LEBANESE AMERICAN UNIVERSITY

D-β-Hydroxybutyrate and lactate mediate the positive effects of exercise on chronic stress by changing the histone modification profile in the hippocampus

By

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A thesis

Submitted in partial fulfillment of the requirements

For the degree of Master of Science in Biological Sciences

School of Arts and Sciences

July 2021

LAU
الجامعة اللبنانية الأميركية
Lebanese American University

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Program: Master of Science in Biological Sciences

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Acknowledgment

First and foremost, I would like to thank my advisor, **Dr. Sama F. Sleiman**, for her constant support, helpful advice, and guidance throughout the past 2 years. Due to Dr. Sleiman's work ethics, critical scientific thinking and knowledge, my passion for research and neuroscience grew considerably stronger. For that, I'm grateful.

I am also very thankful to have been a part of the Sleiman lab team. I want to thank Vanessa Jabre and Mohammad Khalife who helped me, dedicated their time to teach me all the basic techniques, assisted me in certain experiments and motivated me to keep working harder. I'm also thankful for my current lab colleagues Rouba Hobeika, Reine Khoury, Litsa Ghayad, Fadi Eid and Perla El Ahmad who were always ready to lend a hand.

I would like to mention **Dr. Roy Khalaf** and **Dr. Nadine Zeeni** who offered assistance and support throughout my thesis work. Their intriguing questions, coming from different perspectives, triggered my scientific thinking, and encouraged me to read more and gain knowledge.

A special thanks to **Mr. Jean Karam** and **Mr. Elias Abi Ramia**, who taught me how to deal with animals, and were always ready to help with all my *in vivo* experiments whether on weekends or during holidays.

A big thanks to **Ms. Maya Farah** and **Mrs. Helena Bou Farah**, the graduate laboratory supervisors, who were always available to help and provide technical support.

Above all, I cannot but express my love and gratitude to my parents for their unconditional love and continuous support through this entire process. I am grateful for my brothers, boyfriend and friends who were always there for me through all the ups and downs. I'm blessed to have you in my life. Thank you.

D-β-Hydroxybutyrate and Lactate mediate the positive effect of exercise on chronic stress by changing the histone modification profile in the hippocampus

Joelle Saad

Abstract

Depression is a very common mental illness and the leading cause of disability worldwide. Some people are more vulnerable than others; however, the underlying mechanisms behind this vulnerability are not well understood. Exercise has long been studied as a lifestyle factor that can be used to counteract the effect of stress. Our objective was to investigate whether two well-established exercise factors, D-β-Hydroxybutyrate and lactate, mediate its positive effects on resilience to chronic stress and social behavior. For that reason, we first blocked the transport of these metabolites to the brain during exercise and assessed whether this blockage abolished the positive effects of exercise on social behavior. Next, we assessed the effects of D-β-Hydroxybutyrate and lactate and determined whether they promote resilience to chronic stress. To that effect, we induced stress in mice using the chronic social defeat stress paradigm. Mice were subjected to chronic social defeat stress while receiving either D-β-Hydroxybutyrate or lactate. Mice that were subjected to chronic social defeat stress exhibited depressive-like behavior, showed increased susceptibility to stress, and displayed increased social avoidance behavior. D-β-Hydroxybutyrate and lactate promoted resilience to stress and rescued social avoidance behavior. The rescue by D-β-Hydroxybutyrate was associated with an

increase in histone H3 β-Hydroxybutyrylation levels in the hippocampus. Furthermore, we examined the antidepressant properties of these two exercise factors. To assess that, we subjected the mice to chronic social defeat stress, and then administered our treatments. Both D-β-Hydroxybutyrate and lactate acted as antidepressants when administered after the establishment of a depressive phenotype. Finally, we found that a ketogenic diet known to increase the levels of D-β-Hydroxybutyrate in the brain also mimics the effects of these exercise factors and promotes resilience to stress and rescues social avoidance behavior. The molecular basis behind the rescue effect of D-β-Hydroxybutyrate is still being studied.

Keywords: Major depressive disorder, depression, voluntary exercise, epigenetic modification, D-β-Hydroxybutyrate, lactate, β-Hydroxybutyrate, lactylation

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List of Abbreviations

MDD: Major depressive disorder

CSDS: Chronic social defeat stress

SI: Social interaction test

EPM: Elevated plus maze test

DBHB: D-β-Hydroxybutyrate

MCT2: Monocarboxylate transporter

DMSO: Dimethyl Sulfoxide

HDAC: Histone deacetylase

SSRIs: Selective serotonin reuptake inhibitors

SNRIs: Serotonin norepinephrine reuptake inhibitors

SNRIs: Selective norepinephrine reuptake inhibitors

DNA: Deoxyribonucleic acid

BDNF: Brain derived neutrophic factor

HPA: Hypothalamic-pituitary-adrenal axis

PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha

FNDC5: Fibronectin type III domain containing 5

Chapter One

Literature Review

1.1 Major Depressive Disorder

1.1.1 Epidemiology, Etiology & Physiology

Depression also known as Major Depressive Disorder (MDD) is one of the most prevalent mood disorders, and is the leading cause of disability worldwide (Bagot et.al., 2014) (Zhang et.al.,2020). It is characterized by a depressed or irritable mood, reduced interest or loss of pleasure in daily activities, significant weight gain or loss, appetite or sleep disturbance, psychomotor agitation or retardation and feelings of worthlessness. According to the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders DSM-III-R criteria, the lifetime prevalence of MDD is estimated at 17%. Psychiatric epidemiology studies have determined several risk factors associated with MDD. Different childhood experiences, stressful life situations and specific personality traits have been linked to MDD. However, it remains challenging to discriminate between causation and association. Environmental factors such as childhood trauma, medical illness and drug abuse have been associated with an increased risk of MDD, whereas, social support, coping and exercise have been studied as protective factors against MDD. Studies have reported an increased risk for MDD in parents, siblings and offsprings of individuals with MDD versus the whole population indicating that genetic factors in part influence the risk of illness (Fava, M., & Kendler, K. S., 2000). Moreover, MDD is linked to changes in brain regions volumes, specifically in the hippocampus. It is also linked to changes in brain circuits, disturbance of the hypothalamic-pituitary-adrenal axis and disruption of the immune system. Nevertheless, the physiological and anatomical basis of this illness remain the subject of extensive research. Importantly, MDD has been associated with an increased risk of cognitive impairment, disability, obesity, heart disease, diabetes mellitus and cancer (Otte et.al., 2016).

1.1.2 Treatments

There are several treatment approaches used for MDD. Psychotherapy and behavioral therapy are sometimes used; however, they show marginal efficacy. Selective serotonin reuptake inhibitors (SSRIs) have beneficial effects. They block the reuptake of serotonin (5-HT). However, when taken as long-term treatments, they're associated with somnolence or insomnia and apathy. Atypical antidepressants have also been shown to be efficient. They affect norepinephrine (NE), dopamine (DA), and serotonin neurotransmission, but they have an increased risk of sedation. Finally, serotonin norepinephrine reuptake inhibitors and selective norepinephrine reuptake inhibitors (SNRIs) have also been shown to be efficient, but have been corelated to bothersome side-effects when taken for a long period of time (Fava, M., & Kendler, K. S., 2000). Hence, there's an urgent need to find new treatments. Recently, ketamine, an anesthetic, has been shown to have antidepressant effects, and it has been approved as a medication for depression by the United States Food and Drug Administration. Ketamine works in a different manner than the traditional drugs that are used to treat MDD. It triggers glutamate production and allows brain connections to reform. Unlike traditional drugs, it's the effect of ketamine and not its presence that enables it to treat depression. However, since ketamine can become addictive and can have psychological effects, more research needs to be conducted (Shin, C., & Kim, Y., 2020).

1.1.3 Depression & Epigenetics

Depression is a heterogenous illness. Both genetic and environmental factors contribute to the progression of the disease. Hence, epigenetic mechanisms that regulate gene expression have been shown to affect the onset of depression (Nestler, Peña, Kundakovic, Mitchell, & Akbarian, 2016). Epigenetic mechanisms include DNA methylation and post-translational modifications of histones such as acetylation (Covington et.al., 2015), methylation (Lister, 2013), phosphorylation (Aguilar-Valles et.al., 2018), ubiquitylation (Kim. J. H et.al., 2020), lactylation (Izzo et.al., 2019) and β-hydroxybutyrylation (Chen et.al., 2017). Depression is associated with a decrease in H3K14 acetylation in both the hippocampus and the nucleus accumbens (NA). Interestingly, only in the nucleus accumbens, depression was associated with a decrease in the H3K9me2 methylation (Peña, Nestler,. 2018). The decrease in H3K14 acetylation in the hippocampus could be reversed by the antidepressant imipramine. Imipramine increases H3 acetylation in the promoter of the brain derived neutrophic factor (BDNF) leading to an increased BDNF expression in the hippocampus. In the nucleus accumbens, overexpression of HDAC2 or histone methyltransferase G9a acted as antidepressants. On the other hand, overexpression of HDAC5 acted as a pro-depressant. Hence, the significance of histone modifications is region specific (Duclot, Kabbaj, 2015).

1.2 Exercise

1.2.1 Positive effect of Exercise

Physical exercise has long been recognized as a powerful and positive factor affecting physical and mental health. It targets several brain functions including enhancing learning and memory formation, delaying age-related cognitive decline, reducing the risk of neurodegeneration, and alleviating depression (Sleiman and Chao, 2015). These effects are thought to be mediated partially by the activation of BDNF signaling in the hippocampus (Cotman, Berchtold, & Christie, 2007). Recent studies showed that injection of blood from young exercising mice into old mice promoted learning and memory formation by increasing hippocampal BDNF levels (Kim et.al., 2020). Several blood-borne factors released by the liver, muscle and bones have been shown to increase BDNF levels in the brain and hence, mediating the positive effects of exercise. Studying these exercise factors would be beneficial to identify novel therapeutic agents that may alleviate depression by increasing hippocampal BDNF levels. These factors comprise metabolites and proteins released from the muscle, liver, platelets and bones. Some of them have been previously implicated in depression (Chen et.al., 2017; Karnib et.al., 2019), traumatic brain injury and stroke (Stephan, Sleiman, 2021) (Figure 1).

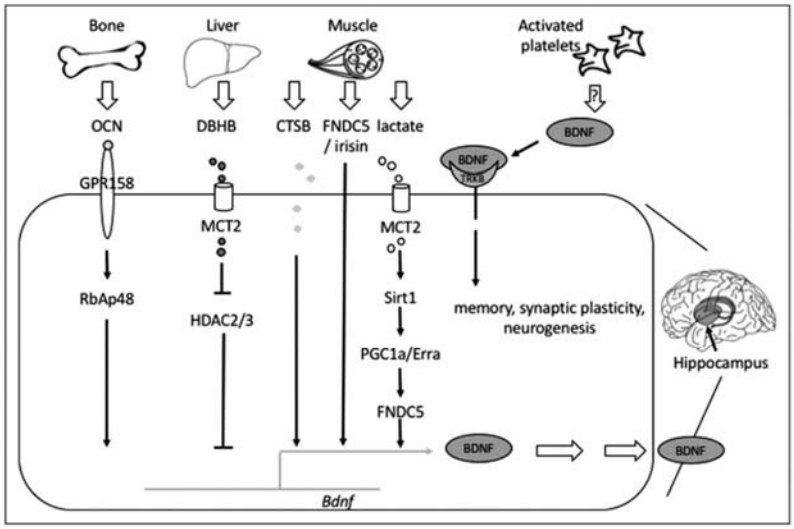


FIGURE 1. Exercise mediates learning and memory formation by inducing the release of multiple peripheral factors that induce *Bdnf* expression and signaling in the hippocampus. The liver releases the ketone body betahydroxy-butyrate that induces hippocampal *Bdnf* expression by inhibiting class I HDACs [5]. The muscle releases lactate that induces hippocampal *Bdnf* expression by activating the SIRT1/PGC1-alpha/FNDC5 expression [4••]. Peripheral FNDC5/irisin can also increase *Bdnf* expression [24]. Bones release osteocalcin that promotes *Bdnf* expression through an epigenetic mechanism involving RbAP48 [74]. Finally activated platelets may also store and secrete BDNF [75••,76]. The roles of these exercise factors in the context of TBI and their potential therapeutic value remains to be assessed. BDNF, brain-derived neurotrophic factor; FDNC5, fibronectin type III domain-containing protein 5; HDACs, histone deacetylases; TBI, traumatic brain injury.

Figure 1 Adapted with permission (Stephan, Sleiman, 2021)

1.2.2 Exercise and Depression

The positive effects of exercise on depression have been extensively studied in the both therapeutic and preventative. contexts. The benefits provided by exercise are similar to those of anti-depressants. However, the underlying molecular mechanisms are poorly understood. Some studies suggest that exercise induces changes in the HPA axis that regulates stress response (Anderson, Shivakumar, 2013). Other studies suggest that exercise induces neurogenesis and an increased growth factor expression in the hippocampus. It is established that exercise increases the expression of Peroxisome proliferator-activated receptor-gamma coactivator PGC-1 α in the muscle, and it is suggested that an increased expression of PGC-1 α in the muscle is associated with a decreased risk of MDD (Agudelo et.al., 2014).

1.3 Exercise Factors: Lactate & DBHB

1.3.1 Brain lactate metabolism

Lactate is an exercise factor that is released by the muscle. It crosses the blood brain barrier and accesses the brain via endothelial monocarboxylate transporters (MCTs) (E et.al., 2013). In our lab, we have recently studied lactate as an exercise factor that improves spatial memory in the hippocampus through the activation of the PGC1a/FNDC5/BDNF signaling pathway (El Hayek et.al., 2019). It is well established that the brain uses astrocytic lactate as a source of energy (Riske et.al., 2017). Studies have shown that astrocytic lactate is also required for long-term memory formation (Alberini et.al., 2018). Researchers have reported that lactate promotes vasodilation, and increases blood flow to the brain. Similarly to other exercise factors, lactate promotes brain health, and enhances neurogenesis (Rice et.al., 2002). It also acts as an antidepressant that promotes resilience to stress by modifying the activity of hippocampal histone deacetylase levels (Karnib et al 2019). Interestingly, recent studies revealed that lactate mediates lactylation of histone lysine residues stimulating gene transcription by remodeling chromatin structure (Zhang et al, 2019). It is still unclear whether this histone modification is regulated by chronic stress and whether lactate's antidepressant effect involves modulating this epigenetic modification.

1.3.2 Brain DBHB metabolism

The brain needs a continuous supply of glucose. Whenever this supply is disturbed, the brain depends on alternative substrates such as ketone bodies like DBHB. DBHB is an exercise factor released by the liver into the blood, and it crosses the blood brain barrier and accesses the brain via the monocarboxylate transporter (MCT2). Once in the hippocampus, DBHB induces the activity of BDNF promoter I leading to an increased BDNF expression through modulating class I histone deacetylase inhibitors (Sleiman et.al., 2016). Ketone bodies and ketogenic diet have been shown to be beneficial for brain health. Ketogenic diet promotes neurogenesis and improve learning and memory formation. DBHB also has been implicated with improved cellular pathologies in Parkinson's disease (Norwitz et.al., 2019). Interestingly, DBHB and ketogenic diets have neuroprotective potential against stroke. In addition, DBHB has antidepressant effects (Kajitani et.al., 2020). The antidepressant effects of DBHB have not been assessed in response to chronic stress and the involvement of epigenetic mechanisms in the antidepressant effect of DBHB have not been carefully deciphered.

1.4 Aim of the study

In this study, we will assess the effect of voluntary exercise on depression and anxiety. We will also address whether lactate and DBHB mediate exercise's positive effects on social behavior by inhibiting their transport to the brain during exercise. We will determine whether both lactate and DBHB mediate resilience to stress and act as antidepressants when given exogenously independent of exercise. We will also assess whether a ketogenic diet can mimic the effects of exercise on social behavior. Finally, we will also start identifying the epigenetic mechanisms by which voluntary exercise promotes resilience to chronic stress and whether DBHB and lactate activate similar epigenetic mechanisms as exercise.

Chapter Two

Materials and Methods

2.1 Experimental Subjects

Exercise Paradigm: Male C57BL/6 4-week-old mice were either individually housed in cages or with a running wheel with food and water *ad libitum* and maintained on a 12-hour light-dark cycle. They were divided into sedentary or exercise groups and further classified based on the treatments they received. They were then housed in a cage divided into two compartment that are separated by a perforated divider. In the next compartment, an aggressor CD1 mouse was housed. Experimental mice underwent chronic social defeat stress for 10 days. Animals were sacrificed and hippocampal tissues were collected on dry ice and stored at -80°C. This work was approved by the Lebanese American University Animal Care and Use Committee (ACUC).

Co-treatment Paradigm: Male C57BL/6 7-week-old mice were housed next to an aggressor CD1 mouse to undergo chronic social defeat stress with simultaneous intraperitoneal (i.p.) injections of DBHB or lactate. Animals were sacrificed and hippocampal tissues were collected on dry ice and stored at -80°C. This work was approved by the Lebanese American University Animal Care and Use Committee (ACUC).

Post-Treatment Paradigm: Male C57BL/6 7-week-old mice were housed next to an aggressor CD1 mouse to undergo chronic social defeat stress. On the 12^{th} day, DBHB or lactate injections start to be administered for 2 weeks. Then, animals were sacrificed and hippocampal tissues were collected on dry ice and stored at -80°C. This work was approved by the Lebanese American University Animal Care and Use Committee (ACUC).

Ketogenic Diet Co-Treatment Paradigm: Male C57BL/6 5-week-old mice were housed in groups of 5. Food (Ketogenic Diet or Standard Diet) was added every day and water was provided. Animals were maintained on a 12-hour light-dark cycle. After 2 weeks, mice were housed next to an aggressor CD1 mouse to undergo chronic social

defeat stress. Animals were sacrificed and hippocampal tissues were collected on dry ice and stored at -80°C. This work was approved by the Lebanese American University Animal Care and Use Committee (ACUC).

2.2 Treatments

Exercise Paradigm: Male mice received intraperitoneal injections of AR-C155858 (1mM), an inhibitor of the monocarboxylate transporter MCT2, or saline + 2%DMS0 after 26 days of voluntary exercise. Animals kept receiving injections during the chronic social defeat stress paradigm.

Co-Treatment Paradigm: Male mice received intraperitoneal injections of either lactate (180mg/kg) (Karnib et.al., 2019) or DBHB (100mg/kg) (Pan et.al., 2020) or saline throughout the chronic social defeat stress paradigm.

Post-Treatment Paradigm: Male mice received intraperitoneal injections of either lactate (180mg/kg) or DBHB (100mg/kg) or saline after the chronic social defeat stress paradigm for 2 weeks.

Ketogenic Diet Co-Treatment Paradigm: Male mice were fed the ketogenic diet or the standard diet 2 weeks prior to chronic social defeat stress and throughout chronic social defeat stress.

2.3. Chronic Social Defeat Stress

The chronic social defeat stress paradigm was performed as previously described (Golden et al., 2011). Briefly, CSDS consists of 3 stages. The first stage involves the screening and selection of the aggressive CD1 mice. Aggressor CD1 mice are housed individually for seven days with food and water. Screening for the CD1 mice consist of three consecutive screening days where the screener mouse is placed in the home cage of the aggressor for three minutes. After three days, aggressor mice are selected upon the initiation of at least one aggression episode within the first minute of the screening session. Aggressor mice were screened before every chronic defeat paradigm and mice that did not meet this criterion were excluded. Selected aggressor mice are next housed individually one day before the first defeat session in a cage with a clear perforated Plexiglas divider. The second stage consists of the ten days where the defeat sessions occur. The experimental mouse and the aggressor are put in physical contact for around seven minutes where the experimental mouse is exposed

to social defeat. The third stage consists of placing the experimental mouse and the aggressor CD1 mouse in sensory contact till the next day allowing only vision, audition and olfaction. Every day, the experimental mouse gets introduced to a novel resident's (aggressor) cage in order to prevent any habituation between the aggressor and the experimental mouse. The control animals are also housed in pairs with an aggressor CD1 mouse and alternated every day for ten days, however no physical contact was allowed between the intruder and resident mouse. On the 11th day, also known as the test day, the mice undergo behavioral tests.

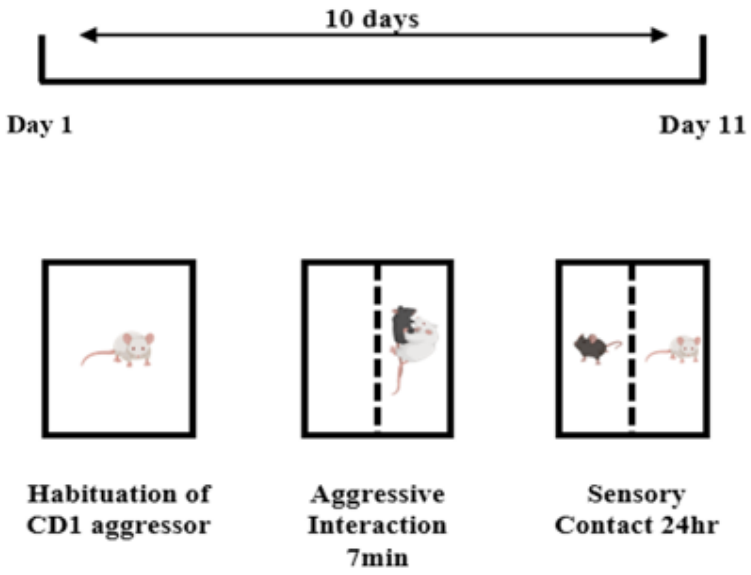


Figure 2 Illustrative schematic of the chronic social defeat stress paradigm

2.4 Social Interaction Test

The social interaction test was performed one day after the last defeat session as previously described (Kaidanovich-Beilin, Lipina, Vukobradovic, Roder, & Woodgett, 2011). The Social Interaction test assesses depressive-like behavior. It consists of placing the experimental mouse in a chamber divided into 3 compartments (Figure 2). The 2 side compartments contain circular wire enclosures. The middle compartment is empty. The first phase is the habituation phase where the experimental mouse is placed in the middle compartment and allowed to explore for 5 minutes. Then, a social stimulus C57BL/6 10-week-old mouse is placed in one of the circular wire enclosures, while the experimental mouse is reintroduced to the middle compartment and allowed to freely navigate the different chambers for 10 minutes.

The movement of the experimental mouse is recorded with a camera mounted to the ceiling, and monitored using a video tracking program known as the ANY-maze which measures the time the mouse spends in each compartment. The choice of the chamber containing the stimulus mouse was alternated between trials and the three chambers were cleaned with 30% isopropyl alcohol after every trial. The total time spent by the experimental mouse in the social compartment was divided by the total time spent by the experimental mouse in the non-social compartment. If this social interaction ratio was greater than 1, then the mouse was considered resilient, and if it was less than 1, then the mouse was considered susceptible to stress.

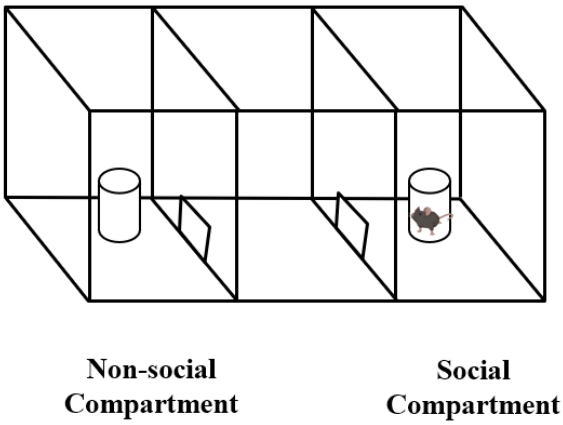


Figure 3 Illustrative schematic of the social interaction test

2.6 Protein Extraction and Immunoblot Analysis

Total cellular proteins were extracted from the tissues by preparing a master mix of lysing buffer RIPA-B (1% Triton X-100, 1% SDS, 50mm Tris-Cl, pH 7.4, 500mm NaCl, and 1mm EDTA), protease (Sigma, BioWORLD), phosphatase (Sigma, BioWORLD), and proteasome (MG-132) inhibitors (Sigma). Samples were boiled in Laemelli buffer and were electrophoresed on an acrylamide gel (Bio-Rad) and then transferred to a PVDF membrane using Trans-Blot SD Semi-Dry transfer cell (Bio-Rad). Membranes were blocked using non-fat milk diluted in TBS-Tween. Membranes were incubated either overnight at 4°C or for 2 hours at room temperature. Primary antibodies against lactylation (PTM Bio), β-Hydroxybutyrylation (PTM Bio), Histone 3 H3 (Santa Cruz Biotechnology) and Actin (Cell Signaling) will be diluted 1:2000, 1:2000, 1:5000, and 1:5000 respectively in blocking buffer. Secondary antibodies (Bio-Rad) were used at a 1:5000 dilution and incubated for 1 hour at room temperature. Three washes of 5 minutes each with TBS-T were performed after primary antibody

incubation and again after secondary antibody incubation. Finally, the proteins were detected by chemiluminescence on ChemiDoc (Bio-Rad) using Clarity Western ECL Substrate (Bio-Rad). Band quantification and analysis was done with ImageJ software.

2.7 Statistical Analysis

Unpaired T-test, one-way or two-way ANOVA followed by the Dunnett, Tukey, or Bonferroni, respectively, were used to assess the statistical significance of the results. $p<0.05$ was considered to be statistically significant. *: $p<0.05$, **: $p<0.01$, ***: $p<0.005$

Chapter Three

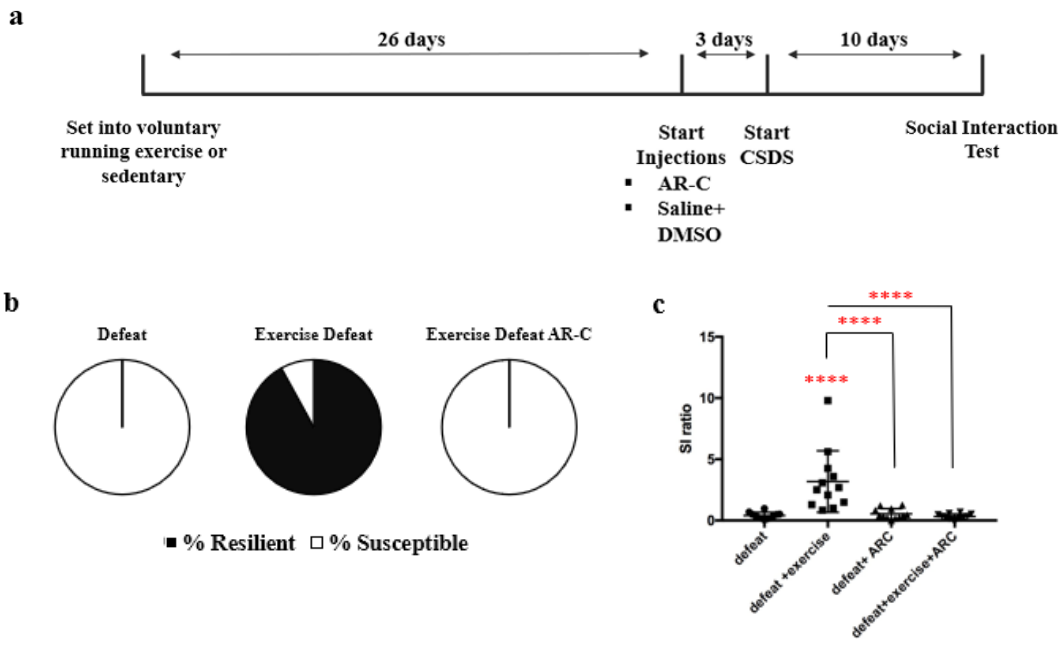
Results

3.1 Voluntary exercise promotes resilience to stress, and the inhibition of the monocarboxylate transporter abolishes the effect.

In order to determine whether voluntary exercise promotes resilience to stress and assess whether DBHB and lactate are necessary for exercise's effects on social behavior, we initially divided our 4-week-old mice into two groups: sedentary or exercise. Animals belonging to both groups were individually housed. Only animals belonging to the exercise group were provided with a running wheel. After 26 days of voluntary exercise, the running wheels were removed and mice either received daily intraperitoneal injections of AR-C155858, an inhibitor of the monocarboxylate transporter MCT2, which is responsible for DBHB and lactate transport to the brain or vehicle (saline and 0.02% DMSO) for the rest of the experiment. After three days of injecting the mice with either AR-C155858 or vehicle, mice underwent chronic social defeat stress. For ten days and prior to every defeat session, mice received injections. Defeated mice were put in physical contact with the aggressors whereas control mice remained in sensory contact with the aggressors. On the 11^{th} day of the chronic social defeat stress paradigm, we performed the social interaction test to assess whether animals were susceptible or resilient to stress. After the completion of the behavioral tests, animals were sacrificed, and hippocampi were harvested for further biochemical analysis (Figure 4a). Studies have established that mice subjected to CSDS can be divided into susceptible mice showing social avoidance behavior and resilient mice showing wild-type social behavior (Krishnan, V., Ming-Hu, H., 2007). The social interaction test results showed that exercise promotes resilience to stress. The percentage of animals that resilient to stress increased from 0% in the defeat group to 100% in the exercise+defeat group. Inhibition of DBHB or lactate uptake into the brain through inhibition of the MCT2 transporter by AR-C155858 abolished the positive effects of exercise. Indeed, 100% of the animals belonging to this group (exercise+defeat+ARC) were susceptible to stress (Figure 4b). In addition, we found

that the defeat mice, defeat+AR-C as well as exercise+defeat+AR-C have a significantly lower social interaction ratio as compared to exercise+defeat mice (Figure 4c). Finally, we found that exercise decreased social avoidance behavior in mice subjected to chronic stress. In contrast, inhibition of DBHB or lactate uptake into the brain abolished exercise's positive effects on social behavior. Indeed, we observed that the control mice spent a significant time interacting with the stimulus mouse as compared to the defeat susceptible mice. This effect was rescued in the defeat mice that exercised. Interestingly, when we inhibited MCT2 transporter in defeat+exercise mice, the rescue was reversed and the positive effects of exercise were abolished (Figure 4d).

Taken together, our results demonstrate that exercise promotes resilience to chronic social defeat stress and rescues social avoidance behavior, and that DBHB and lactate transport to the brain is necessary for this effects.



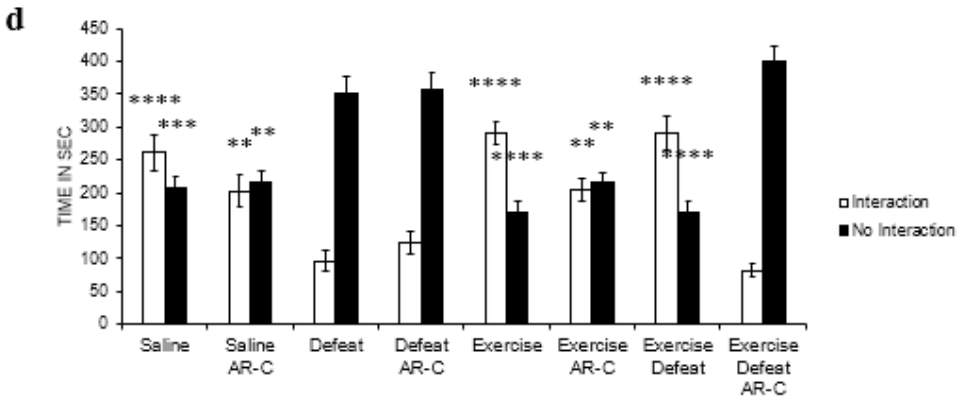


Figure 4 Exercise promotes resilience to stress and inhibition of MCT2 abolished the effect.

(a) The exercise paradigm consists of dividing mice into two groups. The sedentary 4-week-old mice were caged individually and the exercise 4-week-old mice were caged with a running wheel for 26 days. Then, mice were injected with AR-C155858, the inhibitor of the monocarboxylate transporter MCT2, or vehicle (saline+0.02% DMSO). After 3 days, mice were subjected to chronic social defeat stress. 24 hours after the last defeat session, mice underwent the social interaction test to assess depressive-like behavior.

(b) Exercise promotes resilience to stress. Pie charts showing the percentage of resilience in black versus the percentage of susceptibility in white. Results showed an increase in the number of resilient mice from 0/12 in the defeat group to 12/12 in the exercise+defeat group. Introduction of AR-C155858 abolished the effect of exercise as the number of resilient mice was 0/12 in the exercise+defeat+ARC group.

(c) Column scatter graph showing the average social interaction ratio as measured by the social interaction test. Social interaction ratio equals the time the mouse spends interacting with the stimulus divided by the time the mouse spends not interacting with the stimulus. Results showed that the exercise+defeat group had a significantly higher social interaction ratio compared to the defeat, defeat+ARC and exercise+defeat+AR-C group. Statistical significance was measured by 1way Anova followed by Tukey's multiple comparison test; significance was measured versus the defeat group. ****$p<0.0001$; ($F_{3, 44}=13.92$; $p<0.0001$ n=12 for each group.

(d) Bar graph showing the average interaction time in seconds. Exercise+defeat mice spent a significant higher time interacting with the stimulus compared to the defeat susceptible mice. Mice belonging to exercise+defeat+AR-C group spent significantly less time interacting with the stimulus as compared to exercise+defeat mice suggesting that AR-C155858 abolished the positive effect of exercise. Statistical significance was measured by 2way Anova followed by Tukey's multiple comparison test; significance was measured versus the defeat group. ****p<0.0001 and *** p<0.001. Interaction time: interaction: $F_{3,183} = 7.921$, $p < 0.0001$; CSDS: $F_{1,183} = 52.67$, $p < 0.0001$ and treatment: $F_{3,183} = 27.82$, $p < 0.0001$). No interaction time: interaction: $F_{3,183} = 6.48$, $p = 0.0003$; CSDS: $F_{1,183} = 54.08$, $p < 0.0001$ and treatment: $F_{3,183} = 15.23$, $p < 0.0001$) n=12 in each group except n=10 saline AR-C, n=11 exercise and n=10 exercise AR-C.

3.2 DBHB and lactate promote resilience to stress.

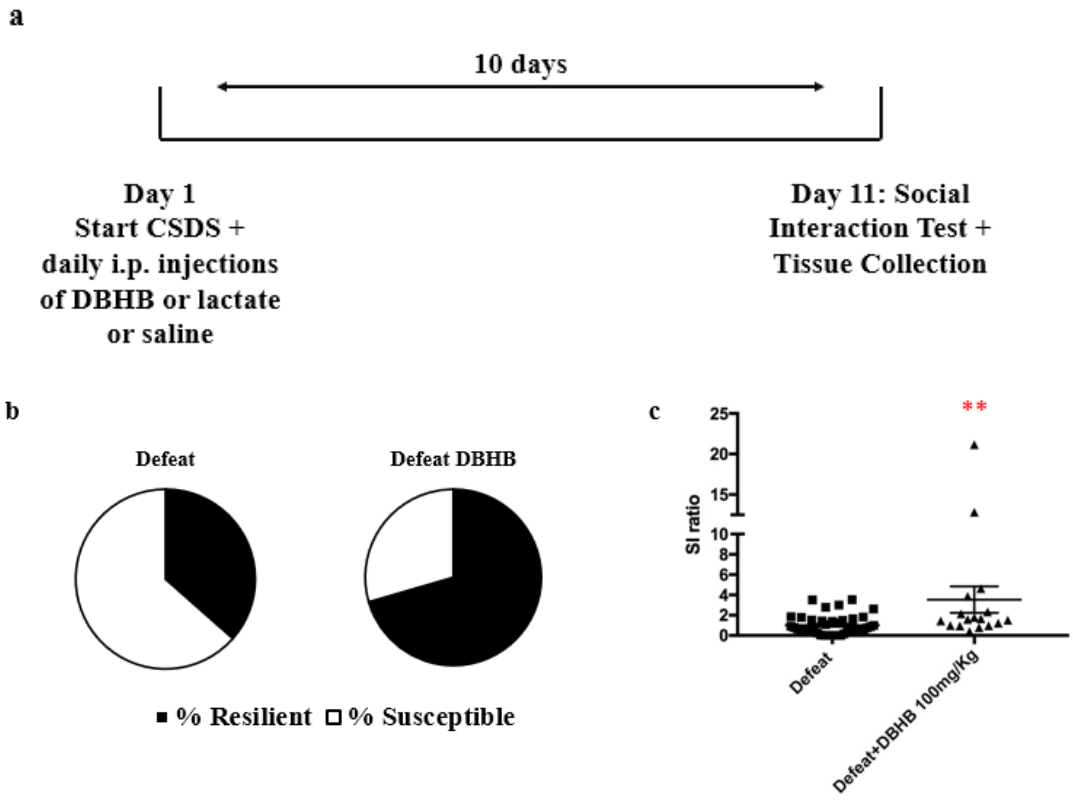
Previous work done in our lab has shown that the concentrations of DBHB and lactate increase in the hippocampus as a result of voluntary exercise (Sleiman, S. F., 2016) (El Hayek, L., 2019). Knowing that DBHB and lactate serve as exercise factors that are transported to the brain through the MCT2 transporter (Pérez-Escuredo, J., 2016), we were interested in determining whether these exercise factors mediate the positive effect of exercise by promote resilience to chronic stress. To assess that, we subjected C57BL/6 7-week-old mice to chronic social defeat stress and simultaneously injected them with either DBHB, lactate or saline. On the 11^{th} day of the chronic social defeat stress paradigm, we performed the social interaction test to assess depressive-like behavior in these mice, and then sacrificed the mice and isolated their hippocampi for further biochemical analysis (Figure 5a).

The social interaction test results showed that DBHB promotes resilience to stress. Defeat mice receiving DBHB were significantly more resilient to stress and had a significantly higher social interaction ratio as compared to defeat mice receiving vehicle (Figure 5b and Figure 5c). Moreover, control mice injected with saline spent a significant time interacting with the stimulus unlike the defeat susceptible mice that spent most of their time not interacting with the stimulus. The group of mice subjected to stress and cotreated with DBHB spent significantly more time interacting with the

stimulus compared to the defeated susceptible group indicating that DBHB rescues social avoidance behavior (Figure 5d).

As we saw in the DBHB co-treatment, lactate also promotes resilience to stress. The defeat mice showed high percentage of susceptibility as compared to the defeat mice receiving lactate. Indeed, all the mice belonging to the defeat+lactate group were resilient to stress (Figure 5e). In addition, the defeat+lactate group had a significantly higher social interaction ratio compared to the defeat group (Figure 5f). Finally, the defeat+lactate group spent significantly more time interacting with the stimulus as compared to the defeat susceptible group demonstrating that lactate rescues social avoidance behavior (Figure 5g).

Our results show that both DBHB and lactate promote resilience to stress and rescue social avoidance behavior.



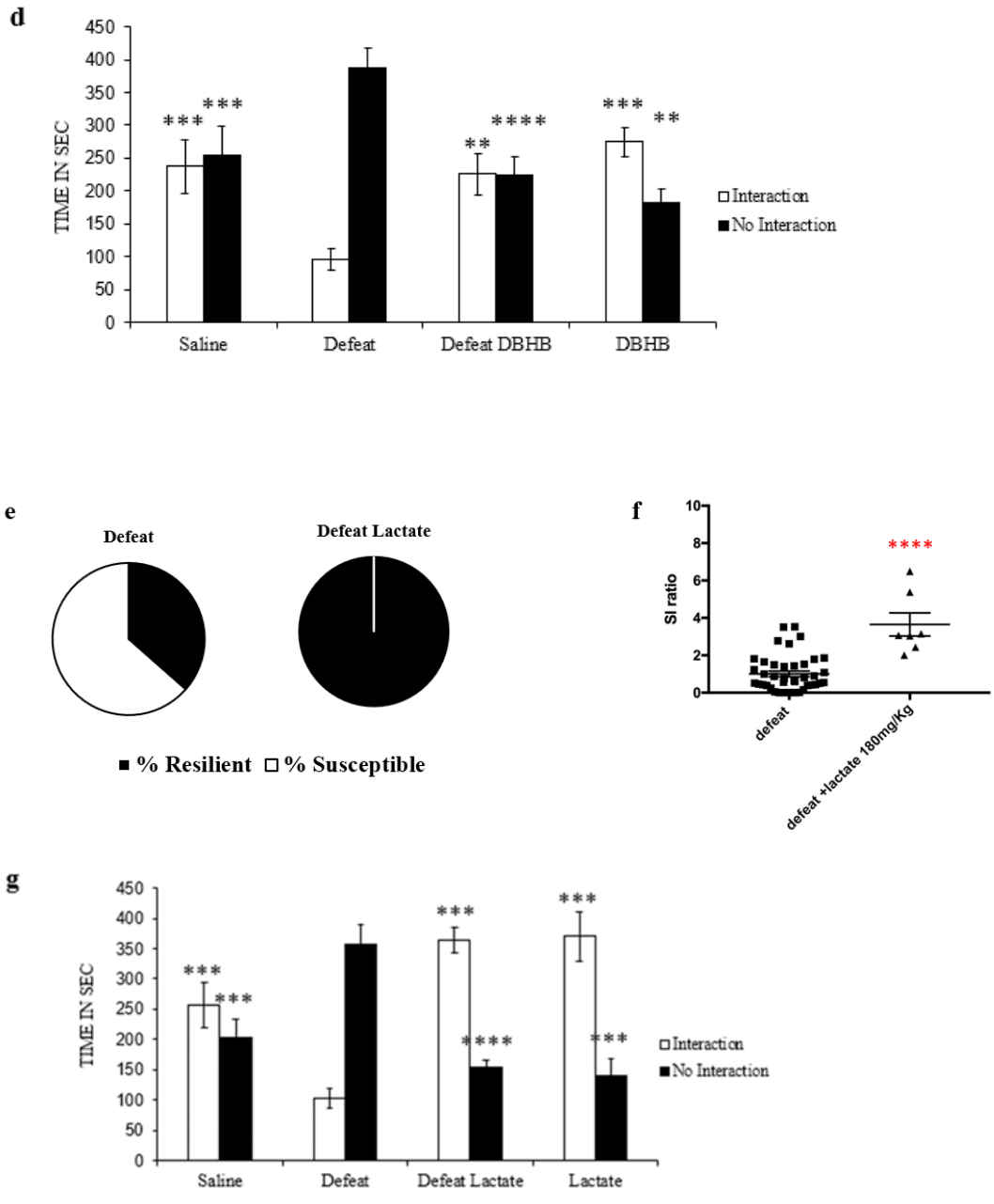


Figure 5 DBHB & lactate promote resilience to stress.

(a) The paradigm consists of male mice undergoing chronic social defeat stress for 10 days. Prior to every defeat session, mice were intraperitoneally injected with either DBHB, lactate or saline. 24 hours after the last defeat session, mice underwent the social interaction test to assess depressive-like behavior.

(b) Pie charts showing the percentage of resilience in black versus the percentage of susceptibility in white. Results showed an increase in the number of

resilient mice from 8/18 in the defeat to 12/17 in the defeat+DBHB mice injected with DBHB.

(c) Column scatter graph showing the average social interaction ratio as measured by the social interaction test. Social interaction ratio equals the time the mouse spends interacting with the stimulus divided by the time the mouse spends not interacting with the stimulus. Results showed that the defeat mice receiving DBHB had a significantly higher social interaction ratio compared to the defeat mice receiving vehicle. n=41 defeat and n=17 for defeat DBHB; Statistical significance was measured by unpaired T-test p=0.045

(d) Bar graph showing the average interaction time. Mice receiving saline spent significantly more time interacting with the stimulus as compared to the defeat mice. The effect was rescued in the mice that were subjected to defeat and receiving DBHB. Statistical significance was measured by 2way Anova followed by Tukey's multiple comparison test; significance was measured versus the defeat group. ****p<0.0001 and *** p<0.001. Interaction time: interaction: $F_{1,55} = 3.765$, $p = 0.0575$; CSDS: $F_{1,55} = 14.15$, $p = 0.0004$ and treatment: $F_{1,55} = 6.875$, $p < 0.0113$. No interaction time: interaction: $F_{1,55} = 4.306$, $p = 0.0427$; CSDS: $F_{1,55} = 14.12$, $p = 0.0004$ and treatment: $F_{1,55} = 8.715$, $p < 0.0046$; n=12 saline, n=18 defeat, n=17 defeat +DBHB and n=12 DBHB

(e) Pie charts showing the percentage of resilience in black versus the percentage of susceptibility in white. Results showed an increase in the number of resilient mice from 8/18 in the defeat group to 7/7 in the defeated mice receiving lactate.

(f) Column scatter graph showing the average social interaction ratio as measured by the social interaction test. Social interaction ratio equals the time the mouse spends interacting with the stimulus divided by the time the mouse spends not interacting with the stimulus. Results showed that the defeat mice receiving lactate had a significantly higher social interaction ratio compared to the defeat mice. n=41 defeat and n=7 for Defeat lactate; Statistical significance was measured by unpaired T-test p<0.0001

(g) Bar graph showing the average interaction time. Mice receiving saline spent significantly more time interacting with the stimulus compared to the defeated susceptible mice. The effect was rescued in the mice that were

subjected to chronic stress and receiving lactate. Statistical significance was measured by 2way Anova followed by Tukey's multiple comparison test; significance was measured versus the defeat group. ****p<0.0001 and *** p<0.001. Interaction time: interaction: $F_{1,40} = 6.422$, $p = 0.0153$; CSDS: $F_{1,40} = 7.453$, $p = 0.0094$ and treatment: $F_{1,40} = 41.48$, $p < 0.0001$. No interaction time: interaction: $F_{1,40} = 5.219$, $p = 0.0277$; CSDS: $F_{1,40} = 7.366$, $p = 0.0098$ and treatment: = 19.07, $p < 0.0001$) n=12 saline, n=18 defeat, n=7 defeat +lactate and n=7 lactate

3.3 DBHB changes the histone modification profile in the hippocampus.

Next, we were interested in determining how exercise and its factors mediate resilience to stress. Since lactate was previously shown to affect histone deacetylase 2 and 3 levels in the hippocampus in response to chronic stress (Karnib et al., 2019), we focused on DBHB and assessed the levels the histone H3 Lysine (K) 9 β-Hydroxybutyrylation in the hippocampus. This is novel histone modification that has been previously detected in mouse models of type I diabetes. It has been associated with active transcription of different metabolic pathways in response to starvation (Huang, H., Zhang, D., 2021). Considering that β-Hydroxybutyrylation has been established to be a mark that links metabolism to gene expression, we tested whether this modification correlates with the resilience to stress phenotype. We compared Histone H3 K9 β-Hydroxybutyrylation levels between defeat susceptible mice and defeat mice that received DBHB. We found that control mice injected with saline showed higher Histone H3K9 β-hydroxybutyrylation levels as compared to the susceptible defeat mice. Interestingly, we observed an increase in the level of β-hydroxybutyrylation of Histone H3 in the hippocampi of defeat mice that received DBHB. These results indicate that DBHB increases histone H3K9 β-hydroxybutyrylation levels in the hippocampus of defeat mice (Figure 6a, b). These results provide a preliminary correlative evidence linking this histone modification to DBHB-induced resilience to chronic stress that warrants further investigation.

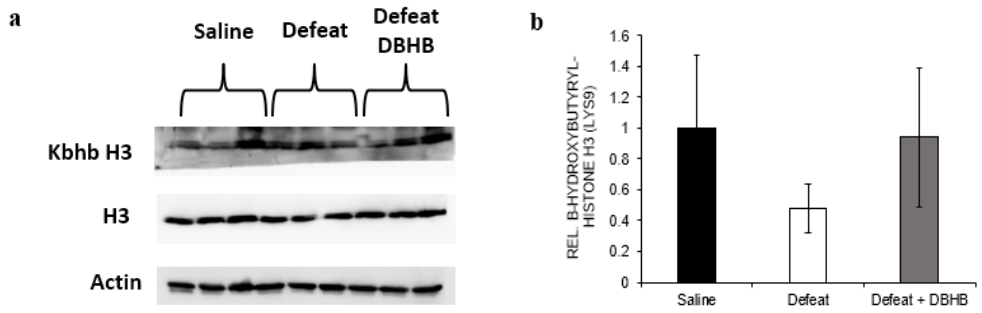


Figure 6 DBHB increases β-Hydroxybutyrylation levels specifically in the hippocampus.

(a) Western blot images showing the effect of DBHB on Histone H3 β-Hydroxybutyrylation levels in the hippocampus of C57BL/6 7-week-old mice.
(b) Relative quantification of the β-Hydroxybutyrylation western blot images.

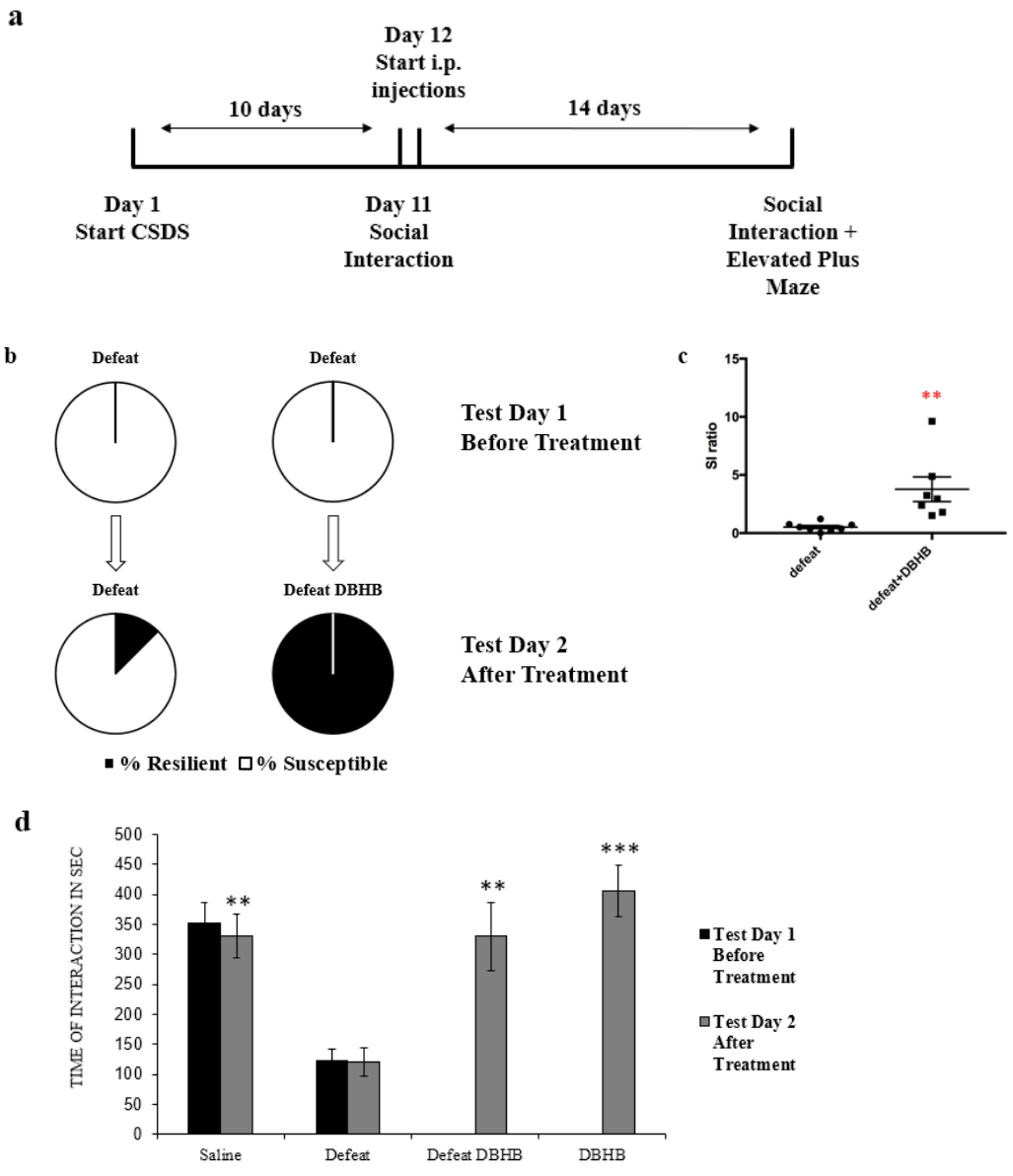
3.4 DBHB and lactate act as antidepressants.

Since DBHB and lactate promote resilience to chronic stress, we were interested in studying if these two endogenous metabolites can act as antidepressants. To evaluate that, we subjected the mice to chronic social defeat stress. On the 11th day, we performed the social interaction test. We divided the mice into two groups based on their social interaction ratio: the susceptible mice and the resilient mice. Then, we administered DBHB or lactate as treatments or saline to the susceptible mice. After 14 days of treatment, we performed the social interaction test and the elevated plus maze test (Figure 7a).

The social interaction test results showed that the defeat mice receiving saline were all susceptible to chronic stress. In contrast, the susceptible defeat mice treated with DBHB all became resilient to stress (Figure 7b). As expected, we found that the defeat mice treated with DBHB had a significantly higher social interaction ratio as compared to the defeat mice receiving saline (Figure 7c). Moreover, the defeat mice treated with DBHB showed significantly higher interaction time with the stimulus as compared to the defeat group treated with saline indicating that DBHB restores social interaction behavior and hence acts as an antidepressant (Figure 7d).

Furthermore, the social interaction test results also showed that lactate also acts as an antidepressant since the susceptible defeated mice treated with lactate showed a

significantly higher percentage of resilience to stress as compared to those treated with saline (Figure 7e). Moreover, we found that the defeat mice treated with lactate had a significantly higher social interaction ratio compared to the defeat mice treated with saline (Figure 7f). By assessing the time spent interacting with the stimulus mouse, we observed that the defeat mice treated with lactate showed a significantly higher interaction time as compared to the defeat group receiving saline further establishing the antidepressant effects of lactate (Figure 7g).



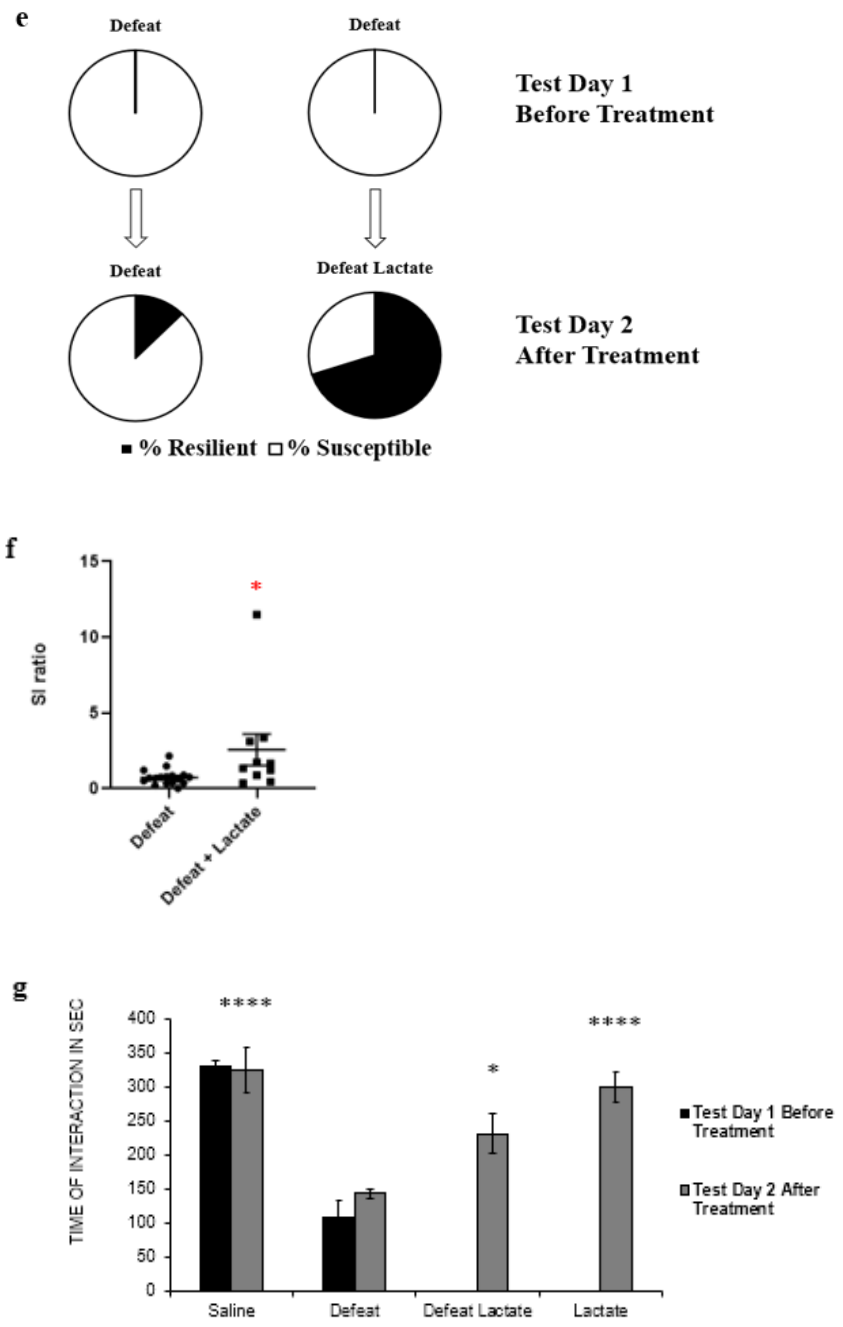


Figure 7 DBHB and lactate have antidepressant properties.

(a) Paradigm consists of subjecting C57BL/6 7-week-old mice to chronic social defeat stress. On the 11th day, mice are divided into susceptible and resilient mice according to their social interaction ratio. Then, DBHB or lactate are administered as treatments or saline as a control. After two weeks of treatment,

animals' behaviors are analyzed through the social interaction test and the elevated plus maze test. Then, mice are euthanized and parts of the brain are extracted for further biochemical analysis.

(b) Pie charts showing the percentage of resilience in black versus the percentage of susceptibility in white. Since susceptible mice are chosen to undergo treatment, pie charts clearly indicate that before treatment, mice were completely susceptible to stress. After treatment, mice injected with saline showed a very high percentage of susceptibility. Results showed an increase in the number of resilient mice from 0/8 in the defeated group treated with saline to 8/8 in the defeated mice treated with DBHB.

(c) Column scatter graph showing the average social interaction ratio as measured by the social interaction test. Social interaction ratio equals the time the mouse spends interacting with the stimulus over the time the mouse spends not interacting with the stimulus. Results showed that the defeated mice treated with DBHB had a significantly higher social interaction ratio compared to the defeated mice treated with saline. Statistical significance was measured by unpaired T-test $p<0.0063$ $n = 8$ per group

(d) Bar graph showing the average interaction time during the first test day before treatment and the second test day after treatment. Mice injected with saline spent a significant time interacting with the stimulus compared to the defeated mice injected with saline. Defeated mice treated with DBHB showed a significantly higher interaction time compared to the defeated mice injected with saline. Statistical significance was measured by 1way Anova followed by Dunnett's multiple comparison test; significance was measured versus the defeat group. ****$p<0.0001$; $F_{3, 31}=7.674$; $p=0.0006$; n=10 Saline, n=8 defeat, n=8 defeat+DBHB and n=9 for DBHB.

(e) Pie charts showing the percentage of resilience in black versus the percentage of susceptibility in white. After treatment, mice injected with saline showed a very high percentage of susceptibility. Results showed an increase in the number of resilient mice from 3/17 in the defeated group treated with saline to 7/10 in the defeated mice treated with lactate.

(f) Column scatter graph showing the average social interaction ratio as measured by the social interaction test. Social interaction ratio equals the time the mouse spends interacting with the stimulus over the time the mouse spends not

interacting with the stimulus. Results showed that the defeated mice treated with lactate had a significantly higher social interaction ratio compared to the defeated mice treated with saline. Statistical significance was measured by unpaired T-test $p<0.33$ $n = 17$ defeat, $n = 10$ defeat lactate

(g) Bar graph showing the average interaction time during the first test day before treatment and the second test day after treatment. Mice injected with saline spent a significant time interacting with the stimulus compared to the defeated mice injected with saline. Defeated mice treated with lactate showed a significantly higher interaction time compared to the defeated mice injected with saline. Statistical significance was measured by 2way Anova followed by Tukey's multiple comparison test; significance was measured versus the defeat group. ****$p<0.0001$ and *** $p<0.001$. Interaction time: interaction: $F_{2,77} = 3.246$, $p = 0.0443$; CSDS: $F_{1,77} = 44.83$, $p < 0.0001$ and treatment: $F_{2,77} = 1.272$, $p=0.2862$). No interaction time: interaction: $F_{2,77} = 1.589$, $p = 0.2108$; CSDS: $F_{1,77} = 21.7$, $p < 0.0001$ and treatment: $F_{2,77} = 2.29$, $p=0.1081$) n=15 saline, n=22 defeat, n=15 defeat +lactate and n=15 lactate

They are also consistent with the hypothesis that DBHB and lactate mediate exercise's effects on social behavior in response to chronic stress.

3.4 The antidepressant effects of lactate require the MCT2 transporter.

To determine whether the MCT2 transporter is also necessary for the antidepressant effects of lactate, we assessed whether inhibition of the MCT2 transporter by AR-C155858 affects the antidepressant effects of lactate. To evaluate that, we subjected the mice to chronic social defeat stress for 10 days. On the 11^{th} day, we performed the social interaction test. We divided the mice into two groups based on their social interaction ratio: the susceptible mice and the resilient mice. Then, we administered lactate+AR-C to the susceptible mice. After 14 day, we performed the social interaction test (Figure 8a).

The social interaction test results showed that AR-C abolishes the positive effects of lactate on chronic stress since the susceptible defeated mice treated with lactate+AR-C showed a significantly lower percentage of resilience to stress as compared to those treated with lactate (Figure 8b). Furthermore, by evaluating the time spent interacting

with the stimulus mouse, we found that the defeat mice that received lactate+AR-C showed a significantly lower interaction time as compared to the defeat group treated with lactate further confirming that AR-C inhibits the antidepressant effects of lactate (Figure 8c).

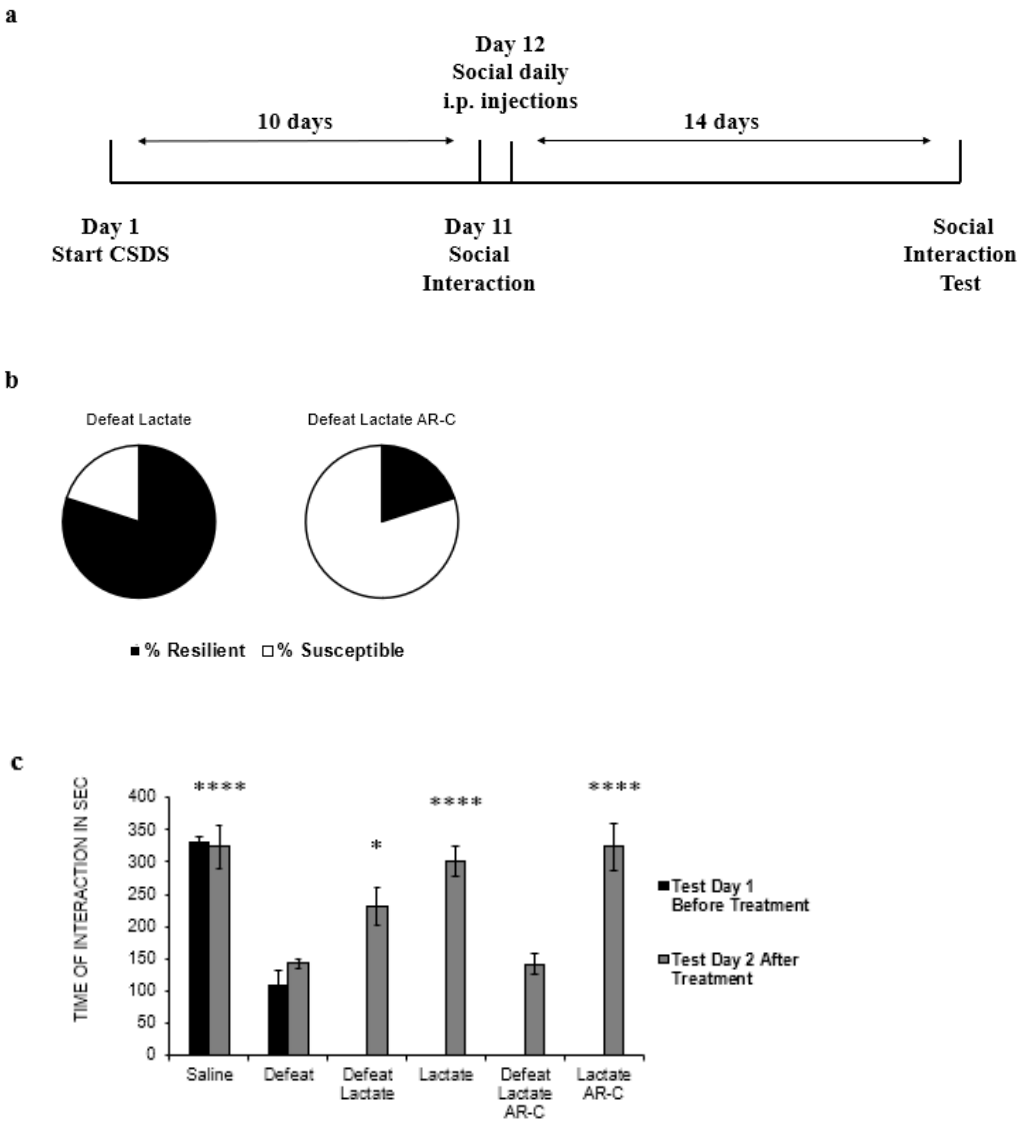


Figure 8 The antidepressant effects of lactate require MCT2

(a) Paradigm consists of subjecting C57BL/6 7-week-old mice to chronic social defeat stress. On the 11th day, mice are divided into susceptible and resilient mice according to their social interaction ratio. Then, lactate or lactate+AR-C are administered as treatments or saline as a control. After two weeks of

treatment, animals' behaviors are analyzed through the social interaction test. Then, mice are euthanized and parts of the brain are extracted for further biochemical analysis.

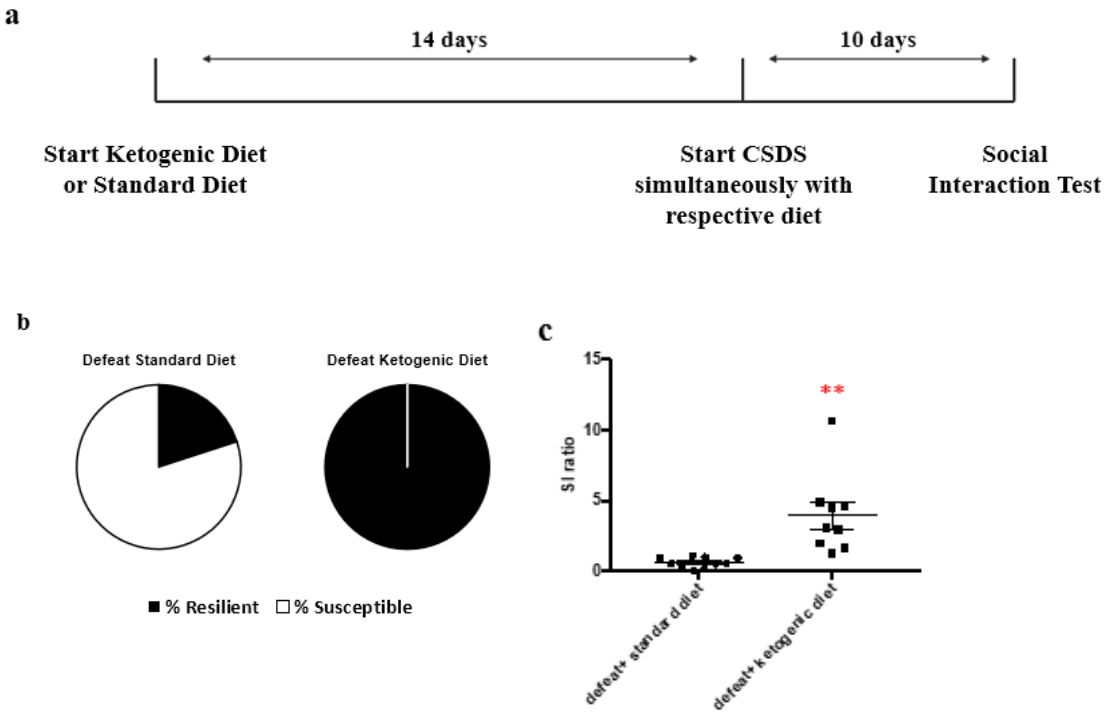
(b) Pie charts showing the percentage of resilience in black versus the percentage of susceptibility in white. Since susceptible mice are chosen to undergo treatment, pie charts clearly indicate that before treatment, mice were completely susceptible to stress. Results showed a decrease in the number of resilient mice from 12/15 in the defeated group treated with lactate to 2/10 in the defeated mice treated with lactate+AR-C.

(c) Bar graph showing the average interaction time during the first test day before treatment and the second test day after treatment. Mice injected with saline spent a significant time interacting with the stimulus compared to the defeated mice injected with saline. Defeated mice treated with lactate showed a significantly higher interaction time compared to the defeated mice injected with saline. Defeated mice treated with lactate+AR-C showed a significantly lower interaction time compared to the defeated mice treated with lactate. Statistical significance was measured by 2way Anova followed by Tukey's multiple comparison test; significance was measured versus the defeat group. ****$p<0.0001$ and *** $p<0.001$. Interaction time: interaction: $F(2,77) = 3.246$, $p = 0.0443$; CSDS: $F(1,77) = 44.83$, $p < 0.0001$ and treatment: $F(2,77) = 1.272$, $p=0.2862$). No interaction time: interaction: $F(2,77) = 1.589$, $p = 0.2108$; CSDS: $F(1,77) = 21.7$, $p < 0.0001$ and treatment: $F(2,77) = 2.29$, $p=0.1081$) n=15 saline, n=22 defeat, n=15 defeat +lactate, n=15 lactate, n=10 defeat lactate AR-C and n=9 lactate AR-C

3.5 Ketogenic diet mimics the effect of DBHB on stress.

Knowing that the ketogenic diet stimulates the formation of ketone bodies in the brain, we were interested in studying the ketogenic diet and whether it mimics the effect of DBHB on resilience to stress. To do that, we started feeding C57BL/6 5-week-old mice either the ketogenic diet or the standard diet for two weeks. Then, we subjected the mice to chronic social defeat stress simultaneously while still feeding them their respective diets. On the 11^{th} day of the chronic social defeat stress paradigm, we performed the social interaction test (Figure 9a). The social interaction test results showed that the defeat group that was fed the ketogenic diet were more resilience to

stress as compared to the defeat group that was fed the standard diet (Figure 9b). In addition, we observed that the defeat mice that were fed the ketogenic diet had a significantly higher social interaction ratio compared to the defeat mice that were fed the standard diet. This indicated that the ketogenic diet promotes resilience to chronic stress (Figure 9c). Finally, we also observed that control mice that were fed the standard diet spent a significantly higher time interacting with the stimulus as compared to the defeat mice that were fed the standard diet. The mice that were fed the ketogenic diet and subjected to chronic stress spent significantly more time interacting with the stimulus compared to the defeat mice that were fed the standard diet showing that ketogenic diet rescues social avoidance behavior (Figure 9d).



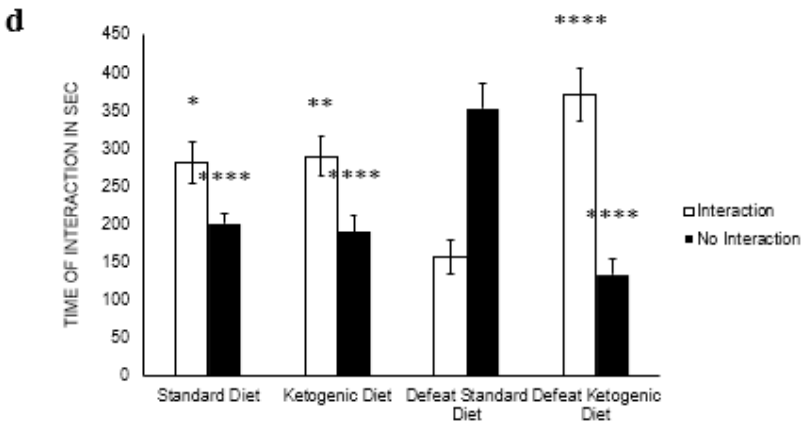


Figure 9 Ketogenic Diet mimics DBHB effect on resilience to stress.

(a) Paradigm consists of feeding 5-week-old C57BL/6 mice ketogenic diet or standard diet for two weeks. Then, mice are subjected to chronic social defeat stress. On the 11th day of the CSDS paradigm, animals' behaviors are analyzed through the social interaction test. Then, mice are sacrificed and parts of the brain are isolated for further biochemical analysis.

(b) Pie charts showing the percentage of resilience in black versus the percentage of susceptibility in white. Results showed an increase in the number of resilient mice increased from 1/6 in the defeat group fed the standard diet to 6/6 in the defeat mice fed the ketogenic diet.

(c) Column scatter graph showing the average social interaction ratio as measured by the social interaction test. Social interaction ratio equals the time the mouse spends interacting with the stimulus divided by the time the mouse spends not interacting with the stimulus. Results showed that the defeat mice that were fed the ketogenic diet had a significantly higher social interaction ratio as compared to the defeat mice that were fed the standard diet. Statistical significance was measured by unpaired T-test $p=0.0018$.

(d) Bar graph showing the average interaction time. Defeat mice that were fed the ketogenic diet spent a significant time interacting with the stimulus compared to the defeat mice that were fed the standard diet. Statistical significance was measured by 2way Anova followed by Tukey's multiple comparison test; significance was measured versus the defeat group. ****$p<0.0001$ and *** $p<0.001$. Interaction time: interaction: $F_{1,35} = 15.58$, $p = 0.0004$; CSDS: $F_{1,35} = 0.7105$, $p = 0.4050$ and treatment: $F_{1,35} = 18.09$, $p = 0.0001$). No

interaction time: interaction: $F_{1,35} = 24.8$, $p < 0.0001$; CSDS: $F_{1,35} = 6.844$, $p = 0.0130$ and treatment: $F_{1,35} = 29.89$, $p < 0.0001$) n=10 standard diet, n=10 defeat standard diet, n=10 defeat + ketogenic diet and n=11 ketogenic diet

Chapter Four

Discussion

MDD is a serious mental illness that interferes with a person's ability to carry out simple daily life activities. According to the American Psychiatric Association, approximately 1 in 15 adults will experience depression in any given year. The United States and countries all over the world are facing a mental health crisis due to the impact of the COVID-19 pandemic. The pandemic has disrupted the economy, education and social relationships. People are facing persistent stress and trauma. Knowing that available antidepressants are of limited efficacy, and only half of the diagnosed patients achieve remission (Al-Harbi, 2012; Kato et.al., 2021), there's an urgent need to study molecular pathways involved in regulating susceptibility and resilience to stress in order to identify novel therapeutic targets.

A vast wealth of evidence connects exercise to positive mental and physical health. Exercise has been shown to increase synaptic plasticity, enhance learning and memory formation, delay age-related cognitive decline, and reduce degeneration. Most importantly, exercise has been shown to play a role in alleviating depression (Cotman, Berchtold, & Christie, 2007). Hence, mimicking the effects of exercise by identifying the molecular mechanisms that are involved and modulating them as therapeutic targets is of great interest to researchers working on MDD.

DBHB and lactate are exercise factors that can cross the blood brain barrier. Both metabolites have been shown to be involved in regulating deacetylases activity, inducing BDNF expression and mediating resilience to stress (Karnib et.al., 2019; Sleiman et.al., 2016). Interestingly, studies suggest that DBHB and lactate induce β-Hydroxybutyrylation and lactylation respectively leading to the activation of gene expression by changing chromatin structure (Xie et.al., 2016; Zhang et.al., 2019). Therefore, we started studying whether these histone modification are affected by chronic stress.

In order to assess this hypothesis, we initially wanted to confirm that exercise and its factors promote resilience to stress. We set our mice to voluntary exercise for 26 days. Then, we injected them with AR-C155858 which is the inhibitor of the

monocarboxylate transporter MCT2. After 3 days, we subjected the mice to chronic social defeat stress and assessed their behavioral changes using the social interaction test. Our results showed that mice subjected to defeat stress and that exercised had a significantly higher SI ratio and a higher interaction time with the stimulus compared to the defeated sedentary mice. Hence, we verified that exercise promotes resilience to stress and rescues social avoidance. Interestingly, the introduction of AR-C-155858 abolished the effect of exercise confirming that the exercise-promoted resilience to stress is dependent on transport through the MCT2, the major transporter that two endogenous metabolites and exercise factors DBHB and lactate use to reach the brain.

Since DBHB and lactate increase in the hippocampus during exercise, we therefore, decided to test whether they mediate the positive effect of exercise on resilience to stress. We administered either DBHB (100mg/kg) or lactate (180mg.kg) intraperitoneally to 7-week-old mice and simultaneously subjected these mice to chronic social defeat stress. The social interaction test results revealed DBHB and lactate increased the percentage of resilient mice, the social interaction ratio, and the social interaction time

After establishing that DBHB and lactate promote resilience to stress and rescue social interaction behavior, we were interested in determining how these exercise factors are mediating the positive effect of exercise. In our work, we focused on DBHB and tested for β-Hydroxybutyrylation of histone 3 on residue lysine 9. As mentioned earlier, β-Hydroxybutyrylation has been associated to an increase in gene expression; so, we wanted to test whether this modification correlates with the resilience to stress phenotype. By looking at the western blot images and their respective quantifications, we saw an increase in the levels of histone H3K9 β-Hydroxybutyrylation upon administering DBHB to mice subjected to defeat stress as compared mice subjected to defeat stress and receiving saline indicating that DBHB restored histone H3 K9 β-Hydroxybutyrylation levels specifically in the hippocampus. These results though promising did not reach statistical significance. As a result, we are currently widening our analysis to include more hippocampi.

We next raised the question of whether DBHB and lactate can act as antidepressants. We subjected 7-week-old mice to chronic social defeat stress. Then, we divided the mice into a susceptible and resilient group based on their social interaction ratio. After

that, we administer either DBHB or lactate intraperitoneally as a treatment for two weeks. To assess the mice's behavior, we performed the social interaction test and the elevates plus maze test. Our results showed that the susceptible defeated mice treated with DBHB or lactate had a higher percentage of resilience, a higher social interaction ratio, and a higher social interaction time compared to the susceptible defeated mice that received saline. This indicates that both DBHB and lactate can act as antidepressants. Moreover, our elevated plus maze results showed no difference in the time the mice spent in the open arms between all four groups indicating that DBHB does not rescue anxiety-like behavior.

Interestingly, we were able to mimic the effects DBHB through a diet known as the ketogenic diet. To do that, we fed 5-week-old mice either the ketogenic diet or the standard diet for two weeks. After that, we subjected the mice to chronic social defeat stress and then assessed their behaviors using the social interaction test. We found that the defeated group that was fed the ketogenic diet showed a significantly higher percentage of resilience, social interaction ratio, and social interaction time compared to the defeated group that was fed the standard diet. As we observed upon injections of DBHB, the ketogenic diet promoted resilience to stress and rescued social avoidance behavior.

In this study, we confirmed that exercise promotes resilience to stress and the inhibition of the monocarboxylate transporter abolishes the effect. We also found that DBHB and lactate can mediate the positive effects of exercise. They both promote resilience to stress by somehow modulating the histone H3 modification profile in the hippocampus. Through this work and Karnib et al 2019, we showed that lactate promotes resilience to stress. And finally, we found that not only do DBHB and lactate promote resilience to stress, but they also act as antidepressants. This research brings us a step closer to creating an exercise pill that could potentially be used as a treatment for depression. Nevertheless, ketogenic diet, as our results have shown, could also be followed as method to alleviate depression.

For future studies, it would be interesting to clearly identify the histone profiles in different regions of the brain. We focused on the hippocampus since we did not have the ability to explore other regions. However, being able to extract the nucleus accumbens would potentially increase our chances in getting better results and being

able to associate our phenotypes to specific regions and molecular pathways. Also, studying histone modifications using whole cell lysates has been a limitation. Being able to look at single cells and analyze results would've been more helpful. Most importantly, by taking a different approach and using Chromatin Immunoprecipitation technique followed by sequencing would be more informative. That way we would obtain a set of genes targeted by a specific histone modification. Hence, we'd be able to identify several molecular pathways correlated to the stress resilience phenotype.

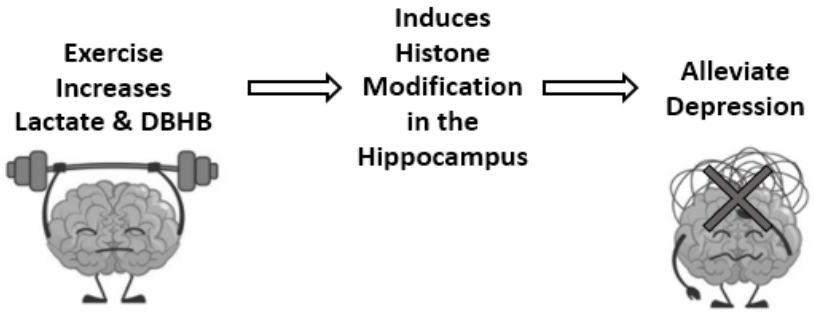


Figure 10 A proposed model by which exercise and its factors mediate resilience to stress

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