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Differential Cortical Neurotrophin and Cytogenetic Adaptation after Voluntary Exercise in Normal and Amnesic Rats

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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 amnesic 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 two-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 [VEGF]) 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 amnesic 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 in the FC. Furthermore, VEx had a selective effect of only recovering oligodendrocyte precursor cells in the FC in PTD rats. These data reveal the therapeutic potential of exercise to restore cortical plasticity in the amnesic brain, and that the FC is one of the most responsive cortical regions to VEx.

Keywords

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

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density on mature neurons (Stranahan et al., 2007), angiogenesis (Swain et al., 2003), and neurotrophin expression (Cotman & Berchtold, 2002; Fabel et al., 2003; Gomez-Pinilla et al., 2002). 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; Greisbach 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 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, 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 in the PTD model of Wernicke-Korsakoff syndrome (WKS, Vetreno et al., 2011). 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; Vetreno & Savage, 2008; Anzalone et al., 2010; Pitel et al., 2012; Roland et al., 2012). Thiamine deficiency produces degeneration of myelinated axons in the frontal, parietal, anterior cingulate, temporal, retrosplenial, occipital and granular insular cortices (Langlais & Savage, 1995; Langlais & Zhang, 1997). There is also a reduction of behaviorally-stimulated acetylcholine (ACh) efflux in the hippocampus, prefrontal cortex, medial FC, and 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 amnesic conditions.

Importantly, it has been revealed that exercise has the ability to upregulate hippocampal and cortical neurotrophin levels (both BDNF and NGF) in the normal and diseased brain (Neeper et al., 1996; Greisbach et al., 2004; Ding 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 cytogetic 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, cortical progenitor cells and newly generated cortical microglia are increased in the cingulate, visual, and motor cortices in control mice (Ehninger & Kempermann, 2003). It has also been found that VEx in mice leads to an increase in the marker for mature oligodendrocytes (GST π) 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, voluntary exercise has been shown to increase the total number of co-expressed BrdU/GFAP (astrocyte) and BrdU/NG2 (oligodendrocyte precursor) cells in the medial 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 proteoglycan neuron-glia 2 protein (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 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 voluntary exercise would enhance spontaneous alternation performance in the PTD model as well as improve cortical neurotrophin expression and cyto genesis. 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 (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 amnesic 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 cyto genetic response to VEx and further determined the cellular phenotypic ratio as a function of cortical region and neuropathology.

General Methods

1. Subjects

Ninety-six adult male Sprague-Dawley rats (Exp. 1 = 64; Exp. 2 = 32) weighing between 275–325 g (Harlan-Teklad Corp., IN) at the beginning of the experiments served as subjects. Rats were maintained on a 12-hr 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.

2. Pyri thiamine-induced Thiamine Deficiency (PTD) and Pair-fed (PF) Treatment

Rats were randomly assigned to one of the following treatments: (1) pyri thiamine-induced thiamine deficiency (PTD: Exp. 1 n=32; Exp. 2 n=16) and (2) pair-fed controls (PF: Exp. 1 n=32; Exp. 2 n=16). PTD rats received daily intraperitoneal (i.p.) injections of pyri thiamine hydrobromide at a dosage of 0.25 mg/kg (Sigma-Aldrich, St. Louis, MO) in conjunction with a thiamine deficient chow (Teklad Diets, Madison, WI). After approximately 15–17 days of treatment, PTD rats displayed neurological symptoms of anorexia, ataxia, loss of righting reflexes, and ultimately opisthotonus. Four hours after opisthotonus, a large bolus of thiamine hydrochloride at a dosage of 100 mg/kg, i.p. (Sigma-Aldrich, St. Louis, MO) was administered to PTD rats. Administration of thiamine and restoration of normal chow arrests

neurological symptoms. The described protocol produces well-documented and reliable diencephalic damage (Vetreno et al., 2011).

PF rats were fed an amount of thiamine deficient chow equivalent to PTD consumption from the previous day to replicate PTD eating habits, as PTD-treated rats consume less food and therefore lose weight (10–15%) as treatment progresses. Furthermore, PF rats received daily 0.4 mg/kg, i.p. injections of thiamine hydrochloride (Sigma-Aldrich Corp., St. Louis, MO) in order to replace thiamine. Upon conclusion of PTD/PF treatment, rats were allowed *ad libitum* access to normal Purina rat chow for a 14-day recovery period. This recovery period was chosen as it has been shown that exercise exposure directly following traumatic brain injury is not beneficial (Griesbach et al., 2004).

3. Voluntary Exercise Paradigm

Rats were randomly divided into the following exercise conditions: (1) voluntary exercise treatment (VEx; Exp. 1: PTD=16; PF=16; Exp. 2: PTD=8; PF=8); (2) stationary control treatment (Stat; Exp. 1: PTD=16; PF=16; Exp. 2: PTD=8; PF=8). Figure 1 displays the design for both experiments. The VEx condition consisted of an opaque home cage (20 cm × 24 cm × 45 cm) with an attached running wheel (Wahmann Manufacturing Company, Baltimore, MD; diameter of 35.5 cm). Revolutions were measured via a counter and were recorded daily. Rats in the Stat condition had an identical apparatus, except the wheel was locked in order to prevent revolutions. In both conditions, rats were pair-housed as it has been found that social isolation can inhibit or delay the beneficial effects of exercise (Stranahan et al., 2006; Leasure & Decker, 2009). Furthermore, all rats were fed a food-restricted diet of 16 g of Purina rat chow per day, as mild food restriction has been shown to increase wheel running (see Sherwin, 1998). Rats were weighed and handled daily, ensuring that body weight remained at 90% of free-feeding weight.

4. Behavioral Testing

Following the conclusion of the VEx and Stat treatment, rats were pair-housed in standard cages (20.5 cm × 22 cm × 45 cm), and tested either acutely (24-h following VEx/Stat; Exp. 1) or after a protracted period (2-wk; Exp. 1, & Exp. 2). Furthermore, rats remained on a food-restricted diet (90% of free feeding weight) until sacrificed following behavioral testing. Rats were tested on a 4-arm spontaneous alternation task. Spontaneous alternation was chosen as it is a reliable measure of spatial working memory that does not require extensive training or reinforcement (Lalonde, 2002; Hughes, 2004). Furthermore, this task is both hippocampal- and FC-dependent, as lesions to either region impairs performance (Lalonde, 2002). Our laboratory has also reliably demonstrated that PTD rats show impairments on this task (Savage et al., 2003, 2007; Roland et al., 2008; Vetreno et al., 2008).

Each rat was brought to the testing room in a covered standard cage and was allowed a 20-min acclimation period. The testing room was rich in visual spatial cues and in the center of the room was a 4-arm maze was constructed of clear Plexiglas sidewalls (12 cm high) and a wood floor painted black. All four arms were of equal distance (55 cm) and the maze was elevated 80 cm above the floor. After the 20-min acclimation period, the rat was placed in the center of the maze. The rat was allowed to traverse the maze freely for an 18-min period, and the number and sequence of arms entered was recorded to determine alternation scores. An alternation score was defined as the number of different arms entered (minimum =1, maximum =4) during a four-choice sequence, dividing the number of different arms by 4, and multiplying by 100. Two types of perseveration behavior were also recorded: (1) re-entry perseveration and (2) response perseveration. An arm re-entry perseveration was defined as when a rat re-entered a previously explored arm within two choices and it is an

index of cognitive inflexibility (see Vetreno et al., 2008). The arm re-entry perseveration percentage was equal to: A sequence of non-overlapping entries into three arms in which there was a repeat entry/(total arm entries – 2) × 100. A response perseveration, defined as a rat making the same body turn (left or right) as the previous trial was determined by counting the number of trials in which a rat made the same consecutive body turn across two trials/(total arm entries – 1) × 100. Upon completion of 18 min of maze testing, rats were transferred back to their home cage.

5. Experiment 1: Mature Neurotrophin Quantification

5.1. Tissue Preparation—Half of the rats were behaviorally tested 24-h after VEx (PF=8; PTD=8)/Stat (PF=8; PTD=8) housing conditions, where the others rats were behaviorally tested 2-wk after VEx (PF=8; PTD=8)/Stat (PF=8; PTD=8) housing. All rats were sacrificed immediately following the conclusion of maze testing. Brains were extracted and placed on ice in order to dissect the FC, RSC, and OC from each hemisphere. Brain regions were then weighed and recorded in order to later determine neurotrophin concentration, and dissected structures were then frozen (–80°C) until ELISA analysis.

5.2. Neurotrophin Quantification—Protein levels of BDNF (Emax[®] Immunoassay system, Promega, Madison, WI), NGF (ELISA rat β-NGF kit, R&D Systems, Minneapolis, MN), and VEGF (Quantikine ELISA rat VEGF Immunoassay kit, R&D Systems, Minneapolis, MN) were determined using the manufacturer's instructions. Regional samples (FC, RSC, OC) were homogenized in ice-cold lysis buffer solution (Promega, Madison, WI) and then centrifuged at 15,000 × g for 20 min. Standard 96-well flat-bottom well plates were coated with either monoclonal anti-BDNF antibody, goat anti-rat β-NGF, or mouse anti-rat VEGF in the respective coating buffer (carbonate buffer for BDNF, and PBS for NGF and VEGF), and incubated overnight at 4 °C (BDNF) or 27 °C (NGF, VEGF). Well plates were then washed with respective buffers (TBST buffer for BDNF; 0.05% Tween[®] 20 in PBS for VEGF and NGF) and blocked with supplied block and sample buffer (BDNF) or BSA reagent diluent (VEGF and NGF) for 1 h at room temperature. Wells were washed again with appropriate buffers, and supplied standards were diluted in blocking buffer to a high value of 500 pg/ml for BDNF and 1000 pg/ml for VEGF and NGF. Serial dilutions were performed with the standards so that the following concentrations were added to the plate in triplicate as follows for BDNF (expressed in pg/ml): 500, 250, 125, 62.5, 31.25, 15.625, 7.8125, and 0 (blocking buffer only). For VEGF and NGF, the dilutions were applied as follows (expressed in pg/ml): 1000, 500, 250, 125, 62.5, 31.25, 15.625, and 0 (reagent diluent only). The samples, diluted to within the range of the standard curve, were also applied in triplicate. Plates were then sealed and incubated with shaking for 2 h (BDNF, NGF, VEGF) at room temperature. For VEGF and NGF, wells were washed and incubated with a supplied detection antibody diluted appropriately with shaking for 2 h at room temperature. For BDNF, wells were washed and incubated with BDNF polyclonal antibody with shaking for 2 h at room temperature. After washing, appropriately diluted anti-IgY HRP conjugate was added to wells for 1 h at room temperature (BDNF), and streptavidin-HRP conjugated to horseradish peroxidase was added to wells for 20 min at room temperature (VEGF and NGF). Following the washing procedure, color development was achieved by applying the supplied TMB One solution with shaking for 10 min at room temperature (BDNF), and supplied substrate solution containing H₂O₂ and tetramethylbenzidine with shaking for 20 min at room temperature (VEGF and NGF). The reaction was stopped with 1N HCl (BDNF) and 2N H₂SO₄ (NGF and VEGF). Absorbance was immediately determined using a Dynex plate reader (Dynex MRX RC Revelation, Vienna, VA) at an optical density of 450 nm (BDNF, VEGF and NGF). Total neurotrophin protein concentrations were determined via regression analysis derived from the standard

curve absorbance values. BDNF/NGF levels were expressed as nanograms per milligram, whereas VEGF levels were expressed as picograms per milligram.

6. Experiment 2: Cytogenesis Labeling and Phenotypic Identification

6.1. 5'-bromo-2'-deoxyuridine (BrdU) Administration—For Exp. 2, all rats received administration of the S-phase mitotic marker 5'-bromo-2'-deoxyuridine (BrdU) at a dosage of 300 mg/kg, i.p. on days 4, 8 and 12 of running wheel exposure (both VEx and Stat groups). BrdU administration at the specified dosage allowed assessment of BrdU+ cells, as the brain becomes fully saturated and labels cells undergoing DNA synthesis (see Cameron & McKay, 2001; Eadie et al., 2005). The number of BrdU+ cells and the phenotype of BrdU cells was assessed 2-wk after VEx/Stat housing. Rats were behaviorally tested on spontaneous alternation and 90-min later their brains were harvested.

6.2. Tissue Preparation—Rats were deeply anesthetized with an i.p. 0.5 mg/kg sodium pentobarbital solution ([26.0% sodium pentobarbital in 7.8% isopropyl alcohol and 20.7% propylene glycol solution], Sleep-Away, Fort Dodge Animal Health, Fort Dodge, IA) and transcardially perfused with phosphate buffered saline (PBS) followed by a 4% paraformaldehyde in PBS solution. Brains were removed and fixed in a 4% paraformaldehyde solution in PBS for 24 h at 4°C followed by a fixation in a 30% sucrose solution for 4 days. Brains were sliced at a thickness of 40 µm using a Leica (Sm2000r; Leica Instruments, Wetzlar, Germany) sliding microtome, and then individually collected in a 96-well plate with cryoprotectant solution (30% glycerol/30% ethylene glycol) and stored at −20°C for later processing.

6.3. Intraventricular Distance—The intraventricular distance (IVD) was used to determine whether VEx had global restorative effect on thalamic mass (extent of lesion) following PTD treatment. A researcher blind to TREATMENT and EXERCISE conditions determined IVD by measuring the distance (in mm) between the floor of the 3rd ventricle and the roof of the 3rd ventricle (see Robinson & Mair, 1992; Roland & Savage, 2009) at the following approximate locations based on the atlas of Paxinos and Watson (1986 [from Bregma: −1.80 mm to −3.30 mm]). Quantitative measures were collected on a Nikon light microscope (Nikon Eclipse E400; Nikon Instruments, Melville, NY, USA) using a Scion 1394 camera JAVA module (Scion Corp; Fredrick, MD, USA) and analyzed using an image analyzer program (IMAGE-J, v.1.34, NIH, Bethesda, MD, USA).

6.4. BrdU Immunohistochemistry—Tissue selection was performed by a random sampling procedure (see Helfer et al., 2009; Vetreno et al., 2011). For BrdU/DAB staining (to assess cell survival), sampling of the FC was every 5th section, for an average of 7 sections per rodent. For the RSC/OC, every 5th section was also collected, for an average of 8 sections/stain per rodent. BrdU/DAB immunohistochemistry allowed for assessment of cell survival in all cortical regions. First, free-floating sections were rinsed in 0.1 M Tris HCl-buffered saline (TBS), followed by a wash in 3% H₂O₂ to quench exogenous peroxidase activity and then rinsed again in TBS. Tissue was then incubated in a 50% formamide solution containing saline-sodium citrate (SSC) for 2 h and rinsed twice in SSC buffer. Next, tissue was incubated in a 2N HCl solution for 30 min at 37°C in order to denature DNA and allow for exposure of BrdU. Subsequently, sections were rinsed in a 0.1 M boric acid/TBS solution (pH=8.6) for 10 min and washed in TBS. Sections were blocked with a goat serum solution (3% normal goat serum/0.2% Triton-X-100 in TBS) for 1 h. Sections were then treated with monoclonal primary antibody (1:200 anti-BrdU; Accurate Chemical, Westbury, NY) in the aforementioned blocking solution for 48 h at 4°C. To ensure specificity of the primary antibody, negative controls were also conducted on random sections from PTD- and PF-treated subjects via omission of the primary antibody step.

Sections were rinsed in TBS and incubated for 2 h in an avidin-biotin complex solution (Vectastain ABC Kit; Vector Laboratories, Burlingame, CA), followed by submersion in a chromagen (Diaminobenzidine, Sigma-Aldrich, Burlingame, MO) to allow for visualization of cells. Sections were mounted on slides and coverslipped using a Permount mounting medium.

6.5. Triple Immunofluorescent Histochemistry—In order to assess cellular phenotypes, BrdU/NG2/Iba-1 and BrdU/NeuN/GFAP triple immunofluorescent stains were performed. For BrdU/NG2/Iba-1 staining, 3 sections per rodent brain were stained in the FC and 4 sections per rodent brain were stained for both the RSC and OC. This same sampling procedure was also conducted for BrdU/NeuN/GFAP triple immunofluorescent staining. Sections were processed for BrdU as previously reported (see above) and sections were incubated in a primary antibody solution of anti-BrdU, anti-NeuN (EMD Millipore Corporation, Billerica, MA), and anti-GFAP (EMD Millipore Corporation, Billerica, MA [1:200, 1:500, 1:1000 dilutions, respectively]) in blocking solution for 48 h at 4°C. Sections were then rinsed in TBS and incubated in a secondary antibody solutions (Jackson ImmunoResearch Laboratories, West Grove, PA) of biotin, Cy3 and AMCA, all at a 1:200 dilution in blocking solution for 24 h at 4°C ensuring no exposure to light. Sections were again rinsed in TBS, and incubated in Cy2-conjugated streptavidin (Jackson ImmunoResearch Laboratories, West Grove, PA) for 3 h. Finally, sections were rinsed in TBS, mounted using Prolong Gold Anti-fade made according to manufacturer specifications (Molecular Probes, Grand Island, NY), and coverslipped. Nail polish was used to seal edges of slides 24 h later and slides were stored in slide boxes to avoid exposure to light.

For BrdU/NG2/Iba-1 co-localization assessment, triple immunofluorescent staining was performed as described for BrdU/NeuN/GFAP, except primary antibodies varied. The primary antibody solution consisted of anti-NG2 (1:100; Millipore Corporation, Billerica, MA), anti-BrdU (1:200; Accurate Chemical) and anti-Iba-1 (1:500; Wako Chemical, Richmond, VA) in blocking solution. Sections were mounted and stored similarly until confocal microscopy analysis.

6.6. Unbiased Stereology—Estimates of BrdU immunopositive cells throughout the FC, RSC, and OC were quantified with unbiased stereology. The FC was sampled between 0.2 mm and −1.4 mm from Bregma based upon the atlas of Paxinos and Watson (1986), while the RSC and OC were sampled between −1.80 mm and −5.30 mm. Sampling parameters were adapted from a paper studying cortical gliogenesis (Helfer et al., 2009). Cell counts were conducted by an investigator that was blind to treatment conditions (coded slides). An image was captured using a Zeiss Plan-Neofluar 5x objective with an attached digital camera (DVC-1310; DVC Company, Austin TX) on a Zeiss microscope (Axioscope 2-Plus) containing a 3-axis motorized stage. A contour around the FC was traced using StereoInvestigator 8.0 software (MicroBrightField, Williston, VT), which was defined as the cingulate 1 (Cg1), cingulate 2 (Cg 2) and area 2 (Fr2) regions. For the RSC, the retrosplenial agranular (RSA) and granular regions (RSG) were included in the contour. For the OC, the mediolateral portion of area 2 (Oc2ML) and lateral portion of area 2 (Oc2L) were included in the contour. The BrdU+ cells were counted with a Zeiss Plan-NEOFLUAR 40x objective using the Optical Fractionator Approach. The fractionator sampling formula consisted of a section sampling fraction ($ssf=1/5$) The equation for determining cell estimates using unbiased stereology was: $N = \sum Q- \times 1/ssf \times 1/asf \times 1/hsf$, where Q constituted the actual number of cells counted in a specimen and N represented the total cell estimate. An average section thickness was calculated by StereoInvestigator and used to estimate the total number of BrdU-immunopositive (thickness sampling fraction, $tsf = 33 \mu\text{m}/\text{section}$). The current study used an identical grid and counting frame size in StereoInvestigator (200×175

μm^2), a guard zone of 2 μm , and a dissector height of 20 μm based on a previous study by Mandyam and colleagues (2007). Overall, for each animal approximately 7 sections were counted for the FC, 8 for the RSC, 8 for the OC, and for each section. The Gundersen-Jensen estimator of the error coefficient (with a smoothness factor = 1) for BrdU+ cells across regions were: FC = 0.08; RSC = 0.08; OC=0.09.

6.7. Confocal Microscopy—Confocal microscopy was employed to determine cellular phenotypes of BrdU+ cells. A LSM 130 Leica SP5 scanning confocal microscope (Leica Instruments, Wetzlar, Germany) was employed to assess phenotypes. Cells were assessed using a 40x/1.3 oil-immersion lens. Single optical sections were collected throughout the section using lasers with excitation and emission wavelengths of: 350 nm/450 nm (AMCA, blue), 492 nm/510 nm (Cy2, green), and 550 nm/570 nm (Cy3, Red). Twenty-five randomly chosen BrdU+ cells were assessed in each cortical region (FC, RSC and OC) and quantification of cellular phenotypes and co-localization was performed by an experimenter who was blind to treatment conditions.

Data Analysis

A mixed model ANOVA was used to determine the distance ran as a function of TREATMENT (PTD vs. PF; between-subjects factor) by DAY (within-subjects factor). Distance ran was pooled across Experiment 1 and Experiment 2 as there were no differences ($p>0.14$). In Experiment 1, neurotrophin levels were examined by a three-factor ANOVA (TREATMENT= PF vs. PTD; EXERCISE= VEx vs. Stat; TIME= 24-h vs. 2-wk) ANOVA. Behavioral data was examined by a two-factor ANOVA (TREATMENT; EXERCISE) for each time point. For Experiment 2, two-factor ANOVAs (TREATMENT; EXERCISE) were performed to assess behavioral performance on the spontaneous alternation task, BrdU+ progenitor cell estimates, and phenotype percentages. When a significant omnibus main effect or interaction was obtained, post-hoc analyses were performed using Fisher's PLSD. Simple regression analyses were performed in order to determine whether neurotrophin levels were correlated with behavior. Data are expressed as mean values $\pm$ the S.E.M. with significance defined as $p \leq 0.05$.

Results

Experiment 1

1. Pair-fed (PF) rats run more than PTD rats—Overall, PF control rats ran greater distances than the PTD-treated rats, as there was a main effect of TREATMENT ($F[1, 598]=11.45, p<0.01$; see Figure 2). Additionally, there was a significant TREATMENT X DAY interaction ($F[13, 598]=9.88, p<0.0001$), which showed that PF rats ran greater distances than the PTD rats toward the end of exercise exposure, relative to the beginning.

2. Voluntary exercise recovers spontaneous alternation behavior in PTD-treated rats only after a 2-wk restoration period—We found that PTD rats performed significantly worse on spontaneous alternation at both the 24-h (main effect of TREATMENT: $F[1,28]=14.58, p<0.01$) and 2-wk period (main effect of TREATMENT; $F[1,28]=10.37, p<0.01$), relative to the PF rats. Voluntary exercise exposure did not affect spontaneous alternation performance in either PF or PTD rats at the 24-h time period (main effect of EXERCISE: $F<1; p>0.70$). At the 24-h period, PTD rats perseverated greater (36.1 ± 3.4) than PF rats (25.7 ± 3.7 ; main effect of TREATMENT: $F[1,28]=4.1, p=0.05$). Exercise did not alter perseveration ($p>0.4$). There were no main effects or interactions in response perseveration (egocentric response turning [i.e., turning left]; all p 's >0.7).

Two weeks after the conclusion of VEx, spontaneous alternation behavior was improved (main effect of EXERCISE: $F[1,28]=7.03, p<0.01$). Specifically, PTD rats that engaged in VEx exhibited increased alternation performance over PTD rats in the Stat condition ($F[1,14]=6.17, p<0.05$), and importantly, their deficits were ameliorated as they did not differ from PF rats ($p>0.7$). However, in PF rats, the effect of VEx was not significant ($p=0.10$).

Examination of re-entry perseveration at the 2-wk time point indicated that PTD rats perseverated greater than the PF rats (main effect of TREATMENT: $F[1,28]=4.8, p<0.05$). Specifically, Stat PTD rats (32.4 ± 4.6) perseverated more than any other condition (PF VEx = 18.1 ± 2.5 , PF Stat = 20.8 ± 4.1 , PTD VEx = 22.6 ± 3.2). However, TREATMENT and/or EXERCISE did not affect response perseveration (all p 's >0.1).

At both 24-h and 2-wk following VEx exposure, we did not observe any notable differences in overall activity on the maze, as measured by the number of arm entries (all p 's >0.08 ; see Figure 3CD).

3. Neurotrophin levels decrease as a function of PTD treatment, but exercise restores neurotrophin levels in some cortical regions—

Table 1 displays mean neurotrophin concentrations $\pm$ S.E.M for each of the cortical regions. The symbols *, ^, ♦ denote significant effects of TREATMENT, EXERCISE, and TIME, respectively. In the FC, all neurotrophins displayed dynamic changes with regard to TREATMENT, EXERCISE, and TIME conditions. First, at all time points, PTD treatment decreased the levels of all three neurotrophins in the FC (main effects of TREATMENT: all F 's $[1,56]>10.5, p<0.01$). Regardless of TREATMENT condition, in the VEx groups, the passage of 2-wk decreased (by about 40%) both BDNF and NGF (main effects of TIME: all F 's $[1,56]>22, p<0.0001$). However, VEx was able to increase all three neurotrophins in both PTD- and PF-treated rats (main effect of EXERCISE: all F 's $[1,56]>10.5, p<0.01$), to comparable degrees. Specifically, VEx changed BDNF levels the greatest degree at both the 24-h (47-41%) and 2-wk (22-21%) time points (EXERCISE X TIME interaction: $F[1,56]=10.12, p<0.01$). The effect of VEx on increasing NGF content over the Stat condition was also prevalent at the 24-h time point (32—30%) and it persisted for 2-wk (18-15% increase; main effect of TIME: $F[1,56]=24.23, p<0.01$). Although VEx increased VEGF levels in the FC at 24-h time point (30-23%— $F[1,28]=7.13, p<0.001$) by the 2-wk time point the increase was marginal (15-7%) and not significant ($p=0.07$). In summary, exercise was able to increase the level of BDNF, NGF, and VEGF in the brains of both PF and PTD rats at the 24-h time point. The greatest rise was seen in BDNF. Importantly, the effect of VEx persisted for 2-wk for both BDNF and NGF (both F 's $>5.39, p<0.05$), but not VEGF.

In the RSC, PTD treatment decreased all neurotrophin levels at both time points (main effects of TREATMENT: all F 's $[1,56]>5.01, p<0.05$). However, regardless of TREATMENT condition, VEx increased neurotrophin content (main effect of EXERCISE: all F 's $[1,56]>10.8, p<0.01$). The passage of time decreased neurotrophin content in the VEx groups (EXERCISE X TIME interaction: $F[1,56]=4.8, p<0.05$). Specifically, in PTD rats, VEx at the 24-h time period increased BDNF levels by 58% ($F[1, 14]>13.39, p<0.01$). Two-weeks following VEx, there was still a small, but significant increase in BDNF in both PF (17%; $F[1, 14]>5.69, p<0.05$) and PTD rats (24%; $F[1, 14]>7.55, p<0.05$). This persistence in elevation following VEx after 2-wks was not seen for NGF in either PF or PTD rats (p 's >0.1), despite that fact that VEx had a robust effect (48% increase in PF rats and 64% increase in PTD rats) of increasing NGF at the 24-h time point (both F 's $[1,14]>52.1, p$'s <0.0001). Exercise also increased VEGF levels in the RSC 24-h following VEx in both PF and PTD rats (EXERCISE X TIME interaction: $F[1,56]>5.22, p$'s <0.05). Interestingly,

only in the PTD group did VEx sustain VEGF levels at the 2-wk time point, beyond Stat treatment ($F[1, 14] > 8.84, p < 0.01$). To summarize, in the RSC, VEx was able to restore BDNF, NGF, and VEGF levels at the 24-h period in PTD rats. The greatest VEx-induced rise was seen for NGF. However, only BDNF persisted in both groups and VEGF persisted in the PTD group at the 2-wk time point.

The OC served as a control region for the effects of VEx. Interestingly, PTD treatment decreased BDNF and NGF content in this cortical region at both time points (main effect of TREATMENT: both F 's $[1, 56] > 6.2, p < 0.05$); whereas VEGF levels were not impacted by PTD treatment ($p > 0.16$). As expected, VEx did not change levels of any neurotrophin at any time point (all F 's $< 2.91, p > 0.09$).

4. Select neurotrophin levels in specific cortical regions correlate with behavior—As shown in Figure 4, panels A–D, simple regression analyses revealed that alternation performance after the 2-wk adaptation period was positively correlated with BDNF ($r = 0.57, p < 0.01$) and NGF levels ($r = 0.42, p < 0.05$) in the FC. The only neurotrophin that displayed persistence in the RSC was VEGF and it correlated with behavior ($r = 0.42, p < 0.05$). In contrast, alternation behavior at the 2-wk time period did not correlate with BDNF and NGF levels in the RSC, nor BDNF, NGF, and VEGF levels in the OC (all p 's > 0.07 ; data not shown). It is important to point out that when spontaneous alternation behavior was assessed 24-hrs after VEx, behavior did not correlate with any neurotrophins in any brain region (all p 's > 0.15 ; data not shown).

Experiment 2

1. Voluntary exercise recovers spontaneous alternation behavior in PTD-treated rats—As a replication of Exp. 1 at the 2-wk time point, the interaction of TREATMENT X EXERCISE was significant ($F[1, 28] = 11.3, p < 0.01$): The Stat PTD rats alternated less than both PF groups and the VEx PTD rats (see Figure 5). Follow-up contrasts revealed that Stat PF rats (PF STAT vs. PTD STAT: $F(1, 14) = 46.1, p < 0.01$) and VEx PTD rats (PTD VEx vs. PTD STAT: $F(1, 14) = 19.0, p < 0.01$) had greater levels of spontaneous alternation than the Stat PTD rats. Furthermore, there were no significant differences in alternation rates between the PF-VEx or PF-Stat groups ($p > 0.33$) and the VEx-PTD rats did not differ from the PF groups ($p > 0.1$), suggesting a recovery of function.

As expected, assessment of re-entry perseveration at this time point revealed a significant TREATMENT X EXERCISE interaction ($F[1, 28] = 19.8, p < 0.01$), indicating that the Stat PTD rats (41.6 ± 2.5) perseverated more than rats in other conditions. Follow-up contrasts revealed that the VEx PTD rats perseverated significantly less than the Stat PTD rats ($[25.3 \pm 2.3]$; $F[1, 14] = 23.2, p < 0.01$) and that there were no statistical differences in perseveration rates between the VEx PTD-treated animals and the VEx PF subjects ($p > 0.5$; $[25.3 \pm 2.3, \text{ and } 22.7 \pm 3.9, \text{ respectively}]$). Similar to Experiment 1, analysis of response perseveration at this time point did not reveal any significant main effects or interactions (p 's > 0.1).

2. Neuroanatomical and cellular changes

2.1. Voluntary exercise does not reverse the reductions in thalamic mass in the PTD model: In order to determine whether the therapeutic effects of voluntary exercise were due to recovery of thalamic volume, measures of intraventricular distance (IVD) were obtained. The analysis revealed a significant main effect of TREATMENT ($F[1, 28] = 6.53, p < 0.02$) as the PTD rats (mean = $2.7 \text{ mm} \pm 0.04$) evidenced reduced thalamic mass relative to PF rats (mean = $2.9 \text{ mm} \pm 0.03$). There was no effect of EXERCISE condition ($p > 0.26$). These data indicate that the therapeutic effects of exercise are not due to a global brain recuperative effect.

2.2. Exercise increased the survival of progenitor cells in the FC and RSC, regardless of treatment condition: In the FC, PTD treatment resulted in decreased BrdU cell numbers (main effect of TREATMENT: $F[1,28]=40.1$, $p<0.01$). In contrast, VEx led to increased numbers of progenitor cells in both PF and PTD rats (main effect of EXERCISE ($F[1,28]=8.3$, $p<0.01$). Both PTD ($F[1,14]=9.35$, $p<0.01$) and PF ($F[1,14]=21.1$, $p<0.01$) VEx rats had higher BrdU+ cell counts than Stat rats, although VEx did not completely restore the BrdU+ cell estimates of PTD rats to normal PF-Stat levels (see Figure 6A).

While BrdU+ cell counts in the RSC had one common overlap to the FC, other significant differences were observed. Similar to what was observed in the FC, PTD rats had lower cell counts compared to PF rats (main effect of TREATMENT ($F[1,28]=6.99$, $p<0.01$). However, EXERCISE only increased BrdU cell numbers in PF rats (Figure 6B). Although there was a main effect of EXERCISE increasing the number of BrdU cells ($F[1,28]=11.6$, $p<0.01$), follow-up analyses revealed that this effect was largely due to a two-fold increase in the number of BrdU cells in the exercising PF group ($F[1,14]=18.7$, $p<0.01$), as there was no effect of VEx in PTD rats ($p>0.3$).

As anticipated, BrdU+ cell counts in the OC (see Figure 6C) were not affected by TREATMENT, EXERCISE or the interaction of those variables (all p 's >0.3). This suggests that selective cortical regions are sensitive to the neuropathology produced By PTD treatment and the enhancement of cytotgenesis produced by exercise.

2.3. Cortical progenitor cell phenotypes are primarily oligodendrocyte precursor cells:

Cellular phenotyping was performed in order to determine whether exercise induced survival of a specific phenotype. Thus, for each region it was determined whether the progenitor cells (BrdU+) co-localized with NG2+ (oligodendrocyte precursor-OLP), Iba-1 (microglia), NeuN (neurons), or GFAP (astrocytes). Images of co-localized cells for each cellular phenotype are displayed in Figure 7. A data summary regarding percentage of BrdU + co-localization is displayed in Table 2.

In the FC, PTD rats had significantly lower numbers of OLPs (BrdU/NG2+ co-localization) compared PF rats (main effect of TREATMENT: $F[1,28]=10.13$, $p<0.01$). A significant main effect of EXERCISE ($F[1,28]=16.5$, $p<0.01$) was observed as there was an approximate 20% increase in OLPs compared to stationary subjects in both PTD and PF rats (both F 's $[1,14]>7.5$, p 's <0.03). Critically, in PTD rats, VEx led to a restoration of OLPs to a level that they were not different from of Stat PF rats ($F[1,14]<1$). However, we did not observe any differences in progenitor cells expressing the neuronal, microglial or astroglial phenotype in this region, emphasizing that oligodendrogenesis is functionally modulated in the FC by VEx. There were no significant TREATMENT or EXERCISE effects of astrocytogenesis (BrdU/GFAP co-localization; all p 's >0.06), microgliogenesis (BrdU/Iba-1 co-localization; all p 's >0.17), or neurogenesis (BrdU/NeuN co-localization; all p 's <0.15).

Surprisingly, in the RSC, despite changes in progenitor cell survival (particularly in PF rats), there were no accompanying changes in cellular differentiation. This was evidenced by neither main effects of TREATMENT nor EXERCISE in oligodendrogenesis, microgliogenesis, astrocytogenesis, or neurogenesis (all p 's >0.11).

As anticipated, the OC did not show any significant changes in the number of co-localized cells. Thus, TREATMENT and EXERCISE effects were not observed in the OC with regard to the number of OLPs, microglia, astrocytes, or neurons that had co-localized with BrdU (all p 's >0.15).

Discussion

Strategically planned exercise has been shown to lead to improved brain recovery in several models of neurological disease (for reviews see Ang et al., 2010; Vaynman & Gomez-Pinilla, 2005). The present study was the first to find that exercise followed by behavioral testing influences plasticity markers (neurotrophin levels and enhanced cytogenesis) in both the FC and RSC. Neurotrophin levels and cytogenesis were assessed and related to behavioral performance on a spontaneous alternation task in normal and amnesic rats. We found that PTD rats engaged in voluntary wheel running, but to a lesser extent than PF rats. However, even this reduced level of exercise (see also Barrientos et al., 2011) led to an increase in several neurotrophins within the FC and RSC, which diminished across time in both treatment conditions (PF and PTD).

It should be noted that all rats were behaviorally tested and neurotrophins can increase in response to behavioral output. A recent paper has found that a single episode of spontaneous alternation testing can acutely increase hippocampal BDNF levels for a period of about 1-hr (Korol et al., 2013). Thus, the experience of behavioral testing could have influenced neurotrophin levels in the current study. However, if behavioral testing, relative to exercise, was the more critical factor driving neurotrophin levels, a reduction in neurotrophins across time should not have been observed, as all rats at each time point were behaviorally tested and then brain samples were collected. Furthermore, neurotrophin levels in any cortical region at the 24-hr period did not correlate with behavior. Thus, given the significant changes in neurotrophins across time in the VEx exposed groups, exercise must be a contributing factor in driving the increase in neurotrophin levels. The neurotrophin findings suggest that the cortex, particularly the FC, retains the capacity for plasticity after damage to the diencephalon and is a potential target for therapeutic strategies to recover some cognitive functions in cases of amnesia and cognitive loss.

In rodents, exercise enhances spatial learning in the Morris water maze and the radial arm maze as well as improves alternation performance on the Y-maze and T-maze (Van der Borgh et al., 2007; Griesbach et al., 2009; Berchold et al., 2010; Marlatt et al., 2012). We found a time-dependent effect of VEx to ameliorate spatial memory impairments observed in the PTD model when assessed by spontaneous alternation. However, control subjects did not exhibit enhanced performance after exercise. This is not surprising given data demonstrating that exercise improvements in young healthy adults is not consistently observed, relative to the benefits of exercise in subjects that are vulnerable to disease, stress, or aging (Barrientos et al., 2011; Dishman et al., 2006; Greenwood et al 2007). Interestingly, the effect of exercise on spontaneous alternation has not been assessed before, despite its wide use to assess spatial working memory (see Lalonde, 2002) and the fact that it evokes the hippocampus (Savage et al., 2003; Pych et al., 2005)—in particular the dentate gyrus (Gobeske et al., 2009). It is likely that stationary PF subjects already perform at maximal (ceiling) levels on this task, whereas the performance of PTD rats without exercise is drastically decreased (30–50% of control rats), producing a window allowing VEx plus a restoration period to rescue performance.

While physical exercise induces acute increases in several neurotrophins, the effects of exercise on some types of behavior are protracted, suggesting a cellular or structural recovery mechanism. The introduction of a delay following exercise exposure has been shown to improve acquisition performance on the radial arm water maze, compared to behavioral testing immediately following exposure, suggestive that some of the cognitive benefits of exercise are dependent on a mechanism that requires weeks to mature (Berchtold et al., 2010). It has been well documented that exercise increases several growth factors that not only enhance neurogenesis, but also improve the cytoarchitecture of mature neurons

(Berchtold et al., 2010; Vivar et al., 2012). Protracted exposure to neurotrophins, such as BDNF and NGF, leads to cytoarchitectural adaptations of synapses and other cell morphology that can contribute to behavioral improvement. The VEx protocol plus a restoration time period in the current study was similar to the time frame in which exogenous neurotrophin delivery recovers cognitive performance in aged rats (Markowska et al., 1996; Frick et al., 1997).

We believe that different neural adaptations explain the unique profile of time-sensitive cognitive improvements after exercise: Rapidly-induced memory improvements are driven by acute BDNF modulation whereas the enhanced learning that emerges after weeks requires alterations in mature neural parameters shaped by prolonged neurotrophin modulation. Conformation of this hypothesis will require mechanistic exploration.

2. Exercise induces acute upregulation of neurotrophin expression in the FC and RSC, but persistent elevation is dependent on cortical region and neuropathology

Various neurotrophins and their signaling cascade markers have different emergence and persistence patterns following exercise (Neeper et al., 1996). Berchtold and colleagues (2005; 2010) have shown that in the hippocampus, BDNF shows an acute robust elevation and slowly decreases back to basal levels 3–4 wks after exercise ends. A study assessing the effects of exercise on VEGF mRNA levels found an mild increase 1-wk following the initiation of exercise and a large increase after 3-wk of exercise (Ding et al., 2004). In the current study, regardless of neuropathology, VEx increased levels of all three neurotrophins in both the FC and RSC, but not the OC. In the FC, BDNF show the largest acute rise and the most persistent elevation following VEx and behavioral testing, but elevated levels of NGF were still detectable in both treatment groups after the 2-wk adaption period. However, in the RSC, the greatest acute VEx-induced rise was seen for NGF; but only BDNF level persisted at the 2-wk time period in both groups and VEGF persisted only in the PTD group.

The present study revealed that an acute, but severe bout of thiamine deficiency results in long-term drastic down-regulation of neurotrophins in all cortical regions assessed. These data indicate that in the PTD model, there may be an overall decrease in brain plasticity. Two factors could account for this: First, PTD could lead to a chronic depletion of neurotrophins in these as a result of direct pathology. Or, more broadly, the brain may globally respond to injury through the modification of factors related to synaptic plasticity, thus remote regions from the site of injury express decreased neurotrophin factor levels (Griesbach et al., 2004). The cellular alterations in the cortex after thiamine deficiency are likely caused by both the degeneration of cortical tracts directly via thiamine deficiency and chronically, but indirectly, through atrophy and necrosis of the thalamus (Langlais & Savage, 1995; Langlais & Zhang, 1997).

Importantly, only behavioral performance at the 2-wk time point, but not the 24-hr time point, correlated with BDNF and NGF levels in the FC, and in the RSC, with VEGF levels. Thus, the experience of behavioral testing alone was not the key factor driving neurotrophin levels. It appears that 2-wks of VEx produces a prolonged period of increased expression of neurotrophins that is related to behavioral recovery. This finding is consistent with other studies assessing neurological diseases, as frontal cortex BDNF levels correlate with working memory in an animal model of Down syndrome (Bimonte-Nelson et al., 2003).

3. Exercise has selective effects on cortical cytogetic phenotypes as a function of brain pathology

Although a handful of previous studies have looked at cytogenesis in the FC, plasticity changes in the RSC after exercise has not been previously assessed. This is somewhat

surprising given the role of the RSC in spatial learning (van Groen et al., 2004) and its integral relationship with the hippocampus and thalamus (Vann & Aggleton, 2002).

Specifically, in both PF and PTD rats, VEx enhanced the survival of progenitor cells in the FC. In contrast, in the RSC, VEx only led enhanced cell survival in the intact brain. Exercise yielded a 20% increase in the number of oligodendrocyte precursor cells in the FC of PTD subjects, but had no effects in the RSC. The RSC in the PTD model maybe less plastic than the FC due to the severe loss of anterior thalamic input after thiamine deficiency. A similar effect was when cholinergic tone was pharmacologically increased in the FC and RSC: Reducing acetylcholine degradation in the RSC was ineffective at restoring behavior, but such a manipulation in the FC led to a full recovery of alternation behavior (Savage, 2012). Thus, the FC still remains adaptable after thiamine deficiency induced pathology to the thalamus.

Other researchers have shown the FC is responsive to exercise. Mandyam and colleagues (2007) found that VEx enhanced gliogenesis within the frontal cortex and increased the number of OLPs. This parallels what we found in the frontal cortex of both control (PF) and PTD rats. While the beneficial effects of exercise on cellular proliferation and survival have been well established in the hippocampus, particularly with regards to neurogenesis (Kempermann & Gage, 1999; Eadie et al., 2005; van Praag et al., 2005), the current data set contribute to the emerging evidence that some cortical regions, particularly the prefrontal and frontal cortical regions, display significant adaptive responses to exercise. It has been suggested that the newly generated OLPs in the frontal cortex during adulthood play a role in remyelination and therefore modulate functions dependent on the PFC, including learning and goal-directed behavior (Mandyam et al., 2007).

3.1. The primary cellular phenotype of BrdU cells in the FC, RSC and OC is gliogenic, specifically oligodendrocyte precursor cells—In all three cortical regions, the cellular phenotype of the newly born cells was primarily gliogenic, specifically OLPs, which is supported by a number of studies. Neurogenesis is not common in the cortex (Helfer et al, 2009; Gould et al., 1999, Mandyam et al, 2007) and newly born astrocytes (2–30%) and microglia (under 2%) occur at low levels in the cortex--if recent neural damage has not occurred. Although several studies have also found that exercise does not significantly alter these glial populations (Helfer et al, 2009; Mandyam et al, 2007), such changes may be difficult to observe given their low frequency.

Both the FC and RSC of rats exposed to VEx displayed a higher number proliferating BrdU + cells compared to Stat subjects, regardless of treatment. In the cortex, as many as 60–75% of BrdU labeled cells express NG2 (Mandyam et al., 2007; Simon et al, 2011). In control PF rats we also found that about 57% of all cortical BrdU+ cells are NG2+ cells, and only in the FC was this was boosted by VEx to 71%. Interestingly, in the PTD brain, the percentage of NG2+ cells drops to below 50% in the FC, and it is the only cortical region that shows a loss of NG2+ cells. Like in control rats, VEx increases the percentage of cells that are NG2+ only in the FC of PTD rats. Importantly, in the FC, VEx PTD rats displayed a recovery of OLP's to the level of sedentary PF rats.

Thus, there is something unique about NG2 response to exercise in the frontal cortex. While it was originally believed that NG2+ cells were multipotent stem cells able to differentiate into neurons and glia, more recently it has been shown that in adult rodents, NG2+ cells develop into myelinating oligodendrocytes in the brain and spinal cord (Kang et al., 2010). NG2+ cells are mitotically active in the adult and acutely respond to neurological insult, ischemia and demyelinating disease (Levine et al., 2001; Richardson et al., 2011). How long this compensatory response lasts is unknown. Our data revealed that VEx can restore the

population of oligodendrocyte precursor cells within the FC in rats with long-term brain pathology (the excitotoxic event in PTD treatment occurred weeks prior to intervention).

It has been proposed that myelinogenesis driven by oligodendrocyte precursor cells may be critical for some forms of neural plasticity (Richardson et al., 2011). In relation to this, evidence is accumulating that some measures of cognition correlate with white matter integrity and it has been postulated that neurodegeneration changes in white matter may be related to alterations in the ability of NG2 cells to proliferate and mature into oligodendrocytes (Richardson et al, 2011).

3.2. The interactions between neurotrophic factors and oligodendrocytes suggest a possible mechanistic role for behavioral recovery—The parallel findings of cellular survival and neurotrophin levels are suggestive of a relation between neurotrophic factors and NG2+ cells. However, at this point there is no direct causal relationship between levels of neurotrophins and extension of NG2 progenitors cells following exercise. Interestingly, OLPs derived from human embryonic stem cells express functional levels of BDNF and have trophic effects on neurons, as evidenced via stimulatory effects on neurite outgrowth (Zhang et al., 2007). Other studies indicate that BDNF is increased as a function of exercise in the spinal cord and accompanied by a parallel increase of oligodendrogenesis (Perreau et al., 2005; Kritiyakiarana et al., 2010). While these studies have focused primarily on the spinal cord, they bear resemblance to the changes we observed in the cortex. Interestingly, in the hippocampus, BDNF has been also shown to induce oligodendrocyte proliferation (Dougherty et al., 2000), and in the basal forebrain it has been shown that oligodendrocytes have a trophic role. For example, NGF and BDNF mRNA expression was found in oligodendrocytes and there is enhanced cholinergic neuronal function when cholinergic cells are co-cultured with oligodendrocytes (Dai et al., 2003). Moreover, these trophic effects were blocked when a tyrosine kinase inhibitor was introduced or neutralization with anti-BDNF antisera, indicating that neurotrophins mediated these effects.

Taken together, these data suggest a mechanism for the behavioral effects observed: The exercise-induced upregulation of neurotrophins accompanied by an increase in oligodendrocyte precursor cell within the FC may contribute to the behavioral recovery in the PTD model through myelination-based plasticity. However, future studies aimed at directly manipulating cortical oligodendrocyte activity and neurotrophin signaling pathways will reveal a complete mechanistic understanding of the role that cortical plasticity has in behavioral recovery.

Summary

Although the core neuropathology associated with diencephalic amnesia (lesion/loss of thalamic mass) was not influenced by exercise, cortical plasticity markers were responsive to exercise. Overall, these data reveal that the FC, and to a more limited degree the RSC, undergo critical plastic changes after exercise exposure even in subjects with extensive neuropathology. Future research is needed to determine the mechanistic interplay between behavior, cortical region and neurotrophin signaling, as well as the role of neurotrophic factors in modulating gliogenesis and specifically the generation of oligodendrocytes in cortical regions.

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Highlights

- Exercise-induced behavioral recovery can take weeks to emerge
- Exercise increased neurotrophins certain cortical regions
- Protracted neurotrophin levels in the frontal cortex correlated with behavior
- Exercise selectively increased precursor oligodendrocytes in the frontal cortex

Figure 1. Schematic of experimental design. Upon completion of treatment (Pair-fed [PF] or Pyrithiamine-induced thiamine deficiency [PTD] for 15–17 days), rats were pair-housed and allowed to recover for 14 days in home cages. Rats were then either exposed to an active running wheel (VEx) or a locked running wheel (Stat) attached to their home cage (paired housed). Rats (pair-housed) were then put back into standard cages and behaviorally tested either a 24-h (Experiment 1) or a 14 days (Experiments 1 & 2) following VEx/Stat conditions.

Figure 2.

Daily cumulative distance ran during the 14 days of running wheel exposure. PTD rats run significantly less than PF rats from sessions 4–14. Distance run is expressed as meters per day $\pm$ S.E.M. for PF- (solid line) and PTD- (dashed line) treated rats.

* indicates a significant effect of TREATMENT, PF>PTD.

Figure 3.

Behavioral data for Experiment 1 is displayed as mean percent alternation $\pm$ SEM (a, b) and activity data is displayed as mean number of arm entries $\pm$ SEM (c, d) during the 18-min spontaneous alternation task. When rats were tested 24-h following VEx there was no significant improvement in alternation performance, although PTD rats were significantly impaired relative to PF rats (a). However, after an additional two-week period, PTD rats that previously exercised had a significant recovery of spontaneous alternation (b); No differences existed in overall activity as a function of TREATMENT, EXERCISE or TIME of testing (c–d). Solid gray bars indicate exercising subjects (VEx) while white bars indicate stationary subjects (Stat). * indicates a significant effect of TREATMENT (PF>PTD), whereas ^ indicates a significant effect of EXERCISE (VEx>Stat).

Figure 4.

Positive correlations ($p < 0.05$) between spontaneous alternation performance and FC BDNF concentration (panel a), FC NGF concentration (panel b), FC VEGF concentration (panel c) and RSC VEGF concentration (panel d). Gray diamonds = PF VEx, whereas black squares = PF Stat, triangles = PTD VEx and X's = PTD Stat. Trend lines on each graph indicate combined regression analyses for PTD and PF groups.

Figure 5. Behavioral data for Experiment 2 is displayed as mean percent alternation $\pm$ SEM (a) and activity data (b) is displayed as mean number of arm entries $\pm$ SEM during the 18-min spontaneous alternation task. After an additional 2-wk period following VEx, PTD rats that previously exercised had a significant recovery of spontaneous alternation (a); No differences existed in overall maze activity levels as a function of TREATMENT, EXERCISE or TIME of testing (b). Solid gray bars indicate exercising subjects (VEx) while white bars indicate stationary subjects (Stat). * indicates a significant effect of TREATMENT (PF>PTD), where as ^ indicates a significant effect of EXERCISE (VEx>Stat).

Figure 6.

Experiment 2 stereological estimates of progenitor cell survival (BrdU+ cells) reported as mean ($\pm$ S.E.M.), in the frontal cortex (FC-panel a), retrosplenial cortex (RSC -panel b) and occipital cortex (OC-panel c) as a function of TREATMENT and EXERCISE conditions. PTD treatment decreased progenitor cell numbers in the FC and RSC. However, the number of progenitor cells increased two weeks following VEx. * indicates a significant effect of TREATMENT, PF>PTD, while ^ indicates a significant effect of EXERCISE.

Figure 7. Confocal microscopy images taken from a Zeiss 510 Meta confocal scope depicting the observed co-localization of BrdU/GFAP, BrdU/NG2, BrdU/Iba-1 and BrdU/NeuN. Cells are shown separated and then merged.

Table 1

Changes in neurotrophin protein levels following VEx in the frontal cortex, retrosplenial cortex and the occipital cortex for BDNF, NGF and VEGF. Overall, PTD treatment decreased neurotrophin content in all three cortical regions. In contrast, voluntary exercise (VEx) leads to robust acute increases in neurotrophin levels in the frontal cortex and retrosplenial cortex. Dependent on the cortical region and neuropathological state neurotrophin can remain elevated even two-weeks following VEx.

	BDNF		NGF		VEGF	
	24 Hours	2 Weeks	24 Hours	2 Weeks	24 Hours	2 Weeks
Frontal Cortex						
<i>PF VEx (n=8)</i>	25.0 ± 2.7 [^]	15.4 ± 0.5 [^] ♦	15.4 ± 1.2 [^]	8.8 ± 0.6 [^] ♦	32.5 ± 4.3	26.7 ± 1.1♦
<i>PF Stat (n=8)</i>	13.3 ± 2.5	12.3 ± 0.5	10.4 ± 1.3	7.2 ± 0.3	25.1 ± 0.9	24.8 ± 1.2
<i>PTD VEx (n=8)</i>	17.0 ± 1.6 [^]	9.9 ± 0.5 [^] ♦	10.9 ± 1.5 [^]	6.8 ± 0.7♦	26.8 ± 2.3 [^]	23.2 ± 1.4♦
<i>PTD Stat (n=8)</i>	10.0 ± 0.9*	8.0 ± 0.6*	7.6 ± 1.9*	5.8 ± 0.5*	18.7 ± 3.0*	19.8 ± 1.2*
Retrosplenial Cortex						
<i>PF VEx (n=8)</i>	28.1 ± 2.1 [^]	16.5 ± 1.0 [^] ♦	20.5 ± 0.8 [^]	9.9 ± 0.9♦	29.8 ± 2.8 [^]	23.2 ± 0.6♦
<i>PF Stat (n=8)</i>	14.4 ± 1.1	13.7 ± 0.6	10.7 ± 1.1	9.1 ± 0.9	22.7 ± 1.9	22.5 ± 0.9
<i>PTD VEx (n=8)</i>	21.4 ± 3.1 [^]	14.5 ± 0.7 [^] ♦	16.6 ± 0.6 [^]	8.7 ± 0.9♦	26.9 ± 2.7 [^]	21.2 ± 0.6 [^] ♦
<i>PTD Stat (n=8)</i>	9.2 ± 1.2*	10.9 ± 1.1*	6.0 ± 1.1*	6.5 ± 0.7*	18.7 ± 2.9*	19.1 ± 0.4*
Occipital Cortex						
<i>PF VEx (n=8)</i>	27.2 ± 2.7	23.6 ± 0.6	11.6 ± 1.2	9.3 ± 0.9	32.1 ± 2.6	28.5 ± 1.7
<i>PF Stat (n=8)</i>	23.6 ± 1.7	23.1 ± 0.7	11.3 ± 1.3	9.5 ± 0.8	30.9 ± 3.7	27.9 ± 1.1
<i>PTD VEx (n=8)</i>	22.3 ± 2.0	22.4 ± 0.7	10.0 ± 1.4	6.9 ± 0.7	29.7 ± 2.0	27.0 ± 0.9
<i>PTD Stat (n=8)</i>	19.5 ± 2.6*	21.2 ± 1.0*	6.2 ± 0.5*	5.9 ± 0.9*	27.8 ± 4.1	25.2 ± 1.4

* denotes a significant effect of TREATMENT (PF>PTD),

[^] denotes a significant effect of EXERCISE (VEx>Stat), while

♦ denotes a decrease across TIME (24-hr>2-wk).

Table 2

Mean percent of BrdU+ cells ($\pm$ S.E.M.) that co-localized with NG2 (oligodendrocytes), GFAP (astrocytes), NeuN (neurons) and Iba-1 (microglia). The majority of BrdU+ cells co-localized with NG2 and exercise increased the NG2 phenotype in the frontal cortex.

	NG2+	GFAP+	NeuN+	Iba-1+
Frontal Cortex				
<i>PF VEx</i>	70.52 $\pm$ 2.59[^]	5.573 $\pm$ 1.22	3.686 $\pm$ 1.49	1.775 $\pm$ 0.68
<i>PF Stat</i>	58.70 $\pm$ 3.39	3.398 $\pm$ 0.96	3.015 $\pm$ 1.64	1.898 $\pm$ 0.72
<i>PTD VEx</i>	61.25 $\pm$ 1.85[^]	6.797 $\pm$ 0.95	4.482 $\pm$ 1.78	3.180 $\pm$ 1.03
<i>PTD Stat</i>	49.57 $\pm$ 3.43[*]	5.061 $\pm$ 0.63	4.168 $\pm$ 1.66	0.897 $\pm$ 0.59
Retrosplenial Cortex				
<i>PF VEx</i>	61.53 $\pm$ 2.70	3.433 $\pm$ 0.67	1.275 $\pm$ 0.62	2.898 $\pm$ 0.99
<i>PF Stat</i>	54.08 $\pm$ 3.47	6.846 $\pm$ 2.92	2.745 $\pm$ 0.99	3.610 $\pm$ 0.95
<i>PTD VEx</i>	55.60 $\pm$ 1.81	7.691 $\pm$ 2.47	2.981 $\pm$ 1.26	3.150 $\pm$ 1.09
<i>PTD Stat</i>	57.37 $\pm$ 3.65	6.569 $\pm$ 2.37	3.168 $\pm$ 1.81	0.413 $\pm$ 0.41
Occipital Cortex				
<i>PF VEx</i>	58.29 $\pm$ 2.95	3.125 $\pm$ 0.78	0.431 $\pm$ 0.43	3.184 $\pm$ 1.88
<i>PF Stat</i>	58.32 $\pm$ 4.71	4.093 $\pm$ 1.41	0.500 $\pm$ 0.50	1.417 $\pm$ 0.69
<i>PTD VEx</i>	55.42 $\pm$ 3.67	4.958 $\pm$ 1.24	1.942 $\pm$ 0.75	0.500 $\pm$ 0.50
<i>PTD Stat</i>	55.58 $\pm$ 2.90	5.811 $\pm$ 1.35	0.937 $\pm$ 0.62	2.042 $\pm$ 0.77

* indicates a significant effect of TREATMENT, PF>PTD, while

[^] (increase) or ^v (decrease) indicates a significant effect of EXERCISE.