Int. J. Devl Neuroscience 31 (2013) 382–390

Contents lists available at SciVerse ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Running-induced epigenetic and gene expression changes in the adolescent brain

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ARTICLE INFO

Article history:
Received 11 September 2012
Received in revised form 7 November 2012
Accepted 9 November 2012

Keywords:
Epigenetic
Exercise
Puberty
Adolescent
Cerebellum
BDNF

ABSTRACT

Physical exercise is associated with positive neural functioning. Here we examined the gene expression consequences of 1 week of voluntary wheel running in adolescent male mice. We assayed expression levels of genes associated with synaptic plasticity, signaling pathways, and epigenetic modifying enzymes. Two regions were examined: the hippocampus, which is typically examined in exercise studies, and the cerebellum, an area directly involved in motor control and learning. After 1 week of exercise, global acetylation of histone 3 was increased in both brain regions. Interestingly this was correlated with increased brain derived neural growth factor in the hippocampus, as noted in many other studies, but only a trend was found in cerebellum. Differences and similarities between the two areas were noted for genes encoding functional proteins.

In contrast, the expression pattern of DNA methyltransferases (*Dnmts*) and histone deacetylases (*Hdacs*), genes that influence DNA methylation and histone modifications in general, decreased in both regions with exercise. We hypothesize that epigenetic mechanisms, involving many of the genes assessed here, are essential for the positive affects of exercise on behavior and suspect these data have relevance for adolescent boys.

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1. Introduction

Over the past 20 years, research conducted in humans and rodents has demonstrated beneficial effects of physical exercise on the brain including enhanced learning and memory, structural plasticity and neuroprotection against neurodegenerative disorders (as reviewed by Cotman and Berchtold, 2002; Thomas et al., 2012; van Praag, 2009). Due to the urgent need to ameliorate symptoms associated with Alzheimer's disease, a significant portion of this research focuses on the aging brain and the ability of exercise to reverse cognitive decline via increased neurogenesis and neural plasticity in the hippocampus (Cotman and Berchtold, 2002; Kramer et al., 2006). Markedly less attention has been devoted to the effects of exercise on the young brain (i.e. children and adolescents). Yet, exercise not only plays an important role in maintaining a healthy brain during aging, but also seems essential in promoting normal brain growth and maturation during development (Tomporowski et al., 2008). For example, studies reviewed by Chaddock et al. (2011), show that low levels of physical activity in children and adolescents correlates with poor cognitive abilities sometimes associated with reduced volume in hippocampal and basal ganglia brain regions. In contrast, acute exercise improves executive function and alleviates symptoms in children with attention deficit hyperactivity disorder (ADHD) (Archer and Kostrzewa, 2012; Chang et al., 2012). Moreover, recent behavioral studies, conducted in children and adolescents, show long term beneficial effects of exercise on spatial memory, visual discrimination and the consolidation of information into long-term memory (Aberg et al., 2009; Coles and Tomporowski, 2008; Fedewa and Ahn, 2011; Herting and Nagel, 2012; Sibley and Etnier, 2003).

Identified mechanisms that presumably underlie improved cognition as a consequence of exercise include neurogenesis, synaptogenesis, synaptic plasticity and mediation by neurotrophic factors (Farmer et al., 2004; Ferreira et al., 2010; Garcia et al., 2012; Lou et al., 2008; Molteni et al., 2002; Uysal et al., 2005; Vaynman et al., 2006). Brain-derived neurotrophic factor (BDNF), a molecule implicated in learning and memory, has shown consistent up-regulation in the hippocampus, dentate gyrus and perirhinial cortex in response to wheel or treadmill running (Adlard et al., 2005; Gomes da Silva et al., 2012; Griffin et al., 2009; Hopkins et al., 2011; Zoladz and Pilc, 2010). Other genes assessed, including those associated with synaptic trafficking and plasticity, signal transduction and transcription regulation, show significant up-regulation in response to exercise (Farmer et al., 2004; Molteni et al., 2002; Tong et al., 2001). However, these studies are confined to adult rat hippocampus. Moreover, the regulatory basis of exercise-induced gene expression is currently under exploration. The powerful and

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http://dx.doi.org/10.1016/j.ijdevneu.2012.11.002

immediate effect of exercise on gene expression changes in the brain likely engages epigenetic mechanisms. In fact, a recent study shows that epigenetic factors regulate hippocampal *Bdnf* expression in response to exercise (Gomez-Pinilla et al., 2011).

In the present study, we used juvenile mice to examine gene expression changes in the brain produced by exercise. We examined genes associated with neuronal function and epigenetic modifications. Along with evaluation of genes that regulate histone acetylation and DNA methylation we asked if total acetylation on histone 3 (H3) was altered by exercise. Finally, we selected to examine gene expression in two very distinct regions of the brain that differ on both a structural and functional level, the cerebellum and hippocampus. This focus allowed us a preliminary look at genes that might be involved in the motoric versus the cognitive affects of exercise. Here we show a complex and regionally specific effect of exercise on a network of genes. Moreover, our results suggest that epigenetic modifications, including histone acetylation and differential histone deacetylase and DNA methyltransferase activity, may underlie many of these gene changes.

2. Methods

2.1. Animals

C57BL/6J mice were born, reared and housed at the University of Virginia School of Medicine Animal Facility in Jordan Hall on a 12:12 h light:dark cycle (lights on at 0600 h). Food (Harlan Teklad Mouse/Rat Sterilizable Diet #7012) and water was provided ad libitum.

2.2. Exercise

We used a voluntary running-wheel model as previously described (Waters et al., 2004). Briefly, 46-day-old male mice were randomized to modified cages equipped with a running wheel. For the exercised group, 10 mice were placed individually in cages with a freely moving wheel attached to a magnetic sensing mechanism which allowed tracking of running activity, as a function of time and distance, by a computer. Mice were given free access to the wheel over a period of 7 days and ran predominately at night. Mice ran on average 8.7 km on Day 1 and steadily increased their distance to an average of 12.2 km by the end of the week. For the sedentary control group, 10 mice were placed individually in cages equipped with the same type of wheel secured to the cage to prevent rotational running activity and were similarly housed in the same room and handled the same way as the exercised mice.

2.3. Brain tissue extraction

Sedentary and exercised mice were randomly removed from cages following 1 week of activity, anesthetized using euthanasol and the brains were rapidly removed. The hippocampus and cerebellum were carefully dissected on ice, frozen on dry ice and pulverized into a fine powder under liquid nitrogen. The powder was then divided into two aliquots and stored at −80 °C until further processing for either histone or RNA extraction.

2.4. Histone extraction and immunoblotting

Histones were extracted and immunoblotting performed as previously described (Tsai et al., 2009). Briefly, mouse brain pulverized tissues were homogenized in RIPA buffer, centrifuged and the pellets containing nuclei were resuspended with sulfuric acid and continuously mixed for 1 h at 4 °C. The samples were again centrifuged and the separated supernatant was precipitated overnight to generate a nuclear pellet, which was resuspended in RIPA buffer. The lysate protein concentrations for each sample were determined by BCA (bicinchoninic acid) protein assays. For immunoblotting, samples (5 μg protein each) were subjected to electrophoresis on 16% polyacrylamide-SDS gels, transferred to nitrocellulose, blocked overnight, rinsed and incubated with the primary antibodies against acetylated K9/14 H3 (Millipore-Upstate in Temecula, CA #06-599; 1:5000) and total H4 (Millipore-Upstate, #07-108). Blots were either incubated for 1 h in a horseradish peroxidase (HRP)-conjugated donkey anti-rabbit IgG secondary antibody and detected on X-ray film with chemiluminescence or IRDye goat-anti-mouse secondary antibody (Licor, Lincoln, NE) and detected with Odyssey® near infrared imager (Licor). For each data point, the amount of acetylated H3 was normalized to total histone H4 to control for differences in the amount of nuclear extract protein loaded on the gel across samples.

2.5. Quantitative real-time PCR (qRT-PCR)

An RNeasy® Lipid Tissue Mini Kit (Qiagen, Valencia, CA) was used to isolate total RNA according to the manufacturer's protocol. The quantity and quality of the RNA were determined using a NanoVue™ Spectrophotometer. cDNA templates were prepared using an AffinityScript qPCR cDNA Synthesis Kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer's protocol. The ABI StepOnePlus real-time PCR system was used to perform qRT-PCR. Either TaqMan® Probe- or SYBR® Green-Based Detection (Applied Biosystems, Carlsbad, CA) were used to detect PCR products of interest. The following TaqMan® Gene Expression Assays were used: estrogen receptor α (*Esr1*, Mm00433149_m1), estrogen receptor β (*Esr2*, Mm00599821_m1), cerebellin 1 precursor protein (*Cbln1*, Mm01247195_m1), c-src tyrosine kinase (*Csk*, Mm00432751_m1), methyl CpG binding protein 2 (*Mecp2*, Mm01193537_g1), fragile X mental retardation syndrome 1 homolog (*Fmr1*, Mm00484415_m1), brain derived neurotrophic factor (*Bdnf*, Mm01334043_m1), calbindin (*Calb1*, Mm00486645_m1), reelin (*Reln*, Mm00465200_m1), calcium/calmodulin-dependent protein kinase IV (*Camk4*, Mm01135329_m1), glutamate receptor, ionotropic, delta 2 (*Grid2*, Mm00515053_m1), synaptophysin (*Syp*, Mm00436850_m1), synapsin I (*Syn1*, Mm00449772_m1*), neuronal calcium sensor 1 (*Ncs1*, Mm00490552_m1). All samples were normalized to either beta-2 microglobulin (*B2m*, Mm00437762_m1) or mouse beta-actin (*Actb*, #4352933E). Oligonucleotide primers were designed for SYBR-Green based analysis using consensus sequences and Blast from the NCBI genomic alignment database and subsequently synthesized by Invitrogen (Carlsbad, CA) as detailed by Table 1. Validation experiments were conducted to test for equally efficient target and endogenous control gene amplification and primers were between 90 and 110% efficient for all amplifications. For TaqMan and SYBR Green based detection, target and endogenous control genes were measured in triplicate for each cDNA sample during each real-time run to avoid inter-sample variance. For SYBR Green based qRT-PCR, a no-reverse transcriptase reaction was run in parallel to cDNA synthesis samples to control for contamination and genomic amplification. Each of these qRT-PCR reactions was verified for a single PCR product of expected size with the disassociation melting curve stage. All genes of interest were analyzed with StepOne™ software using the comparative cycle thresholds method (C_T) method.

2.6. Statistical analyses

Data collected comparing sedentary to exercised mice, was analyzed by Student's *t*-tests. Pearson product–moment or Spearman rank order correlation tests were used to analyze the relationship between two variables. Correlation matrices representing all variables were generated with Sigma Plot and corrected for multiple comparisons.

3. Results

3.1. Voluntary exercise affects expression of neuroplasticity-related genes in the cerebellum and hippocampus

Animals engaged in voluntary exercise through free access to a running wheel for 1 week, a length of time that produces changes in mouse hippocampal *Bdnf* expression (Adlard et al., 2005). Table 2 shows the expression profile of genes related to synaptic plasticity and cell signaling in the cerebellum and hippocampus of exercising animals. This table gives a summary of percent increase or decrease in gene expression, which occurred in exercising, as compared to sedentary, animals. The most dramatic change was a 128% increase in *Bdnf* hippocampal expression ($p < .0001$). A much smaller, (21%) trend ($p < .07$) was noted in the cerebellum of running animals. *Fmr1*, which regulates RNA trafficking to dendrites (Zukin et al., 2009), was increased in both the cerebellum (9%, $p < .03$) and the hippocampus (14%, $p < .001$). Surprisingly, *Ncs*, which encodes a protein that colocalizes with synaptophysin and is involved in synaptic transmission (Noh et al., 2005), was decreased in both brain regions. Interestingly, the expression profiles for a number of genes were not the same in hippocampus and cerebellum in response to exercise. For example, *Syn1* and *Syp*, genes encoding proteins important in synaptic transmission via trafficking and retrieval of synaptic vesicles (Cesca et al., 2010; Kwon and Chapman, 2011) showed increased expression in only the hippocampus. However, *Cbln1*, which encodes a synaptogenic and maintenance protein that predominates in the cerebellum (Yuzaki, 2011), was significantly upregulated in the cerebellum (up 21%, $p < .00$) compared to the hippocampus (down 36%, $p < .05$).

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