VOLUNTARY WHEEL RUNNING ALTERS BRAIN-DERIVED NEUROTROPHIC FACTOR LEVELS IN THE HIPPOCAMPUS OF SENESCENCE ACCELERATED MICE

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ABSTRACT

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Brain-derived neurotrophic factor (BDNF) promotes neuronal development and survival. BDNF and its high affinity receptor, trkB, are extensively expressed in the mammalian hippocampus, a structure susceptible to damage in neurodegenerative disorders such as Alzheimer’s disease. Current research in this field has shown voluntary wheel running to be an effective tool in increasing BDNF protein in the hippocampus, hence promoting cell survival within this structure. The SAMP8 mouse line exhibits accelerated senescence of learning and memory and has been a good model of aging and Alzheimer’s disease. Therefore, the present study investigated the effects of 21 days of voluntary wheel running on total BDNF and free mature BDNF in the hippocampus of young (1-3 months), middle-aged (5-8 months) and old (10 months +) SAMP8 mice. BDNF levels in the SAMP8 hippocampus were measured by sandwich ELISA. In sedentary mice, females showed an increase in total BDNF and mature BDNF protein across the lifespan while male SAMP8 showed a reduction of both in middle-age. Exercise successfully increased total protein content in the hippocampus of young, middle-age, and old female and male SAMP8. Voluntary wheel running also increased mature BDNF at each age group for each sex, similar to total BDNF, with the exception of young males. The increase of BDNF in response to voluntary wheel running declined with age in the females. No significant difference was found between the running distance of males and females in each group. However, young mice ran significantly farther than the middle-aged or old animals and running distance was correlated with total BDNF increase. Overall, aging was shown to be the primary factor in decreasing the effectiveness of exercise to increase BDNF.
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INTRODUCTION

Brain derived neurotrophic factor (BDNF) is a protein produced and secreted in both the central nervous system (CNS) and the periphery (Barde, Edgar, & Thoenen, 1982). It is part of the neurotrophin family of proteins, which encourages cell growth, survival, and synaptic modification (Huang & Reichardt, 2001; 2003). BDNF is widespread and conserved throughout the mammalian CNS and is highly expressed in the developing and adult hippocampus (Hofer, Pagliusi, Hohn, Leibrock, & Barde, 1990; Ip, Li, Yancopoulos, & Lindsay, 1993; Phillips et al., 1990; Webster, Herman, Kleinman, & Weickert, 2006). BDNF mRNA in rat and human hippocampi remain relatively stable during normal aging (Lapchak, et al., 1993; Silhol, Arancibia, Marice, & Tapia-Arancibia, 2007; Webster et al, 2006), although increases have been reported (Narisawa-Saito & Nawa, 1996). However, BDNF protein expression has been shown to increase in healthy rat and mouse hippocampi during their lifespans (Katoh-Semba, Semba, Takeuchi, & Kato, 1998; Narisawa-Saito & Nawa, 1996; Silhol et al., 2007), perhaps as a compensatory mechanism to gradual aging related neuronal changes. One study reported similar BDNF protein levels in sedentary C57 mice across the lifespan (Adlard, Perreau, & Cotman, 2005).

The hippocampus is one of the first structures to be affected by Alzheimer’s disease (AD) in the aging brain. AD is characterized by behavioral impairments in learning and memory, as well as neurobiological changes, including degeneration of the hippocampal neurons and their cholinergic inputs, amyloid plaques and neurofibrillary tangles (Rogan & Lippa, 2002; Schindowski, Belarbi, & Buee, 2008). Lower expression levels of BDNF mRNA (Murray et al., 1994; Phillips et al., 1991) and BDNF protein
(Connor, Young, Yan, Faull, Synek, & Dragunow, 1997; Ferrer et al., 1999; Hock, Heese, Hulette, Rosenberg, & Otten, 2000) have been reported in human post-mortem AD hippocampi. These findings suggest that neurotrophic support may be down-regulated in Alzheimer’s disease. Studies examining the association between plaque load and BDNF levels have been mixed. BDNF increases in mice (Lee et al., 2005) and decreases in BDNF expression levels in humans (Burbach et al., 2004) have both been shown to correlate with plaque load in the temporal and frontal cortices respectively. Stimulation of the BDNF / trkB receptor complex has been shown to promote tau dephosphorylation through inhibition of GSK3β by PI-3Kinase signaling, which is prevented in the presence of β–Amyloid (Elliot, Atlas, Lange, & Ginzburg, 2005; Tong, Balazs, Thornton & Cotman, 2004). Both plaque and tangle formation are associated with disruptions in BDNF levels. Whether the formation of plaques and tangles first impairs neurotrophic support or the dysfunction of neurotrophic maintenance promotes plaque and tangle formation, is yet to be determined (Schindowski et al., 2008). Defining the neurobiological changes responsible for the behavioral impairments is currently an area of active research.

Brain-Derived Neurotrophic Factor

Most research examining BDNF levels within the CNS have been reported in terms of BDNF mRNA or total BNDF protein. However, like other neurotrophins, BDNF protein is divided into two sub-types, mature (13kDa) and pro-BDNF (32kDa). Pro-BDNF is simply the precursor protein of mature BDNF. Within the cell, pro-BDNF is cleaved either by the serine protease, furin, or the prohormone convertase, PC1, while the primary extracellular cleaving enzyme is plasmin, formed when plasminogen is
sectioned via the activity-dependent tissue plasminogen activator, tPA (Lu, Pang, & Woo, 2005).

Mature BDNF binds preferentially to the transmembrane receptor trk tyrosine kinase receptor family (trkB), while pro-BDNF has a high affinity for the pan-neurotrophin receptor (p75NTR) of the tumor necrosis factor receptor family. It is important to understand the role of each protein and receptor complex individually as each is thought to be responsible for diverse neuronal consequences. The mature BDNF / trkB complex promotes cell development, survival, synaptic modification and long-term potentiation (LTP) (Huang & Reichardt, 2001; 2003). In contrast, the pro BDNF / p75NTR protein complex is associated with cell injury, long-term depression (LTD), and programmed neuronal cell death (Huang & Reichardt, 2001; 2003; Teng et al., 2005). In the presence of both trkB and p75 receptors, trkB activation overrides or suppresses the cell death program initiated by p75 receptor binding. Essentially, it is not the presence of the pro-BDNF / p75 complex that yields cell death but rather an absence of trkB activation (Bamji et al., 1998). Furthermore, the absence of trkB activation in neurons that co-express p75 and sortilin increases apoptotic responses (Teng et al., 2005).

Unlike other neurotrophins, the majority of pro and mature BDNF is released from neurons through a regulated secretory pathway, in which the release of BDNF into extracellular space is dependent on neuronal excitation (Farhadi et al., 2000; Goodman et al., 1996; Mowla et al., 1999). In order for BDNF to be processed through the regulated secretory pathway, it must bind to carboxypeptidase E (CPE) intracellularly. Mice deficient in CPE are unable sort and release pro-BDNF and mature BDNF through the regulated secretory pathway (Lou et al., 2005). BDNF can also be released from a
constitutive pathway, which releases pro-BDNF and mature BDNF into the extracellular space. The constitutive pathway contributes to a lesser extent than the regulated secretory pathway and does so in a non-activity dependent yet constant manner (Thomas & Davies, 2005). The ratio of mature/pro-BDNF release from cortical neurons is estimated to be 2:1, while PC12 cells discharge more pro BDNF extracellularly. The ratio of BDNF protein release for hippocampal cells is still undetermined (Lou et al., 2005; Thomas & Davies, 2005).

The unique sorting of BDNF into activity and non-activity pathways allows for an additional level of modulation, allowing for potential therapeutic enhancement of production and subsequent maintaining or improving neuron function, more specifically, hippocampal neurons. Elucidating factors that attenuate the decreases in BDNF observed in neurodegenerative disorders, such as exercise, cognitive or environmental enrichment, and pharmacological treatment, are being examined for their efficacy in up-regulating BDNF.

Exercise

The focus of this study was to investigate one of those factors, voluntary wheel running, on mature and total BDNF protein expression in a mouse model of Alzheimer’s disease, the SAMP8. To date, research has shown that voluntary wheel running can increase BDNF in young healthy mice and rat. Voluntary wheel running has been shown to rapidly increase BDNF mRNA expression in young male rats as demonstrated by increases after 6 hours, (Oliff, Berchtold, Isackson, & Cotman, 1998) 2 days (Neeper, Gomez-Pinilla, Choi, & Cotman, 1996) or 1 week (Vaynman, Ying, & Gomez-Pinilla, 2004) of running. In contrast to BDNF mRNA expression, BDNF total protein
expression, encompassing both pro-BDNF and mature BDNF, requires that an animal spend more time on a voluntary wheel running protocol to see significant increase of BDNF expression. Johnson (Johnson, Rhodes, Jeffrey, Garland, & Mitchell, 2003) showed that one week was not sufficient to see significant increases in total BDNF protein expression. Others demonstrated that at least 14 days of voluntary wheel running was needed to induce significant hippocampal total and mature BNDNF increases over sedentary animal controls (Adlard, Perreau, Engesser-Cesar & Cotman, 2004; Berchtold, Kesslak & Cotman, 2005).

Both BDNF mRNA and mature BDNF protein levels have been correlated with running distance (Adlard et al., 2004; Oliff et al., 1998). Voluntary wheel running has been shown to increase the firing rate of hippocampal pyramidal cells (Czurko, Hirase, Csicsvari & Buzsaki, 1999). These results support the idea that as an animal runs, it increases pyramidal cell firing rate and in turn, induces secretion of BDNF through the regulated secretory pathway.

The extent to which aged animals modulate BDNF stimulation by voluntary wheel running has not been researched extensively. In one study (Adlard et al., 2005), total BDNF was measured in young, middle-aged, and old male C57 mouse hippocampi to determine if total BDNF increases across the lifespan in response to exercise. In animals able to run voluntarily, all age groups exhibited a significant total BDNF increase over the sedentary controls at day 7. Only young animals exhibited exercise-induced increases in total BDNF from sedentary controls at day 21 and day 28. All age groups showed a significant increase in BDNF at day 7 and a drop back to baseline at 14 days. The initial increase during the first week was interpreted as a novelty effect. Therefore, it
is implied that only young animals showed an exercise induced increase in total BDNF over the course of the study at days 21 and 28. The young animals ran significantly farther than the middle-aged and old animals at all time points. However, in the young animals, distance was correlated with exercise induced BNDF levels at day 7 only. This study indicates that middle-aged and old animals may not respond or benefit similarly to voluntary wheel running as young animals do in regards to BNDF production within the hippocampus.

Senescence-Accelerated Mouse

The SAMP8 is an accelerated-aging mouse model developed by Takeda (Takeda et al., 1981). The senescence accelerate mouse (SAM) was derived from Takeda’s laboratory of AKR/J mice, which displayed behavioral characteristics of mice older than that of their actual chronological age. From this observation, Takeda began selectively breeding, based on phenotype, a line of mice prone to accelerated aging (SAMP) and a line of mice resistant to accelerated senescence (SAMR). Since then, studies have found that the SAMP8 demonstrate impairments in learning, memory, increases in amyloid beta protein expression, reduced NMDA receptor activity, and dysfunctional septohippocampal connections (Flood & Morley, 1998; Morley, Farr & Flood, 2002; Nomura & Okuma, 1999; Takemura et al., 1993).

Total BDNF levels of the SAMP8 were lower than that of the control strain, SAM-R1, in old age (Katoh-Simba et al., 1998). SAMP8 mice did not show an increase of total BDNF in the hippocampus after the age of 4 months. This result, like AD subjects, shows a decline or lack of increase across the lifespan in BDNF levels in aged animals when some degree of pathology is present.
Overview

BDNF promotes neuronal development and survival. BDNF is extensively expressed in the mammalian hippocampus, a structure susceptible to damage in neurodegenerative disorders such as Alzheimer’s disease. The SAMP8 mouse line exhibits accelerated senescence of learning and memory and has been a good model for aging and Alzheimer’s disease as the SAMP8 display evidence of many of the same neuropathological changes as observed in AD.

Voluntary wheel running serves as a useful tool to increase BDNF within the hippocampus of young animal models. The goals of this study were three-fold: 1) to determine the age-related changes of total and mature BDNF protein expression in the hippocampus of SAMP8 mice, 2) to assess the effects of voluntary wheel running on hippocampal BDNF levels across the lifespan of SAMP8 mice, and 3) to determine whether the age-related effects of wheel running on BDNF is entirely accounted for by running distance. Whereas research has focused on total BDNF protein expression, this study will assess the effects of age, sex, and wheel running on both total BDNF and free mature BDNF expression.

Understanding if voluntary physical exercise increases BDNF in the SAMP8 is useful both as a preventative measure for individuals with known risk factors and as a therapeutic tool to delay disease associated neuronal loss.
METHOD

The Institutional Animal Care and Use Committee (IACUC) for Bowling Green State University approved the experimental methods involving the animals used during this research.

Animals

One-hundred and twenty three senescence-accelerated mice (SAMP8) were used in this study (60 female and 63 male). All animals were from the Bowling Green State University colony. The initial breeding pair came from a colony under the direction of J.F. Flood (St. Louis, MO, USA). Three groups were used to assess the effects of age: young (1-3 months, \(n = 20\)), middle-aged (5-8 months, \(n = 47\)), and old (10 months and older, \(n = 56\)). Animals were housed in standard cages (17 x 9 x 6.5 in), given food and water ad libitum, and maintained in a 12 hour light / dark cycle.

Exercise

This study examined the effects of voluntary exercise on BDNF protein levels in the hippocampus. Both total and mature BDNF expression were measured. A 2x2x3 experimental design was used with exercise (wheel running or sedentary), sex (male or female) and age (young, middle-aged or old) as between subjects factors. Animals were housed socially prior to the start of the experiment, 2-4 animals per cage, in the SAMP8 colony. Animals were randomly assigned to either of the exercise conditions. At the start of the treatment, animals were transferred to solitary cages in a separate room to avoid variation in vivarium conditions and either given access to a running wheel (diameter, 11.43 cm) or no wheel for 21 days. Both groups of animals could move about the cage at liberty. Running distance was measured by recording wheel revolutions using optical
sensors attached to each cage \((n = 33)\), which sent counts to a customized computer software program (BGSU, 2006).

**Tissue Collection**

BDNF protein levels fluctuate in a circadian cycle, as demonstrated in the rat hippocampus (Schaaf et al. 2000). Peak levels of BDNF protein occur during the onset of the inactive period within a 12-hour light / 12-hour dark circadian cycle. Therefore, all tissue collection took place within two hours after the onset of the light cycle, approximately between seven and nine a.m. After decapitation and rapid removal of the brain, the hippocampus was extracted bilaterally on ice and quickly frozen in dry ice. Eppendorf tubes were weighed pre- and post-tissue to determine wet tissue weight. Samples were stored at -80°C until protein analyse.

**BDNF ELISA Procedure**

The BDNF protein concentration derived from each tissue sample was measured using the BDNF E\(_{\text{max}}\) ImmunoAssay System (Promega, WI, USA), which utilizes an antibody sandwich technique. The assay was conducted with an acid-treated and non-acid treated technique to measure total BDNF and free mature BDNF, respectively. During acidification, it is thought that protein interactions are detached and are available for detection by the antibodies, allowing for quantification of total free BDNF (Okragly, A. & Haak-Frendscho, M., 1997). Detection of free mature BDNF using the non-acid treated technique has been reported previously (Promega, WI, USA; Zhang et al., 2003).

Initially, 100 µl of the anti-BDNF monoclonal antibody and carbonate coating buffer mixture (1:1000) was added to each well of a 96-well flat-bottomed plate (Fisher, USA). Carbonate coating buffer contains 0.025 M of sodium bicarbonate and sodium
carbonate diluted with distilled water. The plate was covered with parafilm and incubated overnight, for approximately 12 hours, in the refrigerator at 4°C. After the initial incubation, the contents of the plate were removed and washed once with Tris-buffered saline / Triton-X100 and Tween (TBST, 20 mM Tris/Triton, 0.15 M NaCl, 0.05% Tween 20). Aliquots of 200 µl of block and sample buffer (available with the kit, 1:5 dilution with distilled water) was added to all wells and incubated for one hour at room temperature.

To prepare the samples, the tissue was homogenized with TBST (1:10), then lysis buffer (2 % NP-40, 40 mM Tris-HCl, 1 mM sodium metavenadate, 2 mM PMSF, 20 % glycerol, 20 µg/ml aprotinin, 2 µg/ml leupeptin, .8006g NaCl) was added, which resulted in a 1:20 dilution. Samples were centrifuged for 10 minutes at 10,000 rpm after which, the maximum extractable amount of supernatant was transferred into a fresh tube. The homogenization and centrifugation process was repeated two more times, the supernatant from each cycle being consolidated. Dulbecco’s phosphate buffered saline (DPBS, 1:50) was added to 100 µl of the supernatant (0.2g KCl, 8g NaCl, 0.2g KH₂PO₄, 1.15g Na₂HPO₄, 0.133g MgCl₂•6H₂O, 0.1g CaCl₂•2H₂O) followed by 5 µl of HCl (HCl omitted for non-acid treatment). Each sample was vortexed and incubated at room temperature for 15 minutes. Finally, 5 µl of NaOH was added to each sample, followed by 750 µl of block and sample buffer. A standard curve was prepared using a serial dilution, which started at 500 pg/ml and was diluted by half until a concentration of 7.8 pg/ml was reached.

At the conclusion of incubation, contents were removed and washed one time with TBST wash buffer. Aliquots of 100 µl of both the standards and samples were
added to the inside wells in triplicate, while 100 µl of the block and sample buffer was
added to outside wells, the plate incubated for two hours with shaking at approximately
110 rpm. Following incubation, the samples and standards were aspirated from the wells,
with care not to disturb the bottom of the well. The plate was washed with TBST wash
buffer three times. Next, 100 µl of the anti-human BDNF polyclonal antibody block and
sample buffer mixture (1:500) was added to each well, while 100 µl of block and sample
buffer was added to the outside wells, the plate was covered with parafilm and incubated
for two hours with shaking at approximately 110 rpm. Following the incubation, the
contents of the wells were removed and washed three times with TBST wash buffer. An
aliquot of 100 µl of the anti-IgY horseradish peroxidase (HRP) in block and sample
buffer mixture (1:200) was added to each well, while 100 µl of block and sample buffer
only was added to the outside wells. The plate was again covered in parafilm and
incubated one hour with shaking at 110 rpm.

After incubation, the liquid was removed and the plate was washed three times
with TBST was buffer. The TMB One Solution was added to all inside wells and one
outside well at 100 µl each, making sure that the TMB was warmed to room temperature
beforehand. The plate was shaken at room temperature for ten minutes (110 rpm).
Finally, 100 µl of HCl was added to each of the wells. The plate was read on a multiskan
plate reader (MTX Lab Systems Inc.) at 450 nm.

Total Protein Procedure

The total protein concentration for each tissue sample was detected using the Bio-
Rad Protein Assay (Bio-Rad Laboratories, California, USA). To prepare the samples,
tissue was homogenized 1:10 with Tris/Triton. 50 µl of the sample was transferred to a
second tube and an additional 50 µl of Tris/Triton was supplemented resulting in a 1:20 dilution. The sample was then centrifuged for 10 minutes at approximately 10,000 rpm. The supernatant was removed and the remaining pellet was again homogenized and centrifuged, the supernatant being consolidated. An aliquot of 30 µl was transferred to a new tube and combined with 270 µl of Tris/Triton, resulting in a 1:200 dilution.

To create the standard curve, bovine serum albumin (BSA) is diluted with distilled water (2 mg/1 ml). There were six concentrations created for the standard curve. The first concentration was comprised only of Tris/Triton, the next contained 50 µl of diluted BSA and 950 µl Tris/Triton. The following concentrations continually added 50 µl to the BSA and subtracted 50 µl from the Tris/Triton until 250 µl of the BSA dilution was reached. After the samples and standards were vortexed well, 10 µl of each was added to the wells in triplicate along with 200 µl of diluted dye (1:4 with distilled water). After approximately five minutes, the plate was read in a multiskan plate reader (MTX Lab Systems Inc.) at 595 nm.

Data Analysis

Statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS for Windows, Version 16, SPSS, Inc., Chicago, IL) To ensure quality control, a linear regression equation of the standard curves (absorbency vs. protein concentration) had to meet a minimum criteria (R= .98) for further use. The equation of this line was used to determine the amount of total protein and BDNF levels within each sample. Total and mature BDNF protein levels were normalized; each value was divided by the total protein in that sample. The normalized values of total and mature BDNF protein were used in further data analyse. Any value of total or mature BDNF protein
that yielded a negative value, which indicated a quantity below the detectable range, was given a value of zero (n= 5, mature BDNF). Upon examining the data used to determine the effects of sex and age on sedentary BDNF levels, a non-normal distribution was discovered and corrected with a square root transformation.

On initial screening, data related to the effects of exercise and aging on BDNF levels displayed non-normal distributions; therefore, a square root transformation of raw values was carried out, which corrected the violation of this assumption.

Running distance was measured 24 hours a day for 21 days in 60 minute blocks. Values were calculated by summing wheel revolutions for each individual over the 21 day running period, multiplying by the circumference of the running wheel and then converting to miles. Screening of data used to determine the effects of exercise and age on BDNF levels, while controlling for running distance, yielded a non-normal distribution. A log transformation of raw values was performed on this data, which resulted in a normal distribution of transformed data.

All results were considered significant at the $p < .05$. Tukey’s post hoc test was used in all one-way analyses of sedentary BDNF levels. Bonferroni’s post hoc test was used to determine effects of exercise for each age within each sex.
RESULTS

BDNF Levels of Sedentary SAMP8 Across the Lifespan

Total BDNF

An important question that needed to be resolved initially was to determine if the effect of aging and sex of the mice on BDNF protein levels in sedentary SAMP8. In order to examine the effects of sex and age group on total BDNF levels within the hippocampus of sedentary animals, a 2x3 factorial analysis of variance (ANOVA) was performed.

Total BDNF levels in sedentary SAMP8 were altered by sex and aging (Fig 1.). ANOVA results indicated a main effect of sex, $F(1, 56) = 5.56, p < .05$, a significant effect of age, $F(2, 56) = 5.66, p < .01$, and a significant interaction between sex and age, $F(2, 56) = 4.89, p < .05$. Post hoc tests showed a significant difference between the middle-aged and old mice, $p < .01$.

In sedentary females, total BDNF increased with age. A one-way analyses confirmed a significant main effect of age in sedentary females, $F(2, 27) = 9.76, p < .01$. Post hoc tests showed that all three age groups of sedentary females were significantly different from each other (young and middle-aged females show a moderate effect, $p = .058$; middle-aged and old females, $p < .05$; young and old females, $p < .01$).

In sedentary males, total BDNF levels decline in middle age and rise again in old age. A one-way analyses confirmed a significant main effect of age in sedentary males, $F(2, 29) = 3.51, p < .05$. Post hoc tests reveal that middle-aged sedentary males have lower total BDNF levels than young sedentary males, the effect is moderate, $p = .052$. 
**Mature BDNF**

To understand the effects of sex and age of the animals on mature BDNF levels within the hippocampus of sedentary animals, a 2x3 factorial analysis of variance (ANOVA) was performed.

Hippocampal mature BDNF protein levels in sedentary mice were altered across their lifespan (Fig. 2). ANOVA results indicate a main effect of age, $F(2, 56) = 4.69, p < .05$, and a significant interaction between sex and age, $F(2, 56) = 5.4, p < .01$. Post hoc tests show a significant difference between young and middle-aged animals, $p < .01$, and middle-aged and old animals, $p < .05$.

In sedentary females, mature BDNF increased with age. A one-way analyses confirmed a significant main effect of age in sedentary females, $F(2, 27) = 8.04, p < .01$. Post hoc tests show that old sedentary females have significantly more mature BDNF than both young, $p < .01$, and middle-aged sedentary females, $p < .05$.

In sedentary males, mature BDNF levels decline in middle age and rise again in old age. A one-way analyses confirmed a significant main effect of age in sedentary males, $F(2, 29) = 5.98, p < .01$. Post hoc tests reveal that the decline in mature BDNF levels from young to middle-aged sedentary males was significant, $p < .01$.

Sedentary females display a similar pattern across aging in that total and mature BDNF increase. Sedentary males also show a consistent pattern in both total and mature BDNF in aging, however, they show a decline in middle-age. Although sedentary females and sedentary males displayed a distinct and significant pattern within sex,
significant differences between the sexes were not found for either total or mature BDNF levels in each age group, all \( F < 4.5, p > .05 \).

Effects of Voluntary Wheel Running on Hippocampal BDNF

*Total BDNF*

To determine the effects of sex, exercise, and age group on total BDNF levels within the SAMP8 hippocampus a 2x2x3 factorial analysis of variance (ANOVA) was performed. Total Hippocampal BDNF levels are increased by exercise but this effect declines with age (Fig. 3). Running proved to be effective in increasing total BDNF across the lifespan of SAMP8, showing a main effect of condition, \( F(1, 111) = 148.56, p < .001 \). A significant main effect of age, \( F(2, 111) = 3.61, p < .05 \), and a significant interaction between condition and age, \( F(2, 111) = 10.81, p < .001 \) were also found. Post hoc analyses of age revealed significant differences between young and middle-aged mice, \( p < .001 \), and young and old mice, \( p < .01 \).

Exercise was effective at increasing total BDNF levels within each sex in all age groups, all \( F > 7.27, p < .012 \). These results indicate that voluntary wheel running for 21 days was sufficient in inducing an increase in total BDNF in the hippocampus in young, middle-age, and old SAMP8 of both sexes.

The interaction between sex and condition was significant, \( F(1, 111) = 12.482, p < .01 \). Although sedentary female SAMP8 had lower levels of BDNF than sedentary males, female response to voluntary wheel running yielded higher BDNF levels than male SAMP8. This significant interaction of sex and condition is primarily due to the margin of difference between young sedentary and young running females as evidenced by the moderate effect of the three-way interaction between sex, condition and age, \( F(2,
111) = 3.05, \( p = .051 \). Taken together, these results imply that total BDNF can be up-regulated significantly by 21 days of access to running wheels. The increases in total BDNF between sedentary females and running females produced a greater margin of increase than sedentary males and running males at each age group. Although females displayed the most benefit from voluntary wheel running in terms of up-regulating BDNF, the margin of difference in both sexes declines across the lifespan indicating that the benefits of voluntary wheel running in old age are not comparable to young.

For each sex, a 2x3 ANOVA was conducted to assess the age-related declines in exercise enhancement on total BDNF. Results show a significant age by condition interaction for female mice, \( F(2, 54) = 12.57, p < .001 \), indicating a significant reduction in the effectiveness of physical exercise to up regulate BDNF in aging. Males did not show an age related decline in total BDNF over the lifespan, \( F(2, 27) = 1.84, p > .05 \).

**Mature BDNF**

To determine the effects of sex, exercise, and age on mature BDNF levels within the SAMP8 hippocampus, a 2x2x3 factorial analysis of variance (ANOVA) was performed. Mature BDNF in SAMP8 showed an increase in response to exercise, which was dependent on sex and age (Fig. 4). Voluntary wheel running proved to be effective in up-regulating free mature BDNF as demonstrated by a significant main effect of condition, \( F(1, 111) = 74.58, p < .001 \). A significant main effect of age, \( F(2, 111) = 4.17, p < .05 \), was also found. Post hoc tests showed a significant difference between young and middle-aged animals, \( p < .01 \). Wheel running significantly up-regulated mature BNDF at each age group for each sex, similar to total BDNF, (all \( F > 14.06, p < .01 \)),
with the exception of young males. There was a significant three-way interaction between sex, condition, and age group on mature BDNF, $F(2, 111) = 6.15, p < .01$.

Assessment of the decline in exercise induced mature BDNF across the lifespan was consistent with the findings for total BDNF, females showed an age related decline, $F(2, 54) = 6.45, p < .01$, while males did not, $F(2, 57) = 2.42, p > .05$.

The results for mature BNDF are similar to total BDNF in that sex and age play important roles in the course of BDNF up-regulation by exercise. Young females exhibited the greatest BDNF increase from voluntary wheel running but up-regulation of BDNF tended to decline across the lifespan for females.

**Recorded Running Distance**

Running distance has been correlated with increased BDNF mRNA and BDNF protein levels (Adlard et al., 2004; Oliff et al., 1998). It has been demonstrated that aging in SAMP8 is associated with less activity in the running wheel (McAuley, Miller, Beck, Nagy, & Pang, 2002). Therefore, an analysis was performed to determine whether the decreased effects of running on BDNF in the aged mouse could be solely due to decreased running. Wheel revolutions were recorded as a measure of locomotor activity to examine whether running distance alters the amount of BDNF induction.

**Running Distance and Aging**

A subset of SAMP8 had their running distances measured ($n = 33$). To examine the effects of sex and age group on total recorded running distance, a 2x3 ANOVA was conducted. Running distance was reduced with age (Fig. 5). The main effect of age was significant, $F(2, 27) = 13.55, p < .001$. Tukey’s post hoc test revealed that young animals
ran significantly farther over 21 days than both the middle-aged and old SAMP8, while the middle-aged and old animals did not differ significantly from each other.

To determine whether running distance changed with time during the 21 days, a repeated measures ANOVA was conducted on weekly running distance with weeks as within subjects factors. Middle-aged and old animals significantly increased their running distance from week 1 to week 2; however, these gains were stabilized by week 3 (Fig 6.).

**Running Distance and BDNF Levels**

As shown above, young animals ran significantly farther than middle-aged and old animals. Running distance shows a moderate correlation with total BDNF levels ($r = 0.471$). Therefore, an analysis of covariance (ANCOVA) was conducted to determine the effect of age on total BDNF levels in the hippocampus, while factoring out total running distance in the analysis. Sex was not included as a factor because it was previously demonstrated not to be a significant factor in running distance. ANCOVA results for total BDNF indicated a moderate effect of age, $F(2, 29) = 3.24, p = .054$. ANCOVA results for mature BDNF did not show a main effect for age group. The results suggest that age, unconfounded by running distance, decreased the effectiveness of running in up-regulating total BDNF but not mature BDNF.
CONCLUSIONS

In this study, the levels of BDNF were examined in sedentary and exercising male and female SAMP8 across their lifespans. In sedentary mice, females displayed an increase of total BDNF and mature BDNF across the lifespan while male SAMP8 exhibited a decline in total and mature BDNF in middle-age. Voluntary wheel running proved to be effective tool in up-regulating total and mature BDNF in the hippocampus of young and aged male and female SAMP8. The increase in BDNF levels in response to exercise is reduced in the aged SAMP8, significantly in the female. It was determined that this decrease in BDNF induction is not entirely accounted for by the reduction of locomotor activity. This suggests that neuronal changes in normal aging and perhaps neuronal pathology specific to the SAMP8 alter the ability to produce and/or release BDNF.

The first goal of this study was to profile BDNF levels within the hippocampus of sedentary SAMP8 across the lifespan. The SAMP8 has been shown to be a good model of accelerated aging and Alzheimer’s disease, therefore, it is presumable that one would expect a reduction in BDNF levels in aging consistent with reductions of BDNF in the AD hippocampus (Connor et al., 1997; Ferrer et al., 1999; Hock et al., 2000). In the case of the male SAMP8, our findings are consistent with this presumption to a degree. Sedentary male SAMP8 do show a significant reduction in both total and mature BDNF in middle-age. However, there was not a significant difference between young or middle-aged and old male SAMP8, indicating a stability of BDNF levels between young and old sedentary males, which is consistent with one study of healthy male C57 mice (Adlard et al., 2005).
In contrast to sedentary males, sedentary female SAMP8 showed an increase in total BDNF and, more importantly, mature BDNF, which is thought to promote cell survival and differentiation (Huang & Reichardt, 2001; 2003). Previous work examining the profile of total BDNF in the female SAMP8 reported no differences between the young and old females (Katoh-Semba et al., 1998). The results in this study are more consistent with prior work reporting increases in total BDNF over the lifespan in male Wistar and female Sprague-Dawley rats, which exhibit normal aging (Katoh-Semba et al., 1998; Silhol et al., 2007). It has also been demonstrated that the female cycle influences BDNF levels. Plasma BDNF levels have been shown to be highest during the luteal phase of fertile women, and amenorrhoeic and postmenopausal women show significantly lower levels of BDNF (Begliuomini et al., 2007). The present study contrasts these findings given that old female SAMP8 (> 10 months) have higher BDNF levels than young female SAMP8. Old female SAMP8 have also been shown to display disrupted estrus cycles (Patton, 2006). Estradiol treatment can reverse the reduction of BDNF mRNA and BDNF protein levels in the hippocampus of female rats with ovariectomy and gonadectomized male rats, respectively (Berchtold, Kesslak, Pike, Adlard, & Cotman, 2001; Scharfman & MacLusky, 2005; Singh, Meyer & Simpkins, 1995; Solum & Handa, 2001). These findings suggest that the differences between BDNF levels in sedentary males and females across the lifespan is not simply due to cycling hormone levels in females.

Levels of BDNF can be altered by the dysfunction of microglial cells, which are highly sensitive to changes in neuronal pathology (Shetty et al., 2004; Kreutzbert et al., 1996). Reduced activation of microglia is correlated to reduced BDNF levels (Shetty et
Impairments in axonal transport by increased hyperphosphorylated tau and plaque load may impair trafficking of BDNF (Schindowski et al., 2008). The extent to which male or female SAMP8 may differ between these factors may account for the different aging patterns of BDNF between the sexes. Studies, which examine BDNF levels in AD patients, are taken from post-mortem tissue, in which AD diagnosis is determined through plaque and tangle load. The extent to which these above factors increase neurotrophin dysfunction in end stage AD may not be parallel to their effects in accelerated aging in the SAMP8.

Consistent with previous work, twenty-one days of exercise was shown to be an effective tool in up-regulating total and mature BDNF levels within the hippocampus of young animals (Berchtold et al., 2005; Adlard et al., 2004). Middle-aged and old SAMP8 also demonstrated exercise induced up regulation of total and mature BDNF. This result contrasts with a previous study that found an up-regulation of BDNF by exercise only in young, but not in aged C57 mice (Adlard et al., 2005). Given that the male SAMP8 in this study did show lower levels of exercise-induced increases in BDNF compared to females, the contrasting results between studies could be attributed to strain. BDNF increases seen in old SAMP8 following exercise, albeit smaller than young mice, suggest that the SAMP8 is a valuable model to evaluate the effectiveness of up-regulating BDNF and reducing age-related cognitive decline and neuronal degeneration.

While young animals showed the greatest increases in total and mature BDNF following wheel running, middle-aged and old animals had reduced benefits from voluntary exercise compared to young SAMP8, particularly old females. Shetty (2004) demonstrated that healthy old animals did not show an increase in BDNF levels to the
same extent as young animals after hippocampal injury. These findings indicate that older animals do not stimulate the production and/or release of BDNF as efficiently as young animals do. Interestingly, stress-related down regulation of BDNF is also reduced in aged rats (Smith & Cizza, 1996). These results suggest that the aged brain may be less able to modulate BDNF in both directions compared to young brains. In regards to exercise, older SAMP8 are associated with reduced locomotor activity (McAuley et al., 2002). Although there are expected aging related declines in locomotor activity, it does not account for all the changes in total BDNF seen in response to voluntary exercise. Therefore, neuronal changes within the hippocampus that attenuate either the production of BDNF or sorting of BDNF into the activity dependent pathway reduce the animals ability to up-regulate BDNF to the extent seen in young SAMP8.

This study examined the profile of total and free mature BDNF in both male and female sedentary SAMP8 across the lifespan. Female SAMP8 showed an increase while males showed in initial decline in middle-age and stabilized in old age. Further research examining the discrepancies between male and female SAMP8 basil levels of BDNF in aging is an area of future research. The present study also demonstrated that the SAMP8 are an excellent model for enhancing BDNF through voluntary exercise, as BDNF up-regulation in old animals was not previously reported. BDNF levels of middle-aged and old SAMP8 were increased in response to voluntary wheel running. The amount of BDNF increase by exercising in SAMP8 is reduced in aging. Therefore, the SAMP8 can serve as a useful model in examining the underlying mechanisms of the aging-related decline in BDNF levels in response to exercise.
REFERENCES


distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain.
*European Journal Molecular Biology Organization, 9*(8), 2459-2464.


Ickes, B. R., Pham, T. M., Sanders, L. A., Albeck, D. S., Mohammed, A. H., &
Granholm, A. (2000). Long-Term Environmental Enrichment Leads to Regional
Increases in Neurotrophin Levels in Rat Brain. *Experimental Neurology, 164*, 45-
52.

neurons show responses to BDNF, NT-3, NT-4, but not NGF. *The Journal of
Neuroscience, 13*(8), 3394-3405.

Hippocampal brain-derived neurotrophic factor but not neurotrophin-3 increases
more in mice selected for increased voluntary wheel running. *Neuroscience, 121*,
1-7.

levels of brain-derived neurotrophic factor in selected brain regions of rats,
normal mice and senescence-accelerated mice: a comparison to those of nerve

in Neuroscience, 19*(8), 312-318.

(1993). BDNF and trkB mRNA expression in the hippocampus formation of

Laske, C., Stransky, E., Leyhe, T., Eschevieler, G. W., Maetzler, W., Wittorf, A.,
Soekadar, S., Richartz, E., Koehler, N., Bartels, M., Buchkremer, G., & Schott, K.
(2007). BDNF serum and CSF concentrations in Alzheimer’s disease, normal
pressure hydrocephalus and healthy controls. *Journal of Psychiatric Research, 41*,
387-394.

Lee, J., Fukumoto, H., Orne, J., Klucken, J., Raju, S., Vanderburg, C. R., Irizarry, M. C.,
Hyman, B. T., & Ingelsson, M. (2005). Decreased levels of BDNF protein in
Alzheimer temporal cortex are independent of BDNF polymorphisms.
*Experimental Neurology, 194*, 91-96.


FIGURE CAPTIONS

Figure 1: Untransformed means of total BDNF levels within the hippocampus of sedentary SAMP8 across the lifespan. In females, total BDNF increases significantly with age. Old Sedentary females have significantly more total BDNF than both young and middle-aged sedentary females. In sedentary males, total BDNF declines significantly in middle age. Error bars represent standard error of the mean.

Figure 2: Untransformed means of mature BDNF levels within the hippocampus of sedentary SAMP8 across the lifespan. Old sedentary females have significantly more mature BDNF than both young and middle-aged sedentary females. Sedentary males show a significant decline in middle-age. Error bars represent standard error of the mean.

Figure 3: Effects of voluntary wheel running on total hippocampal BDNF across the lifespan. Exercise significantly up-regulated total BDNF levels within each sex in all age groups. Female SAMP8 show a significant reduction in the effectiveness of physical exercise to up-regulate total BDNF. Untransformed means of total BDNF levels, error bars represent standard error of the mean.

Figure 4: Effects of voluntary wheel running on mature hippocampal BDNF across the lifespan. Exercise significantly up-regulated mature BDNF levels within each sex in all ages, except young males. Untransformed means of mature BDNF levels, error bars represent standard error of the mean.

Figure 5: Total running distance over 21 days. Young animals ran significantly farther over 21 days than both the middle-aged, $p < .01$, and old SAMP8, $p < .001$. There was not a significant main effect of sex. Error bars represent standard error of the mean.

Figure 6: Running distance did not increase over the 21 days in young SAMP8. Middle-aged and old animals significantly ran farther from week 1 to week 2, $p < .05$. Error bars represent standard error of the mean.
Figure 1

Total Hippocampal BDNF (ng/g)

- Male
- Female

Young | Middle-Aged | Old

0 | 50 | 100 | 150 | 200 | 250 | 300
Figure 2

Mature Hippocampal BDNF (ng/g)

- Male
- Female

Young | Middle-Aged | Old
Figure 3

- Sedentary Male
- Running Male
- Sedentary Female
- Running Female

Total Hippocampal BDNF (ng/g)

Young | Middle-Aged | Old
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[Data points and error bars]

[Graph showing the comparison of total hippocampal BDNF levels across young, middle-aged, and old groups for sedentary and running males and females.]
Figure 4

Mature Hippocampal BDNF (ng/g)

- Sedentary Male
- Running Male
- Sedentary Female
- Running Female

Young | Middle-Aged | Old
Figure 5

Total Distance (Miles)

Young  Middle-Aged  Old

Male  Female

**  ***
Figure 6

Running Distance (Miles)

7 Days
14 Days
21 Days

Young
Middle-Aged
Old

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