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DIFFERENTIAL CORTICAL NEUROTROPHIN AND CYTOGENETIC ADAPTATION AFTER VOLUNTARY EXERCISE IN NORMAL AND AMNESTIC RATS

J. M. HALL, R. P. VETRENO AND L. M. SAVAGE *

Behavioral Neuroscience Program, Department of Psychology, Binghamton University–State University of New York, United States

Abstract—Voluntary exercise (VEx) has profound effects on neural and behavioral plasticity, including recovery of CNS trauma and disease. However, the unique regional cortical adaption to VEx has not been elucidated. In a series of experiments, we first examined whether VEx would restore and retain neurotrophin levels in several cortical regions (frontal cortex [FC], retrosplenial cortex [RSC], occipital cortex [OC]) in an animal model (pyrithiamine-induced thiamine deficiency [PTD]) of the amnestic disorder Wernicke–Korsakoff syndrome. In addition, we assessed the time-dependent effect of VEx to rescue performance on a spontaneous alternation task. Following 2-weeks of VEx or stationary housing conditions (Stat), rats were behaviorally tested and brains were harvested either the day after VEx (24-h) or after an additional 2-week period (2-wk). In both control pair-fed (PF) rats and PTD rats, all neurotrophin levels (brain-derived neurotrophic factor [BDNF], nerve growth factor [NGF], and vascular endothelial growth factor) increased at the 24-h period after VEx in the FC and RSC, but not OC. Two-weeks following VEx, BDNF remained elevated in both FC and RSC, whereas NGF remained elevated in only the FC. Interestingly, VEx only recovered cognitive performance in amnestic rats when there was an additional 2-wk adaptation period after VEx. Given this unique temporal profile, Experiment 2 examined the cortical cytogenetic responses in all three cortical regions following a 2-wk adaptation period after VEx. In healthy (PF) rats, VEx increased the survival of progenitor cells in both the FC and RSC, but only increased oligodendrocyte precursor cells (OLPs) in the FC. Furthermore, VEx had a selective effect of only recovering OLPs in the FC in PTD rats. These data reveal the therapeutic potential of exercise to restore cortical plasticity in the amnestic brain, and that the FC is one of the most responsive cortical regions to VEx.

Key words: exercise, cortex, amnesia, growth factors, cytogenesis.

INTRODUCTION

Exercise has a profound ability to promote neural plasticity in the healthy brain as well as improve brain function after neuropathology. Within the hippocampus, exercise has been shown to increase progenitor cell number, neurogenesis (van Praag et al., 1999), spine density on mature neurons (Stranahan et al., 2007), angiogenesis (Swain et al., 2003), and neurotrophin expression (Cotman and Berchtold, 2002; Gomez-Pinilla et al., 2002; Fabel et al., 2003). Functionally, exercise enhances long-term potentiation (van Praag et al., 1999; Liu et al., 2011) and improves hippocampal-dependent learning and memory (Anderson et al., 2000; Griesbach et al., 2009; Creer et al., 2010). Animal models have shown that exercise also recovers learning/memory performance after prenatal ethanol-exposure (Christie et al., 2005), stroke (Luo et al., 2007), traumatic brain injury (Griesbach et al., 2012), advanced aging (van Praag et al., 2005; Pietrelli et al., 2012) and after the induction of Alzheimer's disease-like neuropathology (Adlard et al., 2005; Nichol et al., 2009; García-Mesa et al., 2011).

The mechanisms that underlie exercise-enhanced learning and memory appear to be regulated in part by neurotrophins. Specifically, blocking the ability of brain-derived neurotrophic factor (BDNF) to bind to the TrkB receptor in the hippocampus during exercise eliminates the effectiveness of exercise to improve spatial learning (Vaynman et al., 2003, 2004; Griesbach et al., 2009). In addition, vascular endothelial growth factor (VEGF) is also necessary for exercise-induced effects on adult hippocampal neurogenesis as blocking VEGF with an adenoviral antagonist abolished VEx-induced neurogenesis (Fabel et al., 2003).

Impaired neurotrophin signaling is a key feature of many neurological disorders (Mufson et al., 1995; Connor et al., 1997; MacLennan et al., 2000; Tapia-Arancibia et al., 2001; Liu et al., 2005). Our laboratory found that there is a chronic reduction in BDNF levels within the hippocampus and frontal cortex

*Corresponding author. Address: Department of Psychology, Binghamton University–SUNY, Binghamton, NY 13902, United States. Tel: +1-607-777-4383; fax: +1-607-777-4890.
E-mail address: lsavage@binghamton.edu (L. M. Savage).

Abbreviations: ACh, acetylcholine; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BrdU, 5′-bromo-2′-deoxyuridine; ELISA, enzyme-linked immunosorbent assay; FC, frontal cortex; GFAP, glial fibrillary acidic protein; HRP, horseradish peroxidase; IVD, intraventricular distance; NG2, proteoglycan neuron-glia 2 protein; NGF, nerve growth factor; OC, occipital cortex; OLPs, oligodendrocyte precursor cells; PBS, phosphate-buffered saline; PF, pair-fed; PFC, prefrontal cortex; PTD, pyrithiamine-induced thiamine deficiency; RSC, retrosplenial cortex; SSC, saline-sodium citrate; TBS, Tris HCl-buffered saline; VEGF, vascular endothelial growth factor; VEx, voluntary exercise; WKS, Wernicke–Korsakoff syndrome.

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(FC) in the PTD model of Wernicke–Korsakoff syndrome (WKS, Vetreno et al., 2011a,b,c). Although the primary site of neuropathology in both PTD-treated rats and WKS patients is the diencephalon, there is also reduced neural activation in the limbic cortices (Reed et al., 2003; Caulo et al., 2005; Anzalone et al., 2010; Pitel et al., 2012; Roland et al., 2008). Thiamine deficiency produces degeneration of myelinated axons in the frontal, parietal, anterior cingulate, temporal, retrosplenial, occipital and granular insular cortices (Langlais and Savage, 1995; Langlais and Zhang, 1997). There is also a reduction of behaviorally-stimulated acetylcholine (ACh) efflux in the hippocampus, prefrontal cortex, medial FC, and retrosplenial cortex (RSC) in PTD-treated rats (Anzalone et al., 2010; Savage et al., 2012). More recently, our laboratory found that amplification of ACh tone in the hippocampus or medial FC, but not RSC, recovered spontaneous alternation performance in PTD rats (Roland et al., 2008; Savage, 2012). Thus, these cortical regions may be critical targets for neuroplasticity and therapeutic intervention in amnestic conditions.

Importantly, it has been revealed that exercise has the ability to upregulate hippocampal and cortical neurotrophin levels (both BDNF and nerve growth factor (NGF)) in the normal and diseased brain (Neeper et al., 1996; Ding et al., 2004; Griesbach et al., 2004). Within the hippocampus levels of BDNF increase after VEx and the elevation can persist for weeks (Berchtold et al., 2010). However, it is unknown how long exercise increases neurotrophin level in other brain regions.

To date, few studies have examined whether exercise alters neurotrophin and cytogenetic plasticity in different cortical regions in the healthy or diseased brain. Although cortical neurogenesis is not found after exercise, gliogenesis after exercise appears to be a significant cortical reaction to exercise. Forced treadmill exercise increases astroglial proliferation in the frontoparietal cortex of rats that persisted for over 3 weeks (Li et al., 2005). After voluntary exercise (VEx), cortical progenitor cells and newly generated cortical microglia are increased in the cingulate, visual, and motor cortices in control mice (Ehninger and Kempermann, 2003). It has also been found that VEx in mice leads to an increase in the marker for mature oligodendrocytes (GSTπ) and a decrease in cells staining for proteoglycan neuron-glia 2 protein (NG2), suggesting that VEx induces glial progenitor cells to more rapidly differentiate into the mature phenotype (Simon et al., 2011). In the rat, VEx has been shown to increase the total number of co-expressed 5′-bromo-2′-deoxyuridine/glial fibrillary acidic protein (BrdU/GFAP) (astrocyte) and BrdU/NG2 (oligodendrocyte precursor) cells in the medial prefrontal cortex (PFC; Mandyam et al., 2007). In sum, exercise appears to increase cortical progenitor cells and these cells express or eventually express the glial phenotypes, but different exercise regimes appear to evoke various glial progenitor phenotypes dependent upon the cortical regions and timeframe.

The major pool of progenitors outside the neurogenic niches of the adult brain express NG2 and have been characterized as oligodendrocyte precursor cells (OLPs; Levine et al., 2001; Karram et al., 2008; Nishiyama et al., 2009). The OLPs are very dynamic and readily respond to insult to the cortex (Simon et al., 2011). Following acute CNS injury, there is an extensive local proliferation of NG2+ cells before such cells differentiate into oligodendrocytes and possibly some astrocytes (Richardson et al., 2011). However, our understanding of the chronic gliogenetic response to cortical pathology and exercise is limited.

In the current study, we sought to determine whether VEx would enhance spontaneous alternation performance in the PTD model as well as improve cortical neurotrophin expression and cytogenesis. We chose the two cortical regions (FC and RSC) that display different degrees of dysfunction in the PTD model (see Anzalone et al. (2010)) and one cortical region (occipital cortex (OC)) as a control to assess the regional specificity of exercise on cortical plasticity. In Experiment 1, we tracked the acute (24-h after VEx) and protracted (2-wk after VEx) exercise-induced changes in neurotrophin levels in the cortex of normal and amnestic rats. We also tested rats on a spontaneous alternation task 24-h or 2-wk after VEx, as it has been suggested that acquisition of spatial learning may be greatest with an additional restoration period (Berchtold et al., 2010). In Experiment 2, we explored the protracted cortical cytogenetic response to VEx and further determined the cellular phenotypic ratio as a function of cortical region and neuropathology.

EXPERIMENTAL PROCEDURES

Subjects

Ninety-six adult male Sprague–Dawley rats (Exp. 1 = 64; Exp. 2 = 32) weighing between 275–325 g (Harlan-Teklad Corp., Madison, WI, USA) at the beginning of the experiments served as subjects. Rats were maintained on a 12-h light/dark cycle, with light onset at 7:00 am in a temperature-controlled (20 °C) colony room. Experimental procedures were conducted in accordance with the National Institute of Health (NIH) guide for the care and use of laboratory animals, and were approved by the Institutional Animal Care and Use Committee (IACUC) at the State University of New York at Binghamton. Care was taken to minimize animal suffering and the number of subjects used.

Pyrithiamine-induced thiamine deficiency (PTD) and pair-fed (PF) treatment

Rats were randomly assigned to one of the following treatments: (1) PTD (Exp. 1 n = 32; Exp. 2 n = 16) and (2) PF controls (Exp. 1 n = 32; Exp. 2 n = 16). PTD rats received daily intraperitoneal (i.p.) injections of pyrithiamine hydrobromide at a dosage of 0.25 mg/kg (Sigma–Aldrich, St. Louis, MO, USA) in conjunction with a thiamine-deficient chow (Teklad Diets, Madison, WI, USA). After approximately 15–17 days of treatment,