TRANSCRANIAL DIRECT CURRENT STIMULATION (TDCS) REVERSES THE INITIAL PARADOXICAL EFFECTS OF PAROXETINE IN OLFACTORY BULBECTOMIZED ADOLESCENT RATS

By © Shannon Waye

A Thesis submitted

to the School of Graduate Studies in partial fulfillment of the

requirements of the degree of

Master of Science, Department of Psychology, Faculty of Science

Memorial University of Newfoundland

October 2019

St. John's, Newfoundland and Labrador

Abstract

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that sends weak electrical current through the skull, resulting in neuroplastic changes. We examined its antidepressant properties using adolescent rats, as current antidepressant drug treatment during pre-pubescent stages often proves ineffective and may also result in worsening of depressive symptoms. We induced a depressive-like phenotype in adolescent Sprague-Dawley rats using olfactory bulbectomy (OBX), a rodent model of depression that results in behavioural and neurochemical changes that are reversed by antidepressant treatment. We examined if acute (two days) or chronic (two weeks) tDCS treatment resulted in reductions of OBX-induced depressive-like behavioural symptoms, including hyperlocomotion in an open field chamber, latency to feed in a novelty suppressed feeding test, immobility in a forced swim test, and deceased sucrose consumption, and if these effects were achievable with tDCS alone or in combination with paroxetine, a selective serotonin reuptake inhibitor. Finally, we examined whether the antidepressant-like activity of tDCS is linked to its capacity to increase the growth-stimulating protein brain-derived neurotrophic factor (BDNF) by collecting blood plasma for an enzyme-linked immunosorbent assay (ELISA). We found that both OBX surgery and paroxetine treatment increased depressive-like behaviours in adolescent rats, and that tDCS reversed these effects. Furthermore, OBX resulted in a decrease in plasma BDNF, an effect that was resistant to both tDCS and paroxetine treatment. Overall, our results suggest that tDCS is an effective adjunct treatment for adolescent depression in combination with antidepressant drugs.

Acknowledgements

First and foremost, I would like to thank my supervisor, Dr. Francis Bambico, for his guidance and assistance throughout the program. His vast depths of knowledge, as well as his generosity in allowing me to travel across the country on multiple occasions to further my education and training, have been critical components in my growth as a person and as a researcher.

I would also like to thank Dr. Darlene Skinner for her many years of mentorship and for teaching me proper experimental and surgical techniques. Without her guidance I would be half the scientist I am today. Also thank you to Dr. Jacqueline Blundell for acting as both my co-supervisor and a member of my committee.

Thank you to the members of TRoN lab for their assistance throughout the project, especially Patric McGrath for helping me prepare samples for transport to the Centre for Addiction and Mental Health (CAMH) in Toronto. I also must thank Dr. Jose Nobrega, Roger Raymond, and Meagan Billyard of CAMH for helping me perform ELISA and other brain analyses, something that would have been incredibly difficult for me to do on my own.

Finally, I would like to thank Marie Wasef, whose dedication, passion, and hard-working attitude has no doubt rubbed off onto me over the years. Her influence has shaped me into a more industrious and conscientious worker…even though she is an egg.

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ADHD	Attention deficit hyperactivity disorder
ASD	Autism spectrum disorder
BDI	Beck depression inventory
BDNF	Brain-derived neurotrophic factor
CaMKII	Ca^{2+} /calmodulin-dependent protein kinase II
CBT	Cognitive behavioural therapy
ChAT	Choline acetyltransferase
CREB	cAMP response element-binding protein
DBS	Deep brain stimulation
DLPFC	Dorsolateral prefrontal cortex
ELISA	Enzyme-linked immunosorbent assay
EPM	Elevated plus maze
fMRI	Functional magnetic resonance imaging
FST	Forced swim test
GFAP	Glial fibrillary acidic protein
LTP	Long term potentiation
NSFT	Novelty suppressed feeding test
N-S-P	Non-OBX, sham stimulation, paroxetine
N-S-S	Non-OBX, sham stimulation, saline
N-T-P	Non-OBX, tDCS, paroxetine
N-T-S	Non-OBX, tDCS, saline
OBX	Olfactory bulbectomy
OFT	Open field test
O-S-P	OBX, sham stimulation, paroxetine
O-S-S	OBX, sham stimulation, saline
O-T-P	OBX, tDCS, paroxetine
O-T-S	OBX, tDCS, saline
PFC	Prefrontal cortex
PND	Post natal day
PSA-NCAM	Polysialylated neuronal cell adhesion molecule
SPT	Sucrose preference test
SSRI	Selective serotonin reuptake inhibitor
tDCS	Transcranial direct current stimulation
VTA	Ventral tegmental area

Transcranial Direct Current Stimulation (tDCS) Reverses the Initial Paradoxical Effects of Paroxetine in Olfactory Bulbectomized Adolescent Rats

Major depressive disorder is a debilitating condition that underlies many other psychiatric disorders and leads as a cause of burden and disability (Ferrari et al., 2013). With a 12-month prevalence rate of 11.3% in adolescents (ages 12-17) and 9.6% in young adults (ages 18-25), it is one of the most common mental health disorders affecting young people, and this rate is suggested to be increasing (Mojtabai, Olfson, & Han, 2016). This high prevalence suggests that depression commonly develops in adolescence and then persists into adulthood (Naicker, Galambos, Zeng, Senthilselvan, & Colman, 2013). However, the nature of depression changes with development, resulting in a heterogeneous disorder that manifests differently in adolescents and adults. For instance, vegetative symptoms, such as weight changes and lethargy, are more common in adolescents while cognitive symptoms, such as concentration problems, are more common in adults (Rice et al., 2019). In addition to divergent symptoms, responses to treatment also vary with age. In contrast to adults, antidepressant drugs show little therapeutic effect among adolescents, and may even worsen depression-related symptoms such as anxiety and suicidality risk (Goldsmith & Moncrieff, 2011; Vitiello & Ordóñez, 2016). These age-dependent differences in depression likely correspond to differences in neural structure and function between depressed adults and adolescents.

Neuroanatomy of Depression

Depression is a complex psychiatric disorder that is associated with abnormalities in the structure, function, and connectivity of various brain regions (Mayberg, 1997; Singh & Gotlib, 2014). Adults and adolescents affected by the disorder share many of

these abnormalities, including altered volume and activity of the prefrontal cortex, limbic areas, and the anterior cingulate cortex (Kerestes, Davey, Stephanou, Whittle, & Harrison, 2014; Pandya, Altinay, Malone, & Anand, 2012; Yang et al., 2010; Yang et al., 2009).

The prefrontal cortex (PFC) is a common area of interest in depression research because of its role in the cognitive regulation of emotion (Kerestes et al., 2014). Indeed, Dunlop et al. (2017) found that larger resting-state functional connectivity scores between prefrontal regions were associated with an increased effectiveness of cognitive behavioural therapy (CBT) but not antidepressant drug treatment, with smaller connectivity scores producing the opposite effect. CBT focuses on changing a patient's perceptions about their own feelings, providing support for the role of the PFC in the cognitive aspect of depression. To further characterize the involvement of the PFC 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 decreased activity in the PFC of depressed patients as compared to healthy controls, a finding that has been replicated across numerous other studies (for review, see Rigucci, Serafini, Pompili, Kotzalidis, & Tatarelli, 2010). Furthermore, this effect is reversed with chronic antidepressant drug treatment, bringing activity of the region back to baseline (Kennedy et al., 2001). Some meta-analyses have even reported reductions of volume in prefrontal areas in depressed patients (Bora, Harrison, Davey, & Yücel, 2012; Kempton et al., 2011). Taken together, these findings strongly suggest that the prefrontal cortex is a key component in the pathology of depression, and as such, has become a primary focus of researchers examining treatment possibilities.

Limbic areas, such as the amygdala and hippocampus, are also implicated in depression research because of their known contributions to emotion and negative affect (for review, see Rolls, 2015). Increased activity in the amygdala correlates positively with depression severity and chronic antidepressant drug treatment often normalizes this hyperactivity (Drevets, Price, & Furey, 2008). Structural abnormalities have also been found in depressed subjects, such as decreased volume of both the hippocampus and amygdala (Bremner et al., 2000; Kronenberg et al., 2009), thought to be due to glucocorticoid-induced neurotoxicity via the glutamatergic connections between the two regions (Sheline, Gado, & Price, 1998).

Finally, the anterior cingulate cortex is comprised of two portions that have differential roles in the pathology of depression. The dorsal portion is important for the cognitive aspects of emotion while the ventral portion, also known as the subgenual cingulate, has bilateral connections with limbic and frontal regions, such as the amygdala and medial prefrontal cortex (for review, see Pandya, Altinay, Malone, & Anand, 2012). Therefore, it acts as a link between the limbic areas that generate emotion and the frontal areas that regulate these emotions, with overactivity of the region being characteristic of depression. Deep brain stimulation of the area is associated with normalization of cerebral blood flow and remission of depressive symptoms (Mayberg et al., 2005), an effect that has been achieved by other treatments as well, including medications (Mayberg et al., 2000) and transcranial magnetic stimulation (Kito, Hasegawa, & Koga, 2011).

Differences in the structure and function of these regions implicated in depression may explain why depressed adolescents differ from adults in response to treatment. An fMRI study by Tao et al. (2012) discovered that depression in adolescents generally

involved similar brain regions as depression in adults, with subjects showing greater activation of limbic areas when presented pictures of fearful faces. However, they also found that adolescents exhibited increased frontal activity, whereas adult studies often show the opposite (Mayberg, 1997). Tao et al. suggest that one possible explanation for this result may be attributed to incomplete neural development. Many areas of the brain remain underdeveloped until adulthood, with myelinogenesis and gamma-aminobutyric acid (GABA)ergic neurotransmission still undergoing maturation in the adolescent brain (Arain et al., 2013). One such area is the PFC, a key moderator in the cognitive regulation of emotion. The developmental disparity between the prefrontal cortex that adjusts emotions and the subcortical limbic areas that generate emotions may predispose adolescents to depression (Kerestes et al., 2014). This is also reflected in response to treatment, as adolescents do not respond to serotonergic and noradrenergic antidepressants as well as adults do, which is most likely due to differences in expression of the serotonin transporter (for review, see Bowman & Daws, 2019) and the underdevelopment of the noradrenergic system (Bylund & Reed, 2007; Mulder, Watkins, Joyce, & Luty, 2003).

Treatments for Depression

Many techniques have been developed to treat patients suffering from depression. Some of these interventions include cognitive behavioural therapy, antidepressant drugs, and stimulation techniques such as deep brain stimulation.

Cognitive behavioural therapy (CBT) is a common type of psychotherapy that guides patients to re-evaluate their perceptions of reality, helping them to replace distorted thoughts with more realistic ones. It is effective in decreasing depressive

symptoms in adults as a stand-alone treatment, but it is more effective when combined with other methods (Cuijpers et al., 2013). Although this combination therapy is also found to be successful in alleviating adolescent depression (Silva, Petrycki, & Curry, 2004), many studies find mixed results with regards to CBT as a stand-alone treatment, often producing little benefit over active controls (for review, see Oar, Johnco, & Ollendick, 2017).

Antidepressant drugs are families of drugs that are administered to reduce depressive symptoms. One of the more commonly prescribed classes, known as selective serotonin reuptake inhibitors (SSRIs), have been found to be moderately useful in treating depressive symptoms in adults, but have a reduced efficacy when used to treat adolescent depression (Sugarman, Loree, Baltes, Grekin, & Kirsch, 2014; Varigonda et al., 2015). However, although they have better tolerability than older antidepressant medications such as monoamine oxidase inhibitors, they are still associated with a variety of side effects such as nausea, headache, weight gain, and sexual dysfunction (Baldwin, 2006). Furthermore, the use of antidepressants in adolescents have been found to result in paradoxical effects. A meta-analysis conducted by Hammad, Laughren, and Racoosin (2006) found that antidepressant drug use in adolescents was associated with a modest increase in suicidality risk, a consequence discovered by other meta-analyses as well (Bridge et al., 2007; Hetrick, McKenzie, Cox, Simmons, & Merry, 2012). These paradoxical effects are also apparent in rodent models. In rats treated with the SSRI fluoxetine, adolescents showed increased passivity in a forced swim test as compared to adults (Homberg et al., 2011). The authors state that this effect is most likely due to changes in neuroplasticity, as fluoxetine was absent in the blood during testing and

immunoreactivity of PSA-NCAM, a neuronal marker of synaptic remodelling, showed increased expression in adolescents and decreased expression in adults. Furthermore, Gomez, Venero, Viveros, and García-García (2015) found that acute treatment of fluoxetine produced anxiogenic effects in adolescent rats as measured by the hole-board test. The causes of these paradoxical side effects are still highly debated; however, it outlines the need for safer, more effective treatments for depression in adolescents.

Deep brain stimulation (DBS) is a last resort technique for severe, treatment-resistant depression. It involves a pair of electrodes that are surgically implanted in the brain and connected to a pulse generator implanted in the chest (Holtzheimer & Mayberg, 2010). Mayberg et al. (2005) applied the technique to white matter tracts adjacent to the subgenual cingulate gyrus, a region found to exhibit hyperactivity in depressed patients. Chronic stimulation reduced cerebral blood flow in this region and in associated areas, and this change was associated with the reversal of depressive symptoms. Additionally, DBS of various brain regions that show depression-related abnormalities, such as the nucleus accumbens, ventral striatum, and medial forebrain bundle, have been found to alleviate depression symptoms, further suggesting the efficacy of DBS as a potential treatment for depression (for review see Drobisz & Damborská, 2019). However, despite these clear beneficial effects, DBS remains a controversial technique because of its 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). Its use in adolescents is even more controversial, with many experts expressing concerns for using the technique to treat mood disorders in these populations (Rabins et al., 2009).

Transcranial Direct Current Stimulation

A potential solution to this problem is the use of transcranial direct current stimulation (tDCS), a non-invasive brain stimulation technique that sends weak electrical current through the scalp to induce changes in the brain. It produces some benefits in cognitive performance and reductions in psychiatric symptoms (Mondino et al., 2014), and has shown promising potential as a treatment for many types of disorders, including major depressive disorder (Alonzo, Chan, Martin, Mitchell, & Loo, 2013). Previous studies have documented the safety, feasibility, and tolerability of tDCS in both adults and adolescents (Boggio et al., 2008; Gillick et al., 2015).

Rather than causing action potentials, tDCS alters resting membrane potentials depending on the polarity of the electrode. Anodal stimulation enhances cortical excitability through neuronal depolarization, while cathodal stimulation reduces cortical excitability through neuronal hyperpolarization (Kuo et al., 2016). The outcomes of tDCS are also reliant on current intensity, as shown by Yu, Li, Wen, Zhang, and Tian (2015), who examined the neuroprotective effects of tDCS in a rat model of Alzheimer Disease. They found that in comparison to sham stimulation, 100μA or 200μA of repetitive anodal tDCS resulted in better acquisition of spatial memory in the Morris water maze, as well as higher density of Nissl bodies, higher expression of ChAT, and lower expression of GFAP in the hippocampus, while rats receiving only 20μA or 60μA failed to show any significant differences.

In addition to altering resting membrane potentials, the effects of tDCS can also be attributed to alterations in brain plasticity and the strength of synaptic transmission, similar to long-term potentiation (LTP) and long-term depression (Nitsche, Müller-

Dahlhaus, Paulus, & Ziemann, 2012), and also to altered transcription of certain genes. Anodal tDCS can increase cerebral concentrations of brain-derived neurotrophic factor (BDNF), a protein that is involved in 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). Furthermore, Yoon, Oh, & Kim (2012) found 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).

tDCS Application in Adult Depression

Although it is not yet approved as a treatment, many clinical studies have examined the therapeutic efficacy of tDCS in adults. In addition to major depressive disorder, its ability to treat various other psychiatric disorders, such as Parkinson's Disease (Broeder et al., 2015) and schizophrenia (Smith et al., 2015), are currently under investigation. Most studies involving the use of tDCS to treat depression target the left dorsolateral prefrontal cortex (dlPFC) because of its role in cognition-related mood regulation through its connections with the limbic system, and also because hypoactivity of this area is associated with depression in adults (for review, see Koenigs & Grafman, 2009).

A randomized, double-blind clinical trial conducted by Boggio et al. (2008) investigated the efficacy of tDCS in treating patients with major depressive disorder.

Patients were divided into three groups: one that received anodal tDCS over the left dlPFC, one that received anodal tDCS over the occipital cortex as an active control, and one that received sham tDCS as a placebo control. Patients were subjected to ten 20-minute sessions over two weeks, with the active tDCS groups receiving 2mA of current. The authors found a significant reduction in depression scores in the dlPFC group as compared to the occipital cortex or sham group, with the beneficial effects of tDCS lasting one month after treatment. Although this supports the antidepressant effects of tDCS, it also highlights the region specificity of the technique. In fact, another study by Ferrucci et al. (2009) also found that anodal tDCS applied to the left dlPFC in patients with drug-resistant major depressive disorder resulted in a significant decrease in depression scores that persisted for one month after treatment. However, this study only included 14 patients and did not include a control group receiving sham stimulation. While it is unlikely that the placebo effect alone is responsible for the treatment outcome, as stimulation parameters were similar to that of Boggio et al., the absence of a control group makes it difficult to separate the effects of tDCS from patient expectations or other extraneous variables.

Although these findings support the use of tDCS as an antidepressant treatment, other studies have failed to produce positive results. Palm et al. (2012) found no difference between active tDCS 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, with many of them taking different combinations of drugs, including antidepressants, antipsychotics, and anxiolytics. The differential interactions between tDCS and these

medications could have masked treatment results. Despite this, active tDCS was successful in increasing subjective ratings of positive emotions as compared to sham tDCS. Loo et al. (2010) also found no significant treatment effects of active tDCS as compared to sham tDCS, despite using stimulation parameters similar to that of previously successful studies. This result may be due to stimulation occurring only three times per week and on alternate days, as well as the use of concurrent antidepressant drug treatment in only a subset of the participants.

These mixed results suggest that while tDCS appears to have antidepressant properties, these properties may only manifest under certain sets of conditions and parameters. A multitude of factors need to be considered before employing tDCS to treat depressive patients, including the personal characteristics of the patient as well as the location, duration, and intensity of the stimulation.

tDCS Application in Adolescent Disorders

Despite the vast body of research for the use of tDCS in adults, there are currently no published studies evaluating the efficacy of tDCS in treating adolescents with depression (U.S. National Library of Medicine, 2019). Comprehensive reviews have found that most of the literature exploring tDCS treatment in adolescents focus on neurodevelopmental disorders such as attention-deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) (Lee, Kenney-Jung, Blacker, Camsari, & Lewis, 2019; Palm et al., 2016). However, interpretation of results from studies of other psychiatric disorders can give some insight on how tDCS could potentially treat pediatric depression as well.

Soff et al. (2017) examined the use of anodal tDCS over the dlPFC in 15 adolescents diagnosed with ADHD. They applied either 1mA of current or sham stimulation for 20 minutes per day for five days. After a 2-week washout period the groups were reversed, and participants underwent the same procedure again but in the opposite condition. However, this 2-week washout period may not have been sufficient to eliminate carryover effects, as the neurological and behavioural changes associated with tDCS have been reported to last for up to 1 month (Ferrucci et al., 2009). Despite this, the researchers found that anodal tDCS reduced clinical symptoms of inattention and impulsivity and also reduced inattention and hyperactivity in a standardized working memory test one week after stimulation.

In a sample of 20 boys aged 5-8 who were diagnosed with ASD, Amatachaya et al. (2014) applied 1mA of anodal or sham tDCS over the dlPFC for 20 minutes over five consecutive days. After a washout period of four weeks, the subjects were then treated with five sessions of the opposite stimulation. They found that tDCS resulted in significant reductions in the severity of ASD symptoms as well as improvements in health and behavioural problem, sociability, and sensory/cognitive awareness subscales.

Notably, the pathology of ADHD, ASD, and depression all involve hypoactivation of the left dlPFC (Amatachaya et al., 2014; Koenigs & Grafman, 2009; Soff et al., 2017), and, as established previously, anodal tDCS over this area has been shown to successfully treat both ADHD and ASD in children, as well as depression in adults. This suggests the feasibility of using anodal tDCS to treat depression in adolescents. However, researchers have also observed an increase in connections between the left dlPFC and the pregenual anterior cingulate cortex in depressed adolescents, a finding that has not been reported in

depressed adults (Davey, Harrison, Yücel, & Allen, 2012; Kerestes et al., 2014). If this finding represents an abnormality specific to pediatric depression, increasing excitability in the dlPFC of depressed adolescents could potentially elicit undesirable results through stimulation of this pathway. It is therefore important for further research to examine this possibility before introducing tDCS into general practice.

A pilot study conducted by Khedr, Elfetoh, Ali, and Noamany (2014) investigated the use of anodal tDCS to treat patients with anorexia nervosa, a condition that is highly comorbid with depression (Hughes, 2012). They applied 2mA of stimulation for 25 minutes over the dlPFC for 10 consecutive days. Out of the seven participants, four were considered adolescents, with ages ranging between 16 and 17. Furthermore, two of these four adolescents were diagnosed with having depression comorbidity, and three of them had been taking SSRIs prior to and during the experiment. After completion of tDCS treatment, the patient with the most severe depression showed significant improvement in the Beck Depression Inventory (BDI) scale, and this effect was even more pronounced one month after the last treatment. Two of the other adolescents that were not reported to have depression comorbidity showed a mild decrease post-treatment but returned to baseline after one month. The fourth patient, diagnosed with mild depression, showed no change in BDI score at any point. Notably, this patient had not been administered SSRIs before or during the study, unlike the other three adolescents. This finding highlights a potential confound in the research: the interaction between tDCS and SSRI treatment. SSRIs have been found to enhance the LTP-like plasticity induced by tDCS (Kuo et al., 2016). Additionally, a study by Nitsche et al. (2009) found that a single dose of the SSRI citalopram enhanced and prolonged tDCS-induced excitability of the motor cortex in

humans. Brunoni et al. (2013) found that after six weeks of treatment, the combination of tDCS plus the SSRI sertraline produced greater reductions in Montgomery-Asberg Depression Rating Scale scores than either treatment alone. Although the results obtained by Khedr et al. cannot be separated from this confound due to their small sample size and lack of control group, in the absence of any other published research on the effects of tDCS in adolescent depression, its finding that tDCS treatment resulted in a decrease of depressive symptoms in adolescents suffering from anorexia nervosa suggests that it may also have merit as a treatment for mood disorders, warranting further investigation.

Animal Model of Depression

The mechanism of both depression and its treatments involve numerous intertwining systems and complex signalling cascades. As a result, animal models are essential for examining the underlying neurological substrates of the disorder and the mechanisms of its treatment.

A commonly used model of depression in rodents is olfactory bulbectomy (OBX), a procedure involving bilateral ablation of the olfactory bulbs that results in behavioural and neurochemical changes that have been found to be reversed by antidepressant treatment (for review, see Kelly, Wrynn, & Leonard, 1997). The most notable behavioural change is an increase in locomotor activity in a novel, brightly lit environment (Klein & Brown, 1969), a change that is most likely the result of some neurological substrate rather than the inability to smell, as this effect on locomotor activity is not seen in anosmia induced by other methods (Sieck and Baumbach, 1974). This hyperactivity is thought to be related to agitated hyposerotonergic depression, which is a major risk factor for suicide in human patients (Klein & Brown, 1969; Lumia,

Teicher, Salchli, Ayers, & Possidente, 1992; Rihmer, 2007). Other behavioural changes that manifest following the OBX procedure include an increase in immobility in a forced swim test (Morales-Medina et al., 2013) and a decrease in sucrose preference (Padilla et al., 2018). Many similarities have been found between the frontal lobes of bulbectomized rats and the brains of depressed human subjects (for review, see Rajkumar & Dawe, 2018). In addition, neural degeneration within the limbic system has been observed in both OBX animals and depressed humans (Drevets, Price, & Furey, 2008; Ramaker & Dulawa, 2017). Furthermore, OBX decreases blood levels of BDNF in rats (Kucera et al., 2019), an effect commonly observed in depressed humans (Molendijk et al., 2011). For these reasons, OBX is an ideal model for preclinical assessment of antidepressant treatments in rodents.

Study Overview

The current study was designed to examine the antidepressant properties of tDCS on olfactory bulbectomized adolescent rats, both with and without concurrent SSRI treatment. Eight different groups were compared, distinguished by whether the animals had received OBX or non-OBX sham surgery (O or N), real or sham tDCS (T or S), and paroxetine or saline injections (P or S). The study was split into two phases, the first of which examined the behavioural outcomes after acute treatment, while the second examined the behavioural and neurobiological outcomes after chronic treatment. These behavioural outcomes included hyperlocomotion in an open field, immobility in a forced swim test, anhedonia in a sucrose preference test, and latency to feed in a novelty suppressed feeding test, while the neurobiological outcomes included blood plasma analysis of BDNF, a marker for brain plasticity. All testing and sample collection were

conducted before PND 60, an age corresponding to late adolescence in rats (Sengupta, 2013).

It was hypothesized that the OBX procedure, as well as paroxetine treatment, would result in depressive-like symptoms as assessed by our behavioural tests at both the acute and chronic time points. It was also hypothesized that the application of tDCS would reverse these effects, and that the combination therapy of tDCS and paroxetine would produce stronger antidepressant effects than tDCS alone. Finally, it was hypothesized that peripheral protein markers related to brain plasticity (BDNF) would be reduced by OBX, but increased by tDCS.

2. Materials and Methods

2.1. Animals

Adolescent male Sprague-Dawley rats (Charles-River Saint-Constant, Quebec, Canada), at PND 20-21, were pair-housed under standard conditions upon arrival. Animals remained pair-housed until electrode placement surgery at PND 35-37. All animals were given ad libitum access to food and water, apart from a 16-hour period of food deprivation on PND 57-58 in preparation for the novelty suppressed feeding test. Animals 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. OBX Surgery

The timeline and distribution of groups for the experiment are displayed in Figure 1. OBX was performed on PND 28-29. 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, Wrynn, & Leonard, 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).

2.3. Electrode Placement

Electrodes were mounted on the skull on PND 35-37. 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. Small holes were drilled on both sides of the skull (AP: +2.0 mm, ML: ±2.0 mm) and behind the cerebellum (AP: -15.0 mm, ML: +1.0 mm) and jeweler screws were tightly twisted into place to act as an anchor for the dental acrylic resin. A male JST connector was placed on the skull such that a

conducting metal plate (2.5±0.25 x 1.5±0.25 mm) attached to the anodal end laid over the medial prefrontal cortex (AP: +2.2 mm to +4.7 mm), based on coordinates obtained from the stereotaxic atlas by Paxinos and Watson (2007). The cathodal end was attached to the cerebellar screw to act as the reference electrode. 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. A schematic of the assembly is displayed in Figure 2. Animals were left to recover for 5-7 days before the beginning of treatment.

2.4. Phase One: Effects of Acute Treatment

To assess the effects of acute antidepressant treatment, 90 adolescent rats received two sessions of treatment before being exposed to a battery of behavioural tests meant to assess depressive-like behaviours.

2.4.1. Antidepressant Treatment

Treatment began on PND 44, consisting of an injection of the SSRI paroxetine (Paxil) immediately followed by administration of transcranial direct current stimulation. A second treatment session occurred on PND 45, 12-16 hours following the first session.

Animals were given an intraperitoneal injection of paroxetine (a kind gift from Dr. Gabriella Gobbi, McGill University) at a dose of 20mg/kg. Twenty grams were dissolved in 100 ml of physiological saline (0.9% NaCl) to obtain an injection volume of 1ml/kg. This dosage was determined to be pharmacologically active and therapeutically effective based on previous studies (Amodeo et al., 2015; Erdemir et al., 2014). Control animals were injected with saline (0.9% NaCl) at the same volume of 1ml/kg.

Following the injection, rats were subjected to 15 minutes of 50μA anodal tDCS (corresponding to a charge density of 13.33A/m^2), delivered from an external current

generator custom-built by Ed Yee (CAMH, Toronto) according to specifications by Drs. José Nobrega and Francis Bambico. This current intensity was determined to be effective based on pilot studies conducted in our lab. Each animal was mildly restrained while being connected to the generator but was promptly placed back in its home cage for the duration of the stimulation. Current intensity was monitored using a multimeter attached to 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.

2.4.2. Behavioural Tests

A battery of tests assessing depressive-like behaviours were conducted beginning five hours following treatment on PND 45 to assess the effects of acute antidepressant treatment. Behavioural tests were performed in the order that they are described, and animals were returned to their colony room for 3-4 hours between each test.

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 two 13-watt lights placed overhead for illumination, as described in previous experiments (Kelly, Wrynn, & Leonard, 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, at PND 43, 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 an indicator of anhedonia (Papp, Willner, & Muscat, 1991). 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.

2.5. Phase Two: Effects of Chronic Treatment

To assess the effects of chronic antidepressant treatment, the same adolescent rats described previously continued to receive antidepressant treatment for 12 additional days before being exposed to another battery of behavioural tests meant to assess depressive-like behaviours. As a result of attrition throughout the experiment's duration, a total of 64 rats completed the entire set of treatments.

2.5.1 Antidepressant Treatment

Treatment protocol followed the same procedure outlined in section 2.4.1. above. Following the initial two sessions on PND 44 and PND 45, treatment was administered every 24 hours for 12 more days, resulting in a total of 14 consecutive daily sessions that ended on PND 57.

2.5.2. Behavioural Tests

A battery of tests assessing depressive-like behaviours were conducted on the days following the completion of treatment to assess the effects of chronic antidepressant treatment.

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). Due to time constraints, this test was not performed at the acute time point. 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 9 food pellets placed in the center. The test was conducted on PND 58 in a novel testing room and, unlike in the OFT, no aluminum foil lined the walls of the arena. 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.

Open Field Test. The OFT was conducted as described previously, five hours following the NSFT on PND 58.

Forced Swim Test. The FST was conducted as described previously, but without the pre-exposure trial, on PND 59.

Sucrose Preference Test. The SPT was conducted for 16 hours, with methods as described previously, five hours following the FST on PND 59.

2.6. Blood Sampling and BDNF Analysis

Blood was collected from the trunk following euthanasia by CO_2 on PND60 in Microvette CB 300 K2E tubes (Sarstedt, Germany). Samples were immediately centrifuged for 10 min at 3000RPM to obtain plasma and stored at -20 ℃ until analysis. BDNF plasma levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Promega, WI, USA) according to the manufacturer's instructions. Plates were coated with an Anti-BDNF mAB + carbonate coating buffer overnight at 4°C. Nonspecific binding was blocked by incubating plates in Block & Sample 1X Buffer for 1 hour at room temperature, followed by a wash with TBST wash buffer. A standard curve was prepared by performing six serial dilutions of BDNF standard in Block & Sample 1X Buffer in columns 11 and 12 of the 96-well plate. The remaining plates were filled with 12.5µl of sample plasma and 87.5µl of Block & Sample 1X Buffer, with plasma from each animal being assayed in duplicate. Plates were incubated with shaking for 2 hours at room temperature and then washed 5 times with TBST wash buffer. 100µl of Anti-Human BDNF pAb mixed with Block & Sample 1X Buffer was then added to each well, followed by a 2-hour incubation with shaking at room temperature and then 5 more washes with TBST wash buffer. Similarly, 100µl of Anti-IgY HRP Conjugate mixed with Block & Sample 1X Buffer was then added to each well, followed by a 1-hour incubation with shaking at room temperature and then 5 more

washes with TBST wash buffer. Each well then received 100µl of TMB One Solution and plates were incubated with shaking for 10 minutes at room temperature. The reaction was stopped by adding 100µl of 1N hydrochloric acid to each well and the absorbance of each plate was recorded at 450nm on a plate reader within 30 minutes of stopping the reaction to determine the concentration of BDNF (pg/ml).

2.7. Statistical Analysis

During the course of treatment, 26 animals were able to remove the head assembly, resulting in an inability to administer the full 14-day treatment protocol. These animals were immediately euthanized, resulting in larger group sizes for the behavioural tests at the acute time point as compared to the chronic time point. Furthermore, behavioural data for some tests were lost due to equipment failure or environmental disturbances. At the acute time point, three animals were excluded from the OFT because of excessive noise in the testing room, one animal was excluded from the FST due to camera failure, and one animal was excluded from the SPT due to overnight bottle leakage. At the chronic time point, failure to completely restrict food availability resulted in the exclusion of two animals from the NSFT, while seven animals were excluded from the FST due to camera failure or FST bin malfunction. Grubb's test determined that two animals were outliers in the BDNF concentration data set, resulting in their exclusion from the analysis.

For validation of the depression model, analyses consisted of independent samples t-tests. For evaluation of treatment effects, analyses consisted of 2x2 ANOVAs with type of stimulation (tDCS or sham) as one factor and type of drug (paroxetine or saline) as the other. As a result of the loss of animals throughout the course of treatment, separate

analyses were conducted for the acute and chronic time points. On all the data sets, non-parametric analyses were used when assumptions of normality and homogeneity of variance were not met. A value of $p < .05$ was considered to be significant.

3. Results

3.1. Validation of Depression Model

To establish OBX as a valid model of depression, we first compared the OBX animals that had received no treatments (O-S-S) to the sham animals that had received no treatments (N-S-S).

3.1.1. Open Field Test

The open field test examined agitated depression and anxiety by measuring hyperlocomotion and time spent in the center of a novel environment. At the acute time point, an independent samples t-test revealed that the distance traveled by OBX animals (*Mean [M]* = 4274.375, *Standard Deviation [SD]* = 1138.895, $n = 12$) was not significantly greater than the distance traveled by sham animals ($M = 3691.445$, $SD = 576.950$, $n = 11$), although a clear trend in this direction was apparent, $t(16.591) = 1.567$, $p = .068$, Glass's $\Delta = 1.010$ (Figure 3). Furthermore, the amount of time spent in the center of the chamber was not significantly different between the two groups (OBX: $M = 44.158$, $SD = 50.732$, $n = 12$; sham: $M = 34.609$, $SD = 29.653$, $n = 11$), $U = 61.500$, $p = .805$, Cohen's $d = .227$ (Figure 4).

At the chronic time point, an independent samples t-test also revealed that the distance traveled by OBX animals ($M = 3450.738$, $SD = 654.521$, $n = 8$) was not significantly greater than the distance traveled by sham animals ($M = 3246.96$, $SD = 474.227$, $n = 10$), $t(16) = .767$, $p = .227$, Cohen's $d = .364$ (Figure 3). Likewise, the

amount of time spent in the center of the chamber was not significantly different between the two groups (OBX: $M = 52.597$, $SD = 64.480$, $n = 8$; sham: $M = 40.100$, $SD = 26.957$, $n = 10$), $U = 37.000$, $p = .829$, Glass's $\Delta = .464$ (Figure 4).

3.1.2. Novelty Suppressed Feeding Test

The novelty suppressed feeding test examined anxiety by measuring latency to feed in an open arena. At the chronic time point, 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: $M = 55.875$, $SD = 46.698$, $n = 8$; sham: $M = 35.800$, $SD = 26.275$, $n = 10$), $U = 30.000$, $p = .398$, Cohen's $d = .548$ (Figure 5A). Additionally, the latency to start eating the food pellets was not significantly different between the two groups (OBX: $M = 315.250$, $SD = 139.552$, $n = 8$; sham: $M = 388.200$, $SD = 102.176$, $n = 10$), $t(16) = -1.282$, $p = .218$, Cohen's $d = -.608$ (Figure 5B). These results suggest that the OBX procedure did not result in any changes to anxiety as measured by the NSFT.

3.1.3. Forced Swim Test

The forced swim test assessed behavioural despair by measuring immobility in an inescapable bin of water. At the acute time point, an independent samples t-test revealed that OBX animals ($M = 139.638$, $SD = 61.876$, $n = 13$) did not spend more time immobile than sham animals ($M = 174.536$, $SD = 77.093$, $n = 11$), $t(22) = -1.231$, $p = .884$, Cohen's $d = -.504$ (Figure 6).

This absence of an effect was seen at the chronic time point as well, as OBX animals ($M = 208.443$, $SD = 135.148$, $n = 7$) did not spend more time immobile than sham animals ($M = 260.800$, $SD = 100.802$, $n = 8$), $t(13) = -.858$, $p = .797$, Cohen's $d = -$

.444 (Figure 6). This suggests that the OBX procedure did not result in an increase in passivity as measured by the FST.

3.1.4. Sucrose Preference Test

The sucrose preference test measured anhedonia by assessing rats' drinking preferences when given bottles containing a sucrose solution or tap water. At the acute time point, an independent samples t-test revealed that OBX animals (M = 82.054, SD = 18.102, n = 13) consumed significantly less sucrose than sham animals (M = 92.100, SD = 4.915, n = 11), U = 34.500, p = .017, Cohen's d = -.729 (Figure 7).

Similarly, at the chronic time point, an independent samples t-test revealed that OBX animals (M = 73.537, SD = 30.280, n = 8) consumed significantly less sucrose than sham animals (M = 95.530, SD = 4.051, n = 10), U = 9.500, p = .004, Glass's Δ = 5.429 (Figure 7). These results suggest that OBX was successful in producing an anhedonia-like response in animals subjected to the procedure.

3.1.5. BDNF in Blood Plasma

Determination of plasma BDNF concentration (pg/ml) was completed after chronic treatment as a measure of depression-induced impairment of brain plasticity, displayed in Figure 8. The data obtained for all BDNF analyses are shown to be reliable and valid upon examination of the standard curve in Figure 8. An independent samples t-test revealed that OBX animals (M = .107, SD = .036, n = 7) did not have lower concentrations of BDNF in blood plasma than sham animals (M = .151, SD = .073, n = 10), although there was a clear trend in this direction, $t(13.799)$ = -1.654, p = .060, Cohen's d = -.727 (Figure 9).

3.2. Evaluation of Treatments in Depressed Animals

To evaluate the effectiveness of the treatment procedures in depressive-like animals, we compared the results of the OBX groups that received tDCS and paroxetine (O-T-P), tDCS and saline (O-T-S), sham stimulation and paroxetine (O-S-P), and sham stimulation and saline (O-S-S).

3.2.1. Open Field Test

Our results suggest that tDCS, but not paroxetine, is effective in reversing the hyperlocomotion characteristic of the OBX procedure after both acute and chronic treatment. However, neither treatment influenced the amount of time spent in the center of the arena. At the acute time point, an ANOVA indicated a main effect of stimulation on distance traveled (tDCS groups: $M = 3303.723$, $SD = 1508.057$, $n = 28$; sham groups: $M = 4290.038$, $SD = 1251.938$, $n = 21$), $F(1,45) = 4.473$, $p = .040$, partial $\eta^2 = .090$. However, there was no main effect of drug (paroxetine groups: $M = 3920.845$, $SD = 1681.193$, $n = 28$; saline groups: $M = 3672.916$, $SD = 1078.803$, $n = 21$), $F(1,45) = .283$, $p = .598$, partial $\eta^2 = .006$, as well as no interaction, $F(1,45) = .216$, $p = .645$, partial $\eta^2 = .005$ (Figure 10A). An ANOVA comparing the amount of time spent in the center of the maze found no main effect of stimulation (tDCS groups: $M = 31.918$, $SD = 37.208$, $n = 28$; sham groups: $M = 54.179$, $SD = 58.819$, $n = 21$), $F(1,45) = 2.477$, $p = .123$, partial $\eta^2 = .052$, and no main effect of drug (paroxetine groups: $M = 45.679$, $SD = 52.341$, $n = 28$; saline groups: $M = 40.418$, $SD = 43.686$, $n = 21$), $F(1,45) = .138$, $p = .712$, partial $\eta^2 = .003$, as well as no interaction, $F(1,45) = 1.092$, $p = .302$, partial $\eta^2 = .024$ (Figure 11A).

Similar effects were found at the chronic time point, as an ANOVA indicated a main effect of stimulation on distance traveled (tDCS groups: $M = 2597.138$, $SD =$

1062.255, n = 16; sham groups: M = 3345.894, SD = 913.179, n = 16), F(1,28) = 4.397, p = .045, partial η^2= .136. However, there was no main effect of drug (paroxetine groups: M = 3020.075, SD = 1065.111, n = 16; saline groups: M = 2922.957, SD = 910.323, n = 16), F(1,28) = .074, p = .788, partial η^2= .003, as well as no interaction, F(1,28) = .738, p = .398, partial η^2= .026 (Figure 10B). An ANOVA comparing the amount of time spent in the center of the maze found no main effect of stimulation (tDCS groups: M = 31.194, SD = 34.603, n = 16; sham groups: M = 52.055, SD = 54.109, n = 16), F(1,28) = 1.644, p = .210, partial η^2= .055, and no main effect of drug (paroxetine groups: M = 41.551, SD = 40.117, n = 16; saline groups: M = 41.699, SD = 48.595, n = 16), F(1,28) = 8.357×10^{-5}, p = .993, partial $\eta^2 < .001$, as well as no interaction, F(1,28) = .003, p = .955, partial $\eta^2 <$ .001 (Figure 11B).

3.2.2. Novelty Suppressed Feeding Test

The results from this test suggest that neither tDCS nor paroxetine affected anxiety as measured by the NSFT. After chronic treatment, an ANOVA revealed that there was no main effect of stimulation on the latency to reach food (tDCS groups: M = 47.215, SD = 26.093, n = 15; sham groups: M = 58.375, SD = 54.814, n = 16), F(1,27) = .486, p = .486, partial η^2= .018. Additionally, there was no main effect of drug (paroxetine groups: M = 49.152, SD = 39.846, n = 15; saline groups: M = 56.438, SD = 41.061, n = 16), F(1,27) = .207, p = .653, partial η^2= .008, as well as no interaction, F(1,27) = .589, p = .450, partial η^2= .021 (Figure 12A). Similarly, another ANOVA revealed that there was no main effect of stimulation on the latency to start eating (tDCS groups: M = 246.643, SD = 145.475, n = 15; sham groups: M = 288.750, SD = 126.003, n = 16), F(1,27) = .739, p = .397, partial η^2= .027, no main effect of drug (paroxetine

groups: $M = 262.268$, $SD = 140.327$, $n = 15$; saline groups: $M = 273.125$, $SD = 131.151$, $n = 16$), $F(1,27) = .049$, $p = .826$, partial $\eta^2 = .002$, as well as no interaction, $F(1,27) = .741$, $p = .397$, partial $\eta^2 = .027$ (Figure 12B).

3.2.3. Forced Swim Test

Our results suggest that no differences in passivity, as measured by the FST, are apparent between the treatment groups. At the acute time point, an ANOVA indicated that there was no main effect of stimulation on time spent immobile (tDCS groups: $M = 154.390$, $SD = 98.451$, $n = 28$; sham groups: $M = 131.958$, $SD = 64.138$, $n = 22$), $F(1,46) = .815$, $p = .371$, partial $\eta^2 = .017$. There was also no main effect of drug (paroxetine groups: $M = 142.018$, $SD = 79.550$, $n = 28$; saline groups: $M = 144.330$, $SD = 83.039$, $n = 22$), $F(1,46) = .009$, $p = .926$, partial $\eta^2 < .001$, as well as no interaction, $F(1,46) = .276$, $p = .602$, partial $\eta^2 = .006$ (Figure 13A).

At the chronic time point, an ANOVA indicated that there was no main effect of stimulation on time spent immobile (tDCS groups: $M = 170.208$, $SD = 106.972$, $n = 14$; sham groups: $M = 224.703$, $SD = 121.323$, $n = 15$), $F(1,25) = 1.614$, $p = .216$, partial $\eta^2 = .061$. There was also no main effect of drug (paroxetine groups: $M = 214.046$, $SD = 117.123$, $n = 15$; saline groups: $M = 180.865$, $SD = 111.172$, $n = 14$), $F(1,25) = .598$, $p = .447$, partial $\eta^2 = .023$, as well as no interaction, $F(1,25) = .0002$, $p = .988$, partial $\eta^2 < .001$ (Figure 13B).

3.2.4. Sucrose Preference Test

Acute administration of paroxetine resulted in an increase of anhedonia-like behaviour in rats, an effect that was blocked when combined with tDCS treatment. However, paroxetine appeared to achieve its intended antidepressant effects after chronic

administration. At the acute time point, an ANOVA indicated a main effect of stimulation on the amount of sucrose consumed (tDCS groups: $M = 82.064$, $SD = 14.054$, $n = 29$; sham groups: $M = 66.877$, $SD = 24.746$, $n = 22$), $F(1,47) = 7.443$, $p = .009$, partial $\eta^2 = .137$, as well as a main effect of drug (paroxetine groups: $M = 66.684$, $SD = 22.631$, $n = 28$; saline groups: $M = 82.257$, $SD = 16.169$, $n = 23$), $F(1,47) = 7.825$, $p = .007$, partial $\eta^2 = .143$. However, there was also a significant interaction, $F(1,47) = 7.050$, $p = .011$, partial $\eta^2 = .130$. Tukey's follow-up tests showed that the O-S-P condition ($M = 51.700$, $SD = 31.389$, $n = 9$) had a lower preference for sucrose than the O-T-P ($M = 81.668$, $SD = 13.873$, $n = 19$), O-T-S ($M = 82.460$, $SD = 14.235$, $n = 10$), and O-S-S conditions ($M = 82.054$, $SD = 18.102$, $n = 13$) (Figure 14A).

At the chronic time point, an ANOVA revealed no main effect of stimulation on the amount of sucrose consumed (tDCS groups: $M = 84.575$, $SD = 11.717$, $n = 16$; sham groups: $M = 81.113$, $SD = 20.456$, $n = 16$), $F(1,28) = .284$, $p = .598$, partial $\eta^2 = .010$. Additionally, although it approached significance, there was no main effect of drug (paroxetine groups: $M = 89.019$, $SD = 8.771$, $n = 16$; saline groups: $M = 76.669$, $SD = 23.402$, $n = 16$), $F(1,28) = 3.614$, $p = .068$, partial $\eta^2 = .114$, as well as no interaction, $F(1,28) = .186$, $p = .670$, partial $\eta^2 = .007$ (Figure 14B).

3.2.5. BDNF in Blood Plasma

Our results suggest that neither tDCS nor paroxetine affected plasma concentrations of BDNF in OBX animals. The data obtained for all BDNF analyses are shown to be reliable and valid upon examination of the standard curve in Figure 8. After chronic treatment, an ANOVA revealed no main effect of stimulation on plasma concentration of BDNF (tDCS groups: $M = .103$, $SD = .049$, $n = 16$; sham groups: $M =$

.120, *SD* = .049, *n* = 15), *F*(1,27) = .865, *p* = .361, partial η^2= .031. There was also no main effect of drug (paroxetine groups: *M* = .113, *SD* = .056, *n* = 16; saline groups: *M* = .110, *SD* = .042, *n* = 15), *F*(1,27) = .016, *p* = .899, partial η^2= .001, and no interaction, *F*(1,27) = 1.630, *p* = .213, partial η^2= .057 (Figure 15).

3.3. Evaluation of Treatments in Non-Depressed Animals

To determine if the treatment procedures had any effect in animals not subjected to the depression paradigm, we compared the results of the non-OBX groups that received tDCS and paroxetine (N-T-P), tDCS and saline (N-T-S), sham stimulation and paroxetine (N-S-P), and sham stimulation and saline (N-S-S).

3.3.1. Open Field Test

Neither tDCS nor paroxetine affected locomotor activity or anxiety in healthy controls as measured by the OFT. At the acute time point, an ANOVA revealed no main effect of stimulation on distance traveled (tDCS groups: *M* = 3586.320, *SD* = 1251.289, *n* = 17; sham groups: *M* = 3767.523, *SD* = 622.404, *n* = 21), *F*(1,34) = .338, *p* = .565, partial η^2= .010. There was also no main effect of drug (paroxetine groups: *M* = 3724.245, *SD* = 976.168, *n* = 19; saline groups: *M* = 3629.598, *SD* = 897.525, *n* = 19), *F*(1,34) = .092, *p* = .763, partial η^2= .003, as well as no interaction, *F*(1,34) = .034, *p* = .855, partial η^2= .001 (Figure 16A). An ANOVA comparing the amount of time spent in the center of the maze found no main effect of stimulation (tDCS groups: *M* = 39.710, *SD* = 40.367, *n* = 17; sham groups: *M* = 40.635, *SD* = 28.481, *n* = 21), *F*(1,34) = .007, *p* = .936, partial $\eta^2 < .001$, and no main effect of drug (paroxetine groups: *M* = 41.847, *SD* = 28.070, *n* = 19; saline groups: *M* = 38.498, *SD* = 40.779, *n* = 19), *F*(1,34) = .087, *p* =

.770, partial η^2= .003, as well as no interaction, $F(1,34) = .588$, $p = .448$, partial η^2= .017 (Figure 17A).

Similar effects were found at the chronic time point, as an ANOVA indicated no main effect of stimulation on distance traveled (tDCS groups: $M = 3405.600$, $SD = 903.804$, $n = 14$; sham groups: $M = 3230.968$, $SD = 529.468$, $n = 18$), $F(1,28) = .456$, $p = .505$, partial η^2= .016. There was also no main effect of drug (paroxetine groups: $M = 3309.381$, $SD = 646.999$, $n = 15$; saline groups: $M = 3327.187$, $SD = 786.273$, $n = 17$), $F(1,28) = .005$, $p = .946$, partial $\eta^2 < .001$, as well as no interaction, $F(1,28) = .003$, $p = .957$, partial $\eta^2 < .001$ (Figure 16B). An ANOVA comparing the amount of time spent in the center of the maze found no main effect of stimulation (tDCS groups: $M = 53.643$, $SD = 37.347$, $n = 14$; sham groups: $M = 54.763$, $SD = 32.787$, $n = 18$), $F(1,28) = .008$, p = .930, partial $\eta^2 < .001$. There was also no main effect of drug (paroxetine groups: $M = 67.091$, $SD = 42.502$, $n = 15$; saline groups: $M = 41.315$, $SD = 27.633$, $n = 17$), $F(1,28) = 4.200$, $p = .050$, partial η^2= .130, as well as no interaction, $F(1,28) = .080$, $p = .780$, partial η^2= .003 (Figure 17B).

3.3.2. Novelty Suppressed Feeding Test

Similar to the results obtained from the OBX groups, neither tDCS nor paroxetine affected anxiety, as measured by the NSFT, in non-depressed animals. After chronic treatment, an ANOVA revealed that there was no main effect of stimulation on the latency to reach food (tDCS groups: $M = 45.643$, $SD = 37.368$, $n = 14$; sham groups: $M = 31.686$, $SD = 18.325$, $n = 17$), $F(1,27) = 1.672$, $p = .207$, partial η^2= .058. Additionally, there was no main effect of drug (paroxetine groups: $M = 39.643$, $SD = 25.895$, $n = 14$; saline groups: $M = 37.686$, $SD = 29.798$, $n = 17$), $F(1,27) = .033$, $p = .857$, partial η^2=

.001, as well as no interaction, $F(1,27) = .890$, $p = .354$, partial $\eta^2 = .032$ (Figure 18A). Similarly, another ANOVA revealed that there was no main effect of stimulation on the latency to start eating (tDCS groups: $M = 295.929$, $SD = 166.132$, $n = 14$; sham groups: $M = 328.743$, $SD = 88.795$, $n = 17$), $F(1,27) = .479$, $p = .495$, partial $\eta^2 = .017$, no main effect of drug (paroxetine groups: $M = 288.500$, $SD = 120.633$, $n = 14$; saline groups: $M = 336.172$, $SD = 134.294$, $n = 17$), $F(1,27) = 1.011$, $p = .324$, partial $\eta^2 = .036$, as well as no interaction, $F(1,27) = 2.258$, $p = .145$, partial $\eta^2 = .077$ (Figure 18B).

3.3.3. Forced Swim Test

Neither tDCS nor paroxetine affected passivity, as measured by the FST, among the non-OBX animals. At the acute time point, an ANOVA indicated that there was no main effect of stimulation on time spent immobile (tDCS groups: $M = 155.321$, $SD = 91.272$, $n = 17$; sham groups: $M = 150.509$, $SD = 70.612$, $n = 22$), $F(1,35) = .033$, $p = .857$, partial $\eta^2 = .001$. There was also no main effect of drug (paroxetine groups: $M = 141.380$, $SD = 88.693$, $n = 20$; saline groups: $M = 164.450$, $SD = 73.192$, $n = 19$), $F(1,35) = .753$, $p = .391$, partial $\eta^2 = .021$, as well as no interaction, $F(1,35) = .883$, $p = .354$, partial $\eta^2 = .025$ (Figure 19A).

At the chronic time point, an ANOVA indicated that there was no main effect of stimulation on time spent immobile (tDCS groups: $M = 219.732$, $SD = 108.044$, $n = 13$; sham groups: $M = 234.686$, $SD = 100.184$, $n = 15$), $F(1,24) = .142$, $p = .709$, partial $\eta^2 = .006$. There was also no main effect of drug (paroxetine groups: $M = 214.111$, $SD = 99.872$, $n = 13$; saline groups: $M = 240.307$, $SD = 108.356$, $n = 15$), $F(1,24) = .437$, $p = .515$, partial $\eta^2 = .018$, as well as no interaction, $F(1,24) = .431$, $p = .518$, partial $\eta^2 = .018$ (Figure 19B).

3.3.4. Sucrose Preference Test

Sucrose preference was unaffected by acute treatment of tDCS or paroxetine. However, chronic treatment of paroxetine was associated with a decrease in sucrose preference. This suggests that long-term administration of paroxetine in non-depressed subjects can elicit anhedonia-like responses, an effect that is blocked when administered with simultaneous tDCS treatment. At the acute time point, an ANOVA revealed no main effect of stimulation on the amount of sucrose consumed (tDCS groups: $M = 89.518$, $SD = 7.346$, $n = 16$; sham groups: $M = 88.678$, $SD = 8.986$, $n = 22$), $F(1,34) = .081$, $p = .777$, partial $\eta^2 = .002$, as well as no main effect of drug (paroxetine groups: $M = 88.239$, $SD = 10.393$, $n = 20$; saline groups: $M = 89.957$, $SD = 5.939$, $n = 18$), $F(1,34) = .340$, $p = .564$, partial $\eta^2 = .010$. There was also no significant interaction, $F(1,34) = 3.021$, $p = .091$, partial $\eta^2 = .082$ (Figure 20A).

At the chronic time point, an ANOVA revealed no main effect of stimulation on the amount of sucrose consumed (tDCS groups: $M = 94.386$, $SD = 5.110$, $n = 14$; sham groups: $M = 93.553$, $SD = 3.980$, $n = 18$), $F(1,28) = .250$, $p = .621$, partial $\eta^2 = .009$, and no main effect of drug (paroxetine groups: $M = 93.788$, $SD = 3.582$, $n = 15$; saline groups: $M = 94.151$, $SD = 5.508$, $n = 17$), $F(1,28) = .047$, $p = .829$, partial $\eta^2 = .002$. However, there was a significant interaction, $F(1,28) = 4.644$, $p = .040$, partial $\eta^2 = .142$, although Tukey's follow-up tests revealed no significant group differences (Figure 20B).

3.3.5. BDNF in Blood Plasma

Neither tDCS nor paroxetine affected plasma concentrations of BDNF in non-OBX rats, although a combination treatment appeared to produce a trend towards a synergistic effect. The data obtained for all BDNF analyses are shown to be reliable and

valid upon examination of the standard curve in Figure 8. After chronic treatment, an ANOVA revealed no main effect of stimulation on plasma concentration of BDNF (tDCS groups: $M = .142$, $SD = .068$, $n = 14$; sham groups: $M = .137$, $SD = .051$, $n = 17$), $F(1,27) = .050$, $p = .824$, partial $\eta^2 = .002$. There was also no main effect of drug (paroxetine groups: $M = .149$, $SD = .058$, $n = 14$; saline groups: $M = .131$, $SD = .061$, $n = 17$), $F(1,27) = .589$, $p = .449$, partial $\eta^2 = .021$, and no interaction, $F(1,27) = 3.879$, $p = .059$, partial $\eta^2 = .126$ (Figure 21).

4.0 Discussion

We examined the antidepressant effects of tDCS both by itself and in combination with paroxetine treatment in a rodent model of depression. This model, the olfactory bulbectomy procedure, was chosen because it is one of the most well-validated models for examining the antidepressant effects of treatments and, in comparison to other common depression models, exhibits very high sensitivity, specificity, reliability, and predictive validity (for review, see Ramaker & Dulawa, 2017).

4.1. Examination of OBX

The validity of OBX as a model for depression in adolescent rats is supported by the results we obtained. At the acute time point, OBX animals showed a marked increase in distance traveled in the OFT in comparison to sham animals, indicative of psychomotor agitation, consistent with findings from other adolescent studies (Flores et al., 2014). Although this result did not achieve significance, the presence of a floor effect may have masked the difference between the two groups. Increased risk-taking behaviours during adolescence is found in many species, and manifests in rodents as increased activity and exploration in novel environments (for review, see Spear, 2000). Therefore, it is possible

that the difference between the OBX and non-OBX groups was obscured by the innate disposition of adolescent rats to explore a new environment. At the chronic time point, the two groups showed more similarity in their locomotor activity, although OBX animals still traveled more than non-OBX animals. In addition to the possibility of the floor effect, this may also be the result of acclimatization to the open field chamber. With animals having already experienced the OFT at the acute time point two weeks prior to this exposure, the environment was no longer a source of novelty, a key component for the manifestation of OBX-induced hyperactivity (Klein & Brown, 1969).

We found no evidence that OBX increases anxious behaviours in adolescent rats, as both groups spent equal time in the center of the arena during the OFT and had similar latencies to both reach and consume food in the NSFT. OBX is known to increase anxiety-like behaviours in adult rats (Pudell et al., 2014), as well as to increase latency of adult mice to feed in the NSFT (Islam, Moriguchi, Tagashira, & Fukunaga, 2014). Similar to the OFT, this absence of an anxiogenic effect may also be due to the increased rate of risk-taking and hyperactivity characteristic of adolescence (Spear, 2000).

Surprisingly, OBX did not result in an increased time spent immobile in the FST. This suggests that the FST is an ineffective test for assessing depressive-like behaviours induced by OBX in adolescent rats, as increased immobility is a commonly found result in adults exposed to the procedure (Morales-Medina et al., 2013). Some component of adolescence may make these animals more resilient to the passivity of the FST paradigm, similar to the hopelessness-resistance seen in the Long Evans strain (Vieyra-Reyes et al., 2008). Alternatively, the time spent swimming, and therefore the time spent immobile,

may have been confounded by the increased locomotor activity characteristic of both adolescence and the OBX procedure.

OBX animals showed a significantly decreased preference for sucrose that persisted across both the acute and chronic time points, mirroring the results commonly reported in adults (Padilla et al., 2018). This indicates a reduced hedonic value of a normally appetitive stimulus, as Counotte, Schiefer, Shaham, & O'Donnell (2014) found that both adolescent and adult rats will lever press to self administer sucrose, and adolescents will consume more sucrose than adult rats relative to body weight. As anhedonia is one of the core symptoms of depression, this provides further validation of the OBX procedure as a model of adolescent depression.

Finally, OBX animals showed a reduction in the concentrations of BDNF in blood plasma. This is consistent with previous research, as other studies have shown that OBX is associated with reduced BDNF in the serum (Kucera et al., 2019), as well as in the hippocampus (Pudell et al., 2013). This peripheral measure can reliably be used to make inferences about the presence of BDNF in the brain, as previous studies have found positive correlations between serum and cortical levels of BDNF in both rats and pigs (Karege, Schwald, & Cisse, 2002; Klein et al., 2011). These results further support the OBX procedure as a valid model of depression, as lowered concentration of BDNF is a commonly reported finding in human patients suffering from depression (Molendijk et al., 2011).

4.2. Examination of Treatments

The purpose of the current study was to test the antidepressant-like properties of tDCS and paroxetine. Using the four OBX groups, we examined the effects of both

individual treatments as well as their combination in a model of adolescent depression. In the OFT, paroxetine had no effect on hyperactivity in these adolescent animals after both acute and chronic administration, despite the well-established finding that chronic SSRI treatment reduces OBX-induced hyperlocomotion in adults (Song & Leonard, 2005). This suggests that paroxetine has reduced efficacy for treating this depressive-like symptom in adolescents, despite its normal success in adults. Conversely, we found that tDCS was effective in reversing novelty-induced hyperactivity after both acute and chronic treatment. Although most antidepressant drugs require chronic treatment to reverse the OBX-induced hyperlocomotion in adults (Song & Leonard, 2005), we found beneficial effects after just two sessions of tDCS. There was no effect of tDCS on locomotor activity in healthy controls, similar to another study conducted in adult rats (Filho et al., 2016), suggesting that this effect of tDCS is specific to reversing OBX-induced hyperactivity.

Neither tDCS nor paroxetine affected anxiety as measured by the amount of time spent in the center of the arena in the OFT and the latency to reach and eat food in the NSFT. While these results may reflect an underlying consequence of OBX-induced hyperlocomotion or the increased risk-taking behaviours characteristic of adolescence, it is also possible that these treatments do not modulate anxious behaviours. Filho et al. (2016) found no effect of tDCS on anxiety as measured by the OFT and elevated plus maze (EPM) in adult rats, while Amodeo et al. (2015) found that paroxetine either increased or had no effect on anxiety in adult rats as measured by the EPM and light/dark box, depending on the dosage. However, it is important to note that based on our previous comparisons, the OBX procedure did not appear to be anxiogenic in adolescent rats.

Perhaps the inability of tDCS and paroxetine to reduce anxiety is due to the absence of any excess anxiety to treat.

Chronic treatment with SSRIs is often found to reduce immobility in the FST in adult rodents (for review, see Cryan, Valentino, & Lucki, 2005), however we found no such effect here. Similar results have been found in previous studies, as Karanges et al. (2011) found that chronic paroxetine treatment reduced immobility in adult but not adolescent rats, while Vorhees, Morford, Graham, Skelton, & Williams (2011) found that neither chronic fluoxetine nor paroxetine treatment affected swimming behaviours in adolescent rats. This may further reflect paroxetine's ineffectiveness at treating depressive symptoms in adolescents, although it must be noted that these studies administered SSRI treatment to healthy animals that were not associated with any depression model. We also found no effect of tDCS, in contrast to Peanlikhit et al. (2017), who found that tDCS significantly reduced immobility in the FST in adult mice. Despite a difference in species, this suggests that tDCS may be ineffective at treating passivity in adolescents as measured by the FST. Alternatively, the swimming behaviour of the animals may have been affected by the hyperlocomotion characteristic of OBX or adolescence, creating a higher baseline and potentially masking the effects of treatment. Based on our previous comparisons and the lack of difference between OBX and non-OBX animals in the FST, it appears that the latter may be the more reasonable explanation.

In line with our hypothesis, acute administration of paroxetine resulted in a sharp decrease of sucrose preference in OBX animals, an indicator of anhedonic depression. Iñiguez, Warren, & Bolaños-Guzmán (2010) found that acute treatment with fluoxetine

resulted in no differences for sucrose preference in young rats. This is most likely due to the use of healthy animals, as their rats were not subjected to any depression paradigm. Indeed, we also found that sucrose preference was unaffected by acute administration of paroxetine to non-OBX animals. Furthermore, the decreased sucrose preference after acute paroxetine treatment in adolescents was completely blocked by concurrent tDCS treatment, possibly due to modulation of the reward system. A single session of tDCS applied over the dorsolateral prefrontal cortex has recently been found to increase dopamine release in the ventral striatum (Fonteneau et al., 2018), an area that mediates the reward network and response to pleasurable stimuli (Der-Avakian & Markou, 2012). Additionally, reduced dopamine activity in the striatum has been found in depressed patients (Shah, Ogilvie, Goodwin, & Ebmeier, 1997), and acute administration of fluoxetine has been found to inhibit the activity of dopaminergic neurons in the ventral tegmental area (VTA) (Prisco & Esposito, 1995). Perhaps tDCS increased sucrose preference in OBX animals through the normalization of reduced dopaminergic activity in the striatum. After chronic treatment, neither tDCS nor paroxetine appeared to affect sucrose preference, although there was a trend towards paroxetine providing a slight antidepressant effect. Amodeo et al. (2015) also found that chronic fluoxetine or paroxetine treatment did not influence sucrose preference in adolescent rats. These results support our hypothesis that antidepressant drug treatment is less efficacious in adolescents, as chronic treatment has been found to reverse depressive-like anhedonia in the sucrose preference test in adults (Casarotto & Andreatini, 2007). The ineffectiveness of tDCS after chronic treatment may be related to the differential effect of SSRIs on dopaminergic function in the VTA. While acute administration of fluoxetine reduces the

activity of dopaminergic neurons in the VTA, this effect has been found to attenuate after chronic treatment (Prisco & Esposito, 1995). This suggests that tDCS functions partly by normalizing dopaminergic activity in the striatum after acute treatment of paroxetine, while ceasing to exert an effect after the VTA becomes tolerant to the inhibitory effect of the SSRI after chronic treatment (Prisco & Esposito, 1995).

Surprisingly, neither tDCS nor paroxetine resulted in changes to BDNF concentration in the plasma of rats subjected to the OBX procedure. While tDCS has been found to increase BDNF mRNA in the stimulated area (Kim et al., 2017), researchers have also shown that altered mRNA expression does not necessarily indicate altered protein levels of BDNF (Jacobsen & Mørk, 2004), signaling the possibility that we were unable to detect any tDCS-induced changes of BDNF. Additionally, the neurotrophic hypothesis of depression suggests that the therapeutic action of antidepressants is regulated by BDNF, as animal models have shown that chronic use of SSRIs increase the expression of BDNF in the hippocampus (for review, see Yu & Chen, 2011). Perhaps our findings reflect that the ineffectiveness of SSRIs to treat depression in adolescents is partially due to a decreased ability to modulate BDNF. Compared to the OBX animals, the non-OBX animals had higher overall levels of plasma BDNF, consistent with the literature (Kucera et al., 2019). Interestingly, it appeared as if chronic administration of either tDCS or paroxetine in these control animals induced stress-like decreases in BDNF, possibly due to the increased emotional vulnerability of adolescents (Spear, 2000). However, the combination of the two treatments appeared to produce a synergistic effect, suggesting that the underlying processes of both treatments may converge to increase BDNF-related plasticity, while each treatment by itself fails to do so.

5. Conclusions

In this study we used an animal model of depression to examine the treatment efficacy of both tDCS and the SSRI paroxetine in adolescent rats. As expected, paroxetine was unable to remedy the depressive-like behaviours induced by the OBX procedure and resulted in the worsening of anhedonia, a core symptom of depression. The concurrent application of tDCS was able to prevent this detrimental effect, suggesting that a combination therapy of both tDCS and paroxetine is more effective than either treatment by itself in reversing the depressive-like phenotype in adolescent rats.

There are several limitations to the current study that may constrain our interpretations of the results. The sample size for each group was relatively small, resulting in insufficient power to determine the true effects of our treatments. The same animals were used to assess both the acute and chronic effects of treatment, so it is possible that exposure to the behavioural tests at the acute time point affected results at the chronic time point. This effect may be seen in the results from the OFT, where habituation to the testing chamber could have resulted in all animals exhibiting less locomotor activity at the chronic time point. Additionally, the nature of the acute treatment assessment required all behavioural testing to be completed within a single day. It is possible that the effects of one test carried over to affect the next, despite the animals being returned to their colony room for 3-4 hours between each test. Finally, we examined only one dose of paroxetine, whereas other studies have shown that the effects of an SSRI can vary based on the dosage (Amodeo et al., 2015). Future experiments should examine the effects of acute and chronic treatment using separate cohorts of

animals, both sexes, and different treatment parameters to generate a wider, more complete spectrum of effects.

There is a pressing need for novel treatments that can effectively relieve depression without causing adverse side effects, especially within the younger population. The results of the current study support the use of tDCS in combination with antidepressant drug therapy for the treatment of adolescent depression. Although much work must be done to examine the underlying effects of tDCS, future research could potentially guide its use as a simple, inexpensive, and non-invasive treatment for depression in adolescents.

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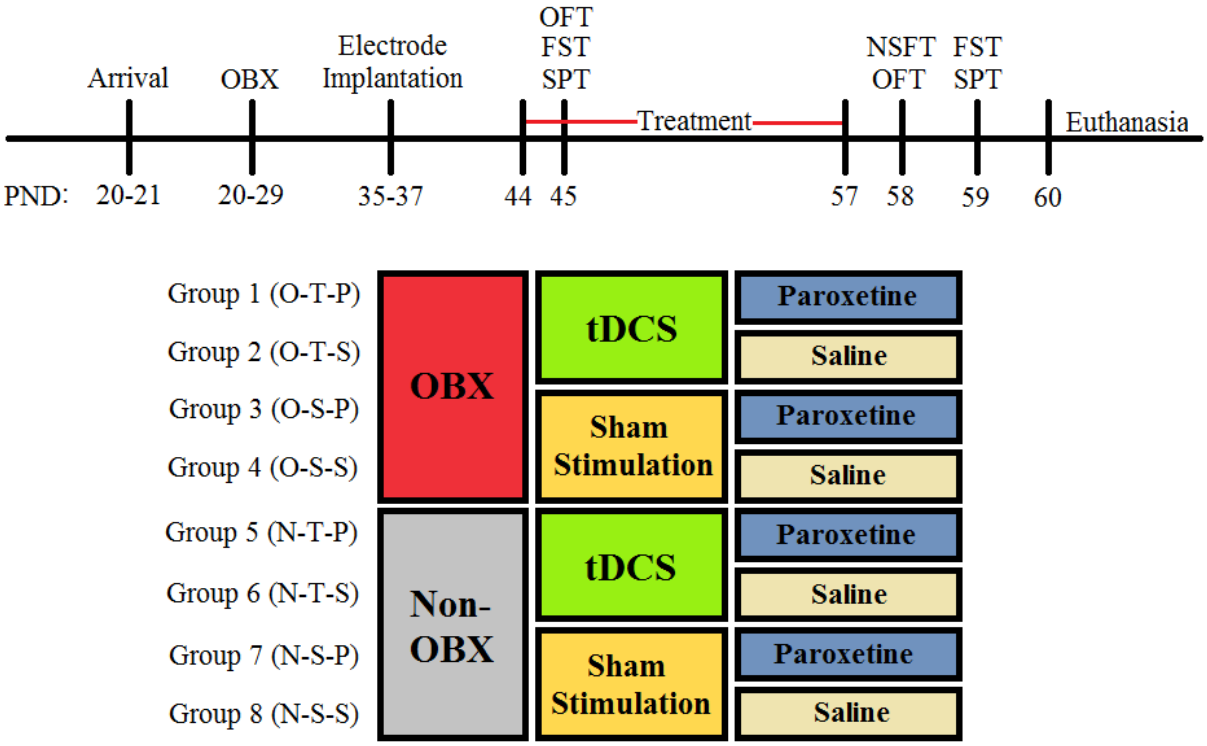

Figure 1. Timeline of experiment

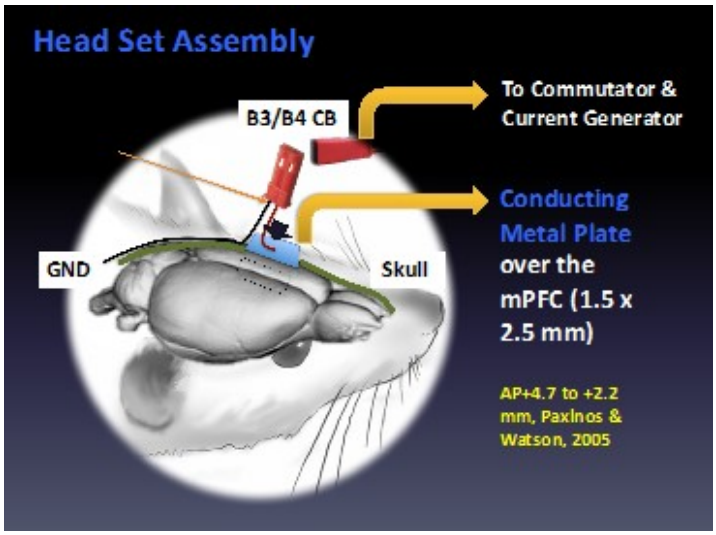

Figure 2. Illustration of electrode placement

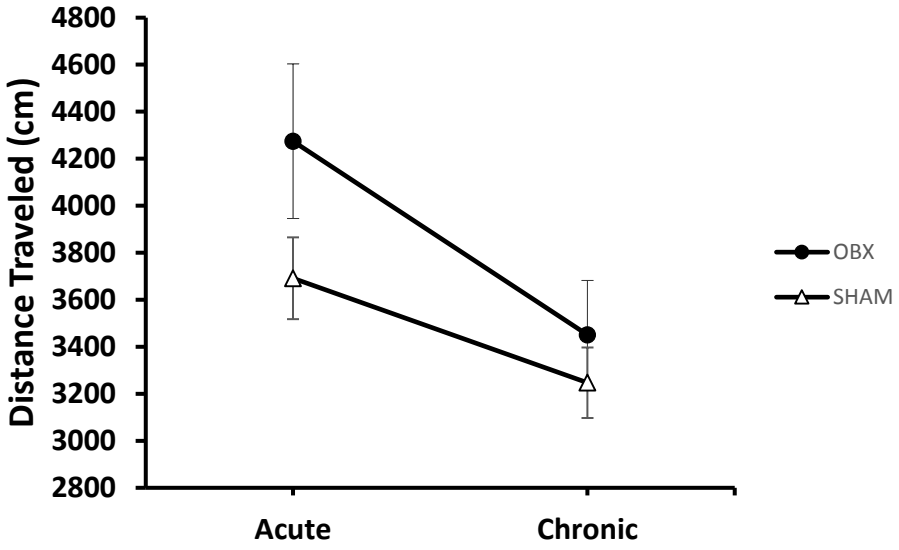

Figure 3. Average (±SEM) distance traveled by O-S-S and N-S-S groups at the acute and chronic time points in the OFT.

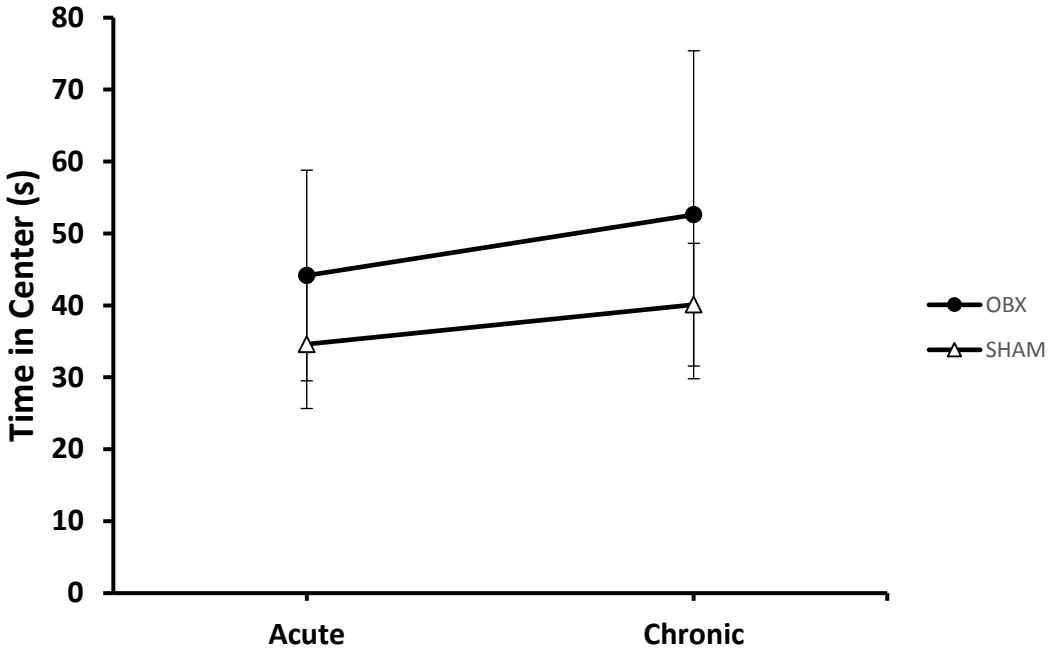

Figure 4. Average (±SEM) time spent in the center of the maze by O-S-S and N-S-S groups at the acute and chronic time points in the OFT.

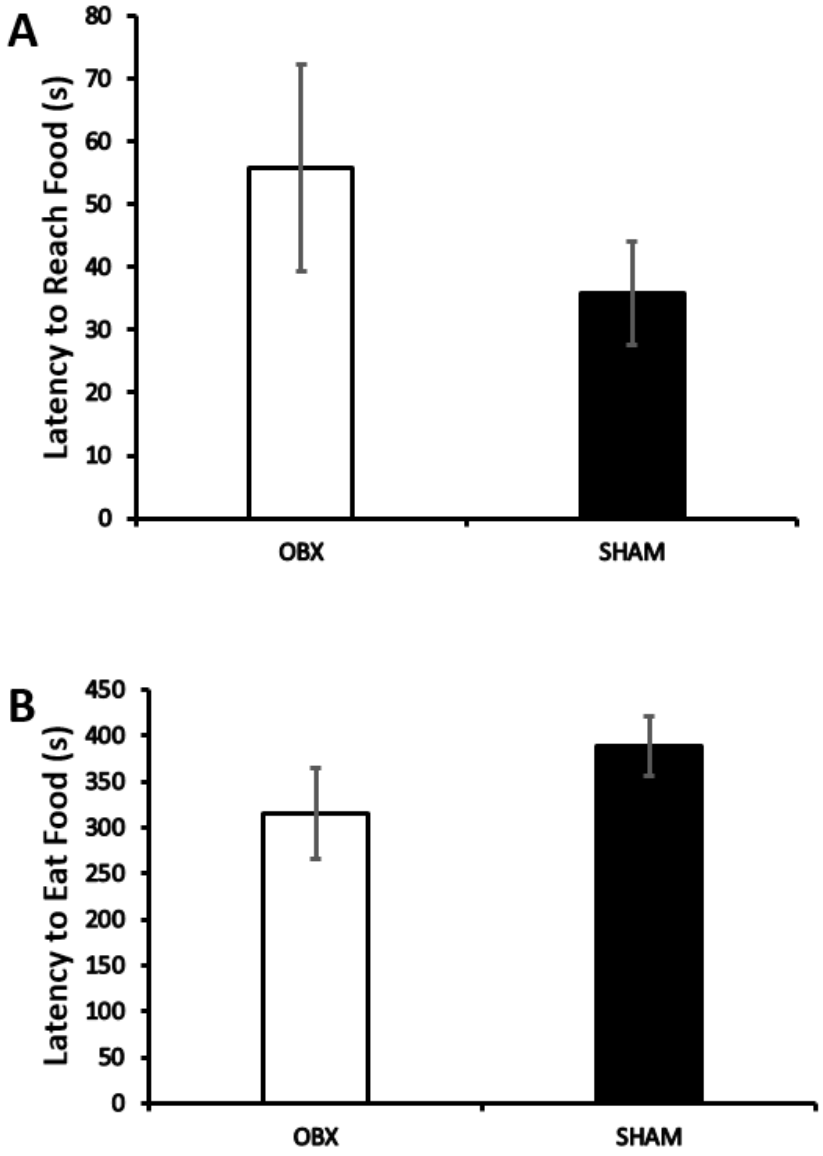

Figure 5. Average (±SEM) latency of O-S-S and N-S-S groups to reach food (A) and start eating (B) in the NSFT.

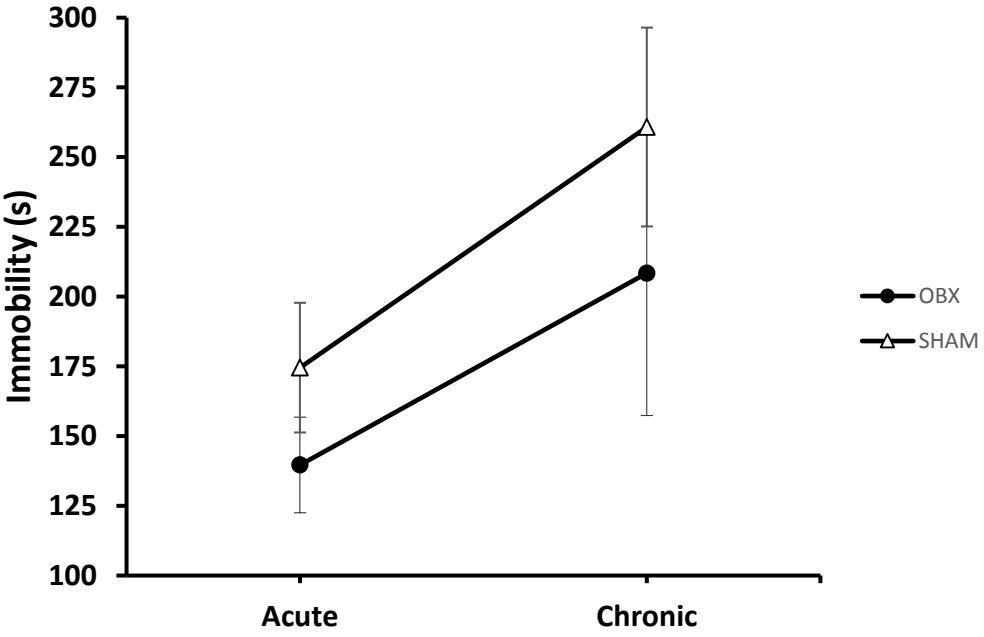

Figure 6. Average (±SEM) time spent immobile by O-S-S and N-S-S groups at the acute and chronic time points in the FST.

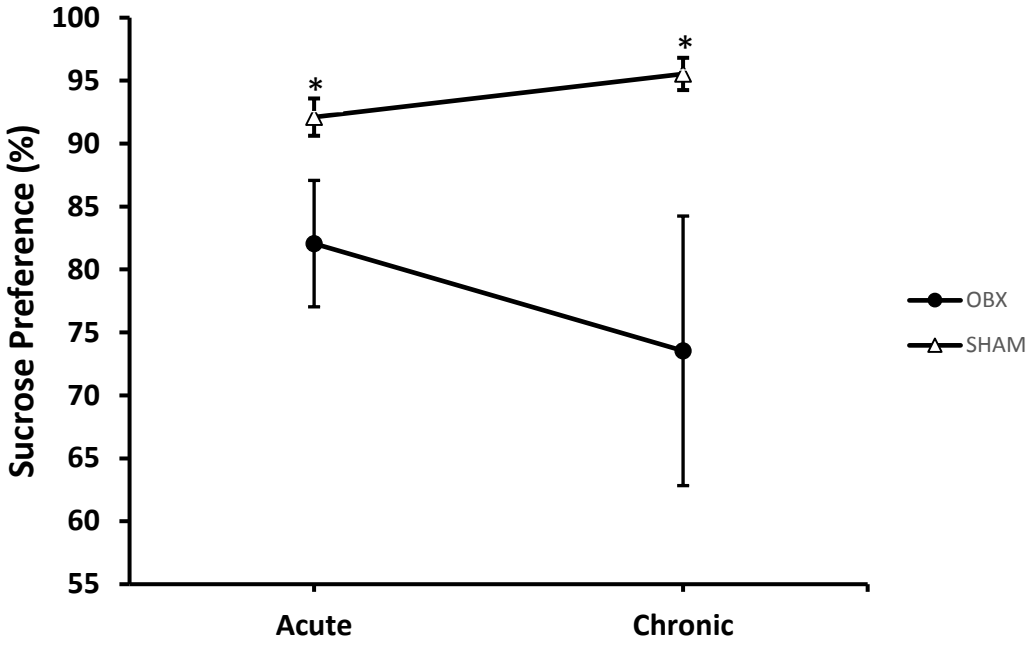

Figure 7. Average (±SEM) sucrose preference by O-S-S and N-S-S groups at the acute and chronic time points in the SPT.

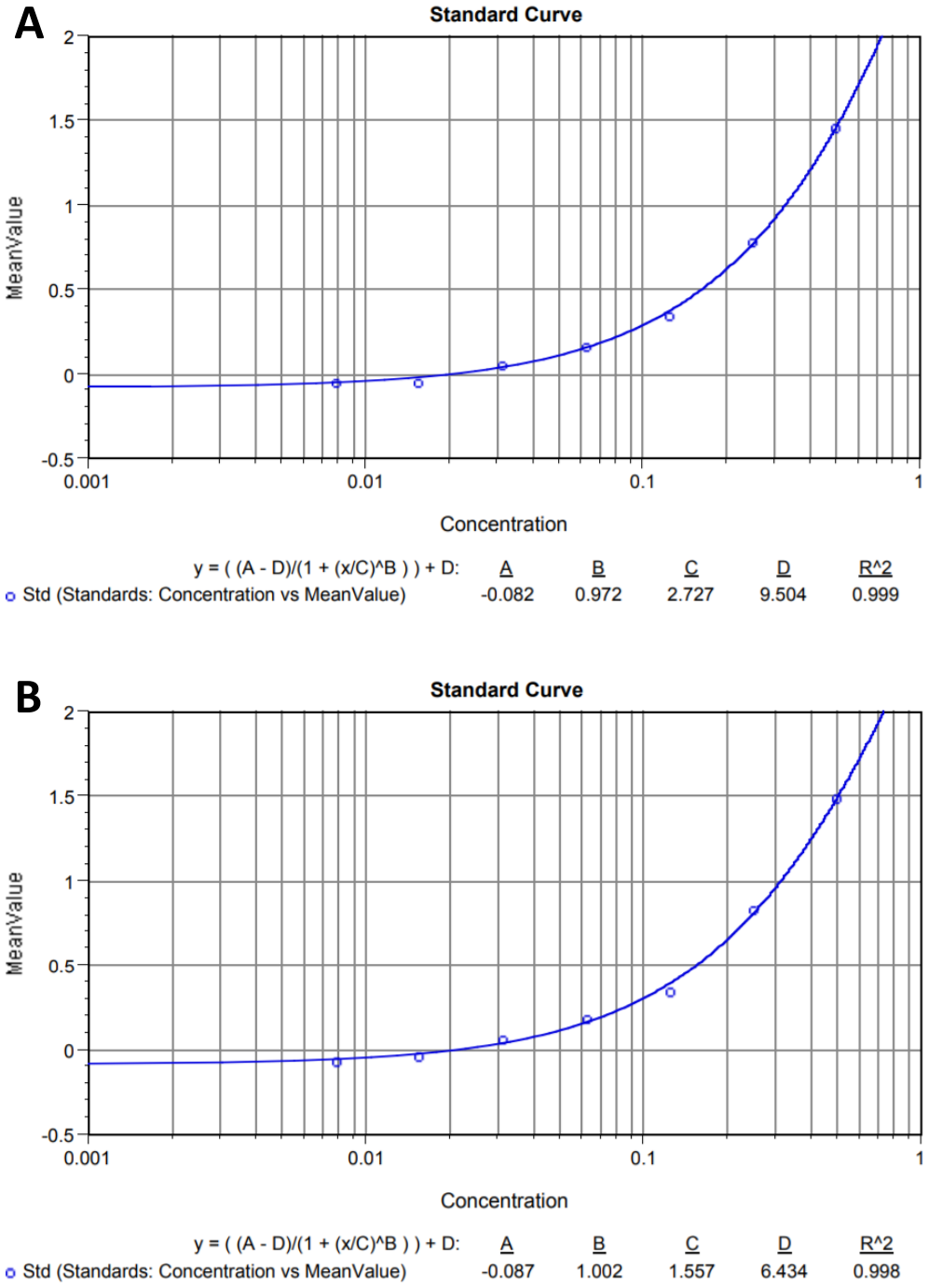

Figure 8. Standard curves for Plate A and Plate B from the ELISA analysis.

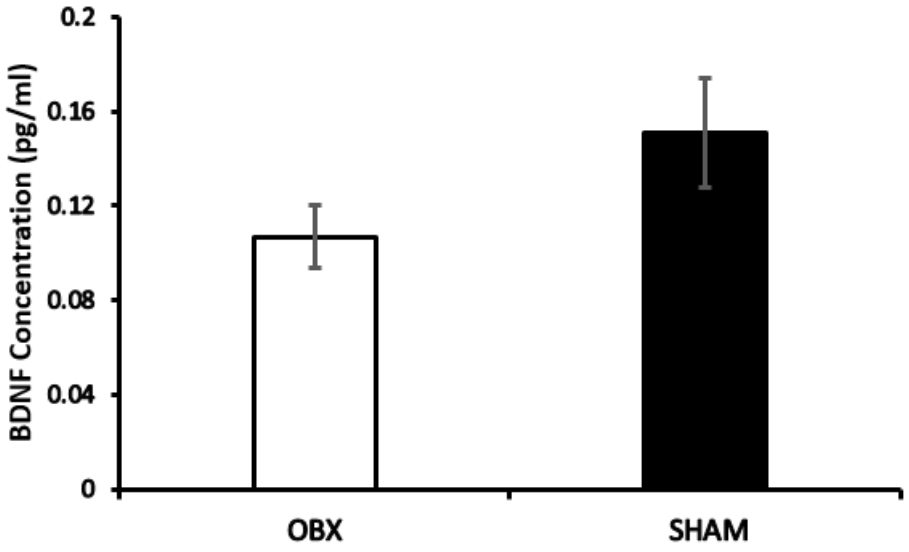

Figure 9. Average (±SEM) plasma concentration of BDNF in O-S-S and N-S-S groups after chronic treatment.

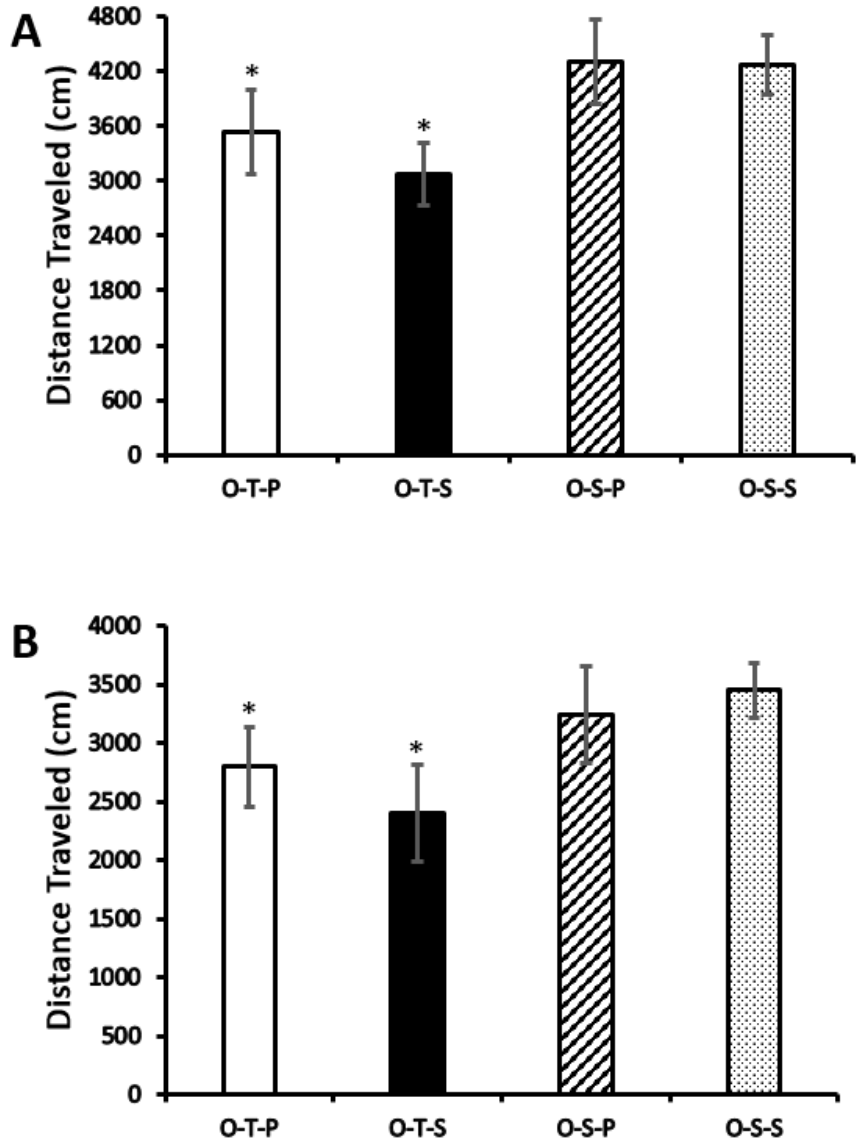

Figure 10. Average (±SEM) distance traveled by all OBX groups after acute (A) and chronic (B) treatment in the OFT.

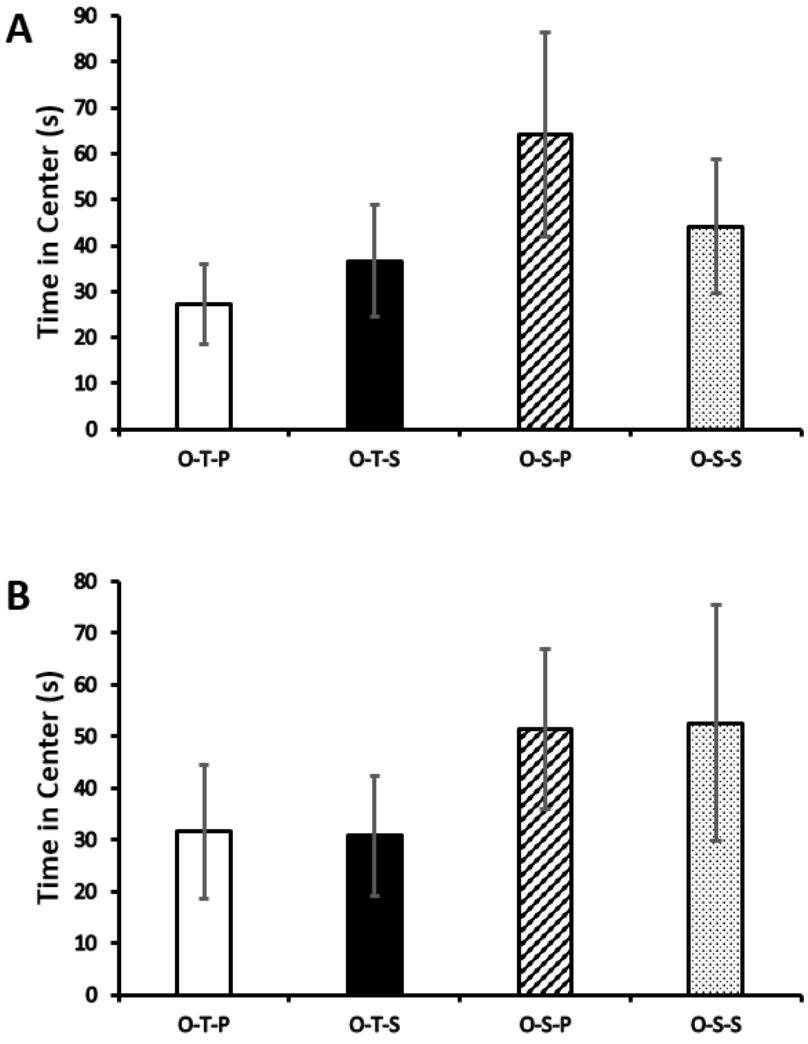

Figure 11. Average (±SEM) time spent in the center of the arena by all OBX groups after acute (A) and chronic (B) treatment in the OFT.

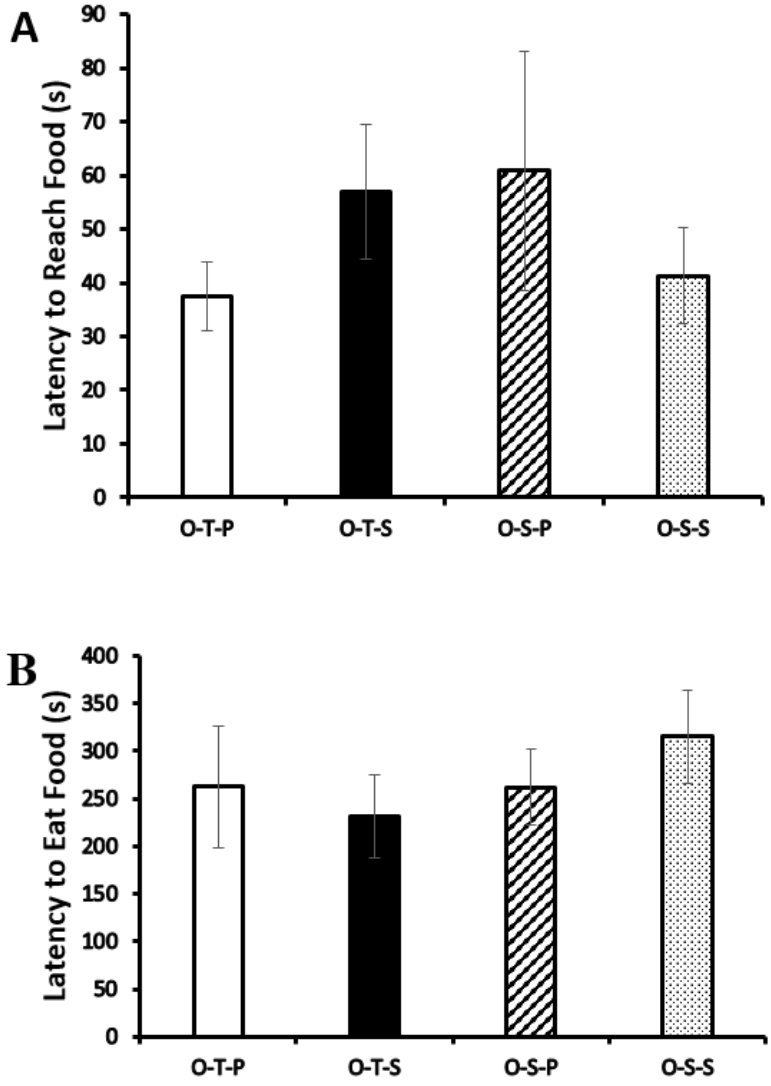

Figure 12. Average (±SEM) latency of all OBX groups to reach food (A) and start eating (B) in the NSFT.

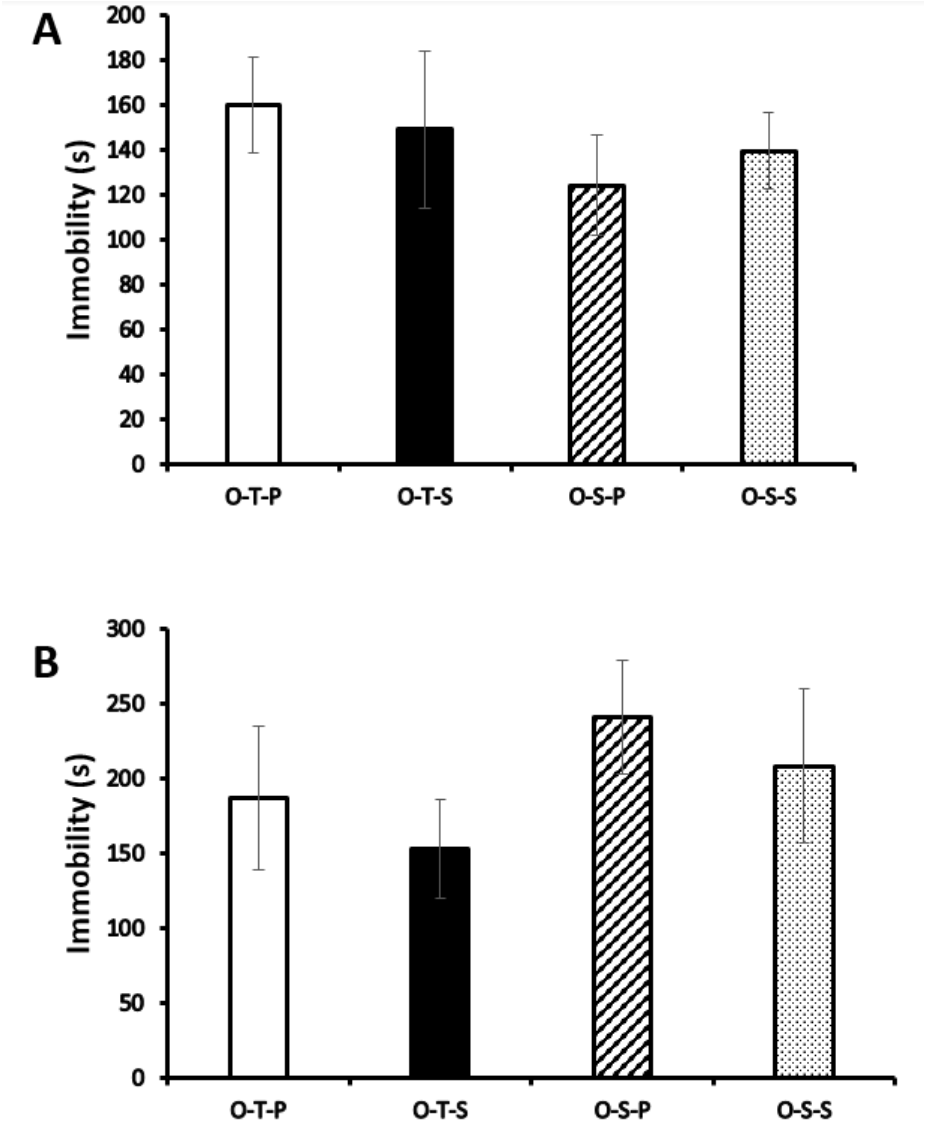

Figure 13. Average (±SEM) time spent immobile by all OBX groups after acute (A) and chronic (B) treatment in the FST.

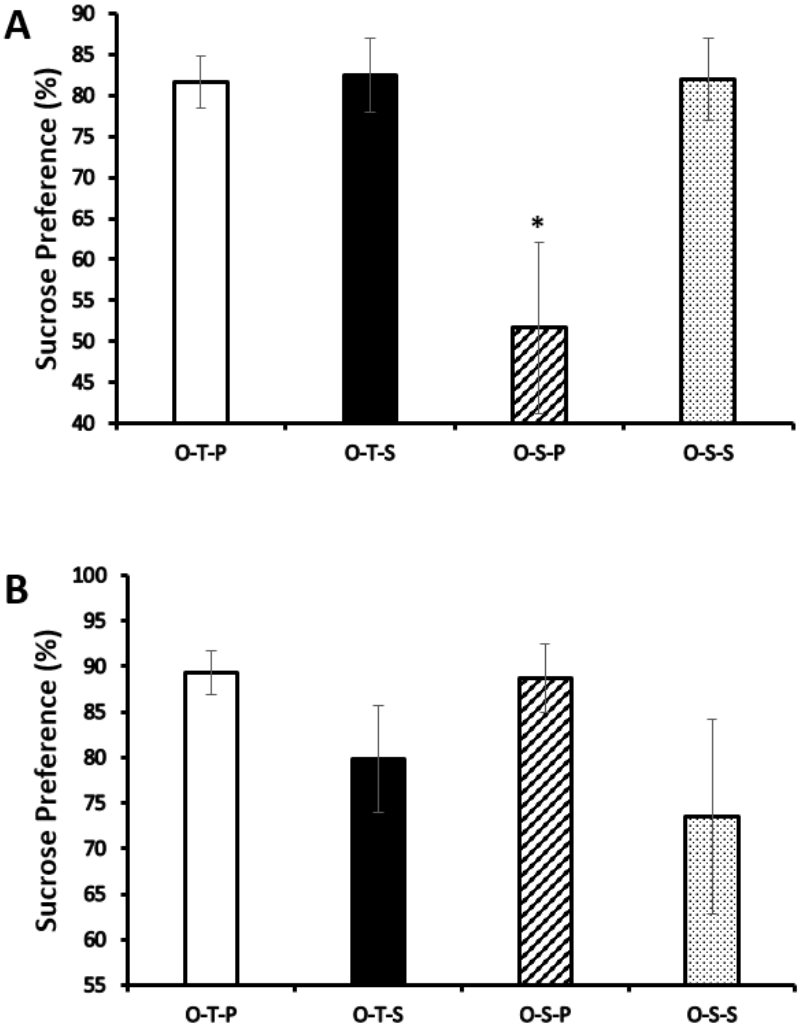

Figure 14. Average (±SEM) sucrose preference by all OBX groups after acute (A) and chronic (B) treatment in the SPT.

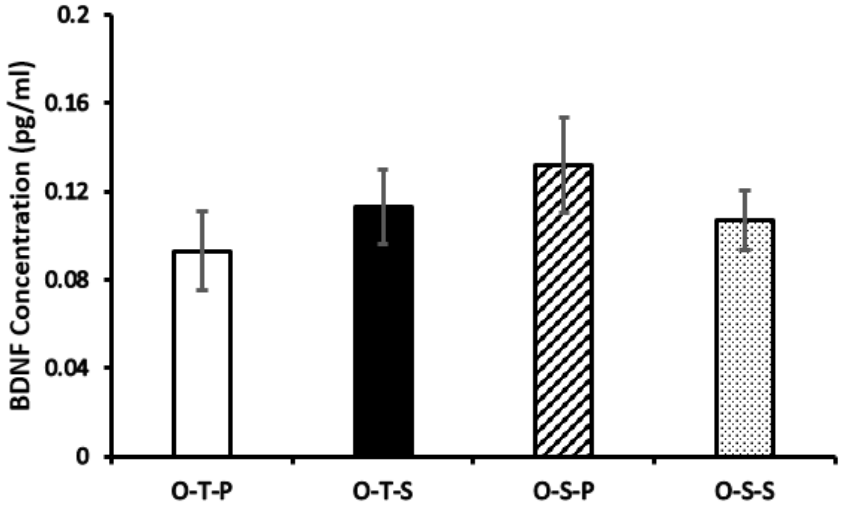

Figure 15. Average (±SEM) plasma concentration of BDNF for all OBX groups after chronic treatment.

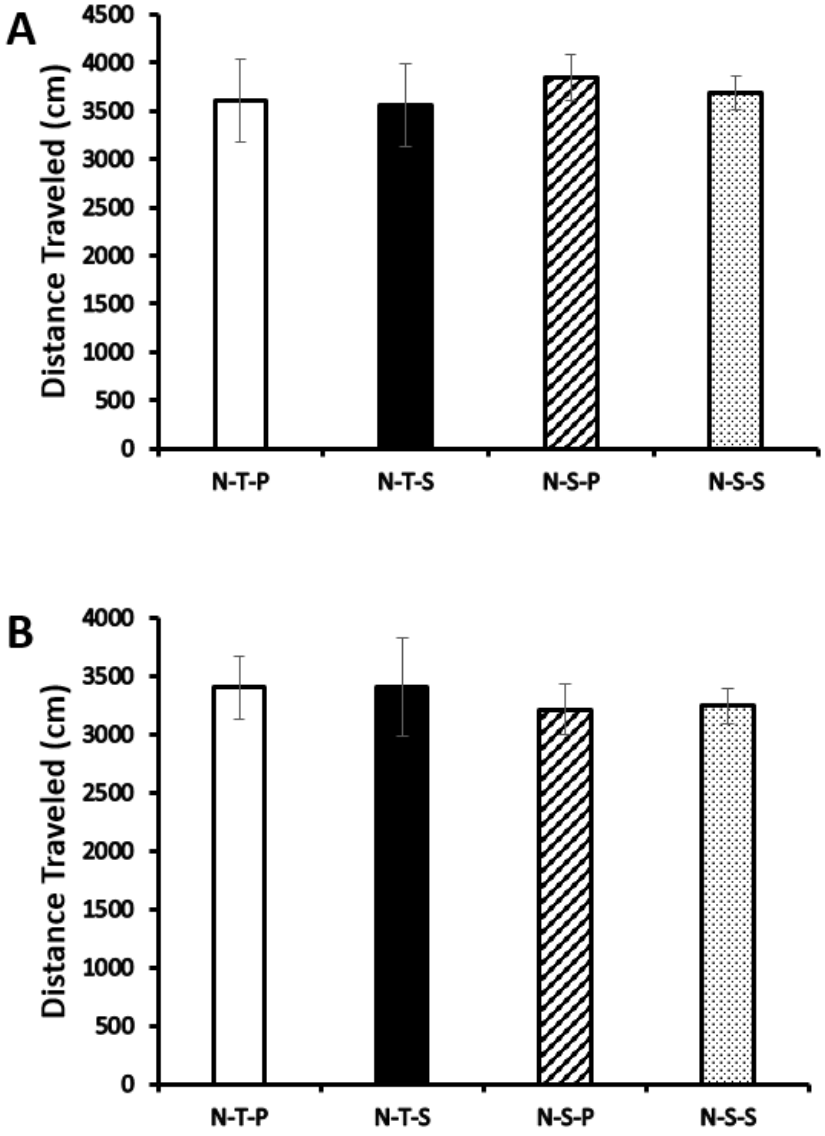

Figure 16. Average (±SEM) distance traveled by all non-OBX groups after acute (A) and chronic (B) treatment in the OFT.

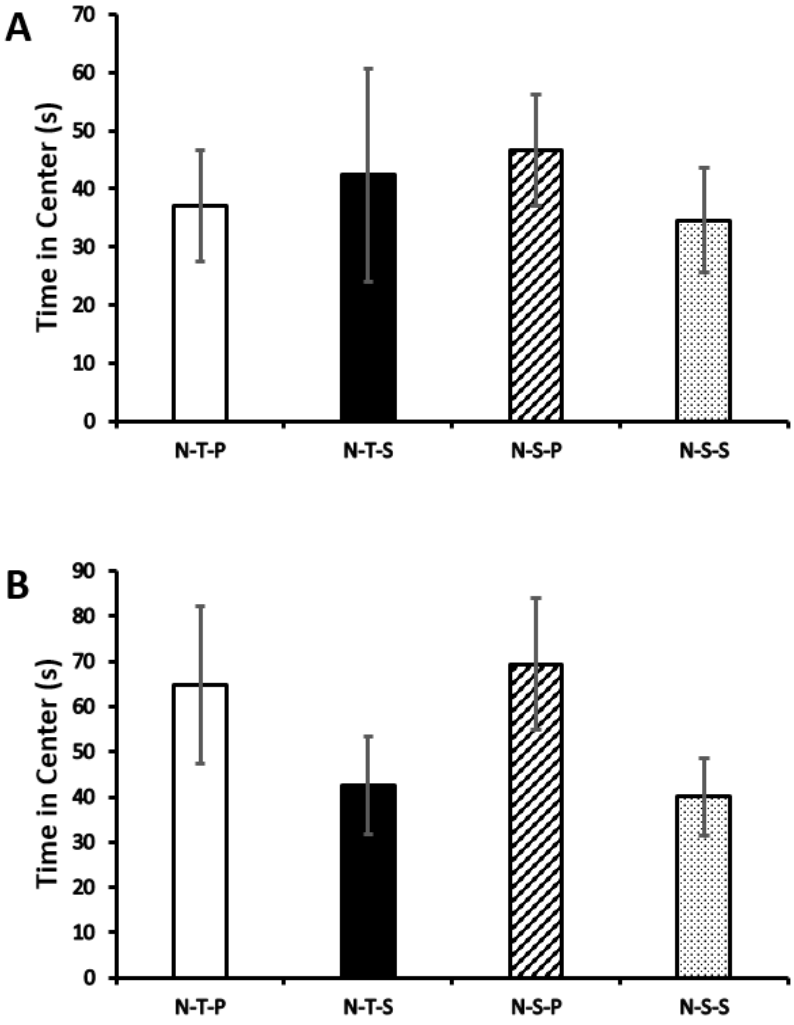

Figure 17. Average (±SEM) time spent in the center of the arena by all non-OBX groups after acute (A) and chronic (B) treatment in the OFT.

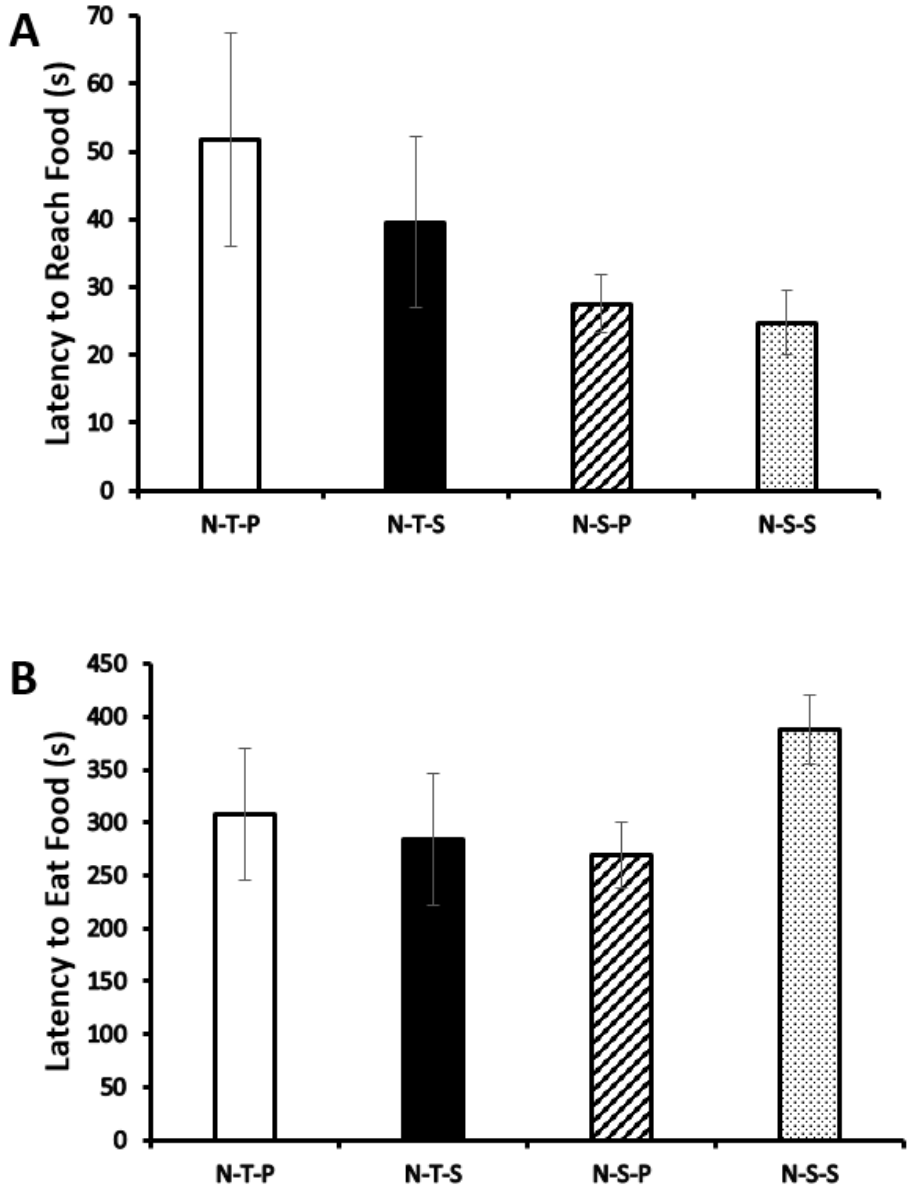

Figure 18. Average (±SEM) latency of all non-OBX groups to reach food (A) and start eating (B) in the NSFT.

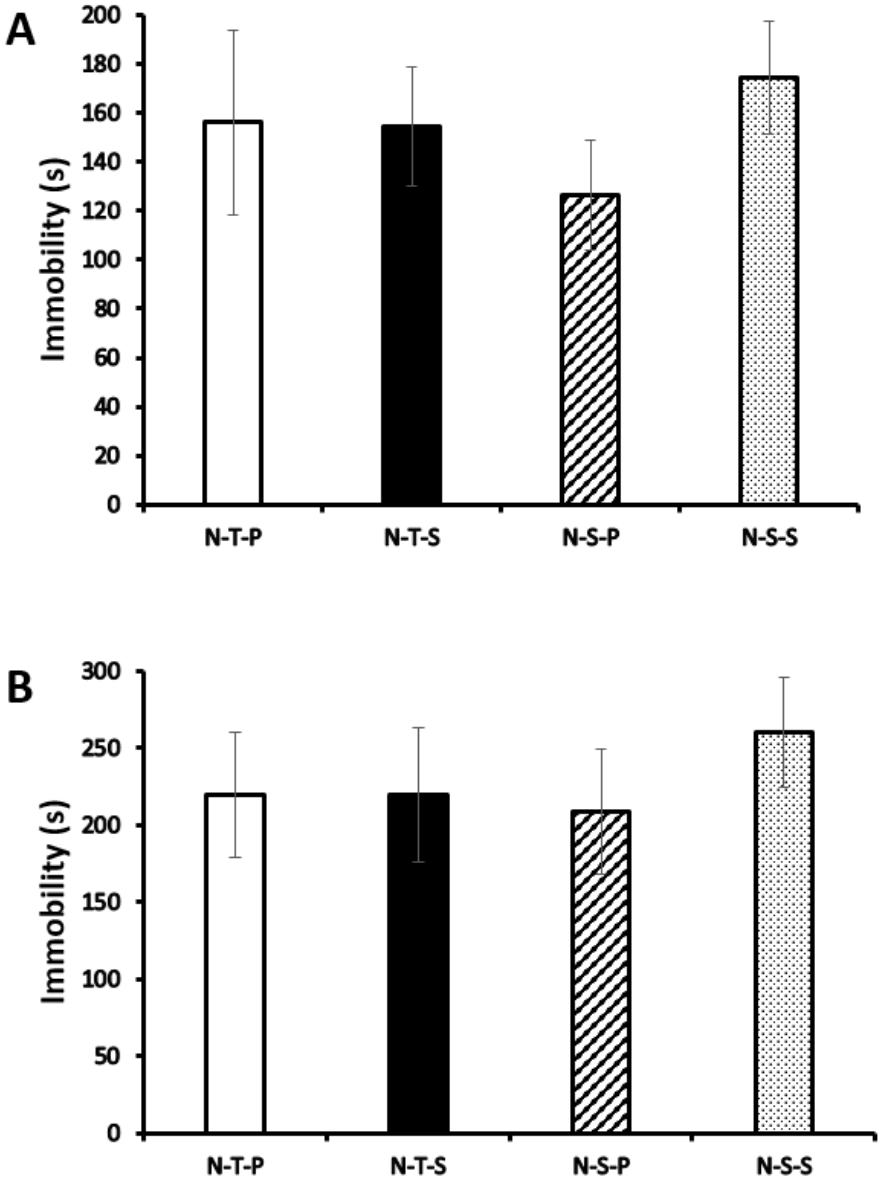

Figure 19. Average (±SEM) time spent immobile by all non-OBX groups after acute (A) and chronic (B) treatment in the FST.

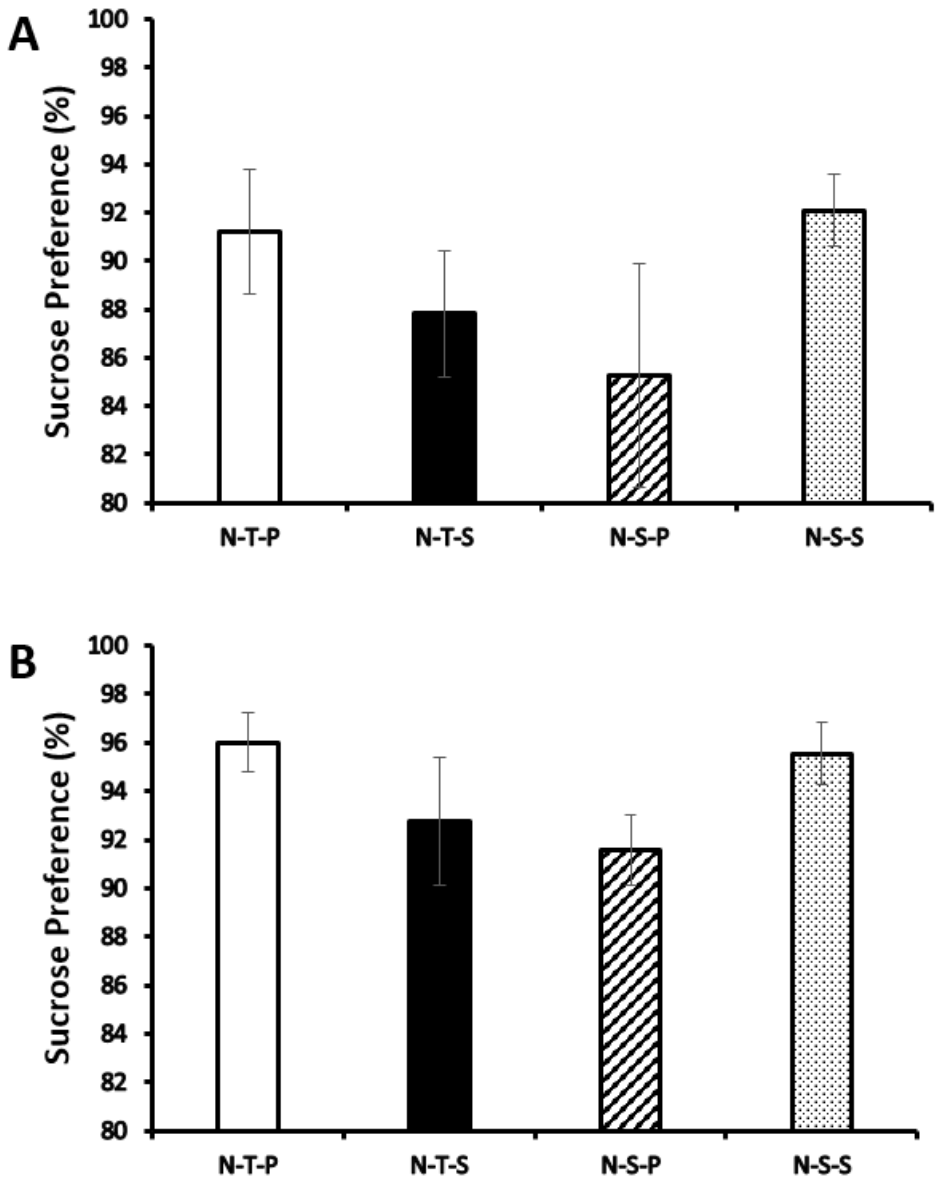

Figure 20. Average (±SEM) sucrose preference by all non-OBX groups after acute (A) and chronic (B) treatment in the SPT.

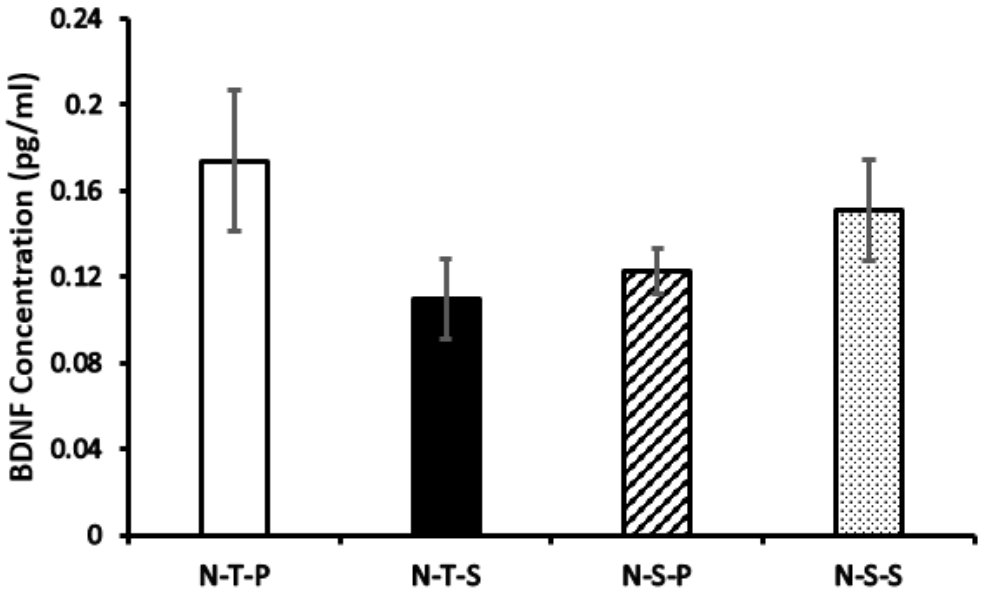

Figure 21. Average (±SEM) plasma concentration of BDNF for all non-OBX groups after chronic treatment.