Exercise Induces Age-Dependent Neuroplastic Changes in Brain Regions Responsible for Learning Memory and Emotional Behavior

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Abstract

During early-life, the brain experiences a variety of developmental adaptions and undergoes a period of immense neural pruning, making the brain more plastic and susceptible to change from external events. Exercise is one such event that produces a milieu of adaptive changes including improved brain plasticity and function, increases in hippocampal brain derived neurotrophic factor (BDNF), and BDNF-mediated enhancements in hippocampal function. Recent work has shown that exercise during early life has can produce long-lasting improvements in brain function that would normally be transient in adulthood. The specific developmental period during which the hippocampus is prone to exercise-induced neuroplastic changes, however, is unknown because previous studies testing the impacts of early life exercise had rats run across multiple developmental stages. The purpose of this study was to determine the impact that exercise has on BDNF if initiated during two different and clearly defined early life developmental periods, the early juvenile period immediately post weaning versus the pubescent period surrounding puberty onset. Hippocampal BDNF mRNA levels were measured following one week of exercise and after 14 days following exercise cessation in juvenile (approx. postnatal day 24), pubescent (approx. postnatal day 40), and in adult rats (approx. postnatal day 7). Exercise produced significant increases in BDNF mRNA compared to sedentary matched counterparts across all ages in the dentate gyrus immediately following one week of exercise. Juvenile rats exhibited a trend towards long-lasting increases in BDNF 14 days after exercise within the dentate gyrus and the CA3. Thus, exercise produced trends toward enduring increases in BDNF when initiated before puberty. These data suggest that the juvenile brain may have the greatest propensity for long term increases in BDNF following exercise.

Introduction

Regular moderate physical activity produces a wide range of positive mental health impacts, including improvements in mood, learning, memory and cognition (Berchtold et al., 2010; Schoenfeld et al., 2011 Van Praag et al., 2005; Zheng et al., 2006). Current research is focused on understanding the exercise-induced neurobiological adaptations within brain regions responsible for mediating these positive affective and cognitive impacts of exercise. The hippocampus is one such region involved in various aspects mood, learning, memory and cognition (Bannerman et al., 2004; McHugh et al., 2005). Exercise can enhance hippocampal function through a range of neural processes such as neurogenesis (Van Praag et al., 2005), synaptogenesis (Nichol et al., 2009), long-term potentiation (Van Praag al., 1999), and by increasing growth factors. Indeed, exercise can increase growth factors, including brain derived neurotrophic factor (BDNF; Adlard et al., 2004; Gomes da Silva et al., 2012). Growth factors, like BDNF, are proteins that activate and modulate signals to promote the survival and maturation of neurons and synapses within various regions of the brain (Barde et al., 1993). BDNF within the hippocampus is implicated in antidepressant- like behavior (Siuciak et al., 1998), improved spatial learning and long-term memory (Vigers et al., 2012; Bekinschtein et al., 2007; Gomes da Silva et al., 2012) and protections against stress-induced cognitive impairments and fear memory (Heldt et al., 2007; Radecki et al., 2005). Furthermore, inhibiting BDNF pathways blocks the exercise-induced neural enhancements that improve cognition (Vaynman et al., 2004). Thus, BDNF is integral in mechanisms by which exercise improves function within regions of the brain associated with mood, learning, memory and cognition.

Exercise-induced increases in BDNF and subsequent BDNF signaling initiates intracellular signaling cascades that promote positive adaptations in neural plasticity and function. BDNF action involves activation of its membrane receptor, tyrosine receptor kinase B (TrkB) and, through several different kinase cascades, induces increases in mRNA levels of certain plasticity-related proteins, including cAMP response element-binding protein (CREB; Vaynman et al., 2003). CREB is a transcription factor that plays a critical role in facilitating the transcription of many other proteins, including synapsin-I (Jovanovic et al., 1996), which maintain and enhance neural and synaptic plasticity (Silva et al., 1998). Synpasin-I is a phosphoprotein involved in vesicular trafficking, and increased levels of synapsin-I indicate increased synaptic vesicle formation and stronger synaptic connections (Greengard et al., 1993). Stronger synapses act to secure and enhance neural connections, and therefore allow for better communication within the brain (Fusi et al., 2005). Furthermore, CREB can increase transcription of BDNF mRNA and its TrkB receptor, resulting in a positive feedback loop that enhances BDNF action (Vaynman et al., 2003). These pathways are important mechanisms by which exercise-induced increases in BDNF produce enhanced neural plasticity and function.

In adult mammals, exercise-induced increases in BDNF and subsequent enhancements in function are transient and only persist as long as exercise is maintained. For instance, Hopkins et al. (2011) demonstrated that the increases in BDNF protein after four weeks of running return back to baseline levels within 14 days following exercise cessation. Similarly, Berchtold et al. (2005) reported that BDNF protein availability decreased after 1 week following exercise cessation. Accordingly, when exercise is initiated during adulthood, it must be sustained in order to maintain the beneficial effects

of increased BDNF on brain plasticity and function. Interestingly, recent work demonstrates that exercise initiated during early, critical periods during development can produce longer lasting increases in BDNF mRNA. Hopkins et al. (2011) has demonstrated that 4 weeks of juvenile onset exercise produces changes in BDNF levels and that are maintained 28 days following exercise, and that these changes are associated with persistent improvements in exercise-dependent recognition learning. These lasting changes were not maintained in adult rats, suggesting the age at which exercise is initiated is important for the duration of neurobiological and behavioral changes. Likewise, unpublished data from our lab has demonstrated that exercise, when initiated during the juvenile period, can produce increases in BDNF that persist 25 days following cessation of exercise (Mika et al., unpublished data). Collectively, these studies suggest that exercise initiated during early stages of development can produce enduring changes that last beyond exercise cessation; however, it is unclear from these data which specific developmental period exercise needs to occur in order to produce these persistent effects. Studies thus far have utilized longer exercise time courses, in that rats begin running during the juvenile period (immediately post weaning) and run across multiple developmental periods into adulthood. Therefore, it is difficult to decipher which early-life period is most critical for exercise to interact with the brain.

Throughout mammalian development, there are discrete developmental periods during which certain events and experiences can have a significantly greater impact upon the organism than that those that occur later in life (Andersen, 2003). These periods are characterized by neural pruning that follow an immense overproduction of synapses and receptors in early life. In this, they act as a vital component in forming the mature

nervous system and promoting the efficacy of synaptic transmission. During these periods, the brain is very plastic and susceptible to influences from external events; such events can produce substantial adaptive or maladaptive changes in brain plasticity and function that persist throughout the lifespan (Nithianantharajah et al., 2006). One of the periods, specifically, occurs in early life, before puberty, and involves pruning of nearly half of chemical synapses within certain regions of the brain (Andersen, 2003). The hippocampus experiences a reduction of nearly 25% of NMDA receptors, which are important ion channel proteins that mediate synaptic plasticity and memory function (Spear et al., 2000). BDNF influences changes in factors that modify synaptic plasticity, and exercise-induced BDNF increases can potentially modulate neural plasticity in the brain during development (Anderson, 2003). Neuroplastic changes that effects BDNF levels are highly impacted by developmental age and seem to be more plastic earlier in life, however, the time course by which this occurs is relatively unclear (de Almeida et al., 2013). Puberty, in particular, is also a critical stage during postnatal development during which exercise could potentially have a greater influence on brain plasticity. Puberty is characterized as period sexual maturation modulated by an immense peak in gonadal steroids (Korenbrot et al., 1977). These steroids can potentially modulate neurotransmitter effectiveness and, overall, alter neural development (Sisk et al., 2005). More specifically, rising levels of androgens during puberty interact and enhance neural growth and function (Andersen et al., 2008; Hebbard et al., 2003; Morrison et al., 2014). Thus, both the early life developmental period of neural pruning and the pubertal spikes in sex steroids could act separately, or interdependently, to influence the enduring exercise-induced changes within the brain.

The purpose of this experiment was to evaluate the persistence of exercise-induced increases in hippocampal BDNF mRNA when exercise was initiated during different developmental periods, in order to determine whether running during particular periods can produce longer lasting increases in BDNF mRNA. The brain is incredibly plastic in early life and exercise has been observed to produce long-lasting neuroplastic changes when initiated in early life as compared to adulthood (Hopkins et al., 2011). However, the particular time course of this development is not well known. The present study will be the first to characterize the specific developmental period at which the brain is most plastic to exercise-induced adaptions in the brain. We assessed BDNF mRNA levels after one week of exercise as well as two weeks after exercise cessation in three different age groups of rats: juvenile (from immediately post weaning until pre-pubescent stage; appx. postnatal day 24), pubescent (during puberty; appx. postnatal day 40), and adult (postnatal day 70), in order to determine the time course of exercise induced BDNF increases within these age groups. We hypothesize that exercise will produce a long-term increases in BDNF mRNA within the hippocampus in either, or both, of the juvenile or pubescent rats due to the sensitive state of the brain in early life. The findings from this experiment can potentially isolate the critical period by which exercise can produce the most enduring, adaptive changes within the hippocampus.

Materials and Methods

Animals

Juvenile (aprrox. postnatal day 24), pubescent (appx. postnatal day 40) and adult (appx. postnatal 70) male Fischer F344 rats (Harlan Laboratiories, IN) were housed in a temperature (22°C) and humidity-controlled environment and were maintained on a

12:12 h light/dark cycle (lights on 05:00-17:00). Rats within the exercise group were allowed access to voluntary running wheels immediately upon arrival and continued to have access to wheels for 1 week. All rats were paired housed in Nalgene Plexiglas cages (45cm x 25.3cm x 14.7cm). Rats were weighed weekly and given *ad libitum* access to food and water. Care was taken to minimize animal discomfort during all procedures. All experimental protocols were approved by the University of Colorado Animal Care and Use Committee.

Experimental Design

Rats in all age groups (juvenile, pubescent, and adult) were randomly assigned to either exercise group or a sedentary group (n = 8/group). Those within exercise groups were given access to a running wheel for one week. One week of exercise is known to produce upregulation of BDNF mRNA across all ages (Neeper et al., 1996). Indeed 2 days of exercise is sufficient to produce exercise-induced BDNF increases (Garza et al., 2004). After one week of exercise, half of the rats within each group were sacrificed (immediate sacrifice group) in order to demonstrate the immediate effects of exercise on growth factor mRNA expression. Running wheels were locked for the remainder of the rats, and this cohort remained in their cages with wheels locked for 14 days until sacrifice (14-day group) in order to investigate the long-lasting effects of exercise on growth factor mRNA expression. The 14-day timepoint following exercise cessation was selected because the exercise-induced elevated levels of BDNF mRNA in adult animals is transient and has been shown to return to baseline levels in less than 14 days after exercise has ended (Berchtold et al., 2005). In young rats, however, elevated levels BDNF mRNA are maintained and remain increased for up to 25 days after exercise has

ended (Mika et al., unpublished data). Thus, 14 days allows us to isolate the long-term effects seen after exercise during the critical developmental periods in early life.

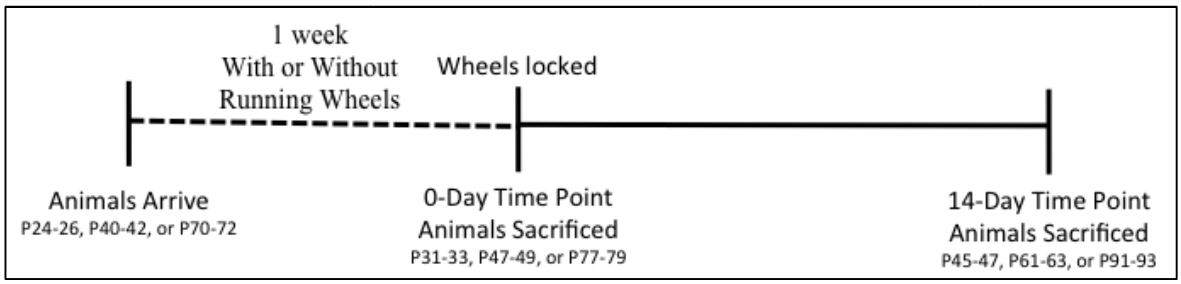


Figure 1: Timeline demonstrates the start and end of exercise, day of sacrifice, and postnatal ages of juvenile (P24-P26), pubescent (P40-P42), and adult groups (P70-P72, respectively.

Voluntary wheel running

Daily wheel revolutions were recorded digitally using Vital View software (Mini Matter, Bend, OR) and running distance was calculated by multiplying the number of revolutions by wheel circumference (1.081m). Running distances are represented mean distance per day. Values for individual rats were estimated by dividing the total daily distance by two since the rats were pair housed.

Verification of Puberty

Balano-preputial separation was analyzed within pubescent male rats following one week of exercise were to determine if they had indeed entered puberty. Puberty in male rats is typically characterized by a short period, approximately occurring around postnatal day 40-55, involving a large surge in gonadal hormones, such as testosterone and DHT, the maturation and increased levels of sperm, and an increase in volume of seminiferous epithelium (Lent et al., 2014). Balano-preputial separation is characterized by the separation of the prepuce and the glans penis. This was assessed by applying

gentle pressure to manually retract the prepuce. This complete separation of the prepuce and the glans penis acts as a significant marker of puberty onset, as it occurs just prior to the surge of testosterone and increased mature sperm count that occur during puberty (Gayton et al., 1988; Korenbrot et al., 1977).

Tissue Processing

Brains were extracted at the time of rapid decapitation and were flash frozen in isopentane with dry ice (-20°C; 4 minutes). Brains were stored at -80°C prior to being sectioned at a thickness of 10 µm with a cryostat (CM 1850, Leica Microsystems, Nussloch, Germany). Slicing occurred at -24°C and rostral-caudal sections of the prefrontal cortex and hippocampus were collected and thaw-mounted onto FisherBrand Colorfrost®Plus slides (Fisher Scientific Company LLC, Denver, CO, USA). Tissue sections were stored at -80°C prior to use in single-label radioactive *in situ* hybridization.

Single Label Radioactive *In Situ* Hybridization

In situ hybridization was conducted according to previously published protocols (Akil, 1996, Greenwood et al., 2011, Greenwood et al., 2012). The probe for BDNF was previously available for this *in situ* hybridization. For hybridization brain tissue slides will be fixed in 4% paraformaldehyde for 1 hour, washed 3 times in 2X saline-sodium citrate (SSC) buffer, acetylated with 0.25% acetic anhydride containing 0.1 M triethanolamine for 10 minutes, and dehydrated with graded ethanol. A previously prepared BDNF complementary DNA probe was transcribed with ATP, CTP, GTP, and S^{35} UTP (radioactive tag) to create a riboprobe. After verification of radioactive labeling, the riboprobes will be mixed with 50% hybridization buffer comprised of 50% high-grade formamide, 10% dextran sulfate, 3X SSC, 1X Denhardt's solution, 0.2 mg/mL

yeast tRNA, and 0.05 M sodium phosphate (pH 7.4). The riboprobe and hybridization buffer mixture was applied directly to slides containing sections of the hippocampus. Slides were incubated overnight at 55°C in humid chambers. After 24 hours, slides were washed 3 times in 2X SSC and incubated for 1 hour in RNase A (200 ug/mL), rinsed in graded concentrations of SSC, washed in 0.1X SSC at 65°C for 1 hour, and dehydrated in graded ethanol. After drying, slides were placed in light-tight autoradiography cassettes and exposed to X-ray films (Kodak or Biomax-MR) for 1 week.

Image Analysis for *In Situ* Hybridization

Levels of BDNF mRNA were analyzed by computer-assisted optical densitometry. Brain section images were captured digitally (CCD camera, model XC-77; Sony, Tokyo, Japan), and the relative optical density of the x-ray film is to be determined using Scion Image Version 4.0 (Scion, Frederick, MD, USA). A macro was written that enabled signal above background to be determined automatically. For each section, a background sample was taken over an area of white matter, and the signal threshold was calculated as mean grey value of background +3.5 standard deviations. The section was automatically density-sliced at this value, so that only pixels with gray values above these criteria were included in the analysis. Results were expressed as a mean integrated density, which reflects both the signal intensity and the number of pixels above assigned background (mean signal above background x number of pixels above background). Each subject's mean integrated density of BDNF at a given level represents the average of between 2 to 4 slices chosen for analysis between the following coordinates: hippocampus from -2.3mm to -3.3mm posterior to bregma based on the atlas by Paxinos and Watson (Paxinos and Watson, 2006). Templates for this region were made to ensure equivalent

areas were analyzed between animals. BDNF levels were quantified in four regions of the hippocampus: dentate gyrus (DG), CA1, CA2, and CA3. Regions can be visualized in Figure 2. Representative slices for different age, exercise, and time point groups can be seen in Figure 5.

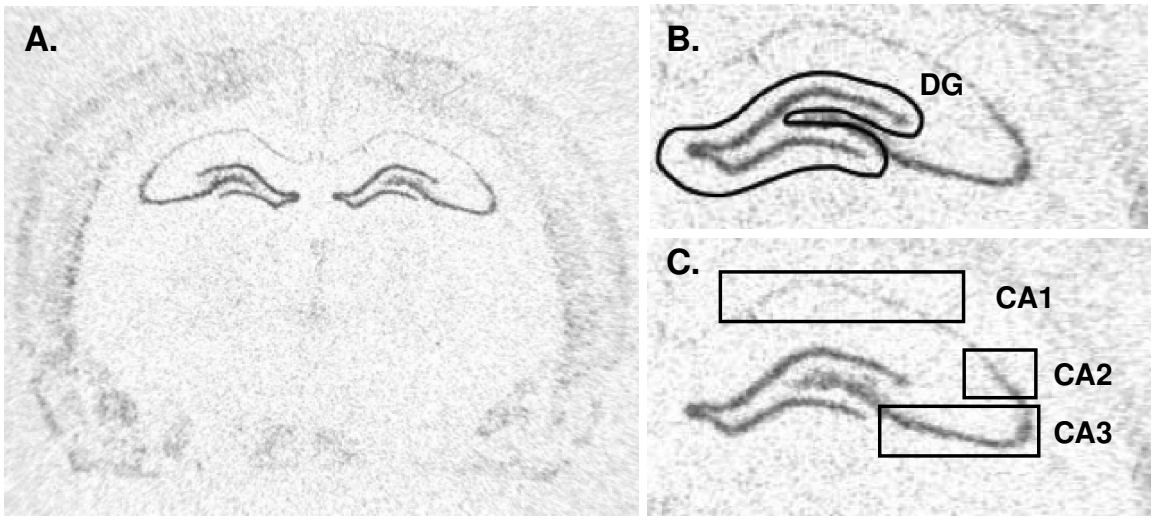


Figure 2: Examples of regions quantified for BDNF mRNA *in situ* hybridization. Regions are within the hippocampus include the dentate gyrus (DG), CA1, CA2 and CA3. (A) A coronal slice of the rat brain, which contains the hippocampus. (B) Region of the hippocampus: dentate gyrus (DG) (C) Regions of the hippocampus: CA1, CA2, and CA3.

Data Analysis

Running distances were analyzed using repeated measures ANOVA. Running distance over the entire week was also compiled and compared using ANOVA. Body weights were similarly analyzed with a 2 x 2 repeated measures ANOVA with Age and Exercise status as between-subjects factors. BDNF mRNA levels in the hippocampus were analyzed with a 3 x 2 x 2 ANOVA, with age (adult, pubescent, juvenile) and exercise (running, sedentary) and timepoint (immediate, 14-day) as factors (n = 8/group). Fisher protected least significant differences (F-PLSD) post hoc analyses were conducted

only when a significant interaction was observed. Significant effects were detected at a p value of 0.05 or less. Data are represented by a means +/- SEM.

Results

Running Distances and Body Weight

Average daily running distance is shown in Figure 3. The mean total daily running distances, estimated per rat, were calculated for adult run, pubescent run, and juvenile run groups (Figure 3A). Age impacted both total running distance ($p=0.0003$), in that pubescent rats ran, on average, more than both juvenile run rats and adult run rats. Additionally, running distance changed significantly across time ($p<0.0001$). Pubescent rats ran, on average, more than both juvenile run rats and adult run rats (Figure 3B). There was a significant interaction between time and age ($p<0.0001$), in that adult run rats displayed typical running behavior of Fischer 344 rats (Greenwood et al., 2009) as they increased running distance throughout the week of exercise until the final day. In contrast, pubescent run and juvenile run rats reached a greater running distance earlier than adult rats and then leveled off the rest of the week. Running data was not reported for day 7 because rats were sacrificed halfway through their running day.

All adult run, pubescent run, and juvenile run groups gained weight throughout the experiment ($p<0.0001$) (Figure 3C). Overall, the adult run rats weighed more than the pubescent run rats, which weighed more than the juvenile run rats ($p<0.0001$). ANOVA revealed a significant interaction between time and age ($p<0.0001$). There was a slight difference between runners and their sedentary counterparts ($p=0.05$), however there was no interaction between exercise status and age ($p=0.73$) or time and exercise status ($p=0.15$).

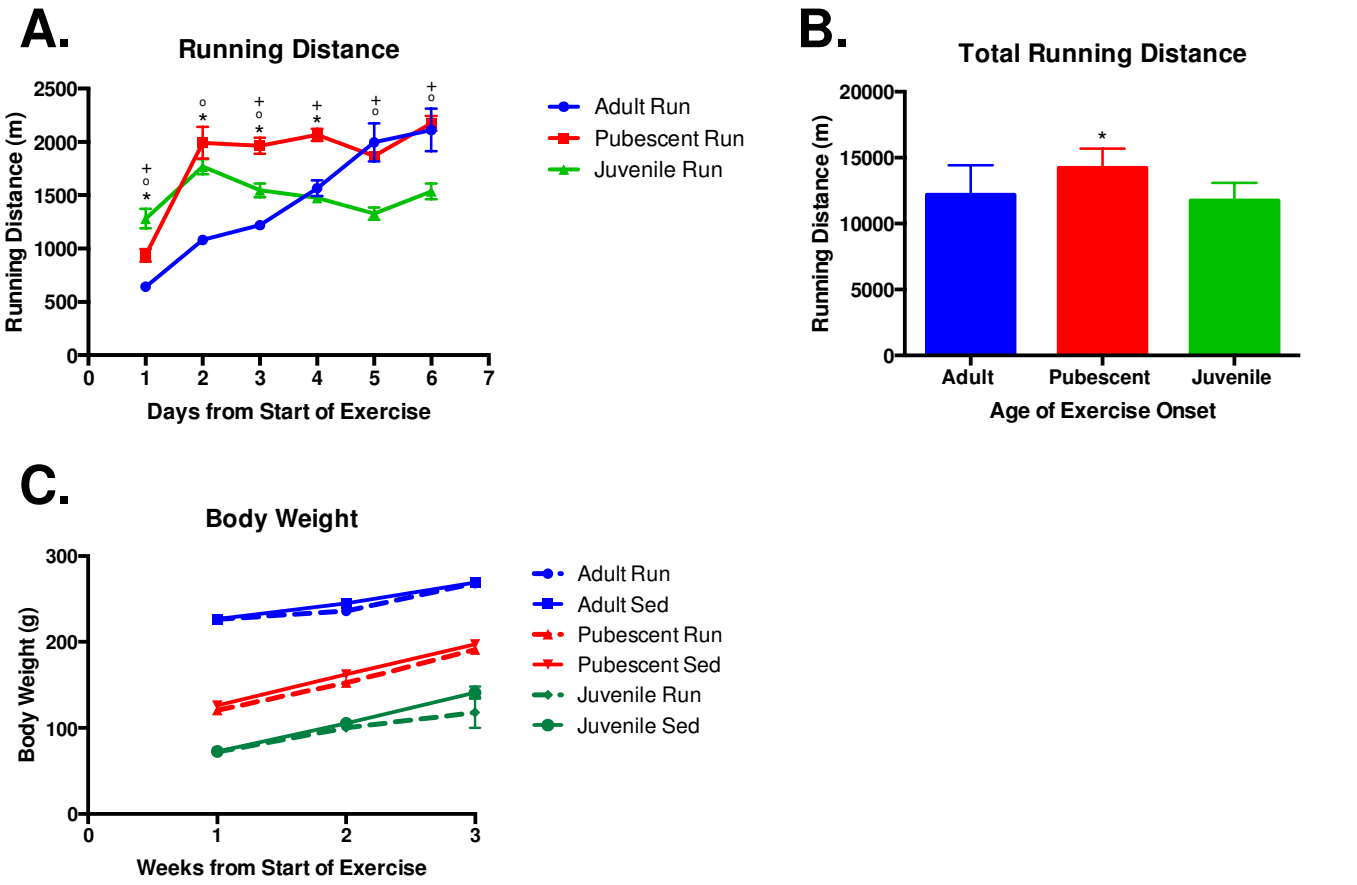
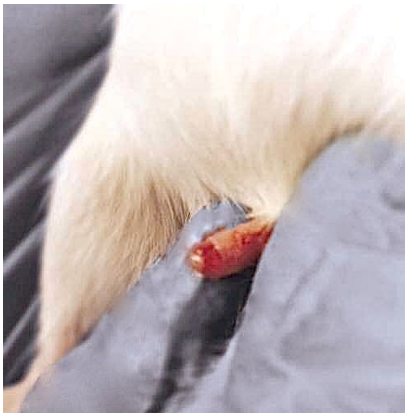


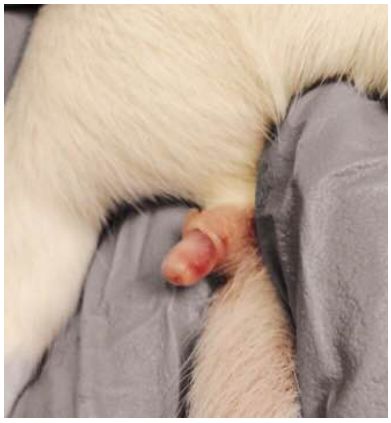
Figure 3: Running distance and body weight. (A) Daily total running distance across one week of exercise, estimated per rats (double housed). (B) Total running distances per day. Pubescent rats ran significantly more than other age groups (p=0.0003). (C) Weekly body weight across three weeks of duration of the experiment per 2 rats (double housed). Adults weighed more than pubescent rats, which ran more than juvenile rats. There was no main effect of exercise status. Data are represented as mean ± SEM; * denotes a significant difference between pubescent and juvenile runners ($p<0.05$). ^{o} denotes a significant difference between adult and juvenile runners ($p<0.05$). $^{+}$ denotes a significant difference between pubescent and adult runners ($p<0.05$)

Onset of puberty in Pubescent Rats

Balano-preputial separation was observed in all but one pubescent rat runners after one week of exercise. In the remainder of the rats, balano-preputial separation was either complete or almost complete. Figure 4 demonstrates a rat penis before and after preputial separation.



Before



After

Figure 4:
Male pubescent rat penis before and after balano-preputial separation. Note that the prepuce can be completely retracted to expose the glans penis.

BDNF mRNA in the dentate gyrus:

Within the dentate gyrus (Figure 6A), ANOVA revealed a significant main effect of time ($p=0.0006$) and exercise status ($p=0.0007$). Post-hoc analyses revealed that exercise increased BDNF mRNA at the immediate time point across all ages. This increase, however, was not maintained at the 14-day time point any rats, although there was a trend toward a significant increase in juvenile runner versus juvenile sedentary rats.

BDNF mRNA in the CA1:

Within the CA1 (Figure 6B), there was a significant main effect of time ($p=0.011$) and exercise status ($p=0.017$). Post-hoc analyses for time point and exercise status revealed that exercise increased BDNF mRNA at the immediate time point in pubescent run rats, but not in juvenile run and adult run rats. There were no significant increases in BDNF mRNA at the 14-day time point.

BDNF mRNA in the CA2:

Within the CA2 (Figure 6C), there was a significant main effect of time (p=0.005). Post-hoc analysis revealed that exercise increased BDNF mRNA at the immediate time point in pubescent run rats, but not in juvenile run and adult run rats. There were no significant increases in BDNF mRNA at the 14-day time point.

BDNF mRNA in the CA3:

Within the CA3 (Figure 6D), ANOVA revealed a significant main effect of time (p=0.0002) and exercise status (p=0.015). Post-hoc analyses revealed that exercise increased BDNF mRNA at the immediate time point in juvenile run rats, but not in pubescent run and adult run rats. There were no significant increases in BDNF mRNA at the 14-day time point, although there was a trend towards a significant increase in juvenile runner versus juvenile sedentary rats.

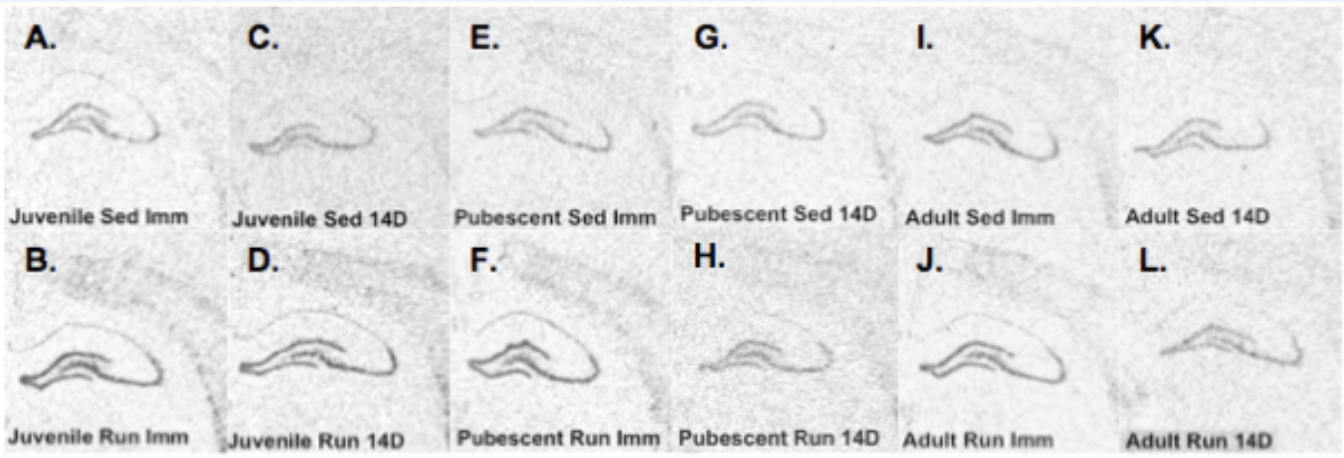


Figure 5: Representative autoradiographic coronal sections through the hippocampus show relative levels of brain derived neurotropic factor (BDNF) mRNA in in juvenile, pubescent, and adult rats, for sedentary (Sed) and exercise (Run) conditions, at the immediate time point (Imm) and 14-day time point (14). The dentate gyrus, CA1, CA2, and CA3 regions of the hippocampus can be seen within these sections.

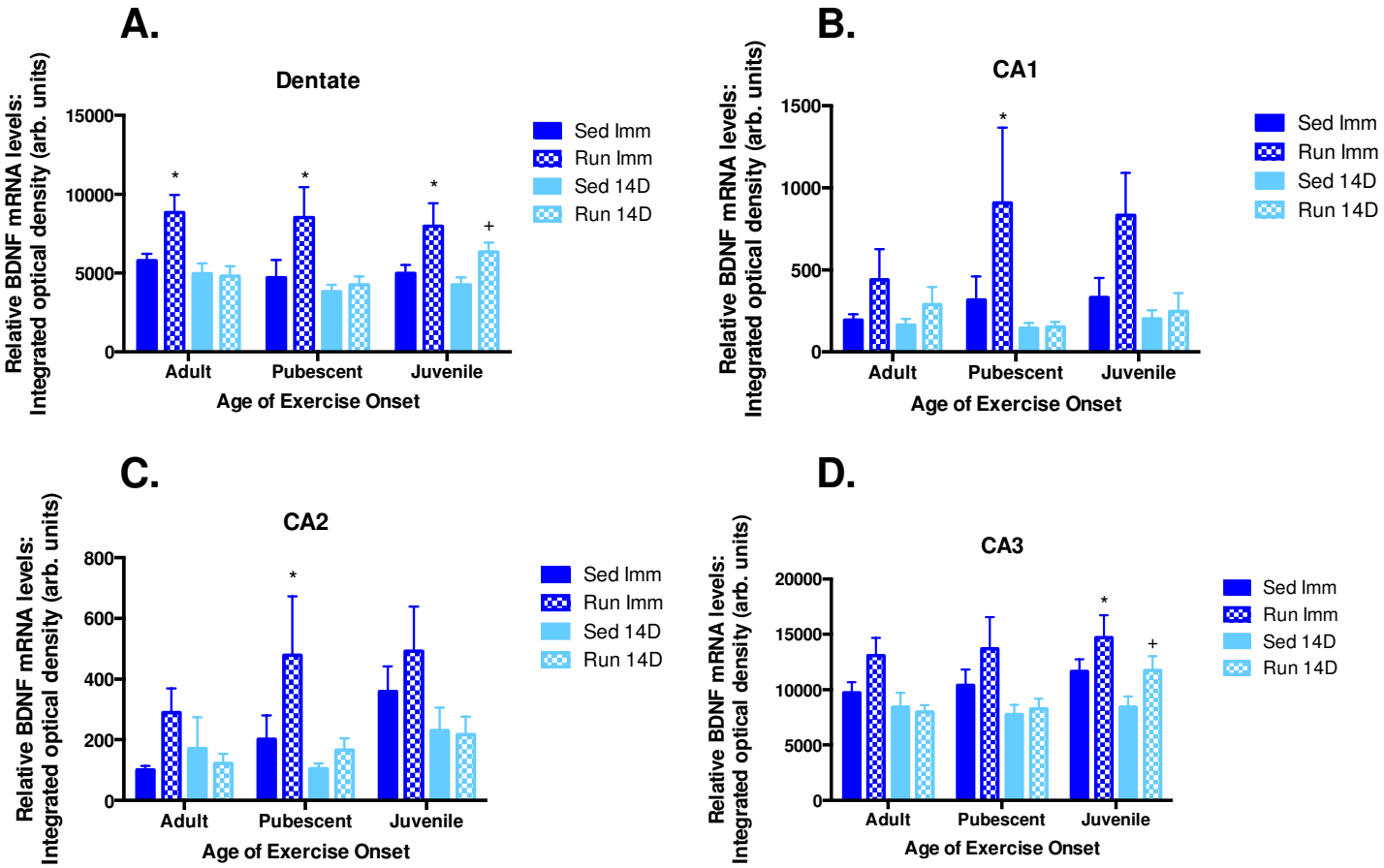


Figure 6: BDNF mRNA in hippocampal regions, dentate gyrus (A), CA1 (B), CA2 (C), and CA3 (D), for adult, pubescent, and juvenile rats within exercise and sedentary condition at the immediate (Imm) or 14-day (14D) timepoint. Post-hoc (F-PLSD) analyses for repeated measures ANOVA revealed increased BDNF in exercise rats as compared to sedentary rats at the immediate timepoint in adult run, pubescent run, and juvenile run in the DG, pubescent run in the CA1, pubescent run in the CA2, and juvenile run in the CA3. There were no significant effects of age on BDNF with exercise conditions or time points; however, juveniles show a trend towards elevated BDNF mRNA within the DG and the CA3 at 14 days. * denotes significance at $p<0.05$ between exercise and sedentary groups. $^{+}$ represents a trend in exercise groups from sedentary groups.

Discussion

The present study examines several ages across the development of male rats in order to isolate the critical developmental period at which exercise produces lasting

increases in BDNF mRNA within the hippocampus. Recent work has shown that 4-6 weeks of exercise initiated during the juvenile period can produce lasting increases in BDNF expression; however, it is unclear which stage during early postnatal development is critical for this enduring effect. Rats were assigned to three different age groups whereupon they initiated one week of wheel running either during the juvenile period, during puberty or in adulthood. Exercise produced significant changes in BDNF mRNA across all ages in the dentate gyrus immediately following one week of exercise, and juvenile rats only exhibited a trend towards long-lasting increases in BDNF 14 days after exercise cessation in the dentate gyrus and the CA3. These data indicate that the brain is more susceptible to persistent increases in BDNF when exercise is initiated earlier in life.

Exercise rats had significantly upregulated BDNF mRNA as compared to sedentary rats immediately following exercise in the dentate gyrus across all ages. Juvenile and pubescent runners also exhibited similar increases in BDNF mRNA across other regions as well. These data agree with those in previous literature, which show increases in BNDF mRNA following one week of exercise (Neeper et al., 1996). Two weeks after exercise cessation, juvenile run rats had a trend towards elevated BDNF mRNA in the dentate gyrus and the CA3, while pubescent run and adult run rats did not show any significant changes as compared to their sedentary counterparts. This is relatively consistent with previous data from our lab, which demonstrated that exercise, when initiated in early life as compared to adulthood, produces significantly increased levels of BDNF mRNA in the dentate gyrus up to 25 days after six weeks of exercise (Mika et al., unpublished data). In this study, the trend in increasing BDNF mRNA in juvenile run rats following exercise cessation suggests that the early-life developmental

period plays an important role in neuroplastic changes following exercise. Indeed, the juvenile brain may be the most susceptible for long term increases in BDNF following exercise.

The lack of a persistent effect in the pubescent rats is somewhat surprising given the established research on puberty and development. The spike in gonadal hormones during puberty plays a significant role in modulating neuron development (Andersen et al., 2008; Morrison et al., 2014). For example, androgens can regulate long-term depression mechanisms and, in this, effect neural function (Hebbard et al., 2003). We limited exercise duration to one week within the three ages groups so that only pubescent rats would run through puberty. This duration was valuable in isolating developmental periods since puberty is brief and only lasts for several days in rats (Lent et al., 2014). As such, we predicted that running during puberty would produce a more significant increase following exercise cessation. However, it is possible that puberty plays a different role in neural development, and could even be distinguished as the *end* of the critical developmental window of neural pruning in early life (Andersen et al., 2003). If this were the case, we would expect juveniles to maintain elevated levels of BDNF mRNA after 14 days following exercise cessation as compared to pubescent rats, as we saw in our data. Puberty did not demonstrate a direct effect on BDNF following exercise, which indicates that exercise produces the most beneficial neural adaptions when started before puberty.

There is an age-dependent increase in BDNF following exercise, which modulates and enhances neural plasticity through a variety of potential mechanisms (Hopkins et al., 2011; Mika et al., unpublished data). Possible mechanisms for the relationship between BDNF levels, development, and plasticity involve epigenetics, the chemical

modifications made to the chromatin. The epigenome is particularly sensitive to environmental influences during early life; therefore, changes in the epigenetics of neural factors could play an important role in maintaining long-term effects of exercise on the brain (Roth et al., 2011). One epigenetic change involves the demethlylation of specific promoter regions on the BDNF gene following exercise. Abel et al. (2013) determined that running in young mice induced a decrease in a DNA methyltransferases in the hippocampus: Dnmt1, Dnmt3a and Dmnt3. The down regulation of DNA methyltransferases allows for demethylation and activation to occur for transcription in the BDNF gene, therefore leading to an upregulation of BDNF mRNA. Moreover, the BDNF gene can be modulated post-transcriptionally through acetylation of H3 histone in the chromatid complex. This opens the complex and allows for enhanced transcription of BDNF DNA and ultimately an upregulation of BDNF mRNA. Exercise can also increase acetylated H3, allowing for enhanced transcription (Gomez-Pinilla et al., 2011). Furthermore, exercise elicited a down regulation of certain histone deacetylases, HDACs, which regulate the acetylation of H3 histone and compact the chromatid structure (Abel et al., 2013). It is unclear whether the increased H3 histone affected HDAC numbers, or whether the reverse is true; however, the two only further enhance the overall upregulation of BDNF mRNA. Acetylation of H3 and demethylation mechanisms both serve as possible epigenetic changes that could explain longer lasting changes in BNDF following early-life exercise through altering the genetics of BDNF. Our data did not represent these long-lasting changes, but the trends towards long-term upregulation of BDNF indicate a potential permanent change in BDNF gene regulation.

There are many other potential mechanisms in which exercise can produce long lasting improvements in learning, memory, and stress-resistant behaviors. In particular, there are subsequent means beyond epigenetic changes in the BDNF gene that modulate enduring increases in BDNF and its downstream factors. The actions and rate at which transcription of BDNF occurs is not necessarily similar to that of BDNF mRNA translation, thus post-transcriptional regulation could contribute significantly to long-term adaptions from exercise (Vaynman et al., 2003). As various transcript forms of BDNF mRNA regulate BDNF protein levels, it is plausible that exercise could modulate BDNF mRNA translation in a time dependent manner (Adlard et al., 2004). Furthermore, exercise may even regulate pro-BDNF levels, the precursor to BDNF protein, through upregulation of proteolytic genes that cleave pro-BDNF to the mature form of BDNF (Sartori et al., 2011). Alternatively, exercise increases synaptic proteins that are regulated by BDNF, including CREB and synapsin-I, and these proteins could induce more permanent changes in synaptic strength and neural plasticity. (Vaynman et al., 2003; Vaynman et al., 2006; Yoshii et al., 2010). Ultimately, exercise can modulate BDNF action through a number of different mechanisms that enhance neural plasticity and thus produce long-term improvements in cognition and behavior.

In order to fully understand the effects of early-life exercise on BDNF and neural plasticity, we need to expand our research to observe several other important variables, including the downstream factors of BDNF action and other regions in which BDNF mediates enhancements like cognition and behavior. This experiment will continue beyond this thesis to analyze regions outside of the hippocampus to other areas of the brain involved in cognition, learning, and memory, including the medial prefrontal cortex

and the perihrinal cortex (Hopkins et al., 2011). Ideally, we will be able to confirm enhanced neural plasticity in these regions with behavior tasks associated with these regions. For example, we can use object in place task to observe enhanced memory associated with the hippocampus and possibly the medial prefrontal cortex (Barker et al., 2011). We will also expand our research to look at BDNF mechanisms through quantifications of mRNA for TrkB, synapsin-I and CREB, which are all down-stream targets for BDNF action and help strengthen synaptic joints and enhance neural communication. Increases in neural factors modulated by BDNF could act as the missing link to permanent changes in neural plasticity following early-life exercise. Since the brain is more plastic during early life due to neural pruning, elevated levels of BDNF have the potential to increase the factors such as CREB and synapsin-I, and therefore, strengthen and protect chemical synapses and dendritic connections from pruning. This would elicit a more permanent structural change in dendritic morphology that could be responsible for long-lasting neural enhancements. These potential effects can be confirmed by analysis and quantification of dendritic morphology. There is still more to be explored regarding the mechanisms behind the upregulation of BDNF mRNA, as well as other down-stream factors, and the behavior produced by exercise initiated in early life.

Conclusion

One week of wheel running in male rats produced a trending increase in mRNA for the juvenile age groups, but not pubescent or adult age groups. The data do not imply a direct role of puberty in the long-lasting increases in BDNF following exercise, but instead indicate that exercising produces the most beneficial enhancements within the hippocampus when initiated earlier in life. The experiment will continue past this thesis

to thoroughly examine the effects of exercise of BDNF mRNA in several other regions associated with behavior and cognition, as well as several down-stream factors of the BDNF pathway. Ultimately, these data from this thesis allow us to glimpse into the microbiological mechanisms of the interactions between critical brain development in early life and exercise.

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