Neurobiology of Learning and Memory 149 (2018) 28–38

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Running exercise mitigates the negative consequences of chronic stress on dorsal hippocampal long-term potentiation in male mice

Roxanne M. Miller[a], David Marriott[a], Jacob Trotter[b], Tyler Hammond[b], Dane Lyman[a], Timothy Call[b], Bethany Walker[b], Nathanael Christensen[b], Deson Haynie[b], Zoie Badura[a], Morgan Homan[b], Jeffrey G. Edwards[a,b,*]

[a] Brigham Young University, Department of Physiology and Developmental Biology, Provo, UT 84602, USA
[b] Brigham Young University, Neuroscience Center, Provo, UT 84602, USA

ARTICLE INFO

Keywords:
LTP
Dorsal hippocampus
Dopamine 5 receptor
RT-qPCR
LTD
Corticosterone

ABSTRACT

In the hippocampus, learning and memory are likely mediated by synaptic plasticity, known as long-term potentiation (LTP). While chronic intermittent stress is negatively correlated, and exercise positively correlated to LTP induction, we examined whether exercise could mitigate the negative consequences of stress on LTP when co-occurring with stress. Mice were divided into four groups: sedentary no stress, exercise no stress, exercise with stress, and sedentary with stress. Field electrophysiology performed on brain slices confirmed that stress alone significantly reduced dorsal CA1 hippocampal LTP and exercise alone increased LTP compared to controls. Exercise with stress mice exhibited LTP that was significantly greater than mice undergoing stress alone but were not different from sedentary no stress mice. An ELISA illustrated increased corticosterone in stressed mice compared to no stress mice. In addition, a radial arm maze was used to examine behavioral changes in memory during 6 weeks of stress and/or exercise. Exercised mice groups made fewer errors in week 2. RT-qPCR was used to examine the mRNA expression of components in the stress and exercise pathways in the four groups. Significant changes in the expression of the following targets were detected: BDNF, TrkB, glucocorticoid, mineralocorticoid, and dopamine 5 receptors. Collectively, exercise can mitigate some of the negative impact stress has on hippocampal function when both occur concurrently.

1. Introduction

Plasticity is a unique characteristic of the nervous system. Following environmental stimuli or experiences, neuronal synaptic connections in the brain are modified. The most common form of synaptic modification observed *ex vivo* is known as long-term potentiation (LTP) and is one phenomenon used to quantify learning and memory. Synaptic plasticity occurring in the hippocampus has become the leading theory of the mechanism for memory formation and recall (Malenka & Bear, 2004).

One factor that has a dramatic impact on hippocampal learning and memory in rodents is stress (McEwen & Sapolsky, 1995). There are various types of stress induction techniques (McCarty, 2017). Regarding acute stress, it is a single stress incident that can be adaptive in rodents and enhance memory behavioral performance (Maras & Baram, 2012; Pignatelli et al., 2017) as well as synaptic activity and LTP (Blank, Nijholt, Eckart, & Spiess, 2002), or alternatively decrease LTP (Foy, Stanton, Levine, & Thompson, 1987; Garcia, Musleh, Tocco, Thompson, & Baudry, 1997). Acute stress particularly affects LTP in the dorsal hippocampus, which is thought to be mediated by the glucocorticoid receptors (Cazakoff & Howland, 2010; Howland & Wang, 2008), and has been reviewed previously (Howland & Wang, 2008). The effects of acute stress on LTP can be reversed over time (Garcia et al., 1997). However, chronic/chronic intermittent stress are ongoing stress incidents that are more maladaptive and their effects are harder to reverse over time (Artola et al., 2006; Joels & Krugers, 2007). In behavioral studies, chronic intermittent stress decreases the ability of rodents to form and recall spatial memories (McEwen, 1999) and hinders performance in the Morris water maze (Kim, Lee, Han, & Packard, 2001) and novel object recognition (Baker & Kim, 2002). Chronic stress

Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic; AMPK, adenosine monophosphate-activated protein kinase; BDNF, brain-derived neurotrophic factor; ENS, exercise no stress; EWS, exercise with stress; GR, glucocorticoid receptor; LTD, long term depression; LTP, long term potentiation; NMDA, N-methyl-D-aspartate; MR, mineralocorticoid receptor; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction; SNS, sedentary no stress; SWS, sedentary with stress

* Corresponding author at: Brigham Young University, Physiology and Developmental Biology, Neuroscience Center, 4005 LSB, Provo, UT 84602, USA.
E-mail address: Jeffrey_Edwards@byu.edu (J.G. Edwards).

https://doi.org/10.1016/j.nlm.2018.01.008
Received 19 June 2017; Received in revised form 30 November 2017; Accepted 21 January 2018

also decreases neurogenesis and can induce neuronal cell death (McEwen, 1999). As our study employed various chronic stress methods, the factor most pertinent to this study is that chronic stress reduces CA1 hippocampal LTP in rodents (Artola et al., 2006).

The connection between hippocampal plasticity and the aforementioned behavioral deficits have been reviewed and discussed extensively (Howland & Wang, 2008; Kim & Diamond, 2002; Kim & Yoon, 1998; McEwen, 1999; McEwen & Sapolsky, 1995; Sandi & Pinelo-Nava, 2007). The glucocorticoids are important hormones released during stress. In rodents, corticosterone is a glucocorticoid that is released. Corticosterone binds to both glucocorticoid and mineralocorticoid receptors in the central nervous system. Glucocorticoid and mineralocorticoid release are increased during chronic stress and have been implicated in causing changes in hippocampal plasticity (Conrad, 2008; McEwen, 2012). Studies have shown that corticosterone acting on glucocorticoid and mineralocorticoid receptors alter alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptor function and trafficking, as well as induces changes in synaptic plasticity (Krugers & Hoogenraad, 2009; Xiong et al., 2016). While many things remain unclear, it is consistent that chronic stress impedes the ability for neurons to experience LTP and has profound effects on memory.

Conversely, studies have found that mice performing voluntary physical exercise has the opposite effect on the mechanisms that are impaired by stress (Salmon, 2001). Rodents that exercise show robust performance in maze navigation and have increased hippocampal LTP in the dentate gyrus (van Praag, Christie, Sejnowski, & Gage, 1999). Exercise also has anxiolytic and antidepressant effects by increasing brain-derived neurotrophic factor (BDNF) levels (Duman, Schlesinger, Russell, & Duman, 2008). BDNF is a protein that promotes neural survival, growth, and differentiation of new neurons and synapses. It is known that voluntary exercise increases BDNF (Kim et al., 2001; Russo-Neustadt, Ha, Ramirez, & Kesslak, 2001; Tong, Shen, Perreau, Balazs, & Cotman, 2001). BDNF and other proteins in the BDNF pathway are thought to be the major contributors for enhancing memory (Bekinschtein et al., 2008) and increasing LTP (Lu, Christian, & Lu, 2008; Martinez-Moreno, Rodriguez-Duran, & Escobar, 2011). Recent research illustrates that BDNF activates mTOR, which regulates the expression of AMPA receptors to increase memory and LTP (Slipczuk et al., 2009). Studies showed chronic stress downregulated BDNF (Zagaar, Dao, Levine, Alhaider, & Alkadhi, 2013) and upregulated interneuron activity (Schoenfeld, Rada, Pieruzzini, Hsueh, & Gould, 2013), while exercise prevented these changes. However, these studies were performed in the ventral hippocampus and not the dorsal hippocampus, which is an important distinction since the different hippocampal subfields have different neural projections and functions (Fanselow & Dong, 2010). The dorsal hippocampus has been studied less in regards to stress and exercise compared to the ventral hippocampus.

While many of the molecular, physiological, and behavioral effects of stress and exercise on rodents have been studied in isolation, they are rarely studied concurrently. Despite the evidence that exercise and stress influence brain health and plasticity in opposite ways, there is a paucity of data that connects the effects these two factors might have in the dorsal hippocampus when experienced by the same animal. Additionally, the mechanism by which exercise could potentially reduce the negative effects of stress is not completely understood (Salmon, 2001). Therefore, we examined whether exercise occurring concurrently with stress could alleviate the negative impact of stress on dorsal hippocampal plasticity. Using behavioral interventions, such as exercise, to combat learning deficits due to chronic stress could be a safe, cost-effective treatment that could improve cognitive function and quality of life for many individuals. Furthermore, considering the neurotoxic effects of chronic stress and the shown benefits of exercise, our results could add to the body of literature seeking to understand and prevent neurodegenerative disorders associated with chronic stress.

2. Materials and methods

2.1. Treatment groups

Adult male C57BL/6 mice were used in this study. Mice were housed in approved conditions with a 12-h light-dark cycle. The experiments had ethical approval and were conducted in accordance with the Brigham Young University Institutional Animal Care and Use Committee standards and National Institute of Health guidelines to minimize pain and suffering of the mice. The four treatment groups utilized in this study were sedentary no stress (control; SNS), sedentary with stress (SWS), exercise with stress (EWS), and exercise no stress (ENS). The average ages of the mice used were 87 days for the electrophysiology experiments and 145 days for RT-qPCR and behavioral experiments. The difference in ages between these groups is due to the radial arm maze assay lasting 6 weeks. The mice continued to run during the duration of behavioral testing, and were sacrificed at the conclusion of this memory assay. Their brains were extracted and hippocampi then isolated for RT-qPCR testing as described below. All four groups of mice used in electrophysiology experiments were of similar age. The RT-qPCR and behavioral experiments also used age-matched mice from all four groups.

2.2. Exercise and stress protocols

Mice from all four groups were housed solitarily with the same type of bedding to ensure that those mice with access to running wheels had accurate distance measurements as well as to maintain a consistent social environment to prevent uncontrolled variables. SNS and SWS mice did not have locked running wheels in their cages and were housed in slightly smaller cages than the exercise cages, and therefore were under slightly more impoverished conditions. No enrichment was provided to the SNS and SWS mice. This is a potential limitation in our methods, though we still note differences in SNS and SWS mice in LTP studies, which were housed identically. ENS and EWS mice were allowed to run *ad libitum* in a cage with a running wheel purchased from Lafayette Instrument Co and the distance was tracked by software provided by the same company on a portable computer. The average distance run by all exercise mice (stress and no stress) was 5.42 ± 0.32 km per day, which was slightly higher, but still comparable to the average of approximately 4.5 km per day (4.1 & 4.8 km/day) others saw using the same strain of mice (van Praag et al., 1999; Marlatt, Potter, Lucassen, & van Praag, 2012). ENS and EWS mice ran at least an average of 2 km per day; no mice ran less than this so no exercise mice were excluded from our study. Mice were exercised for a minimum of 4 weeks before being used for any experimentation (electrophysiology, PCR, and behavior). The mice also were at least 30 days old before being moved into running cages. Surprisingly, there were differences in average daily running distance between ENS and EWS mice used for electrophysiology (ENS = 6.31 ± 0.52 km, EWS = 4.40 ± 0.53 km, *t* test $p < 0.05$). This is surprising as running occurred for one month while stress was only the last three days and running distances were not significantly changed after stress. This difference appears to be random based on which mice were selected for entry into stress procedures or not. However, this caveat could influence differences in LTP noted between the two in the results section. No differences in running distance were noted between ENS and EWS for behavioral/PCR experiments (5.18 ± 0.75 km and 4.69 ± 0.97 km day; $p > 0.5$).

Electrophysiology SWS and EWS mice experienced three consecutive days of stressors to create chronic intermittent/variable stress. We used similar variable stressors from a prior report (Katz, Roth, & Carroll, 1981), with some slight modifications to the stressors and shorter stress duration. Another group (DeVallance et al., 2017) also modified the original Katz et al. protocol to a shorter duration of 5 days. We shortened the stress protocol because we wanted chronic stress physiological changes in the shortest amount of time. The stressors