The Potential Benefits of Exercise in the Mouse Hippocampus Using an Immobilization Chronic Restraint Stress Model

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2009 Rhodes Institute for Regional Studies

Abstract

Exercise has a multitude of benefits for the human body and the brain. Exercise has been shown to decrease the likelihood to develop Alzheimer's or Parkinson's Disease, increases neurogenesis, and preserve cognitive function that is diminished during aging. Since exercise has neuroprotective effects, we examined the effect of exercise in protection of neurons from the toxic effects of glucocorticoids (GCs) that are released during physiological stress. Chronically sustained levels of glucocorticoids have been shown to cause apoptosis in the hippocampus. Exercise has been shown to increase neurotrophic factors, which protect neurons in the hippocampus from ischemic damage. Therefore, we hypothesize that chronic restraint stress will increase neurodegeneration in the brain as measured by apoptosis and reactive astrogliosis, and that exercise will decrease the expression of these. To test this, female mice were randomly assigned to standard housing (SH) or in cages equipped with running wheels (Ex). Half of the mice from each group were also exposed to chronic restraint stress for two hours each day for two weeks prior to sacrifice. To analyze neurotoxic effects, sections of the hippocampus were labeled for glial activation (GFAP) and apoptosis (cleaved caspase-3 and Bax), and expression of the markers was quantified. Preliminary data suggests that chronic restraint stress induced apoptosis involves Bax but not Caspase-3 mediated signaling. As well, quantification of astrocyte numbers indicates that exercise significantly decreases the amount of astrocytes in mouse hippocampus regardless of stress. We are continuing to investigate the toxic effects of stress in the hippocampus, and the potential neuroprotective effects of exercise in this model.

Introduction

Stress is a ubiquitous aspect of life and so it is important that the body can effectively counteract it with maximum damage protection. Fortunately, the human brain has built-in mechanisms for triggering a stress response when it senses internal or external stimuli disruptions that veer the body away from homeostasis (Joel, 2008). These natural auto-protective responses for adapting to stress are important to survival (Madrigal et al, 2006). Acutely, the stress response provides immediate positive responses in the hippocampal region of the brain, thus stress enhances long-term potentiation (LTP) and learning through catabolic processes that suppress normal

systemic functions and promote diversion of energy and nutrients to target tissues, resulting in heighten cognitive ability (Wiegert et al., 2006; Sapolsky, 1992). Nonetheless, as too much of anything does pose dangerous risks to the body, prolonged or chronic stress is detrimental to physiological functions and homeostatic balance (Joels, 2008). The extent of its deleterious effects on the brain are of serious interest as the brain controls the functions of the whole body and ultimately behavior. Exercise, a non-pharmacological tool, has been studied as a preventative and therapeutic measure in reducing and possibly even inhibiting neuronal loss due to stress. Moderate, voluntary exercise has been suggested in maximizing the aforementioned benefits because it allows the brain to create robust structural changes that will fight the damages of stress and subsequent stress more efficiently.

Chronic Stress

Research studies have shown that the effects of chronic stress are not mere negligible sequelae but can cause considerable behavioral and anatomical changes in the brain. This is a result of glucocorticoids (stress hormones) easily crossing the blood brain barrier and activating glucocorticoid receptors that are predominantly in the hippocampus (Sapolsky, 1992). The hippocampus structure of the limbic system is prominent for its role in memory and learning and is also one of the most vulnerable areas to the toxic affects of stress (Sapolsky, 1992). The hippocampus is greatly saturated with two steroid receptors: mineralcorticoid receptors which have a high affinity for glucocorticoids and are normally activated when the body is at homeostasis and glucocorticoid receptors which have a lower affinity for glucocorticoids and are only activated when stress is perceived by way of elevated release of glucocorticoids from the adrenal gland

(McEwen, 1995). This creates an excess release of Ca^{2+} ions and eventual arrest in delivery of glucose/energy to neurons and glia due to an impaired energy system, resulting in neuronal death (apoptosis) (Stein-Behrens et al., 1994, Selye, 1936). Additionally, this prolonged exposure to stress exacerbates production of free radicals and other oxidants leading to oxidative damage of neurons (Liu and Mori, 1999). Thus, if chronic stress exposure is leading to neuronal atrophy particularly in the hippocampus, returning to homeostasis is particularly challenging. When glucocorticoid receptors are actively saturated in the hippocampus, it is the hippocampus itself that is responsible in discontinuing any further release of glucocorticoids in the hypothalamic-pituitary-adrenal (HPA) cascade stress response by terminating the release of corticotrophin release hormone (CRH) and adrenocorticotrophic hormone (ACTH) (Vreugdenhil et al., 2001). If this feedback mechanism is impaired due to hippocampal damage, then there is a continual release of glucocorticoids causing increased neuronal compromise (McEwen, 1995; Porter, 1998).

Research has shown that stress can cause significant structural changes in the hippocampus, such as a reduction of dendritic spines, alteration in terminal synaptic structure, suppression of neurogenesis in the dentate gyrus, increase in necrotic processes, myelin alterations, and altered astrocytic foot processes (Magarinos, 1997; Avila-Costa et al., 1999 ; Nino-Cabrera et al., 2001). These structural changes may underlie observed behavioral changes such as reduced response to stress, cognitive ability, as well as reduction in learning and memory, indicating that stress compromises brain plasticity necessary for these events (Zhao, 2007 and Radak, 2001). This degeneration is a component of neuropsychiatric disorders such as Alzheimer's disease (AD) and

depression. Consequently, it is pertinent to determine preventative methods that can minimize the effects of stress on the hippocampal brain region that largely influence behavioral outcome.

Benefits of Exercise

The advantages of exercise for overall health have been promoted repeatedly, but the exact mechanisms underlying the effect remain to be elucidated. The knowledge of its role in brain health has been increasing extensively. Exercise inherently augments the number of reactive oxygen species (ROS) in the body due to the increase in oxygen consumption from physical activity, amplifying the number already made from aerobic metabolism. ROS is necessary for maintaining homeostasis under baseline conditions (Davies et al, 1982). Oxidative stress, however, results when the number of ROS surpasses the body's capability to supply anti-oxidants to inactivate their unstable nature (Pratico & Delanty, 2000), This can result in oxidative damage to tissues and make the brain more susceptible to neurodegenerative diseases and general brain insult (Somani, 1995). Thus while exercise does increase ROS in the body and theoretically increases likelihood of oxidative stress, it rather paradoxically improves the physiological function of the brain by increasing its resistance to oxidative stress via "exercise-induced adaptation" (see review Radak, 2008).

This concept of exercise-induced adaptation is the body's mode of adjusting to the internal changes from the stressor. Moderate amounts of exercise actually activate the body's anti-oxidant system and the necessary biomolecules to prompt the oxidative damage repair/elimination system. This is especially critical for the brain because it, comparatively to other body organs, has a lower anti-oxidant defense system (Floyd,

1999). This hormesis theory suggests that by exposing the body to a low-dose stimulating stressor like exercise in this case, it allows a high response in inhibition and resistance to subsequent stress toxins because the body has previously become familiar with these factors and knows how to respond appropriately with efficiency (Calabrese & Baldwin, 2003). Thus, exercise can potentially prevent the negative effects of glucocorticoid steroid hormones through production of neuroprotective resources, and, in turn, reduce neurodegenerative effects such as mental attenuation.

Experiments have observed the benefits of exercise against stress in animal rodent models by way of increase in protective factors. In a mice exercise study conducted by Adlard & Cotman (2004), it was found that stress hormones mediate changes in brain-derived neurotrophic factor protein (BDNF) production. BDNF is a neurotrophic and neuroprotective factor, whose signaling up-regulates protein resources important for synaptic transmission for increased brain plasticity (Cotman & Berchtold, 2002). Control sedentary mice given stress were compared to exercising mice given stress to see what the differences were in BDNF production. Both groups did have a significant increase in corticosterone levels, but BDNF production was different. There was an elevated amount of BDNF protein in animals allowed to exercise for 3 weeks prior to the stressor, showing the positive effects of exercise. Non-exercising controls subjected to stress, however, did have a stress-induced decrease in BDNF protein levels (Adlard & Cotman, 2004). Thus exercising animals can overcome the negative effects of stress (Adlard & Cotman, 2004). The protective effects of BDNF requires, however, IGF-1 (insulin growth factor-1) and VEGF (vascular endothelial growth factor), which are both also elevated after exercise and considered critical to neurogenesis and cell proliferation in hippocampal regions

(Trejo et al., 2001; Fabel et al., 2003). This study suggests that exercise generates neuroprotective factors that can overcome negative consequences of stress and, thereby, reduce negative cognitive impairment induced by stress.

Additional research has shown that exercise retards the effects of stress in rodents. In a study immobilizing rats for 8 hours, the stress had significantly reduced their level of GS (glutamine synthetase) activity, meaning less uptake of glutamate, which is suggested to promote oxidative damage (Radak, 2001). The group allowed to have a single bout of exercise afterwards, however, had their GS level restored comparable to that of control groups and their cognitive and behavioral performance was also improved by the single bout of exercise (Radak, 2001). This illustrates the powerful and immediate results of even periodic exercising. Furthermore, this same study showed that immobilized mice with no exercise did have an increase in oxidative damage as indicated by a significant increase level of lipid peroxidation, reactive carbonyl derivatives (RCD), and DNA nuclear damage (Radak, 2001). Other studies support the aforementioned results of exercise and enriched environments mitigating the negative effects of stress and supporting augmentation of cell proliferation and neurogenesis in hippocampal regions (Cotman & Berchtold, 2002; Henriette van Praag et al, 1999; Veena, J. et al, 2008).

Proposed Study

Chronic stress combined with oxidative stress are considered to underlie major health issues such as Alzheimer's Disease due to oxidative damage done particularly in the hippocampus structure of the brain. This neuronal network compromise is suggested as a result of accumulation of glucocorticoid exposure over the years (Sapolsky, 1992), making oxidative damage more vulnerable for the elderly. The stronger the body is in

defending itself from toxins via anti-oxidant system, then the compound effect of damage done by chronic stress and oxidative stress can be reduced. It is proposed that exercise promotes positive brain health by diminishing the deleterious effects caused by stress and can consequently increase individuals' quality of life. The mechanisms, however, remain to be elucidated. By further examining how stress and exercise interact and the brain's response to them in isolation and combined, a new approach can be taken to diminish neurodegenerative effects.

Stress markers such as reactive gliosis and apoptosis allow for assessing quantitatively the extent of stress on the brain due to a stressor. Exercise has been suggested to reduce the number of the aforementioned oxidative markers due to an increase in neuroprotective factors (Cotman & Berchtold, 2002). The hypersecretion of glucocorticoids is associated with more neurodegeneration as assessed by astrogliosis, which are critical for providing metabolic needs to neurons. Consequently, it is hypothesized that in damaged tissue there is an increase in astrogliosis via inflammatory response in attempt to accommodate for the needs of impaired neuronal tissue by removing cellular debris and releasing neurotrophic factors (Hui-Ming et al, 2003). Thus, the number of astrocytes should be increased in the standard housing conditions compared to the exercise model due to an inflammatory response system set up to repair neuronal damage (Issa et al, 1990). In addition, the level of apoptosis will be examined in each condition to determine if exercise conditioned mice have reduced apoptosis compared to sedentary. By assessing the number of astrocytes and Bax expressive markers in the hippocampal region, it is possible to see the effects of exercise and stress in the CA regions.

MATERIALS AND METHODS

Animals

Thirty-two, eight-week-old, female mice (C57/B16J) were obtained from Jackson Laboratories (Bar Harbor, ME) and kept in a room at constant temperature (68 ± 2°C) and humidity with a light set to a 12-hour light/12-hour dark cycle (6AM-6PM). Two mice were placed in each cage and given standard mouse chow and water *ad libitum* while in home cages. Mice were monitored daily to ensure maintenance of proper living conditions. Six mice were randomly assigned to each experimental condition: standard housing (SH), standard housing plus stress (SH + S), exercise (Ex), and exercise plus stress (Ex + S). Mice in the SH and SH + S conditions were housed in a standard colony cage and mice in the Ex and Ex + S conditions were housed in standard breeding cages equipped with two running wheels for voluntary exercise. At all times the animals were maintained and handled according to guidelines approved by the Rhodes Institutional Animal Care and Use Committee and the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Chronic Restraint Stress Paradigm

The mice were habituated into their new living conditions for two weeks. Stress was administered by placing the mice in Broome Restraint tubes (Harvard Apparatus; Holliston, MA) for two hours a day for fourteen consecutive days. Each day, during the time the other mice were being stressed, the mice not receiving stress were picked up in order to avoid confounding effects of handling stress.

Immunocytochemistry Techniques

Tissue Preparation

After the two weeks of experimental manipulation, mice were euthanized using tribromoethanol (250 mg/kg (i.p.)). After euthanization the brains were transcardially perfused with 4% paraformaldehyde in 0.1M phosphate buffered saline (PBS; pH 7.4). Whole brains were extracted and postfixed overnight at 4°C in the same fixative for preservation purposes. The next day the brain was processed for embedding in paraffin for sectioning. A microtome was used to make eight-micron-thick sections in the coronal plain which were mounted into SuperFrost Plus Slides (Fisher Scientific; Atlanta, GA).

GFAP Staining Technique

Stains that mark the presence of glial fibrillary acidic protein (GFAP) were used to visualize astrocytes. Once the hippocampus was clearly visible, every third slide was selected for staining. The slides were then deparaffinized in xylenes and rehydrated in graded alcohols before being rinsed and rehydrated in PBS-Tween (PBS-T; PBS containing 0.05% Tween 20). After removing the excess PBS-T around the tissue, the tissue was encircled using a PAP pen (Research Products International; Mt. Prospect, IL) and blocked in with PBS-T/Blocking Buffer (PBS-T/BB; 0.5% Bovine Serum Albumin, 0.3% Triton, in PBS) for 30 minutes at room temperature. After blocking, slides were incubated in the primary antibody (anti-GFAP; clone G-A-5; Sigma Chemical Company; St. Louis, MO) at a 1:1000 dilution in PBS-T/BB overnight at 4°C.

The next day the slides were washed in three changes of PBS-T for five minutes each. The secondary donkey anti-mouse antibody (Jackson ImmunoResearch Laboratories; West Grove, PA) was added to the slides in a 1:250 dilution in PBS-T/BB and incubated in a humid chamber for two hours at room temperature. The slides were washed in PBS-T as before and visualized by peroxidase-mediated deposition of diaminobenzidine (DAB; DAB Immunoperoxidase Substrate kit; Vector Laboratories; Burlingame, CA) per manufacturer's instructions. The slides were washed in running tap water for 10 minutes and dehydrated though a series of graded alcohols and xylenes before being coverslipped using Permount.

Bax Staining Technique

Bax was used to detect apoptosis. The slides were deparaffinized and rehydrated though xylenes and graded alcohols and placed in tap water for 5 minutes. Antigen retrieval was performed by gently boiling the slides in a 10mM citrate buffer (0.1M citric acid, 0.1M sodium citrate, distilled water) for 15 minutes. The slides were allowed to cool to room temperature and were then rehydrated in PBS-T for 30 minutes. The tissue was encircled using a PAP pen as described above. Non-specific binding was blocked by pre-incubating sections for 30 minutes at room temperature in blocking buffer [TNB (0.1M TrisHCL, 0.15M NaCl, 5% blocking buffer); pH 7.5; Renaissance TSA-Indirect kit, NEN Life Science Products; Boston, MA]. The primary anti-Bax antibody (clone 6A7; BD Biosciences; San Jose, CA) was diluted 1:250 in TNB and placed on the slides which were incubated overnight in a humid chamber at 4°C.

The slides were washed in PBS-T as before. The same secondary donkey anti-mouse antibody was diluted 1:250 in TNB and added to the slides before incubating for 2 hours in a humid chamber at room temperature. The slides were washed in PBS-T as previously described. In order to amplify the stain, the slides were incubated in biotinyl tyramide (BT; 1:50 dilution in Amplification Diluent from the Renaissance TSA-Indirect kit) for 10 minutes at room temperature. The slides were washed in PBS-T as before and incubated in a 1:100 dilution of streptavidin conjugated to HRP in TNB for 1 hour at room temperature. Again the slides were washed in PBS-T and visualized with DAB per instructions on the kit. The slides were next counterstained by placing the slides in hematoxylin for 15 seconds followed by 10 minutes in running tap water and 30 seconds in acid alcohol (3% acetic acid in 70% EtOH). The slides were then dehydrated through graded alcohols and xylenes and coverslipped using Permount.

Data Analysis

To quantitatively analyze the amount of astrocytes detected with GFAP staining, images of the hippocampus on the prepared slides were taken with Leica microscope equipped with a digital camera. Five images of tissue sections containing CA1-3 subfields were randomly chosen from each animal in each condition. The images were coded so as to make the quantification process blind to the raters. Astrocytes in each image were counted by three independent reporters and their data compiled to achieve an average amount of astrocytes for each image. These averages were used to perform statistical analysis to determine if there were significant differences among the variables. Statistical Program for Social Sciences (SPSS) was used to perform a 2 x 2 factorial

Analysis of Variance (ANOVA) and a one-way ANOVA with Tukey and LSD post-hoc analyses.

Images of the CA fields of the hippocampus were also taken of the prepared tissue stained with Bax. The slides photographed were chosen in the same way as GFAP imaging. These images were qualitatively analyzed for high, medium, or low levels of apoptotic nuclei marked by the expression of the Bax protein.

RESULTS

Astrogliosis in the Hippocampus

One marker of degeneration in the hippocampus is enhanced glial expression. To assess glial recruitment, photomicrographs were taken of the CA fields of animals from each condition, and the number of GFAP labeled astrocytes was assessed (Figure 1). The numbers observed from three independent reporters were averaged for each condition and the marginal means (±SEM) were: SH = 36.80 (±5.02); SH + S = 46.40 (±3.55); Ex = 51.30 (±4.46); Ex + S = 39.80 (±4.24) (Figure 2). There were no significant main effects in either the housing or the stress conditions, [$F(1, 85) = 0.820$, $p = 0.368$; $F(1,85) = 0.045$, $p = 0.832$]. A factorial ANOVA revealed a significant housing by stress interaction [$F(1,85) = 5.87$, $p<.05$]. In order to further examine the interaction, the factorial ANOVA was followed up with a one-way ANOVA and post hoc analyses using Fisher LSD and Tukey HSD for pair-wise comparisons. The second series of tests revealed that the only significant simple effect was between the SH condition and the Ex condition ($p<0.05$). Mice in the Ex condition expressed significantly more astrocytes ($M = 51.26$) than mice in the SH condition ($M = 36.80$).

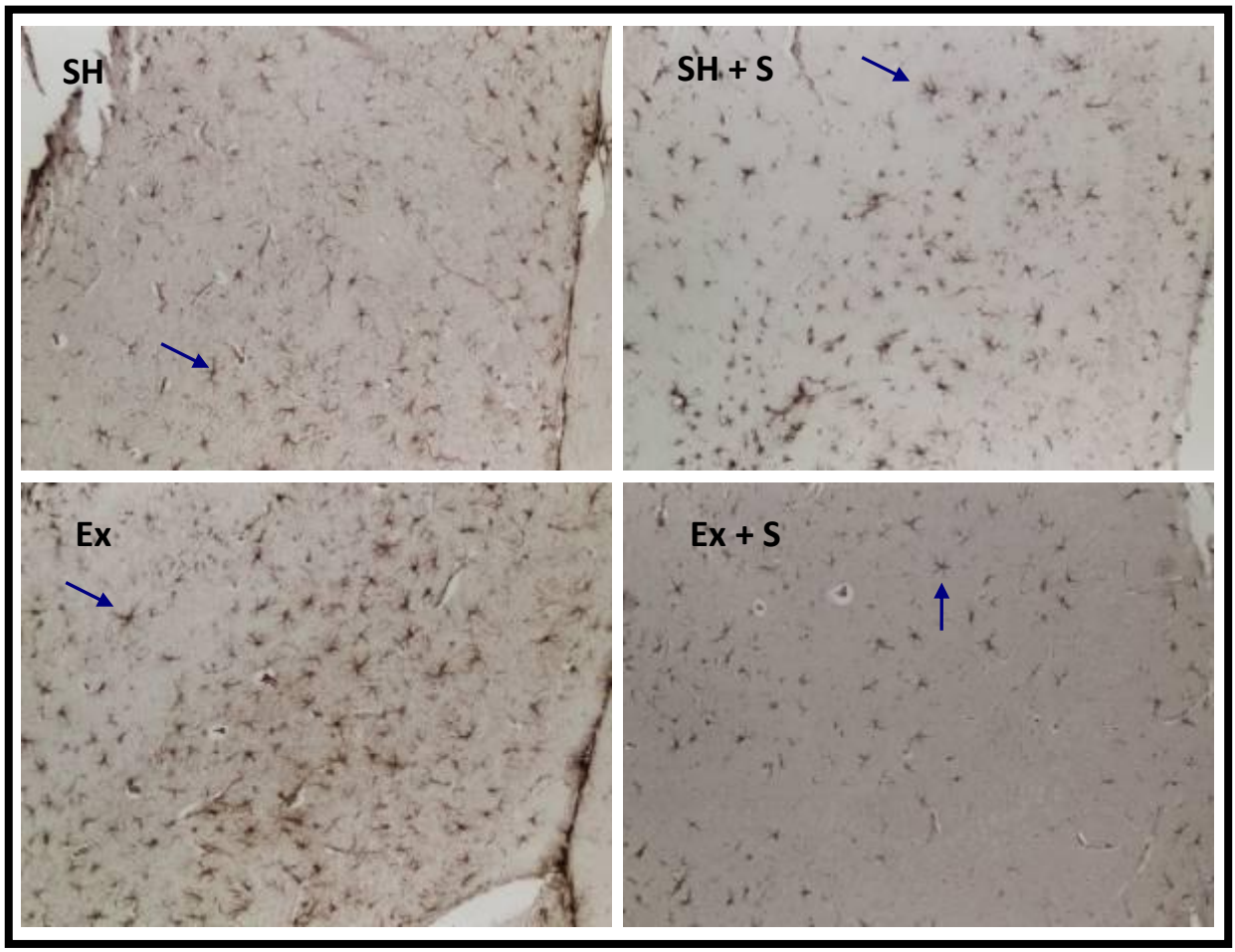

Figure 1: Low power photomicrographs (10X) of GFAP labeled astrocytes in the CA fields of the hippocampus. The number of astrocytes in each image is representative of the average for each group and arrows point to an astrocyte with well defined cell soma and processes.

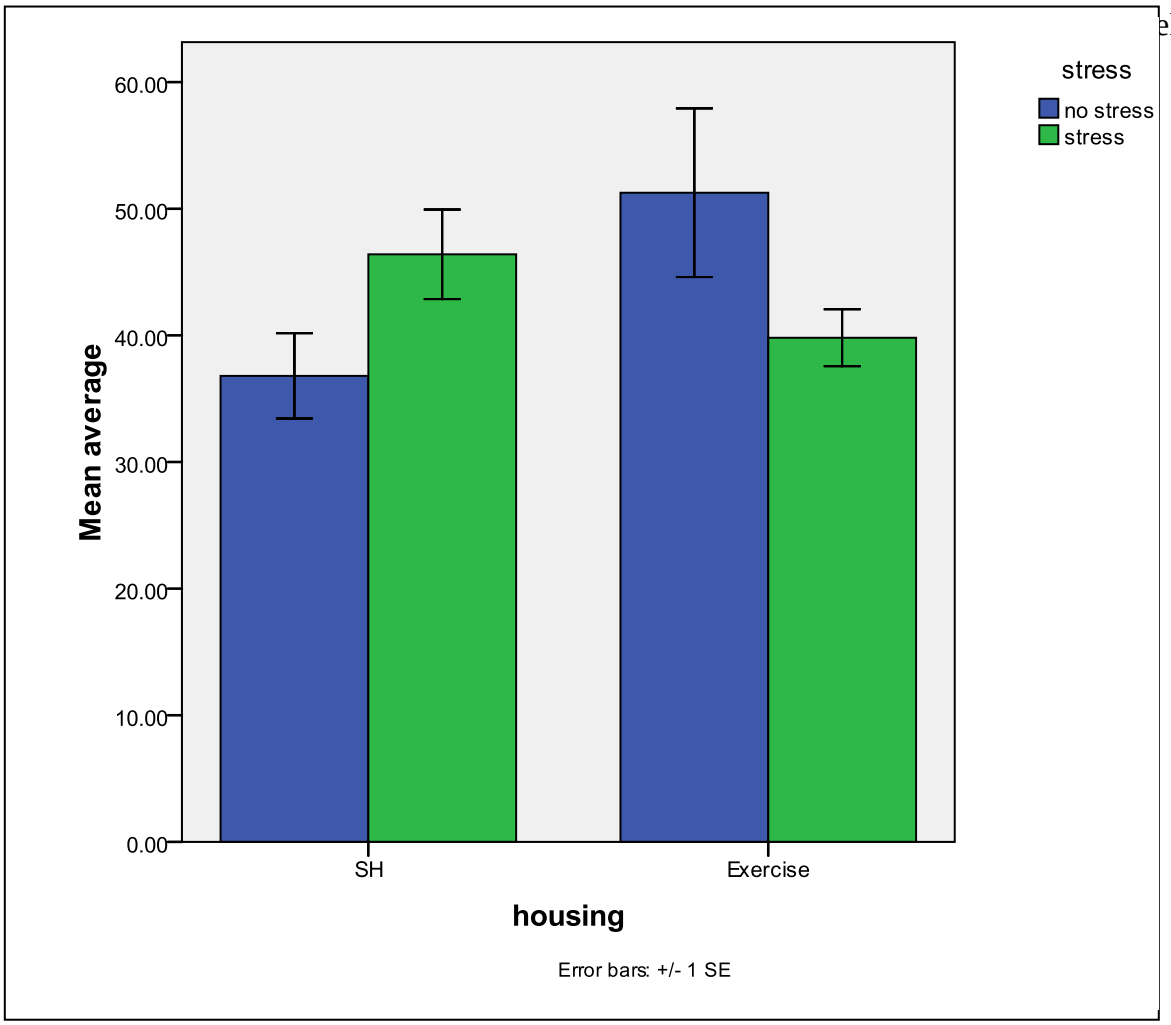

Figure 2: A bar graph showing the average number of astrocytes expressed in the CA region of the hippocampus of mice in each experiemental condition. A factorial ANOVA revealed a significant housing by stress interaction. Bars represent means ± SEM; * represents $p<0.05$ as compared to standard housing.

Expression of the Apoptotic Marker Bax in the Hippocampus

Initial observation seemed to indicate that the Bax staining was optimized and appropriate; however, upon further analysis the staining proved to be inconsistent and therefore unquantifiable. Qualitative analysis was attempted but since the quality of the staining varied between slides from the same animal, the results were inconclusive. Further optimization is needed to obtain quantifiable results. Selected images are

representative of slides with appropriate staining for Bax labeled neurons (Figures 3 and 4).

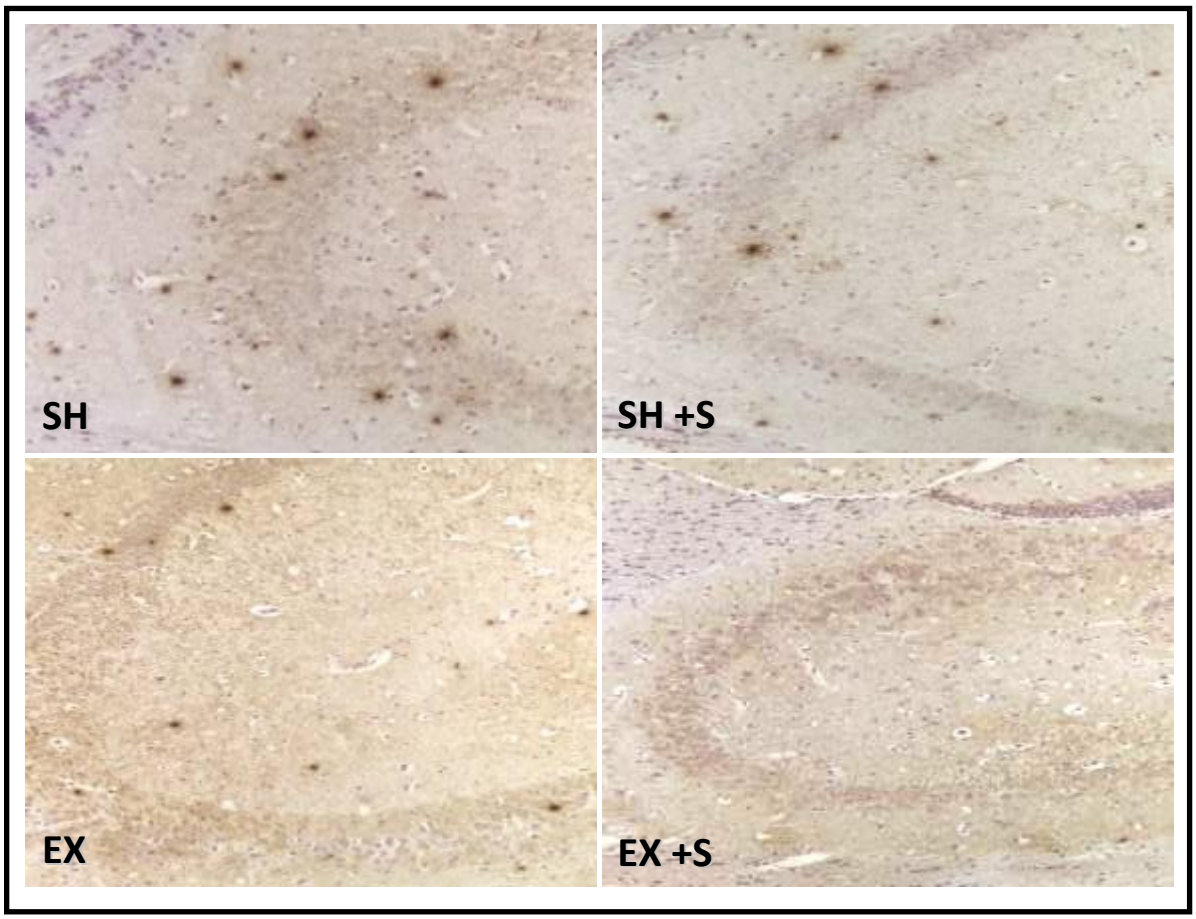

Figure 3: Low power photomicrograph (10X) of Bax labeled apoptotic nuclei in the CA fields of the hippocampus.

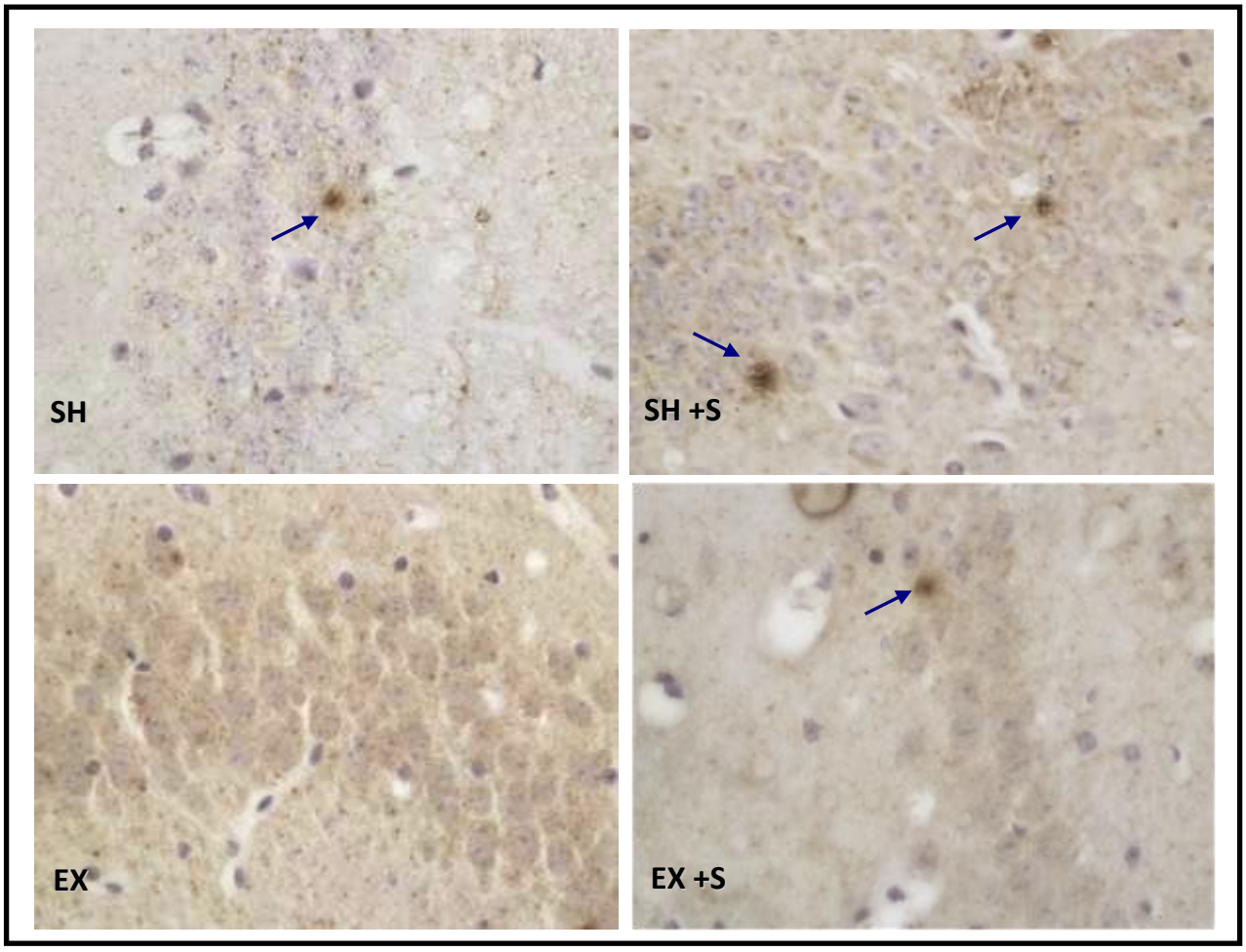

Figure 4: High power photomicrograph (40X) of Bax labeled apoptotic neurons in the CA fields of the hippocampus. Arrows point to cells that phenotypically appear to be prominent apoptotic neurons.

Discussion

These results did not support the initial hypothesis that the number of astrocytes should be significantly less in exercise stressed animals (Ex+S) compared to standard housing stressed (SH+S) animals. There was no statistical significance between SH+S and Ex+S indicating that exercise did not enhance cellular mechanisms for combating stress. Statistical analysis, surprisingly, showed that the only significant interaction ($p=.034$) was between no stress SH condition and no stress Ex condition with no other main effects.

The significant finding that exercise alone does increase the number of astrocytes in exercise animals was supported by research conducted by Radak (2001; 2008). According to the hormesis theory, exercise does increase the amount of ROS and this is necessary for the increase in anti-oxidant activity (Radak, 2008; Davies et al, 1982; 1986). This concept of moderately stressing the body to produce stress is critical to the adaptation theory. Exercise is only effective when the physical activity acts as a stressor and fatigues the body. This muscular adaptation to the stressor via restructuring networks increases the oxygen blood supply to the brain regions. In the brain, it is suggested that there is a similar mechanism such that because there is a heightened metabolic activity due to the oxygen uptake in the brain, there is an increased activity in antioxidant defense systems and repairing enzymes (Cotman and Berchtold, 2002; Fabel et al., 2003; Radak et al., 2001a, 2006). Along with this increase due to exercise is augmentation of glial transport (Kandel and O'dell, 1992) which shuttles more glucose from capillaries to neurons. This transport is necessary to provide more energy for the increased networking and task of eliminating dead neural tissue (Rosenzweig et al, 1972). In addition, exercise

activates the peripheral immune system, instigating activation of inflammatory mediators in the central nervous system including astrocytes which provide neuroprotective sources (Little et al., 2002; Miller and O'Callaghan, 2005). Astroglial activation has multiple roles including release of neurotrophic factors called glia-derived neurotrophic factor (GDNF), removal of toxic debris, and repairing tissue (Hui-Ming et al., 2003; Cotman and Berchtold, 2002). The astrocytes strengthen the brain and allow for more resistance to toxins. Consequently, the correlation of significant increase of astrocytes in exercise animals compared to standard housing is suggested to be true. Conversely, in standard housing animals where physical activity is minimal, there is no need for excess stimulation of astrocytes.

Using only one stress-marker is not sufficient enough to look at this study holistically. Another indicator is necessary to examine the role of astrocytes since astrocytes are increased after exercise to promote brain plasticity but are also increased to facilitate in the recovery process from neuronal damage as occurs during chronic stress. Inflammatory-mediated activation of astrocytes contributes to meeting the cellular demands of the brain during these times of metabolic need. Having a second marker would verify the role of astrocytes.

Bax-expressed protein markers provide more information in how gliosis responds to exercise and stress. Bax staining would have observed the degree of apoptotic neurons, displayed initially by pyknotic nuclei and/or morphological changes in the hippocampal region, indicating the extent of neuronal insult caused by stress and exercise both isolated and combined. Unfortunately, the staining showed too inconsistent to provide either quantitative or good qualitative analysis. It may be that by looking at the

brain 24 hours after last stressor is too long of a period for analysis of short term effects because Bax expression may normalize by then. Thus looking at the brain 1-6 hours after last stressor may provide better quality in Bax expression (Krajewska et al, 1995). Determining how to optimize Bax immunoreactivity in the future would be beneficial in providing valuable information on the degree of apoptotic cell death by clarifying the role of astrocytes. Additionally, perfusion may have interfered with the quality of Bax expression, and so it is worthwhile in the future to take fresh brain tissue and perform Western blotting to look at the protein content. The downside to Western blotting, however, is its inability to give protein locations but only protein concentrations. Nonetheless, it may provide more reliable results for Bax-expressed proteins compared to immunocytochemical staining.

Other issues within the study could have prevented other significant findings from being found. One is the unequal sampling size variation among the conditions. This could have skewed the data and given unreliable results. Thus, future work should include equal sample sizes and ideally at least 4 animals' brain tissue for each condition. Due to time constraints in this research study, however, this was not feasible.

Altering the time frame mice are under chronic stress may provide more information in how exercise affects stress-induced activities. Exercise mice had ample time to build up adaptation to exercise before given the stressor because they were allowed to exercise during the habituation period along with the actual 2-week experimental period. It could be possible the 2-week period of stress was only acute and so did not have much impact on the brains and so a longer period of chronic stress is necessary to see the effects of exercise. In a study conducted in tree shrews, it showed

that one month of social stress is not enough to cause neuronal loss but required a longer stress period to begin seeing significant neuronal damage (Vollmann-Honsdorf et al., 1997). In another study, 3 weeks of repeated restraint stress did not even show any significant alterations in neurotrophic expression such as BDNF (Kuroda and McEwen, 1998). Thus maybe recovery was normalized quickly after the stressor, showing no neuronal damage and subsequently no astrocytic stimulation. This could possibly explain why there was no significant increase in astrocytic levels in standard housing animals given stress. Consequently, possibly prolonging the stress period could allow observations of exercising counteracting stress because neuronal damage will have taken place.

Although no conclusive results were found illustrating the beneficial effects of exercise against stress in this particular study, it is still of great importance to emphasize the importance of moderate exercise for improved quality of life. Too little exercise or even too much exercise can cause harm to the body (Radak, 2008). Inactivity is a predominant problem now in the Unites States where susceptibility to numerous common health problems including diabetes and heart attacks is high. The body needs a physical stressor in order to ward off dangerous toxins and oxidants that enter the body and prevent health problems. It is important, however, to also understand that too much exercise is dangerous for the body (Radak, 2008).

A common misunderstanding that many may associate with exercise and health is that there is a direct correlation saying an increase in exercise must cause an increase in health, which is not necessarily true. Prolonged exercise of high intensity can lead to increased cortisol levels, compromising the immune system leading to more

susceptibility to sickness (Okutsu et al., 2005; Smith and Myburgh, 2006). Illness, in turn, leads to activation of microglia, which causes the release of pro-inflammatory and neurotoxic factors such as lipopolysaccharide (LPS). LPS directly affects dopamine-containing neurons by inducing degeneration (Hui-Ming et al., 2004). Additionally, free radicals are produced from activated microglia and can contribute significantly to oxidative damage in the brain (Hui-Ming et al., 2003). Consequently, overstressing the body can lead to deleterious effects in the brain as a result of the inflammatory response.

Studies have shown the detrimental effects of extreme mechanical stress on other regions such as skeletal muscle, causing apoptosis and necrosis to cells as a result of irreparable sarcomere structural damage (Armand et al., 2003). This does not provide enhancement to physiological functions and causes more harm. This perpetuates the oxidative damage cycle by initiating the inflammatory response system and causing release of neurotoxic factors. So it suggested that overtraining the body, a problem for endurance athletes, is more damaging than beneficial. Moderate exercise provides time for rest in between exercise whereas overtraining does not leave enough time for the body to recuperate from the stressor and adapt to the changes (Radak et al, 2001). Moderation provides maximum efficiency and minimal overall damage to body and brain health.

Future Implications

Stress has a huge impact on the lives of many individuals and contributes to neurodegenerative illnesses. Prevention is necessary but more in-depth understanding is required about the roles and mechanisms of stress and exercise in the brain. Developing a scientific understanding on how a simple tool like exercise can positively alter the

effects of chronic stress can do invaluable good for the population as a whole and so it is worthwhile to ascertain what can make this study better in the future to obtain better results and make meaningful clinical applications. Finding the pathways of how exercise is combating stress could lead to better interventions. In the meantime, there is extensive support for the advocacy of moderate exercise, so the practice of regular exercise should continue.

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