AEROBIC EXERCISE: POTENTIAL ROLES IN THE TREATMENT AND PREVENTION OF DEPRESSION

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Abstract

Depression is one of the most common mental illness with over 300 million people affected worldwide. Additionally, research suggests that many fail to seek or decline treatment for multiple reasons. Antidepressants, the most common form of treatment, are highly successful in managing depression, but are accompanied by multiple drawbacks that reduce treatment availability for some individuals. To that end, aerobic exercise may serve viable alternative treatment as it is known to reduce depressive-like behaviors and increase prefrontal cortex and hippocampal brain derived neurotrophic factor (BDNF) levels, properties similar to those of antidepressants. This study looked to further assess the benefits of aerobic exercise in reducing and preventing depression. Wistar rats, treated with chronic unpredictable stress (CUS), and Wistar-Kyoto rats were subject to aerobic exercise followed by open field (OFT) and forced swim (FST) behavioral testing. Tissue histology further examined the quantity of BDNF containing cells in the medial prefrontal cortex (mPFC) and hippocampal CA3 region. Aerobic exercise increased OFT exploratory activity and increased FST swimming across strains and increased BDNF containing cells in the CA3 of WKY exercise-treated rats. These findings support current literature suggesting the beneficial role of exercise in reducing depression. Therefore, exercise may provide an alternative to antidepressants for some individuals in the treatment of depression.

Keywords: depression, aerobic exercise, BDNF, WKY rat model

List of Abbreviations:

BDNF = Brain-Derived Neurotrophic Factor

CUS = Chronic Unpredictable Stress

OFT = Open Field Test

FST = Forced Swim Test

mPFC = Medial Prefrontal Cortex

NIMH = National Institute of Mental Health

DSM-V = The Diagnostic and Statistical Manual of Mental Disorders

APA = American Psychological Association

MDD = Major Depressive Disorder

WHO = World Health Organization

WKY = Wistar-Kyoto

Akt = Protein Kinase B

MAPK = Mitogen-Activated Protein Kinase

TrK = Tyrosine-Kinase Receptor

FDA = Food and Drug Administration

SSRI = Selective Serotonin Reuptake Inhibitor

TCA = Tricyclic Antidepressant

MAOI = Monoamine Oxidase Inhibitor

SNRI = Serotonin-Norepinephrine Reuptake Inhibitor

PPHN = Persistent Pulmonary Hypertension of the Newborn

LTP = Long Term Potentiation

HIIT = High Intensity Interval Training

AD = Alzheimer's Disease

IACUC = Institutional Animal Care and Use Committee

TBS = Tris-Buffered Saline

PB = Phosphate Buffer

PBS = Phosphate Buffered Saline

DG = Dentate Gyrus

RT = Ventral Tegmental Area

NAc = Nucleus Aucubas

VEGF-A = Vascular Endothelial Growth Factor

PLCγ/PKC = Phospholipase Cγ

KYN = Kynurenine

KYNA = Kynurenic Acid

BBB = Blood Brain Barrier

3-HK = 3-Hydroxykynurenine

QUIN = Quinolinic Acid

KAT = Kynurenine Aminotransferase

ADN = Adiponetin

AMPK = AMP-Activated Protein Kinase

PI3K-Akt = Phosphoinositide-3-Kinase–Protein Kinase B/Akt

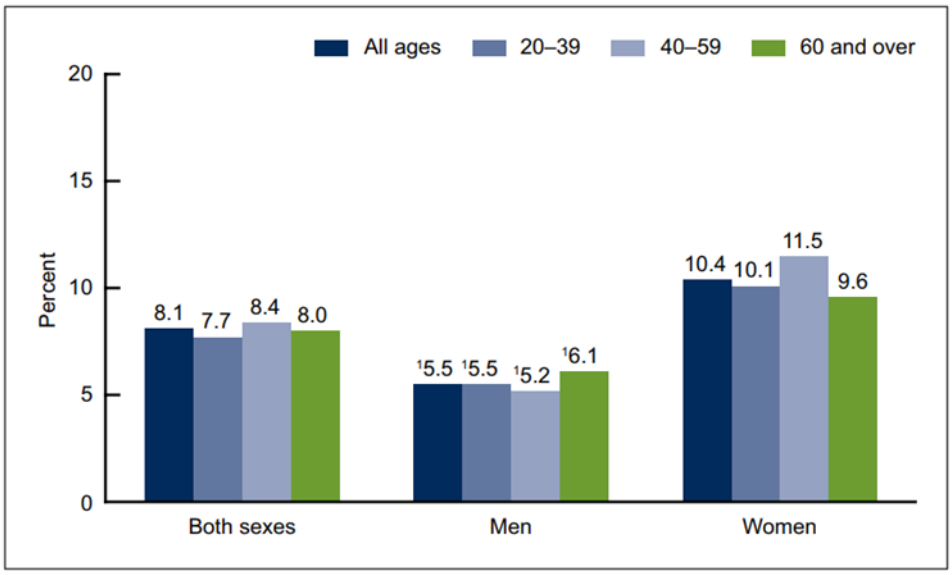
Introduction

Depression, a mood disorder characterized by changes in an individual's thoughts, feelings, and behaviors, is one of the most common mental illnesses in the United States (National Institute of Mental Health (NIMH), 2018). Depression impacts individuals of every age, sex, race, social class, and financial class and comes with high costs to society and higher reported functional impairments than many chronic diseases like diabetes and arthritis (Brody, Pratt, & Hughes, 2018). The manifestation of depression is thought to be influenced my multiple factors including genetics, environmental stressors, and psychological factors (NIMH, 2018). Collectively, the literature indicates that depression may have roots within the brain and may be, in part, due to an imbalance of neurotransmitters, especially serotonin (Duman & Li, 2012).

The Diagnostic and Statistical Manual of Mental Disorders (5th ed., DSM-V) divides depression into multiple types including Major Depressive Disorder and Persistent Depressive Disorder (American Psychiatric Association (APA), 2013). Major Depressive Disorder (MDD) is characterized by a period of two weeks or longer in which a person reports either a depressed mood or a loss of interest/pleasure with at least four other symptoms as denoted in the DSM-V (APA, 2013). Persistent Depressive Disorder (also called Dysthymia) is characterized by a depressed mood that lasts for at least two years in which a person experiences episodes of major depression and episodes of less severe depression (APA, 2013).

Over 300 million people worldwide are estimated to suffer from depression (World Health Organization (WHO), 2018). From 2013-2016, 8.1% of Americans experienced a depressive disorder (Figure 1; Brody et al., 2018). The prevalence of depression differs based on factors such as age, sex, race, and family income. Considering sex, for example, a 2013-2016 report showed that females were almost twice as likely to develop depression compared to males

(Female: 10.4%, Males: 5.5%) (Brody et al., 2018). Over 50% of depressed individuals reported a difficulty with work, home, or social activities due their depressive symptoms, making depression the leading cause of disability worldwide (Brody et al., 2018; WHO, 2018). Major depression, a highly debilitating classification of depression, is the most prevalent form of depression in the United States population. In 2016, the National Institute of Mental Health (NIMH) reported that 6.7% of adults suffered from major depression, an incidence that was nearly double that value in adolescents (NIMH, 2017).



[1]Significantly different from females in same age group.
NOTES: Depression was defined as a score greater than or equal to 10 on the Patient Health Questionnaire. Access data table for Figure 1 at: https://www.cdc.gov/nchs/data/databriefs/db303_table.pdf#1.
SOURCE: NCHS, National Health and Nutrition Examination Survey, 2013–2016.

Figure 1. Percentage of individuals with depression in the United States (2013-2016) by age and sex (Brody et al., 2018).

While the overall prevalence of depression is cause for alarm, depression is a treatable illness. Common treatments for depression include medication (antidepressants), psychotherapy, and electroconvulsive therapy (NIMH, 2018). Unfortunately, and possibly most alarming, treatment is not attainable or not sought after by individuals with depression (NCHS Data Brief). Fewer than 50% of individuals with depression received treatment worldwide, with rates below

10% in some countries (WHO, 2018). In 2016, the NIMH and the National Survey on Drug Use and Health (NSDUH) reported that of Americans with depression, 63% of adults (18+ years old) (Figure 2A), 44% in young adults (18-25 years old), and 40% of adolescents (12-17 years old) (Figure 2B) received treatment for depression (NIMH, 2017; Substance Abuse and Mental Health Services Administration, 2017).

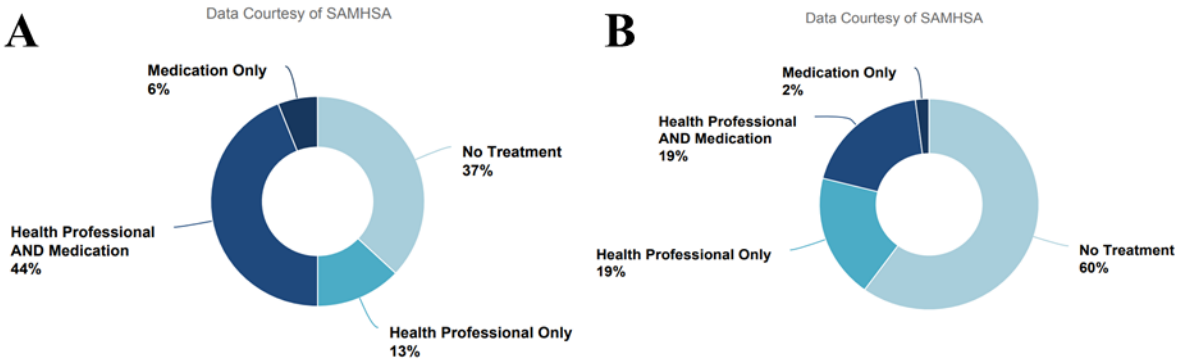


Figure 2. Distribution of treatment received among individuals with Major Depressive Disorder (2016). A: Adults; B: Adolescents (NIMH, 2017; Substance Abuse and Mental Health Services Administration, 2017).

The low treatment seeking rates may be, among other factors, due to the stigma surrounding depression and mental illness (Gulliver, Griffiths, & Christensen, 2012). This is of concern for many reports as this stigma may lead to the underreporting of depression, potentially making current statistics on depression inaccurate and vastly understated (Bharadwaj, Pai, & Suziedelyte, 2017). This stigma also concerns medical officials as it may reduce treatment-seeking behaviors for a disorder that is already one of the leading causes of suicide worldwide (Bharadwaj et al., 2017; WHO, 2018).

Rodent models are a primary method of studying depression *in vivo* (Duman & Aghajanian, 2012). The primary rodent models used in depression research are the chronic unpredictable stress (CUS) model (Duman & Aghajanian, 2012; Zheng et al., 2006) and the Wistar-Kyoto (WKY) rat strain model (Kurtz & Morris, 1987; Will, Arid, & Redei, 2003). The

CUS model works by consistently exposing rodents to randomized chronic mild stressors (e.g. damp bedding, tilted cage, reduced temperature, empty cage) which lead to the development of behaviors associated with anxiety and depression (Gibson, Klatzkin, & Littlefield, 2012; Rotzinger, Lovejoy, & Tan, 2010). The WKY rat strain, another common model for examining depression in rodents was originally developed by Okamoto and Aoki (1963) to be a model of hypertension but was later found to express depressive behavioral patterns. The strain has been examined for its reliability and validity as a model of depression. Kurtz and Morris (1987) and Will et al. (2003) showed that the WKY rat stain was a reliable model of endogenous depression and that the strain exhibited common behavioral symptoms of depression on tests such as the Open Field Test (OFT) and Forced Swim Test (FST). While these findings were consistent across much of the literature, Will et al. (2003) suggested that there may be high variability within the WKY rat strain which may impact the findings of studies using the strain. While this may be of concern, this potential variability may serve as a strength of the model given the heterogeneity of depression in the human population. It is important to note that both of these models of depression are behavioral models and were not designed through gene knockout.

Two prominent regions of the brain imperative for higher-level human functioning are the hippocampus and prefrontal cortex. The hippocampus, part of the larger limbic system within the brain, is a medial structure deep under the cortex of the brain that is responsible for memory consolidation and recovery (Preston & Eichenbaum, 2013). The prefrontal cortex, part of the frontal cortex, is the most anterior region of the brain and plays a major role in planning, decision making, and aspects of personality (Preston & Eichenbaum, 2013).

In depression, both the hippocampus and prefrontal cortex undergo major structural changes (Duman & Li, 2012; Miguel-Hidalgo & Rajkowska, 2002). Neuronal death is a common

occurrence within the brain as neural connections undergo dynamic changes due to an individual's specific experiences. Processes such as pruning (the removal of damaged or defective neurons), sprouting (the growth of new connections), and reorganization (the modification of neural connections) all work to optimize brain performance when functioning normally (Yamahachi, Marik, McManus, Denk, & Gilbert, 2009). When this neuronal death becomes excessive and erroneous, atrophy, a common neurological factor expressed in depressed individuals, often occurs (Duman & Li, 2012). Live imaging and post-mortem studies of the brains of depressed humans show substantial levels of atrophy in the hippocampus and prefrontal cortex (Duman & Aghajanian, 2012; Duman & Li, 2012; Erickson, Miller, & Roecklein, 2012; Miguel-Hidalgo & Rajkowska, 2002), a finding also seen in rodents (Zheng et al., 2006). Closer examinations of human hippocampi and prefrontal regions found that individuals with depression exhibited decreased grey matter tracks, indicating the presence of substantial neuronal death and suggesting that neuronal death may occur inside the brains of depressed individuals (Miguel-Hidalgo & Rajkowska, 2002).

Structural correlates of depression can also be seen in individual neurons (Duman & Aghajanian, 2012). Dendrites (the projections off the neural cell body that typically receive signals from an adjacent neuron), axons (the major projection carrying the neural signal to the axon terminal), and synapses (the junction between two neurons where the neural message is passed chemically) all show alterations in depressed individuals (Duman & Aghajanian, 2012; Duman & Li, 2012). Post-mortem studies in both humans and rodents with depression documented reductions in neuron size, dendrite and axon branching, axon length, and quantity of terminal synapsing (Duman & Aghajanian, 2012; Duman & Li, 2012).

Neurotrophic factors, small proteins within the brain that promote the survival of neurons and glia, the growth of neurons, and neuronal differentiation, are also disrupted in individuals with depression (Duman & Li, 2012; Skaper, 2012). Brain-derived neurotrophic factor (BDNF) is one neurotrophic factor responsible for maintaining a healthy neural system within the brain. BDNF, found in high concentrations in the hippocampus and prefrontal cortex of the brain, aids in the survival of neurons by serving many neural-promoting functions including the regulation of neurogenesis, neural dendrite length, and neural spine density (Duman & Li, 2012; Erickson et al., 2012; Groves, 2007). Clinical trials and post-mortem studies in humans showed reduced BDNF levels in brains of depressed individuals (Erickson et al., 2012; Groves, 2007), particularly in the hippocampus and prefrontal cortex (Duman & Li, 2012; Zheng et al., 2006). Given its role in promoting neuron survival, this reduction in BDNF may be an underlying factor in the cellular disruptions associated with depression.

Researchers developed the Neurotrophic Hypothesis of depression to better understand and explain the underlying mechanism of depression by focusing on the role of neurotrophic factors in the brain and their altered levels during depression (Duman & Aghajanian, 2012; Duman & Li, 2012; Groves, 2007). The basis of the Neurotrophic Hypothesis suggests the role of reduced neurotrophic support in the development and maintenance of depression, a notion that is supported by the discovery that the induction of BDNF can induce antidepressant-like effects (Duman & Li, 2012). Therefore, clinically, BDNF has potential as a biomarker, a biological indicator of the presence of a disease, for depression (Duman & Li, 2012). BDNF deletion studies in mice showed neuronal atrophy and decreased dendrite length and branching in the hippocampus and prefrontal cortex, both of which are commonly seen in the brains of depressed individuals (Duman & Aghajanian, 2012; Duman & Li, 2012). By infusing male Sprague

Dawley rats with BDNF, Shirayama, Chen, Nakagawa, Russell, and Duman (2002) reversed these neuronal deficits.

BDNF and other neurotrophic factors share general neurotrophic cellular mechanisms seen in Figure 3 (Skaper, 2012). Neurotrophic factors, like BDNF, bind to cell surface tyrosine kinase receptors, and dimerization of the two receptor parts leads to the activation of multiple downstream pathways *via* phosphorylation, a common molecular energy transfer step involving the addition of a phosphoryl group (PO_3). This phosphorylation, in turn, activates multiple secondary messengers which contribute to the activation of the protein kinase B (Akt) and mitogen-activated protein kinase (MAPK) pathways (Skaper, 2012). Activation of the Akt pathway, a highly conserved signaling pathway among mammals mediating many cellular functions (angiogenesis, metabolism, growth, survival, and protein synthesis) through gene transcription regulation, leads to the upregulation of pro-survival genes that promote neuronal growth and survival (Hemmings & Restuccia, 2012). Activation of the MAPK pathway, a highly conserved signaling pathway among mammals composed of serine/threonine protein kinases regulating multiple cellular functions (neuronal proliferation, differentiation, and survival) activates multiple downstream transcription factors that increase neuronal differentiation (Zhang & Liu, 2002).

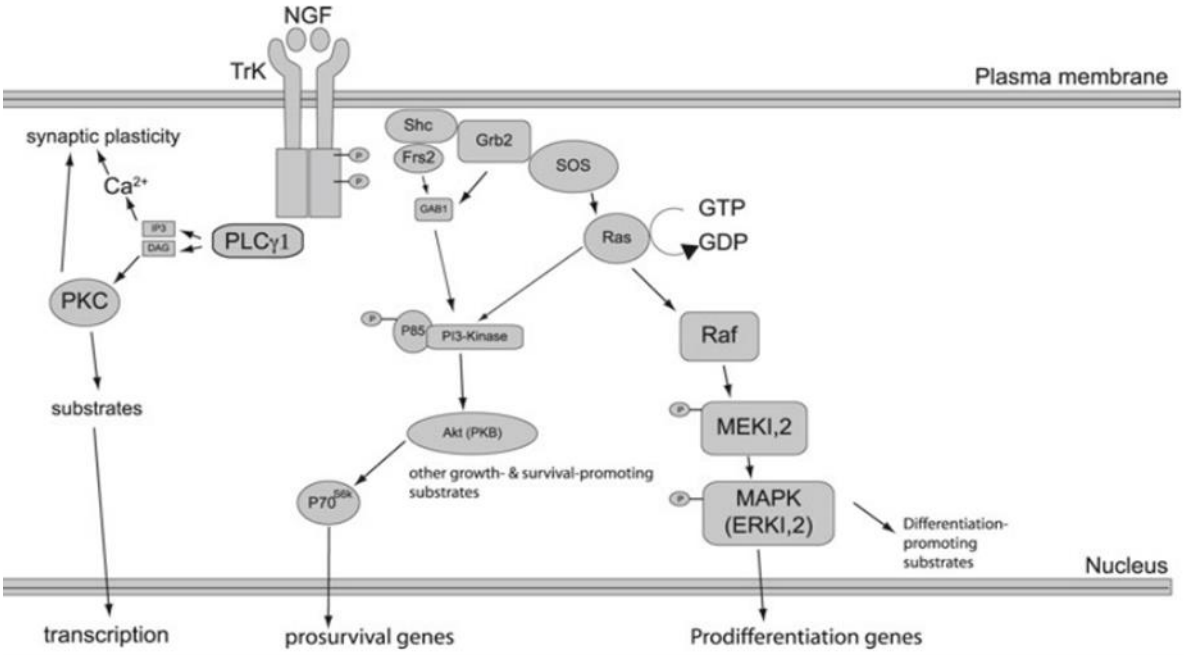


Figure 3. Generalized neurotrophin signaling pathway. Interactions depicted are of NGF (example neurotrophin). NGF binding with tyrosine-kinase receptor (TrK) initiates dimerization and autophosphorylation. Multiple intracellular molecules form a complex that activates Ras, by replacing GDP with GTP, which further activates the Atk and MAPK pathways. Downstream nuclear translocation causes phosphorylation of transcription factors that promote neuronal differentiation, survival, and growth, as well as increase synaptic plasticity (Skaper, 2012).

Currently, the one of the most common treatment courses for depression is pharmaceuticals (Food and Drug Administration (FDA), 2017). There are multiple classifications of antidepressant drug treatments including selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and serotonin-norepinephrine reuptake inhibitors (SNRIs), each having their own specific mechanisms of action (FDA, 2017). Each category of antidepressant contains multiple drugs that all have different efficacies. The most common class of antidepressants is the SSRIs due to their relatively low side effects (FDA, 2014).

SSRIs reestablish the balance of serotonin in the brain by blocking the serotonin transporter 5-HTT on presynaptic neurons and astrocytes, a support glial cell in the brain,

increasing the extracellular concentration of serotonin (Oberlander, Gingrich, & Ansorge, 2009). Researchers suggest that serotonin neurotransmission directly impacts downstream neurotrophic factors, specifically BDNF (Zheng et al., 2006). Duman and Li (2012) and Zheng et al. (2006) showed that antidepressant treatments increase BDNF in the hippocampus. Further, Miguel-Hidalgo and Rajkowska (2002) demonstrated that antidepressant treatments combat hippocampal atrophy by increasing the number and complexity of neurons within the hippocampus. Antidepressant treatments may function to promote hippocampal survival, a process that may be mediated by antidepressants' increase of BDNF (Duman & Li, 2012; Miguel-Hidalgo & Rajkowska, 2002). Both stress and antidepressant therapies impact BDNF levels within the brain. Elevated cortisol and other stress responses reduce BDNF levels and, consequently, neuronal proliferation, survival, and plasticity in the hippocampus leading to decreased overall hippocampal function and mood (Groves, 2007). The impacts of antidepressant treatments, in comparison to a stress model of depression, on BDNF and its downstream effects can be seen in Figure 4 (Groves, 2007).

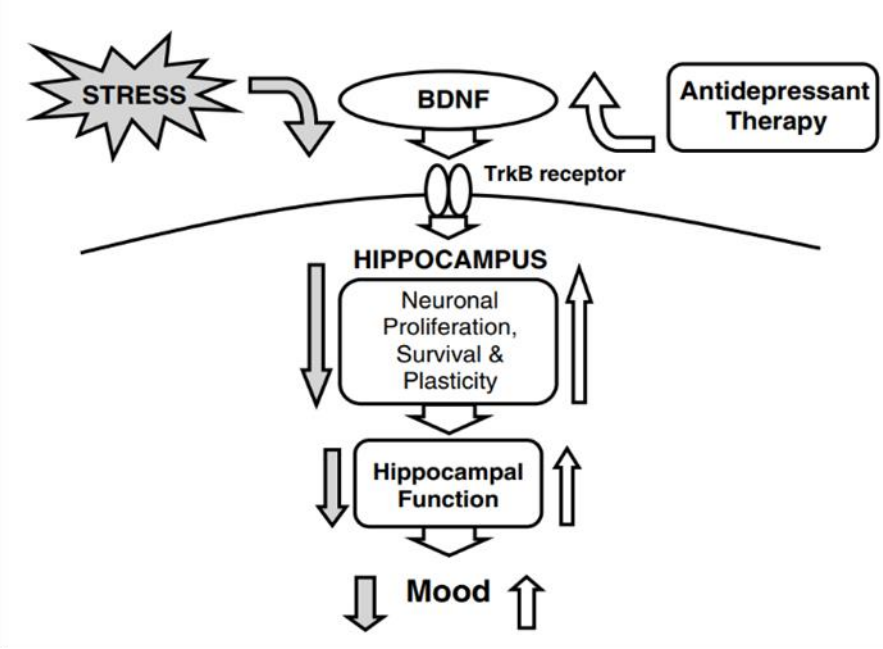


Figure 4. Summary of opposing roles of stress and antidepressant therapy on hippocampal BDNF, hippocampal functioning, and resulting mood. Abbreviations: TrkB receptor, tyrosine kinase receptor B (Groves, 2007).

Despite the successes of antidepressants like SSRIs in combating depression, they carry potential drawbacks, such as variable treatment efficacies, rigorous drug schedules, side effects, cost, and stigma, all of which might limit people from obtaining effective treatment. Duman and Li (2012) demonstrated that only about one-third of patients respond to their first antidepressant prescribed, thus highlighting the low efficacy of antidepressant therapies. Antidepressants effects may not be noticeable until three to four weeks after the start of medication as the drug must build up within the system before having an impact, often leaving patients with depression for multiple weeks after the start of the treatment (Duman & Li, 2012). Further complicating treatments, research and warning labels note that abruptly stopping antidepressants may lead to symptoms of withdrawal and/or the return of the depression which poses an added challenge for patients who need to switch medications if the initial drug is not effective (FDA, 2017; Keks, Hope, & Keogh, 2016). To switch antidepressants, patients must taper off the first medication,

potentially leaving patients without treatment and at risk of the return of their depressive symptoms for a period, before starting the second medication (FDA, 2017; Keks et al., 2016).

Antidepressants also come with the potential for side effects. Cascade, Kalail, and Kennedy (2009) demonstrated that patients taking SSRIs most commonly reported sexual dysfunction, sleep disturbances, and weight gain as negative effects. Taking antidepressants may also increase suicidal thoughts in adolescents and adults (FDA, 2017; Gibbons et al., 2007). Recent research has also focused on antidepressant use during pregnancy, and whether their use may lead to birth defects, deficits in motor development, and/or persistent pulmonary hypertension of the newborn (PPHN) (Casper et al., 2003; FDA, 2018).

The cost of antidepressant medications is the first hurdle that patients face. An analysis of depression patient costs suggests that a six-month SSRI treatment supply can cost upwards of $1,700 (Sheehan, Eaddy, Shah, & Mauch, 2005). While medical insurance may cover some expenses, antidepressant treatments are often life-long treatments and, as a result, individuals with low incomes or poor prescription drug coverage may be unable to obtain consistent antidepressant treatment (Chisholm, Sanderson, Ayuso-Mateos, & Saxena, 2004). Also, due to the stigma related to depression, individuals with depression showed reduced treatment seeking behaviors toward medical professionals and reduced antidepressant drug adherence after obtaining professional medical treatment (Gulliver et al., 2012; Sirey et al., 2001).

Given the drawbacks of antidepressants, alternative therapies need to be explored to replace or supplement the need for medications. Aerobic exercise may serve as a good alternative as it addresses many of the potential problems of drug treatments (i.e. low cost, personalized schedule, low stigma, total body health benefits; Dunn, Trivedi, Kampert, Clark, & Chambliss, 2005; Hatziandreu, Koplan, Weinstein, Caspersen, & Warner, 1988; Warburton,

Nicol, & Bredin, 2006). Aerobic exercise reduces depression and has been shown work as effectively as common SSRIs antidepressant treatments (van Pragg, 2009). Studies of high school students, a population with an abnormally high susceptibility to and prevalence of depression, suggested that aerobic exercise and sport participation were linked to increased psychological and social well-being and decreased depression (Moti, Birncaum, Kubik, & Dishman, 2004; Sanders, Field, Diego, & Kaplan, 2000). Nabkasorn et al. (2005) expanded on this research in college students, another at-risk population for depression (Eisenberg, Gollust, Golberstein, & Hefner, 2007), and demonstrated that aerobic exercise was effective in improving depressive symptoms.

When considering aerobic exercise as an effective treatment for depression, metabolic rate and energy expenditure may influence the effectiveness of the exercise treatment. A 2005 study of adults suggested that exercise was successful at reducing major depressive disorder in adults when energy expenditure was at or above the public health recommended dose of 17.5 kcal/kg/week (Dunn et al., 2005).

Erickson et al. (2012) found that aerobic exercise increased hippocampal volume in adult humans, a region of the brain that undergoes atrophy during depression, an effect rooted in the neurogenic benefits of aerobic exercise. Similarly, multiple rodent studies, have documented that voluntary aerobic exercise increased neurogenesis in the hippocampus (Eadie, Redila, & Christie, 2005; Erickson et al., 2012; Ernst, Olson, Pinel, Lam, & Christie, 2006; Kempermann, Kuhn, & Gage, 1997; van Pragg, Christie, Sejnowski, & Gage, 1999). Kempermann et al. (1997) showed that aerobic exercise promoted the survival of neural precursor cells, stem cells with the ability to regenerate and differentiate into mature neural cells, leading to increased hippocampal volume. Aerobic exercise may also play a role in increasing dendritic branching and synaptic

plasticity. For example, Eadie et al. (2005) found that voluntary exercise in rats increased dendritic complexity and connectivity within the cortex. Further, multiple studies documented that aerobic exercise increased synaptic plasticity by promoting long-term potentiation (LTP), the process by which consistent excitatory synaptic stimulation strengthens synaptic connections, throughout the cortex (Eadie et al., 2005; Farmer et al., 2004).

The structural changes in the brain that arise consequent to aerobic exercise may be rooted in the impact that exercise has on BDNF levels. Multiple rodent studies found that aerobic exercise increased BDNF mRNA and overall BDNF levels in the hippocampus and cortex (Erickson et al., 2012; Marlatt, Potter, Lucassen, & van Pragg, 2002; Zheng et al., 2006). The intensity and timing of aerobic exercise may also play a role in the effectiveness of the exercise treatment. Naghibzadeh, Ranjbar, Tabandeh, and Habibi (2018) found that high intensity interval training (HIIT), short intense anaerobic exercise periods followed by short rest periods, increased BDNF transcription more than continuous aerobic exercise in rodents.

Additionally, prior work suggests exercise may act as a protective factor against the development of depression. García-Mesa et al. (2011) showed that exercise served a protective role against the development of neurological disorders such as Alzheimer's disease (AD), a neurological disorder characterized by severe hippocampal and cortex damage leading to memory loss, and Parkinson's disease (García-Mesa et al., 2011; Paillard, Rolland, & Barreto, 2015), and psychiatric disorders such as depression (Cotman & Engesser-Cesar, 2002) in mouse models. Exercise also delayed the onset of AD and protected against the onset of depression in human clinical trials (Cotman, Berchtold, & Christie, 2007).

Depression is rooted in the brain as depressed individuals show signs of atrophy, abnormalities in neuron projections, and reduced BDNF (Duman & Aghajanian, 2012; Duman &

Li, 2012; Erickson et al., 2012; Miguel-Hidalgo & Rajkowska, 2002). For many patients, pharmaceutical treatments constitute the main therapy for depression. Unfortunately, antidepressants often fall short as they have variable efficacies, rigorous drug schedules, and potential side effects (Cascade et al., 2009; Duman & Li, 2012; Keks et al., 2016). Aerobic exercise has been shown to increase hippocampal volume (by promoting hippocampal neurogenesis), BDNF, and hippocampal neural projection complexity (Eadie et al., 2005; Erickson et al., 2012; Marlatt et al., 2002). Clinically, aerobic exercise also reduces depressive symptoms in adolescents with depression (Moti et al., 2004; Nabkasorn, 2005; Sanders et al., 2000). Therefore, exercise may serve as a viable treatment option for depression and may even protect against the development of the disease. In the current study, we hypothesize that aerobic exercise will reduce the behavioral and neurological correlates with depression and will reduce the severity of the development of depression. WKY rats were trained to voluntarily run in a gradually elongating lane and Wistar rats, a parent strain of the WKY strain, were trained to run on a rodent treadmill during a five-week aerobic exercise protocol. After cessation of the exercise regimen, OFT and FST behavioral tests and neurological assessments of BDNF and neuronal atrophy were conducted to determine the role of aerobic exercise as a treatment and protective factor in depression.

Materials & Methods

Animals

WKY (n = 8) and Wistar (n = 9) rat strains were included in the study. The WKY strain was initially developed by Okamoto & Aoki (1963) as a hypertensive model but later studies found WKY rats exhibited depressive behaviors and the strain is currently seen as an acceptable rodent model for endogenous depression. Due to vastly different exercise capabilities between the strains and the inability of the WKY strain to meet the treadmill training criteria – both strains moved directionally on the stationary treadmill, but the WKY would not move directionally on the treadmill moving at 0.5 mph – the strains were divided into two separate studies. Table 1 and Figure 5 depict the study breakdown and experimental timeline, respectively.

Table 1. Experiment breakdown by rat strain and number of animals per treatment group.

Experiment	Rat Strain	Exercise	Non-Exercise
1	WKY	4 (2 Female, 2 Male)	4 (3 Female, 1 Male)
2	Wistar	4 (2 Female, 2 Male)	4 (2 Female, 2 Male)

Figure 5. Live-animal exercise and behavioral testing protocol duration and layout. The initial exercise (WKY) and exercise and CUS (WIS) protocol was carried out for 35 days with control animals not receiving exercise. Following exercise and CUS, OFT and FST 1 testing was conducted. An extension of the exercise and CUS protocols were added, along with FST 2 testing before euthanasia and perfusions were conducted.

All animals were bred in-house (parent strains: Charles River) and breeding took place in a 12:12-hour light cycle in which the lights were on from 8:00 am to 8:00 pm. Upon birth, breeding mothers and pups were transferred to a 12:12-hour reverse light-dark cycle in which the lights were on from 8:00 pm to 8:00 am so not to interfere with the gestation period of the breeding mothers. The animals were maintained on the reverse light cycle and all procedures took place during the dark phase of the cycle. All animals were provided rodent chow and water *ad libitum* and environmental enrichment (one five-inch sterilized ¾-inch cut PVC pipe per cage). After rodents matured to 3 months, rodent chow was restricted (20 g per day) to maintain a healthy weight. Temperature was maintained at 70°F-72°F and cage cleaning was conducted weekly. Animal weights were measured weekly to monitor weight gain/loss due to exercise participation. Animals were monitored during and after exercise for limb damage and were removed from the study if signs of injury were visible. All procedures were conducted at Washington College, MD and were approved by the Washington College Institutional Animal Care and Use Committee (IACUC; Protocol: F18-003).

Experiment 1: Role of Exercise as a Treatment for Depression

Animals. WKY rats (n = 8, 5 female & 3 male), 14-19 weeks of age, were used as an endogenous model of depression. Post-weaning, animals were matched by sex and divided evenly into exercise (WKY-E; n = 4, 2 female & 2 male) and non-exercise control (WKY-C; n = 4, 3 female and 1 male) groups. Animals in the exercise and non-exercise groups were housed separately in pairs by sex (if possible) or singly if no sex-match was available in the group.

Exercise. Animals within the exercise group engaged in voluntary exercise for 35 days (29 exercise days, 6 off days total). Exercise consisted of walking back-and-forth in an enclosed custom lane (72 x 5.5 x 7 in, Figure 6). Animals were removed from their cage, placed into the

lane individually, and allowed to move freely for the duration of the exercise session (three minutes). Exercise sessions occurred once per day, except on designated rest days. To motivate the animals to walk, small Fruit Loop pieces were continuously placed at the ends of the lane. The duration of the 35 days of exercise was divided into 6 intervals. At each new interval, the distance between the ends of the lane was increased by moving the removable divider that blocked the full distance of the lane. This was done to familiarize the animals with the lane and ease the them into the exercise procedure. Table 2 depicts the exercise protocol. As the animals became accustomed to the exercise, Fruit Loop pieces were provided at the end of the lane on a fixed interval of every 3 full lane crossings. At the end of each exercise session, animals were returned to their cages and the lanes were sanitized. Animals in the non-exercise group remained in their cages throughout the 35 days. Animal handling was minimized in both groups to reduce the impact of handling on the animals.

Figure 6. Custom exercise lanes (72 x 5.5 x 7 in).

Table 2. WKY exercise protocol.

Interval	Days	Length (ft)	Duration (min)
1	1-2	2	3
	3	Rest	-
2	4-8	3	3
	9	Rest	-
3	10-14	4	3
	15	Rest	-
4	16-20	5	3
	21	Rest	-
5	22-26	6	3
	27	Rest	-
6	28-32	6	3
	33	Rest	-
	34-35	6	3

Experiment 2: Role of Exercise as a Protective Factor in the Development of Depression

Animals. Wistar rats (n = 9, 4 female & 5 male), 19 weeks of age, were used. Post-weaning, animals were matched by sex and divided evenly into exercise (Wistar-E; n = 5, 2 female & 3 male) and non-exercise control (Wistar-C; n = 4, 2 female & 2 male) groups. Due to a refusal to exercise, one animal was removed (n = 8) from the Wistar-E treatment group leaving the group with four animals (Wistar-E: n = 4, 2 female & 2 male) for the remainder of the procedures. All animals were subject to the CUS procedure. Animals housed individually as per the CUS model and additional animal monitoring occurred in conjunction with the CUS procedures.

Exercise. Animals within the exercise group engaged in forced exercise for 35 days (29 exercise days, 6 off days). Exercise consisted of running on a custom rodent treadmill (Figure 7)

that was constructed based off the model proposed and tested by Boughanim and Bergdahl (2017). The custom treadmill used was constructed using a standard treadmill (Weslo Crosswalk 5.2t). A custom enclosed acrylic chamber (30 x 16 x 4.5 in) with an open floor was built to sit on the treadmill just above the moving belt. The chamber was separated into 3 lanes (30 x 5.25 x 4.5 in) to allow animals to run individually. The rear 4.5 inches of the lanes hung off the end of the treadmill belt and was filled using a wooden block to create a safe standing place for the animals. Exercise sessions occurred once per day, except on designated rest days. The sound of a paintbrush and/or flyswatter rubbing against the moving treadmill was used to motivate the animals to run on the treadmill. If needed, the gentle probe with a paintbrush and/or flyswatter was also used. The duration of the 35 days of exercise was divided into 6 intervals. The speed of the treadmill gradually increased by 0.1 mph (0.045 meters per second) over the 6 intervals. The different sessions within the interval consisted of either 3x30 second exercise bouts or 3x45 second exercise bouts alternating with 3x30 second rest periods. Table 3 depicts the exercise protocol. Animals were closely monitored for difficulty or failure at an exercise speed and duration. This was commonly seen as constant motivation to continue running, ignoring motivation and falling to the rear platform, or attempting to exit the chamber. If this occurred, the speed was reduced to the most recent successfully completed speed and time. At the end of each exercise session, animals were returned to their home cage and given a Fruit Loop as a reward and the treadmill lanes were sanitized. Animals in the non-exercise group remained in their cages throughout the 35 days. Animal handling was minimized in both groups to reduce the impact of handling on the animals.

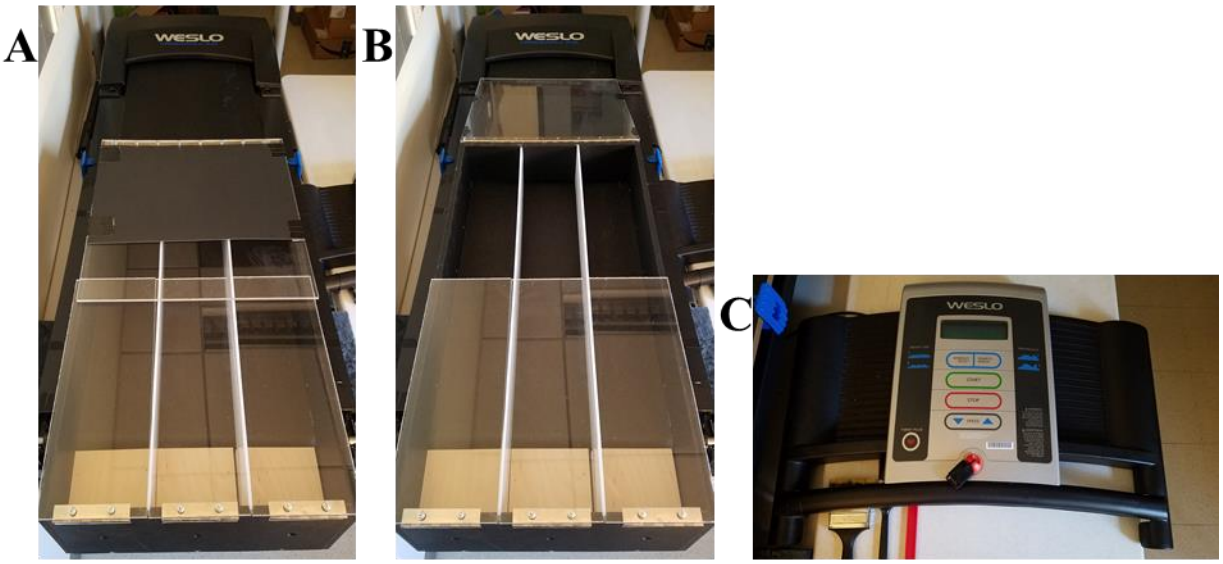


Figure 7. Custom exercise treadmill. Constructed based off the model proposed by Boughanim and Bergdahl (2017). Standard treadmill with custom acrylic chamber (30 x 16 x 4.5 in) divided into three lanes (30 x 5.25 x 4.5 in) including a rear 4.5 inch safe-zone. Treadmill speed increased at 0.1 mph (0.045 m/sec). A: Closed acrylic chamber; B: Open acrylic chamber; C: Control panel.

Table 3. Wistar exercise protocol.

Interval	Days	Speed (mph)	Speed (meter/min)	Duration Exercise (sec)	Duration Rest (sec)
1	1-2	0.6	16.1	45	30
	3	Rest	Rest	-	-
2	4-5	0.7	18.8	30	30
	6-8	0.7	18.8	45	30
	9	Rest	Rest	-	-
3	10-11	0.8	21.5	30	30
	12-14	0.8	21.5	45	30
	15	Rest	Rest	-	-
4	16-17	0.9	24.1	30	30
	18-20	0.9	24.1	45	30
	21	Rest	Rest	-	-
5	22-23	1.0	26.8	30	30
	24-26	1.0	26.8	45	30
	27	Rest	Rest	-	-
6	28-29	1.1	29.5	30	30
	30-32	1.1	29.5	45	30
	33	Rest	Rest	-	-
	34-35	1.1	29.5	45	30

Chronic Unpredictable Stress (CUS). Animals were exposed to daily mild stressors modeled off the chronic mild stress protocol proposed by Gibson et al. (2012). Animals were first transferred from group housing to single housing cages, causing social isolation distress. Over the course of the following 35 days, three additional stressors were administered in a randomized fashion. Damp bedding was caused my pouring 150 mL of water over the normal bedding placed in the cage. To cause the tilted cage stressor, blocks were securely placed under one side of the cage, causing the cage to sit at an angle. Finally, to create the empty cage stressor, all bedding and enrichment was removed from the cage, while water and food remained available. Table 4 shows the sequence of stressors used in the CUS protocol. All animals were monitored for signs of severe distress and weighed weekly to monitor for abnormal changes in weight.

Table 4. Wistar CUS treatment sequence of events.

Days	Start Day / Duration	Stressor
1-3	Day 1 / 3 Days	Damp Bedding
4-5	Day 4 / 2 Days	Tilted Cage
6-7	Day 6 / 2 Days	Empty Cage
8-10	Day 8 / 3 Days	Tilted Cage
11-12	Day 11 / 2 Days	Damp Bedding
13-14	Day 13 / 2 Days	Tilted Cage
15-17	Day 15 / 3 Days	Empty Cage
18-20	Day 18 / 3 Days	Damp Bedding
21-23	Day 21 / 3 Days	Tilted Cage
24-25	Day 24 / 2 Days	Empty Cage
26-27	Day 26 / 2 Days	Tilted Cage
28-30	Day 28 / 3 Days	Damp Bedding
31-32	Day 31 / 2 Days	Tilted Cage
33-35	Day 33 / 3 Days	Empty Cage

Behavioral Assessments

Open Field Test (OFT). The OFT, a measure of locomotor activity and anxiety, is a reliable measure for measuring the behavioral symptoms associated with depression in rodents

(Rittenhouse, López-Rubalcava, Stanwood, & Lucki, 2002; Shirayama et al., 2002). Marlatt et al. (2002) and Shirayama et al. (2002) found that depressed rodents showed reduced total activity and exploration behaviors, both of which returned to normal after antidepressant treatment (Marlatt et al., 2002; Shirayama et al., 2002). Animals were brought into the dimly lit testing room and allowed 30 minutes to acclimate. OFT testing took place 2 days after the cessation of the exercise and stress procedures. OFT testing was conducted using the Photobeam Activation System (San Diego Instruments) which tracked the total, center, peripheral, and rearing movement of the animal. The beams collected the quantity of beam crossings, resulting in total, peripheral, center, and rearing activity (Figure 8A). Animals were removed from their home cage and placed into the testing chamber (standard rodent housing cage) containing a thin layer of fresh bedding. The 5-minute session began upon the first beam break. Upon completion of the trial, the animal was removed and returned to its home cage. The used bedding from the testing chamber was discarded and the chamber was sanitized before adding fresh bedding for subsequent trials. Session data from each animal was extracted using a custom Microsoft Access report. Animals within each experiment were compared. Independent samples t-tests were used to compare mean total, perimeter, center, and rearing OFT activity between the exercise and non-exercise groups. For the rearing analysis of the WKY strain, one animal (non-exercise) was removed due to an improbably high rearing behavior, potentially due to an error in data collection.

Forced Swim Test (FST). The FST is a common reliable measure of depressive-like behavior in rodents (Rittenhouse et al., 2002; Shirayama et al., 2002). During the FST, depressed rodents swam less and showed increased susceptibility to learned helplessness, the phenomena in which an animal no longer attempts escape from a stressor, both of which return to normal after

antidepressant treatment (Fukumoto et al., 2017; Rittenhouse et al., 2002; Shirayama et al., 2002). FST testing took place 3 days after the cessation of the exercise and stress procedures. Animals were allowed 30 minutes to acclimate to the dimly lit testing room. FST testing was conducted in a plexiglass cylinder under normal light in water maintained at 25℃-30℃ (Figure 8B). Singly, animals were gently placed into the water and latency for no longer searching for an exit or failing to stay afloat was recorded. Each trial lasted a maximum of 2 minutes. At the completion of the trial, animals were retrieved from the water and towel dried before being returned to their home cage. Water was changed after 4 consecutive animals and was regularly cleaned of fecal matter between trials. Animals within each experiment were compared. Independent samples t-tests were used to compare mean latency to surrender between the exercise and non-exercise groups.

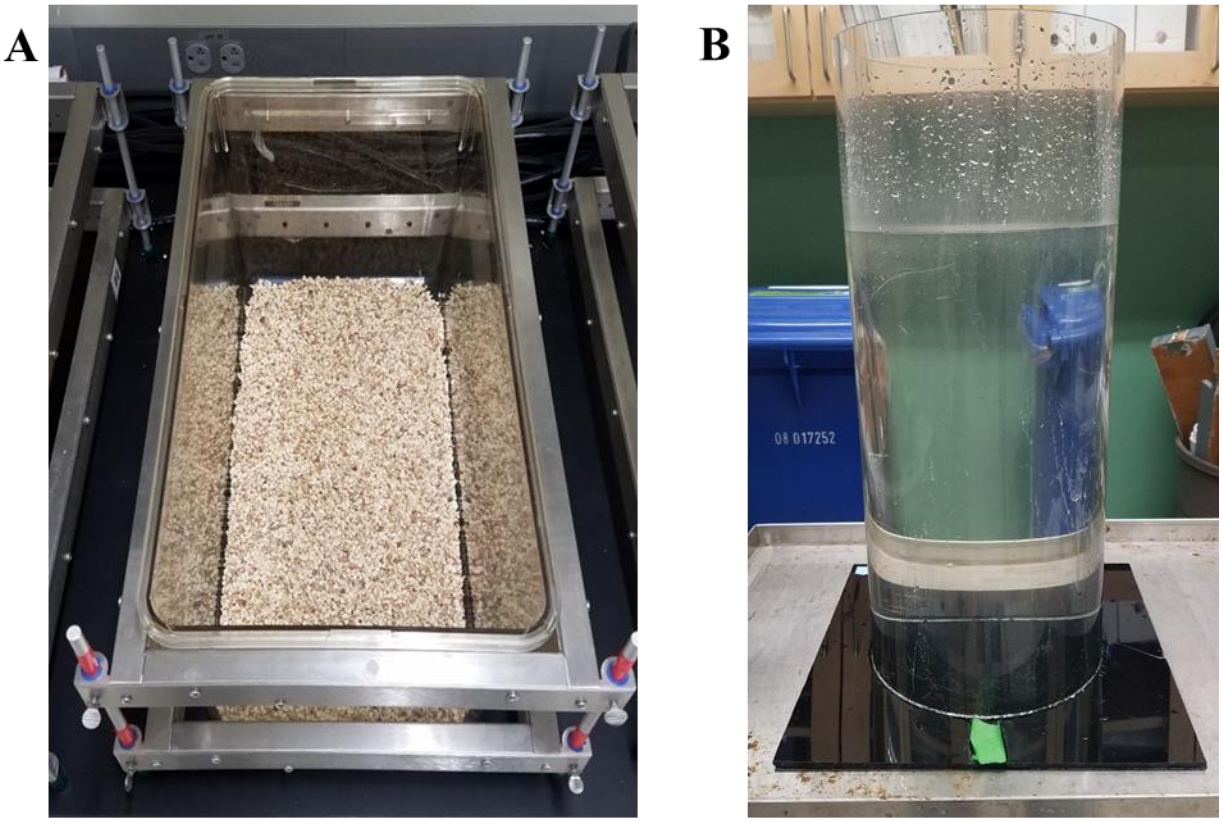


Figure 8. Apparatuses for assessment of depressive behaviors. A: Photobeam Activation System (PAS) OFT apparatus used to measure total, center, peripheral, and rearing activity. B: FST apparatus used to measure latency to surrender (min), cessation of searching or swimming behaviors.

An unforeseen delay for euthanasia procedures extended time until euthanasia from 3 days to 9 days post-exercise. To compensate for this time change, exercise and CUS procedures were reinstated 5 days after original exercise cessation and lasted for an additional 5 days until euthanasia. For both rodent strains, exercise was conducted at the final exercise interval as seen in the original exercise protocol. The Wistar strain received tilted cage (2 days) and damp bedding (3 days) as CUS stressors during the extension period. On the third day of the extension, a second FST trial was conducted at a 5-minute maximum (four days after the initial trial) due to the low spread in the data as all animals swam for the full 2 minutes or close to the full 2 full

minutes. On the fifth day of the extension, euthanasia and tissue collection began in conjunction with the final exercise/CUS protocols sessions.

Euthanasia & Tissue Collection/Preparation

Euthanasia & Perfusion. Euthanasia and tissue collection began on the final day of the protocol extension period and took place over four consecutive days. Animals received intraperitoneal injections of sodium pentobarbital (196mg/kg). Once the animal no longer perceived pain, assessed by hand pinch of the skin between the toes of the animal, the chest cavity was surgically opened to expose the heart and a luer stub needle (22 ga x 12 mm) was inserted in the left ventricle. Saline was pumped throughout the vascular system. The right atrium was cut to allow blood to flow out of the system. Once the blood was removed from the animal, paraformaldehyde (4%) was perfused until the tissue was fixed. The stiffening of the neck was used as an indicator that fixation occurred successfully in the brain. Upon completion, the head of the animal was severed from the body using a rat guillotine and the brain was removed from the skull. Excavated brains were submerged singly in glass jars containing paraformaldehyde (4%) for 3-6days before being transferred to jars containing tris-buffered saline (TBS; 1X) and refrigerated pending brain sectioning.

Brain Sectioning. Tissue slicing for histological analysis was conducted in TBS using a Leica VT1000S vibratome (Leica Biosystems). Coronal slices (60 micrometers) of the prefrontal cortex and hippocampus were collected and stored at -20℃ in FD Section Storage Solution (FD Neuro Technologies, Inc.) to preserve the tissue until staining.

Staining. Staining procedure was modeled off the Vector VECTASTAIN Elite ABC Peroxidase (HRP) Kit (Vector Laboratories Inc.) procedure. Blocking serum, biotinylated secondary antibody, Reagent A (Avidin), and Reagent B (Biotinylated HRP) were provided in

the kit. Sections were washed 3x10 minutes in TBS at room temperature on a Labnet Shaker Orbit 300 (Labnet International Inc.) on high shake (50 rpm). Between each wash, the wash solution was removed and fresh TBS was added. After washing and thawing to room temperature, the tissue was incubated at 45℃ in 1 mL of blocking serum (1.5%) for 30 minutes followed by 30 minutes at room temperature with low shake (30 rpm). The blocking serum was removed, and the tissue was incubated at 45℃ in 750 µL of primary anti-BDNF antibody [ERP1292] (Abcam) at 1:750 in fresh phosphate buffer (PB) for 1 hour. Incubation continued overnight at 3℃ on low shake.

The following day, the tissue was washed 4x15 minutes in fresh phosphate buffered saline (PBS) at room temperature and high shake. After washing, the tissue was incubated at 45℃ in 1 mL of biotinylated secondary antibody at 1:200 in blocking serum and PBS for 30 minutes followed by 30 minutes at room temperature with low shake. The tissue was then washed 3x10 minutes in fresh PBS at room temperature. The VECTISTAIN Elite ABC Reagent was made by combining the following contents in the VECTISTAIN Elite ABC Kit Peroxidase (HRP) procedure at the designated 1:50 concentration: Reagent A (100 µL), Reagent B (100 µL), and PBS (5 mL). The reagent was incubated at 45℃ for 20-30 minutes before its use. After the final wash solution was removed, the tissue was incubated at 45℃ in 1 mL of the VECTISTAIN Elite ABC Reagent for 1 hour followed by 1 hour at room temperature with low shake. The tissue was then washed 2x10 minutes in PBS and 1x10 minutes in Tris at room temperature with high shake.

The Vector DAB Peroxidase Substrate Kit (Vector Laboratories Inc.) was used to produce a brown reaction product in the presence of the HRP enzyme. The working peroxidase solution was prepped using the Vector DAB Peroxidase Substrate Kit procedure: 2 drops

(approximately 84 µl) of Buffer Stock Solution, 4 drops (approximately 100 µl) of DAB Stock Solution, and 2 drops (approximately 80 µl) of Hydrogen Peroxide Solution, and 5 ml of distilled water. Tissue sections were completely submerged in the peroxidase solution at room temperature until the optimal development (45 seconds-1 minute) was acquired and then transferred to cold Tris for washing to halt the reaction. The tissue was then mounted onto Proteinase-Resistant Microscope Slides (75 x 25mm; FD Neuro Technologies, Inc.) and dried overnight. Slides were then washed with fresh 95% and 100% ethanol 3x5 minutes each, followed by 3x15 minute washes in Safeclear Tissue Clearing Agent (Fischer Scientific), and mounted with coverslips using Permount (Fisher Scientific).

Imaging. Slides were visualized on a Nikon Optiphot-2 microscope (Nikon Instruments Inc.; Reference: 520831) using a Leica DFC295 photo adaptor (Leica Microsystems) and the Leica FireCam imaging program. Images of the medial prefrontal cortex (mPFC), hippocampus CA1, hippocampus CA3, and hippocampus dentate gyrus (DG) were collected. Locations of the mPFC, CA1, CA3, and DG in tissue sections were modeled off Grüter et al., 2015 (Figure 9A-D). Images of each region were acquired at 40x, 100x, and 400x magnification (Figure 9E-G).

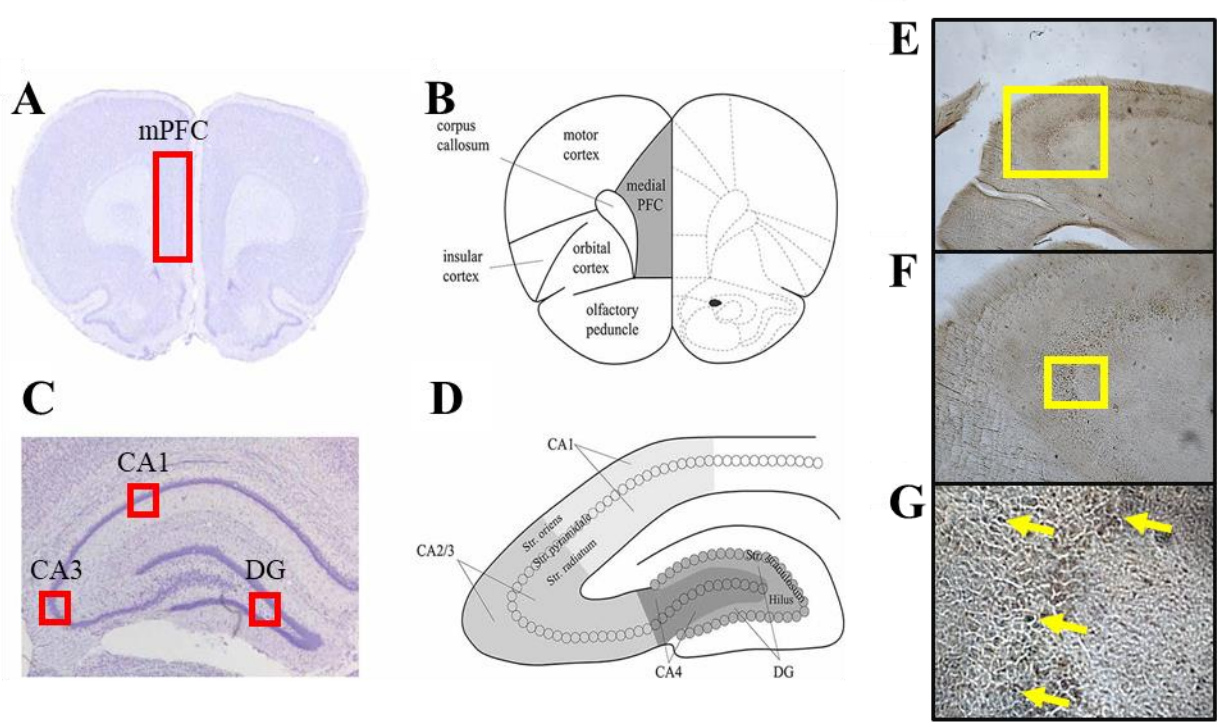


Figure 9. Regions of interest across prefrontal cortex and hippocampal coronal slices and BDNF containing cell count procedure. A-B: Sample rat prefrontal cortex coronal section (A) and labeled schematic (B) showing regions within the prefrontal cortex (modified from Grüter et al., 2015). Box indicates the medial prefrontal cortex (mPFC). C-D: Sample rat hippocampal section (C) and labeled schematic (B) showing regions within the hippocampus (modified from Grüter et al., 2015). Boxes indicate the CA1, CA3 , and dentate gyrus (DG). E-G: Sample images of CA3 at 40x (E), 100x (F), and 400x (G). Boxes indicate CA3 region of interest. Arrows indicate stained BDNF containing cells.

Neurological Assessments

Brain-Derived Neurotrophic Factor (BDNF). Cell counts were acquired from each region (mPFC, CA1, CA3, and DG) from images at 400x magnification. Multiple tissue sections and multiple sample locations per tissue section from each animal were counted to obtain a more representative cell count for each region. Cells were counted using ImageJ Cell counter. Image contrast and color balance were adjusted for optimal cell visualization. Cell counts were averaged within each region for each animal and then averaged within the exercise and non-

exercise groups. Independent samples t-tests were used to compare mean BDNF containing cells within each region between the exercise and non-exercise groups.

Results

Open Field Test (OFT)

Within the Wistar strain, independent samples t-tests revealed no significant differences in total, peripheral, center, or rearing OFT activity between the exercise and non-exercise groups (Figure 10A, Table 5). Within the WKY strain, there was a significant difference in mean peripheral OFT activity between the exercise (M = 119.00, SD = 14.90) and non-exercise (M = 97.75, SD = 15.31) groups, $t(6) = 1.9897$, $p < 0.05$, $\eta^2 = 0.3975$, indicating that exercise increased peripheral OFT activity, a treatment that accounted for 39.75% of the variance between the groups. A significant difference was also found for mean rearing OFT activity between the exercise (M = 27.50, SD = 3.70) and non-exercise (M = 17.33, SD = 1.53) groups, $t(5) = 4.4046$, $p < 0.01$, $\eta^2 = 0.7951$, indicating that exercise increased rearing OFT activity, a treatment that accounted for 79.51% of the variance between the groups. A trend toward significance was found for mean total OFT activity between exercise and no-exercise animals ($t(6) = 1.7655$, $p = 0.064$, $\eta^2 = 0.3419$). Together, these indicate that the exercise group had increased exploratory behaviors and OFT activity. No significant differences were found between exercise (M = 91.75, SD = 16.80) and non-exercise (M = 85.50, SD = 9.98) groups for mean center OFT activity (Figure 10B).

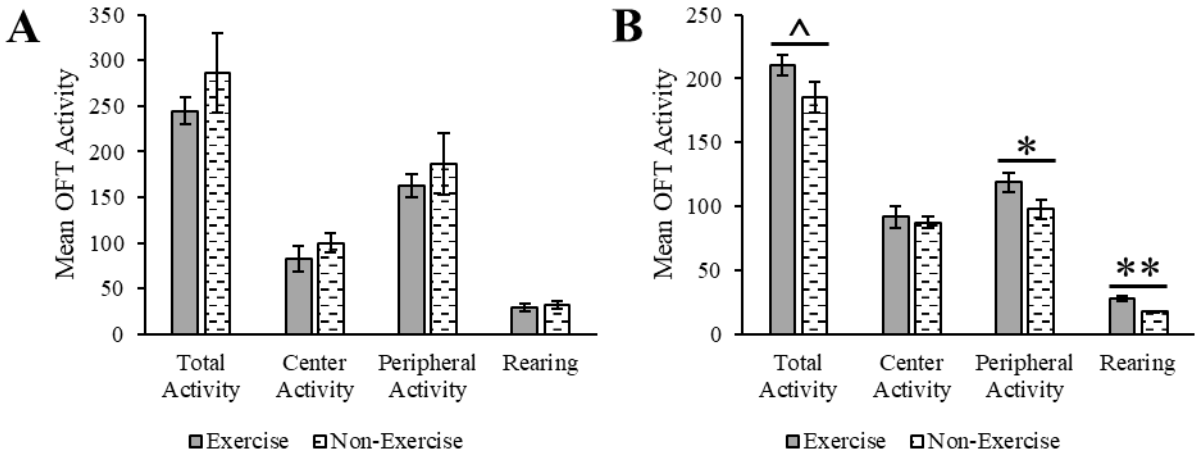


Figure 10. Comparison of OFT activity between exercise and non-exercise groups. Total activity, center activity, peripheral activity, and rearing data was collected using the quantity of beam crossings within the testing chamber. Individual sessions began upon the first beam break and continued for five minutes. Session data was extracted using a custom Microsoft Access report. OFT activity for each measure was averaged within the exercise and non-exercise groups. A: Wistar (n = 8). B: WKY (n = 8). Exercise significantly increased mean peripheral activity ($p < 0.05$ *) and rearing (n = 7, $p < 0.01$ **) compared to the non-exercise group. A clear trend toward significance was found indicating that exercise increased mean total activity ($p = 0.06$ ^) compared to the non-exercise group. Error bars represent ± 1 standard error of the mean.

Table 5. OFT results for Wistar rat strain (n = 8). Total activity, center activity, peripheral activity, and rearing data were collected using the quantity of beam crossings within the testing chamber. Individual sessions began upon the first beam break and continued for five minutes. Session data was extracted using a custom Microsoft Access report and averaged for each measure within the exercise and non-exercise groups.

OFT Activity	Exercise Treatment	Mean (*X*)	Standard Deviation (*SD*)
Total	Yes	244.75	29.23
	No	286.25	86.30
Peripheral	Yes	82.50	28.99
	No	100.00	20.31
Center	Yes	162.25	24.99
	No	186.25	66.70
Rearing	Yes	29.00	9.52
	No	31.75	8.42

Forced Swim Test (FST)

Within the Wistar strain, an independent samples t-test for mean latency to surrender between the exercise (M = 277.00, SD = 46.00) and non-exercise (M = 223.75, SD = 47.13) groups exhibited a potential trend toward significance, $t(6) = 1.6172$, $p = 0.0785$, $\eta^2 = 0.3036$, indicating that indicating that exercise increased latency to surrender, a treatment that accounted for 30.36% of the variance between the groups (Figure 11A). Within the WKY strain, no difference was found for mean latency to surrender between the exercise (M = 122.25, SD = 31.61) and non-exercise (M = 123.50, SD = 62.96) groups (Figure 11B).

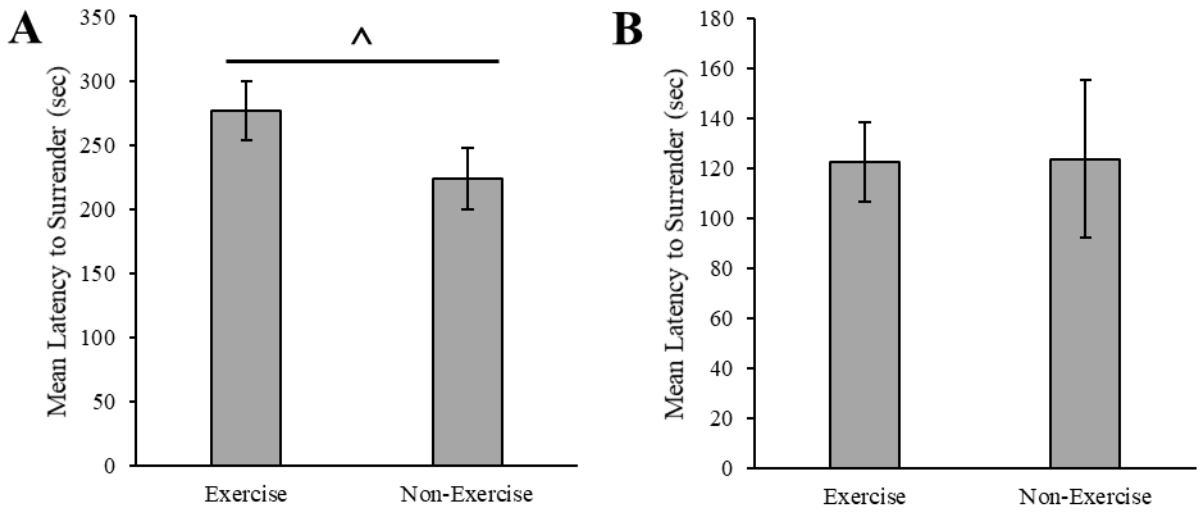


Figure 11. Comparison of FST activity between exercise and non-exercise groups. Latency to surrender (sec), stop searching or swimming behaviors, was collected for each animal and then averaged across each treatment to acquire mean latency to surrender values. Trials were conducted individually in a plexiglass chamber containing water (25°C - 30°C) for a maximum of five minutes. Latency to surrender times were averaged within the exercise and non-exercise groups. A: Wistar (n= 8). A trend toward significance was found suggesting that exercise increased mean latency to surrender (p=0.08 ^) compared to the non-exercise group. B: WKY (n = 8). Error bars represent ± 1 standard error of the mean.

Brain-Derived Neurotrophic Factor (BDNF)

Due to low tissue availability and tissue damage, cell counts were not conducted in the CA3 region for the Wistar strain and the CA1 or DG of the hippocampus for either strain. Within

the Wistar strain, no significant difference in mean BDNF containing cells in the mPFC was found between the exercise (n = 4, M = 152.08, SD = 34.74) and non-exercise (n = 3, M = 136.89, SD = 29.94) groups (Figure 12A). Within the WKY strain, independent samples t-tests were used to compare the number of BDNF containing cells within the mPFC and CA3. While no difference was found between the exercise (n = 4, M = 133.56, SD = 15.82) and non-exercise (n = 4, M = 102.75, SD = 40.04) groups within the mPFC, a significant difference was found within the CA3 between the exercise (n = 3, M = 205.17, SD = 27.59) and non-exercise (n = 4, M = 141.25, SD = 46.56) groups, $t(5) = 2.0889$, $p < 0.05$, $\eta^2 = 0.4660$, indicating that exercise significantly increased BDNF expression in the CA3 of the hippocampus, a treatment that accounted for 46.60% of the variance between the groups (Figure 12B).

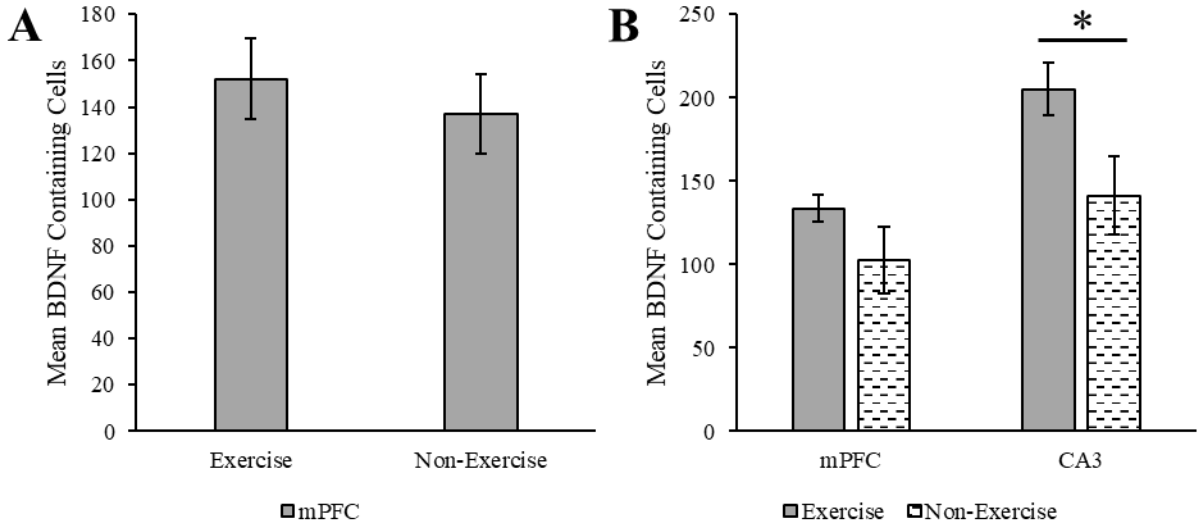


Figure 12. Comparison of BDNF expressing cells between exercise and non-exercise groups. Cells containing brain derived neurotrophic factor (BDNF), visualized using the Vector VECTISTAIN Elite ABC Kit Peroxidase (HRP) and DAB Peroxidase Substrate Kit, were counted at 40x magnification using ImageJ Cell Counter. Cell counts were acquired from the medial prefrontal cortex (mPFC) and hippocampus CA3 from multiple tissue sections and multiple sample locations per tissue section from each animal. Cell counts were averaged for each animal and then within the exercise and non-exercise groups. A: Wistar (n = 7, 4 exercise and 3 non-exercise). B: Kyoto (mPFC: n = 8; CA3: n = 7, 3 exercise and 4 non-exercise). Exercise significantly increased mean BDNF containing cells in the CA3 of the hippocampus ($p < 0.05$ *). Error bars represent ± 1 standard error of the mean.

Discussion

This study examined the efficacy of aerobic exercise on depressive behaviors and BDNF expression in two rodent models of depression. These studies examined the potential benefits of aerobic exercise in either protecting against the development of depression, by administering exercise and CUS to Wistar rats simultaneously, or reducing depression, by administering exercise to WKY rats.

The results of this study partially supported aerobic exercise's ability to reduce depressive symptoms in rodents. In the CUS-treated Wistar strain, aerobic exercise had no noticeable effect in reducing depressive behaviors in the OFT, showed a trend toward reducing depressive behaviors in the FST, and did not significantly impact expression of BDNF containing cells. In the WKY strain, aerobic exercise reduced depressive behaviors in the OFT, showed no effect in reducing depressive behaviors in the FST, and increased BDNF producing cells in the CA3 region of the hippocampus. These findings imply that the aerobic exercise employed in the study may not have been effective in protecting against the onset of depression or that the CUS protocol may not have been sufficient to induce depression since a depressive state was not verified, as evidenced by the lack of effect in the FST.

Aerobic exercise appeared to reduce activity and anxiety-related depressive behaviors in the WKY strain in the OFT. Increased exploratory behavior accompanied exercise-treated animals compared to non-exercise treated animals on all OFT activity measures, specifically rearing, peripheral, and total activity, a finding consistent with prior work (Erickson et al., 2012; Marlatt et al., 2002; Shirayama et al., 2002). While this suggests that aerobic exercise may be effective in reducing depressive behaviors in the WKY rodent model of depression, it is important to consider that the increase may be an artifact of the nature of the exercise. The

exercise procedure capitalized on the rat's motivation to search for food, therefore, the exercise may have unintentionally rewarded exploratory behaviors which may have translated to the novel OFT environment resulting in increased exploration as the rats searched for a food reward. Contrary to recent findings, no difference in OFT activity was found across exercise treatments of the CUS-treated Wistar rats. This discrepancy may have been influenced by a small effect of CUS and exercise programs. It is necessary to consider that the CUS and exercise treatments may not have been sufficient enough to produce a depressive state and then reduce depressive symptoms, respectively, therefore no difference should be detectable.

In the FST test, aerobic exercise showed a trend toward reducing depressive behaviors, specifically learned helplessness, in the CUS-treated Wistar strain. As seen in prior work, depressed rats showed a reduced latency to surrender compared to exercise-treated rats (Rittenhouse et al., 2002; Shirayama et al., 2002). While this may be the case, the Wistar rodents may have had greater stamina due to aerobic exercise and, therefore, may have performed better on the FST since the assessment is also a measure of stamina (Duman, Schlesinger, Russel, & Duman, 2008; Trejo, LLorens-Martín, & Torres-Alemán, 2008). Particularly if the CUS treatment did not produce a depressive state in the Wistar rats, the trend found here may be better explained by the rodents' increase in stamina due to exercise. FST latency to surrender did not differ in WKY rats based on exercise treatment, though current literature suggests that WKY rats are very susceptible to learned helplessness and often swim for a shorter duration in FST (Rittenhouse et al., 2002; Will et al., 2003). This may suggest that the exercise treatment may not have had the intended impact, possibly due to its reduction in difficulty at the start of the study, and that the exercise program should be revised. This may also suggest that the entire WKY strain benefited equally from the minimal handling or that the rats used was not as depressed as

other WKY rats. Moving forward, learned helplessness could also be further assessed using other common behavioral assessments like the tail suspension test or sucrose preference test (Fukumoto et al., 2017; Rotzinger et al., 2010).

Taken together, these behavioral results uncover an interesting and valuable notion regarding depression. Both rat strains exhibited reductions in depressive symptoms differently depending on the strain. While strain difference could play a role, this occurrence may highlight the heterogeneity of depression and variability in depressive symptoms in rodent models, a concept commonly understood in the human population as contributing to difficulties in both depression research and treatment (Jaffee et al., 2002).

A closer examination of BDNF within the prefrontal cortex and hippocampus of the animals revealed promising results for aerobic exercise. Within the WKY strain, exercise-treated animals had increased expression of BDNF containing cells within the CA3 region of the hippocampus, suggesting an increase in BDNF. Despite no observable change in prefrontal cortex, this finding is consistent with previous literature suggesting that exercise works to increase BDNF in the hippocampus as a mechanism for combating depression (Erickson et al., 2012; Marlatt et al., 2002; Zheng et al., 2006). Overall, this study supports that aerobic exercise may be beneficial in reducing depression. The lack of evidence within the mPFC of the WKY strain may suggest that the exercise program may not have been sufficient enough to elicit a response in the cortex, suggesting a regional hierarchy in the molecular impacts of exercise beginning within the hippocampus. In the Wistar strain, increased BDNF expression was not identified within the mPFC. Unfortunately, the study was unable to assess the hippocampus to determine any change in BDNF expressing cells. Therefore, it is inconclusive to suggest that exercise exposure may protect against depression, especially without clear OFT or FST support.

Similarly, it is again important to consider that the CUS and exercise treatments may not have had their intended effects within the Wistar strain.

Current literature suggests variability within WKY rats and, while that may be beneficial in modeling the heterogeneity of depression, the variability may contribute to difficulty in studying depression (Will et al., 2003). The study expands on how motivation impacts the strain's ability to exercise. In current study, prior to the division into two separate studies, several attempts were made to motivate the WKY rats to engage in exercise. The rats were initially trained on the treadmill, but they failed to directionally walk once the treadmill was moving and immediately climbed out of the apparatus. Follow-up attempts included the use of a free-moving rodent exercise balls and a rodent harness/leash, but the rats were content on remaining stationary when in the exercise ball and visibly panicked when placed in the harness. During the training and multiple exercise attempts, it was noticed that the WKY rats responded well to food motivation (Fruit Loops). This was used to the study's advantage as the rats would travel up to six feet for the food reward. This led to the division of the rat strains into separate studies. Through a better understanding of the WKY strain's motivations, better individualized exercise models can be developed to more accurately assess the strain.

The findings of this study must be considered within the study's limitations. While the major strength of this study's ability to consider each strain's motivation to exercise, effectively individualizing each exercise procedure to the strain, the exercise may not have produced the intended impact. The current study did not contain a control to test the effectiveness of the exercise procedures, despite being modelled off existing protocols (Erickson et al., 2012; Kempermann et al, 1997; Naghibzadeh et al., 2018), so the efficacy of the exercise treatment was not confirmed. The exercise protocols may not have elicited adequate energy expenditure within

the rats (Dunn et al., 2005) or they may have been limited in duration. The exercise protocol may have also induced stress in some of the animals as previous work suggested that forced exercise may lead to an increase in the stress response in mice (Cook et al., 2013). This increase in stress response may, therefore, exacerbate depressive symptoms instead of contributing to a reduction. The impact of novel and enriched environments may also have contributed to the results found in the study as the exercise groups were consistently transferred between their home cage and the exercise environment. This consistent transfer may have increased BDNF alone, a result similar to results seen in current literature suggesting that novel and enriched environments increase BDNF in the brain (Boulle et al., 2012; Sun et al., 2010). In the Wistar strain, the current study did not test the effectiveness of the CUS procedure despite being modelled off existing protocols (Gibson et al., 2012) and, therefore, may not have created depressive state within the Wistar strain.

It is important to consider literature from multiple avenues to create a more complete picture of the interaction between aerobic exercise and depression. While substantial research has focused on BDNF as a potential factor in the manifestation and maintenance of depression, some research suggests that BDNF may not play a significant role in depression (Groves, 2007; Krishnan & Nestler, 2008). Monteggia et al. (2007) found that the conditional forebrain knockout of BDNF or its receptor or the selective disruption of BDNF gene or BDNF-receptor gene regulation, leading to a reduction in BDNF, did not result is depressive-like behaviors in male mice. Berton et al. (2006) and Krishnan et al. (2007) showed that an increase in BDNF in the ventral tegmental area (VTA) or nucleus aucubas (NAc) regions of the brain, due to chronic stress or direct BDNF infusion, increased depressive-like behaviors. Finally, genetic variant studies found that a single-nucleotide polymorphism (G196A; Val 66 3 → Met 66) of the BDNF

gene, a single nucleotide change rendering the BDNF gene non-functional, did not alter genetic vulnerability to depression in rodents (Chen et al., 2006; Egan et al., 2003). These findings suggest the presence of gaps in the current literature regarding the role(s) of BDNF in depression, further highlighting the complexity of depression, prompting researchers to examine the roles of other molecular mechanisms in the treatment and prevention of depression connected with depression.

Researchers have turned to examining vascular endothelial growth factor A (VEGF-A) due to increasing evidence that blood flow is altered within the brain during depression (Duman & Li, 2012; Mackenzie & Ruhrberg, 2012). Miguel-Hidalgo and Rajkowska (2002) showed that depression decreased blood flow to the prefrontal cortex and increased blood flow to the amygdala, the emotion center of the limbic system potentially contributing to prefrontal cortex atrophy and the atypical emotional activity of depressed individuals (Miguel-Hidalgo & Rajkowska, 2002).

VEGFs are small polypeptides that increase angiogenesis, the formation of new blood vessels, throughout the body (Duman & Li, 2012; Holmes & Zachary, 2005; Jensen, Pilegaard, Neufer, & Hellsten, 2004). Recent research suggested that VEGF-A may have a role in protecting against damage within the brain, particularly damage associated with depression (Duman & Li, 2012). By delivering oxygen and nutrients to the high metabolically active tissue of the brain, VEGF-A promotes neurogenesis, increases cell soma size, and increases axon length in some regions (Mackenzie & Ruhrberg, 2012). This may be highly important in regions such as the hippocampus that experience significant neuronal atrophy with depression (Duman & Li, 2012). Berent, Macander, Szemraj, Orzechowska, and Galecki (2014) and Mackenzie & Ruhrberg (2012) showed that VEGF-A levels are elevated in the hippocampus of depressed

individuals and that, after successful depression treatments, VEGF-A levels returned to normal suggesting that VEGF-A may work to protect and heal the damaged brain. Prior research highlighted the neuroprotective role of VEGF-A as infusions increased neurogenesis, the formation of new neurons, in the hippocampus and cortex (Mackenzie & Ruhrberg, 2012). Whether the neurogenic effects of VEGF-A are direct or indirect is currently at debate within the literature (Mackenzie & Ruhrberg, 2012). Falk, Gonzalez, and Sherman (2010) described the potential cellular mechanism of VEGF-A as seen in Figure 13. VEGF-A binds to cell surface VEGF receptors (VEGFR1-3) and, after dimerization, activates downstream Akt and PLCγ/PKC pathways via phosphorylation, which leads to transcriptional gene regulation (the increase or decrease of specific gene transcription) of genes involved in angiogenesis, neuroprotection, and neurogenesis (Falk et al., 2010).

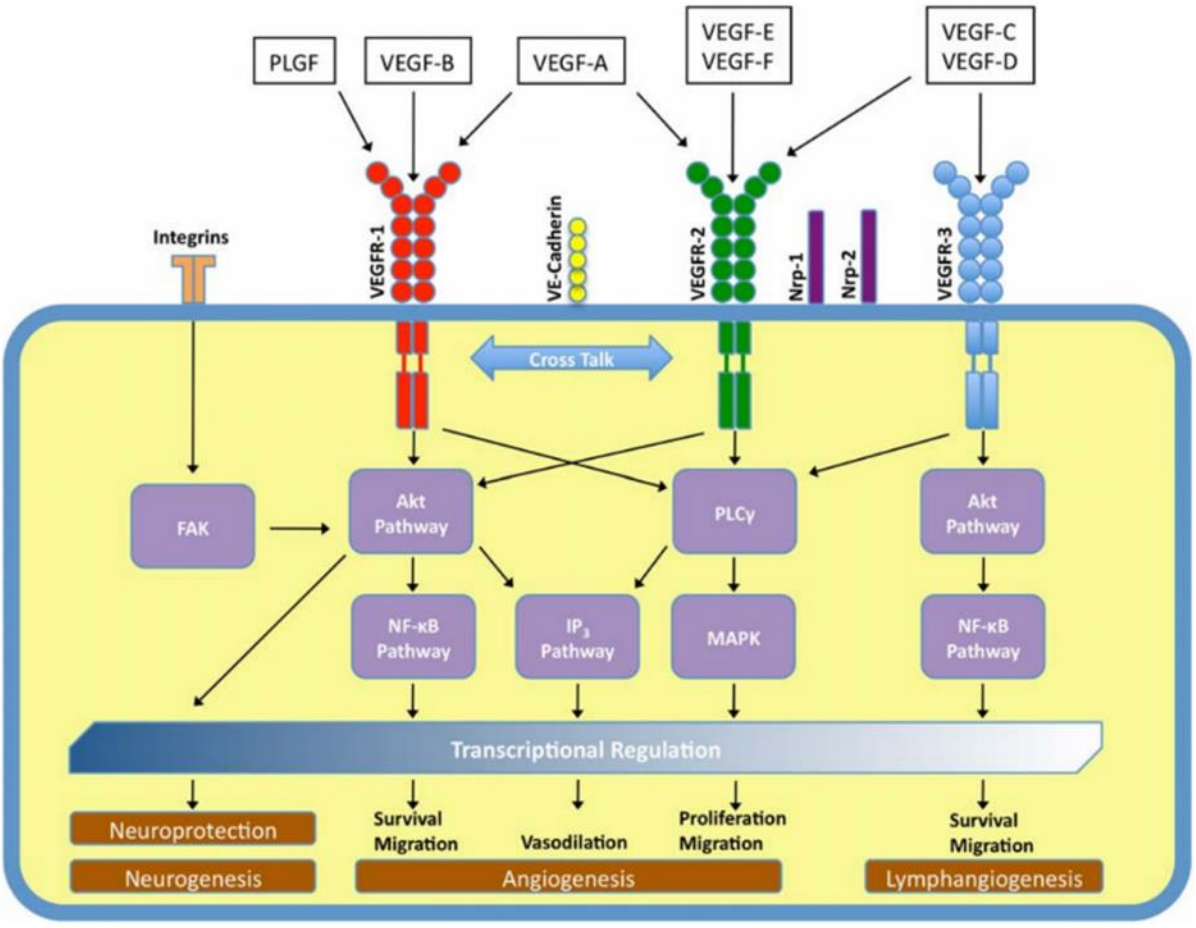


Figure 13. Signaling pathways activated by VEGF-isoforms. VEGF-A binds to VEGFR-1 and VEGFR-2 receptors, activates the Atk pathway, and induces transcriptional regulation leading to the increase of neuroprotection and neurogenesis. VEGF-A, along with other isoforms, also activate the Atk, PLCγ, NF-κB, IP_3, and MAPK pathways leading to transcriptional regulation and increases in angiogenesis and lymphangiogenesis. Abbreviations: FAK: focal adhesion kinase; Akt: also known as protein kinase B (PKB); PLCγ: phospholipase Cγ; IP_3: inositol 1,4,5-trisphosphate; NF-κB: nuclear factor κ-light-chain-enhancer of activated B cells; MAPK: mitogen-activated protein kinase (Falk et al., 2010).

One of the beneficial roles of aerobic exercise in combating depression may arise from the ability of exercise to increase vascularization within the brain (Jin et al., 2002). In addition to increasing vascularization and VEGF mRNA and total VEGF protein in skeletal muscles, Tang, Xia, Wagner, and Breen (2010) showed that aerobic exercise increased VEGF-A transcription in the hippocampus and cortex of the brain in rodents. Prior work also found that VEGF-A

increased angiogenesis, promoted neurogenesis, and served other neurotrophic-like functions similar to BDNF within the hippocampus and cortex (Jin et al., 2002).

Another group of molecules of recent interest to researchers are kynurenine (KYN) and its metabolite, kynurenic acid (KYNA). KYN, KYNA, and other molecules in the kynurenine family are derived from the amino acid tryptophan, similar to the well-studied neurotransmitter serotonin (Schwarcz, Bruno, Muchowski, & Wu, 2012). KYN, normally identified as harmless, is found throughout the body's periphery, but it causes problems when it enters the brain (Agudelo et al., 2014). Research by Agudelo et al. (2014) suggested that, once KYN crosses the blood brain barrier (BBB), it activates stress-induced mechanisms, such as neuronal death, neuroinflammation, and glutamate transmission, that are linked with depression. These occur as KYN is metabolized by microglia and astrocytes, two types of glial cells within the brain, into 3-hydroxykynurenine (3-HK) and KYNA, respectively (Schwarcz et al., 2012). 3-HK is further metabolized to quinolinic acid (QUIN), a molecule linked to excitotoxic neuronal damage (Schwarcz et al., 2012). The KYN pathway may play an even greater role with the induction of stress. Agudelo et al. (2014) suggested that tryptophan conversion is shifted highly toward KYN in the presence of stress, thus increasing KYN's influx into the brain and, consequently, increasing QUIN and downstream neurotoxic pathways leading to depression-inducing conditions. Prior work using individuals with clinically-diagnosed depression, found higher levels of KYN in individuals with depression indicating this shift in conversion during stress (Schwarcz et al., 2012). Broader research concerning dysregulation of the KYN pathway has been linked to both neurological disorders (i.e. Huntington's disease and Alzheimer's disease) and psychiatric disorders (i.e. depression and schizophrenia) in clinical populations (Schwarcz et

al., 2012). A simplified schematic of the KYN synthesis and metabolism depicting KYN conversion can be seen in Figure 14A (Agudelo et al., 2014).

Since most KYN enters the brain from the periphery, researchers are exploring methods of preventing it from crossing the BBB (Agudelo et al., 2014; Schwarcz et al., 2012). KYN's major metabolite, KYNA, is unable to cross the BBB and enter the brain (Agudelo et al., 2014; Schwarcz et al., 2012). Agudelo et al. (2014) found that aerobic exercise increases skeletal muscle expression of kynurenine aminotransferases (KATs), a group of enzymes that convert KYN to KYNA, in mice. By increasing the expression of KATs in the periphery (the same enzyme that astrocytes carry to convert KYN to KYNA in the brain), KYN is more readily converted to KYNA and is, therefore, unable to cross the BBB and induce depression-involving pathways (Agudelo et al., 2014; Figure 14B).

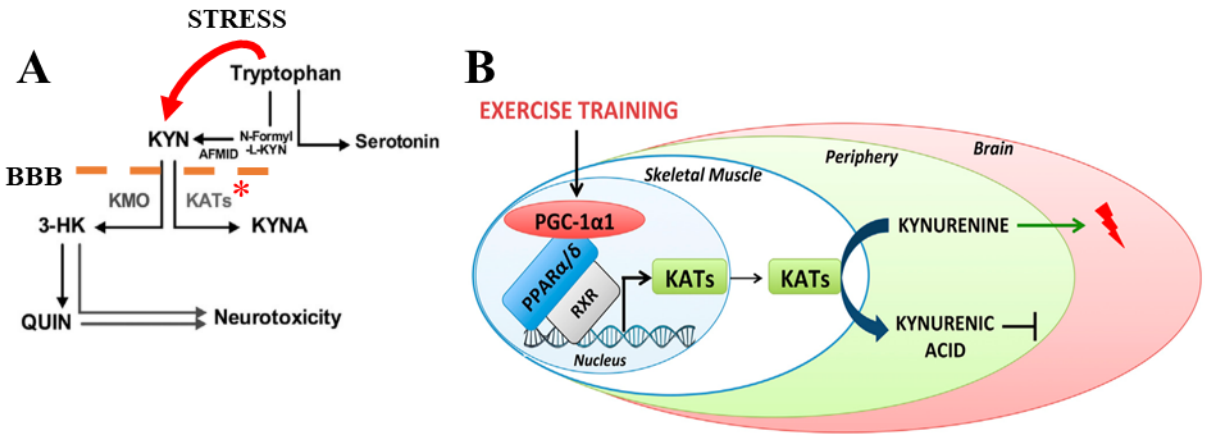


Figure 14. Kynurenine (KYN) and kynurenic acid (KYNA) roles in depression-related pathways. A: Simplified representation of the kynurenine (KYN) pathway. Tryptophan is enzymatically converted to kynurenine (KYN) and serotonin. KYN is further metabolized into 3-hydroxykynurenine (3-HK) by kynurenine 3-monooxygenase (KMO) and kynurenic acid (KYNA) by kynurenine aminotransferases (KATs). In the brain, 3-KT is converted to quinolinic acid (QUIN) which leads to neurotoxicity. Red arrow indicates tryptophan conversion shift to KYN. Dashed line depicts KYN metabolism steps occurring within the brain. Asterisk (*) indicates that KYN to KYNA conversion via KATs can occur in both the brain and periphery. B: Molecular mechanism for exercise-induced peripheral shift of KYN to KYNA. Exercise upregulates the expression transcription factor PGC-1α1 and transcriptional coactivators, PPARα/δ and RXR, in skeletal muscles, leading to the increased transcription of KATs. Free periphery KYN is converted by KATs at skeletal muscles to KYNA, a molecule unable to enter the brain (modified from Agudelo et al., 2014).

Yau et al. (2014) identified another molecule that may play a role in exercise-mediated benefits in combating depression. Adiponectin (ADN) is a protein hormone responsible for mediating glucose and lipid uptake in skeletal muscles and the liver (Thundyil, Pavlovski, Sobey, & Arumugam, 2012). ADN readily crosses the BBB and acts on its receptors (AdipoR1, AdipoR2, and T-cadherin) located in brain regions such as the hypothalamus, pituitary gland, cortex, and hippocampus (Thundyil et al., 2012; Yau et al., 2014). Yau et al. (2014) found reduced ADN levels in patients with depression. While Thundyil et al. (2012) noted that several studies have examined the role of ADN in neurological pathologies, a mechanism is largely unknown. A study conducted by Yau et al. (2014) examined the role of ADN in the brain and

uncovered that it is essential for the exercise-mediated benefits (increased neurogenesis, cell proliferation, and other antidepressant effects) in combating depression. While *Adipo*$^{-/-}$ mice, ADN knockout mice, did not exhibit altered basal hippocampus neurogenesis levels, they showed significantly reduced running-induced hippocampal neurogenesis, cell proliferation, and other running-induced antidepressant effects (Yau et al., 2014). When examining a potential mechanism, it was found that *Adipo*$^{-/-}$ mice were unable to trigger running-induced phosphorylation of AMP-activated protein kinase (AMPK) within the PI3K-Akt signaling pathway in hippocampal tissue, a protective pathway within the hippocampus, suggesting that the PI3K-Akt signaling pathway may play a role in ADN-mediated exercise-induced benefits (Yau et al., 2014).

Conclusion

In conclusion, this study partially supports the role of aerobic exercise in reducing depression. Consistent with recent literature, aerobic exercise reduced some depressive-like behaviors and increased hippocampal CA3 BDNF in the WKY strain. Therefore, aerobic exercise may be beneficial for those seeking treatment for depression, yet continued research is needed to understand the extent of its treatment abilities. The study further contributes to the growing understanding of the difficulty researching a complex heterogeneous disorder such as depression. Overall, aerobic exercise shows promise in its ability to reduce depression, a quality similar to antidepressant medications. While continued research is needed to further understand the molecular mechanisms underlying the benefits of aerobic exercise, current research suggests it may serve as a useful alternative or supplement to antidepressant medications.

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References

American Psychiatric Association (APA). (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Washington, DC: American Psychiatric Association.

Agudelo, L. Z., Femenía, T., Orhan, F., Porsmyr-Palmertz, M., Goiny, M., Martinez-Redondo, V., … & Ruas, G. L. (2014). Skeletal muscle PGC-1α1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. *Cell, 159*(1), 33-45. doi: 10.1016/j.cell.2014.07.051

Berent, D., Macander, M., Szemraj, J., Orzechowska, A., & Galecki, P. (2014). Vascular endothelial growth factor A gene expression level is higher in patients with major depressive disorder and not affected by cigarette smoking, hyperlipidemia or treatment with statins. *Acta Neurobiologiae Experimentalis, 74*(1), 82-90.

Berton, O., McClung, C. A., DiLeone, R. J., Krishnan, V., Renthal, W., Russo, S. J., … & Nestler, E. J. (2006). Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science, 311*(5762), 864-868. doi: 10.1126/science.1120972

Bharadwaj, P., Pai, M. M., & Suziedelyte, A. (2017). Mental health stigma. *Economics Letters, 159*, 57-60. doi: 10.3386/w21240

Boulle, F., van der Hove, D. L. A., Jakob, S. B., Rutten, B. P., Hamon, M., van Os, J., … & Kenis, G. (2012). Epigenetic regulation of the *BDNF* gene: implications for psychiatric disorders. *Molecular Psychiatry, 17*(6), 584-596. doi: 10.1038/mp.2011.107

Brody, D. J., Pratt, L. A., & Hughes, J. P. (2018). *Prevalence of depression among adults aged 20 and over: United States, 2013–2016* (NCHS Data Brief No. 303). Hyattsville, MD: National Center for Health Statistics.

Cascade, E., Kalail, A. H., & Kennedy, S. H. (2009). Real-world data on SSRI antidepressant side effects. *Psychiatry (Edgmont), 6*(2), 16-18.

Casper, R. C., Fleisher, B. E., Lee-Ancajas, J. C., Gilles, A., Gaylor, E., DeBattista, A., & Hoyme, E. (2003). Follow-up of children of depressed mothers exposed or not exposed to antidepressant drugs during pregnancy. *The Journal of Pediatrics, 142*(4), 402-408. doi: 10.1067/mpd.2003.139

Chen, Z. Y., Jing, D., Bath, K. G., Ieraci, A., Siao, C. J., Herrera, D. G., … & Lee, F. S. (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science, 314*(5796), 140-143. doi: 10.1126/science.1129663

Chisholm, D., Sanderson, K., Ayuso-Mateos, J. L., & Saxena, S. (2004) Reducing the global burden of depression: Population-level analysis of intervention cost-effectiveness in 14 world regions. *British Journal of Psychiatry, 184*, 393-403. doi: 10.1192/bjp.184.5.393

Cook, M. D., Martin, S. A., Williams, C., Whitlock, K., Wallig, M. A., Pence, B. D., & Woods, J. A. (2013). Forced treadmill exercise training exacerbates inflammation and causes mortality while voluntary wheel training is protective in a mouse model of colitis. *Brain, Behavior, and Immunity, 33*, 46-56. doi: 10.1016/j.bbi.2013.05.005

Cotman, C. W., Berchtold, N. C., & Christie, L. A. Exercise builds brain health: Key roles of growth factor cascades and inflammation. *Trends in Neurosciences, 30*(9), 464-472. doi: 10.1016/j.tins.2007.06.011

Cotman, C. W. & Engesser-Cesar, C. (2002). Exercise enhances and protects brain function. Exercise and Sport Sciences Reviews, 30(2), 75-79.

Duman, C. H., Schlesinger, L., Russell, D. S., & Duman, R. S. (2008). Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. *Brain Research, 1199*(14), 148-158. doi: 10.1016/j.brainres.2007.12.047

Duman, R. S. & Aghajanian, G. K. (2012). Synaptic dysfunction in depression: Potential therapeutic targets. *Science, 338*(6103), 68-72. doi: 10.1126/science.1222939

Duman, R. S. & Li, N. (2012). A neurotrophic hypothesis of depression: role of synaptogenesis in the actions of NDMA receptor antagonists. *Philosophical transactions of the Royal Society, 367*, 2475-2484. doi: 10.1098/rstb.2011.0357

Dunn, A. L., Trivedi, M. H., Kampert, J. B., Clark, C. G., & Chambliss, H. O. (2005). Exercise treatment for depression: Efficacy and dose response. *American Journal of Preventative Medicine, 28*(1), 1-8. doi: 10.1016/j.amepre.2004.09.003

Eadie, B. D., Redila, V. A., & Christie, B. R. (2005). Voluntary exercise alters the cytoarchitecture of the adult dentate gyrus by increasing cellular proliferation, dendritic complexity, and spine density. *The Journal of Comparative Neurology, 486*, 39-47. doi: 10.1002/cne.20493

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., … & Weinberger, D. R. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*(2), 257-269. doi: 10.1016/S0092-8674(03)00035-7

Eisenberg, D., Gollust, S. E., Golberstein, E., & Hefner, J. L. (2007). Prevalence and correlates of depression, anxiety, and suicidality among university students. *American Journal of Orthopsychiatry, 77*(4), 534-542. doi: 10.1037/0002-9432.77.4.534

Erickson, K. I., Miller, D. L. & Roecklein, K. A. (2012). The aging hippocampus: Interactions between exercise, depression, and BDNF. *Neuroscientist, 18*(1), 82-97. doi: 10.1177/1073858410397054

Erickson, K., I., Raji, C., A., Lopez, O., L., Becker, J., T., Rosano, C., & Newman, A., B. (2010). Physical activity predicts gray matter volume in late adulthood: The cardiovascular health study. *Neurology, 75*(16), 1415-1422. doi: 10.1212/WNL.0b013e3181f88359

Ernst, C., Olson, A. K., Pinel, J. P. J., Lam, R. W., & Christie, B. R. (2006). Antidepressant effects of exercise: Evidence for an adult-neurogenesis hypothesis? *Journal of Psychiatry Neuroscience, 31*(2), 84-92.

Falk, T., Gonzalez, T. T. & Sherman, S. J. (2010). The Yin and Yang of VEGF and PEDF: Multifaceted Neurotrophic Factors and Their Potential in the Treatment of Parkinson's Disease. *International Journal of Molecular Sciences, 11*(8). doi: 10.3390/ijms11082875

Farmer, J., Zhao, X., van Praag, H., Wodtke, K., Gage, F. H., & Christie, B. R. (2004). Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats in vivo. *Neuroscience, 124*, 71-79. doi: 10.1016/j.neuroscience.2003.09.029

Food and Drug Administration (FDA). (2014). *Selective serotonin reuptake inhibitors (SSRIs) information*. Retrieved from https://www.fda.gov/Drugs/DrugSafety/InformationbyDrugClass/ucm283587.htm

Food and Drug Administration (FDA). (2017). *Depression: FDA-approved medications may help*. Retrieved from https://www.fda.gov/forconsumers/consumerupdates/ucm095980.htm

Food and Drug Administration (FDA). (2018). *FDA drug safety communication: Selective serotonin reuptake inhibitor (SSRI) antidepressant use during pregnancy and reports of a rare heart and lung condition in newborn babies*. Retrieved from https://www.fda.gov/Drugs/DrugSafety/ucm283375.htm

Fukumoto, K., Toki, H., Iijima, M., Hashihayata, T., Yamaguchi, J., Hashimoto, K., & Chaki, S. (2017) Antidepressant potential of (R)-Ketamine in rodent models: Comparison with (S)-Ketamine. *Journal of Pharmacology and Experimental Therapeutics, 361*(1), 9-16. doi: 10.1124/jpet.116.239228

García-Mesa, Y, López-Ramos, J. C., Giménez-Llort, L., Revilla, S., Guerra, R., Gruart, A., … & Sanfeliu, C. (2011). Physical exercise protects against Alzheimer's disease in 3xTg-AD mice. *Journal of Alzheimer's Disease, 24*, 421-454.

Gibbons, R. D., Brown, C. H., Hur, K., Marcus, S. M., Bhaumik, D. K., Erkens, J. A., … & Mann J. J. (2007). Early evidence on the effects of regulators' suicidality warnings on ssri prescriptions and suicide in children and adolescents. *American Journal of Psychiatry, 164*(9), 1356-1363. doi: 10.1176/appi.ajp.2007.07030454

Gibson, C. J., Klatzkin, R., & Littlefield, L. M. (2012). Modeling depression. Research readings and statistical exercises using SPSS and Excel. Office of Teaching Resources in Psychology. Retrieved from http://www.teachpsych.org/otrp/resources/gibson/index/php

Groves, J. O. (2007). Is it time to reassess the BDNF hypothesis of depression? *Molecular Psychiatry, 12*, 1079-1088. doi: 10.1038/sj.mp.4002075

Gulliver, A., Griffiths, K. M., & Christensen, H. (2012). Barriers and facilitators to mental health help-seeking for young elite athletes: a qualitative study. *BMC Psychiatry, 12*, 157. doi: 10.1186/1471-244X-12-157

Grüter, T., Wieschollеck, V., Dubovyk., Aliane, V., & Manahan-Vaughan, D. (2015). Altered neuronal excitability underlies impaired hippocampal function in an animal model of psychosis. *Frontiers in Behavioral Neuroscience, 9*(117), 1-15. doi: 10.3389/fnbeh.2015.00117

Harrington, M. E. (2012) Neurobiological studies of fatigue. *Progress in Neurobiology, 99*(2), 93-105. doi: 10.1016/j.pneurobio.2012.07.004

Hatziandreu, E. I., Koplan, J. P., Weinstein, M. C., Caspersen, C. J., & Warner, K. E. (1988). A cost-effectiveness analysis of exercise as a health promotion activity. *American Journal of Public Health, 78*(11), 1417-1421.

Hemmings, B. A. & Restuccia, D. F. (2012). PI3K-PKB/Akt pathway. *Cold Spring Harbor Perspectives in Biology, 4*(9), a011189. doi: 10.1101/cshperspect.a011189

Holmes, D. I. & Zachary, I. (2005). The vascular endothelial growth factor (VEGF) family: Angiogenic factors in health and disease. *Genome Biology, 6*(2), 209. doi: 10.1186/gb-2005-6-2-209

Jaffee, S. R., Moffitt, T. E., Caspi, A., Fombonne, E., Poulton, R., & Martin, J. (2002). Differences in early childhood risk factors for juvenile-onset and adult-onset depression. *Archives of General Psychiatry, 59*(3), 215-222. doi: 10.1001/archpsyc.59.3.215

Jensen, L., Pilegaard, H., Neufer, D., & Hellsten, Y. (2004). Effect of acute exercise and exercise training on VEGF splice variants in human skeletal muscle. *American Journal of Regulatory, Integrative and Comparative Physiology, 287*(2), R397-R402. doi: 10.1152/ajpregu.00071.2004

Jin, K., Zhu, Y., Sun, Y., Mao, X. O., Xie, L., & Greenberg, D. A. (2002). Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. *Proceedings of the*

National Academy of Sciences of the United States of America, 99(18), 11946-11950. doi: 10.1073/pnas.182296499

Keks, N., Hope, J., & Keogh, S. (2016). Switching and stopping antidepressants. *Australian Prescriber, 39*(3), 76-83. doi: 10.18773/austprescr.2016.039

Kempermann, G, Kuhn, H. G., & Gage, F. H. (1997). More hippocampal neuron in adult mice living in an enriched environment. *Nature, 386*(6624), 493-495. doi: 10.1038/386493a0

Krishnan, V., Han, M. H., Graham, D. J., Berton, O., Renthal, W., Russo, S. J., ... & Nestler, E. J. (2007). Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell, 131*(2), 391-404. doi: 10.1016/j.cell.2007.09.018

Krishnan, V. & Nestler, E. J. (2008). The molecular neurobiology of depression. *Nature, 455*(7215), 894-902. doi: 10.1038/nature07455

Kurtz, T. W. & Morris, Jr. C. (1987). Biological variability in Wistar-Kyoto rats: Implications for research with the spontaneously hypertensive rat. *Hypertension, 10,* 1, 127-131. doi: 10.1161/01.HYP.10.1.127

Mackenzie, F. & Ruhrberg, C. (2012). Diverse roles for VEGF-A in the nervous system. *Development, 139*(8), 1371-1380. doi: 10.1242/dev.072348

Marlatt, M. W., Potter, M. C., Lucassen, P. J., & van Praag, H. (2002). Running throughout middle-age improves memory function, hippocampal neurogenesis and BDNF levels in female C57Bl/6J mice. *Developmental Neurobiology, 72*(6), 943-952. doi: 10.1002/dneu.22009

Miguel-Hidalgo, J. J. & Rajkowska, G. (2002). Morphological brain changes in depression. *CNS Drugs, 16*(6), 361-372. doi: 10.2165/00023210-200216060-00001

Monteggia, L. M., Luikart, B., Barrot, M., Theobold, D., Malkovska, I., Nef, S., … & Nestler, E. J. (2007). Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. *Biological Psychiatry, 61*(2), 187-197. doi: 10.1016/j.biopsych.2006.03.021

Morris, G., Arkadir, D., Nevet, A., Vaadia, E., & Bergman, H. (2004). Coincident but distinct messages of midbrain dopamine and striatal tonically active neurons. *Neuron, 43*(1), 133-143. doi: 10.1016/j.neuron.2004.06.012

Moti, R. W., Birncaum, A. S., Kubik, M. Y., & Dishman, R. K. (2004). Naturally occurring changes in physical activity are inversely related to depressive symptoms in early adolescence. *Psychosomatic Medicine, 66*, 336-342. doi: 10.1097/01.psy.0000126205.35683.0a

Nabkasorn, C., Miyai, N., Sootmongkol, A., Junprasert, S., Yamamoto, H., Arita, M., & Miyashita, K. (2005). Effects of physical exercise on depression, neuroendocrine stress hormones and physiological fitness in adolescent females with depressive symptoms. *European Journal of Public Health, 16*(2), 179-184. doi: 10.1093/eurpub/cki159

Naghibzadeh, M., Ranjbar, R., Tabandeh, M. R., & Habibi, A. (2018). Effects of two training programs on transcriptional levels of neurotrophins and glial cells population in hippocampus of experimental multiple sclerosis. *International Journal of Sports Medicine, 39*(8), 604-612. doi: 10.1055/a-0608-4635

National Institute of Mental Health (NIMH). (2017). *Major Depression*. Retrieved from https://www.nimh.nih.gov/health/statistics/major-depression.shtml

National Institute of Mental Health (NIMH). (2018). *Depression*. Retrieved from https://www.nimh.nih.gov/health/topics/depression/index.shtml

Oberlander, T. F., Gingrich, J. A., & Ansorge, M. S. (2009). Sustained neurobehavioral effects of exposure to SSRI antidepressants during development: Molecular to clinical evidence. *Clinical Pharmacology & Therapeutics, 86*(6), 672-677. doi: 10.1038/clpt.2009.201

Okamoto, K. & Aoki, K. (1963). Development of a strain of spontaneously hypertensive rats. *Japan Circulation Journal, 27*, 282–293.

Paillard, T., Rolland, Y., & de Souto Barreto, P. Protective effects of physical exercise in Alzheimer's Disease and Parkinson's disease: A narrative review. *Journal of Clinical Neurology, 11*(3), 212-219. doi: 10.3988/jcn.2015.11.3.212

Preston, A. R. & Eichenbaum, H. (2013). Interplay of hippocampus and prefrontal cortex in memory. *Current Biology, 23*(17), R764-R773. doi: 10.1016/j.cub.2013.05.041

Rittenhouse, P. A., López-Rubalcava, C., Stanwood, G. D., & Lucki, I. (2002). Amplified behavioral and endocrine responses to forced swim stress in the Wistar–Kyoto rat. *Psychoneuroendocrinology, 27*, 303-318. doi: 10.1016/S0306-4530(01)00052-X

Rotzinger, S., Lovejoy, D. A., & Tan, L. A. (2010). Behavioral effects of neuropeptides in rodent models of depression. *Peptides, 31*(4), 736-756. doi: 10.1016/j.peptides.2009.12.015

Sanders, C. E., Field, T. M., Diego, M., & Kaplan, M. (2000). Moderate involvement in sports is related to lower depression levels among adolescents. *Adolescents, 35*(140), 793-797.

Schwarcz, R., Bruno, J. P., Muchowski, P. J., & Wu, H. Q. (2012). Kynurenines in the mammalian brain: When physiology meets pathology. *Nature Reviews Neuroscience, 13*(7), 465-477. doi: 10.1038/nrn3257

Sheehan, D. V., Eaddy, M. T., Shah, M. B., & Mauch, R. P. (2005). Differences in total medical costs across the SSRIs for the treatment of depression and anxiety. *American Journal of Managed Care, 11*(12), S354-S361.

Shirayama, Y., Chen, A. C. H., Nakagawa, S., Russell, D. S., & Duman, R. S. (2002). Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. *The Journal of Neuroscience, 22*(8), 3251-3261. doi: 10.3410/f.1005737.68355

Sirey, J. A., Bruce, M. L., Alexopoulos, G. S., Perlick, D. J., Friedman, S. J., & Myers, B. S. (2001). Stigma as a barrier to recovery: Perceived stigma and patient-rated severity of illness as predictors of antidepressant drug adherence. Psychiatric Services, 52(12), 1615-1620. doi: 10.1176/appi.ps.52.12.1615

Skaper, S. D. (2012). The neurotrophin family of neurotrophic factors: an overview. *Methods in Molecular Biology, 846,* 1-12. doi: 10.1007/978-1-61779-536-7_1

Substance Abuse and Mental Health Services Administration. (2017). *Key substance use and mental health indicators in the United States: Results from the 2016 National Survey on Drug Use and Health* (HHS Publication No. SMA 17-5044, NSDUH Series H-52). Rockville, MD: Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration. Retrieved from https://www.samhsa.gov/data/sites/default/files/NSDUH-FFR1-2016/NSDUH-FFR1-2016.htm#mde

Sun, H., Zhang, J., Zhang, L., Liu, H., Zhu., & Yang, Y. (2010). Environmental enrichment influences BDNF and NR1 levels in the hippocampus sand restores cognitive impairment in chronic cerebral hypoperfused rats. *Current Neurovascular Research, 7*(4), 268-280. doi: 10.2174/156720210793180819

Tang, K., Xia, F. C., Wagner, P. D., & Breen, W. C. (2010). Exercise-induces VEGF transcriptional activation in brain, lung, and skeletal muscle. *Respiratory Physiology & Neurobiology, 170*(1), 16-22. doi: 10.1016/j.resp.2009.10.007

Thundyil, J., Pavlovski, D., Sobey, C. G., & Arumugam, T. V. (2012). Adiponectin receptor signaling in the brain. *British Journal of Pharmacology, 165*(2), 313-327. doi: 10.1111/j.1476-5381.2011.01560.x

Trejo, J. L., LLorens-Martín, & Torres-Alemán, J. (2008). The effects of exercise on spatial learning and anxiety-like behavior are mediated by an IGF-I-dependent mechanism related to hippocampal neurogenesis. *Molecular and Cellular Neuroscience, 37*(2), 402-411. doi: 10.1016/j.mcn.2007.10.016

van Praag, H., Christie, B. R., Sejnowski, T. J., & Gage, F. H. (1999). Running enhance neurogenesis, learning, and long-term potentiation in mice. *Proceedings of the National Academy of Sciences of the United States of America, 96*(23), 13427-13431. doi: 10.1073/pnas.96.23.13427

van Pragg, H. (2009). Exercise and the brain: something to chew on. *Trends in Neuroscience, 32*(5), 283-290. doi: 10.1016/j.tins.2008.12.007

Vercoulen, J. H. M. M., Bazelmans, E., Swanink, C. M. A., Fennis, J. F. M., Galama, J. D. M., Jongen, P. J. H., … & Bleijenberg, G. (1997) Physical activity in chronic fatigue syndrome: Assessment and its role in fatigue. *Journal of Psychiatric Research, 31*)(6), 661-673. doi: 10.1016/S0022-3956(97)00039-3

Warburton, D. E. R., Nicol, C. W., & Bredi, S. S. D. (2006). Health benefits of physical activity: the evidence. *Canadian Medical Association Journal, 174*(6), 801-809. doi: 10.1503/cmaj.051351

Will, C. C., Arid, F., & Redei, E.E. (2003). Selectively bred Wistar-Kyoto rats: an animal model of depression and hyper-responsiveness to antidepressants. *Molecular Psychiatry, 8*(11), 925-932. doi: 10.1038/sj.mp.4001345

World Health Organization (WHO). (2018). *Depression*. Retrieved from http://www.who.int/news-room/fact-sheets/detail/depression

Yamahachi, H., Marik, S. A., McManus, J. N. J., Denk, W., & Gilbert, C. D. (2009). Rapid axonal sprouting and pruning accompany functional reorganization in primary visual cortex. *Neuron, 64*(5), 719-729. doi: 10.1016/j.neuron.2009.11.026

Yau, S. Y., Li, A., Hoo, R. L. C., Ching, Y. P., Christie, B. R., Lee, T. M. C., … & So. K. F. (2014). Physical exercise-induced hippocampal neurogenesis and antidepressant effects are mediated by the adipocyte hormone adiponectin. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(44), 15810-15915. doi: 10.1073/pnas.1415219111

Yoshii, A. & Constantine-Paton, M. (2010). Post-synaptic BDNF-TrkB signaling in synapse maturation, plasticity and disease. *Developmental Biology, 70*(5), 304-322. doi: 10.1002/dneu.20765

Zhang, W. & Liu, H. T. (2002) MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Research, 12*(1), 9-18. doi: 10.1038/sj.cr.7290105

Zheng, H., Liu, Y., Li, W., Yang, B., Chen, D., Wang, X., … & Halberg, F. (2006). Beneficial effects of exercise and its molecular mechanisms on depression in rats. *Behavioral Brain Research, 168*(1), 47-55. doi: 10.1016/j.bbr.2005.10.007