Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Neurobiol Learn Mem. Author manuscript; available in PMC 2019 March 01.

Published in final edited form as:

Neurobiol Learn Mem. 2018 March ; 149: 28–38. doi:10.1016/j.nlm.2018.01.008.

Running Exercise Mitigates the Negative Consequences of Chronic Stress on Dorsal Hippocampal Long-Term Potentiation in Male Mice

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

Brigham Young University, Department of Physiology and Developmental Biology, Provo, UT 84602 USA

[#]Brigham Young University, Neuroscience Center; Associate Director, Provo, UT 84602 USA

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.

Keywords

LTP; dorsal hippocampus; dopamine 5 receptor; RT-qPCR; LTD; corticosterone

---

[*]Corresponding Author Dr. Jeffrey G. Edwards, Brigham Young University, Physiology and Developmental Biology; Neuroscience Center, 4005 LSB, Provo, UT 84602, Phone: 801-422-8080, Fax: 801-422-0004, Jeffrey_Edwards@byu.edu.

Author Contributions: DM, RMM and JGE designed experiments, acquired and analyzed data and wrote the paper. JT, TH, DL, TC, BW, NC, SH ZB and MH acquired and analyzed data and edited the paper.

Conflict of Interest: There is no conflict of interest for any of the authors.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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 and Bear 2004).

One factor that has a dramatic impact on hippocampal learning and memory in rodents is stress (McEwen and 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 and Baram 2012, Pignatelli, Umanah et al. 2017) as well as synaptic activity and LTP (Blank, Nijholt et al. 2002), or alternatively decrease LTP (Foy, Stanton et al. 1987, Garcia, Musleh et al. 1997). Acute stress particularly affects LTP in the dorsal hippocampus, which is thought to be mediated by the glucocorticoid receptors (Howland and Wang 2008, Cazakoff and Howland 2010), and has been reviewed previously (Howland and Wang, 2008). The effects of acute stress on LTP can be reversed over time (Garcia, Musleh 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, von Frijtag et al. 2006, Joels and 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 et al. 2001) and novel object recognition (Baker and Kim 2002). Chronic stress 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, von Frijtag et al. 2006).

The connection between hippocampal plasticity and the aforementioned behavioral deficits have been reviewed and discussed extensively (McEwen and Sapolsky 1995, Kim and Yoon 1998, McEwen 1999, Kim and Diamond 2002, Sandi and Pinelo-Nava 2007, Howland and Wang 2008). 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 and Hoogenraad 2009, Xiong, Cassé et al. 2016). While many things remain unclear, it is clear 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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

in the dentate gyrus (van Praag, Christie et al. 1999). Exercise also has anxiolytic and antidepressant effects by increasing brain-derived neurotrophic factor (BDNF) levels (Duman, Schlesinger et al. 2008). BDNF is a protein that promotes neural survival, growth, and differentiation of new neurons and synapses. It is clear that voluntary exercise increases BDNF (Kim, Lee et al. 2001, Russo-Neustadt, Ha et al. 2001, Tong, Shen et al. 2001). BDNF and other proteins in the BDNF pathway are thought to be the major contributors for enhancing memory (Bekinschtein, Cammarota et al. 2008) and increasing LTP (Lu, Christian et al. 2008, Martinez-Moreno, Rodriguez-Duran et al. 2011). Recent research illustrates that BDNF activates mTOR, which regulates the expression of AMPA receptors to increase memory and LTP (Slipczuk, Bekinschtein et al. 2009). Studies showed chronic stress downregulated BDNF (Zagaar, Dao et al. 2013) and upregulated interneuron activity (Schoenfeld, Rada et al. 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 and 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.

MATERIALS AND METHODS

Treatment groups

Adult male C57BL/6 mice were used in this study. Mice were housed in approved conditions with a 12-hour 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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

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.

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 kilometers per day, which was slightly higher, but still comparable to the average of approximately 4.5 kilometers per day (4.1 & 4.8 km/day) others saw using the same strain of mice (van Praag, Christie et al. 1999, Marlatt, Potter et al. 2012). ENS and EWS mice ran at least an average of 2 kilometers 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 et al. 1981), with some slight modifications to the stressors and shorter stress duration. Another group (DeVallance, Riggs 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 included: a 5-minute cold (2-8° C) water swim on day one, a 30-minute elevated platform stress on day two, and a 60-minute restraint with a 1 second 10 mA tail shock once per minute on day three. During shock stressing, mice were put in restraining plastic tubes. Copper electrodes, in the form of toothless alligator clips, were clamped on the taped down mice tails. Electrode gel was applied before electrode attachment. Mice were sacrificed on the third day of stress 1-2 hours following the completion of the last stress. Each stressor was only performed once.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

SWS and EWS mice used for behavioral and RT-qPCR experiments were stressed once per day beginning at the start of behavioral testing, which occurred immediately before performing in the radial arm maze and continued with one daily stress to create chronic intermittent stress until they were sacrificed for RT-qPCR experiments six weeks later. For ENS/EWS groups, exercise began 30 days prior to use for memory behavior testing. It is important to note that tail shock stress was not used with these mice, instead a 30 minute tube restraint with no shock was used. Stressing started on day 1 of the radial arm maze assay. During the radial arm maze, stressors were alternated Monday through Friday between the 5-minute cold-water swim, 30-minute elevated platform stress, or 30-minute restraining stress in a tube for the entire 6 weeks of the behavioral experiments. Mice were not stressed on the weekends during the duration of the radial arm maze assay.

Field Slice Electrophysiology

The physiology methods used were similar to those described previously by our lab (Bennion, Jensen et al. 2011). All mice were anesthetized with isoflurane using a vapomatic chamber and decapitated. After decapitation, the brains were removed rapidly and placed in ice-cold, oxygenated artificial cerebral spinal fluid (ACSF). Next, 400 μm coronal slices were cut using a vibratome and then transferred to a holding chamber containing oxygenated ACSF at room temperature.

Following an interval of at least 1 hour, slices were transferred to a submerged recording chamber and perfused with oxygenated ACSF at a temperature on average of 30° C. Slices were continuously perfused with ACSF at a flow rate of 2-3 ml/min. A bipolar stainless steel stimulating electrode was placed in the stratum radiatum in the CA1 to stimulate the Schaffer Collateral pathway at 8-50 μA for 100 μsec once every ten seconds in order to sample at 0.1Hz. Stimulation intensity was adjusted to elicit an excitatory post-synaptic potential (EPSP) of ~0.8 mV at the beginning of each experiment. Recordings were performed in current clamp mode to measure excitatory postsynaptic potentials using an Axopatch 200B or MultiClamp 700B amplifier (Molecular Devices). Field recording electrodes were borosilicate glass patch pipettes (2-3 MΩ) filled with 1 M NaCl. Theta burst stimulation was used to invoke LTP, which consisted of two bursts with each burst consisting of 10 sets of 5 pulses, each pulse lasting 100 μsec and applied at 100 Hz with 200 ms between each set. There was a 20 sec delay between the two bursts. (S)-3,5-dihydroxyphenylglycine (DHPG; Tocris), was used to induce long-term depression (LTD).

For analysis, the value of the EPSPs slopes was calculated using pClamp10.4 Clampfit software (Molecular Devices). EPSPs initially measured every 10 seconds were averaged into 1-minute intervals. EPSP normalized slope values were compared for significance 20-25 minutes post-theta burst stimulus. The time points of acute depression at 26-30 minutes as well as long-term depression at 41-45 minutes and 81-85 minutes were analyzed from the DHPG experiments for statistical significance between the groups. For the paired pulse ratios, the last 5 minutes of baseline and 30-35 minutes for post-conditioning were used. Only one experiment was performed per slice, with the reported n-value being the number of slices not the number of animals and one to three slices were used per mouse.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Microsoft Excel and Origin (North Hampton, MA) software were used to organize, average, graph, and perform statistical analysis on the data.

Solutions and Chemicals

Artificial cerebrospinal fluid (ACSF; in mM): NaCl, 119; $NaHCO_3$, 26; KCl, 2.5; NaH_2PO_4, 1.0; $CaCl_2$, 2.5; $MgSO_4$, 0.6; glucose, 11; saturated with 95% O_2, 5% CO_2 (pH 7.4). Salts were purchased from Sigma-Aldrich, Mallinkrodt-Baker, or Fisher Scientific and dissolved in double distilled water.

Radial arm maze

An eight arm radial maze was constructed out of 7 mm thick opaque, white, plastic. The dimensions of the maze were a 27 inch (685.8 mm) diameter, 3.5 inch (88.9 mm) arm width, 6.5 inch (165.1 mm) arm height, 9 inch (228.6 mm) arm length, and 1 inch (25.4 mm) hole diameter at the end of each arm for food baiting.

Our protocol was designed similarly to previous researchers that used the radial arm maze, showing that food deprivation was one of the most common methods for encouraging rodents to explore the maze (Hodges 1996). Mice were given food ad libitum until testing was initiated and then food was restricted to 4 hours a day starting at approximately 6 pm Monday through Thursday. Feeding restrictions ensured that the mice would search the maze for food. The mice had ad libitum access to food Friday night through Sunday night since no testing was done over the weekends. Mice were weighed at the beginning and end of each week during the testing period to verify they maintained a healthy body weight. If mice lost more than 15% of their body weight or seemed lethargic, they were allotted additional time to feed. Only a few mice needed extra time to feed, which would only occur within the first two weeks.

Testing consisted of 5 trials per day, 5 days a week for 6 weeks. Week 1 was an acclimation week where all eight arms were baited with small pieces of cheese that the mice could eat quickly without biasing the time each trial took to complete. For weeks 2-6, only four arms were baited instead of eight and those same four arms were baited for every trial. A trial was considered complete after the mouse found all four bates or if the timer reached 5 minutes.

The data recorded by researchers from each trial were the time, the number of reference (long-term) memory errors, and the working (short-term) memory errors. A reference memory error was recorded when the mouse would go down an arm that never had food in it. A working memory error was recorded when the mouse would go down an arm it had already been down during the same trial. Videos of each trial were recorded using a GoPro Hero 3 camera and analyzed using ANY-maze software (Stoelting Co.; Wood Dale, IL; version 4.99m).

Reverse Transcriptase Quantitative PCR (RT-qPCR)

DNA and RNA sequences for each gene were downloaded from PubMed and used for primer design. Exon sequences from each gene were selected and compared to the RNA sequence to ensure that the intended sequence did not undergo alternative splicing. Primers

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

were designed using Primer Express (Applied BioSystems), such that when possible primer sets (forward and reverse primers) would lie on either side of an exon-intron boundary. This prevented amplification of genomic DNA. However, there were five targets whose forward and reverse primers were on the same exon: BDNF, EIF4BP1, DRD1, DRD5, and 18S. Table 1 contains all the sequences and provides information about which exons the primers bind to, along with where they are located in the RNA sequence. We used ensembl.org and Primer xpress to determine the exons where the primers bound. The BDNF primers were both located on exon 9, which is the common coding exon (Aid, Kazantseva et al. 2007). This is because exon 9 is included in all BDNF transcripts while other exons may or may not be included. This was done to ensure we were examining all potential BDNF transcripts and not missing some BDNF variants. DRD5 has only one exon, and DRD1 and EIF4BP1 have one major exon and other smaller exons that proved more difficult for good functioning primers across exon-intron boundaries. 18S was designed for a site that is specific for both rat and mouse, and keeps our control gene consistent in both. The design parameters were defined with an optimal annealing temperature range from 55-59° C. The range of GC content was set for 40-60%, with primer lengths ranging from 18-30 base pairs. The range for amplicon length was set for 100-150 base pairs. ThermoFisher Scientific/Life Technologies manufactured final primer set sequences. All primers, including 18s, were efficiency tested using serial dilutions of whole mouse brain cDNA template, and adjusted to be at 90-95% efficiency. The 18S primers used had been previously designed by our laboratory (Merrill, McNeil et al. 2012).

The entire hippocampi from both hemispheres were removed from the brain and the dorsal portions were separated from the rest of the hippocampi. Dorsal hippocampal tissue was homogenized and mRNA extracted using TriZOL (Invitrogen; Carlsbad, CA) as per manufacturer instructions. After extraction, samples were placed into a reverse transcription mixture containing iScript reaction mix and reverse transcriptase (BioRad). This mixture was cycled in a C1000 Thermocycler (BioRad) according to the iScript reaction protocol, which was 25° C for 8 minutes, 42° C for 60 minutes, and then 70° C for 15 minutes. The cDNA was stored at 4° C.

For the quantitative PCR procedure, cDNA from the iScript reverse transcriptase reaction described above was used. Each target was run individually in triplicates (triplicate values were averaged together for analysis). Each sample was run on a CFX96 qPCR machine (BioRad) using Sso Fast EvaGreen Supermix (BioRad) according to the following protocol: 95° C hot start for 3 minutes, followed by 50 cycles of 95° C for 15 seconds, 57° C for 20 seconds, and 72° C for 25 seconds. Amplification was measured using FAM (excitation at 488 nm, absorption at 494 nm, and emission at 518 nm) by detecting increased relative fluorescence during each cycle. A cycle threshold (Ct) value was assigned to each target using BioRad CFX Manager software. The 18S ribosomal gene was the housekeeping control gene used for expression comparison. Samples from each target were also examined using 4% agarose gel electrophoresis to verify amplicon size (Figure 4C). Relative quantities of gene expression were determined using Microsoft Excel and the Livak and Schmittgen delta delta Ct/Cq method (Livak and Schmittgen 2001). For each individual target, all four mice groups were run on the same plate for more accurate comparison. If a sample failed to have the majority of three replicates show up, then we did not use it and thus there are

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

varying sample sizes for the different targets. Samples were rerun if pipetting errors occurred during the first run.

Corticosterone Competitive ELISA

To determine differences in corticosterone levels, a corticosterone ELISA kit was purchased from Enzo Life Sciences. Whole blood was collected between 8 – 11 AM from the electrophysiology mice subsequent to isoflurane anesthesia and decapitation. For each sample, 1 mL of blood was added to a plastic tube containing 100 μL of heparin to prevent clotting. The blood samples were stored at -80° C until the ELISA was performed. Blood samples were brought to room temperature and processed according to the manufacturers specifications. The plate was read at a 405 nm optical density on a BioTek Synergy HT plate reader using the BioTek Gen5 1.11 software. Triplicates were made of all samples, including controls. Control values were then plotted into a logarithmic graph using Microsoft Excel. The equation obtained from the best-fit line in the graph was used to determine the corticosterone concentrations of the whole blood samples from the four treatment groups.

Statistical Analysis

The results in the graphs are presented as mean ± S.E. The n values for all the experiments are reported in the figure captions. The LTP, RT-qPCR, and ELISA data were analyzed using a two-way (2×2) ANOVA and one-way ANOVA with Bonferroni's post-hoc test. For the LTP data, since our major comparison was between the EWS group to all other 3 groups, it was appropriate to also use a two-tailed unequal variance Student's T-test for pairwise comparisons between groups to compare if the means between EWS and all other groups was significantly different. The radial arm maze data as a whole were analyzed using a mixed model that is similar to a regression, but incorporated a random effect since not all observations were independent since there were multiple data points for each mouse and post-hoc Tukey's tests were also used after confirmation of normal distribution. The 2×2 ANOVAs were also performed. When analyzing just the second week of the radial arm maze data, one-way ANOVAs were used for a comparison of the means between the test groups. Paired pulse ratios were analyzed with Wilcoxon rank sum tests to compare baseline or post-conditioning within a group. A one-way ANOVA was used to compare the baseline paired pulse ratios between all of the groups. A p value of <0.05 was considered statistically significant for all tests performed. A p value between 0.06 and 0.15 was considered a trend.

RESULTS

First, it was necessary to demonstrate that our stress and exercise models could effectively alter LTP as noted by others. We used a form of chronic stress, lasting for 3 days with a different stress each day. We waited at least an hour after stress before sacrificing the mouse to perform the electrophysiology experiments. As others have shown differences in LTP between control, exercise, and stress, our primary goal was to note whether exercise could mitigate the negative effects of stress (i.e. is the exercise with stress LTP significantly bigger than stress alone). The sedentary with stress (SWS) group experienced smaller (144.3±4.9%) LTP compared to the sedentary no stress group (SNS; 171.6±4.7%; Figure 1A). In addition, as expected, the exercise no stress (ENS) group experienced LTP

(192±7.3%) that was larger than the SWS group, demonstrating that our exercise protocol was sufficient to induces changes in LTP mechanisms. Important to this study, exercise with stress (EWS) mice, the novel group in this experiment, had significantly greater ($p<0.05$; 165.8±3.7%) LTP than SWS mice and was extremely similar to SNS mice, but still significantly smaller ($p<0.05$) than ENS LTP (Figure 1A). A one-way ANOVA with a Bonferroni post hoc test was performed and showed significant differences between the groups ($F_{3, 56}$=5.471, p=0.002). To further examine interactions between stress and exercise, we also performed analysis using a two-way (2×2) ANOVA and demonstrated a significant effect of exercised and non-exercised groups ($F_{1, 56}$ =3.804, p=0.05) and between stressed and non-stressed groups ($F_{1, 56}$ =9.403, p=0.003), again confirming that stress and/or exercise have a significant effect on LTP. The average daily running distances between ENS (6.31 +/- 0.52 km) and EWS (4.40 +/- 0.53) mice was significant (T-test p=0.01). Collectively, this demonstrates that not only do our exercise and stress methodology have effects on plasticity, but also that exercise significantly mitigated the negative effect of stress on LTP.

We used a linear regression to examine whether there was a relationship between distance mice ran and percentage of LTP achieved in both the EWS and ENS groups (i.e. does the amount of exercise correlate to the amount of LTP), but there was not a correlation. This suggests that there is a ceiling on the effect exercise can have on LTP after a minimum amount of exercise.

Considering that stress may also alter another form of synaptic plasticity, long-term depression (LTD), we examined whether there was stress and exercise induced changes in metabotropic glutamate receptor (mGluR)-dependent LTD (Figure 1B). DHPG, a type 1 mGluR agonist, was applied to the bath to induce LTD. No significant changes to mGluR-LTD were mediated by the stress or exercise protocols used in this study.

Paired pulse ratios from LTP experiments were examined to determine if exercise or stress were having a pre-synaptic effect on plasticity (Figure 2). Wilcoxon rank sum tests showed no significant difference between baseline and post-conditioning ratios within the groups. A one-way ANOVA showed no significant differences between the paired pulse ratios of the baselines or post-conditioning between the four groups.

A competitive ELISA was used to determine whole blood concentrations of the stress hormone corticosterone among the groups after three days of stress. Significant differences were observed in corticosterone concentrations among the four groups (Figure 3). The ENS group had significantly less ($p<0.05$) corticosterone in their whole blood compared to the SWS group. The SWS group also had significantly more ($p<0.05$) corticosterone than the SNS group. The 2×2 ANOVA ($F_{1, 27}$ =15.797, p=0.0004) also revealed that there was a significant difference in corticosterone between stressed and not stressed groups. These data demonstrate our stress methods were successful in evoking physiological changes in corticosterone, and interestingly it is suggestive that corticosterone alone cannot account for differences in plasticity.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Since a difference in plasticity was noted, we wanted to examine whether behavioral changes could be seen in these four groups using the memory assay, the radial arm maze. However, one issue was that in order accomplish this we would have to increase the duration of our stress model to the 6 weeks required to perform the radial arm maze in mice. While this is a different stress and thus not comparable to plasticity data, we still thought it important to examine potential changes between all the groups behaviorally as no one had compared exercise with stress to stress alone in the radial arm maze to our knowledge. Therefore, the physiology and behavioral data are not directly comparable, but provide mutual support for one another by examining the behavioral effect of stressed mice that concurrently exercise versus those that do not.

The radial arm maze tests the spatial short-term and long-term memory between the treatment groups. Reference (long-term) memory errors, working memory (short-term) errors, total distance traveled per trial, and total time to complete each trial were measured (Figure 4). The chronic stress protocol for these mice lasted the duration of the behavioral experiments as well as the running. The behavioral ENS and EWS data were extremely close to each other and the SNS and SWS data were very similar to each other as well. The exercise groups made significantly fewer ($F_{1, 40}$=2.901, p=0.04) reference memory errors than the sedentary groups during the second week of the maze. Trends (p=0.06-0.15) were also observed in the working memory errors between the exercise and sedentary groups, with exercise groups making less errors than sedentary groups. In conclusion, exercise increased learning and memory capabilities in the observed mice during the second week of testing. A 2×2 ANOVA did not show any significance between the relation of stress, exercise, or stress and exercise between the groups.

Next, we examined molecular adaptations that could potentially be involved in the molecular mechanisms of stress and exercise effects on the brain. To do this, RT-qPCR was used to study specific mRNA expression levels in the dorsal hippocampus. Primers were designed for fifteen different targets. Targets included elements involved in the exercise/BDNF pathway, which were BDNF, mTOR, TrkB receptor, EIF4EBP1, and p70s6K. TrkB is the receptor that binds BDNF. EIF4EBP1, mTOR, and p70s6K are downstream targets of the BDNF pathway. Stress targets included glucocorticoid and mineralocorticoid receptors, which bind corticosterone. Dopamine 1 and dopamine 5 receptors were also examined since other researchers have demonstrated that these play a role in the BDNF pathway in the pre frontal cortex (Perreault, Jones-Tabah et al. 2013) and can modulate LTP and LTD (Lemon and Manahan-Vaughan 2006). The N-methyl-D-aspartate (NMDA) receptor plays a vital role in synaptic plasticity, especially in LTP, so the expression levels of the different NMDA receptor subunits and the NMDA anchoring protein postsynaptic density protein 95 (PSD-95) were examined. Lastly, the A1 and A2 subunits of 5’ adenosine monophosphate-activated protein kinase (AMPK) were inspected to discover whether AMPK expression in the hippocampus increased with voluntary running exercise. The reason why we chose to look at AMPK is because other researchers have discovered increases in AMPK expression in skeletal muscle and the brain due to exercise and AMPK is thought to be crucial to energy metabolism (Pedersen 2013); AMPK is also thought to affect the BDNF pathway in the hippocampus (Huang, Cao et al. 2015).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

The dorsal portion of the hippocampus was separated from the rest of the brain before isolating mRNA. The mice used for RT-qPCR were the same ones that performed in the radial arm maze in order to reduce animal numbers used. The whole hippocampus was not used because it is known that the dorsal and ventral portions of the hippocampus have differing response to stress (Fanselow and Dong 2010). As a result, the PCR data cannot be directly correlated to the behavioral data of intact mice. As an important note, parallel processing of all four groups avoided aberrant variations between mRNA isolation or PCR runs. The dorsal hippocampus had several targets that showed significant differences from one-way ANOVAs ($p<0.05$) in expression level among the treatment groups. The targets that had significant differences in expression levels were BDNF ($F_{3, 24}=4.918$, $p=0.008$), TrkB receptor ($F_{3, 27}=3.319$, $p=0.035$), glucocorticoid receptor ($F_{3, 22}=6.404$, $p=0.003$), mineralocorticoid receptor ($F_{3, 29}=7.542$, $p=0.001$), and dopamine 5 receptor ($F_{3, 26}=14.527$, $p=0.0004$) (Figure 5A). In general, the ENS mice displayed trends of increased expression of almost all the targets examined with some being significant when compared to one or more of the other three groups, indicating that exercise alone has the greatest effect for increasing mRNA expression levels of the targets we studied.

Two-way (2×2) ANOVAs were also performed on each of the targets. This analysis demonstrated that other targets besides the ones that were significant in the one way ANOVAs had differences in expression levels among the treatments. The three targets that showed the most significant differences between the treatments in the 2×2 ANOVA were the dopamine 5 receptor, glucocorticoid receptor, and mineralocorticoid receptor. Dopamine 5 receptor showed a significant difference between stress ($F_{1, 30}=35.955$, $p=0.0004$), exercise ($F_{1, 30}=5.316$, $p=0.029$), and a trend ($F_{1, 30}=3.669$, $p=0.066$) for exercise and stress combined. Glucocorticoid receptor had a significant difference between stress ($F_{1, 26}=14.739$, $p=0.001$) and stress and exercise combined ($F_{1, 26}=5.347$, $p=0.03$). Mineralocorticoid receptor showed a significant change between exercise ($F_{1, 33}=5.263$, $p=0.029$), stress ($F_{1, 33}=13.635$, $p=0.001$), and exercise and stress combined ($F_{1, 33}=4.023$, $p=0.05$). Other targets also had some significant differences in the 2×2 ANOVA, which was due to stress groups having decreased expression: BDNF ($F_{1, 28}=8.177$, $p=0.009$), EIF4EBP1 ($F_{1, 24}=6.345$, $p=0.02$), NMDA1 ($F_{1, 29}=4.863$, $p=0.037$), NMDA2A ($F_{1, 33}=6.890$, $p=0.014$), p70s6K ($F_{1, 28}=7.727$, $p=0.01$), and PSD95 ($F_{1, 29}=6.566$, $p=0.017$). In the 2×2 ANOVA, TrkB was the only target besides the dopamine 5 receptor and mineralocorticoid receptor that showed significant changes ($F_{1, 31}=5.978$, $p=0.021$) due to exercise, because both exercise groups had greater expression than both sedentary groups.

DISCUSSION

Overall, the data demonstrate that exercise mitigates some of the negative effects chronic stress has on LTP and memory. The novel part of our electrophysiology data revealed that if exercise and stress occur concurrently, the exercise is able to combat the stress so that the dorsal hippocampus can experience normal levels of LTP. Our ELISA data illustrate that corticosterone is less likely playing a role in the effects on LTP that we observed among the four treatment groups and suggest a neuroprotective effect from exercise by another target. Our radial arm maze data showed that exercise was having positive effects on spatial memory when initially learning a new task. The RT-qPCR data identified some potential

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

targets for future examination that exercise, stress or a combination of the two can modify. While the stress paradigms differed between our LTP, behavior and PCR experiments (i.e. due to different stress duration, variation in animal age upon experimentation, etc.), they collectively demonstrate that exercise when occurring concurrently with stress can alter the outcomes of stress alone.

The novel finding of our study is the fact that exercise was able to mitigate the negative effects stress has on dorsal CA1 LTP, bringing the animal back to normal (control) levels of LTP. In a somewhat related study, another group examining ventral hippocampus demonstrated that exercise alleviated chronic sleep deprivation decreases in LTP (Zagaar, Dao et al. 2013). Our data in combination with their data show that exercise is able to make positive changes in multiple portions of the hippocampus even while experiencing stress. Our SWS electrophysiology data support that chronic stress reduces CA1 LTP particularly in the dorsal portion, which adds to LTP data obtained by other groups in the CA1 (Artola, von Frijtag et al. 2006, Zagaar, Dao et al. 2013) and dentate gyrus (Alfarez, Joels et al. 2003) showing that stress alone reduces LTP levels. We also saw that exercise enhances dorsal CA1 LTP, supporting what others have shown indicating that exercise alone increases LTP in the hippocampus, as occurs in the dentate gyrus (van Praag, Christie et al. 1999). Based on the previous published literature and our electrophysiology data, the ideal situation for improving hippocampal LTP and thereby learning and memory would be to experience no stress and to exercise. However, since stress is unavoidable throughout life, knowing that exercise can likely combat the deleterious effects stress has on dorsal CA1 hippocampal LTP is a significant finding. Since none of the paired pulse data had any significant changes, this suggests that the differences we observe in LTP due to exercise and/or stress are more than likely being caused by an alteration to a standard postsynaptic CA1 LTP mechanism.

Our ELISA data demonstrate that exercise could be having a neuroprotective effect on the dorsal hippocampus, which has been suggested by others (Wiegert, Pu et al. 2005, Pu, Krugers et al. 2007, Martin, Henley et al. 2009, Kvarta, Bradbrook et al. 2015, Xiong, Cassé et al. 2016), despite increased levels of corticosterone. Exercise mitigation of stress effect is a mechanism independent of corticosterone as there were no differences in corticosterone between EWS and SWS mice even though there was a difference between their LTP. Not seeing a difference between EWS and SWS corticosterone levels supports what has been reported by Campeau et al. where exercise does not have an effect on the HPA response if severe stressors are used Campeau, Nyhuis et al. 2010). Our ELISA data are in line with other studies that show that chronic stress increases glucocorticoid release and suppresses LTP in the hippocampus (McEwen and Sapolsky 1995, Magarinos, Verdugo et al. 1997, Pu, Krugers et al. 2007, Martin, Henley et al. 2009) and that chronic increases of glucocorticoids can damage the hippocampus and hinder its functionality (Wiegert, Pu et al. 2005, Conrad 2008), supporting our methodology. The lower levels of corticosterone in ENS mice could be why we saw elevated mRNA expression of mineralocorticoid and glucocorticoid receptors in this group, but there are also other glucocorticoids and stress hormones that we did not measure that could be causing the increased stress receptor mRNA expression levels observed in the ENS mice.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

While no changes in LTD were observed among the groups, other studies found alterations in hippocampal LTD between acute stressed rats and control rats using DHPG, and that higher levels of corticosterone contributed to the change in mGluR-dependent LTD (Chaouloff, Hemar et al. 2007, Chaouloff, Hemar et al. 2008, Pignatelli, Vollmayr et al. 2013). The difference between our study and theirs could be due to differences in stress techniques because our model was chronic stress and not acute stress like the Chaouloff group. Furthermore, the same pathways that are altered by exercise may not affect this type of LTD. Therefore, it cannot be ruled out that exercise and the type of stressors we used could have an effect on other forms of LTD, such as NMDA-dependent LTD, since other groups (Artola, von Frijtag et al. 2006, Martin, Henley et al. 2009) have shown that chronic stress can alter AMPA and NMDA dependent LTD.

To examine the effects exercise and stress have on spatial memory, we used the radial arm maze since it is commonly used in the literature to test spatial memory. While these experiments are not directly comparable to the LTP experiments, our behavior data support the notion that exercise rescues some of the changes caused by chronic stress. In relation to reference memory errors, the EWS mice performed as well as the ENS mice, and both the exercise groups performed better than the sedentary groups of mice showing that exercise improves learning and memory of a new task. Others have shown that just being sedentary reduces brain functionality (Vaynman and Gomez-Pinilla 2006), which is probably why both sedentary groups performed similarly in our maze. Exercise enhanced the ability of the mice to learn the maze, since exercise mice made significantly fewer reference memory errors in their second week in the radial arm maze. This is similar to data obtained by some researchers who noted memory assay changes using the Morris water maze in either stress or exercise models (van Praag, Christie et al. 1999, Marlatt, Potter et al. 2012, Kim and Leem 2016), however in contrast, others noted no changes in Morris water maze performance in exercised, stressed, and control female mice (Marlatt, Potter et al. 2012). Kim et al compared male chronic stressed with exercise mice to sedentary chronic stressed mice using the Morris water maze and saw that the exercise with stress mice traveled less distance and found the platform faster than the sedentary with stress mice. Though most publications used the Morris water maze to test spatial memory, we chose not to because of the forced swim aspect of this assay, which is why we chose the radial arm maze instead. Also, while other researchers have demonstrated stress (He, Zhang et al. 2008) or exercise (Berchtold, Castello et al. 2010) induced effects using the radial arm maze, as far as we know, we are the only group that has performed the radial arm maze on male mice that were stressed and exercised concurrently. Particularly important to note in this study is that exercise, when occurring with stress, eliminated the increased reference memory errors at the beginning of the assay associated with stress alone. This data indicate that exercise can help expedite learning new tasks, regardless of being stressed and fasted. It is important to note that while we had to house mice individually in order to track the exercise amount of each animal accurately, and therefore mice needed to be housed individually to reduce variables in our study, individual housing can create stress. Also, it could be suggested that the exercised mice had enrichment with the running cage. Therefore, our data must be considered with these caveats and that control mice may have had added stress, which may have reduced our overall stress effect compared to others. Despite this added stress to all the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

groups of mice, we still saw differences in the radial arm maze, though not as dramatic as other studies that used Morris water maze.

In addition, it was anticipated that RT-qPCR would identify definitive targets that were altered in exercise, stress, or the combination of the two. Some potential candidates that may play significant roles in molecular changes in the brain due to exercise and stress are dopamine 5, glucocorticoid, and mineralocorticoid receptors since they showed the most significant changes between ENS and the other treatment groups in one-way ANOVAs, and all showed differences when stress and exercise were both examined using 2×2 ANOVAs. The dopamine 5 receptor was the only target that showed a significant difference between the SNS and SWS groups, with the SWS group having lower mRNA levels. Dopamine 5 receptors are coupled to adenylyl cyclase and alter the BDNF pathway in the prefrontal cortex (Perreault, Jones-Tabah et al. 2013) and could be doing something similar in the dorsal hippocampus. As exercise increases dopamine 5 receptor mRNA expression and stress decreases it, dopamine 5 receptor is an interesting target to examine the changes due to exercise. Regarding dopamine 1 receptor mRNA levels, we did not see significant changes in expression, which correlates to another study examining the effects of exercise in the caudate putamen of rats (Rabelo, Horta et al. 2017). In summary, as far as we know, the discoveries we have shown on how the dopamine 5 receptor dorsal hippocampal mRNA expression changes due to exercise and stress are novel and have not been shown before, which adds to the possibility of dopamine 5 receptor playing a role in memory changes.

In general, ENS mice had enhanced expression of several targets that were not elevated in the other three groups. The BDNF pathway was indeed more activated in the dorsal hippocampus in the ENS group, which is similar to the western blot data obtained by another group (Fang, Lee et al. 2013). The BDNF pathway is a likely candidate for the positive hippocampal changes in all the targets examined due to exercise since BDNF was expressed more in our RT-qPCR data. The TrkB receptor was the only target that had higher expression levels in our data in both exercise groups compared to both sedentary groups, which indicates that the BDNF pathway activity could be helping exercise mitigate the negative consequences of stress. These observations support what has been shown by others, which is that the BDNF pathway is more activated due to exercise and can have many positive effects on the brain by acting on other pathways and receptors (Cunha, Brambilla et al. 2010, Fang, Lee et al. 2013) and that BDNF expression levels are elevated (Vaynman and Gomez-Pinilla 2006, Duman, Schlesinger et al. 2008) due to exercise. Specifically one group showed that the BDNF pathway was interacting with increased AMPK levels to cause the positive changes to learning and memory (Marlatt, Potter et al. 2012) and we also saw a trend ($p<0.15$) of increased AMPK A1 subunit expression in our ENS mice compared to the stressed and sedentary groups. It could also be presumed that the NMDA receptor subunits 2A or 1, and the NMDA receptor anchoring protein PSD-95 could also be involved in the changes in spatial memory caused by ENS, since we did show a trend ($p<0.15$) of increased expression in those plasticity elements in our ENS mice. Two other groups of researchers also showed increases in PSD-95 protein in exercised rodent hippocampi compared to sedentary (Fang, Lee et al. 2013, Kim and Leem 2016). The increased expression levels of these proteins involved in synaptic plasticity could be affected by the increased activation of the BDNF pathway, which has been suggested by other researchers (Tong, Shen et al. 2001,

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Cotman and Berchtold 2002, Gomez-Pinilla, Ying et al. 2002, Cotman, Berchtold et al. 2007).

As a note, while it has been shown that EWS mice will voluntarily run less than ENS mice (DeVallance, Riggs et al. 2017), we did not have a significant difference (p=0.21) in our mice used for behavior/PCR, only in our electrophysiology exercise mice (p=0.01). Therefore, decreased exercise may not account for mRNA expression differences between EWS and ENS. A possible reason why we did not see a significant difference, only a partial difference could be because all the behavior/PCR mice underwent food deprivation, which is a stress, and could have caused the ENS mice to not run as much. Collectively, the data still suggest there is probably more than one molecular pathway and/or many receptors involved in any effect exercise has on mitigating stress changes. However, it is still uncertain how many molecular targets are specifically interacting between exercise and stress when stress and exercise are experienced concurrently in the same animal. One last caveat to consider is that mRNA levels do not always correlate to protein expression or activation of signaling pathways involved; mRNA expression means that a certain gene was transcribed more, but this does not indicate simultaneous increased translation. Therefore, we cannot exclude any of the pathways we examined based on PCR data for their potential involvement.

CONCLUSIONS

Overall, our data does suggest, even with the differing stress protocols, that exercise may be a legitimate and cost-effective treatment or adjunctive therapy for mitigating the negative effects stress has on dorsal CA1 hippocampal LTP and spatial memory function. Exercise can improve spatial memory and hippocampal LTP when compared to being sedentary. Therefore, our study suggests understanding the interactions between exercise and stress when co-occurring is an important consideration.

Acknowledgments

The authors would like to thank David Thomson for his assistance and for loaning us the running cages used to collect our data. We would also like to thank Jonathan Wisco for use of the AnyMaze software, David Vogelsang for building our radial arm maze, and Kaylea Drake for helping with the statistical analysis on the data we collected. Thank you to other members of the Jeffrey Edwards laboratory for assistance in collecting data for this project.

FUNDING

National Institute of Health Grant R15NS078645 supported this work. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Neurological Disorders and Stroke or the National Institutes of Health.

Mentoring Environment Grants from Brigham Young University also funded this research.

References

Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T. Mouse and rat BDNF gene structure and expression revisited. J Neurosci Res. 2007; 85(3):525–535. [PubMed: 17149751]

Alfarez DN, Joels M, Krugers HJ. Chronic unpredictable stress impairs long-term potentiation in rat hippocampal CA1 area and dentate gyrus in vitro. European Journal of Neuroscience. 2003; 17(9): 1928–1934. [PubMed: 12752792]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Artola A, von Frijtag JC, Fermont PCJ, Gispen WH, Schrama LH, Kamal A, Spruijt BM. Long-lasting modulation of the induction of LTD and LTP in rat hippocampal CA1 by behavioural stress and environmental enrichment. Eur J Neurosci. 2006; 23(1):261–272. [PubMed: 16420435]

Baker KB, Kim JJ. Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. Learning & Memory. 2002; 9(2):58–65. [PubMed: 11992016]

Bekinschtein P, Cammarota M, Katche C, Slipczuk L, Rossato JI, Goldin A, Izquierdo I, Medina JH. BDNF is essential to promote persistence of long-term memory storage. Proc Natl Acad Sci U S A. 2008; 105(7):2711–2716. [PubMed: 18263738]

Bennion D, Jensen T, Walther C, Hamblin J, Wallmann A, Couch J, Blickenstaff J, Castle M, Dean L, Beckstead S, Merrill C, Muir C, St Pierre T, Williams B, Daniel S, Edwards JG. Transient receptor potential vanilloid 1 agonists modulate hippocampal CA1 LTP via the GABAergic system. Neuropharmacology. 2011; 61(4):730–738. [PubMed: 21645527]

Berchtold NC, Castello N, Cotman CW. Exercise and time-dependent benefits to learning and memory. Neuroscience. 2010; 167(3):588–597. [PubMed: 20219647]

Blank T, Nijholt I, Eckart K, Spiess J. Priming of long-term potentiation in mouse hippocampus by corticotropin-releasing factor and acute stress: Implications for hippocampus-dependent learning. Journal of Neuroscience. 2002; 22(9):3788–3794. [PubMed: 11978854]

Campeau S, Nyhuis TJ, Sasse SK, Kryskow EM, Herlihy L, Masini CV, Babb JA, Greenwood BN, Fleshner M, Day HE. Hypothalamic pituitary adrenal axis responses to low-intensity stressors are reduced after voluntary wheel running in rats. J Neuroendocrinol. 2010; 22(8):872–888. [PubMed: 20406350]

Cazakoff BN, Howland JG. Acute stress disrupts paired pulse facilitation and long-term potentiation in rat dorsal hippocampus through activation of glucocorticoid receptors. Hippocampus. 2010; 20(12):1327–1331. [PubMed: 20043285]

Chaouloff F, Hemar A, Manzoni O. Acute stress facilitates hippocampal CA1 metabotropic glutamate receptor-dependent long-term depression. J Neurosci. 2007; 27(27):7130–7135. [PubMed: 17611266]

Chaouloff F, Hemar A, Manzoni O. Local facilitation of hippocampal metabotropic glutamate receptor-dependent long-term depression by corticosterone and dexamethasone. Psychoneuroendocrinology. 2008; 33(5):686–691. [PubMed: 18321654]

Conrad CD. Chronic Stress-Induced Hippocampal Vulnerability: The Glucocorticoid Vulnerability Hypothesis. Rev Neurosci. 2008; 19(6):395–411. [PubMed: 19317179]

Cotman CW, Berchtold NC. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci. 2002; 25(6):295–301. [PubMed: 12086747]

Cotman CW, Berchtold NC, Christie LA. Exercise builds brain health: key roles of growth factor cascades and inflammation. Trends in Neurosciences. 2007; 30(9):464–472. [PubMed: 17765329]

Cunha C, Brambilla R, Thomas KL. A Simple Role for BDNF in Learning and Memory? Front Mol Neurosci. 2010; 3

DeVallance E, Riggs D, Jackson B, Parkulo T, Zaslau S, Chantler PD, Olfert IM, Bryner RW. Effect of chronic stress on running wheel activity in mice. PLoS ONE. 2017; 12(9):e0184829. [PubMed: 28926614]

Duman CH, Schlesinger L, Russell DS, Duman RS. Voluntary Exercise Produces Antidepressant and Anxiolytic Behavioral Effects in Mice. Brain Res. 2008; 1199:148–158. [PubMed: 18267317]

Fang ZH, Lee CH, Seo MK, Cho H, Lee JG, Lee BJ, Park SW, Kim YH. Effect of treadmill exercise on the BDNF-mediated pathway in the hippocampus of stressed rats. Neuroscience Research. 2013; 76(4):187–194. [PubMed: 23665137]

Fanselow MS, Dong HW. Are the dorsal and ventral hippocampus functionally distinct structures? Neuron. 2010; 65(1):7–19. [PubMed: 20152109]

Foy MR, Stanton ME, Levine S, Thompson RF. Behavioral stress impairs long-term potentiation in rodent hippocampus. Behavioral and Neural Biology. 1987; 48(1):138–149. [PubMed: 2820370]

Garcia R, Musleh W, Tocco G, Thompson RF, Baudry M. Time-dependent blockade of STP and LTP in hippocampal slices following acute stress in mice. Neuroscience Letters. 1997; 233(1):41–44. [PubMed: 9324235]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Gomez-Pinilla F, Ying Z, Roy RR, Molteni R, Edgerton VR. Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. J Neurophysiol. 2002; 88(5):2187–2195. [PubMed: 12424260]

He WB, Zhang JL, Hu JF, Zhang Y, Machida T, Chen NH. Effects of glucocorticoids on age-related impairments of hippocampal structure and function in mice. Cell Mol Neurobiol. 2008; 28(2):277–291. [PubMed: 17710532]

Hodges H. Maze procedures: the radial-arm and water maze compared. Cognitive Brain Research. 1996; 3:167–181. [PubMed: 8806020]

Howland JG, Wang YT. Synaptic plasticity in learning and memory: stress effects in the hippocampus. Prog Brain Res. 2008; 169:145–158. [PubMed: 18394472]

Huang W, Cao J, Liu X, Meng F, Li M, Chen B, Zhang J. AMPK Plays a Dual Role in Regulation of CREB/BDNF Pathway in Mouse Primary Hippocampal Cells. J Mol Neurosci. 2015; 56(4):782–788. [PubMed: 25645685]

Joels M, Krugers HJ. LTP after Stress: Up or Down? Neural Plast. 2007; 2007

Katz RJ, Roth KA, Carroll BJ. Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. Neurosci Biobehav Rev. 1981; 5(2):247–251. [PubMed: 7196554]

Kim DM, Leem YH. CHRONIC STRESS-INDUCED MEMORY DEFICITS ARE REVERSED BY REGULAR EXERCISE VIA AMPK-MEDIATED BDNF INDUCTION. Neuroscience. 2016; 324:271–285. [PubMed: 26975895]

Kim JJ, Diamond DM. The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci. 2002; 3(6):453–462. [PubMed: 12042880]

Kim JJ, Lee HJ, Han JS, Packard MG. Amygdala is critical for stress-induced modulation of hippocampal long-term potentiation and learning. J neurosci. 2001; 21(14):5222–5228. [PubMed: 11438597]

Kim JJ, Yoon KS. Stress: metaplastic effects in the hippocampus. Trends in Neurosciences. 1998; 21(12):505–509. [PubMed: 9881846]

Krugers HJ, Hoogenraad CC. Hormonal Regulation of AMPA Receptor Trafficking and Memory Formation. Front Synaptic Neurosci. 2009; 1

Kvarta MD, Bradbrook KE, Dantrassy HM, Bailey AM, Thompson SM. Corticosterone mediates the synaptic and behavioral effects of chronic stress at rat hippocampal temporoammonic synapses. J Neurophysiol. 2015; 114(3):1713–1724. [PubMed: 26180121]

Lemon N, Manahan-Vaughan D. Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. J Neurosci. 2006; 26(29): 7723–7729. [PubMed: 16855100]

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001; 25:402–408. [PubMed: 11846609]

Lu Y, Christian K, Lu B. BDNF: A Key Regulator for Protein-synthesis Dependent LTP and Long-term Memory? Neurobiol Learn Mem. 2008; 89(3):312–323. [PubMed: 17942328]

Magarinos AM, Verdugo JMG, McEwen BS. Chronic stress alters synaptic terminal structure in hippocampus. Proc Natl Acad Sci U S A. 1997; 94(25):14002–14008. [PubMed: 9391142]

Malenka RC, Bear MF. LTP and LTD: An embarrassment of riches. Neuron. 2004; 44(1):5–21. [PubMed: 15450156]

Maras PM, Baram TZ. Sculpting the hippocampus from within: stress, spines, and CRH. Trends in Neurosciences. 2012; 35(5):315–324. [PubMed: 22386641]

Marlatt MW, Potter MC, Lucassen PJ, van Praag H. Running throughout middle-age improves memory function, hippocampal neurogenesis and BDNF levels in female C57Bl/6J mice. Dev Neurobiol. 2012; 72(6):943–952. [PubMed: 22252978]

Martin S, Henley JM, Holman D, Zhou M, Wiegert O, van Spronsen M, Joels M, Hoogenraad CC, Krugers HJ. Corticosterone Alters AMPAR Mobility and Facilitates Bidirectional Synaptic Plasticity. PLoS ONE. 2009; 4(3)

Martinez-Moreno A, Rodriguez-Duran LF, Escobar ML. Late Protein Synthesis-Dependent Phases in CTA Long-Term Memory: BDNF Requirement. Front Behav Neurosci. 2011; 5

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

McCarty R. Optimizing laboratory animal stress paradigms: The H-H* experimental design. Psychoneuroendocrinology. 2017; 75:5–14. [PubMed: 27768983]

McEwen BS. Stress and hippocampal plasticity. Annu Rev, Neurosci. 1999; (22):105–122. [PubMed: 10202533]

McEwen BS. The Ever-Changing Brain: Cellular and Molecular Mechanisms for the Effects of Stressful Experiences. Dev Neurobiol. 2012; 72(6):878–890. [PubMed: 21898852]

McEwen BS, Sapolsky RM. Stress and cognitive function. Current Opinion in Neurobiology. 1995; 5(2):205–216. [PubMed: 7620309]

Merrill CB, McNeil M, Williamson RC, Poole BR, Nelson B, Sudweeks S, Edwards JG. Identification of mRNA for endocannabinoid biosynthetic enzymes within hippocampal pyramidal cells and CA1 stratum radiatum interneuron subtypes using quantitative real-time PCR. Neuroscience. 2012; 218:89–99. [PubMed: 22609938]

Pedersen BK. Muscle as a secretory organ. Compr Physiol. 2013; 3(3):1337–1362. [PubMed: 23897689]

Perreault ML, Jones-Tabah J, O'Dowd BF, George SR. A physiological role for the dopamine D5 receptor as a regulator of BDNF and Akt signalling in rodent prefrontal cortex. Int J Neuropsychopharmacol. 2013; 16(2):477–483. [PubMed: 22827965]

Pignatelli M, Umanah GK, Ribeiro SP, Chen R, Karuppagounder SS, Yau HJ, Eacker S, Dawson VL, Dawson TM, Bonci A. Synaptic Plasticity onto Dopamine Neurons Shapes Fear Learning. Neuron. 2017; 93(2):425–440. [PubMed: 28103482]

Pignatelli M, Vollmayr B, Richter SH, Middei S, Matrisciano F, Molinaro G, Nasca C, Battaglia G, Ammassari-Teule M, Feligioni M, Nistico R, Nicoletti F, Gass P. Enhanced mGlu5-receptor dependent long-term depression at the Schaffer collateral-CA1 synapse of congenitally learned helpless rats. Neuropharmacology. 2013; 66:339–347. [PubMed: 22709946]

Pu Z, Krugers HJ, Joels M. Corticosterone time-dependently modulates beta-adrenergic effects on long-term potentiation in the hippocampal dentate gyrus. Learn Mem. 2007; 14(5):359–367. [PubMed: 17522027]

Rabelo PCR, Horta NAC, Cordeiro LMS, Poletini MO, Coimbra CC, Szawka RE, Soares DD. Intrinsic Exercise Capacity in Rats Influences Dopamine Neuroplasticity Induced by Physical Training. J Appl Physiol. 2017; 1985 jap 00506 02017.

Russo-Neustadt A, Ha T, Ramirez R, Kesslak JP. Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. Behav Brain Res. 2001; 120(1):87–95. [PubMed: 11173088]

Salmon P. Effects of physical exercise on anxiety, depression, and sensitivity to stress: A unifying theory. Clinical Psychology Review. 2001; 21(1):33–61. [PubMed: 11148895]

Sandi C, Pinelo-Nava MT. Stress and Memory: Behavioral Effects and Neurobiological Mechanisms. Neural Plast. 2007; 2007

Schoenfeld TJ, Rada P, Pieruzzini PR, Hsueh B, Gould E. Physical Exercise Prevents Stress-Induced Activation of Granule Neurons and Enhances Local Inhibitory Mechanisms in the Dentate Gyrus. J Neurosci. 2013; 33(18):7770–7777. [PubMed: 23637169]

Slipczuk L, Bekinschtein P, Katche C, Cammarota M, Izquierdo I, Medina JH. BDNF Activates mTOR to Regulate GluR1 Expression Required for Memory Formation. PLoS ONE. 2009; 4(6)

Tong L, Shen H, Perreau VM, Balazs R, Cotman CW. Effects of exercise on gene-expression profile in the rat hippocampus. Neurobiol Dis. 2001; 8(6):1046–1056. [PubMed: 11741400]

van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A. 1999; 96(23):13427–13431. [PubMed: 10557337]

Vaynman S, Gomez-Pinilla F. Revenge of the "sit": how lifestyle impacts neuronal and cognitive health through molecular systems that interface energy metabolism with neuronal plasticity. J Neurosci Res. 2006; 84(4):699–715. [PubMed: 16862541]

Wiegert O, Pu Z, Shor S, Joëls M, Krugers H. Glucocorticoid receptor activation selectively hampers N-methyl-d-aspartate receptor dependent hippocampal synaptic plasticity in vitro. Neuroscience. 2005; 135(2):403–411. [PubMed: 16125856]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Xiong H, Cassé F, Zhou M, Xiong ZQ, Joels M, Martin S, Krugers HJ. Interactions between N-Ethylmaleimide-sensitive factor and GluA2 contribute to effects of glucocorticoid hormones on AMPA receptor function in the rodent hippocampus. Hippocampus. 2016; 26(7):848–856. [PubMed: 26766634]

Zagaar M, Dao A, Levine A, Alhaider I, Alkadhi K. Regular Exercise Prevents Sleep Deprivation Associated Impairment of Long-Term Memory and Synaptic Plasticity in The CA1 Area of the Hippocampus. Sleep. 2013; 36(5):751–761. [PubMed: 23633758]

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

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

HIGHLIGHTS

- Exercise is neuroprotective against the negative effects of stress on CA1 dorsal hippocampal long-term potentiation (LTP), in a likely corticosterone-independent manner.
- Exercise with stress mice performed just as well on the radial arm maze spatial memory assay as exercise no stress mice, while performing better than both groups of sedentary mice.
- Changes in glucocorticoid, mineralocorticoid, and dopamine 5 receptors mRNA expression were noted between exercise and stress groups.
- Exercise is a viable method to protect learning and memory mechanisms from the negative cognitive impact of chronic intermittent stress on the brain.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

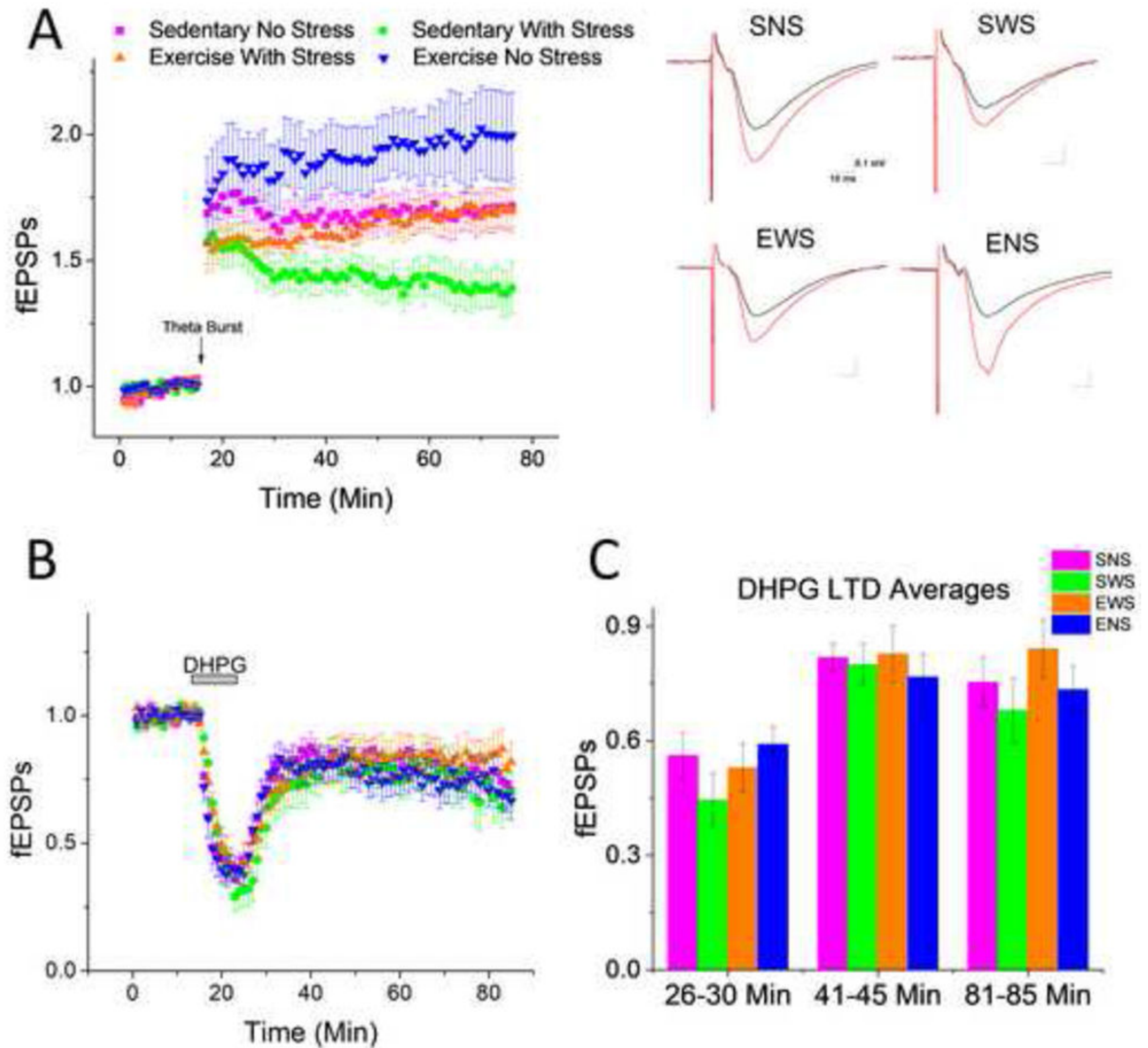

Figure 1.

Exercise and stress significantly alter theta burst induced LTP, but not DHPG-mediated LTD. EPSP normalized slope values were compared for significance using a one-way ANOVA with Bonferroni's posthoc analysis and a 2×2 ANOVA. A) The important comparison was that exercise with stress mice had greater theta burst-induced (arrow) LTP as measured using fEPSPs than sedentary with stress mice ($p < 0.05$). Exercise no stress mice exhibited the largest levels of LTP that were greater than exercise with stress, whereas sedentary with stress significantly decreases LTP compared to sedentary no stress ($p < 0.05$). Exercise with stress mice have similar levels of LTP as sedentary no stress mice ($p > 0.05$). The biggest difference in LTP was between the exercise no stress and sedentary with stress groups ($p=0.004$). Sedentary No Stress (SNS, n=14); Sedentary With Stress (SWS, n=19); Exercise With Stress (EWS, n=12); Exercise No Stress (ENS, n=15). Percent changes for LTP when

compared to pre-conditioning baseline: SNS = 171.6±4.7%, SWS = 144.3±4.9%, EWS = 165.8±3.7%, ENS = 192±7.3%. Inset: average of 10-15 traces taken just before (black) and 28-30 minutes after (red) the conditioning theta burst stimulation. B) This dot graph represents the average slopes throughout the DHPG experiments. There were no significant differences in metabotropic glutamate receptor (mGluR)-dependent LTD induced by 10 minutes of DHPG (100 μM) application among the four groups as analyzed by one-way ANOVAs (SNS n=15, SWS n=11, EWS n=17, ENS n=17). C) This bar graph compares the average slopes of all the groups at multiple time points during the DHPG experiments and one-way ANOVAs determined that there were no significant differences at any of the three time points measured.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

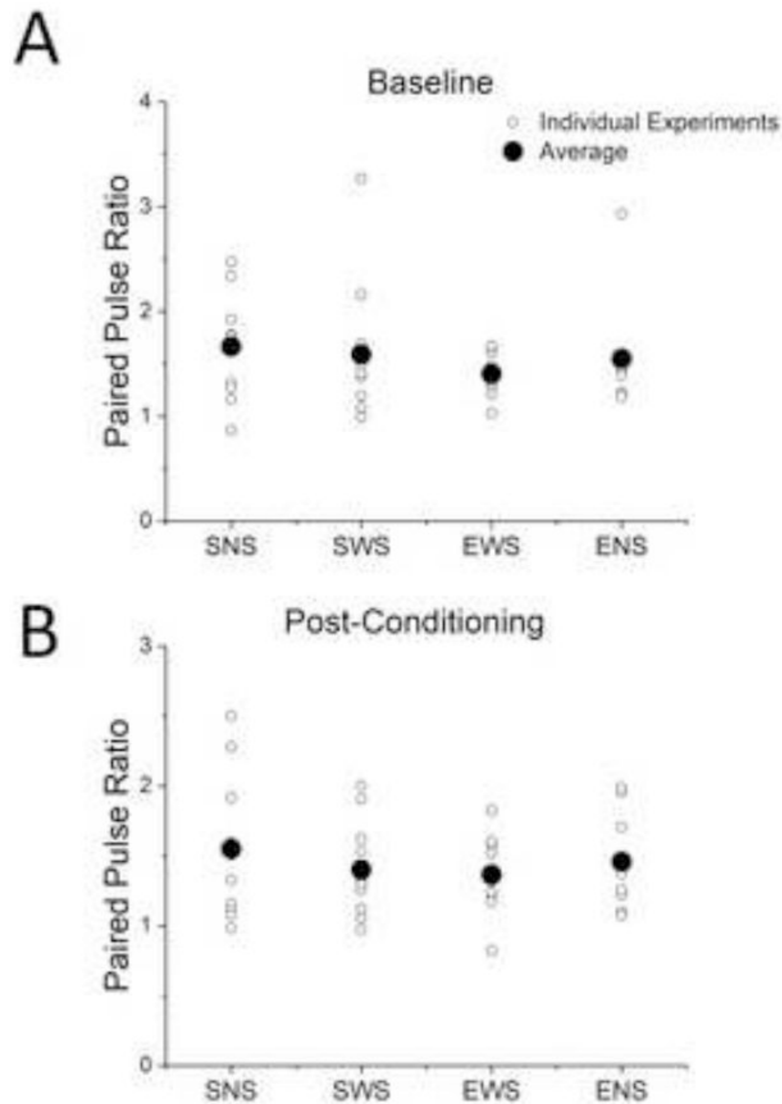

Figure 2.

Paired pulse ratios of field electrophysiology experiments of all four groups showed no significant changes. A) A plot of all the baseline ratios for all four groups (ENS n=8, EWS n=9, SWS n=11, SNS n=11). A one-way ANOVA showed no significant differences between the paired pulse ratios of the baselines between the four groups. B) A plot of all the postconditioning ratios for all four groups. A one-way ANOVA showed no significant differences between the paired pulse ratios between the four groups. This indicates that stress and exercise were not likely having a presynaptic effect on LTP.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

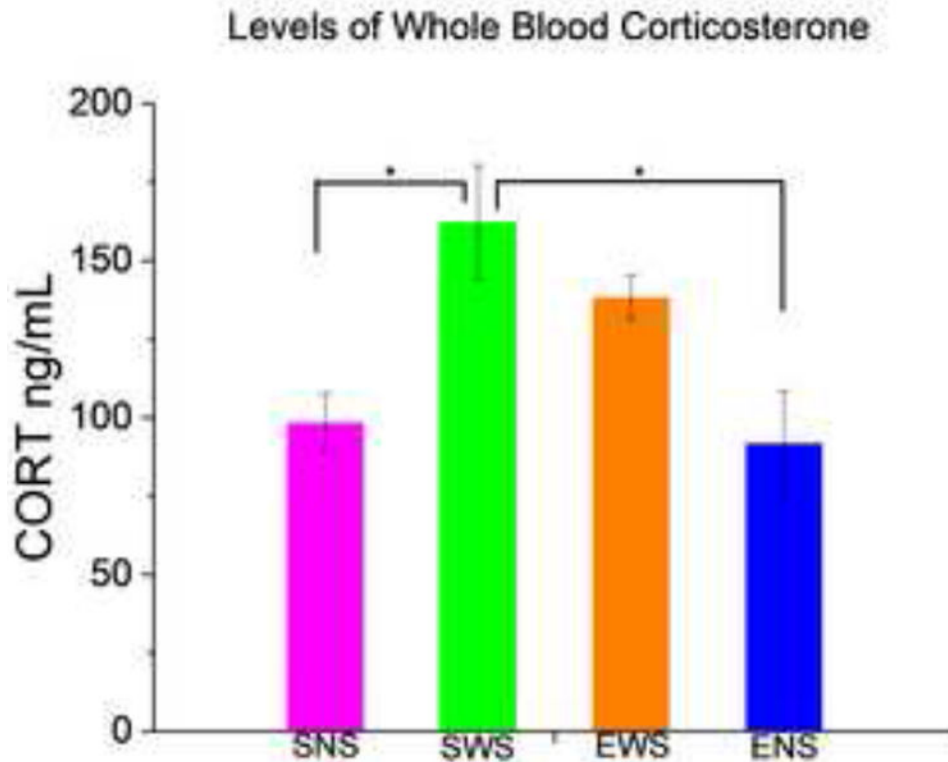

Figure 3.
Corticosterone blood concentrations for all four groups measured by an enzyme-linked immunosorbent assay (ELISA). A one-way ANOVA ($F_{3, 27}$ =6.098, p=0.003) showed significant differences in corticosterone concentrations with a Bonferroni post-hoc test. SWS mice had significantly more corticosterone compared to SNS mice ($p< 0.05$). ENS mice had significantly less corticosterone than SWS ($p< 0.05$). There were trends ($p<0.15$) in differences of corticosterone levels for EWS vs SNS and EWS vs ENS, with EWS having increased levels. A 2×2 ANOVA indicate that corticosterone levels were significantly different ($F_{1, 27}$ =15.797, p=0.0004) between stressed groups and not stressed groups. (SNS n=9, SWS n=9, EWS n=8, ENS n=5). An asterisk (*) here denotes $p < 0.05$.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

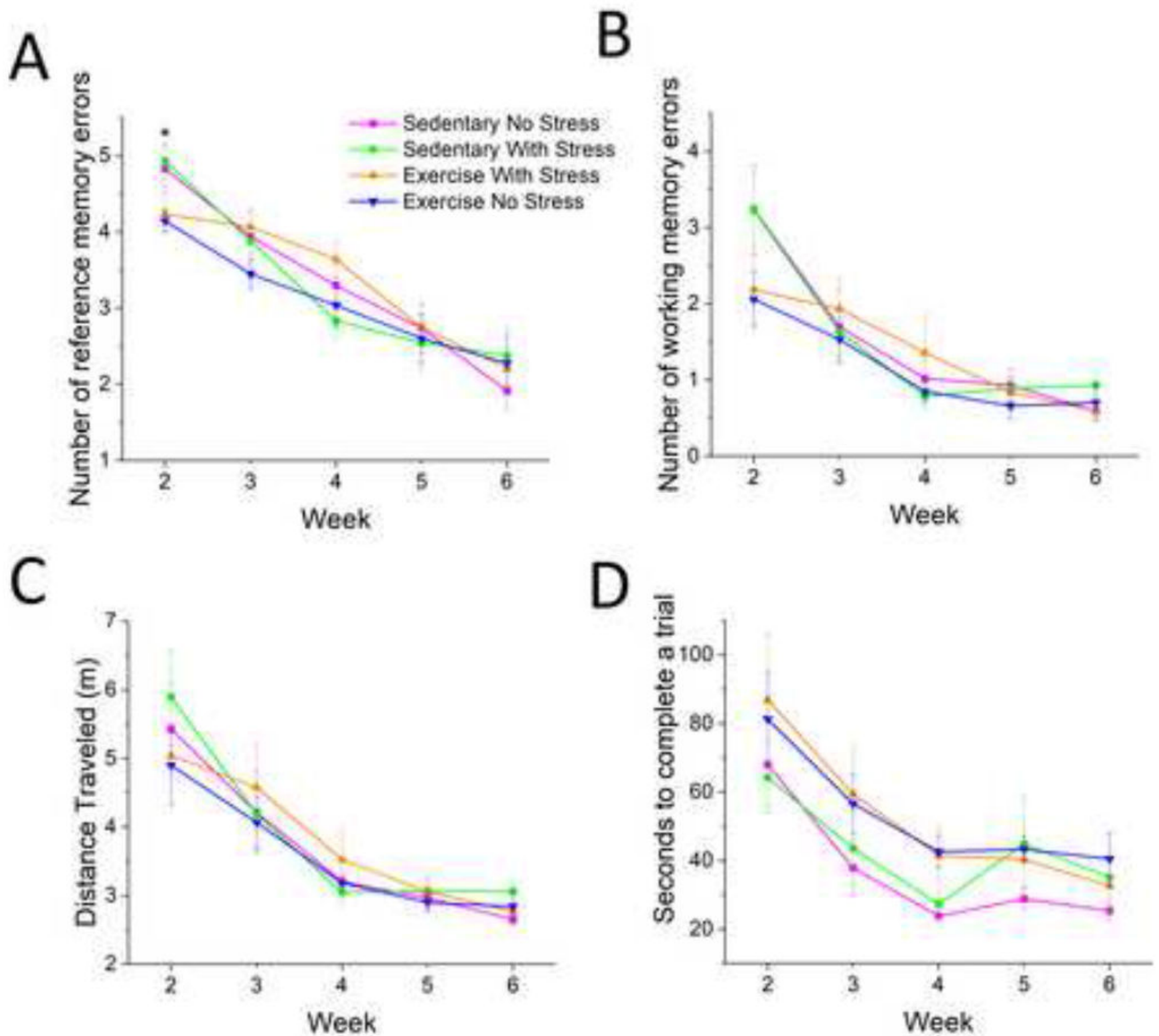

Figure 4.
Radial arm maze results indicating learning and memory differences between exercise and stressed mice during the second week of testing. In all the graphs, the mixed model statistics (see methods section for details) showed that there was a significant difference ($p<0.05$) between weeks for all groups showing that all groups improved in maze performance from one week to the next (n=11 for all groups and graphs). 2×2 ANOVAs revealed no significant differences in A-D. A) Average reference (long-term) memory errors per trial for all groups over the course of 5 weeks. A one-way ANOVA revealed significant differences ($F_{3, 40}$ =2.901, p=0.04) in week 2 between exercised and sedentary mice, with the exercise mice making fewer errors. No significant difference was noted between sedentary or exercise mice (SNS vs SWS $p > 0.5$; EWS vs ENS $p > 0.5$), but significant ($p < 0.05$) differences were importantly noted between SWS vs EWS as well as SNS vs ENS, and SWS vs ENS.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

B) Average working (short-term) memory errors. Week 2 differences between exercised and sedentary mice were not significant according to a one-way ANOVA ($F_{3, 40}$ =1.857, $p< 0.15$,), but instead illustrate a trend of exercise mice making fewer errors. Several trends were noted between specific groups (SNS vs EWS p=0.15, SNS vs ENS p=0.10, SWS vs EWS p=0.12, SWS vs ENS p=0.06), with no differences noted between stress or exercise groups (SNS vs SWS p=0.99, EWS vs ENS p=0.82). C) There was no significant ($p > 0.05$) difference between the distances traveled in each trial between the four groups. D) There were no significant ($p > 0.05$) difference in time to complete the trial between the four groups.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

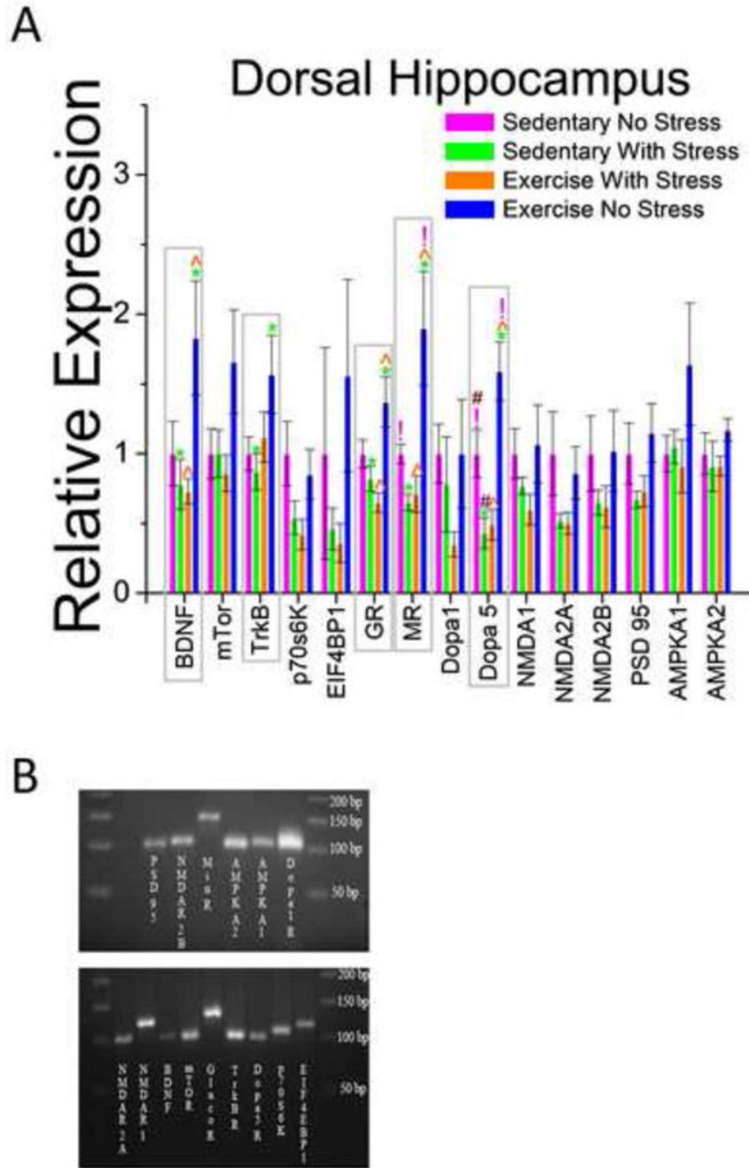

Figure 5.

The expression of various mRNA targets involved in the exercise and stress pathways, as well as synaptic plasticity. A) Dorsal hippocampus RT-qPCR results: one-way ANOVAs showed significant difference in expression levels for brain-derived neurotropic factor (BDNF), TrkB, glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and dopamine 5 receptor. SNS n=7-9, SWS n=5-8, EWS n=5-7, ENS n=5-8; Boxes highlight targets with significant changes in mRNA expression: * indicates $p<0.05$ between ENS and SWS groups; ^ indicates $p<0.05$ between ENS and EWS; ! indicates $p<0.05$ between ENS and SNS; # indicates $p<0.05$ between SNS and SWS. B) Gels illustrating that the primers used were specific for the designed target as indicated by the appearance of single bands at the correct base pair amplicon length. For specific amplicon base pair lengths and primer

sequences, refer to table 1. The gel illustrating 18S was noted in a prior publication (Merrill, McNeil et al. 2012).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Primer sequences designed for the genes of interest. The table includes what part of the ribonucleic acid sequence the nucleotides were modeled from, the forward and reverse primer sequences, the amplicon length in base pairs, and what exon the primers bind.

Mouse Gene/Target	RNA Sequence (F,R)	Forward (F) sequence	Reverse (R) Sequence	Amplicon	Exon
mTor	3376-3393, 3456-3475	AACAGCCAAGGCCGAATC	CAATCGGAGGCAACAACAAG	100 bp	F=exon 19, R=exon 20
Ntrk2 (TrkB Receptor)	2186-2210, 2266-2286	GTTATTGAAAACCCCCAGTACTTTG	TCCCTCTTCAGAACGATGTTG	101 bp	F=exon 22, R=exon 23
BDNF (transcript: bdnf- 211, exon 2)	534-556, 615-643	GACATCACTGGCTGACACTTTTG	CAAGTCCGCGTCCTTATGG	110 bp	F=exon 9, R=exon 9
EIF4EBP1	623-646, 717-737	TCTGTCTATACCGAGCTGCTACTG	GCTAGGATTGCCAGATCATTC	115 bp	F=exon 6, R=exon 6
Rps6kb1 (p70s6K)	628-654, 714-733	GTGGAGGAGAACTATTTATGCAGTTAG	TGATGTAAATGCCCCAAAGC	106 bp	F=exon 8, R=exon 12
NR3C1 (Glucocorticoid Receptor)	2193-2212, 2300-2322	GGCAGCGGTTTTATCAACTG	TCAGCTAACATCTCTGGGAATTC	130 bp	F=exon 11, R=exon 12
NR3C2 (Mineralocorticoid Receptor)	2543-2564, 2667-2686	GCATCAGTCTGCCATGTATGAG	CTCTTGAGGCCATCTTTTGG	144 bp	F=exon 13, R=exon 14
Grin1 (NMDAR Subunit 1)	1240-1259, 1337-1359	CCACGAGCTCCTAGAAAAGG	TCTGCATACTTGGAAGACATCAG	120 bp	F=exon 5, R=exon 6
Grin2A (NMDAR Subunit 2A)	2331-2350, 2409-2430	CGCTCTGCTCCAGTTTGTTG	GCTGCTCATCACCTCATTCTTC	100 bp	F=exon 11, R=exon 12
Grin2B (NMDAR Subunit 2B)	2373-91, 2453-2474	TGGTATCACGCAGCAATGG	CAGAGACAATGAGCAGCATCAC	102 bp	F=exon 15, R=exon 16
DLG4 (PSD-95)	1864-1886, 1945-1963	AGAAGACTCGGTTCTGAGCTATG	ATCGTTGGCACGGTCTTTG	100 bp	F=exon 20, R=exon 21
PRKAA1 (AMPK Subunit A1)	326-348, 410-429	TGTCTCTGGAGGAGAGCTATTTG	AATAATCCACACCGGAAAGG	104 bp	F=exon 3, R=exon 4
PRKAA2 (AMPK Subunit A2)	350-373, 431-449	AGCACTCCGACAGACTTTTTTATG	GCGCTTCCACCTCTTCAAC	100 bp	F=exon 3, R=exon 4
DRD1 (Dopamine Receptor 1A)	2836-2868, 2916-2936	TGACTGTCCAGGATTAAGATGTG	AGATGAAGAACCAGCGATGAG	101 bp	F=exon 3, R=exon 3
DRD5 (Dopamine Receptor 1B/5)	2416-2439, 2494-2515	ACAATGGTCCTGCTTGCTAGATAG	TCGCTCTGAAACACCCTAGATG	100 bp	F=exon 1, R=exon 1
18S	1331-1351, 1440-1463	GTGCATGGCCGTTCTTAGTTG	GCCACTTGTCCCTCTAAGAAGTTG	133 bp	F=exon 2, R=exon 2