CHANGES IN mRNA LEVELS FOR BRAIN-DERIVED NEUROTROPHIC FACTOR

AFTER WHEEL RUNNING IN RATS SELECTIVELY BRED FOR

HIGH- AND LOW-AEROBIC CAPACITY

by

JESSICA LEE GROVES-CHAPMAN

(Under the Direction of Rod K. Dishman)

ABSTRACT

Voluntary wheel running by rodents increases mRNA expression of BDNF, a neurotrophic protein in the hippocampus that is important in learning, memory, and prophylaxis against depression. Evidence from single and repeated exposures to activity wheel running is unclear about whether there is a dose-response relation between running distance and the induction of BDNF expression. After three weeks of exposure to activity wheels, rats bred for high or low intrinsic aerobic running capacity had similar increases in BDNF mRNA in Ammon's horn area 1 (CA1) of the hippocampus. Furthermore, cumulative running distance was not related to BDNF mRNA. The results, observed across a wider range of running distances than reported in prior studies, do not support a putative dose-response relationship between wheel running and BDNF expression in the hippocampus. Research is needed to further clarify the threshold of wheel running that induces BDNF transcription and mechanisms that explain this neurotrophic effect.

INDEX WORDS: brain-derived neurotrophic factor, activity wheel running, hippocampal formation, selective breeding

CHANGES IN mRNA LEVELS FOR BRAIN-DERIVED NEUROTROPHIC FACTOR AFTER WHEEL RUNNING IN RATS SELECTIVELY BRED FOR HIGH- AND LOW-AEROBIC CAPACITY

by

JESSICA LEE GROVES-CHAPMAN

B.A., Wesleyan College, 2008

A Thesis Submitted to the Graduate Faculty of the University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2010

CHANGES IN mRNA LEVELS FOR BRAIN-DERIVED NEUROTROPHIC FACTOR AFTER WHEEL RUNNING IN RATS SELECTIVELY BRED FOR HIGH- AND LOW-AEROBIC CAPACITY

by

JESSICA LEE GROVES-CHAPMAN

Major Professor: Rodney K. Dishman

Committee: Philip V. Holmes
Patrick J. O'Connor

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
July 2010

DEDICATION

This thesis is dedicated to the memory of my younger cousin, Paul Joseph Mula. I have been fortunate to have a second chance at higher education, to better myself and contribute in whatever way I may to the continuation of scientific research. My final year of this degree was not easy due to the sudden loss of such a dear friend and member of my family. It was Paul's strength and dedication to changing his own life that pushed me through the most difficult times. He never got to see the fulfillment of his dreams, so I dedicate the fulfillment of mine to him.

ACKNOWLEDGMENTS

I would like to acknowledge everyone that contributed to this achievement in my life. First, I would like to thank my patient and supportive husband, Marvin E. Chapman, III. Without your encouragement, positive attitude, financial backing, and love this would not have been possible. You always had the perfect words to make any situation brighter, and for every moment I doubted my abilities, you were there reminding me of how much you believed in me. Thank you for being such strength in my life, and my biggest fan. I look forward to the next challenge, with you by my side.

To my major professor, Dr. Dishman, thank you for accepting me as a student. Thank you for your patience with my missed "deadlines" and various other life interruptions. I am grateful for every opportunity I have been given while working with you, and I look forward to a few more years of scientific exploration and improvements to my writing under your direction. You are an excellent mentor, and I am fortunate to have you as my advisor.

I would like to thank Dr. Holmes for introducing me to *in situ* hybridization and the many joys of working with animals. I have truly enjoyed my time in your lab, and I look forward to a few more years of learning under your direction. I appreciate all of the extra advisement you provided as I was planning for my next academic step forward, it paid off big time!

Thank you, Dr. O'Connor, for agreeing to be a part of my committee even though I did not include human subjects. I always appreciate your perspective on my research with animals, and the reminder that things should not always be looked at from one direction. Thank you for always being available to answer questions, regarding topics from class or just personal research interests.

To Dr. Koch and Dr. Britton at the University of Michigan, thank you for the opportunity to work with these unique animals, and for your collaboration with our lab at the University of Georgia.

I would also like to acknowledge my family, specifically my parents. There have been many times in my life that I am certain you were unsure about my future. But despite your doubts you provided me with every opportunity to get me back on track. Well, here I am, and it has a lot to do with the second chance you gave me. I love you both and I am extremely grateful that you did not give up on me. Be proud of yourselves, this is your accomplishment too.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Physical activity, both single and repeated sessions, has been accompanied by neurotrophic and neuroprotective effects in rats and mice that might be beneficial for human neurological disorders and insults including depression, Parkinson's disease, Alzheimer's dementia, and ischemic stroke (Dishman et al., 2006; Holmes, Yoo, & Dishman, 2006; Reiss, Dishman, Boyd, Robinson, & Holmes, 2009; Waters et al., 2008). The induction of neuropeptides, neurotransmitters, and other neurotrophic factors in studies involving exercise models suggests that exercise has a vital role in alterations to neurobiological systems. One neurotrophic factor that has received attention in regards to the influence of exercise and physical activity on expression is brain-derived neurotrophic factor (BDNF).

BDNF, a trophic factor with similar qualities to nerve growth factor, is highly expressed within the hippocampal formation (Binder & Scharfman, 2004). The primary binding of BDNF occurs on the tyrosine receptor kinase B (trkB), which is responsible for regulating neuronal survival, promoting neurite outgrowth, and maintaining synaptic connectivity within the CNS (Zhang & Ko, 2009). BDNF's role in supporting neuronal survival, differentiation, connectivity, and neurogenesis, specifically activity-dependent synaptic plasticity has been widely established throughout the literature (Binder & Scharfman, 2004; Johnson, Rhodes, Jeffrey, Garland, & Mitchell, 2003; Scharfman et al., 2005; Zhang & Ko, 2009). BDNF is also a key player in neuronal development, promoting survival and growth of dorsal root ganglion, hippocampal, and cortical neurons (Binder & Scharfman, 2004). The location of BDNF within the hippocampus suggests a role in learning and memory, and BDNF induction has been observed in the

hippocampus during contextual learning models. Blocking BDNF leads to impairment in spatial learning, and decreased BDNF expression has been linked to neurological disease and pathology (Binder & Scharfman, 2004). Exogenous application of BDNF promotes the function and sprouting of 5-HT neurons in rats and effective antidepressants increase BDNF messenger ribonucleic acid (mRNA), an indication of a possible role for BDNF in the development and/or treatment of depression (Binder & Scharfman, 2004). Evidence also supports a combination of anti-depressant therapy and physical activity, which has been indicated as having possible additive effects on depression symptoms and levels of BDNF mRNA (Russo-Neustadt, Beard, & Cotman, 1999; Russo-Neustadt, Ha, Ramirez, & Kesslak, 2001; Russo-Neustadt, Beard, Huang, & Cotman, 2000).

Like other neuropeptides (i.e. galanin), BDNF protein and mRNA levels can be elevated following physical activity, both forced and voluntary (Adlard & Cotman, 2004; Berchtold, Chinn, Chou, Kesslak, & Cotman, 2005; Berchtold, Kesslak, Pike, Adlard, & Cotman, 2001; Chen & Russo-Neustadt, 2005; Duman, Schlesinger, Russell, & Duman, 2008; Garza, Ha, Garcia, Chen, & Russo-Neustadt, 2004; F. Gomez-Pinilla, Vaynman, & Ying, 2008; Johnson & Mitchell, 2003; Kim et al., 2005; Kitamura, Mishina, & Sugiyama, 2003; Klintsova, Dickson, Yoshida, & Greenough, 2004; Neeper, Gomez-Pinilla, Choi, & Cotman, 1996; Rhodes et al., 2003; A. Russo-Neustadt, et al., 1999; A. Russo-Neustadt, et al., 2001; A. A. Russo-Neustadt, Alejandre, Garcia, Ivy, & Chen, 2004; A. A. Russo-Neustadt, et al., 2000; Soya et al., 2007; Tong, Shen, Perreau, Balazs, & Cotman, 2001; Van Hoomissen, Chambliss, Holmes, & Dishman, 2003; Shoshanna Vaynman, Zhe Ying, & Fernando Gomez-Pinilla, 2004; Zheng et al., 2006). More recent evidence suggests that BDNF levels are not only induced with exercise, but are correlated to running distance, with higher levels of BDNF mRNA expression observed in

animals that run for a longer duration or farther distance (Adlard, Perreau, & Cotman, 2005; Bjornebekk, Mathe, & Brene, 2005; Griesbach, Hovda, Gomez-Pinilla, & Sutton, 2008; Griesbach, Hovda, Molteni, Wu, & Gomez-Pinilla, 2004; Johnson & Mitchell, 2003; Johnson, et al., 2003; Oliff, Berchtold, Isackson, & Cotman, 1998; Widenfalk, Olson, & Thoren, 1999).

To date, literature that has evaluated exercise-induced BDNF expression has provided contradictory evidence for the correlation between running distance and BDNF protein or mRNA levels. Specifically, of the 7 studies that have provided evidence of a correlation between distance run and BDNF levels (Adlard, et al., 2005; Bjornebekk, et al., 2005; Griesbach, et al., 2008; Griesbach, et al., 2004; Johnson & Mitchell, 2003; Johnson, et al., 2003; Oliff, et al., 1998), wheel exposures ranged from a few hours to a few weeks, nearly half the correlations reported had a 95% confidence interval that included zero, and only 4 studies evaluated continuous running effects without the confounding of additional treatments or manipulations (Adlard, et al., 2005; Johnson & Mitchell, 2003; Johnson, et al., 2003; Oliff, et al., 1998).

The purpose of this study was to evaluate the current evidence regarding a dose-response relationship between distance run and exercise-induced changes in BDNF expression. Although other studies have evaluated this theorized dose-response relationship and indicated correlations with and without the influence of confounding manipulations, discrepancies in amount of running wheel activity measured (acute versus chronic exercise) and a discontinuation of the dose-response effect beyond a certain point of exposure prevent a concise assessment of this effect (Adlard, et al., 2005; Johnson & Mitchell, 2003; Johnson, et al., 2003; Oliff, et al., 1998). Therefore, the following review and experiment will address the limitations of current research and provide the results from an original investigation of BDNF mRNA expression after activity wheel or sedentary assignment in rats selectively bred for high or low aerobic running capacity.

CHAPTER 2

REVIEW OF LITERATURE

It has been established that BDNF is important in neuronal functioning, neuroplasticity, and neurogenesis; and key to understanding exercise effects on depression and cognitive functioning. The following review will present evidence supporting an exercise-induced increase in BDNF protein and mRNA within the hippocampal formation of the rat and mouse brain; as well as the contradictory evidence of a dose-response effect of distance run on levels of exercise-induced BDNF.

Exercise-induced Increases in BDNF

Brain-derived neurotrophic factor and nerve growth factor (NGF) can increase activities of free-radical scavengers, increasing protection against free-radical damage (Neeper, et al., 1996). In a 1996 study by Neeper et al., Sprague-Dawley rats were allowed 0, 2, 4, or 7 nights of free-access to a running wheel. BDNF mRNA and NGF were measured following the cessation of the exercise condition. A significant elevation in BDNF mRNA was observed following 2 days of running compared to sedentary controls, and this significance was maintained at about 20% above controls through 7 nights of wheel running. Hybridization of BDNF mRNA primarily occurred in the pyramidal layers of Ammon's horn and in the dentate gyrus of the hippocampus. In CA1 and CA4 a significant increase in BDNF mRNA was reported after 7 nights. Hybridization increased to 80% above controls in CA1 and 40% above controls in CA4. NGF peaked at 30% above controls after 2 nights, but returned to levels similar to controls after 4 and 7 nights. NGF mRNA was elevated to 20 – 30 % above controls after night 7 in both the DG and CA4. An increase in BDNF mRNA was also observed in the neocortex and cerebellum,

but the largest increase was limited to the hippocampus. Neeper et al. (1996) identified the importance of the location of exercise effects as being on brain areas associated with cognitive function (hippocampus and caudal cortex), not directly related to the motor system.

Molteni, et al. (2004) compared the effects of running wheel exercise on rats exposed to regular and high fatty diets. BDNF mRNA levels increased 135% over sedentary controls in animals with 2 months access to exercise and on a regular diet ($p < 0.01$). In sedentary animals fed a high fat diet, BDNF mRNA levels reportedly decreased to 76% compared to controls; however, this decrease was reduced to only 91% of controls when high fat diet animals were allowed 2 months access to running wheels. BDNF protein levels increased from 100 to 185% in exercising animals on regular diets, and 61% to 141% in exercising high fat diet animals (both increases displayed a significance level of $p < 0.01$). Molteni et al (2004) also reported a decrease in synapsin I in the high fat diet-sedentary group. Synapsin I was measured because of its role in modulating neurotransmitter release by BDNF. The authors indicated that this decrease in synapsin I was reversed with the application of exercise. In the high fat diet exercising group, synapsin I mRNA was increased from 76% to 105% ($p < 0.01$). The authors concluded that exercise was sufficient to reverse the negative effects of a high fat diet on BDNF mRNA and synapsin I mRNA levels (Molteni et al., 2004).

In a study conducted by Engressar-Cessar, Anderson, and Cotman (2007) the effects of anti-depressant drugs and exercise on BDNF protein levels were compared. Female mice were assigned to a fluoxetine-only treatment condition, an exercise-only treatment condition, and a combination of fluoxetine-exercise treatment condition. Low (5 mg/kg), medium (10 mg/kg) or high (25 mg/kg) doses of fluoxetine were evaluated with and without a 21 day exercise condition. Throughout the duration of the study the high dose group's running distance became

significantly lower than the other running groups. In addition, the high dose group weighed significantly more at the 3 week end point, a possible explanation for the reduction in running distances. Contrary to other exercise models, exercise-only control mice did not yield significant increases in BDNF protein levels. In the fluoxetine-only group, a significant increase in hippocampal BDNF was recorded in the high dose group only (a 496% increase). No interaction was found for exercise and drug treatment in any of the exercise and drug treatment combination groups, and no additive effects of drug treatment were observed. Fluoxetine-only groups increased in cytogenesis and dentate gyrus volume; however, the combination group did not yield any additional effects. Insulin-like growth factor protein was also measured within the hippocampus with no increases in BDNF protein, IGF-1 protein, or neurogenesis reported (Engesser-Cesar, Anderson, & Cotman, 2007).

Russo-Neustadt, et al. (2004) conducted an investigation of norepinephrine involvement in BDNF mRNA expression. Sprague-Dawley rats were placed into one of five treatment settings: exercise-only, Reboxetine-only (norepinephrine reuptake inhibitor), combined Reboxetine and exercise, Citalopram- only (SSRI), or combined Citalopram and exercise. BDNF mRNA levels were measured across several time points and found to be elevated significantly in the Reboxetine-only treatment group, exercise-only group, and combination group after 2 days. Citalopram-only increased BDNF mRNA significantly; however the combination of Citalopram and exercise produced no significant changes in BDNF mRNA after 2 days. At the 7 day time point Reboxetine-only significantly increased BDNF in CA1 of the hippocampus, exercise only significantly increased BDNF in all areas except CA1, and the combined effects of Reboxetine and exercise increased BDNF significantly in all areas of the hippocampus. Citalopram-only treatment had no effect on BDNF mRNA at the 7 day time point,

yet both the exercise-only and combination group showed significant increases in BDNF mRNA. Russo-Neustadt et al. (2004) indicates that this is an exercise effect at the 7 day time point. After 14 days of voluntary wheel running the Reboxetine-only treatment group displayed no significant increases in BDNF mRNA, while both the exercise-only and combination groups maintained significant increases in BDNF m RNA. Citalopram-only treatment resulted in no significant increase in BDNF mRNA after the 14 day time point, although a significant increase was recorded in the combination group. Overall, Russo-Neustadt, et al. (2004) reported that the combination of exercise and Reboxetine yielded more widespread and longer-lasting effects on BDNF mRNA. Reboxetine also appeared to have an effect on activity levels indicated by significantly higher running distance after 14 days of running compared to the Citalopram-only group. The Citalopram treatment was less consistent, with results appearing only after 14 days of treatment. With both drug treatment groups, more robust results were achieved with the combination groups, indicating a large role of exercise in the increased BDNF levels (Russo-Neustadt, et al., 2004).

In the Russo-Neustadt et al. (2004) investigation of NE involvement in BDNF induction it was reported that the NE reuptake-inhibitor, exercise, and the combination of these two treatments elevated BDNF mRNA. To further clarify NE involvement in BDNF signaling, Chen & Russo-Neustadt (2005) provided an abbreviated explanation of the PI-3 kinase pathway. NE-induced activation of PKA leads to increased neural activity (induced by either antidepressant or exercise). This causes an increase in NE which then binds to G-protein coupled receptors. The receptors activate PKA and CREB phosphorylation, yielding BDNF transcription and exocytosis. BDNF binds to autophosphorylating trkB leading to CREB phosphorylation by way of the PI-3 kinase pathway to activate BDNF transcription in a cyclical manner. The PI-3 kinase pathway

can also be transactivated directly through the NE – G-protein coupled receptor binding. Increased BDNF has also been associated with enhanced activity of cyclic AMP response element binding protein (CREB) (Chen & Russo-Neustadt, 2005). In this study the effects of voluntary wheel running and/or tranylcypromine (a MAOI antidepressant) treatment on the pro-survival PI-3 kinase signaling pathway were assessed. Chen & Russo-Neustadt (2005) found that BDNF immunoreactivity and trk phosphorylation were significantly higher in the tranylcypromine-exercise combination group when compared to tranylcypromine-only and sedentary control groups. Trk phosphorylation was also elevated in the exercise-only group. CREB phosphorylation increased 60 times above controls in the exercise-only group ($p = 0.05$), and 90 times above controls in the tranylcypromine-exercise group ($p = 0.0053$). Chen & Russo-Neustadt (2005) reported that an increase in intracellular signaling increased BDNF transcription which regulates synaptic plasticity in an activity-dependent manner.

Farmer, et al. (2004) explored the mechanisms involved in exercise-induced neurogenesis in Sprague-Dawley rats by evaluating BrdU-positive cells and BDNF mRNA expression. The numbers of BrdU-positive cells in the dentate gyrus of the hippocampus of adult rats were significantly increased following voluntary wheel running. Specifically, runners were found to have significantly more new neurons ($p< 0.05$). In addition to higher numbers of new neurons, runners presented with significantly more long-term potentiations (LTP, $p< 0.05$) following pulse stimulation, and the induction threshold for LTP was reduced. Farmer et al. (2004) reported significant increases in BDNF, GluR5, and NR2b mRNA expression within the dentate gyrus (DG) of the hippocampal formation. Both GluR5 and NR2b were measured to evaluate molecular mechanisms underlying changes in the DG of exercising animals. The authors

concluded that voluntary exercise increases both neurogenesis and LTP of synaptic efficacy in the DG of adult rats (Farmer et al., 2004).

It was hypothesized in one investigation that exercise enhances NMDA receptors in the hippocampus which subsequently yield enhanced BDNF production and neurogenesis in mice (Kitamura, et al., 2003). Kitamura, et al. (2003) proposed that BDNF, an activity-induced gene, was dependent upon transcriptional regulation that is initiated by Ca+ increase generated through activation of NMDA receptors or voltage-sensitive calcium channels. NMDA receptor ε1 subunit knockout (KO) mice and wild type (WT) mice were allowed access to running wheels, followed by an analysis of cell proliferation and neurogenesis. A 2-fold difference in running distance was observed between KO and WT mice, with KO mice running significantly less. This difference was controlled for by adding a third group of wild type mice that received reduced exercise access (reduced-WT). Results indicated that exercise increased cellular proliferation in WT, but not KO mice. In the reduced-WT group, BrdU positive cells (markers for cell proliferation) still increased, indicating a role for the NMDA receptor in running distance. BDNF levels increased by 77% compared to controls in the wild type group and 65% compared to controls in the reduced-WT group. No significant difference was recorded between BDNF levels in the KO exercise group compared to controls. Kitamura et al. (2003) concluded that basal proliferation was the same for WT and KO mice, but exercise-induced proliferation differed. An exercise-induced increase in BDNF was determined to be dependent upon NMDA receptors, but basal BDNF was not (Kitamura, et al., 2003).

While many exercise studies have thoroughly evaluated continuous voluntary running, one study compared daily and intermittent running wheel access (Berchtold, et al., 2005). A third group was included in this project to evaluate long-term running effects, and these animals

were given 3 months of uninterrupted running wheel access. BDNF protein levels were measured in all groups and a significant increase was found with daily access $F(7, 78) = 6.71$, $p < 0.0001$ and intermittent access $F(4, 54) = 4.54$, $p < 0.0001$. In the daily running group, BDNF protein levels increased gradually and reached a level of significance above controls at 14 days (150%, $p < 0.005$); which was subsequently maintained at a significant level after 28 days (174%, $p < 0.0001$), and continued to increase after 90 days (222%, $p < 0.0001$) In the intermittent group a small but significant increase was reported after 14 days (124%, $p = 0.057$), 21 days (145%, $p < 0.0001$), and after 28 days (159%, $p < 0.0001$). At 28 days the daily and intermittent groups reached equivalent levels of BDNF protein. BDNF protein levels remained elevated following the cessation of running wheel access, although levels decreased more rapidly in the intermittent group compared to the daily group. Berchtold, et al. (2005) indicated an association between total amount of exercise days and the rate of decline in BDNF protein levels. An additional experiment was performed to assess the rate of increase in BDNF protein following a second bout of exercise. The authors concluded that once exposed to exercise a lower threshold of exercise was required for an increase in BDNF protein levels to occur (Berchtold, et al., 2005).

Presumed Functions of BDNF Also Influenced by Exercise

BDNF's role in the survival, growth, and maintenance of neurons is well represented throughout the literature and emphasized in the neuroprotective benefits of exercise-induced upregulation of BDNF protein and mRNA. However, BDNF's role in behavior and cognition, specifically learning and depression models, are also important in understanding exercise-induced improvements to this system. One study, conducted by Vaynman, et al. (2004) investigated exercise-induced cognitive enhancement and BDNF's role in this process. Utilizing the Morris Water Maze (MWM) task, the authors found that exercise enhanced learning

acquisition and resulted in shorter escape latencies in rats placed into the MWM. Additionally, exercised rats displayed enhanced recall ability and spent significantly more time in the correct quadrant. To evaluate BDNF's role in this learning and spatial memory model, hippocampal BDNF was blocked with a microbead injection of IgG during exercise. IgG effectively prevented exercise-induced increases in BDNF mRNA, trkB receptor mRNA, and other end products of BDNF action (CREB and synapsin I). Exercised rats receiving IgG did not display similar learning and memory enhancements, and took significantly longer to locate the platform than the other exercising group. Exercise-IgG rats displayed performance on the MWM that was not significantly different from that of the control group. The authors conclude that blocking BDNF was specific for exercise-induced enhancements because sedentary animals receiving IgG did not reflect similar deficits in task acquisition (Vaynman, Z. Ying, & Gomez-Pinilla, 2004).

Adlard & Cotman (2004) investigated the relationship between corticosterone and BDNF with an experiment involving immobilization stress and exercise in mice in which it was determined that exercise counteracts the deleterious effects of stress (i.e. the reduction in BDNF protein). Corticosterone (CORT) was measured because of its relationship with decreased BDNF expression; specifically, high levels of CORT have been associated with low levels of BDNF. Significantly different levels of CORT were reported between control and stressed sedentary animals up to 1 hour $p < 0.05$, 2764% at 0 hr and 576% at 1 hr). BDNF protein levels differed significantly between the control and stressed animals at the 5 (11.2% compared to controls) and 10 hour (8.9% of controls) time points. Exercise was shown to elevate CORT levels above sedentary non-stressed controls for all exercise groups ($p < 0.05$), and exercise displayed a protective effect against stress-induced decreases in BDNF protein expression despite an increase in CORT levels. Adlard & Cotman (2004) reported a 684% increase in

CORT levels of control-exercising animals and a 683% increase in stressed-exercising animals at the 0 hour time point, followed by a 184% increase in the non-stressed-exercise group and 202% increase in the stressed-exercise at the 10 hour time point. In the non-stressed-exercise group BDNF protein increased significantly ($p < 0.05$) relative to sedentary controls, while stress alone decreased BDNF significantly ($p < 0.05$) at the 10 hour time point compared to sedentary controls. Stressed and non-stressed exercising animals displayed significant increases in BDNF protein levels at both time points ($p < 0.05$) compared to control and stressed sedentary animals. The authors concluded that CORT regulates stress associated decreases in BDNF protein, but it is not a primary modulator for exercise-induced increases in BDNF (Adlard & Cotman, 2004).

In addition to stress, depressive symptoms have also been associated with decreased levels of BDNF mRNA (Duman, et al., 2008). In an evaluation of exercise effects on depression and BDNF mRNA, Duman, et al. (2008) observed the effects of 3 to 4 weeks of voluntary wheel running on performance during a learned helplessness task, forced swim task (FST), and tail suspension test in mice. These tasks are often used as models for depression-like responses in animals (Duman, et al., 2008). Exercise produced a significant decrease in escape latencies and decreased the number of escape failures in the learned helplessness task. Compared to chronic amitriptylline treatment (an antidepressant administered in the drinking water daily), exercise produced similar results. In the forced swim task, both exercise and the antidepressant desipramine significantly decreased immobility, no significant difference was reported between groups. In the tail suspension test desipramine reduced immobility to a greater degree than exercise ($p = 0.05$ for the exercise group, $p = 0.02$ for the desipramine group). BDNF mRNA was measured and an increase was found in CA1 of the hippocampus after 3 weeks of exercise, and after 1 or 3 weeks in the DG. Duman et al. (2008) further evaluated the role of BDNF in the

antidepressant effects of exercise with a comparison of exercise effects on FST performance in wild type mice and BDNF (+/-) heterozygous knockout mice. BDNF (+/-) mice have half the normal complement of BDNF and performance in the FST was not altered by exercise in this group compared to sedentary BDNF (+/-) mice. Exercising wild type mice did reduce immobility in the FST, providing evidence for the role of BDNF in the antidepressant effect of exercise. No significant differences in running distances were determined between groups, suggesting that the difference in FST performance was not due to distance run (Duman, et al., 2008).

Dose-Response: Running Distance and BDNF protein and mRNA levels

While many studies support an exercise-induced increase of BDNF protein and BDNF mRNA, the range of running distances reported creates a problem for determining the amount of running required to yield this induction. The following evidence, also presented in Table 3.1, indicates a dose-response effect of running distance and BDNF induction, supported by moderate to strong correlations and effect sizes.

In order to evaluate the neurogenesis and neuroplasticity actions of BDNF, Griesbach, Hovda, Molteni, Wu, & Gomez-Pinilla (2004) investigated the effects of exercise on traumatic brain injury in male Sprague-Dawley rats. Rats were divided into 4 groups: acute exercise fluid percussion injury (FPI), acute exercise-Sham injury, delayed exercise FPI (DFPI), delayed exercise Sham injury, and sedentary control groups matched for injury status. In the delayed onset exercise condition access to running wheels was delayed for 14 days post-injury. As in other studies, exercise increased BDNF protein levels and enhanced performance on the MWM. Specifically, the authors reported a significant elevation in BDNF protein levels in the DFPI-exercise group, $F(1, 64) = 4.98$ ($p < 0.05$), compared to FPI-sedentary animals, and a significant

elevation in BDNF in the Sham-exercise group, $F(1, 64) = 3.712$ ($p < 0.05$), compared to the Sham-sedentary group. A significant difference was found between the Sham-exercise and FPI-exercise groups, $F(1, 64) = 4.84$ ($p < 0.05$). These results represent the effects on the hippocampus ipsilateral to the injury site. In the contralateral hippocampus a significant interaction effect between exercise x injury x time was reported as $F(1, 64) = 6.3$, $p < 0.05$. A dose-response of running distance on BDNF protein levels was observed in acute and delayed Sham-exercise animals ($r^2 = 0.76$, $p<0.0005$, $n = 15$). A correlation for distance run and BDNF protein induction was also recorded in the DFPI-exercise group for both ipsilateral ($r^2 = 0.308$) and contralateral ($r^2 = 0.386$) to the injury site. No dose-response was recorded for CREB or synapsin I levels. MWM performance indicated a significant increase in latency to reach platform for the acute FPI-exercise group; however, delayed onset exercise showed improvement in latency for this group (Griesbach, et al., 2004).

In a 2008 study involving voluntary exercise and amphetamine treatment, Griesbach, et. al (2008) again investigated the effects of traumatic brain injury on cognitive performance. Male Sprague-Dawley rats ($N = 48$) were assigned to one of eight conditions: sedentary or exercise (RW), saline (S) or amphetamine (AMPH), Sham or controlled cortical impact (CCI). The exercise condition involved free access to running wheels for 7 days. The authors reported running differences between the sham and CCI groups, but indicated no drug effects on overall running performance. Increases in BDNF protein were found for both exercise and amphetamine in the Sham and CCI groups; however, the combination of exercise with drug treatment eliminated this effect. Synapsin I levels were also influenced by exercise or drug treatment, and the combination of the two treatments removed this effect. A correlation between total running distance and BDNF protein levels was indicated for the Sham-S-RW group ($r = 0.74$, $n = 6$), and

a smaller correlation was found for the Sham-AMPH-RW group ($r = 0.47$, $n = 6$) (Griesbach, et al., 2008).

Studies evaluating BDNF mRNA levels in animal models of depression indicate that reduced levels of BDNF are associated with depressive symptoms (Engesser-Cesar, et al., 2007; Russo-Neustadt, et al., 2004). A 2005 study conducted by Bjornebekk, Mathe, and Brene evaluated the effects of voluntary running wheel access on two selectively bred strains of rats: Flinders Sensitive Line (FSL, an animal model of depression) and Flinders Resistant Line (FRL, a control for FSL rats). No differences in basal BDNF mRNA levels were found between the two strains of rats. In FRL, running increased BDNF mRNA within the dorsal and ventral blade of the DG ($p< 0.01$). No increase in BDNF mRNA was observed in the FSL. Basal levels of cell proliferation were recorded for FSL and FRL, and levels were indicated as being significantly lower in the FSL compared to the FRL ($p< 0.05$). Running increased FSL cell proliferation by 450% ($p< 0.05$), brining FSL to cell proliferation levels equivalent to FRL levels. The authors reported a correlation between BDNF mRNA levels and running distance in the FRL rats, with greater running distance yielding higher BDNF mRNA expression (Bjornebekk, et al., 2005).

The Bjornebekk, et al. (2005) study was not the first to evaluate different rat strains for exercise effects on neurochemistry and the antidepressant effects of exercise. A 1999 study by Widenfaulk et al. utilized a spontaneously hypertensive rat strain to evaluate the impact of running distance on reported exercise effects. In this study spontaneously hypertensive rats, reported to run longer distances (9000 to 21,000m/day) were divided into groups permitted to run either 3200 or 8500 meters a night. A separate group was given unlimited access to running wheels, followed by cessation of running wheel access after 5 weeks exposure. Increased

running activity led to increased levels of BDNF and trkB mRNA in the hippocampus in the 8500m group only. Widenfaulk, et al. (1999) suggests increases in BDNF mRNA in the 8500m group provide evidence for a dose-response effect of running in spontaneously hypertensive rats, although no correlational data is provided in support of this theory. The authors reported that removing running wheel access after 5 weeks of continuous running leads to a long-term decrease in both BDNF and trkB mRNA within the hippocampus and cortex cerebri (a 50% decrease compared to controls). This decrease was observed after 5 and 10 days of exercise interruption. In addition, the authors observed a correlation between the down regulation of BDNF mRNA and level of wheel running activity, where long-distance runners displayed a more pronounced decrease ($r = 5.907$, $p < 0.05$) (Widenfalk, et al., 1999).

An investigation of age and exercise effects on neurochemistry in mice was conducted using young (2 months), late middle-aged (15 months), and old (24 months) mice (Adlard, et al., 2005). Each age-group was divided into two conditions: 4 weeks of voluntary running or 4 weeks sedentary control. Average running distance increased significantly in the younger mice over the 4 week period, and the older mice ran significantly less than the other groups. All groups had a significant increase in BDNF protein levels after 7 days of running wheel access, and all groups displayed similar baseline levels. At the 14 day time point all groups displayed a marked decrease in BDNF protein levels back to baseline levels. At the 21 and 28 day time points, young and late middle-aged mice displayed significant increases in BDNF protein ($p < 0.02$ for both groups) that was not observed in the older mice. A significant difference was reported between groups at the 21 day time point ($p < 0.02$), and a correlation between distance run and BDNF protein levels was observed after 7 days for young mice only ($r = 0.959$, $p = 0.053$) (Adlard, et al., 2005).

Many studies evaluating exercise-induced increases in BDNF protein and mRNA include confounding elements such as additional treatments, manipulations, or strain/age differences. Oliff, Berchtold, Isackson, and Cotman (1998) presented the results of voluntary wheel running on BDNF mRNA levels and other components important for the upregulation of BDNF (i.e. Exons I- IV) in male, Fisher-344, rats without the inclusion of other manipulations/treatments. Rats were divided into 4 groups: 6 hours wheel running, 12 hours wheel running, and time-matched sedentary controls for both time points. Both BDNF mRNA and Exon III were significantly elevated within the hippocampal regions analyzed in rats after a 3 hour training run followed by 10 days of no running. BDNF mRNA and Exon 1 were significantly elevated after 6 hours of running (post training run and 10 day exercise lapse), while Exon IV decreased. Following 12 hours of running, BDNF mRNA and Exons III and IV levels were no different from controls; although Exon I and II expression increased at the 12 hour time point. A positive correlation was indicated for total distance run and BDNF mRNA levels after 6 hours of running ($n = 6$). Specific areas within the hippocampal formation were indicated as displaying this correlation, including the hilus region ($r = 0.866$, $p<0.003$), CA1 ($r = 0.894$, $p<0.001$), and CA3 ($r = 0.704$, $p<0.050$) (Oliff, et al., 1998).

Dose-Response: Running Distance, Related Outcomes of BDNF, and Other Neuropeptides

BDNF protein and BDNF mRNA are important markers of neuroplasticity and neuron survival following insult or injury. However, many studies also evaluate related outcomes and end-products of BDNF induction following exercise. The following studies indicate a dose-response for running distance and related BDNF outcomes, as well as other neuropeptides.

In a study conducted by Vaynman, Ying, Yin, & Gomez-Pinilla (2006) it was proposed that exercise uses BDNF-mediated mechanisms to change specific properties of hippocampal

synapses at the presynaptic membrane by selectively increasing protein levels of synapsin I and synaptophysin, but not syntaxin. Synapsin I is involved in vesicle clustering and is important for sustaining release of neurotransmitter under high levels of activity, synaptophysin is important for endocytosis, and syntaxin is involved in exocytosis within the hippocampus. Vaynman, Ying, Yin, & Gomez-Pinilla (2006) reported a correlation between hippocampal synapsin I and running distance over a 3 day period of voluntary running wheel access ($r = 0.9$, $p < 0.05$), and a positive trend (not significant, $p = 0.22$) was found between synaptophysin and running distance. Additionally a positive correlation was determined between synapsin I and synaptophysin in the exercising animals ($r + 0.91$, $p < 0.05$) (Vaynman, Ying, Yin, & Gomez-Pinilla, 2006)

Exercise induction of BDNF and related outcomes of BDNF protein and BDNF mRNA have not only been assessed within the brain. Gomez-Pinilla, Ying, Roy, Molteni, & Edgerton (2002) investigated induction of BDNF, its receptor, and related outcomes within the lumbar spinal cord of rats exposed to 3 or 7 days of voluntary wheel running. The authors reported exercise-induced increases in BDNF protein at the 7 day time point, and increases in BDNF mRNA and TrkB mRNA within the lumbar spinal cord at both the 3 and 7 day time points. The authors also indicated a positive correlation between distance run and BDNF mRNA levels ($r = 0.91$, $p<0.01$, $n = 6$). Synapsin I protein and mRNA levels were also elevated in exercise rats at both time points. In a separate experiment, the soleus muscle was injected with botulinum toxin type A (BTX-A) to determine the role of neuromuscular activity on BDNF induction. The BTX-A injections reduced BDNF and synapsin I mRNA levels in sedentary rats, and exercise following injection was not found to improve this condition. The authors conclude that this suggests a minimum level of neuromuscular activity for BDNF induction to occur (F. E. Gomez-Pinilla, Ying, Roy, Zhong, & Edgerton, 2002).

Exercise enhancements to neurochemistry are not limited to BDNF and its end products. In a 2006 study by Holmes, Yoo, and Dishman, the neuropeptide galanin (Strohle et al.) was measured in the locus coeruleus (LC) following exercise or clomipramine (tricyclic antidepressant) treatment. A significant interaction was reported between exercise and drug treatment, with both exercise-only and clomipramine-only groups showing significantly increases in prepro GAL mRNA levels ($p = 0.02$). A correlation between running distance (at 4 weeks) and prepro GAL mRNA within the LC of exercise-only rats ($r = 0.65$, $p < 0.01$) that was independent of drug therapy was also reported. The authors conclude that this provides evidence that the effect of exercise on GAL gene expression is dose dependent (Holmes, et al., 2006).

Selective Breeding for Running

Further analysis of dose-dependent induction of BDNF protein and mRNA has been presented in studies that have evaluated strain differences in running distance. Johnson & Mitchell (2003) presented evidence that strain differences due exist among rats commonly used in exercise models. Specifically, Johnson & Mitchell (2003) recorded running distances, BDNF protein, and NT-3 among 4 strains of rats: Brown Norway (BN), Dark Agouti (DA), PVG, and Sprague-Dawley (SD). Each strain was divided into four groups: 1 night running wheel access, 1 night sedentary control, 7 nights running wheel access, and 7 nights sedentary control. A total of 128 animals were included, 8 per strain per group. Johnson & Mitchell (2003) found strain differences in running distance after 7 nights of running, with PVG and DA strains running significantly farther than the other strains. The authors also indicated a dose-response effect of running distance on BDNF protein levels (see Table 3.1).

This same group of authors presented another study in 2003 investigating the impact of selective breeding for increased aerobic capacity on exercise-induced BDNF protein levels

(Johnson, et al., 2003). Utilizing a selectively bred strain of distance running mice (S mice from the 25th generation of a selectively bred strain of distance running mice) and a control group of standard lab mice, Johnson et al. (2003) evaluated baseline hippocampal BDNF protein levels and running distance. Baseline levels did not differ significantly between S mice and controls. After 7 nights of running wheel access a significant difference was found in the distance run between S mice and controls. Additionally, BDNF protein increased by 171% relative to baseline in S mice, while a non-significant increase of 20% relative to baseline was observed in control mice. A significant interaction between condition (exercise versus sedentary) and strain (S versus control mice) was observed, and running distance was correlated with BDNF protein levels regardless of strain. Johnson, et al. (2003) measured neurotrophin-3 levels due to its proposed association with BDNF and found no alteration in NT-3 levels with exercise in either group of mice. The authors suggest that the increase in running distance is due to more short-fast bursts of running in the S mice, and that speed, not distance, is correlated with a greater increase in BDNF levels (Johnson, et al., 2003).

In another study involving mice selectively bred for high levels of wheel running, neurogenesis and BDNF levels were assessed in female mice obtained from the 27th generation of selectively bred runners (S mice) (Rhodes, et al., 2003). S mice ran approximately 18,000 meters a day on average, which was 2 times more than the WT mice used as controls in this study. Rhodes, et al. (2003) reported that Brd-U positive cells increased more in S mice than in the WT mice (5-fold increase in S mice, 4-fold increase in WT mice), and this increase was related to running distance. There was no difference in Brd-U positive cells recorded in sedentary control mice. Running wheel access increased DG volume for both groups (20% in WT, 17% in S mice). BDNF levels also increased significantly for both groups, 56% for WT

and 38% for S mice, but no correlation was observed between BDNF levels and running distance. The authors propose that the positive correlation between running distance and neurogenesis and BDNF levels lost in the S mice is due to a ceiling effect for running distance. Baseline levels for BDNF and BrdU cell counts were not provided (Rhodes, et al., 2003).

More recent investigations involving selectively bred runners utilize a strain of rats developed by Koch and Britton at the University of Michigan. These rats are selected for intrinsic aerobic capacity and then selectively bred across generations to develop two distinct groups: rats with higher than normal aerobic capacity (HCR) and rats with lower than normal aerobic capacity (LCR) (L. G. Koch & Britton, 2001; Waters, et al., 2008). Waters, et al. (2008) obtained female rats from generations 9 and 10 of this strain to evaluate the effects of running on corticosterone and dopamine levels. The HCR and LCR rats displayed a 471% difference in aerobic capacity as measured by a forced treadmill condition. HCR rats ran significantly more than LCR rats throughout the experiment and accessed wheels more. A trend to run faster, longer, and further than LCR rats was observed in the HCR group. Individual endurance scores were not related to wheel-running distance, duration, or intensity. LCR rats weighed more at the beginning and end of the experiment compared to HCR rats, although weight gained throughout the experiment by both groups did not reach significance. Baseline corticosterone levels were reported as similar for both groups. After 8 weeks of wheel running, HCR rats displayed markedly lower corticosterone levels than the LCR group. Initial levels of DA were higher in the HCR than the LCR group, as assessed by higher striatal dopaminergic activity; however, running wheel access abolished this difference (no significance in changes, HCR indicated a slight decrease and LCR a slight increase in DA). Overall wheel running increased from week 2 to week 4 for both groups, peaked at week 4 and leveled off (Waters, et al., 2008).

The investigation by Waters, et al. 2008 of LCR and HCR rats indicated the possibility of genetic influences on distance run, as well as the physiological and neurochemical differences present in different strains of rats. While the literature presented supports a dose-response effect for distance run and BDNF mRNA levels, this has not been evaluated in rats selectively bred for higher and lower aerobic capacity. The idea behind the inclusion of such animals is that higher aerobic capacity rats will run significantly more than their lower aerobic capacity counterparts, expanding the range of activity wheel exposure beyond prior dose-response studies. In the following experiment, an investigation of exercise-induced BDNF mRNA expression between HCR and LCR rats was evaluated. Specifically, the relationship between running distance and BDNF MRNA expression was assessed in an attempt to determine if the hypothesized dose-response effect exists.

CHAPTER 3

CHANGES IN mRNA LEVELS FOR BRAIN-DERIVED NEUROTROPHIC FACTOR AFTER WHEEL RUNNING IN RATS SELECTIVELY BRED FOR HIGH- AND LOW-AEROBIC CAPACITY*

*Groves-Chapman, J.L., Murray, P.S., Stevens, K.S., Monroe, D., Koch, L.G., Britton, S.L., Holmes, P.V., & Dishman, R.K. To be submitted to *Brain Research*.

Abstract

We evaluated levels of exercise-induced brain-derived neurotrophic factor (BDNF) messenger RNA (mRNA) expression within the hippocampal formation of rats selectively bred for 1) high intrinsic (i.e., untrained) aerobic capacity (High Capacity Runners, HCR) and 2) low intrinsic aerobic capacity (Low Capacity Runners, LCR) and 3) unselected Sprague-Dawley (SD) rats with and without free access to running wheels for three weeks. The specific aim of the study was to determine whether a dose-response relationship exists between cumulative running distance and levels of BDNF mRNA. No additional treatments or behavioral manipulations were used. HCR, LCR, and SD rats were blocked by strain and randomly assigned to sedentary or activity (voluntary access to activity wheel) conditions. Animals were killed after 21 days of exposure to the assigned conditions. A main effect for time was observed for running distance in all strains ($p=0.005$), and a time by strain quadratic trend was also found to be significant ($p=0.036$). In general, HCR rats ran more than LCR rats, but not more than SD rats. Levels of BDNF mRNA were increased in wheel runners in Ammon's horn area 1 (CA1), and this increase persisted after adjustment for age ($p=0.040$). There were no differences according to strain, regardless of exercise assignment. Linear regression analysis revealed no relationship between total running distance and BDNF mRNA expression in any region. The results of this study support that BDNF mRNA expression is increased by unlimited access to activity wheel running for 3 weeks but is not dependent upon accumulated running distance.

Key words: brain-derived neurotrophic factor, hippocampus, activity wheel running, selective breeding

Introduction

Brain-derived neurotrophic factor (BDNF), a neurotrophin that functions similarly to nerve growth factor, has widespread effects throughout the central nervous system. Experimental evidence indicates BDNF's role in supporting neuronal survival, differentiation, connectivity, and neurogenesis, specifically activity-dependent synaptic plasticity (Binder & Scharfman, 2004; Johnson, et al., 2003; Scharfman, et al., 2005; Zhang & Ko, 2009). BDNF is also a key player in neuronal development, promoting survival and growth of dorsal root ganglion, hippocampal, and cortical neurons (Binder & Scharfman, 2004). BDNF is located throughout the CNS, with a high expression found within the hippocampal formation (Binder & Scharfman, 2004). The primary binding of BDNF occurs on the tyrosine receptor kinase B (trkB) which is responsible for regulating neuronal survival, promoting neurite outgrowth, and maintaining synaptic connectivity within the CNS (Zhang & Ko, 2009).

The location of BDNF within the hippocampus suggests a role in learning and memory, and BDNF induction has been observed in the hippocampus during contextual learning models. Blocking BDNF leads to impairment in spatial learning, and decreased BDNF expression has been linked to neurological disease and pathology (Binder & Scharfman, 2004). Exogenous application of BDNF promotes the function and sprouting of 5-HT neurons in rats and effective antidepressants increase BDNF messenger ribonucleic acid (mRNA), an indication of a possible role for BDNF in the development and/or treatment of depression (Binder & Scharfman, 2004). Evidence also supports a combination of anti-depressant therapy and physical activity, which has been indicated as having possible additive effects on depression symptoms and levels of BDNF mRNA (Russo-Neustadt, et al., 1999; Russo-Neustadt, et al., 2001; Russo-Neustadt, et al., 2000).

Physical activity, in general, has been associated with potentially neuroprotective benefits for neurological disorders and insults other than depression; including, Parkinson's disease, Alzheimer's, and ischemic stroke (Dishman, et al., 2006; Holmes, et al., 2006; Reiss, et al., 2009; Waters, et al., 2008). The induction of BDNF, as well as other neuropeptides and neurotransmitters, in studies involving exercise suggests that BDNF protein and mRNA levels can be elevated following physical activity, both forced and voluntary (Adlard & Cotman, 2004; Berchtold, Chinn, Chou, Kesslak, & Cotman, 2005; Berchtold, Kesslak, Pike, Adlard, & Cotman, 2001; Chen & Russo-Neustadt, 2005; Duman, Schlesinger, Russell, & Duman, 2008; Garza, Ha, Garcia, Chen, & Russo-Neustadt, 2004; Gomez-Pinilla, Vaynman, & Ying, 2008; Johnson & Mitchell, 2003; Kim et al., 2005; Kitamura, Mishina, & Sugiyama, 2003; Klintsova, Dickson, Yoshida, & Greenough, 2004; Neeper, Gomez-Pinilla, Choi, & Cotman, 1996; Rasmussen et al., 2009; Rhodes et al., 2003; Russo-Neustadt, et al., 1999; Russo-Neustadt, et al., 2001; Russo-Neustadt, Alejandre, Garcia, Ivy, & Chen, 2004; Russo-Neustadt, et al., 2000; Soya et al., 2007; Tong, Shen, Perreau, Balazs, & Cotman, 2001; Van Hoomissen, Chambliss, Holmes, & Dishman, 2003; Vaynman, Ying, & Gomez-Pinilla, 2004; Zheng et al., 2006). Further evidence suggests that BDNF levels are correlated to running distance, with higher levels of BDNF mRNA expression observed in animals that run for a longer duration or farther distance (Adlard & Cotman, 2004; Bjornebekk, Mathe, & Brene, 2005; Griesbach, Hovda, Gomez-Pinilla, & Sutton, 2008; Griesbach, Hovda, Molteni, Wu, & Gomez-Pinilla, 2004; Johnson & Mitchell, 2003; Johnson, et al., 2003; Oliff, Berchtold, Isackson, & Cotman, 1998; Widenfalk, Olson, & Thoren, 1999).

To date, literature that has evaluated exercise-induced BDNF expression has provided contradictory evidence for the correlation between running distance and BDNF protein or mRNA

levels. Specifically, of the 7 studies that have provided evidence of a correlation between distance run and BDNF levels (Adlard & Cotman, 2004; Bjornebekk, et al., 2005; Griesbach, et al., 2008; Griesbach, et al., 2004; Johnson & Mitchell, 2003; Johnson, et al., 2003; Oliff, et al., 1998; Widenfalk, et al., 1999) (Table 3.1), only 4 studies evaluated continuous running effects without the confounding of additional treatments or manipulations (Adlard & Cotman, 2004; Johnson & Mitchell, 2003; Johnson, et al., 2003; Oliff, et al., 1998). Also, for nearly half the reported correlations a 95% confidence interval would include zero. Activity wheel exposures varied widely from a few hours to several weeks, and running distances ranged from 250 meters to 7,000 meters per day in rats, or were not reported (Oliff et al., 1998).

The purpose of this study was to evaluate, using rats that are heterogeneous on intrinsic running capacity, the possibility of a dose-response relationship between distance run and increases in BDNF mRNA expression within the hippocampus without the confounding influences of additional manipulations or treatments. Other studies have evaluated this dose-response relationship and indicated correlations without the influence of confounding manipulations, but discrepancies in amount of running wheel activity measured (acute versus chronic exercise) and a discontinuation of the dose-response effect beyond a certain point of exposure prevent a concise assessment of this effect (Adlard, et al., 2005; Johnson & Mitchell, 2003; Johnson, et al., 2003; Oliff, et al., 1998) In this study, three strains of rats were used for evaluation: Sprague-Dawley derived outbred rats, high capacity runners (HCR) selectively bred for high intrinsic aerobic capacity, and low capacity runners (LCR) selectively bred for low intrinsic aerobic capacity. The selectively bred strains of rats, obtained from the University of Michigan, were included for two reasons: to increase the range of running distance among rats

and to test the impact of intrinsic running capacity on activity wheel running distance and BDNF mRNA levels.

Running capacity can be operationally divided into two components: 1) intrinsic i.e., inborn capacity that operates in the sedentary untrained state and 2) adaptational, that acquired in response to activity, Recently, an animal model system was developed, by selectively breeding rats to express greater or lesser intrinsic aerobic capacity (Koch & Britton, 2005; Koch & Britton, 2001; Koch & Britton, 2008). These high capacity and low capacity rat strains (HCR and LCR, respectively) differ widely in their capacity to run on a treadmill to the point of exhaustion (Koch & Britton, 2001; Koch & Britton, 2008) and demonstrate a substantial divergence in running speed, duration, and maximal oxygen uptake (Høydal, Wisloff, Kemi, & Ellingsen, 2007). These strain differences may be associated with several traits subordinate for exercise performance including a greater capacity of HCR to deliver and utilize O_2 in skeletal muscle (Howlett et al., 2008). Here we use the LCR/HCR rat model system to investigate whether intrinsic (untrained) running capacity influences exercise-induced upregulation of BDNF. Previous evidence indicating differences in running distances among rats of different strains has been reported (Bjornebekk, et al., 2005; Johnson & Mitchell, 2003; Waters, et al., 2008; Widenfalk, et al., 1999). Therefore, it was hypothesized that rats bred for higher-aerobic capacity would have higher average voluntary running distances in activity wheels than the other two strains.

Age also modifies exercise exposures and outcomes in rats. Average running distances decrease with age; such that older rats maintain a constant or decreasing level of activity over time, whereas younger rats increase average running distance across time (Adlard, et al., 2005).

Because age and exercise can have differential effects on the expression of activity-related protein in the brain, the present experiments included and analyzed age as a covariate.

It was hypothesized that all animals (HCR, LCR, and Sprague-Dawley rats) in the activity wheel groups would show higher levels of BDNF mRNA within the hippocampal structures analyzed than sedentary controls of the same strain. In addition, it was hypothesized that running distance would be correlated with levels of exercise-induced BDNF mRNA, such that higher levels of running would result in higher levels of BDNF mRNA.

Materials and Methods

Animals and Experimental Design

Adult, male rats of 3 strains were housed individually in 30x30x30 polycarbonate cages (HCR, n=22 LCR, n=18, SD, n=31) in a temperature and humidity-controlled environment on a 12-hour light/dark schedule. Food and water were available *ad libitum* and animals were weighed weekly. Selectively bred HCR and LCR rats were obtained from the University of Michigan. The mean treadmill running duration and daily distance range, provided by the University of Michigan for phenotype of intrinsic fitness variability between strains, were as follows: HCR rats had a mean best time of 64 ± 3.23 minutes (mean ±SD) and a daily running distance mean of 1,642.64 ± 137.58 meters, compared to a mean best time of 18 ± 1.72 minutes and daily distance mean of 251.7 ± 31.1 meters for LCR rats. For comparison, out bred Sprague-Dawley rats (SD; aged 60 to 321 days) supplied by Harlan were also studied; ages for the LCR and HCR rats ranged from 121 to 218 days. All rats were randomly separated into wheel running and sedentary groups. Wheel running groups consisted of 9 HCR, 9 LCR, and 11 SD rats. All animals underwent a 2 week quarantine and facility adaptation period prior to

assignment to activity wheel or sedentary conditions. All procedures were conducted in accordance with NIH Guide for Care and Use of Laboratory Animals.

Exercise Protocol

Rats were blocked by strain (HCR, LCR, and Sprague-Dawley) and randomly assigned to either activity wheel (AW) or sedentary (SED) condition. To increase statistical power and because of wheel availability, SD rats were assigned at 2:1 ratio of sedentary to wheel running groups. Activity wheels (MiniMitter) with a circumference of 105 cm were placed in cages and attached to a magnetic revolution counter. Home cages of sedentary rats did not contain an activity wheel. AW rats were given unlimited access to activity wheels for 21 days. Wheel revolutions were recorded and daily distances determined by multiplying the circumference (105 cm) of the activity wheel by the number of revolutions. Previous evidence regarding enriched environment versus activity wheel access and BDNF induction indicates that benefits derived from activity wheel access exceed enriched conditions; therefore this study did not include an enriched environment control condition (Gobbo & O'Mara, 2005; Olson, Eadie, Ernst, & Christie, 2006).

In Situ Hybridization Histochemistry

Animals were killed by rapid decapitation at the cessation of the 21 day exercise or control exposure immediately after the 12-hour light cycle. Brains were extracted and stored at -80◦C. Brains were sliced into 12 μm sections at the level of the dorsal hippocampal formation (HF) using a Microm cryostat (Carl Zeiss, Waldorff, Germany) and thaw-mounted to gelatin coated microscope slides. In situ hybridization methods used are reported in detail elsewhere (Van Hoomissen, et al., 2003). Briefly, slides were fixed in 4% formaldehyde in 0.12M sodium phosphate-buffered saline, rinsed in PBS, and placed in 0.25% acetic anhydride. Sections were

then be dehydrated through a series of ethanol washes, delipidated in chloroform, rinsed again in ethanol, and allowed to dry.

Oligonucleotide probes were obtained from Oligos Etc (Wilsonville, OR). The BDNF oligonucleotide probe is complimentary to bases 650-699 of the mouse BDNF mRNA as previously described by Van Hoomissen, et al. (2003). Column separation was utilized to separate unicorporated nucleotides from the probes. Sections were hybridized with radiolabeled probes in solution containing formamide, NaCL, Tris-HCl, EDTA, sodium pyrophosphate, sodium dodecyl sulfate, and dextran sulfate. Sections were incubated in hybridization solution, followed by a series of washes to reduce nonspecific binding. Microscope slides were rinsed in deionized water and allowed to dry. Hybridized brain sections were exposed to autoradiographic film for a period of 3 weeks and then developed.

Film Analysis

Autoradiographic film analysis was conducted with ImageJ (National Institutes of Health, Bethesda, MD), a coputerized analysis system to determine optical density (OD) within the HF; specifically the dentate gyrus (DG), Ammon's horn area 1 (CA1), Ammon's horn area 2 (CA2), and Ammon's horn area 3 (CA3).

Data Analysis

Running distance over time was analyzed with a 3 group (HCR vs. LCR vs. Sprague-Dawley) x 3 time (weeks 1-3) mixed-model repeated measures analysis of variance (RM-ANOVA that was also adjusted for age (RM-ANCOVA). The Huynh-Feldt ε correction for sphericity violation was used. Optical densities from the in situ hybridization analyses for each brain region were compared using 3 group (HCR vs. LCR vs. Sprague-Dawley) x 2 condition (activity wheel vs. sedentary) ANOVA that was also adjusted for age by ANCOVA. Bonferroni

followup contrasts were used. Linear regression analysis adjusted for age was utilized to assess the effects of daily running distance on mRNA for BDNF. All analyses were conducted using SPSS Windows version 17.0 (SPSS, Inc., Chicago, IL).

Results

Running Distance

Weekly running distance was highly reliable, ICC (2, 3) =0.96, 95% CI, 0.93-0.98, and increased over time, $F(2,52)=7.497$, $\varepsilon = .694$, $p=0.005$. There was a main effect of strain, $F(2, 26)=4.426$, $p=0.022$, whereby HCR rats ran more than LCR rats, $p=0.022$, but not more than SD rats, $p=0.158$. There was also a strain by quadratic trend effect of time, $F(2, 26)=3.8$, $p=0.036$ (Figure 3.1). Adjustment for an age by time effect ($p=0.008$) gave similar results for the time effect, $F=12.459$, $\varepsilon = .768$, $p<0.001$, and the strain by quadratic trend effect of time, $F(2, 25)=3.67$, $p= 0.040$. To further assess the strain by quadratic time effect, each strain was analyzed individually with age as a covariate. LCR rats daily running distances indicated a quadratic increase, $F(1,7) = 6.24$, $p = .036$, between the first (380 ± 193) and third (672 ± 323; p=0.017) weeks. SD rats increased running distances linearly, $F(1,9)=4.86$, $p=.055$, across all three weeks (week 1: 1112 ± 945, week 2: 1695 ± 2404, week 3: 2293 ± 3461). There was a non-significant increase, $F(1,7) = 4.10$, $p=.083$, across weeks for HCR (week 1: 3152 ± 2155, week 2: 4502 ± 2374, week 3: 4726 ± 3220).

Based on a median split across strains, high runners averaged (mean ±SD) 4073 ± 3431 meters per day across the three weeks and 5022 ± 4438 meters per day during week three, while low runners averaged 394 ± 233 meters per day across the three weeks and 533 ± 379 meters per day during week three.

Hippocampal BDNF mRNA Expression

CA1

The results for the ANCOVA of hippocampal BDNF mRNA expression was statistically significant only within Ammon's horn region 1 (CA1) for the exercise assignment, $F(1, 61)=17.003$, $p=0.040$ (Figure 3.2). Age did contribute as a covariate to differences in BDNF mRNA expression within this region of the hippocampal formation, $F(1, 61)=3.93$, $p=0.052$. There was no significant effect for strain on BDNF mRNA expression, $F(2, 61)=5.359$, $p=0.089$, and no interaction effect for strain and exercise assignment, $F(2, 61)=.164$, $p= 0.849$.

Regression analysis revealed no significant relation between total distance run and BDNF mRNA expression within the CA1 region, $R=0.266$, $p=0.189$ (Figure 3.3), and this remained non-significant after controlling for age and strain, $\beta=0.40$, $p=0.173$. There were no interaction effects by age or strain with distance. An additional regression analysis using running distance during the last week also revealed no relation with mRNA expression, $p=0.925$, and this finding remained after controlling for age and strain, $p=0.950$. BDNF in CA1 was not different between the top half of all runners (designated as high runners, median split) 73.8 ± 19.7 vs. the bottom half of all runners (designated as low runners) 72.8 ± 13.8, $t(25) = .157$, $p = .876$, or after adjustment for age, $p = 0.705$ (Figure 3.5).

Dentate Gyrus, CA2, and CA3

The omnibus F test of BDNF mRNA expression within the dentate gyrus was not significant for exercise assignment, $F(1, 62)=1.412$, $p=0.355$, or strain, $F(2, 62)=1.615$, $p=0.382$ (Figure 3.2) and there was no interaction of exercise assignment and strain. Null effects of exercise and strain remained after controlling for a significant effect for age on BDNF mRNA in the dentate gyrus region, $F=13.095$, $p=0.001$. A regression analysis for the DG was also

conducted, and revealed so significant relation between mRNA expression and total running distance (Figure 3.4). BDNF in DG was not different between high runners (median split) 82.0 ± 11.2 vs. low runners 80.1 ± 17.0, t(25) = .345, $p = 0.733$ or after adjustment for age, $p = 0.915$ (Figure 3.5).

Results for BDNF mRNA expression within Ammon's horn regions 2 and 3 (CA2 and CA3) were also found to be non–significant and were similar to the levels obtained for the DG analysis (data not reported).

Discussion

We observed no dose-response relation between accumulated running distance and BDNF mRNA expression in the hippocampus, despite daily running distances for HCR rats that were 7 times higher than LCR rats, and 2 times higher than SD rats, by the third week of wheel access. BDNF mRNA within the hippocampus was elevated by access to an activity wheel regardless of strain; although a significant increase was limited to Ammon's horn area 1 (CA1). The increase in BDNF mRNA after wheel running exposure remained significant after adjusting for age, which was correlated with running distance and mRNA expression Effects of activity wheel running in the DG and Ammon's horn areas 2 and 3 (CA2 and CA3) remained non–significant after adjustment for age.

Previous research also found an age effect for both running distance and BDNF mRNA expression (Adlard, et al., 2005), similar to our present findings. Adlard, et al. (2005) reported an increase in BDNF levels after only one week of exercise, that proceeded to decrease to baseline levels following the second week of activity wheel exposure. The authors also indicated that younger animals tended to have higher levels of BDNF expression, in conjunction with linear increases in running behavior across the three week study duration (Adlard, et al., 2005).

Running data for the present study indicated that running distance was significantly different among strains and increased over time, both with and without adjusting for age.

In the present study, BDNF mRNA expression was significantly increased in only one area of the hippocampal formation evaluated, Ammon's horn area 1 (CA1). Previous research has reported that this region is a key component in the consolidation of long-term memories, matching sensory input recall to contextual memory, and order of recall for visual stimuli (Farovik, Dupont, & Eichenbaum, 2010; Hoge & Kesner, 2007; Remondes & Schuman, 2004). These findings pair well with the Greenwood et al. (2009) study that reported improved contextual memory consolidation following activity wheel running, and that this was mediated by neurochemical and trophic changes observed within the hippocampus (i.e. increased BDNF mRNA expression). The role of BDNF within the hippocampus is clearly established as being involved in cognitive and behavioral improvements, neuronal outgrowth, and learning and memory; but there remains a debate as to which is more influenced by physical activity. The current experiment did not include behavioral or cognitive evaluations, so as to reduce the influence of additional maipulations on running behavior and BDNF mRNA expression.

As CA1 has been primarily implicated in memory, it is doubtful that any influences on BDNF mRNA expression were due to environmental enrichment instead of exercise. Evidence provided by Gobbo & O'Mara (2005) supports that increases in BDNF brought about by exercise yielded neuroprotective benefits that could not be replicated by environmental enrichment alone In their evaluation, exercise provided improvements in learning following a neurodegenerative insult, in addition to increasing BDNF levels (Gobbo & O'Mara, 2005). A review by Olson and colleagues (2006) suggests that enriched environments influence neurogensis via a different mechanism than voluntary exercise. The different pathways in which voluntary exercise and

enriched enviroment lead to cell proliferation and differentiation may partially explain why studies, such as the one conducted by Gobo & O'Mara (2005), do not always yield similar results for both exposure conditions (Olson et al., 2006). An enriched environmental control was not included in this experiment because of these discrepancies in the evidence supporting the influence of enriched environmental conditions.

The present study is not the first to indicate changes in only one region of the hippocampus. In a study conducted in 1998 by Oliff and colleagues, BDNF mRNA expression increased after only 6 hours of activity wheel exposure and only within CA1 (Oliff, et al., 1998). A 2004 study by Farmer and colleagues reported an increase in BDNF mRNA after 10 days of activity wheel exposure, but only within the dentate gyrus (Farmer et al., 2004). Finally, a 2009 study conducted by Greenwood and colleagues found that 6 weeks of activity wheel running increased BDNF mRNA within the dentate gyrus and CA1 (Greenwood, Strong, Foley, & Fleshner, 2009). With such an array of exposure times and differenences in regions that express BDNF mRNA, it is evident that current literature remains uncertain as to the required amount of running exposure necessary for induction, and which hippocampal area is primarily influenced by exercise. The effect sizes (Hedge's d) reported here for CA1 (d= 0.4139) and DG (d=0.3495), both adjusted for age, were lower than reported in several of the past studies of the dose-response relation between wheel running and BDNF expression (see Table 3.2) but were within the 95% confidence intervals (random effects model) of several of those studies.

The primary goal of this investigation was to evaluate the putative dose-response relation between BDNF mRNA expression within the hippocampus and running distance. While we observed the expected differences in running distances between the strains used (HCR daily distances ranged from 3700m to 4200m, SD ranged from 1100m to 4300m, and LCR ranged

from 400m to 700m across the three week exposure), no dose-response relation was observed for any region of the hippocampus. Previous dose-response evaluations (Table 3.1) reported differences in running distance between strains of selectively bred animals that reached 1 to 3 times the lowest reported distance within each study (Adlard, et al., 2005; Bjornebekk, et al., 2005; Griesbach, et al., 2008; Griesbach, et al., 2004; Johnson & Mitchell, 2003; Johnson, et al., 2003), and one study reported a dose-response without including running distance comparisons (Oliff, et al., 1998). We report here a 7-fold difference in running distances between the selectively bred HCR and LCR lines, with SD rats falling at about half the distance of the HCR running group. This wide range permitted a fuller evaluation of the dose-response relation than provided by prior reports. During week 3, the mean running distance per day ranged from 57 meters to 13,800 meters.

Additionally, we report here an increase in BDNF mRNA expression following a minimum of approximately 600 meters/day of running. Some earlier studies also reported increased BDNF mRNA with similarly small exposures to activity wheel running, but a minimum distance has yet to be established for BDNF mRNA induction. Many studies have not reported running distances, so in addition to duration of exposure, future studies are needed to determine the threshold of activity required for mRNA induction and to distinguish the effects of running distance from other features of activity wheel exposure.

The studies described in Table 3.1 varied in exposure times from 6 or 12 hours to 28 and 35 days (Adlard, et al., 2005; Bjornebekk, et al., 2005; Oliff, et al., 1998). The majority of studies, however, provided 1 to 7 days of activity wheel access (Griesbach, et al., 2008; Griesbach, et al., 2004; Johnson & Mitchell, 2003; Johnson, et al., 2003). We reasoned that this short duration of exposure might not allow full consolidation of wheel running behavior, which

we have observed previously after 3 weeks (Holmes et al., 2006; Reiss et al., 2009; Van Hoomissen et al., 2003). Further evaluation of running exposure revealed that many 'acute' studies included a pre-exposure novelty check, followed by a 10-day activity cessation period. This protocol, presented in both Oliff et al. (1998) and Neeper et al. (1996), involves exposing animals to 3 days of activity wheel access to contol for novelty effects. All animals were then removed from the activity wheel for 10 days, and subsequently divided into sedenteray or activity conditions for a set time point of exposure. This would appear to account for any influence of a novel environment or stimuli on BDNF expression. However, Oliff and colleagues reported that BDNF levels were increased immediately after the 10-day cessation period in animals that had received the 3 days of pre-training when compared to controls that had not received pre-training. This suggests that the BDNF effects observed in this study after 6 hours of activity wheel exposure might have been the result of the previous training or, conversely, stimuli exposure effects rather than the result of acute running.

In additon to a wider range in running behavior and activity wheel exposure, the present study included 70 animals of three strains for comparison, with 9 to 11 animals from each strain in the exercise condition. Many of the studies presented in Table 3.1 include only 5 to 8 (with most being in the 5 – 6 range) animals per group. Finally, this experiment was free of confounding manipulations which allowed for a specific evaluation of running behavior and mRNA induction due to exercise manipulation only.

In summary, the findings from this experiment confirm a dose-independent effect of exercise on BDNF mRNA in region CA1 of the rat hippocampus. However, the results indicate that BDNF mRNA induction is independent of daily or total running distance, despite daily running distances for HCR rats that were 7 times higher than LCR rats, and 2 times higher than

SD rats, by the third week of wheel access. The conflicting prior evidence of dose-response can be partly explained by discrepancies in the amount or timing of running wheel exposure, regions of the brain that were examined, and the confounding of wheel exposure with other manipulations in several studies. Future research is needed to better clarify the threshold of running wheel exposure and its features (e.g., distance, timing, or novelty) sufficient to elicit BDNF transcription in the hippocampus and to determine the molecular mechanisms that explain the effect of wheel running on BDNF expression.

References

Adlard, P. A., & Cotman, C. W. (2004). Voluntary exercise protects against stress-induced decreases in brain-derived neurotrophic factor protein expression. *Neuroscience, 124*(4), 985.

Adlard, P. A., Perreau, V. M., & Cotman, C. W. (2005). The exercise-induced expression of BDNF within the hippocampus varies across life-span. *Neurobiology of Aging, 26*(4), 511-520.

Berchtold, N. C., Chinn, G., Chou, M., Kesslak, J. P., & Cotman, C. W. (2005). Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. *Neuroscience, 133*(3), 853-861.

Berchtold, N. C., Kesslak, J. P., Pike, C. J., Adlard, P. A., & Cotman, C. W. (2001). Estrogen and exercise interact to regulate brain-derived neurotrophic factor mRNA and protein expression in the hippocampus. *European Journal of Neuroscience, 14*(12), 1992-2002.

Binder, D. K., & Scharfman, H. E. (2004). Brain-derived neurotrophic factor. *Growth Factors, 22*(3), 123-131.

Bjornebekk, A., Mathe, A. A., & Brene, S. (2005). The antidepressant effect of running is associated with increased hippocampal cell proliferation. *International Journal of Neuropsychopharmacology, 8*(3), 357-368.

Chen, M. J., & Russo-Neustadt, A. A. (2005). Exercise activates the phosphatidylinositol 3-kinase pathway. *Brain Research Molecular Brain Research, 135*(1-2), 181-193.

Dishman, R. K., Berthoud, H. R., Booth, F. W., Cotman, C. W., Edgerton, V. R., Fleshner, M. R. (2006). Neurobiology of exercise. *Obesity (Silver Spring), 14*(3), 345-356.

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

Farmer, J., Zhao, X., van Praag, H., Wodtke, K., Gage, F. H., & Christie, B. R. (2004). Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats in vivo. *Neuroscience, 124*(1), 71-79.

Farovik, A., Dupont, L. M., & Eichenbaum, H. (2010). Distinct roles for dorsal CA3 and CA1 in memory for sequential nonspatial events. *Learning and Memory, 17*, 12-17.

Garza, A. A., Ha, T. G., Garcia, C., Chen, M. J., & Russo-Neustadt, A. A. (2004). Exercise, antidepressant treatment, and BDNF mRNA expression in the aging brain. *Pharmacology Biochemistry and Behavior, 77*(2), 209-220.

Gobbo, O. L., & O'Mara, S. M. (2005). Exercise, but not environmental enrichment, improves learning after kainic acid-induced hippocampal neurodegeneration in association with an increase in brain-derived neurotrophic factor. *Behavioral Brain Research, 159*(1), 21-26.

Gomez-Pinilla, F., Vaynman, S., & Ying, Z. (2008). Brain-derived neurotrophic factor functions as a metabotrophin to mediate the effects of exercise on cognition. *European Journal of Neuroscience, 28*(11), 2278-2287.

Greenwood, B. N., Strong, P. V., Foley, T. E., & Fleshner, M. (2009). A Behavioral Analysis of the Impact of Voluntary Physical Activity on Hippocampus-Dependent Contextual Conditioning. *Hippocampus, 19*(10), 988-1001.

Griesbach, G. S., Hovda, D. A., Gomez-Pinilla, F., & Sutton, R. L. (2008). Voluntary exercise or amphetamine treatment, but not the combination, increases hippocampal brain-derived neurotrophic factor and synapsin I following cortical contusion injury in rats. *Neuroscience, 154*(2), 530-540.

Griesbach, G. S., Hovda, D. A., Molteni, R., Wu, A., & Gomez-Pinilla, F. (2004). Voluntary exercise following traumatic brain injury: brain-derived neurotrophic factor upregulation and recovery of function. *Neuroscience, 125*(1), 129-139.

Hoge, J., & Kesner, R. (2007). Role of CA3 and CA1 subregions of the dorsal hippocampus on temporal processing of objects. *Neurobiology Of Learning And Memory, 88*(2), 225-231.

Holmes, P. V., Yoo, H. S., & Dishman, R. K. (2006). Voluntary exercise and clomipramine treatment elevate prepro-galanin mRNA levels in the locus coeruleus in rats. *Neuroscience Letters, 408*(1), 1-4.

Howlett, R. A., Kirkton, S. D., Gonzalez, N. C., Wagner, H. E., Britton, S. L., Koch, L. G. (2008). Peripheral oxygen transport and utilization in rats following continued selective breeding for endurance running capacity. *Journal of Applied Physiology, 106*, 1819-1825.

Høydal, M. A., Wisloff, U., Kemi, O. J., & Ellingsen, O. (2007). Running speed and maximal oxygen uptake in rats and mice: practical implications for exercise training. *European Journal of Cardiovascular Prevention and Rehabilitation, 14*(6), 753-760.

Johnson, R. A., & Mitchell, G. S. (2003). Exercise-induced changes in hippocampal brain-derived neurotrophic factor and neurotrophin-3: effects of rat strain. *Brain Research, 983*(1-2), 108-114.

Johnson, R. A., Rhodes, J. S., Jeffrey, S. L., Garland, T., Jr., & Mitchell, G. S. (2003). Hippocampal brain-derived neurotrophic factor but not neurotrophin-3 increases more in mice selected for increased voluntary wheel running. *Neuroscience, 121*(1), 1-7.

Kim, M. W., Bang, M. S., Han, T. R., Ko, Y. J., Yoon, B. W., Kim, J. H. (2005). Exercise increased BDNF and trkB in the contralateral hemisphere of the ischemic rat brain. *Brain Research, 1052*(1), 16-21.

Kitamura, T., Mishina, M., & Sugiyama, H. (2003). Enhancement of neurogenesis by running wheel exercises is suppressed in mice lacking NMDA receptor epsilon 1 subunit. *Neuroscience Research, 47*(1), 55-63.

Klintsova, A. Y., Dickson, E., Yoshida, R., & Greenough, W. T. (2004). Altered expression of BDNF and its high-affinity receptor TrkB in response to complex motor learning and moderate exercise. *Brain Research, 1028*(1), 92-104.

Koch, L., & Britton, S. (2005). Divergent selection for aerobic capacity in rats as a model for complex disease. *Integrative and Comparitive Biology, 45*(3), 405.

Koch, L. G., & Britton, S. L. (2001). Artificial selection for intrinsic aerobic endurance running capacity in rats. *Physiological Genomics, 5*(1), 45-52.

Koch, L. G., & Britton, S. L. (2008). Development of animal models to test the fundamental basis of gene-environment interactions. *Obesity (Silver Spring), 16 Suppl 3*, S28-32.

Neeper, S. A., Gomez-Pinilla, F., Choi, J., & Cotman, C. W. (1996). Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Research, 726*(1-2), 49-56.

Oliff, H. S., Berchtold, N. C., Isackson, P., & Cotman, C. W. (1998). Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus. *Brain Research Molecular Brain Research, 61*(1-2), 147-153.

Olson, A. K., Eadie, B. D., Ernst, C., & Christie, B. R. (2006). Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. *Hippocampus, 16*(3), 250-260.

Rasmussen, P., Brassard, P., Adser, H., Pedersen, M. V., Leick, L., Hart, E. (2009). Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Experimental Physiology, 94*(10), 1062-1069.

Reiss, J. I., Dishman, R. K., Boyd, H. E., Robinson, J. K., & Holmes, P. V. (2009). Chronic activity wheel running reduces the severity of kainic acid-induced seizures in the rat: possible role of galanin. *Brain Research, 1266*, 54-63.

Remondes, M., & Schuman, E. M. (2004). Role for a cortical input to hippocampal area CA1 in the consolidation of a long-term memory. *Nature, 431*(7009), 699-703.

Rhodes, J. S., van Praag, H., Jeffrey, S., Girard, I., Mitchell, G. S., Garland, T., Jr. (2003). Exercise increases hippocampal neurogenesis to high levels but does not improve spatial

learning in mice bred for increased voluntary wheel running. *Behavioral Neuroscience, 117*(5), 1006-1016.

Russo-Neustadt, A., Beard, R. C., & Cotman, C. W. (1999). Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. *Neuropsychopharmacology, 21*(5), 679-682.

Russo-Neustadt, A., Ha, T., Ramirez, R., & Kesslak, J. P. (2001). Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. *Behavioral Brain Research, 120*(1), 87-95.

Russo-Neustadt, A. A., Alejandre, H., Garcia, C., Ivy, A. S., & Chen, M. J. (2004). Hippocampal brain-derived neurotrophic factor expression following treatment with reboxetine, citalopram, and physical exercise. *Neuropsychopharmacology, 29*(12), 2189-2199.

Russo-Neustadt, A. A., Beard, R. C., Huang, Y. M., & Cotman, C. W. (2000). Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus. *Neuroscience, 101*(2), 305-312.

Scharfman, H., Goodman, J., Macleod, A., Phani, S., Antonelli, C., & Croll, S. (2005). Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. *Experimental Neurology, 192*(2), 348-356.

Soya, H., Nakamura, T., Deocaris, C. C., Kimpara, A., Iimura, M., Fujikawa, T. (2007). BDNF induction with mild exercise in the rat hippocampus. *Biochemical and Biophysical Research Communications, 358*(4), 961-967.

Tong, L., Shen, H., Perreau, V. M., Balazs, R., & Cotman, C. W. (2001). Effects of exercise on gene-expression profile in the rat hippocampus. *Neurobiology of Disease, 8*(6), 1046-1056.

Van Hoomissen, J. D., Chambliss, H. O., Holmes, P. V., & Dishman, R. K. (2003). Effects of chronic exercise and imipramine on mRNA for BDNF after olfactory bulbectomy in rat. *Brain Research, 974*(1-2), 228-235.

Vaynman, S., Ying, Z., & Gomez-Pinilla, F. (2004). Exercise induces BDNF and synapsin I to specific hippocampal subfields. *Journal of Neuroscience Research, 76*(3), 356-362.

Waters, R. P., Renner, K. J., Pringle, R. B., Summers, C. H., Britton, S. L., Koch, L. G. (2008). Selection for aerobic capacity affects corticosterone, monoamines and wheel-running activity. *Physiology and Behavior, 93*(4-5), 1044-1054.

Widenfalk, J., Olson, L., & Thoren, P. (1999). Deprived of habitual running, rats downregulate BDNF and TrkB messages in the brain. *Neuroscience Research, 34*(3), 125-132.

Zhang, H. N., & Ko, M. C. (2009). Seizure activity involved in the up-regulation of BDNF mRNA expression by activation of central mu opioid receptors. *Neuroscience, 161*(1), 301-310.

Zheng, H., Liu, Y., Li, W., Yang, B., Chen, D., Wang, X. (2006). Beneficial effects of exercise and its molecular mechanisms on depression in rats. *Behavioral Brain Research, 168*(1), 47-55.

Table Captions

Table 3.1 Dose-Response: Running Distance in meters per day and BDNF protein/mRNA for studies included for comparison.

Table 3.2 Effect size and 95% confidence intervals for BDNF measures in dose-response studies.

Table 3.1

Study	Subjects (n)	Running Distance (m/day)	Methods/Treatments	Dose-Response: BDNF mRNA/protein
Adlard, Perreau, & Cotman (2005)	Male; C57 B16 mice; age range 2 mos. to 24 mos. (n=5/age/condition)	7 to 28 days of running; range: Young = 5000m – 10,000m, Middle-age = 1800m – 4800m, Old = 2400m – 3800m	None	Young group (2 mos.), 7 days only: r = 0.959 (n=5); 95% CI: 0.499 to 0.997
Bjornebekk, Mathe, & Brene (2005)	Male; Flinders Sensitive and Resistant Lines rats (n=8/strain/condition)	35 days of running; range: FSL = 1600m – 2600m, FRL = 2500m – 5600m	Forced swim test & BrdU administration	Last week of running: r = 0.69 (n =14); 95% CI: 0.252 to 0.893
Johnson & Mitchell (2003)	Male; 4 strains (7-8 wk/old): Brown Norway (BN), Dark Agouti (DA), PVG, & Sprague-Dawley (SD); (n = 8/strain/conditon, 4 groups)	Voluntary; 1 and 7 night time points; Running distance: no distance differences among strains at 1 night (range ~750m to 1500m, including SE), strain differences found after 7 nights – PVG: 1000m to 7000m, DA: 500 to 3000m, SD: 500 to 1500m, BN: 2500 to 2200m	None	BDNF levels correlated with distance run among all strains $p<0.001$ r = .53 (n=32); 95% CI: 0.393 to 0.644, after 7 nights running, similar slopes between strains.

Griesbach, Hovda, Molteni, Wu, & Gomez-Pinilla (2004)	Male; Sprague-Dawley rats (n=6-10/condition)	7 days of running; acute and DSham-RW groups range: 500m – 3400m, acute FPI-RW range: 250m – 2100m, DFPI-RW range: 800m – 2300m	FPI vs. Sham & Water maze training	DSham-RW: r = 0.872 (n = 15), 95% CI: 0.65 to 0.956; DFPI-RW: r = 0.555 (n =8) (ipsilateral) (n = 8), 95% CI: -0.245 to 0.905, and r = 0.621 (contralateral), 95% CI = -0.148 to 0.922
Oliff, Berchtold, Isackson, & Cotman (1998)	Male; Fisher-344 rats (3-4 mos. n=7-8/condition)	6 or 12 hrs of running following 3 day activity wheel training period and 10 day activity cessation period; distance not reported	None	6 h running only: (Hilus) r = 0.866, (CA1) r = 0.894, (CA3) r = 0.704 (n = 7); 95% CI: (Hilus) 0.325 to 0.979, (CA1) 0.432 to 0.984, (CA3) -0.104 to 0.952
Griesbach, Hovda, Gomez-Pinilla, & Sutton (2008)	Male; Sprague-Dawley rats (n=6/condition)	7 days of running; Sham-S-RW and Sham-AMPH-RW groups range = 1000m – 1800m, CCI-S-RW ad CCI-AMPH-RW groups range = 20m to 1250m	CCI vs. Sham; Amphetamine (AMPH) vs. Saline (S)	Sham-S-RW: r = 0.74 (n = 6), 95% CI: -0.179 to 0.969; Sham-AMPH-RW: r = 0.47 (n = 6). 95% CI: -0.552 to 0.927
Johnson, Rhodes, Jeffrey, Garland, & Mitchell (2003)	Male; gen. 25 mice selectively bred for high voluntary running wheel behavior (n=8-16/condition)	1 or 7 nights of running; S mice range = 2900m – 8600m, C mice range = 1000m – 3400m	None	Correlation for combined C and S mice, $p < 0.05$ (n = 64), r-value not reported

Table 3.2

Study	Brain Region	Effect Size	95 % Confidence Interval
Bjornebekk, Mathe, & Brene (2005)	DG	1.9396	0.7514, 3.1283
	DG	0.4240	-0.5626, 1.4197
Oliff, Berchtold, Isackson, & Cotman (1998)	Hilus	1.7557	0.6022, 2.9092
	CA1	3.3427	1.8255, 4.8599
	CA3	2.1946	0.9542, 3.4350
Adlard, Perreau, & Cotman (2005)	HF	1.2675	-0.0909, 2.6259
Griesbach, Hovda, Molteni, Wu, & Gomez-Pinilla (2004)	HF	1.0039	0.0235, 1.9843
Griesbach, Hovda, Gomez-Pinilla, & Sutton (2008)	HF	1.5988	0.2989, 2.8987

Figure Captions

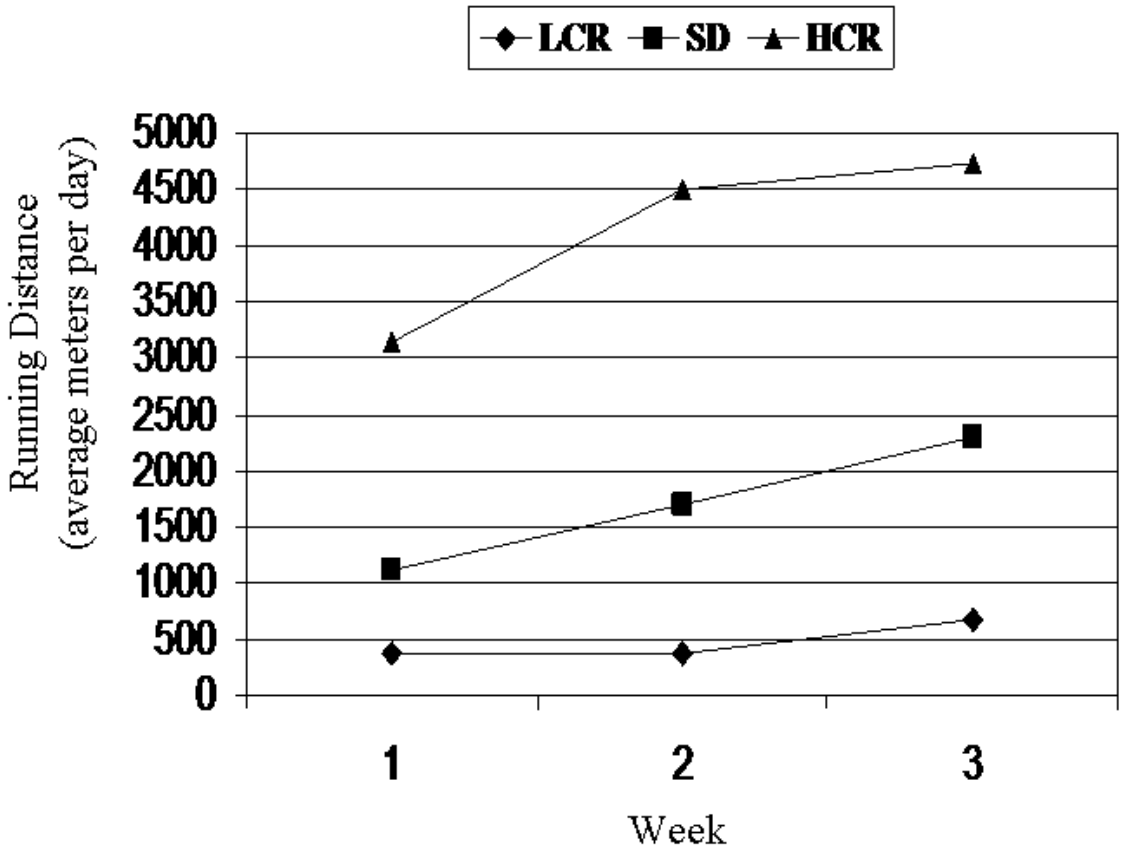
LCR
SD
HCR
Running Distance
(average meters per day)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
1
2
3
Week

Figure 3.1

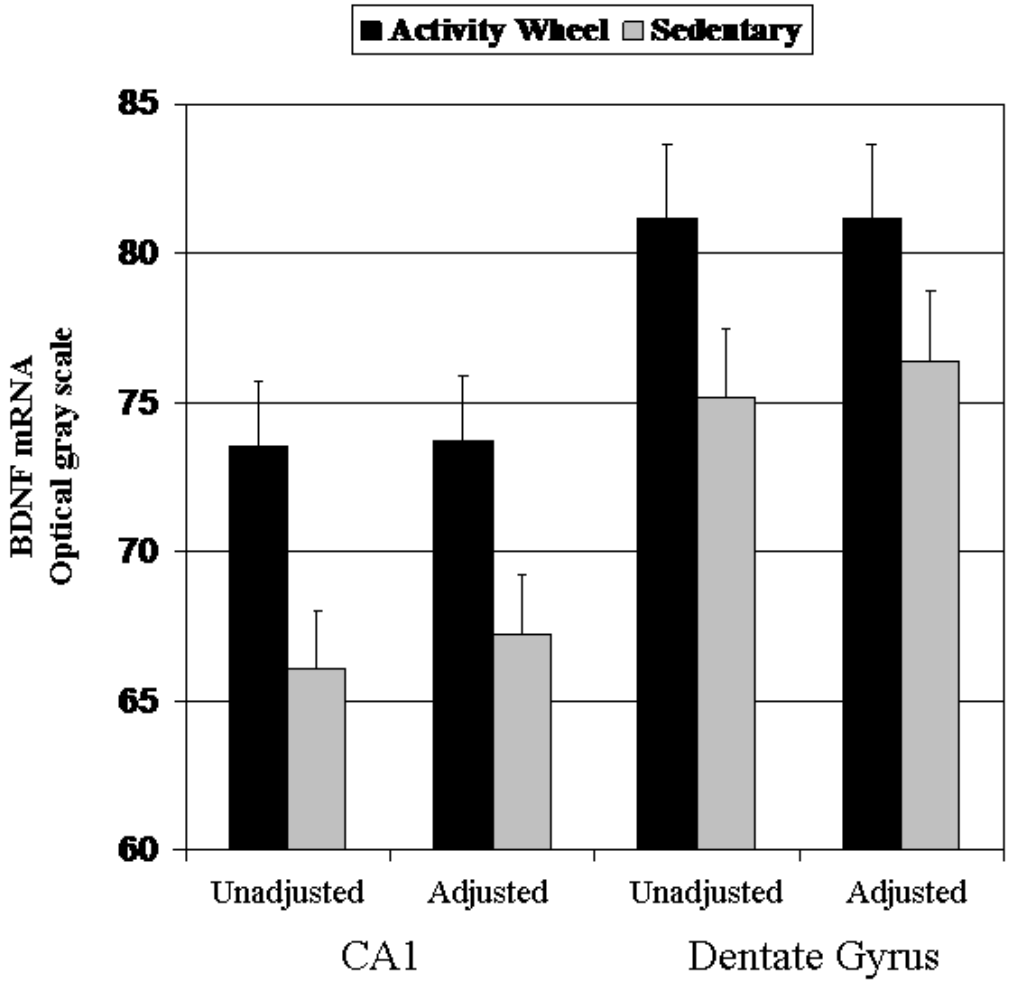
Activity Wheel
Sedentary
BDNF mRNA
Optical gray scale
85
80
75
70
65
60
Unadjusted
Adjusted
Unadjusted
Adjusted
CA1
Dentate Gyrus

Figure 3.2

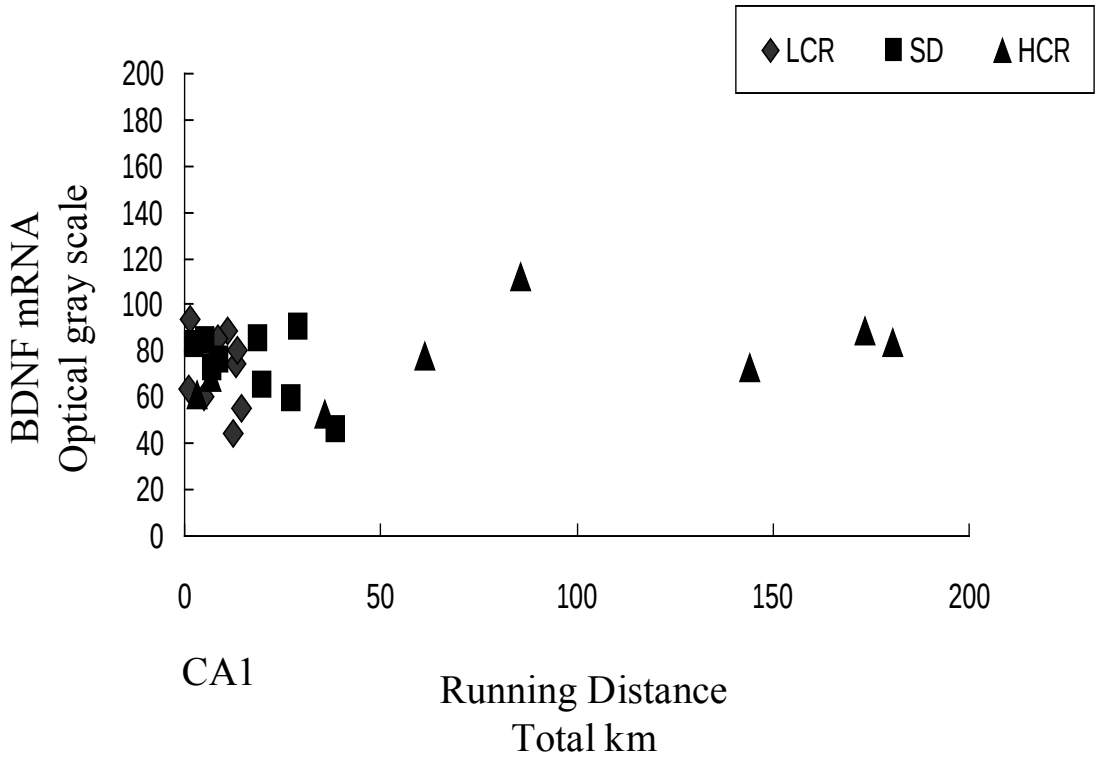
LCR
SD
HCR
BDNF mRNA
Optical gray scale
200
180
160
140
120
100
80
60
40
20
0
0
50
100
150
200
CA1
Running Distance
Total km

Figure 3.3

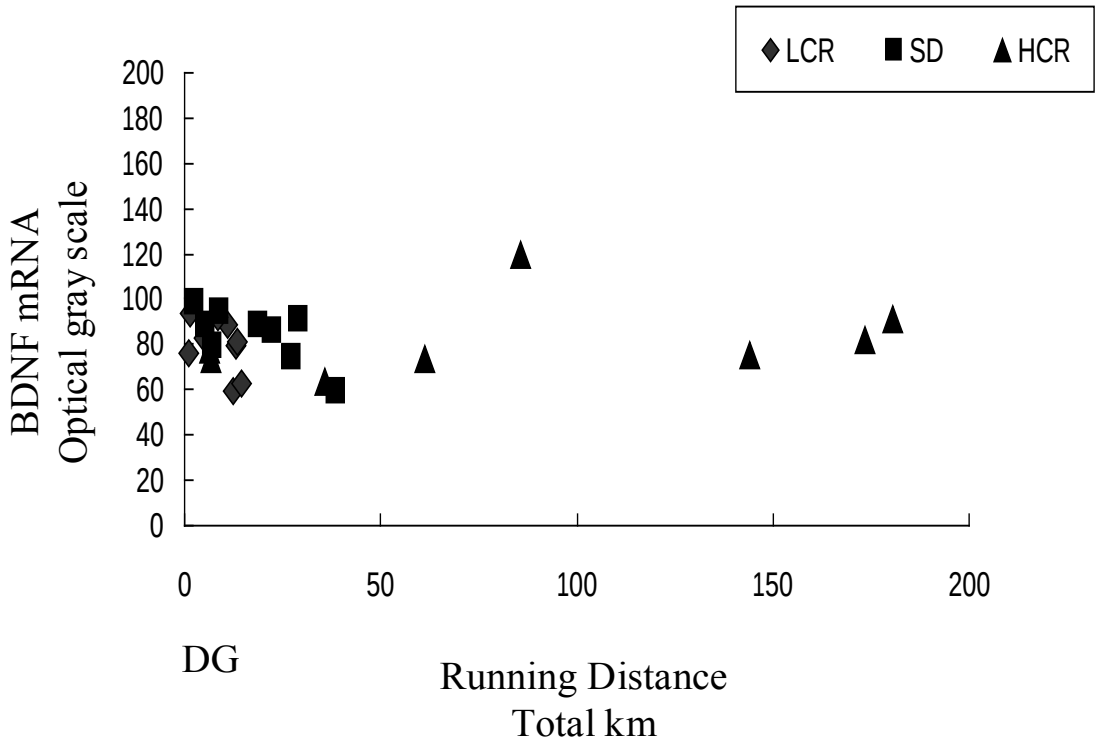
LCR
SD
HCR
BDNF mRNA
Optical gray scale
200
180
160
140
120
100
80
60
40
20
0
0
50
100
150
200
DG
Running Distance
Total km

Figure 3.4

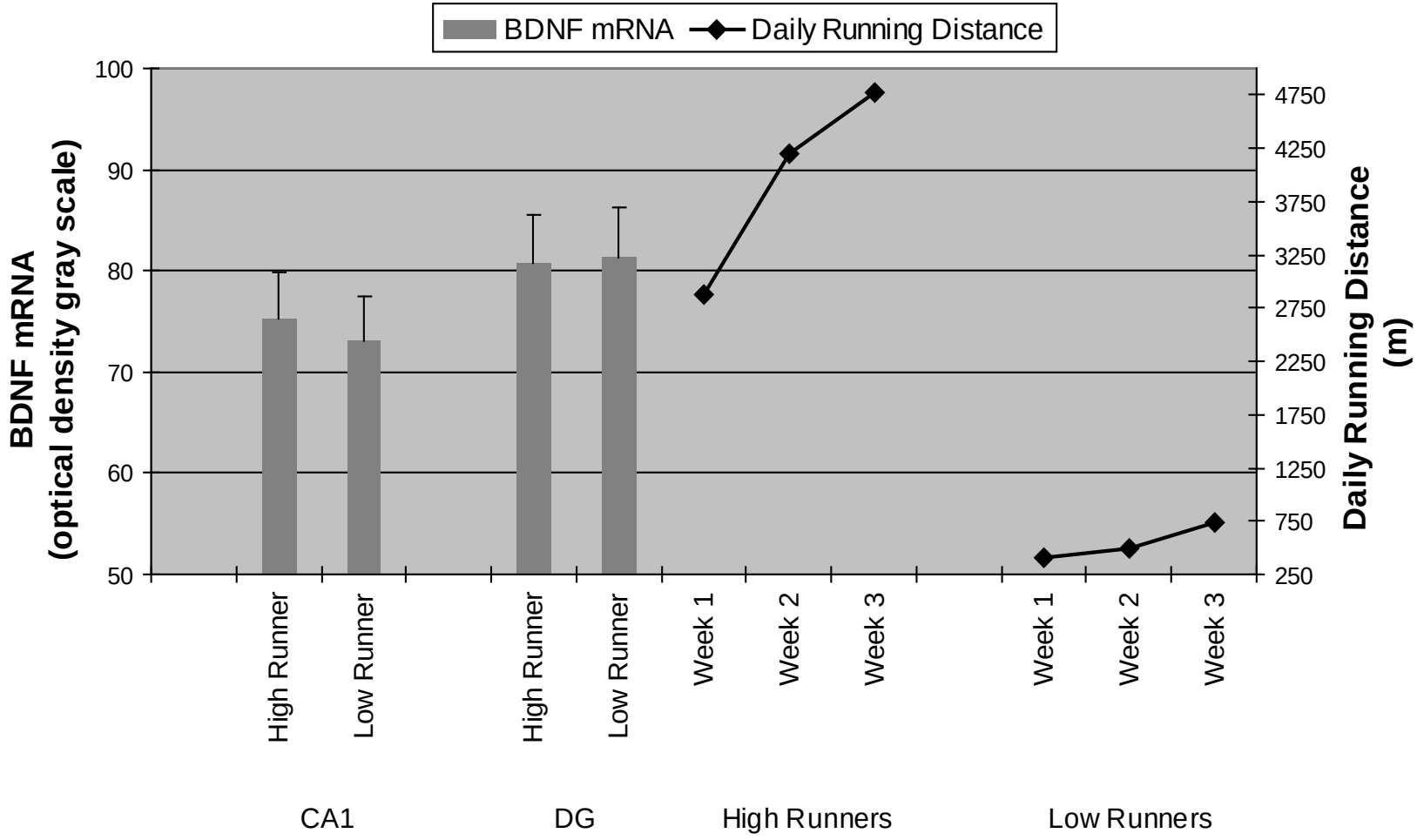
BDNF mRNA
Daily Running Distance
BDNF mRNA (optical density gray scale)
Daily Running Distance (m)
100
90
80
70
60
50
4750
4250
3750
3250
2750
2250
1750
1250
750
250
High Runner
Low Runner
High Runner
Low Runner
Week 1
Week 2
Week 3
Week 1
Week 2
Week 3
CA1
DG
High Runners
Low Runners

Figure 3.5

CHAPTER 4

SUMMARY

The results of this study indicate that activity wheel running increases BDNF mRNA within CAI of the hippocampus, but that effect does not depend on cumulative running distance. The effect persisted after adjusting for age, which was inversely related to both BDNF expression and running distance. BDNF expression was increased by exposure to voluntary activity wheel running, regardless of differences in intrinsic aerobic running capacity and a wide range in running distance. The increase in BDNF mRNA seen in rats with low intrinsic running capacity and that ran between 400m and 600m daily was similar to the increase seen in rats with high intrinsic running capacity and that ran 7 times farther. Future research is needed to clarify the minimum running wheel exposure that is sufficient to induce hippocampal BDNF expression, and to determine the mechanisms that explain exercise-induced BDNF transcription.

REFERENCES

Adlard, P. A., & Cotman, C. W. (2004). Voluntary exercise protects against stress-induced decreases in brain-derived neurotrophic factor protein expression. *Neuroscience, 124*(4), 985.

Adlard, P. A., Perreau, V. M., & Cotman, C. W. (2005). The exercise-induced expression of BDNF within the hippocampus varies across life-span. *Neurobiology of Aging, 26*(4), 511-520.

Berchtold, N. C., Chinn, G., Chou, M., Kesslak, J. P., & Cotman, C. W. (2005). Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. *Neuroscience, 133*(3), 853-861.

Berchtold, N. C., Kesslak, J. P., Pike, C. J., Adlard, P. A., & Cotman, C. W. (2001). Estrogen and exercise interact to regulate brain-derived neurotrophic factor mRNA and protein expression in the hippocampus. *European Journal of Neuroscience, 14*(12), 1992-2002.

Binder, D. K., & Scharfman, H. E. (2004). Brain-derived neurotrophic factor. *Growth Factors, 22*(3), 123-131.

Bjornebekk, A., Mathe, A. A., & Brene, S. (2005). The antidepressant effect of running is associated with increased hippocampal cell proliferation. *International Journal of Neuropsychopharmacology, 8*(3), 357-368.

Chen, M. J., & Russo-Neustadt, A. A. (2005). Exercise activates the phosphatidylinositol 3-kinase pathway. *Brain Research Molecular Brain Research, 135*(1-2), 181-193.

Dishman, R. K., Berthoud, H. R., Booth, F. W., Cotman, C. W., Edgerton, V. R., Fleshner, M. R. (2006). Neurobiology of exercise. *Obesity (Silver Spring), 14*(3), 345-356.

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

Duman, C. H., Schlesinger, L., Terwilliger, R., Russell, D. S., Newton, S. S., & Duman, R. S. (2009). Peripheral insulin-like growth factor-I produces antidepressant-like behavior and contributes to the effect of exercise. *Behav Brain Res, 198*(2), 366-371.

Engesser-Cesar, C., Anderson, A. J., & Cotman, C. W. (2007). Wheel running and fluoxetine antidepressant treatment have differential effects in the hippocampus and the spinal cord. *Neuroscience, 144*(3), 1033-1044.

Farmer, J., Zhao, X., van Praag, H., Wodtke, K., Gage, F. H., & Christie, B. R. (2004). Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male Sprague-Dawley rats in vivo. *Neuroscience, 124*(1), 71-79.

Farovik, A., Dupont, L. M., & Eichenbaum, H. (2010). Distinct roles for dorsal CA3 and CA1 in memory for sequential nonspatial events. *Learning and Memory, 17*, 12-17.

Garza, A. A., Ha, T. G., Garcia, C., Chen, M. J., & Russo-Neustadt, A. A. (2004). Exercise, antidepressant treatment, and BDNF mRNA expression in the aging brain. *Pharmacology Biochemistry and Behavior, 77*(2), 209-220.

Gobbo, O. L., & O'Mara, S. M. (2005). Exercise, but not environmental enrichment, improves learning after kainic acid-induced hippocampal neurodegeneration in association with an increase in brain-derived neurotrophic factor. *Behavioral Brain Research, 159*(1), 21-26.

Gomez-Pinilla, F., Vaynman, S., & Ying, Z. (2008). Brain-derived neurotrophic factor functions as a metabotrophin to mediate the effects of exercise on cognition. *European Journal of Neuroscience, 28*(11), 2278-2287.

Gomez-Pinilla, F. E., Ying, Z., Roy, R. R., Zhong, H., & Edgerton, V. R. (2002). Role of neuromuscular activity in maintaining basal levels of neurotrophins in the spinal cord. *Society for Neuroscience Abstract Viewer and Itinerary Planner, 2002*, Abstract No. 732.739.

Greenwood, B. N., Strong, P. V., Foley, T. E., & Fleshner, M. (2009). A Behavioral Analysis of the Impact of Voluntary Physical Activity on Hippocampus-Dependent Contextual Conditioning. *Hippocampus, 19*(10), 988-1001.

Griesbach, G. S., Hovda, D. A., Gomez-Pinilla, F., & Sutton, R. L. (2008). Voluntary exercise or amphetamine treatment, but not the combination, increases hippocampal brain-derived neurotrophic factor and synapsin I following cortical contusion injury in rats. *Neuroscience, 154*(2), 530-540.

Griesbach, G. S., Hovda, D. A., Molteni, R., Wu, A., & Gomez-Pinilla, F. (2004). Voluntary exercise following traumatic brain injury: brain-derived neurotrophic factor upregulation and recovery of function. *Neuroscience, 125*(1), 129-139.

Hoge, J., & Kesner, R. (2007). Role of CA3 and CA1 subregions of the dorsal hippocampus on temporal processing of objects. *Neurobiology Of Learning And Memory, 88*(2), 225-231.

Holmes, P. V., Yoo, H. S., & Dishman, R. K. (2006). Voluntary exercise and clomipramine treatment elevate prepro-galanin mRNA levels in the locus coeruleus in rats. *Neuroscience Letters, 408*(1), 1-4.

Howlett, R. A., Kirkton, S. D., Gonzalez, N. C., Wagner, H. E., Britton, S. L., Koch, L. G. (2008). Peripheral oxygen transport and utilization in rats following continued selective breeding for endurance running capacity. *Journal of Applied Physiology, 106*, 1819-1825.

Høydal, M. A., Wisloff, U., Kemi, O. J., & Ellingsen, O. (2007). Running speed and maximal oxygen uptake in rats and mice: practical implications for exercise training. *European Journal of Cardiovascular Prevention and Rehabilitation, 14*(6), 753-760.

Johnson, R. A., & Mitchell, G. S. (2003). Exercise-induced changes in hippocampal brain-derived neurotrophic factor and neurotrophin-3: effects of rat strain. *Brain Research, 983*(1-2), 108-114.

Johnson, R. A., Rhodes, J. S., Jeffrey, S. L., Garland, T., Jr., & Mitchell, G. S. (2003). Hippocampal brain-derived neurotrophic factor but not neurotrophin-3 increases more in mice selected for increased voluntary wheel running. *Neuroscience, 121*(1), 1-7.

Kim, M. W., Bang, M. S., Han, T. R., Ko, Y. J., Yoon, B. W., Kim, J. H. (2005). Exercise increased BDNF and trkB in the contralateral hemisphere of the ischemic rat brain. *Brain Research, 1052*(1), 16-21.

Kitamura, T., Mishina, M., & Sugiyama, H. (2003). Enhancement of neurogenesis by running wheel exercises is suppressed in mice lacking NMDA receptor epsilon 1 subunit. *Neuroscience Research, 47*(1), 55-63.

Klintsova, A. Y., Dickson, E., Yoshida, R., & Greenough, W. T. (2004). Altered expression of BDNF and its high-affinity receptor TrkB in response to complex motor learning and moderate exercise. *Brain Research, 1028*(1), 92-104.

Koch, L., & Britton, S. (2005). Divergent selection for aerobic capacity in rats as a model for complex disease. *Integrative and Comparitive Biology, 45*(3), 405.

Koch, L. G., & Britton, S. L. (2001). Artificial selection for intrinsic aerobic endurance running capacity in rats. *Physiological Genomics, 5*(1), 45-52.

Koch, L. G., & Britton, S. L. (2008). Development of animal models to test the fundamental basis of gene-environment interactions. *Obesity (Silver Spring), 16 Suppl 3*, S28-32.

Molteni, R., Wu, A., Vaynman, S., Ying, Z., Barnard, R. J., & GÃ³mez-Pinilla, F. (2004). Exercise reverses the harmful effects of consumption of a high-fat diet on synaptic and behavioral plasticity associated to the action of brain-derived neurotrophic factor. *Neuroscience, 123*(2), 429.

Neeper, S. A., Gomez-Pinilla, F., Choi, J., & Cotman, C. W. (1996). Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Research, 726*(1-2), 49-56.

Oliff, H. S., Berchtold, N. C., Isackson, P., & Cotman, C. W. (1998). Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus. *Brain Research Molecular Brain Research, 61*(1-2), 147-153.

Olson, A. K., Eadie, B. D., Ernst, C., & Christie, B. R. (2006). Environmental enrichment and voluntary exercise massively increase neurogenesis in the adult hippocampus via dissociable pathways. *Hippocampus, 16*(3), 250-260.

Rasmussen, P., Brassard, P., Adser, H., Pedersen, M. V., Leick, L., Hart, E. (2009). Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Experimental Physiology, 94*(10), 1062-1069.

Reiss, J. I., Dishman, R. K., Boyd, H. E., Robinson, J. K., & Holmes, P. V. (2009). Chronic activity wheel running reduces the severity of kainic acid-induced seizures in the rat: possible role of galanin. *Brain Research, 1266*, 54-63.

Remondes, M., & Schuman, E. M. (2004). Role for a cortical input to hippocampal area CA1 in the consolidation of a long-term memory. *Nature, 431*(7009), 699-703.

Rhodes, J. S., van Praag, H., Jeffrey, S., Girard, I., Mitchell, G. S., Garland, T., Jr. (2003). Exercise increases hippocampal neurogenesis to high levels but does not improve spatial learning in mice bred for increased voluntary wheel running. *Behavioral Neuroscience, 117*(5), 1006-1016.

Russo-Neustadt, A., Beard, R. C., & Cotman, C. W. (1999). Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. *Neuropsychopharmacology, 21*(5), 679-682.

Russo-Neustadt, A., Ha, T., Ramirez, R., & Kesslak, J. P. (2001). Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behavior in an animal model. *Behavioral Brain Research, 120*(1), 87-95.

Russo-Neustadt, A. A., Alejandre, H., Garcia, C., Ivy, A. S., & Chen, M. J. (2004). Hippocampal brain-derived neurotrophic factor expression following treatment with reboxetine, citalopram, and physical exercise. *Neuropsychopharmacology, 29*(12), 2189-2199.

Russo-Neustadt, A. A., Beard, R. C., Huang, Y. M., & Cotman, C. W. (2000). Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus. *Neuroscience, 101*(2), 305-312.

Scharfman, H., Goodman, J., Macleod, A., Phani, S., Antonelli, C., & Croll, S. (2005). Increased neurogenesis and the ectopic granule cells after intrahippocampal BDNF infusion in adult rats. *Experimental Neurology, 192*(2), 348-356.

Soya, H., Nakamura, T., Deocaris, C. C., Kimpara, A., Iimura, M., Fujikawa, T. (2007). BDNF induction with mild exercise in the rat hippocampus. *Biochemical and Biophysical Research Communications, 358*(4), 961-967.

Tong, L., Shen, H., Perreau, V. M., Balazs, R., & Cotman, C. W. (2001). Effects of exercise on gene-expression profile in the rat hippocampus. *Neurobiology of Disease, 8*(6), 1046-1056.

Van Hoomissen, J. D., Chambliss, H. O., Holmes, P. V., & Dishman, R. K. (2003). Effects of chronic exercise and imipramine on mRNA for BDNF after olfactory bulbectomy in rat. *Brain Research, 974*(1-2), 228-235.

Vaynman, S., Ying, Z., & Gomez-Pinilla, F. (2004). Exercise induces BDNF and synapsin I to specific hippocampal subfields. *Journal of Neuroscience Research, 76*(3), 356-362.

Vaynman, S., Ying, Z., & Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *European Journal of Neuroscience, 20*(10), 2580-2590.

Vaynman, S. S., Ying, Z., Yin, D., & Gomez-Pinilla, F. (2006). Exercise differentially regulates synaptic proteins associated to the function of BDNF. *Brain Research, 1070*(1), 124-130.

Waters, R. P., Renner, K. J., Pringle, R. B., Summers, C. H., Britton, S. L., Koch, L. G. (2008). Selection for aerobic capacity affects corticosterone, monoamines and wheel-running activity. *Physiology and Behavior, 93*(4-5), 1044-1054.

Widenfalk, J., Olson, L., & Thoren, P. (1999). Deprived of habitual running, rats downregulate BDNF and TrkB messages in the brain. *Neuroscience Research, 34*(3), 125-132.

Zhang, H. N., & Ko, M. C. (2009). Seizure activity involved in the up-regulation of BDNF mRNA expression by activation of central mu opioid receptors. *Neuroscience, 161*(1), 301-310.

Zheng, H., Liu, Y., Li, W., Yang, B., Chen, D., Wang, X. (2006). Beneficial effects of exercise and its molecular mechanisms on depression in rats. *Behavioral Brain Research, 168*(1), 47-55.