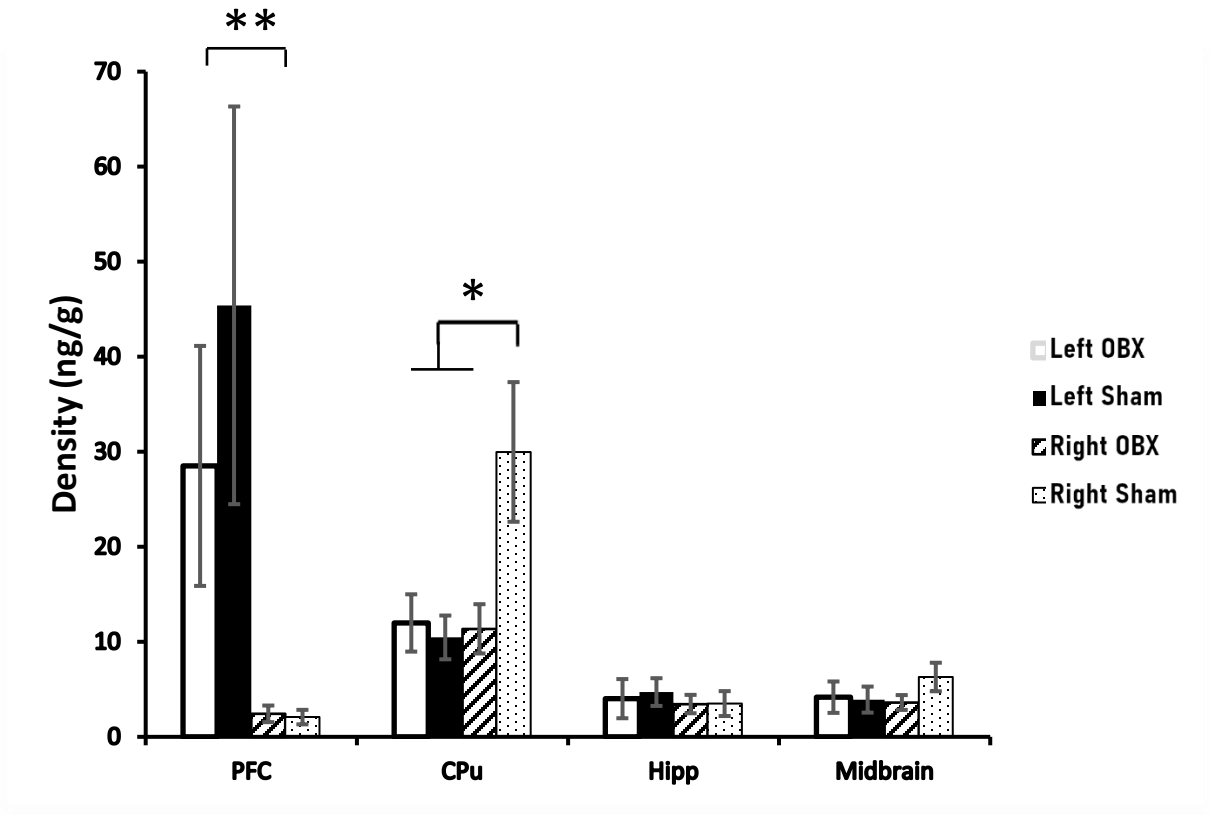


Figure 17: Average (±SEM) density of Serotonin in the left and right hemispheres across each brain region following OBX. The results indicate hemispheric differences in 5-HT density, with higher concentrations being observed in the left PFC and right CPu, n = 11-12/group, *$p < .05$, **$p < .01$

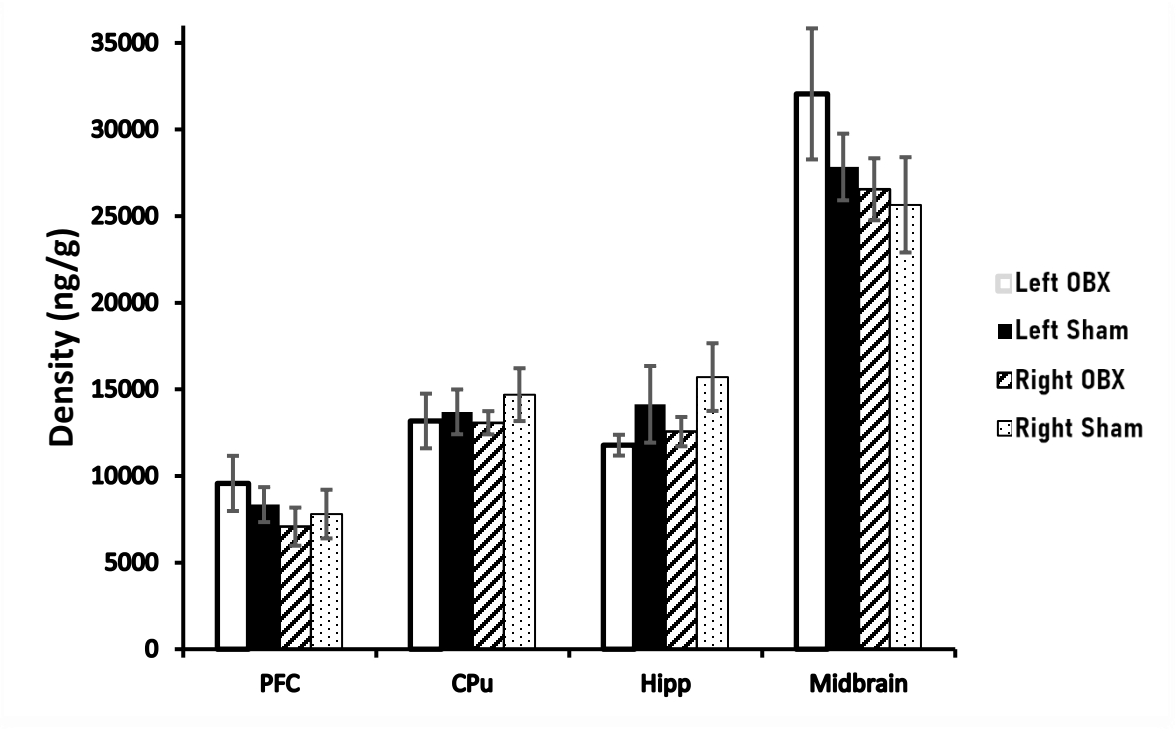


Figure 18: Average (±SEM) density of Tryptophan in the left and right hemispheres across each brain region following OBX. No significant differences were found between groups on this measure, *n* = 11-12/group.

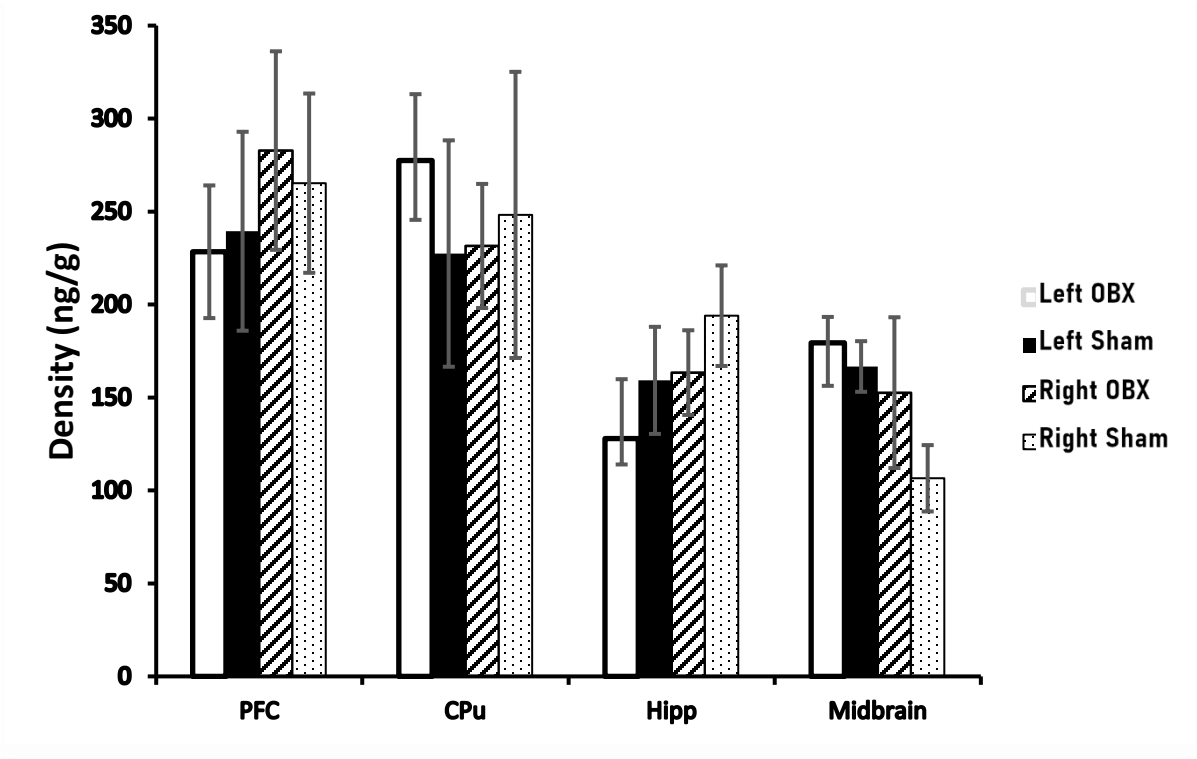


Figure 19: Average (±SEM) density of 5-hydroxyindoleacetic acid in the left and right hemispheres across each brain region following OBX. No significant differences were found between groups on this measure, *n* = 11-12/group.

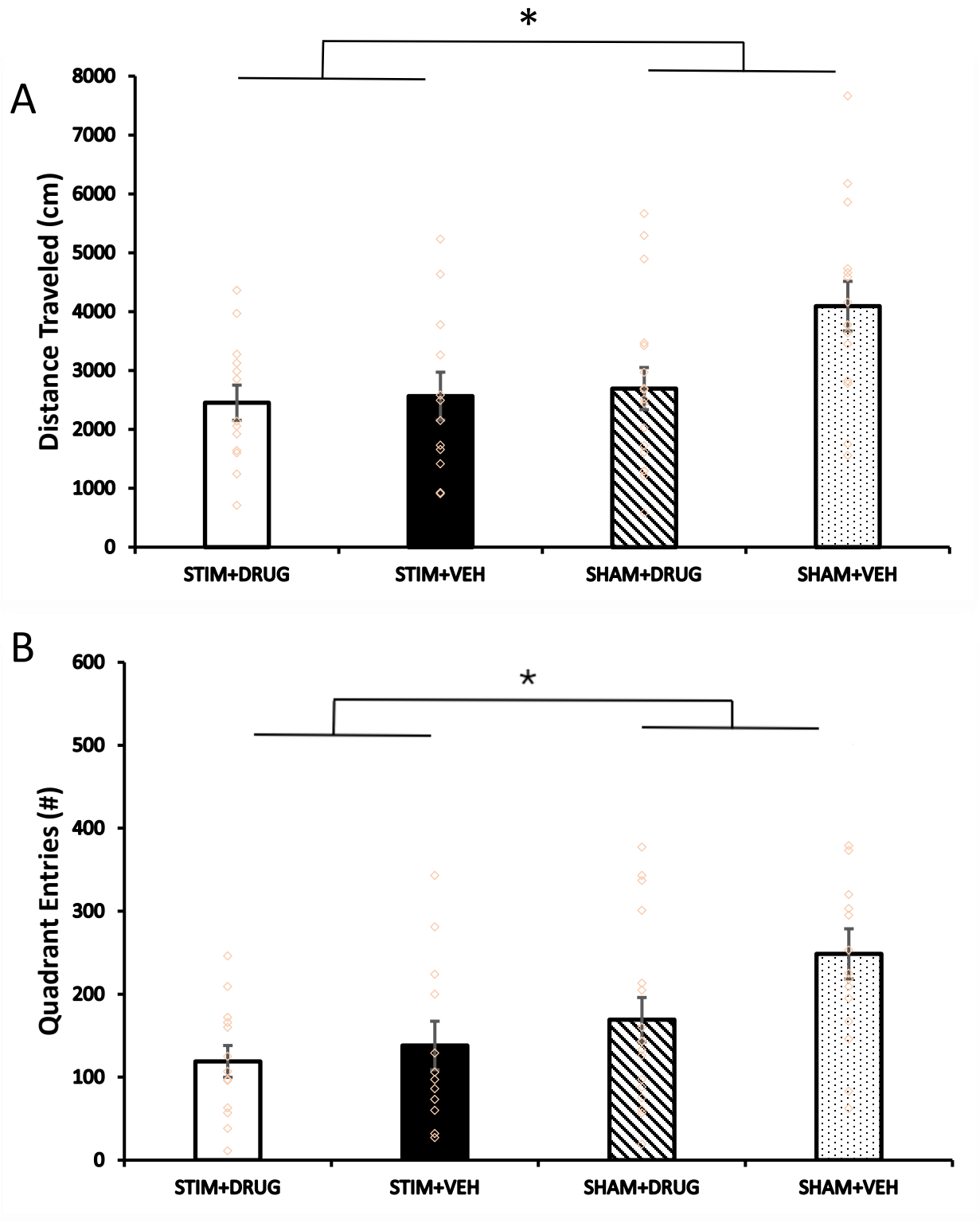


Figure 20: Average (±SEM) distance traveled (A) and quadrant entries (B) in the OFT in response to EC Stimulation. The results indicate that tDCS, but not NS8593, reduced hyperlocomotion as assessed by distance traveled and quadrant entries in the OFT, n = 12-17/group, *$p < .05$

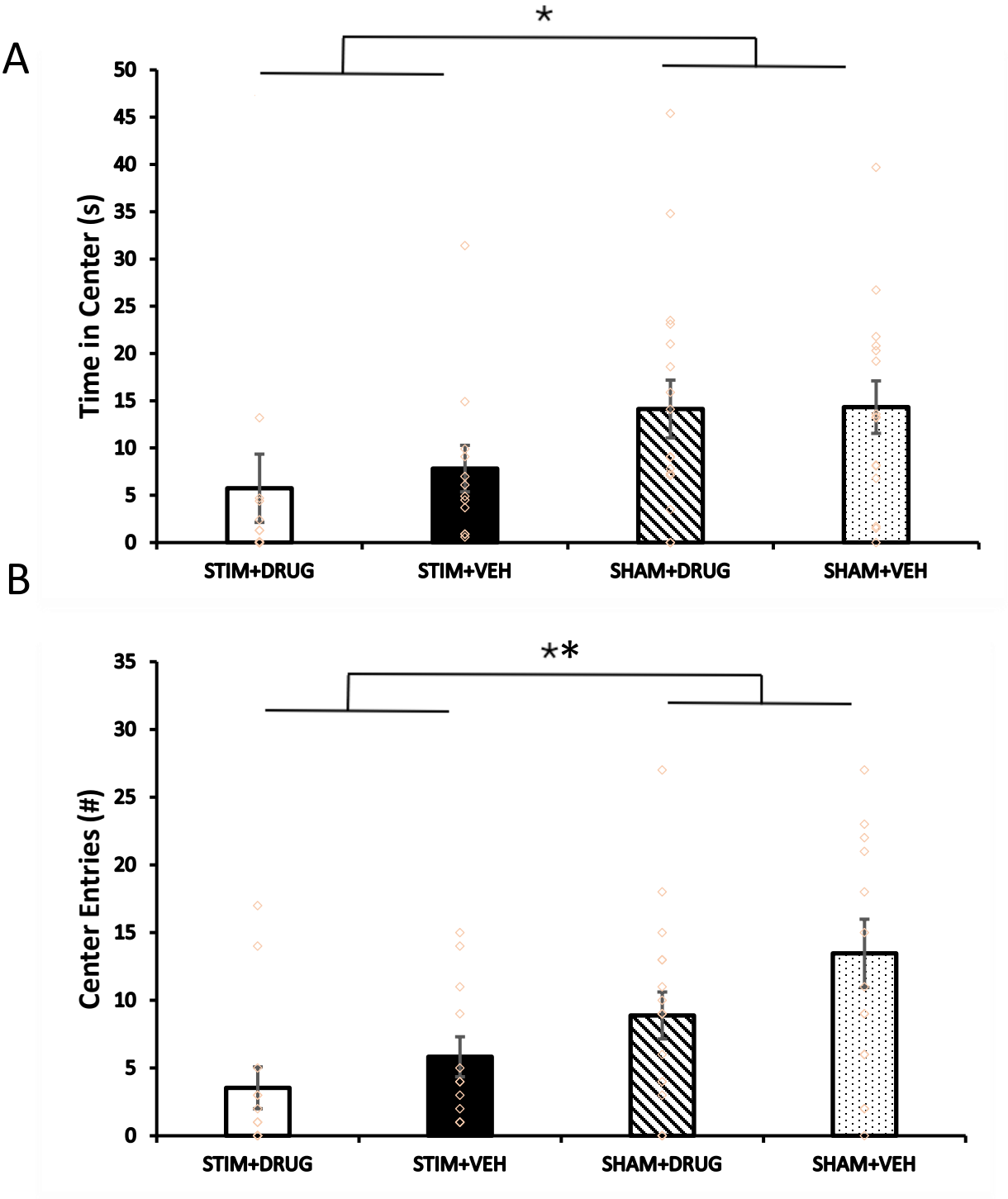


Figure 21: Average (±SEM) time spent in the center (A) and center entries (B) in the OFT in response to EC Stimulation. The results suggest that tDCS, but not NS8593, increases anxiety-like behaviour by reducing the cumulative time and entries into the center of the OFT chamber, n = 12-17/group, *$p < .05$, **$p < .01$

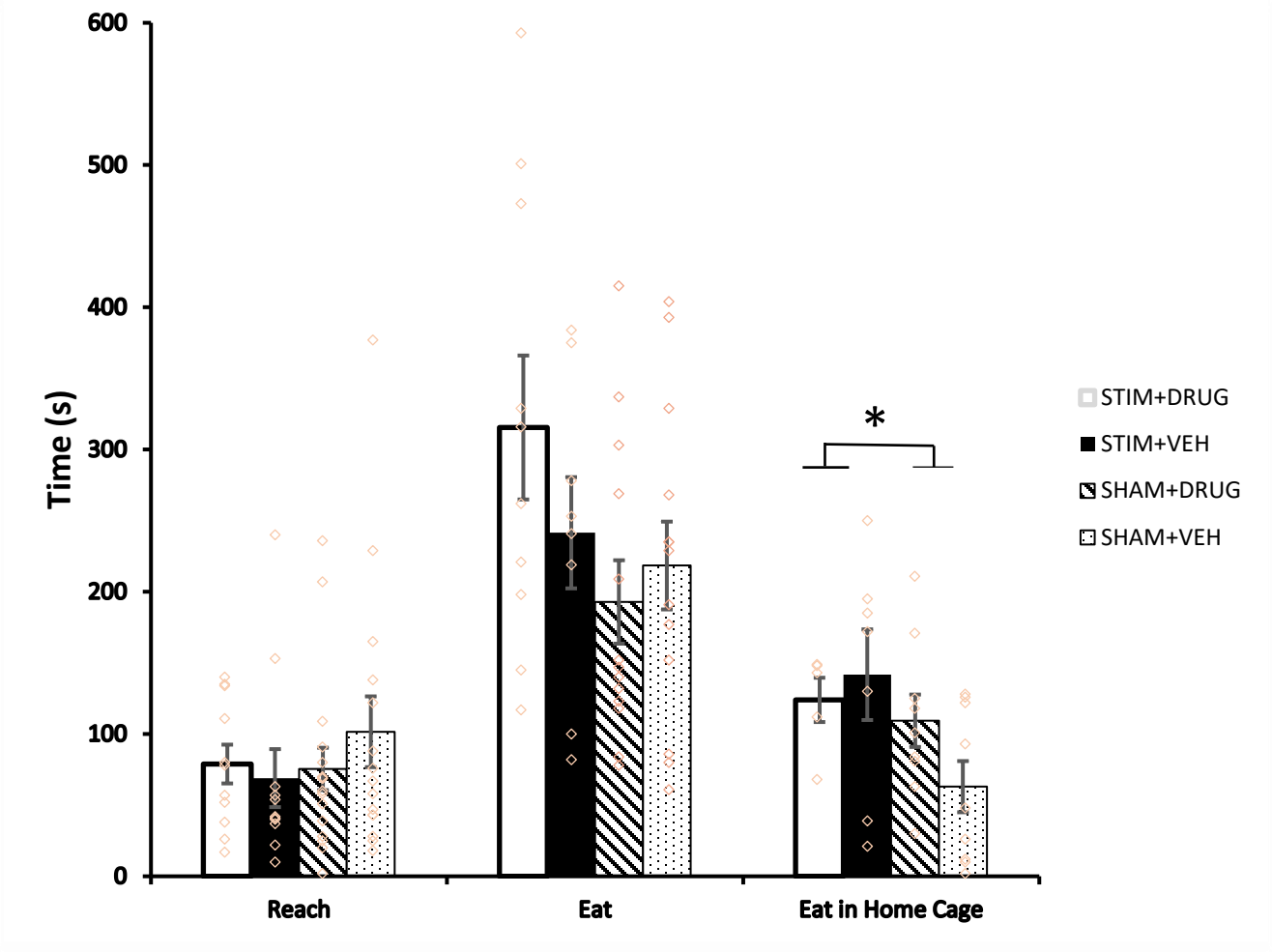


Figure 22: Average (±SEM) latency to reach food, commence eating in the arena, and commence eating in the home cage in the NSFT in response to EC Stimulation. The results indicate that tDCS, but not NS8593, increases the latency to feed within the home cage, suggesting an anxiogenic effect of stimulation, n = 5-17/group, *p = .05

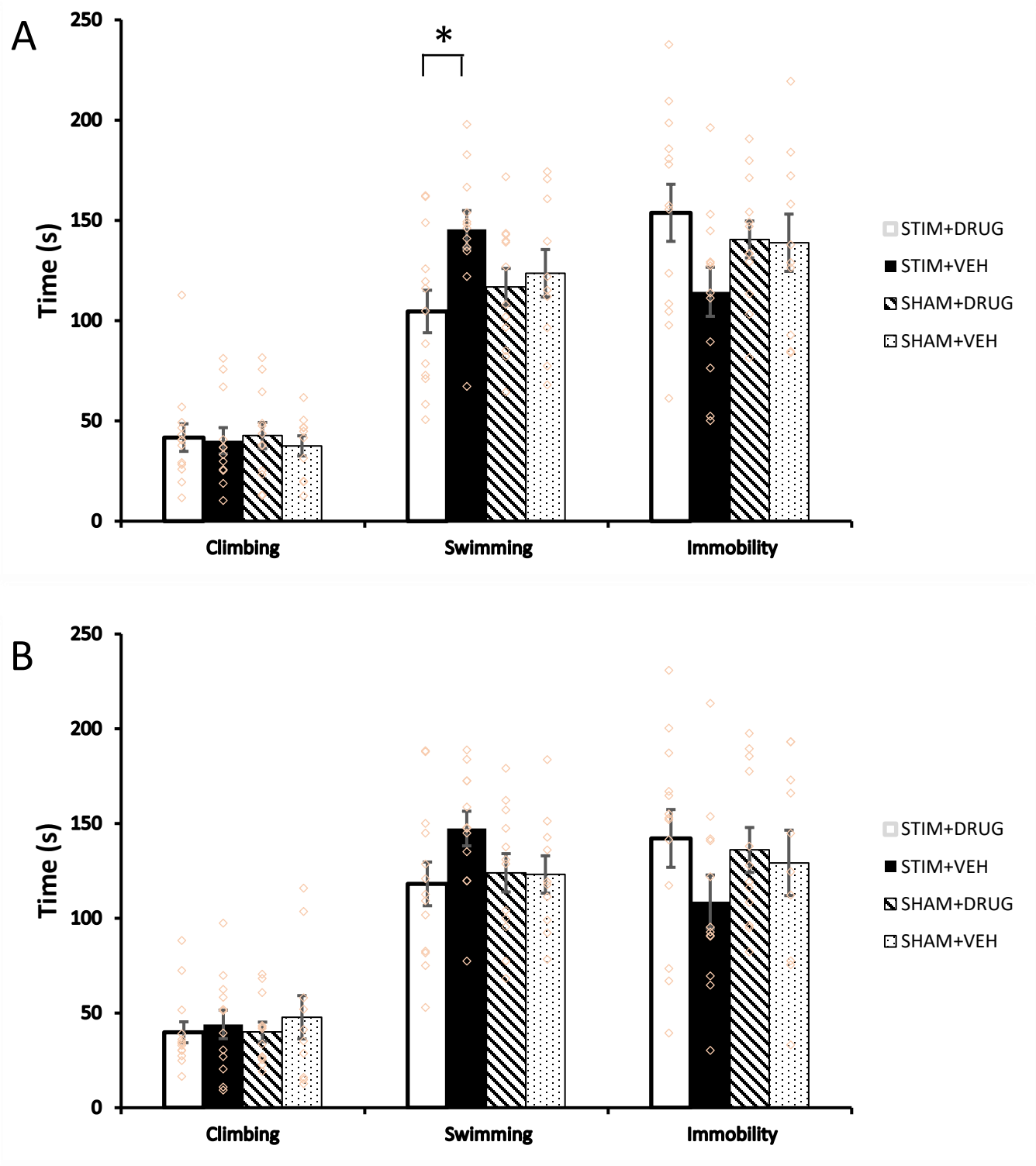


Figure 23: Average (±SEM) climbing, swimming, and immobility time in the FST in response to EC Stimulation on week 1 (A) and week 2 (B) of testing. The results indicate that tDCS does not affect passivity in the FST, but NS8593 reduces swimming behaviour following acute treatment only, n = 10-13/group, *$p < .05$

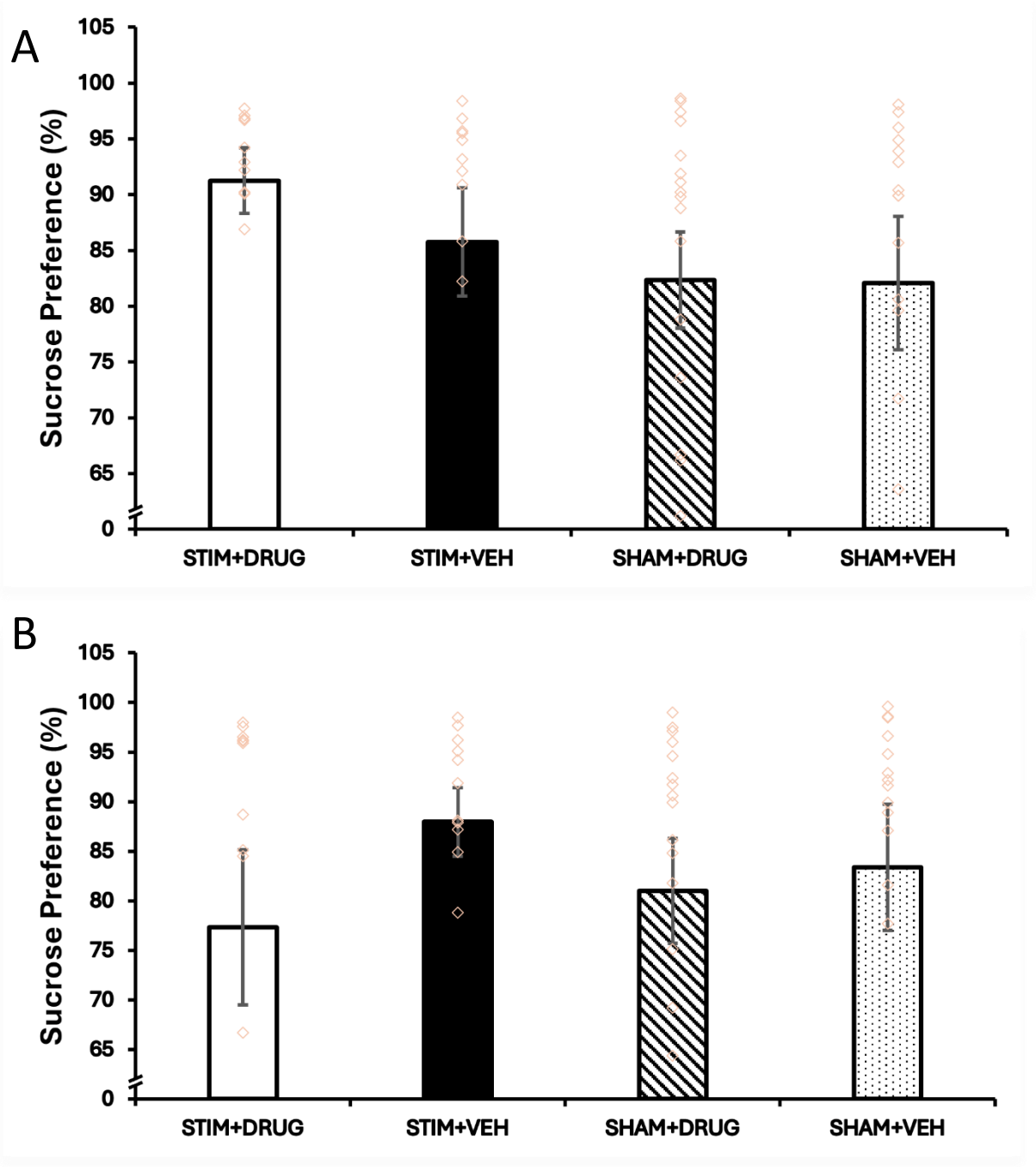


Figure 24: Average (±SEM) sucrose preference in the SPT in response to EC Stimulation on week 1 (A) and week 2 (B) of testing. No significant differences were found between groups on this measure, *n* = 12-17/group.

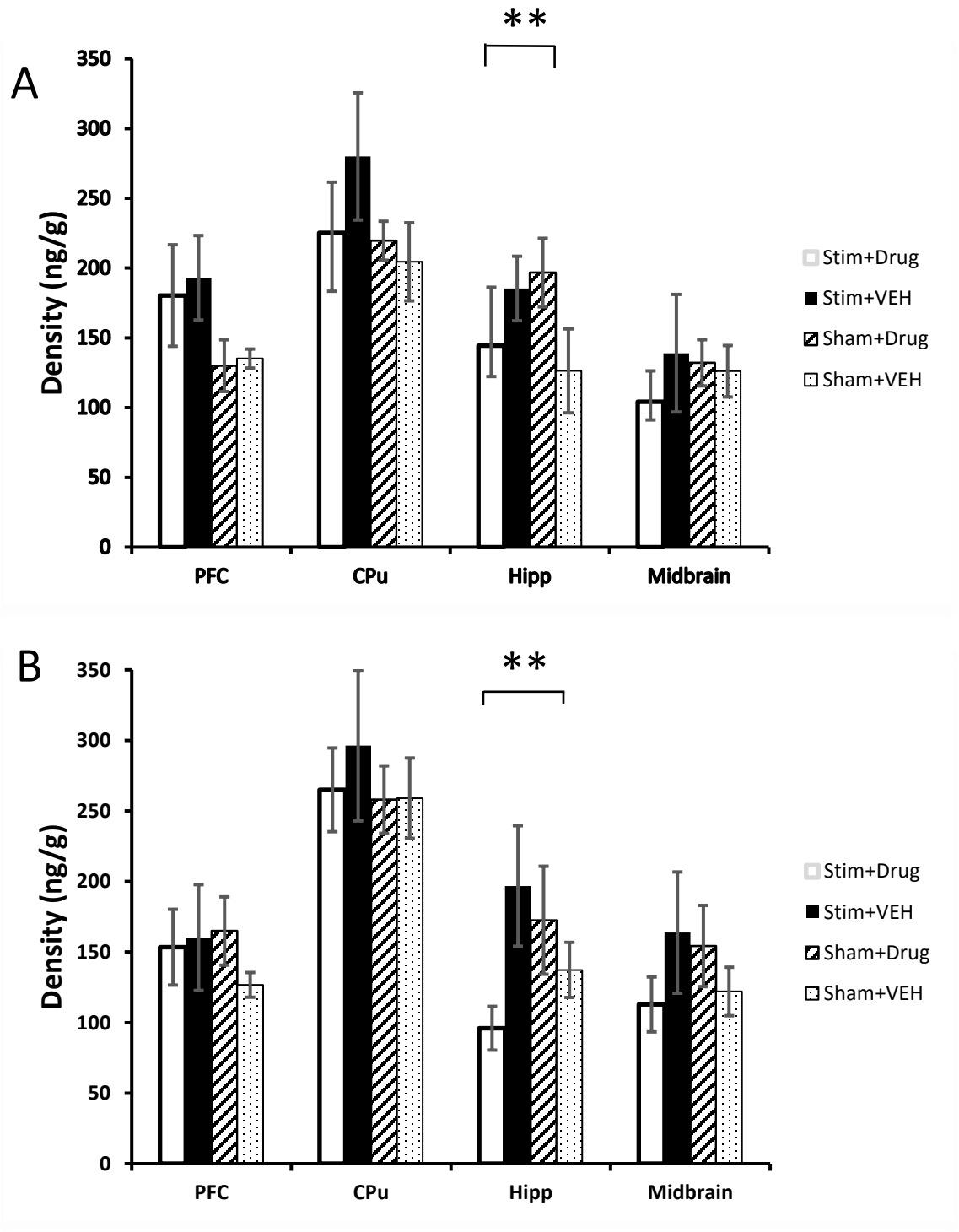


Figure 25: Average (±SEM) density of Dopamine in the left (A) and right (B) hemispheres across each brain region following EC Stimulation. The results indicate that EC stimulation, but not tDCS or NS8593 alone, reduced dopamine concentration within the hippocampus, n = 23-24/group, **$p < .01$

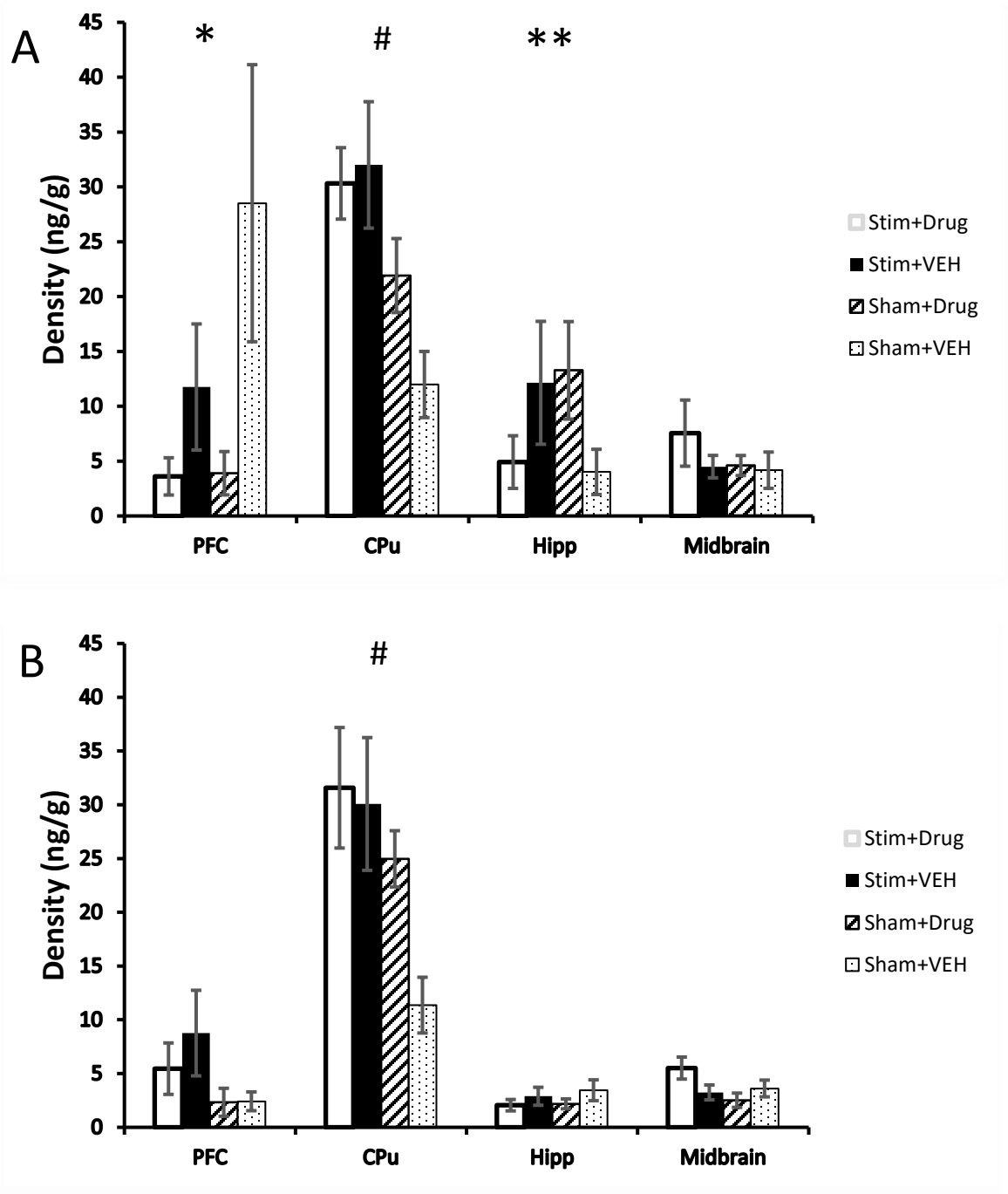


Figure 26: Average (±SEM) density of Serotonin in the left (A) and right (B) hemispheres across each brain region following EC Stimulation. The results indicate a number of alterations in 5-HT following treatment. First, NS8593 reduces 5-HT concentration in the left PFC, but not the right. Second, tDCS increases 5-HT concentration in the CPu in both the left and right hemispheres. Finally, both tDCS and NS8593 increase 5-HT density within the left hippocampus, but this effect is blocked with combinative EC stimulation, n = 23-34/group, *p < .05, **p < .01, $^{\#}$p < .001

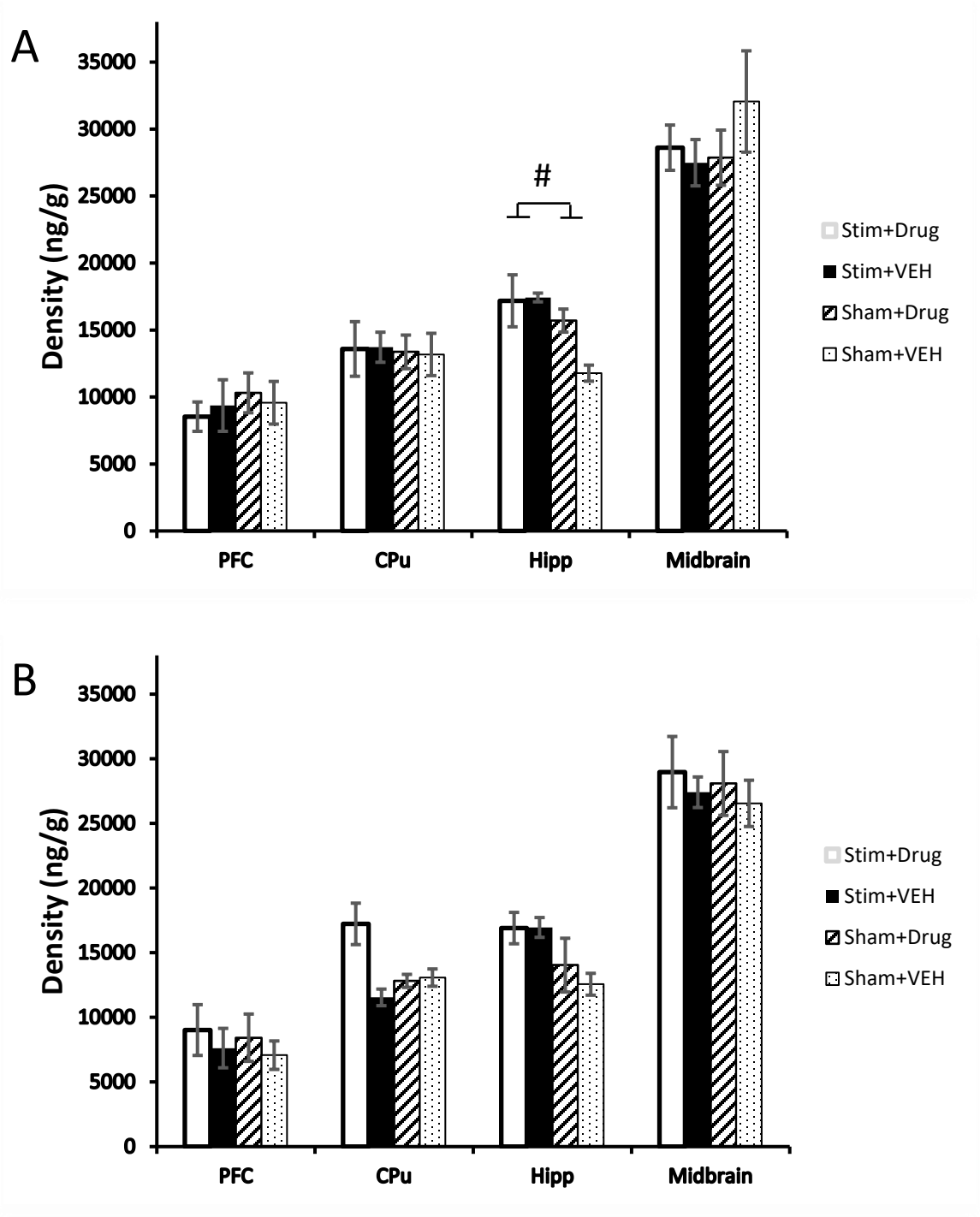


Figure 27: Average (±SEM) density of Tryptophan in the left (A) and right (B) hemispheres across each brain region following EC Stimulation. The results indicate that stimulation increases TRP concentration within the hippocampus, n = 23-24/group, $^{\#}p < .001$

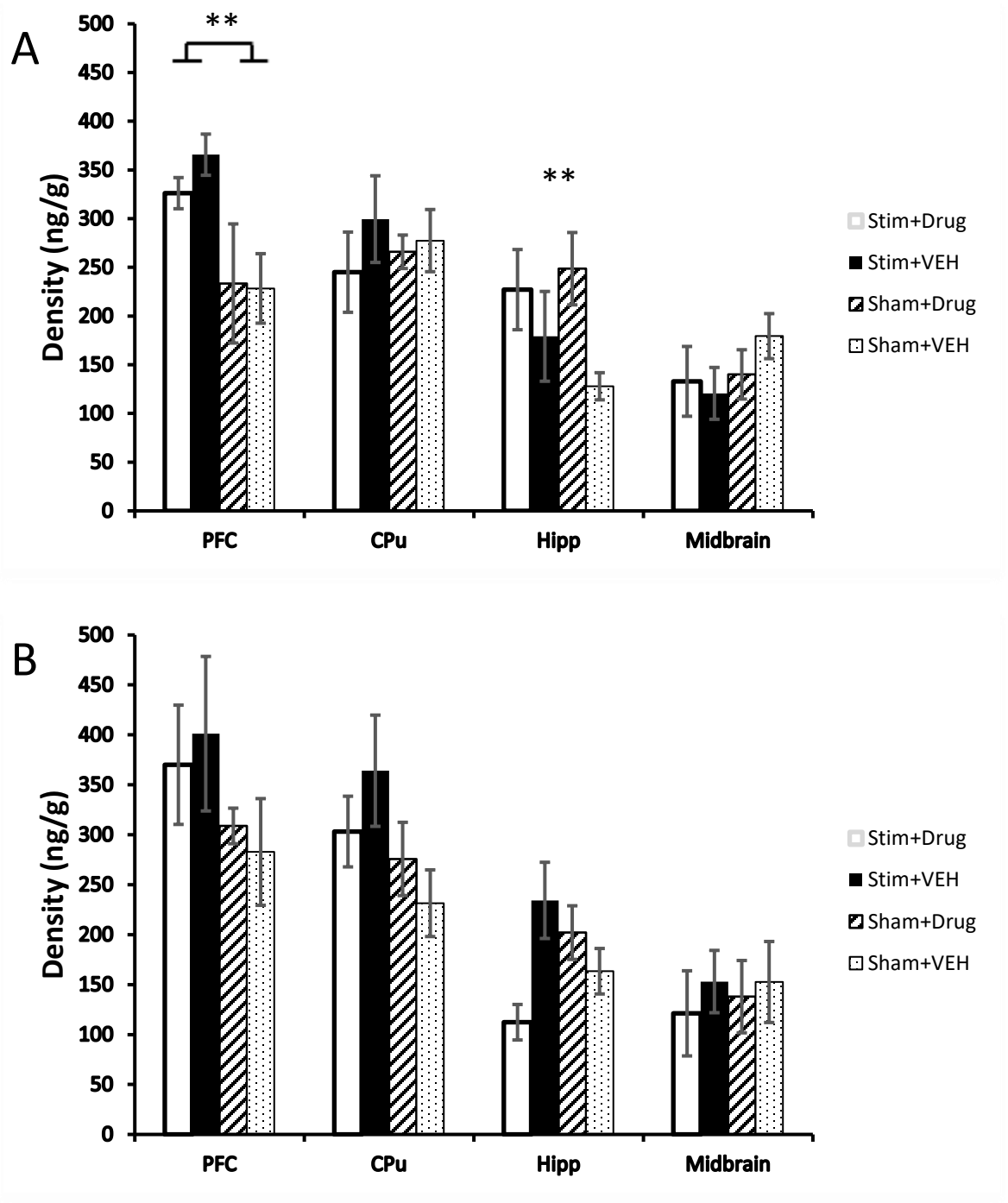


Figure 28: Average (±SEM) density of 5-hydroxyindoleacetic acid in the left (A) and right (B) hemispheres across each brain region following EC Stimulation. The results indicate that tDCS increases 5-HIAA concentration within the left PFC, while NS8593 increases 5-HIAA concentration within the left hippocampus, n = 23-24/group, **$p < .01$

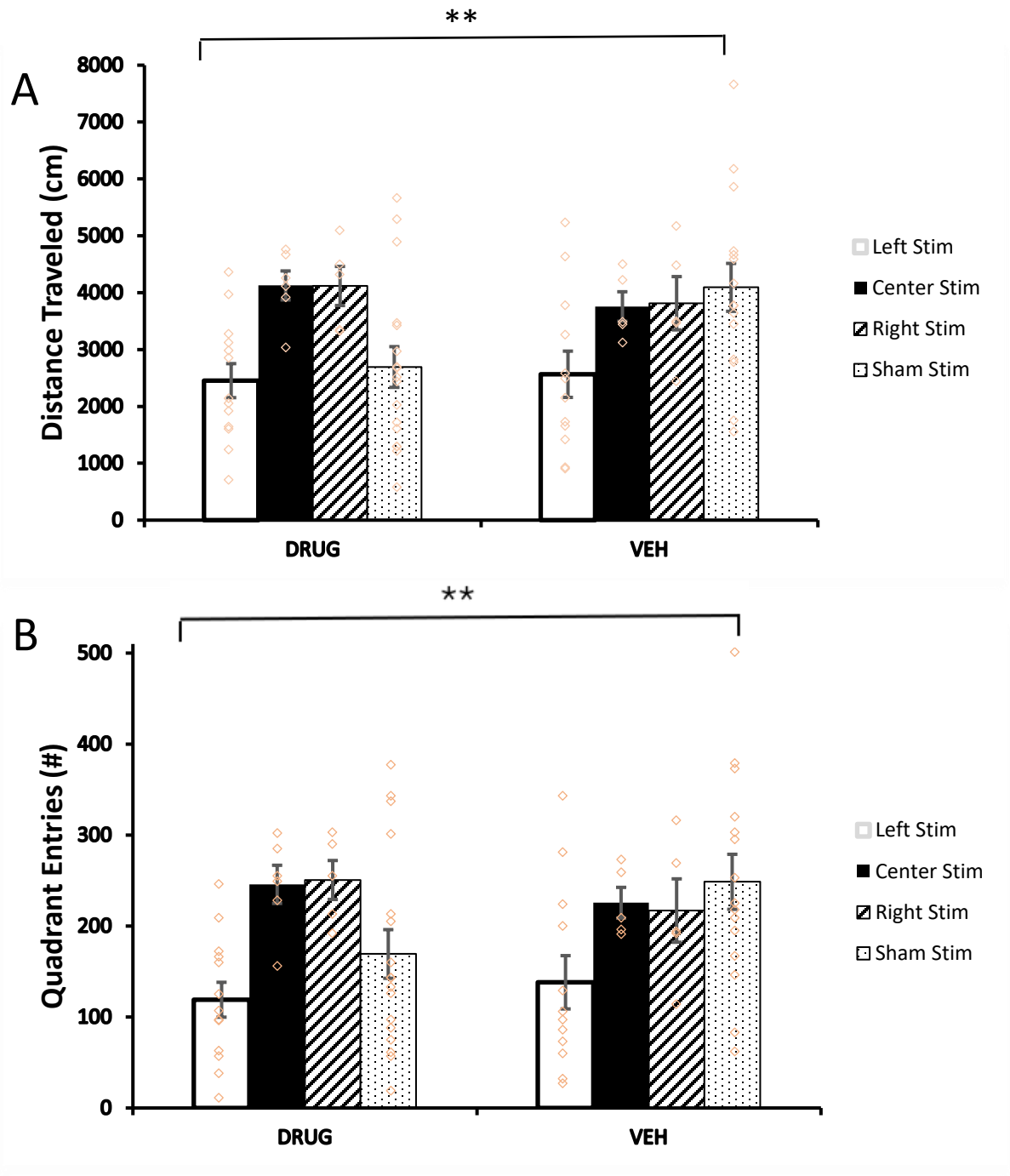


Figure 29: Average (±SEM) distance traveled (A) and quadrant entries (B) in the OFT in response to hemispheric EC Stimulation. The results indicate that left tDCS, but not center or right tDCS, decreases the distance traveled and number of quadrants entered. This suggests that stimulation of the right or center mPFC is incapable of reversing OBX-induced hyperactivity, *n* = 5-17/group, **p < .01

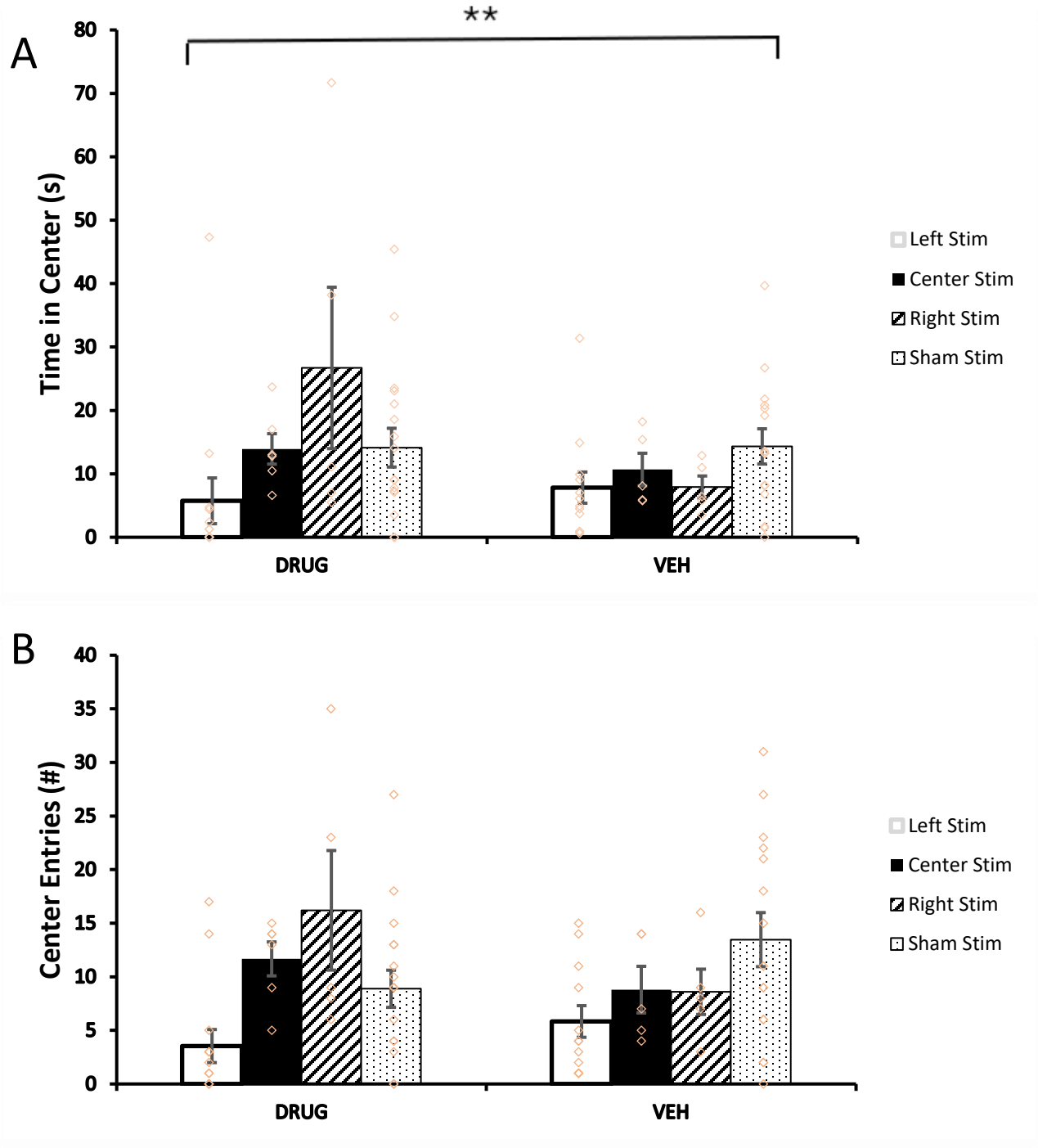


Figure 30: Average (±SEM) time spent in the center (A) and center entries (B) in the OFT in response to hemispheric EC Stimulation. The results indicate that left tDCS, but not center or right tDCS, reduces the amount of time spent in the center of the maze, suggesting that only stimulation of the left mPFC elicits an increased anxiety response, *n* = 5-17/group, **p < .01

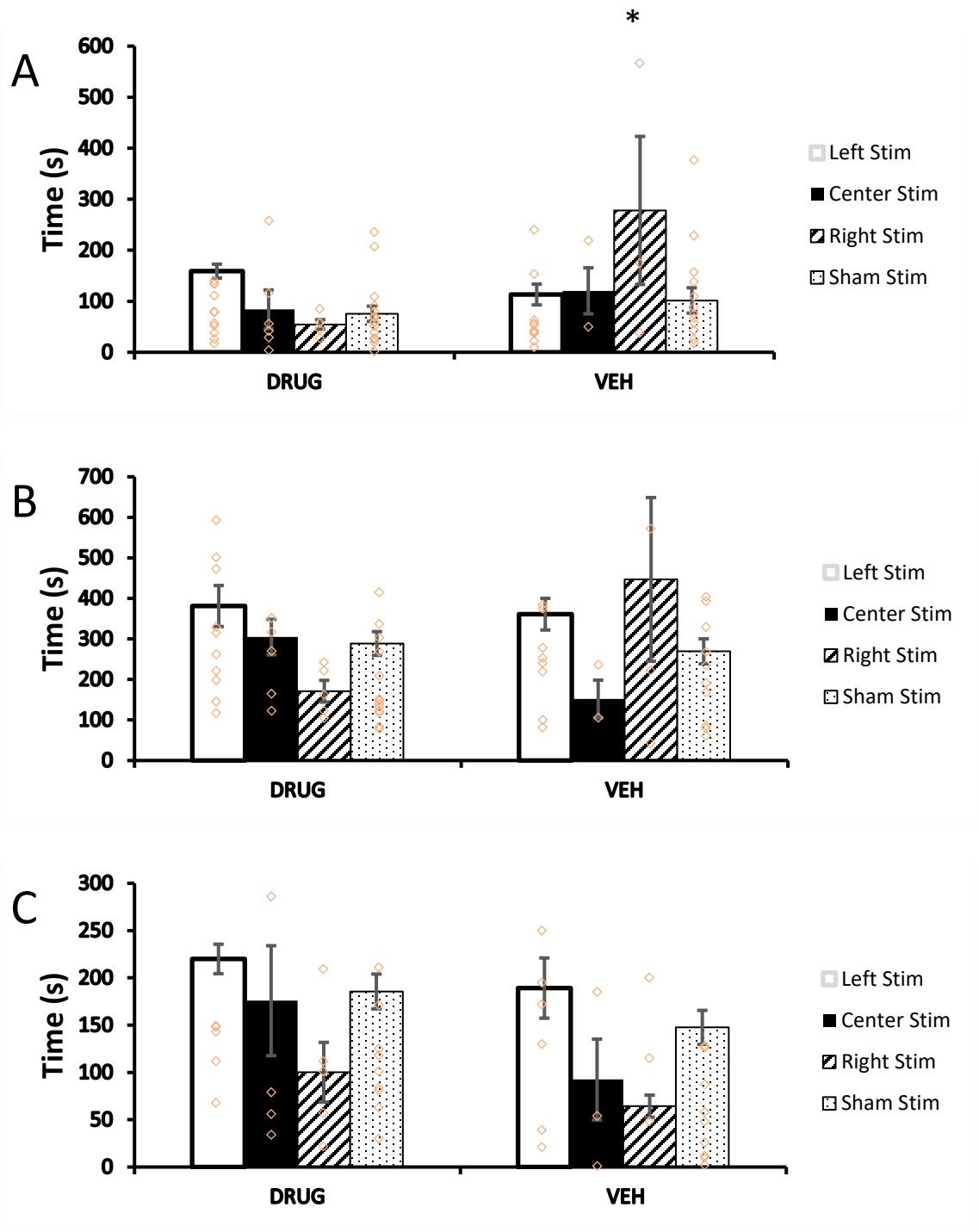


Figure 31. Average (±SEM) latency to reach food (A), commence eating in the arena (B), and commence eating in the home cage (C) in the NSFT in response to hemispheric EC Stimulation. Our results indicate that right tDCS increases the latency to approach food in the NSFT, suggesting an increase in anxiety-like behaviour that is blocked by concurrent NS8593 treatment. Many animals within the right and center tDCS groups exhibited high levels of anxiety and failed to complete the test within the 10-min trial, resulting in small group sizes, n = 2-17/group, *$p < .05$

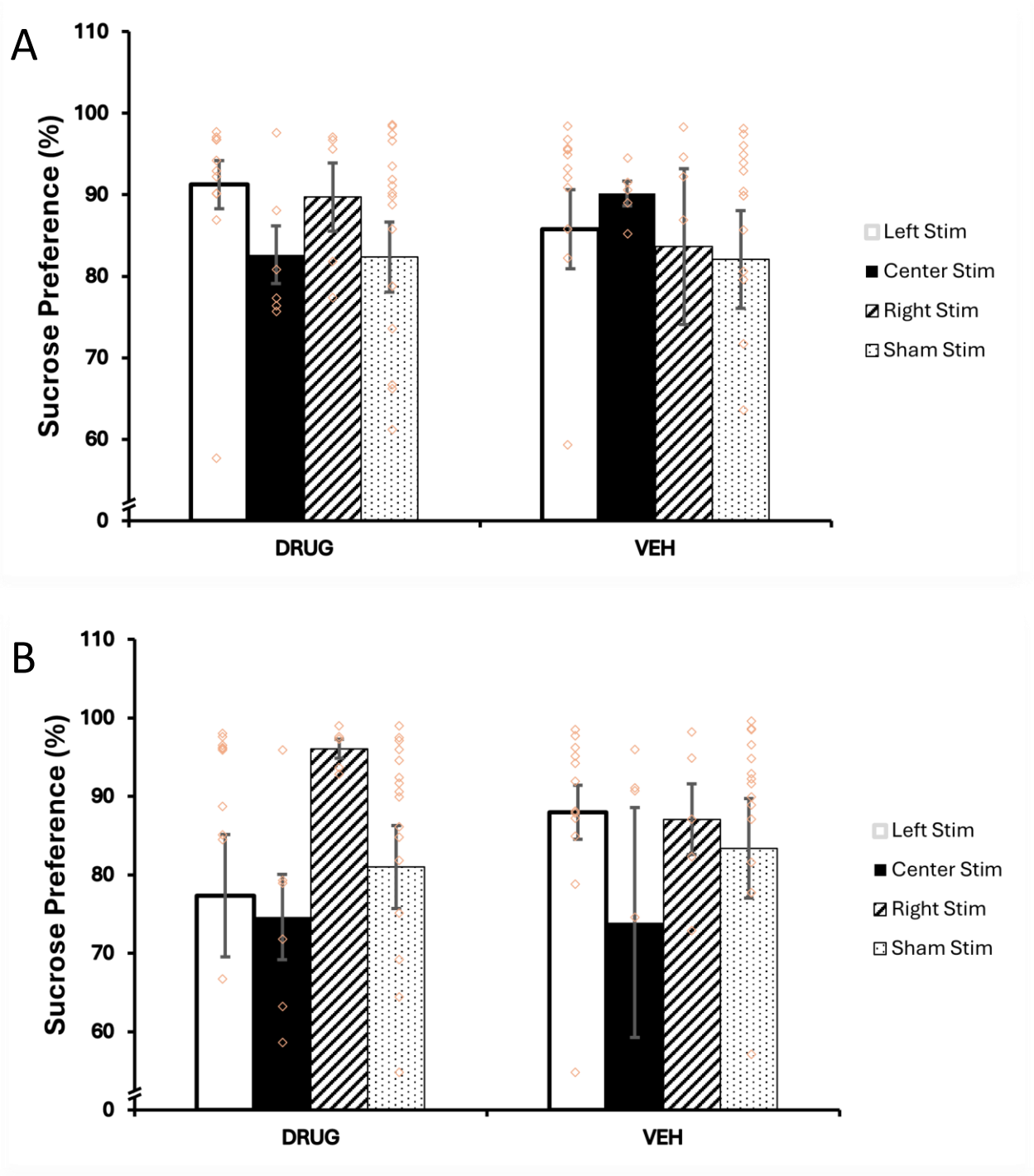


Figure 32. Average (±SEM) sucrose preference in the SPT in response to hemispheric EC Stimulation on week 1 (A) and week 2 (B) of testing. No significant differences were found between groups on this measure, *n* = 5-17/group.

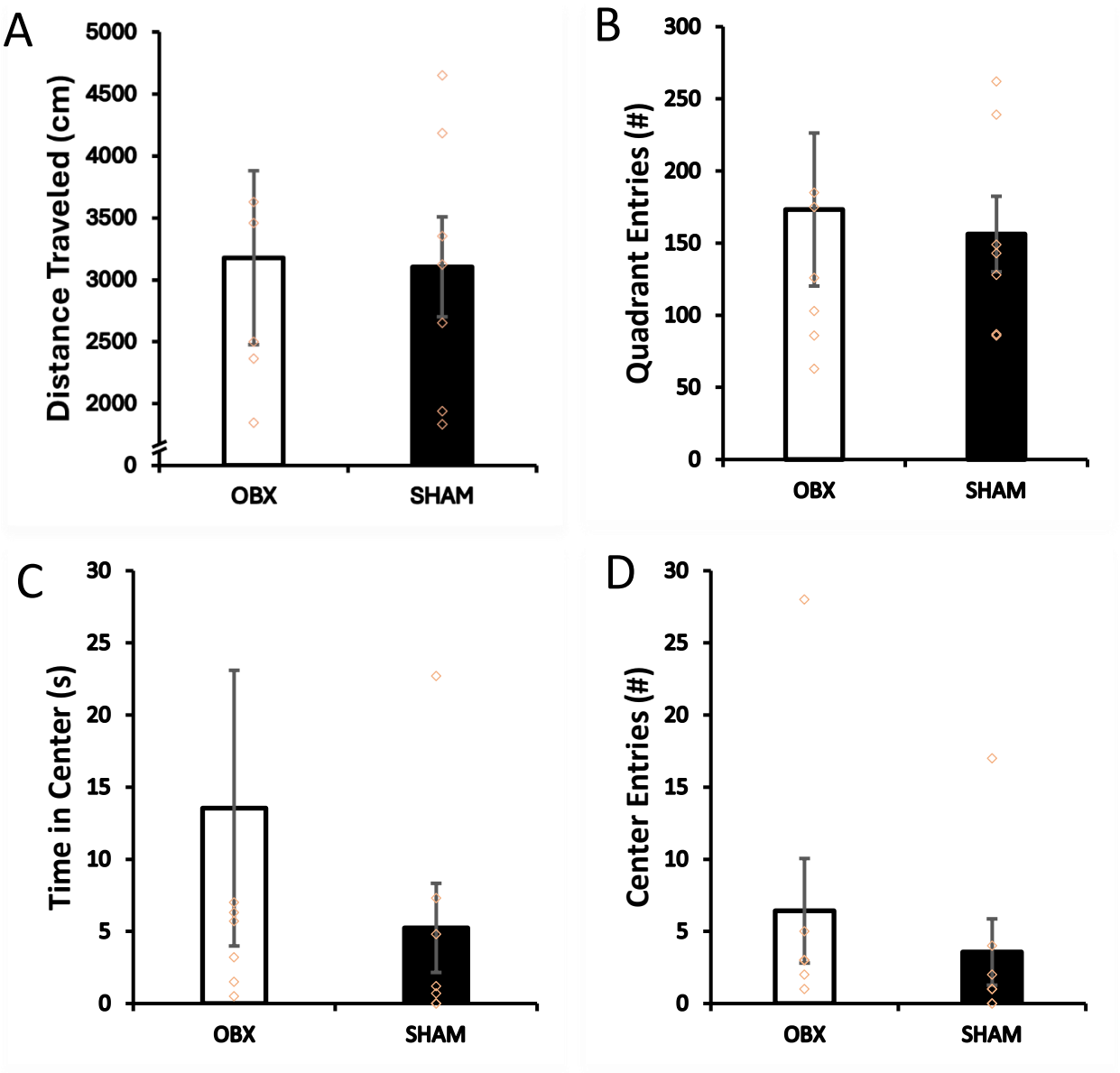


Figure 33: Average (±SEM) distance traveled (A), number of quadrants visited (B), time spent in the center of the arena (C), and number of center entries (D) in the OFT by female rats following OBX. No significant differences were found between groups on these measures, *n* = 7/group.

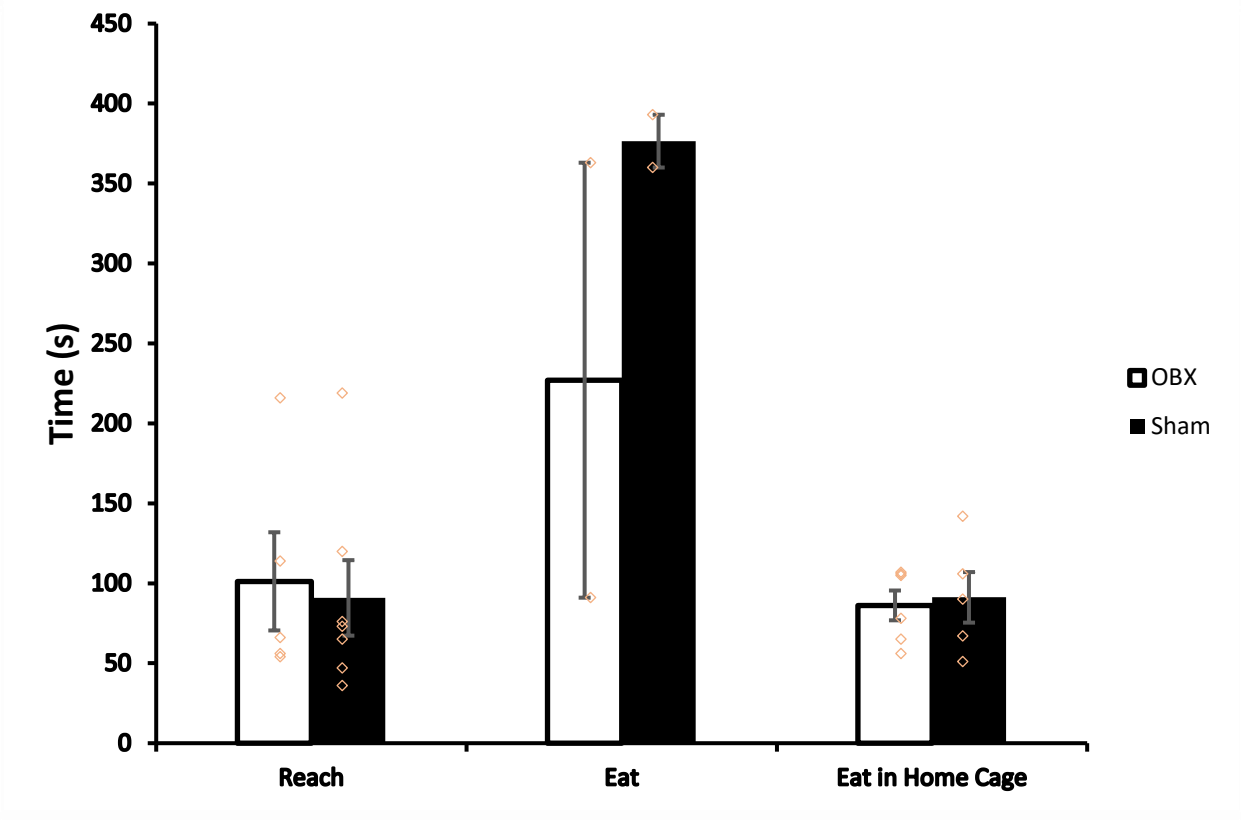


Figure 34: Average (±SEM) latency to reach, eat within the chamber, and eat within the home cage in the NSFT by female rats following OBX. No significant differences were found between groups on these measures. Many animals exhibited high levels of anxiety and failed to complete the test within the 10-min trial, leading to small group sizes, *n* = 2-7/group.

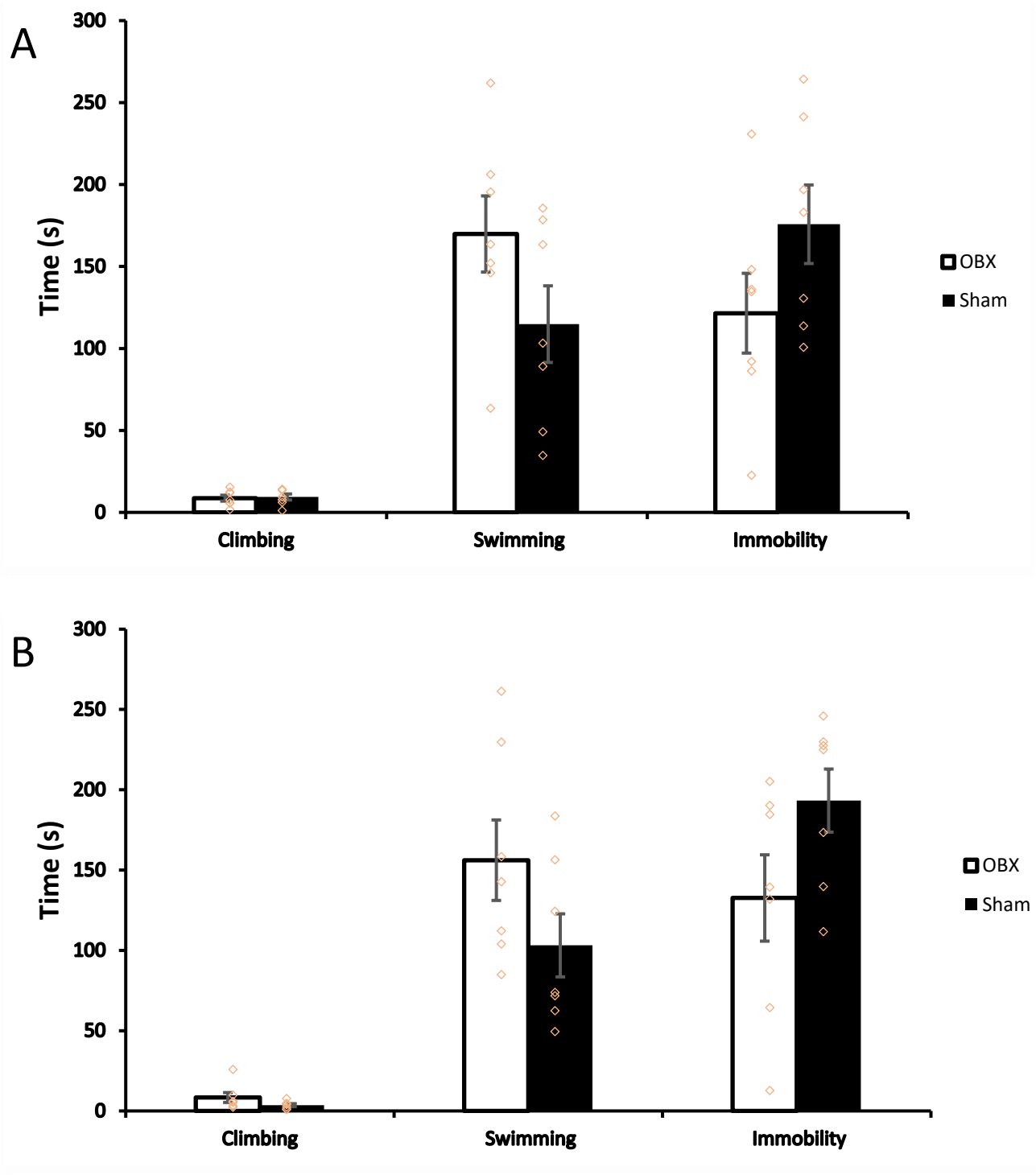
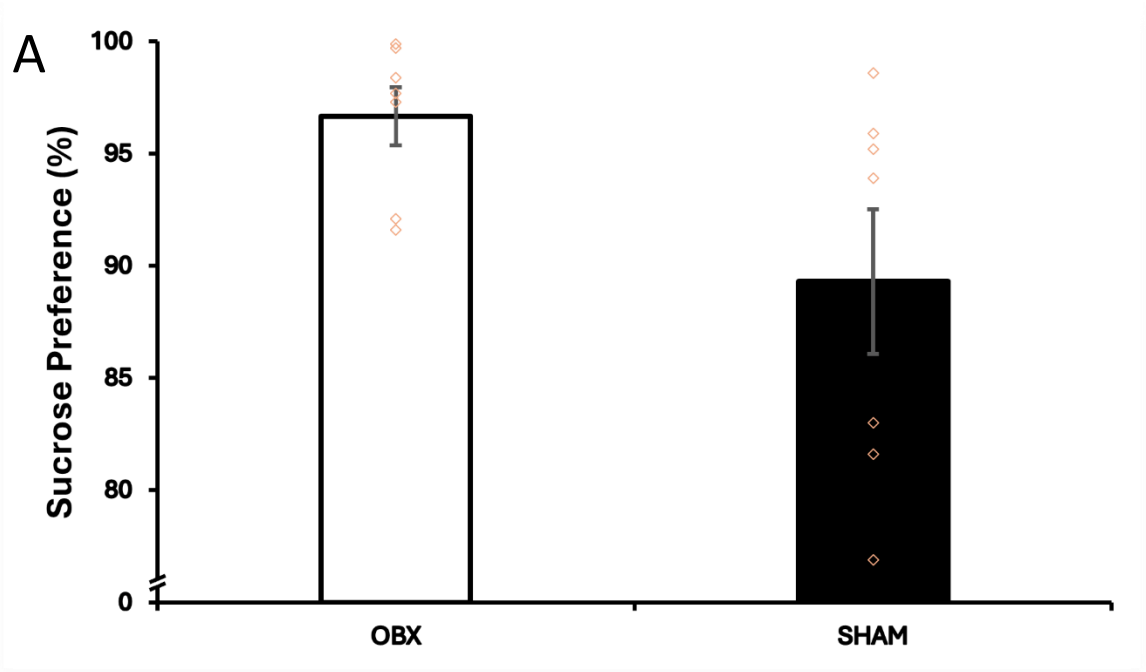


Figure 35: Average (±SEM) climbing, swimming, and immobility time in the FST by female rats following OBX on week 1 (A) and week 2 (B) of testing. No significant differences were found between groups on these measures, *n* = 7/group.



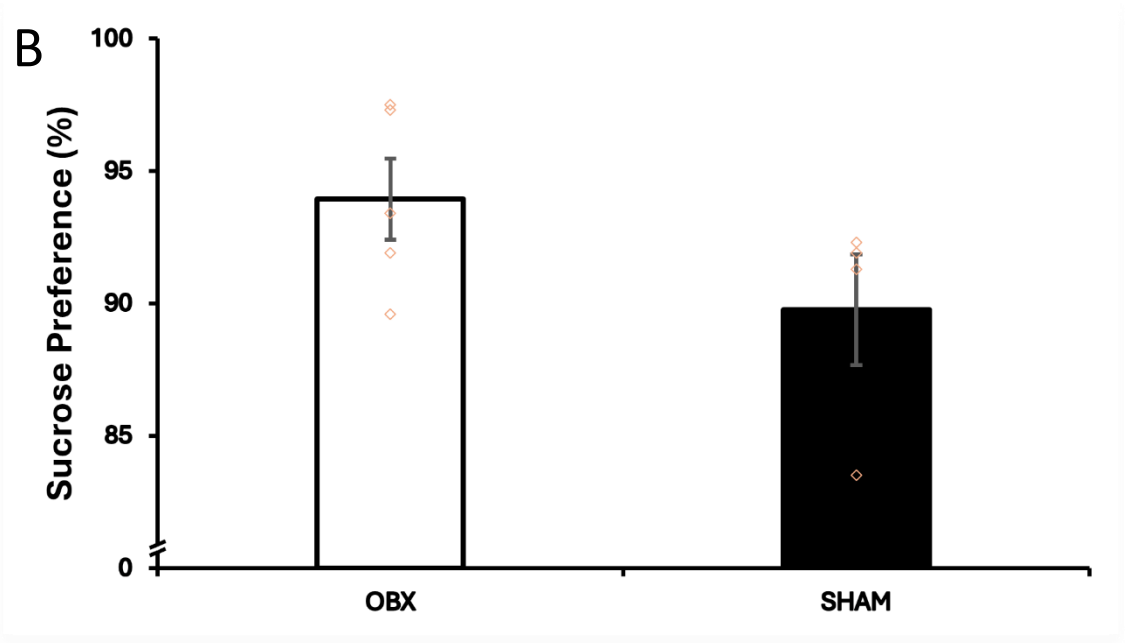


Figure 36: Average (±SEM) sucrose preference in the SPT by female rats following OBX on week 1 (A) and week 2 (B) of testing. No significant differences were found between groups on this measure, *n* = 4-7/group.

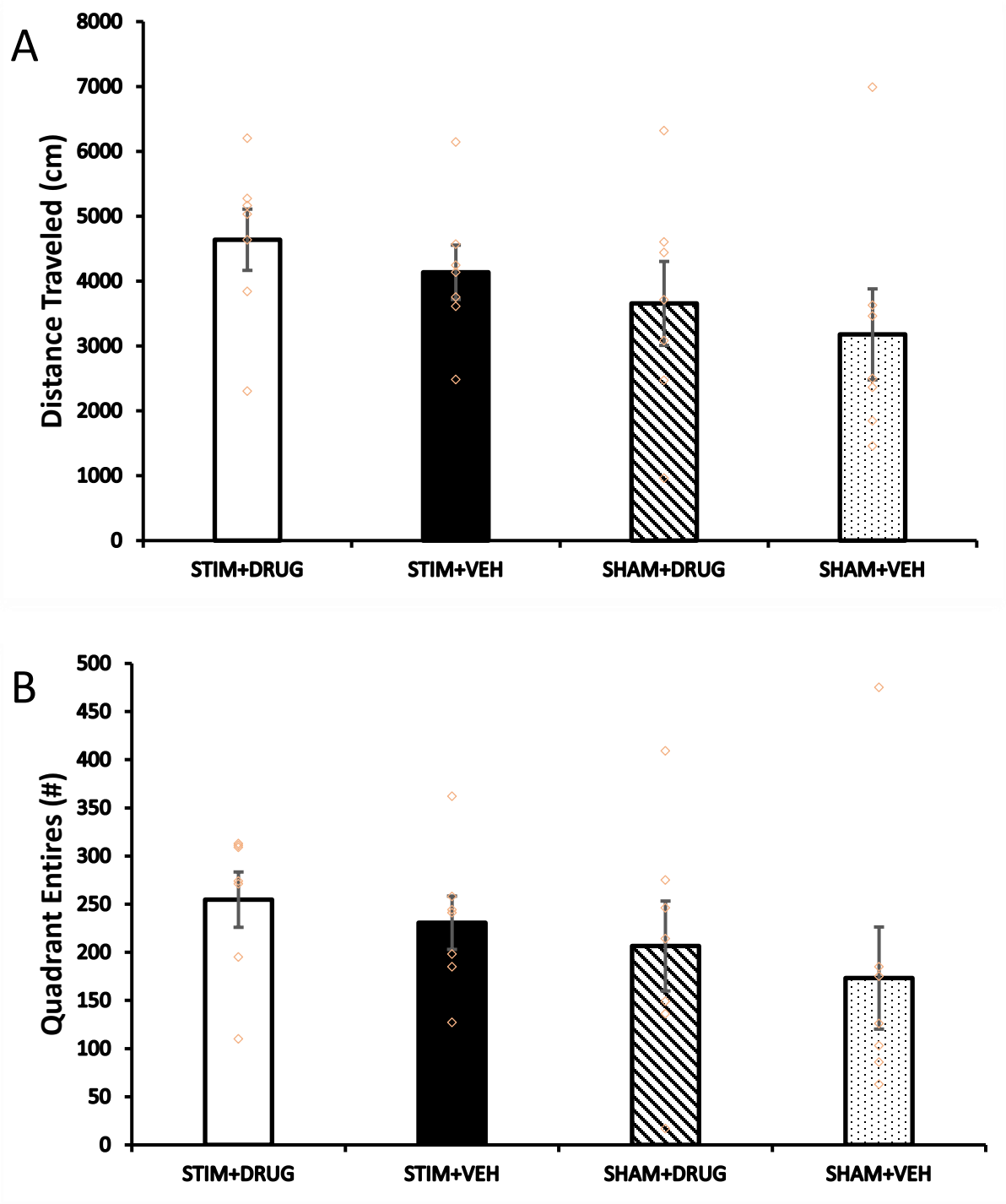


Figure 37: Average (±SEM) distance traveled (A) and quadrant entries (B) in the OFT by female rats in response to EC Stimulation. No significant differences were found between groups on these measures, *n* = 7/group.

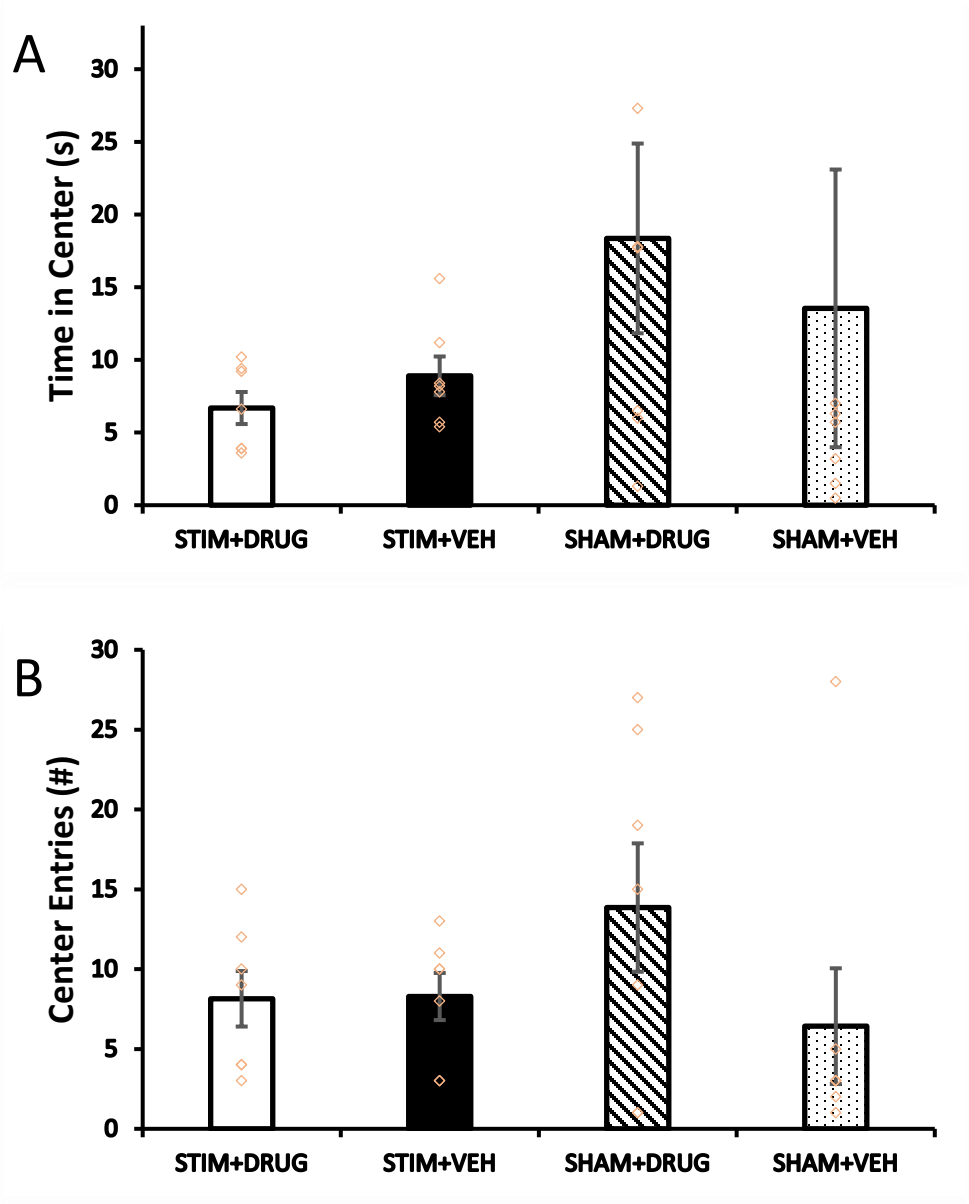


Figure 38: Average (±SEM) time spent in the center (A) and center entries (B) in the OFT by female rats in response to EC Stimulation. No significant differences were found between groups on these measures, *n* = 7/group.

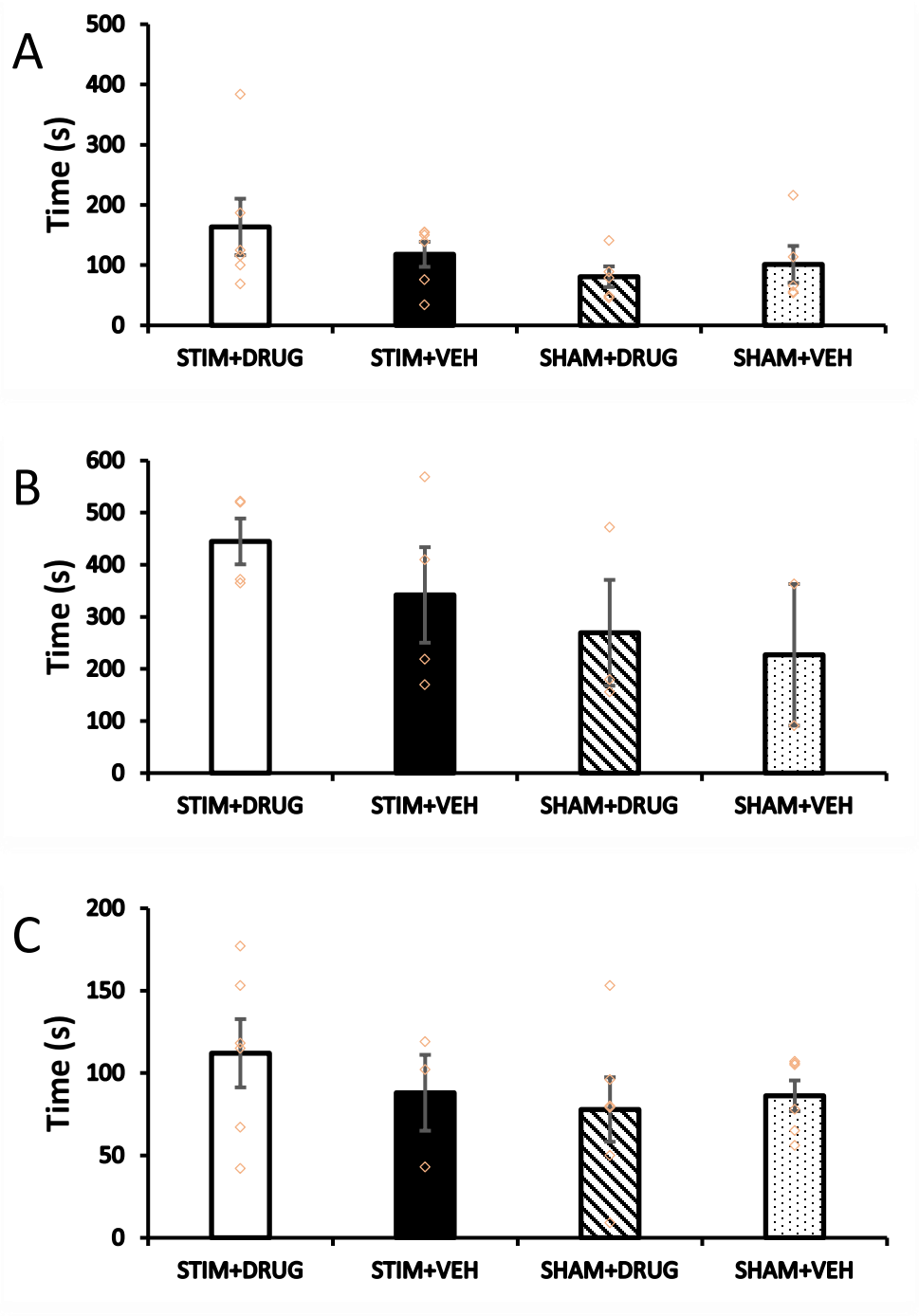


Figure 39: Average (±SEM) latency to reach food (A), commence eating in the arena (B), and commence eating in the home cage (C) in the NSFT by female rats in response to EC Stimulation. No significant differences were found between groups on these measures. Many animals exhibited high levels of anxiety and failed to complete the test within the 10-min trial, leading to small group sizes, *n* = 2-6/group.

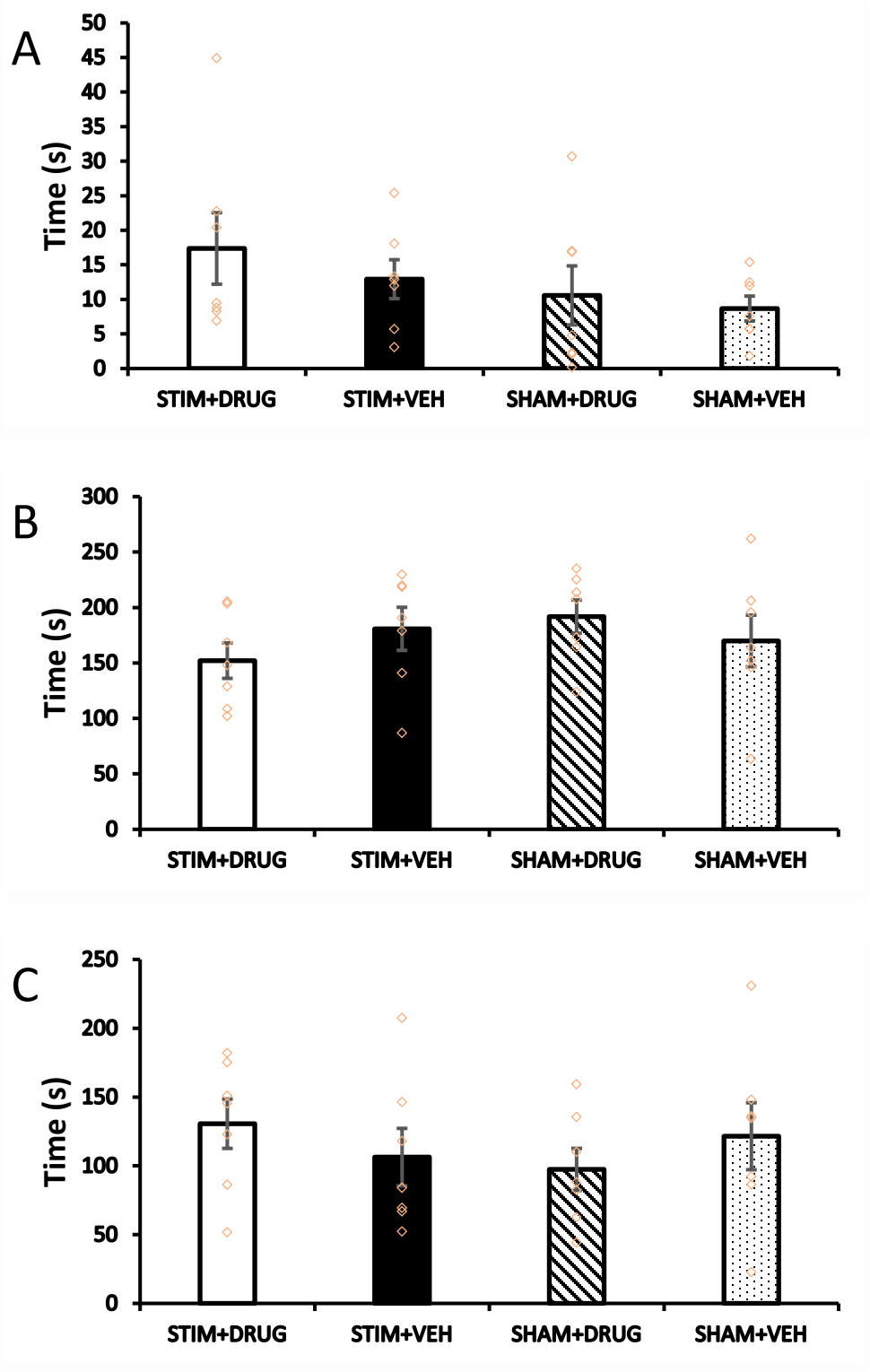


Figure 40: Average (±SEM) climbing (A), swimming (B), and immobility time (C) in the FST by female rats in response to EC Stimulation on week 1. No significant differences were found between groups on these measures, *n* = 7/group.

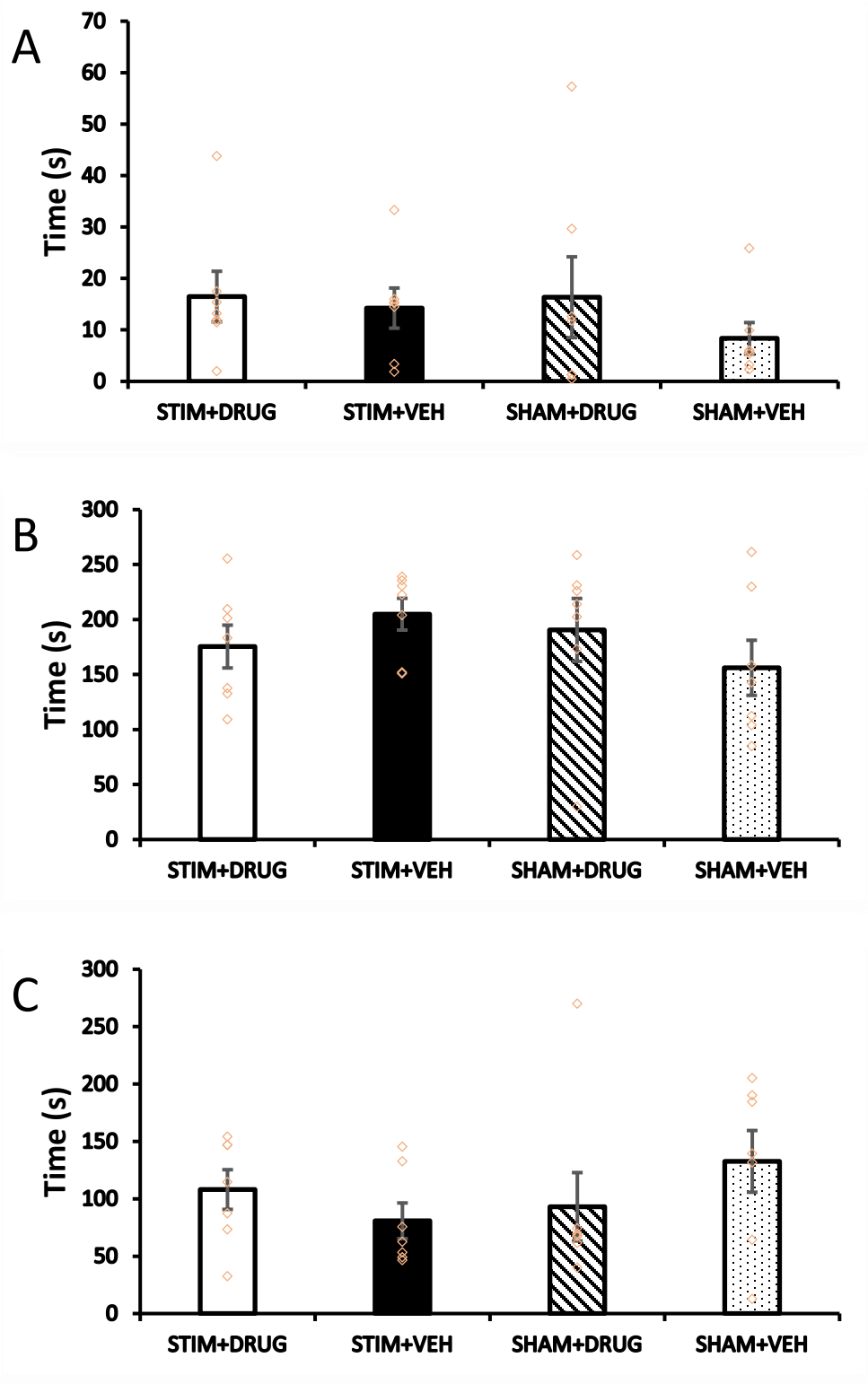


Figure 41: Average (±SEM) climbing (A), swimming (B), and immobility time (C) in the FST by female rats in response to EC Stimulation on week 2. No significant differences were found between groups on these measures, *n* = 7/group.

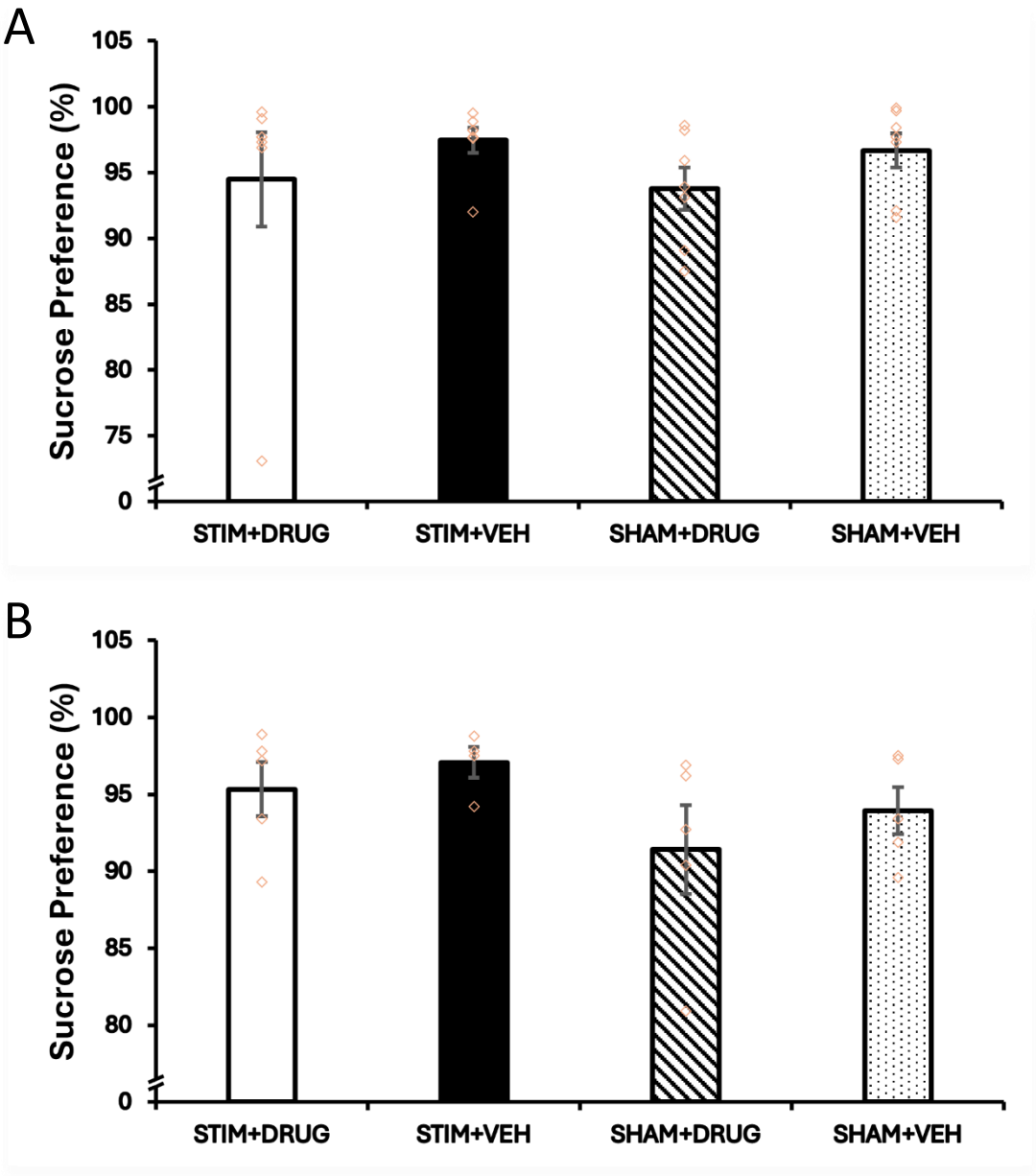


Figure 42: Average (±SEM) sucrose preference in the SPT by female rats in response to EC Stimulation on week 1 (A) and week 2 (B) of testing. No significant differences were found between groups on these measures, *n* = 4-7/group.