Neuroscience 125 (2004) 129–139

VOLUNTARY EXERCISE FOLLOWING TRAUMATIC BRAIN INJURY: BRAIN-DERIVED NEUROTROPHIC FACTOR UPREGULATION AND RECOVERY OF FUNCTION

G. S. GRIESBACH,[a]* D. A. HOVDA,[a,b] R. MOLTENI,[c] A. WU[c] AND F. GOMEZ-PINILLA[a,c]

[a]*David Geffen School of Medicine at UCLA, Division of Neurosurgery, Los Angeles, CA 90095-7039, USA*

[b]*Department of Molecular and Medical Pharmacology, UCLA, Los Angeles, CA 90095, USA*

[c]*Department of Physiological Sciences, UCLA, Los Angeles, CA 90095, USA*

Abstract—Voluntary exercise leads to an upregulation of brain-derived neurotrophic factor (BDNF) and associated proteins involved in synaptic function. Activity-induced enhancement of neuroplasticity may be considered for the treatment of traumatic brain injury (TBI). Given that during the first postinjury week the brain is undergoing dynamic restorative processes and energetic changes that may influence the outcome of exercise, we evaluated the effects of acute and delayed exercise following experimental TBI. Male Sprague–Dawley rats underwent either sham or lateral fluid-percussion injury (FPI) and were housed with or without access to a running wheel (RW) from postinjury days 0–6 (acute) or 14–20 (delayed). FPI alone resulted in significantly elevated levels of hippocampal phosphorylated synapsin I and phosphorylated cyclic AMP response element-binding-protein (CREB) at postinjury day 7, of which phosphorylated CREB remained elevated at postinjury day 21. Sham and delayed FPI-RW rats showed increased levels of BDNF, following exercise. Exercise also increased phosphorylated synapsin I and CREB in sham rats. In contrast to shams, the acutely exercised FPI rats failed to show activity-dependent BDNF upregulation and had significant decreases of phosphorylated synapsin I and total CREB. Additional rats were cognitively assessed (learning acquisition and memory) by utilizing the Morris water maze after acute or delayed RW exposure. Shams and delayed FPI-RW animals benefited from exercise, as indicated by a significant decrease in the number of trials to criterion (ability to locate the platform in 7 s or less for four consecutive trials), compared with the delayed FPI-sedentary rats. In contrast, cognitive performance in the acute FPI-RW rats was significantly impaired compared with all the other groups. These results suggest that voluntary exercise can endogenously upregulate BDNF and enhance recovery when it is delayed after TBI. However, when exercise is administered to soon after TBI, the molecular response to exercise is disrupted and recovery may be delayed. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: fluid-percussion injury, synapsin I, CREB, plasticity, rehabilitation, vulnerability.

Exercise has been shown to be beneficial to overall health and has been linked with a decrease in cognitive decay (Friedland et al., 2001; Laurin et al., 2001). Animal studies have found that exercise leads to protection against ischemia (Stummer et al., 1994), enhanced hippocampal neurogenesis (van Praag et al., 1999) and improved learning capabilities (Fordyce and Wehner, 1993; Samorajski et al., 1985). Given this, exercise has been considered as a therapeutic tool for traumatic brain injury (TBI; Booth et al., 2002; Grealy et al., 1999; Vitale et al., 1996). Currently, there is no accepted treatment to ameliorate neurological disabilities in victims surviving in over two million head injuries occurring annually in the United States.

It is likely that the beneficial effects of exercise on the brain are mediated by select neurotrophins, such as brain-derived neurotrophic factor (BDNF). BDNF has well-defined effects on neuronal survival, growth and synaptic plasticity (Barde, 1989). It has also been associated with improving cognitive and neurological deficits due to ischemia (Almli et al., 2000; Schabitz et al., 1997; Yamashita et al., 1997). Exercise leads to an increase of BDNF and its downstream effectors on synaptic transmission in select brain regions (Molteni et al., 2002; Neeper et al., 1995). For example, exercise in rats activates hippocampal cyclic AMP response element binding protein (CREB) and the mitogen-activated protein kinase (MAP-K) pathway (Shen et al., 2001). The MAP-K cascade facilitates the phosphorylation of CREB (Finkbeiner et al., 1997) and synapsin I (Gottschalk et al., 1999; Hicks et al., 1997; Jovanovic et al., 2000). Synapsin I is involved in synaptic vesicle clustering and release (Greengard et al., 1993; Li et al., 1995; Llinas et al., 1991; Melloni et al., 1994; Pieribone et al., 1995). CREB plays a role in long-term plasticity (Abel and Kandel, 1998) and memory (Silva et al., 1998), and its phosphorylation is involved in the activation of its target genes, including BDNF (Finkbeiner, 2000).

Despite of the therapeutic potential of BDNF, TBI has been nonresponsive to exogenous BDNF administration (Blaha et al., 2000). Assuring the delivery of BDNF to critical regions of the injured brain has been problematic. We propose that endogenous upregulation of BDNF and associated molecular systems through exercise can lead

*Correspondence to: G. S. Griesbach, 18-228A NPI, UCLA Medical Center, Box 957039, Los Angeles, CA 90095, USA. Tel: +1-310-794-5250; fax: +1-310-794-2147.
E-mail address: ggriesbach@mednet.ucla.edu (G. S. Griesbach).

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CREB, cyclic AMP response element binding protein; DFPI, delayed fluid-percussion injury; DSham, delayed sham; ELISA, enzyme-linked immunosorbent assay; FPI, fluid-percussion injury; MAP-K, mitogen-activated protein kinase; MWM, Morris water maze; RW, running-wheel; Sed, sedentary; TBI, traumatic brain injury; TBST, 20 mM Tris–HCl (pH 7.6), 150 mM NaCl, 0.05% Tween 20.

0306-4522/04$30.00+0.00 © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2004.01.030

to a better TBI outcome. However, the therapeutic effect of exercise is likely to be influenced by dynamic changes occurring after TBI. Following TBI, surviving cells are in a state of energy crisis and vulnerable to secondary activation (Lee et al., 1999; Signoretti et al., 2001; Zanier et al., 2003). Therefore, it is the possible that premature exercise after TBI may reduce the capacity for plasticity of the brain. We set out to determine the post-traumatic time window during which exercise can increase BDNF and associated proteins in the brain. In addition, the effects of voluntary exercise on spatial learning and memory were evaluated. Here we report that whereas exercise can lead to an increase in plasticity-related proteins following injury, it resulted in a behavioral and molecular dysfunction when administered prematurely.

EXPERIMENTAL PROCEDURES

Subjects

A total of 161 male Sprague–Dawley adult rats (mean weight: 312 g) were utilized in these experiments. Rats underwent lateral fluid-percussion injury (FPI; $n=89$) or sham ($n=72$) injury and were housed with or without access to a running wheel (RW) at different postinjury times. In order to control for motor impairments that may have an effect on RW activity, rats were tested for motor skills before and after injury. All animals were continually monitored and cared for by an Institutional Association Animal Care and Use Committee-approved veterinary care staff upon arrival at UCLA. During the experiments, rats were single housed in opaque plastic bins (20×10×10 inches), which were lined with bedding material. Each animal was weighed upon inclusion into the study and checked for weight loss. All procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the UCLA Animal Research Committee. The suffering and the number of animals were minimized in all experimental conditions.

Lateral fluid percussion injury

Rats were initially anesthetized with 4% isoflurane (in 100% O_2) and then maintained to 1.5–2% isoflurane (in 100% O_2). The head was secured in a stereotactic frame, shaved and prepped with betadine and ethanol. Body temperature was monitored and maintained at 37 °C with a heating pad (Braintree Scientific Inc., Braintree, MA, USA). A midline sagittal incision was made with the aid of a microscope (Wild, Heerburg, Switzerland) and a 3-mm diameter craniotomy was made with a high-speed drill (Dremel, Racine, WI, USA) 3-mm posterior to bregma and 6-mm lateral to the midline, on the left side. A plastic injury cap was placed over the craniotomy with silicone adhesive, cyanoacrylate and dental cement. When the dental cement hardened, the cap was filled with 0.9% saline solution. Anesthesia was discontinued and the animal was removed from the stereotaxis device. The injury cap was attached to the fluid percussion device. At the first sign of hind-limb withdrawal to a paw pinch, a mild fluid percussion pulse (1.5 atm) was administered. Apnea times were determined as the time from injury to the return of spontaneous breathing. Respiratory support was provided after 30 s of apnea. Time of unconsciousness was determined by the return of the hind-limb withdrawal reflex. Sham animals underwent an identical preparation with the exception of the FPI. Immediately upon responding to a paw pinch, anesthesia was restored, the injury cap removed, and the scalp was sutured. Neomycin was applied on the suture and the rat was placed in a heated recovery chamber for approximately 1 h before returning to its cage.

Voluntary wheel exercise

Rats were individually caged with or without access to a RW from postinjury days 0–6 (acute) or 14–20 (delayed). Rats had *ad libitum* access to food and water and were maintained on a 12-h light/dark cycle. Exercising animals were placed in standard cages equipped with RWs (diameter=31.8 cm, width=10 cm) that rotate against a resistance of 100 g. Sedentary (Sed) animals were left undisturbed in their home cages. The number of wheel revolutions per hour was recorded by a computer using VitalViewer Data Acquisition System software (Mini Mitter company, Inc., Sunriver, OR, USA). The mean number of revolutions was calculated for each night, given that this was the most active period.

Water-maze training

On post-injury day 7 or 21, animals were behaviorally tested after random assignment to the following acute (FPI-RW, $n=10$; FPI-Sed, $n=10$; ASham-RW, $n=9$; ASham-Sed, $n=9$) or delayed housing conditions (delayed fluid-percussion injury [DFPI]-RW, $n=8$; DFPI-Sed, $n=8$; delayed sham [DSham]-RW, $n=6$; DSham-Sed, $n=7$). The water maze consisted of a 1.5-m-diameter, 0.6-m-height tank filled with white opaque organic paint (Stechler, Albuquerque, NM, USA). The water level was kept at 2 cm above an escape platform and maintained at 20 °C. The platform dimensions were 15×15 cm. Rats received two training blocks per day for 5 days, with of an interblock interval ranging from 25 to 30 min. Each block consisted of four consecutive trials. Animals were released from four predetermined points around the water maze in random order and were given 45 s to locate the platform. Once they reached the platform, they remained on it for 1 min before proceeding to the next trial. If they failed to locate the platform, they were guided toward it. A 1 min probe trial was performed a week after training. In the probe trial, the platform was removed and the rat was released from the tank center. Behavior variables (latency, speed, trials to reach criterion and time spent in target areas) were recorded with the SMART tracking system (San Diego Instruments).

Protein immunoassay and Western data

We measured hippocampal protein levels given that we were primarily interested in the influence that these could have on the behavioral outcome. In addition, the hippocampus a strong activity-induced increase in BDNF has been observed in this region (Neeper et al., 1995). Protein levels were analyzed for Sed and exercised rats following acute (FPI, $n=21$; sham, $n=16$) or delayed housing conditions (DFPI, $n=18$; sham, $n=17$). All rats in the acute group were killed at post-injury day 7, whereas rats in the delayed groups were killed at post-injury day 21. After being killed by decapitation, left and right hippocampal tissue was dissected and homogenized in homogenization buffer (137 mM NaCl, 20 mM Tris–HCl pH 8.0, 1% NP40, 10% glycerol, 1 mM PMSF, 10 μg/ml aprotinin, 0.1 mM benzethonium, 0.5 mM sodium vanadate). Supernatants were collected and protein concentration was determined according to the Micro BCA procedure (Pierce, Rockford, IL, USA) using bovine serum albumin as standard. Mean values for BDNF protein were expressed as picograms per milligram of total protein.

BDNF protein was quantified using an enzyme-linked immunosorbent assay (ELISA) and standard protocols (BDNF Emax ImmunoAssay System kit; Promega Inc., Madison, WI, USA). Each hippocampal side was processed in a different plate with appropriate controls. Briefly, Nunc MaxiSorp 96 well plates were coated with 0.1 ml of a monoclonal antibody against BDNF in a buffer containing 0.025 M sodium bicarbonate and 0.025 M sodium carbonate (pH 9.7) for 16 h at 4 °C. After washing in TBST [(20 mM Tris–HCl (pH 7.6), 150 mM NaCl, 0.05% Tween 20)], wells were incubated with 0.2 ml of a blocking buffer at room

temperature for 1 h and then washed in TBST again. Samples, six serial dilutions of a BDNF standard (500 pg/ml), and a blank (no BDNF) were added in triplicate into separate wells. Plates were incubated for 2 h at room temperature and washed five times in TBST. A polyclonal antibody against BDNF (1:500 dilution) was added into each well and plates were incubated for 2 h at room temperature. After five washes in TBST, 0.1 ml of a secondary anti-IgY antibody with a horseradish peroxidase conjugate was added to each well and plates were incubated for 1 h at room temperature. Wells were washed five times with TBST. A hydrogen peroxidase solution with a peroxidase substrate was added and incubated for 10 min at room temperature. Reactions were stopped with 1 M phosphoric acid and absorbance at 450 nm was measured using an automated microplate reader. Standard curves were plotted for each plate. Triplicates were averaged and values were corrected for total amount of protein in the sample.

Synapsin and phospho-synapsin were analyzed by Western blot and normalized for actin levels. Separate blots, including FPI- and sham-injured rats, were performed for each region and side. Protein samples were separated by electrophoresis on an 8% polyacrylamide gel and electrotransferred to a nitrocellulose membrane. Non-specific binding sites were blocked in TBS overnight at 4 °C with 2% BSA and 0.1% Tween-20. Membranes were rinsed for 10 min in buffer (0.1% Tween-20 in TBS) and then incubated for 1 h at room temperature with anti-actin and either anti-synapsin or anti-phospho-synapsin (1:2000; Santa Cruz Biotechnology), anti-CREB and anti-phospho CREB (1:1000; New England Biolabs Inc., Beverly, MA, USA). After rinsing in buffer three times for 10 min, membranes were incubated with anti-goat IgG horseradish peroxidase-conjugate for actin and synapsin I or phospho-synapsin I (1:2000; Santa Cruz Biotechnology Inc.). Immunocomplexes were analyzed by chemiluminescence using the ECL kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) according to manufacturer instructions.

Immunohistochemistry

Animals were injected with a lethal dose of sodium pentobarbital (200 mg/kg i.p.) and transcardially perfused with heparinized PBS followed by 4% paraformaldehyde solution in 0.1 M phosphate buffer. Serial coronal sections (25 μm) were cut using a cryostat, mounted on gelatin-coated slides and processed for immunohistochemistry as previously described (Gomez-Pinilla et al., 2001). A 1:1000 dilution was used for the rabbit polyclonal anti-BDNF (Chemicon International Inc., Temecula, CA, USA).

Fluro-Jade B staining

Additional FPI-RW (n=4), FPI-Sed (n=4) and Sham-Sed rats (n=4) were perfused at postinjury day 7 and tested for neuronal degeneration with Fluoro-Jade staining. Briefly, 25 μm coronal sections were cut in a cryostat and incubated in alcohol, water, and potassium permanganate (0.6%). After rinsing, sections were then incubated in the dark with 0.001% Fluoro-Jade solution, made with 0.01% acetic acid, for 30 min. Slides were then rinsed in three changes of water, air-dried, cleared in xylene, and coverslipped. The number of Fluoro-Jade positive cells was counted on sections spanning the hippocampus under a fluorescence microscope with a FITC filter. Alternate sections were stained with Cresyl Violet for qualitative assessment of tissue integrity.

Statistical analysis

Analysis of variance (ANOVA) was conducted and a P value of <0.05 was considered statistically significant. Acute and DSham data were combined, given that no significant differences were found between these groups.

Hippocampal BDNF protein was quantified at postinjury days 7 and 21 following exercise or Sed conditions. Absolute values were obtained through an ELISA. The ratio of BDNF concentration/total protein concentration was analyzed. Mean values for BDNF protein levels were computed for each group and compared using a three-way ANOVA [injury: (FPI vs Sham), time: (acute vs delayed) and exercise: (RW vs Sed). Interaction effects were further analyzed by performing means comparisons in where desired contrast weights were specified. Separate ANOVAs were performed for each hippocampal side. Analysis of correlation (linear regression) was performed between the amount of exercise and BDNF protein levels for each exercised rat.

Western blots were corrected by actin. It has been reported that actin levels change following TBI; however, it is mostly restricted to the first 24 h after brain injury (Bareyre et al., 2001). Each gel included the Sham-Sed group, thus allowing the normalization of groups in different gels. The percent change from the corresponding Sham-Sed mean value was obtained for each blot. Data were then analyzed through a three-way ANOVA [injury: (FPI vs Sham), time: (acute vs delayed) and exercise: (RW vs Sed)]. Interaction effects were further analyzed by performing means comparisons in where desired contrast weights were specified. Separate ANOVAs were performed for each hippocampal side. Mean values for behavior were analyzed through a repeated three-way ANOVA [injury: (FPI vs Sham), time: (acute vs delayed) and exercise: (RW vs Sed)]. Interaction effects were further analyzed by performing means comparisons in where desired contrast weights were specified. Data were also analyzed by acquisition of criterion. This criterion was defined as the ability to locate the platform in 7 s or less for four consecutive trials. The probe test was analyzed by determining the percentage of time swimming near the platform area for the first 30 s. Platform area consisted of 8% of the tank area. Morris water maze (MWM) criterion and probe test values were compared among groups with a three-way ANOVA.

RESULTS

Animals sustaining injury exhibited a period of unconsciousness ranging from 15 to 180 s (mean: 82 s; standard deviation: 54 s) and apnea time ranging from 5 to 50 s (mean: 14 s; standard deviation: 11 s). All animals displayed normal behavior after recovery from anesthesia. Motor impairments were not observed in the injured rats. Injured rats that were exposed to exercise acutely tended to exercise less than the sham animals during the first night, thus this did not reach statistical significance. Exercise levels became normal at the second night of RW exposure. No Fluoro-Jade-stained hippocampal neurons were detected in the sham and FPI rats. Fluoro-Jade B is an anionic tribasic fluorochrome that has been extensively used to detect neurodegeneration in response to insults (Schmued and Hopkins, 2000). In addition, no gross cell loss was detected in Cresyl Violet-stained sections.

Effects of exercise on hippocampal BDNF upregulation

Ipsilateral hippocampus. An exercise-induced increase in BDNF was not found in all the exercised groups in the side ipsilateral to the injury. This was indicated by a significant "Exercise×Injury×Time" interaction [$F(1, 64)=4.00$, $P<0.05$]. Means comparison analysis demonstrated that exercise significantly increased BDNF levels in the Sham-RW rats compared with the Sham-Sed rats [$F(1)=3.712$, $P<0.05$]. Acute and DShams were pooled for this comparison given that no significant differences in

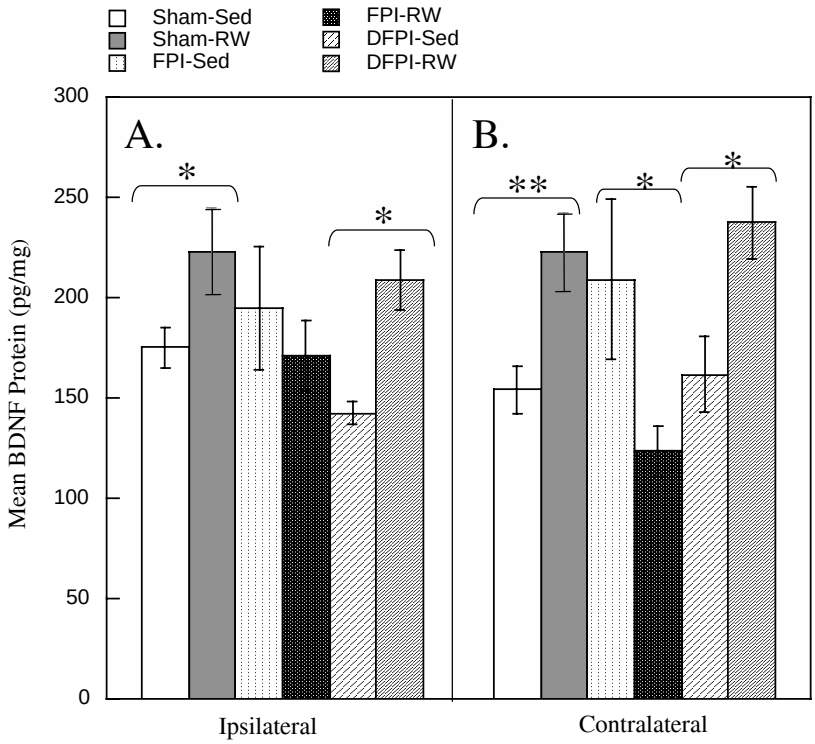

Fig. 1. Effects of exercise following FPI on BDNF protein. Sham and FPI rats were housed with or without access to a RW from postinjury days 0–6 (acute) or 14–20 (delayed). BDNF protein levels within the hippocampus were measured by ELISA. Mean values were analyzed through a repeated three-way ANOVA [injury: (FPI vs. Sham), time: (acute vs. delayed) and exercise: (RW vs. Sed)]. Interaction effects were further analyzed by performing means comparisons in where desired contrast weights were specified. Graph demonstrates BDNF levels, ipsilateral and contralateral to the injury. Each value represents the mean±S.E.M. Significant comparisons between groups are indicated by brackets (* $P<0.05$; ** $P<0.005$). (A) Side ipsilateral to the injury. (B) Side contralateral to the injury.

BDNF levels were observed between these groups. In addition, significant increases in ipsilateral hippocampal BDNF were found in the DFPI-RW rats compared with the D-FPI-Sed group [$F(1)=4.98$, $P<0.05$] and in the Sham-RW rats compared with the FPI-RW rats [$F(1)=4.84$, $P<0.05$; Fig. 1A]. All this was supported by a main effect for exercise [$F(1, 64)=4.42$, $P<0.05$].

Contralateral hippocampus. Analysis of BDNF levels, in the hippocampal side contralateral to the injury, revealed a significant "Exercise×Injury×Time" interaction [$F(1, 64)=6.3$, $P<0.05$]. Means comparisons indicated that BDNF levels were elevated in the Sham-RW compared with the Sham-Sed [$F(1)=8.42$, $P<0.005$] and in DFPI-RW compared with DFPI-Sed [$F(1)=6.45$, $P<0.05$]. No significant differences were observed between acute and DSham rats. In addition means comparisons indicated that contralateral hippocampal BDNF levels decreased in the FPI-RW compared with the FPI-Sed rats [$F(1)=8.2$, $P<0.05$]. A significant BDNF increase in Sham-RW compared with FPI-RW was also found in the contralateral hippocampus [$F(1)=17.75$, $P<0.0005$]. All these effects were supported by a significant main effect for in the contralateral hippocampus [$F(1, 64)=3.96$, $P<0.05$; Fig. 1B].

Exercise was quantified by recording the mean number of nightly wheel revolutions. Acute and DSham-RW rats were pooled given that no significant differences in the amount of exercise and BDNF was found between these groups. Analysis of correlation (linear regression) between the amount of exercise and BDNF protein levels indicated that the increase in hippocampal BDNF levels was proportional to the amount of exercise in the sham animals ($y=0.08x+107.11$, R^2: 0.76, $P<0.0005$; Fig. 2A). This correlation was not found in rats that were exercised immediately following FPI [(ipsilateral; $y=0.003x+165.58$, R^2: $3.19E^{-4}$), (contralateral: $y=0.014x+131.32$, R^2: 0.02; Fig. 2B].

However, there was a positive correlation between exercise and BDNF upregulation [(ipsilateral; $y=0.036+102.325$, R^2: 0.308), (contralateral: $y=0.073x+129.827$, R^2: 0.386; Fig. 2B] for DFPI-RW rats (Fig. 2C). The DFPI-RW rats had a qualitative increase in BDNF immunostaining of the CA3 and dentate gyrus compared with DFPI-Sed and the CA3 compared with Sham-Sed (Fig. 3).

Effects of exercise on hippocampal synapsin I

Acute and DSham groups were pooled for means comparisons given that no significant differences in total and phosphorylated synapsin I were observed. No significant effects were found for total synapsin I in the ipsilateral and contralateral hippocampus.

Ipsilateral hippocampus. Analysis of phosphorylated synapsin I levels, revealed a significant

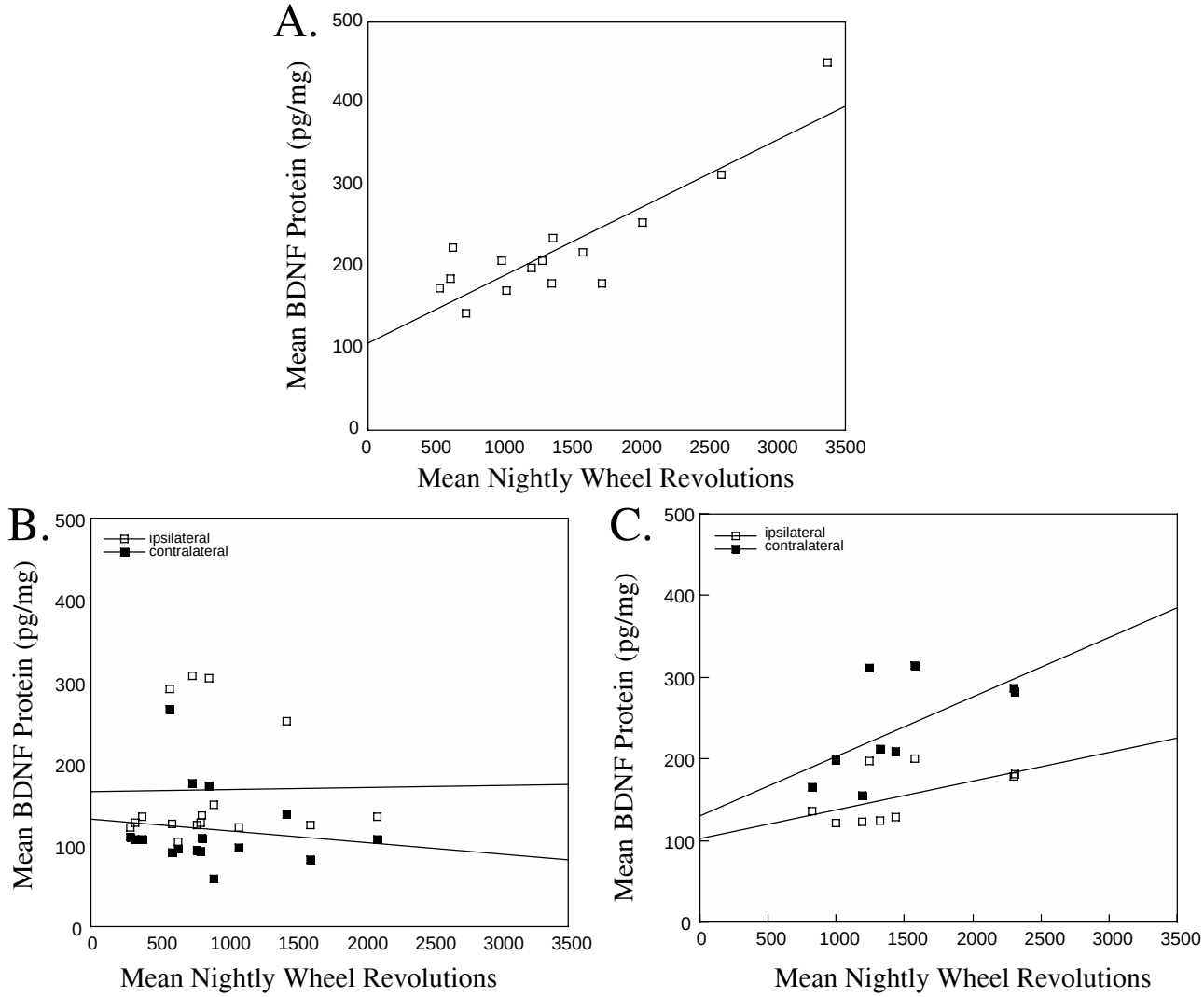

Fig. 2. Correlation between exercise and BDNF. Rats were exercised from postinjury days 0–6 or 14–20. Data points in plots indicate the amount of hippocampal BDNF protein and the mean number of nightly revolutions per rat. (A) Acute and DSham-RW data were pooled given that no significant differences were found between these two groups. A significant positive correlation between the amount of exercise and BDNF levels was found ($P<0.0005$, R^2: 0.76). Right and left sides were combined due to no side differences. (B) Exercise-dependent BDNF upregulation was not observed in FPI-RW rats exercised from postinjury days 0–6 (Ipsilateral: R^2: $3.19E^{-4}$; Contralateral: R^2: 0.01). (C) DFPI-RW rats, that were exercised from postinjury days 14–20, showed a positive correlation between the amount of exercise and BDNF levels (Ipsilateral: R^2: 0.308; Contralateral: R^2: 0.386).

"Exercise×Injury×Time" interaction [$F(1, 30)=6.59$, $P<0.05$]. No significant differences were observed between acute and DSham rats. Means comparisons indicated that synapsin I levels were significantly elevated in the Sham-RW [$F(1)=12.88$, $P<0.005$] and in the FPI-Sed [$F(1)=17.2$, $P<0.0005$] groups compared with Sham-Sed. Means comparisons also revealed a significant decrease of phosphorylated synapsin I in the FPI-RW rats compared with the FPI-Sed rats [$F(1)=23.65$, $P<0.0005$; Fig. 4A]. These effects were also, supported by a significant "Injury×Exercise" interaction [$F(1, 30)=25.06$, $P<0.0005$]. No significant comparisons were observed for DFPI-Sed and DFPI-RW groups.

Contralateral hippocampus. A significant "Exercise×Injury" interaction [$F(1, 30)=5.15$, $P<0.05$] was found in the contralateral hippocampus for phosphorylated synapsin I. No significant differences were observed between acute and DSham rats. Means comparisons indicated that synapsin I levels were significantly elevated in Sham-RW compared with Sham-Sed [$F(1)=4.87$, $P<0.05$; Fig. 4B].

Effects of exercise on hippocampal CREB

Acute and DSham groups were pooled for means comparisons given that no significant differences in total and phosphorylated CREB were observed.

Ipsilateral hippocampus. Analysis of total CREB levels revealed a significant "Exercise×Injury" interaction [$F(1, 27)=35.56$, $P<0.0005$]. No significant differences

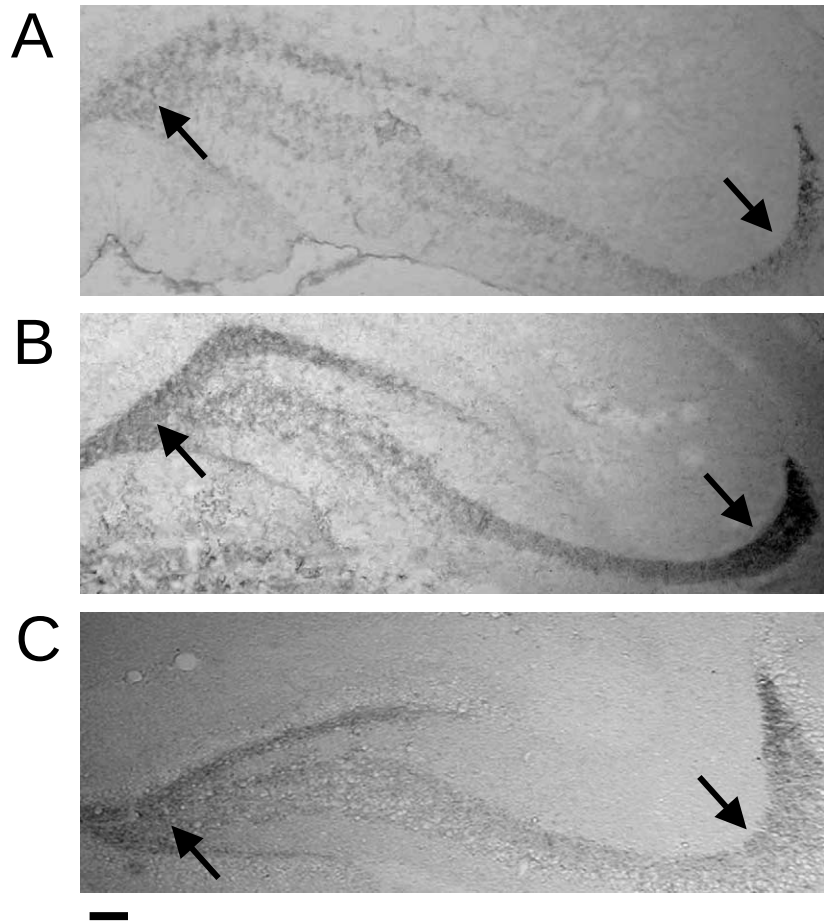

Fig. 3. Immunostaining for BDNF at postinjury day 21. (A) DFPI-Sed, (B) DFPI-RW, (C) Sham-Sed. BDNF labeling was predominantly distributed in the dentate gyrus and CA3 region of the hippocampus. Exercise resulted in a qualitative increase of BDNF. Scale bar=250 μm.

were observed between acute and DSham rats. Means comparisons indicated that total CREB levels were significantly elevated in Sham-RW compared with Sham-Sed [$F(1)=14.9$, $P<0.0005$]. Means comparisons also revealed a significant decrease of total CREB in FPI-RW compared with FPI-Sed [$F(1)=17.21$, $P<0.0005$] and in DFPI-RW compared with DFPI-Sed [$F(1)=6.53$, $P<0.05$; Fig. 4C].

A significant "Exercise×Injury" interaction [$F(1, 30)=5.15$, $P<0.05$] was found for phosphorylated CREB. No significant differences were observed between acute and DSham rats. Means comparisons indicated that phosphorylated CREB levels were significantly elevated in Sham-RW compared with Sham-Sed [$F(1)=7.49$, $P<0.05$]. Significant increases of phosphorylated CREB were also found in DFPI-Sed [$F(1)=6.26$, $P<0.05$] and DFPI-RW [$F(1)=11.15$, $P<0.005$] compared with Sham-Sed (Fig. 4E).

Contralateral hippocampus. A significant "Exercise×Injury" interaction [$F(1, 27)=13.97$, $P<0.005$] was found for total CREB. No significant differences were observed between acute and DSham rats. Means comparisons indicated that total CREB levels were significantly elevated in Sham-RW compared with Sham-Sed [$F(1)=9.99$, $P<0.005$]. In addition, significant decreases of total CREB were found in FPI-Sed [$F(1)=9.78$, $P<0.005$], FPI-RW [$F(1)=17.59$, $P<0.0005$] and DFPI-RW [$F(1)=15.21$, $P<0.0005$] compared with Sham-RW (Fig. 4D). These effects were supported by a significant main effect for "Injury" [$F(1, 27)=9.24$, $P<0.05$].

A significant "Exercise×Injury" interaction [$F(1, 29)=9.1$, $P<0.05$] was found for phosphorylated CREB. No significant differences were observed between acute and DSham rats. Means comparisons indicated that phosphorylated CREB levels were significantly elevated in Sham-RW compared with Sham-Sed [$F(1)=8.56$, $P<0.05$]. In addition, significant increases of phosphorylated CREB were found in FPI-Sed [$F(1)=17.98$, $P<0.0005$], DFPI-Sed [$F(1)=7.05$, $P<0.05$] and DFPI-RW [$F(1)=12.7$, $P<0.005$] compared with Sham-Sed (Fig. 4F). These effects were supported by a significant main effect for "Injury" [$F(1, 29)=8.87$, $P<0.05$].

Behavior

Rats were cognitively assessed in the MWM from postinjury days 7–11 or 21–25. A significant "Exercise×Injury×Time" interaction [$F(1, 60)=5.04$, $P<0.05$] indicated that FPI post-injury time had an effect on latency to find the hidden platform. Means comparison analysis demonstrated that

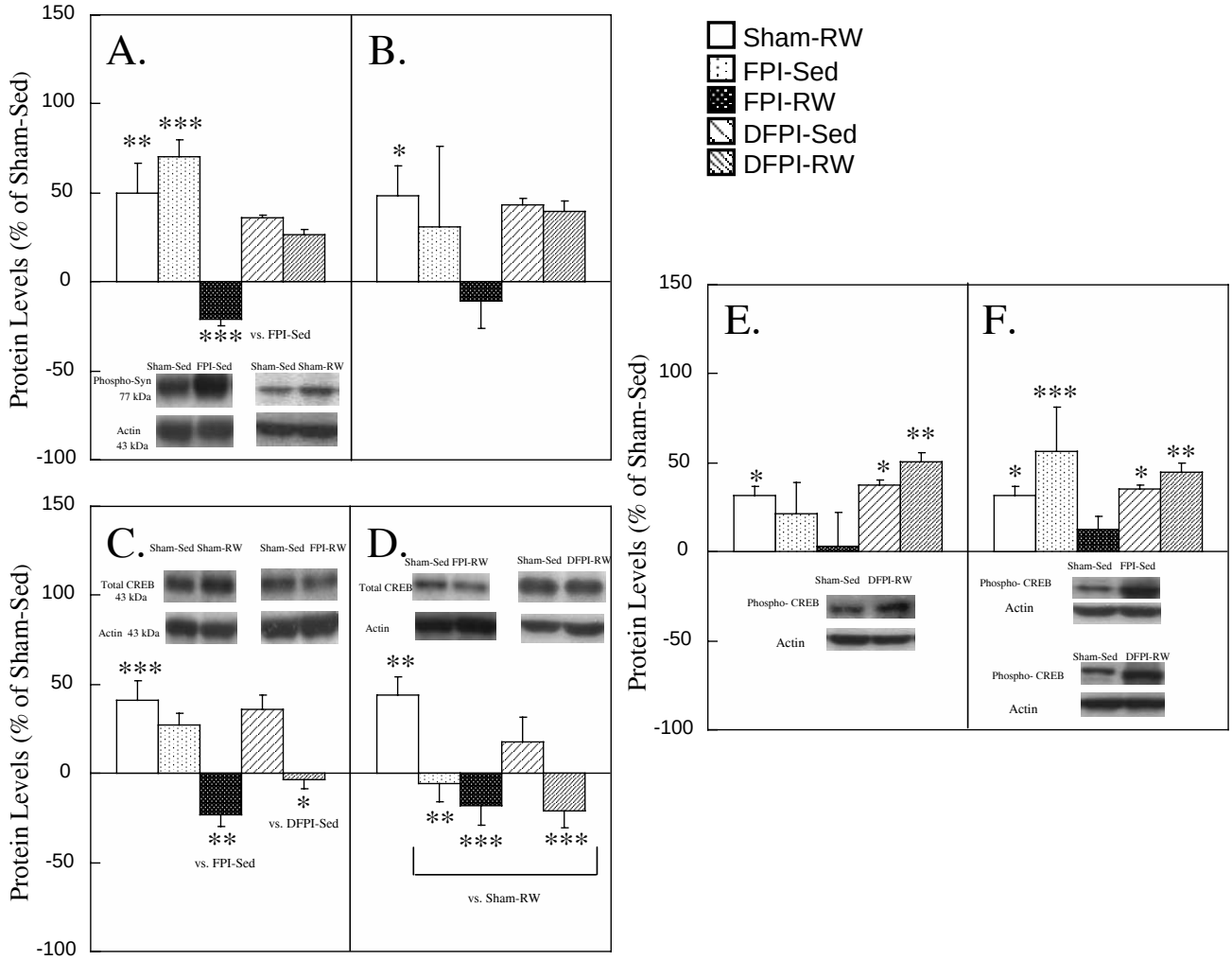

Fig. 4. Effects of exercise following FPI on synapsin I and CREB. Sham and FPI rats were housed with or without access to a RW from postinjury days 0–6 (acute) or 14–20 (delayed). Protein was obtained from the hippocampus on postinjury day 7 or 21. Each value represents the percent change from the Sham-Sed mean value±S.E.M. Mean values were analyzed through a repeated three-way ANOVA [injury: (FPI vs. Sham), time: (acute vs. delayed) and exercise: (RW vs. Sed)]. Interaction effects were further analyzed by performing means comparisons in where desired contrast weights were specified. Acute and DSham data were pooled given that no significant differences were found between these two groups. Significant comparisons are against Sham-Sed unless otherwise indicated (* $P<0.05$; ** $P<0.005$, *** $P<0.0005$). (A) Phosphorylated synapsin I on the side ipsilateral to the injury. (B) Phosphorylated synapsin I on the side contralateral to the injury. (C) Total CREB on the side ipsilateral to the injury. (D) Total CREB on the side contralateral to the injury. (E) Phosphorylated CREB on the side ipsilateral to the injury. (F) Phosphorylated CREB on the side contralateral to the injury. Representative immunoblots are shown for values that reached a significance of <0.005. Actin was used as an internal standard for Western blots.

latency to reach the platform was significantly increased in the FPI-RW rats compared with the FPI-Sed [$F(1)=6.42$, $P<0.05$]. No significant differences were detected between the DFPI-Sed and DFPI-RW groups, as well as between acute and DShams. In addition, means comparison indicated that the latency to reach the platform was significantly increased in the FPI-RW rats compared with the other groups combined [$F(1)=13.78$, $P<0.0005$; Fig. 5A]. These findings were supported by a significant main effect for injury [$F(1,60)=8.45$, $P<0.005$]. No significant differences in swimming speed were observed.

A significant "Exercise×Injury" interaction [$F(1,60)=3.79$, $P<0.05$] was found in the analysis of the number of trials to reach criterion. Means comparison analysis demonstrated Sham-RW rats needed significantly less trials to reach criterion compared with the other groups combined [$F(1)=18.63$, $P<0.0005$]. Means comparisons also demonstrated that DFPI-RW needed less trials to criterion compared with DFPI-Sed [$F(1)=4.95$, $P<0.05$] and FPI-RW [$F(1)=7.77$, $P<0.05$] groups. These effects were also supported by significant main effects for exercise [$F(1,60)=10.93$, $P<0.005$] and injury [$F(1,60)=6.44$, $P<0.05$; Fig. 5B].

A week after MWM training, a probe trial was performed to test for long-term memory deficits. A significant main effect for injury [$F(1, 46)=7.4$, $P<0.01$] was present in the first half of the probe trial. Means comparison demonstrated that Sham-RW rats spent significantly more time

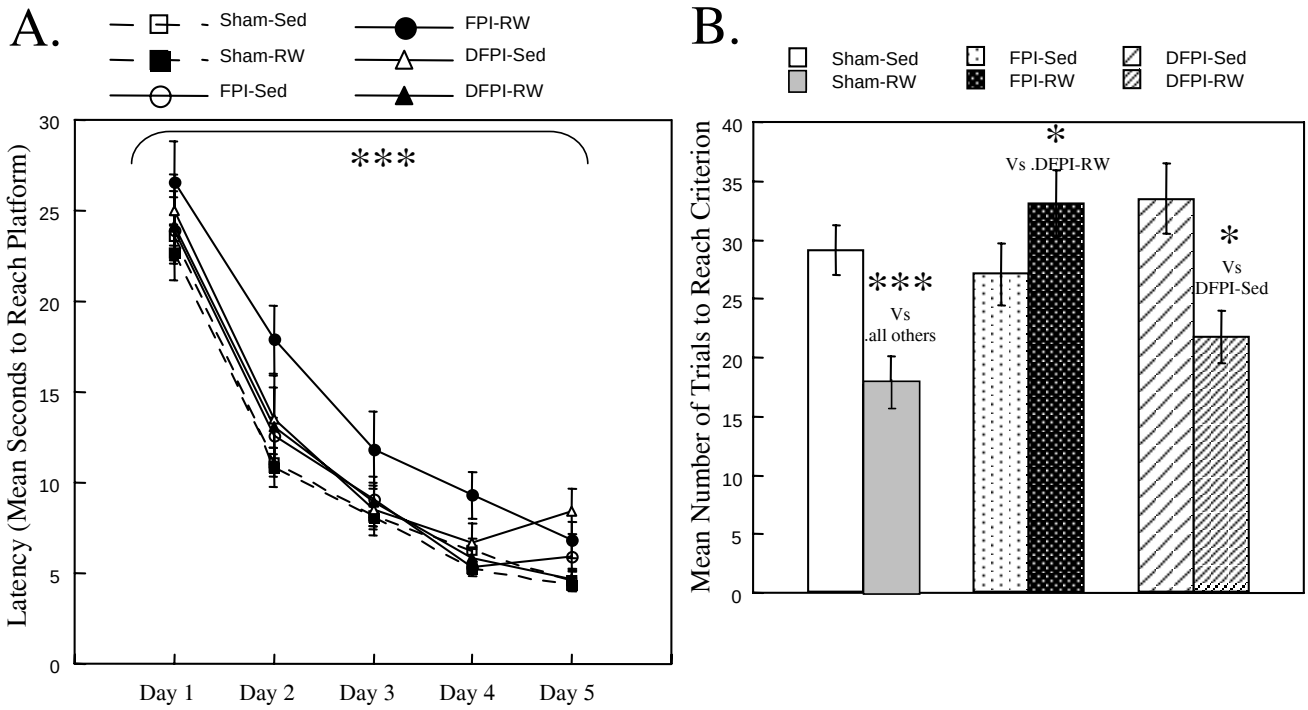

Fig. 5. Effects of exercise immediately following FPI on MWM performance. Sham and FPI rats were housed with or without access to a RW from postinjury days 0–6 (acute) or 14–20 (delayed) and trained in the MWM on postinjury day 7 or 21. Mean values were analyzed through a repeated three-way ANOVA [injury: (FPI vs. Sham), time: (acute vs. delayed) and exercise: (RW vs. Sed)]. Interaction effects were further analyzed by performing means comparisons in where desired contrast weights were specified. (A) Bracket indicates that FPI-RW rats spent significantly more time to reach the platform compared with all the other groups combined. Each value represents the mean latency±S.E.M. (B) Mean number of trials to reach criterion. Criterion was defined as the ability to locate the platform in 7 s or less for four consecutive trials. Each value represents the mean±S.E.M. (* $P<0.05$, *** $P<0.0005$.)

swimming in the platform area than all the other groups combined [$F(1)=8.5$, $P<0.005$; Fig. 6A]. A significant effect for injury was also found in the second half of the probe trial [$F(1,46)=6.28$, $P<0.05$; Fig. 6B]. No significant means comparisons were found.

DISCUSSION

The beneficial effects of exercise on the healthy brain are well recognized; however, the action of voluntary exercise on the injured brain remains largely unexplored. The suitability of exercise to help the injured brain is complex due to dynamic neurochemical and metabolic alterations elicited by TBI that may interfere with the effects of exercise. The present results indicate that in order for exercise to prove beneficial, it must be administered at the appropriate post-injury time window. Indeed these findings demonstrated that premature physical activity, after a mild FPI, was associated with impairments in the acquisition of a cognitive task, and a disruption of the molecular response to exercise. However, exercise when applied with a delay after TBI resulted in the endogenous upregulation of BDNF and enhanced performance in the MWM task.

Voluntary exercise at the appropriate post-injury time window seems beneficial for brain plasticity

Voluntary exercise results in an increase of BDNF and its downstream effectors on synaptic plasticity, CREB and synapsin I, in the hippocampus of intact rats (Molteni et al., 2002; Neeper et al., 1995). Our current findings indicate the incapacity of exercise to increase the same molecular systems when applied immediately after a mild FPI. Whereas, hippocampal BDNF in the sham animals increased proportionally to the amount of exercise, the acutely exercised FPI rats failed to show BDNF upregulation. Exercise also decreased levels of CREB and phosphorylated synapsin I when provided immediately after FPI.

It is likely that the incapacity of exercise to promote neuroplasticity-associated molecular changes in the acute phase of TBI is related to the metabolic alterations that take place during this post-injury period. Moreover, recent findings indicate that cortical stimulation to the brain following lateral FPI elicits a metabolic response and may also act as a secondary injury by increasing cortical degeneration (Ip et al., 2003). During the first postinjury week, the brain is undergoing metabolic changes that may strongly influence the outcome of therapies based on the use of activity (Fineman et al., 1993; Ginsberg et al., 1997; Hovda, 1996; Moore et al., 2000). Studies suggest that following TBI there is a lower concentration of the primary source of cellular energy, ATP (Lee at al. 1999; Signoretti et al., 2001), as well as structural alterations of the mitochondria (Lifshitz et al., 2003). Untimely exercise may accelerate ATP loss or divert it from needed functions such as producing synaptic plasticity molecules, by introducing

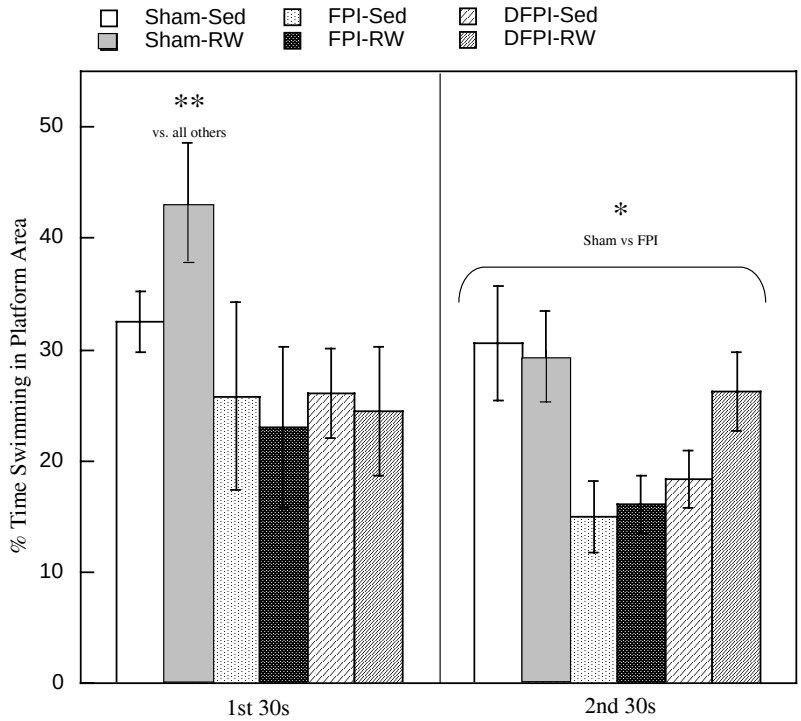

Fig. 6. Effects of postinjury exercise on delayed probe test. One week after training in the MWM the platform was removed and a 60 s probe test was performed to test for long-term memory deficits. Mean values of time spent swimming in the target area were analyzed through a repeated three-way ANOVA [injury: (FPI vs. Sham), time: (acute vs. delayed) and exercise: (RW vs. Sed)]. Interaction effects were further analyzed by performing means comparisons in where desired contrast weights were specified. Target area consisted of 8% of the tank area. Each value represents the mean±S.E.M. (* $P<0.05$, ** $P<0.005$.)

an increase in metabolic demand at a time when the brain is energetically compromised. Indeed, exercise increases energy demands primarily in the hippocampus, motor cortex and striatum (Vissing et al., 1996). In addition exercise increases regional cerebral blood flow (Gross et al., 1980; Orgogozo and Larsen, 1979), extracellular lactate (De Bruin et al., 1990) and hippocampal discharge rates (Czurko et al., 1999).

Neurochemical and metabolic disruptions that extend over a period of days after the initial insult normalize after approximately 10 days (Bergsneider et al., 1997; Kawamata et al., 1995; Yoshino et al., 1991). In a similar manner, the lack of an exercise-induced increase in BDNF following FPI was transient. Levels of BDNF were elevated when FPI rats were exposed to exercise from post-injury days 14–20. It is conceivable that the increase in BDNF may be related to the restoration of a normal energetic response following TBI.

Changes in BDNF and synaptic plasticity resulting from TBI and exercise may affect cognitive performance

The acute FPI-RW group performed worse than all the other groups in the MWM, a hippocampal dependent memory task. In comparison, MWM acquisition was enhanced in the sham and FPI rats that underwent delayed RW exposure. These rats also had elevated levels of BDNF and phosphorylated CREB. Given the involvement of BDNF in hippocampal-dependent learning (Mizuno et al., 2003; Tyler et al., 2002), it is likely that it had an influence on the MWM performance of the exercised sham and delayed FPI-RW rats. Functional recovery following brain injury has been correlated with synaptic changes that enhance connectivity (Kolb, 1999). Thus it seems that in order for exercise to have a beneficial effect following TBI, the brain must be capable of increasing BDNF and associated proteins in response to activation.

While the exact mechanism underlying the acquisition impairment found in the acutely exercised injured rats is unknown, it is possible that the lack of an activity-induced increase of BDNF, synapsin I and CREB is reflective of alterations in select molecular systems that have an influence on cognitive performance. Alterations of synapsin I and CREB may indicate a disruption of ongoing restorative processes, such as changes in connectivity reported following brain injury. Indeed, phosphorylated synapsin I was increased in the hippocampus of FPI-Sed rats, and acute exercise resulted in its decrease. Similarly acute exercise decreased levels of CREB. The injury-induced increase of synapsin I supports the idea that synaptic modification occurs after TBI (Albensi, 2001; Ivanco and Greenough, 2000; Kolb, 1999). In addition elevated levels of BDNF were found in the contralateral hippocampus following FPI alone. Contralateral changes that have an impact on neural plasticity have been observed after unilateral somatosensory cortex injury in humans (Chu et al., 2000) and animal injury models (Griesbach et al., 2002; Ip et al.,

2002; Jansen and Low, 1996; Kozlowski and Schallert, 1998).

It must be noted that a long-term deficit was found in the probe test for all brain injury groups, including those exposed to the RW at immediate and delayed post-injury time windows. This was a more cognitively demanding task, being that it tapped into long-term memory. It remains unknown if exercise at a later post-injury window will prove to be beneficial in this long-term memory task. Although phosphorylated synapsin I and CREB levels did not decrease as a result of delayed exercise in the FPI rats, these proteins had not returned to control levels at postinjury day 21 as compared with the levels in shams. Given that the delayed exercise led to an increase of BDNF, it is possible that an even later post-injury time-window for exercise might result in more robust increases of CREB and synapsin I, which may have an influence on the probe test performance. In addition, the present study has focused on exercise provided for 1 week, and it is possible that exercise provided for a longer period could have a more beneficial effect.

Implications

Exercise-induced upregulation of endogenous BDNF has a strong therapeutic potential considering the difficulties that have been observed with exogenous neurotrophin delivery. Its high molecular size, basic nature and widespread expression of its receptor, trkB, limit the diffusion of BDNF in the brain. However, the possibility that exercise (even voluntary) provided at the wrong time may have detrimental effects to the recovery process requires careful planning of the use of rehabilitative environmental stimulation during the acute phase of TBI. The fact that exercise was associated with an increase of BDNF levels and an enhanced performance in the MWM task provides important leads for the design of therapeutic applications. Experimental studies, in non-TBI models of brain injury, suggest that there is a period of vulnerability to secondary injury due to premature activation. For example, forced exercise following a unilateral sensorimotor cortex injury leads to anatomical and behavioral dysfunction (Humm et al., 1999; Kozlowski et al., 1996). The information provided by these studies emphasizes the importance of the timing for resumption of activity after brain injury and, hopefully, can nurture more studies to determine the exact conditions required for the optimization of the use of exercise to help the injured brain.

REFERENCES

Abel T, Kandel E (1998) Positive and negative regulatory mechanisms that mediate long-term memory storage. Brain Res Brain Res Rev 26:360–378.

Albensi BC (2001) Models of brain injury and alterations in synaptic plasticity. J Neurosci Res 65:279–283.

Almli CR, Levy TJ, Han BH, Shah AR, Gidday JM, Holtzman DM (2000) BDNF protects against spatial memory deficits following neonatal hypoxia-ischemia. Exp Neurol 166:99–114.

Barde YA (1989) Trophic factors and neuronal survival. Neuron 2:1525–1534.

Bareyre FM, Raghupathi R, Saatman KE, McIntosh TK (2001) DNase I disinhibition is predominantly associated with actin hyperpolymerization after traumatic brain injury. J Neurochem 77:173–181.

Bergsneider M, Hovda DA, Shalmon E, Kelly DF, Vespa PM, Martin NA, Phelps ME, McArthur DL, Caron MJ, Kraus JF, Becker DP (1997) Cerebral hyperglycolysis following severe traumatic brain injury in humans: a positron emission tomography study. J Neurosurg 86:241–251.

Blaha GR, Raghupathi R, Saatman KE, McIntosh TK (2000) Brain-derived neurotrophic factor administration after traumatic brain injury in the rat does not protect against behavioral or histological deficits. Neuroscience 99:483–493.

Booth FW, Chakravarthy MV, Gordon SE, Spangenburg EE (2002) Waging war on physical inactivity: using modern molecular ammunition against an ancient enemy. J Appl Physiol 93 (1):3–30.

Chu D, Huttenlocher PR, Levin DN, Towle VL (2000) Reorganization of the hand somatosensory cortex following perinatal unilateral brain injury. Neuropediatrics 31:63–69.

Czurko A, Hirase H, Csicsvari J, Buzsaki G (1999) Sustained activation of hippocampal pyramidal cells by 'space clamping' in a running wheel. Eur J Neurosci 11:344–352.

De Bruin LA, Schasfoort EM, Steffens AB, Korf J (1990) Effects of stress and exercise on rat hippocampus and striatum extracellular lactate. Am J Physiol 259 (4 Pt 2):R773–779.

Fineman I, Hovda DA, Smith M, Yoshino A, Becker DP (1993) Concussive brain injury is associated with a prolonged accumulation of calcium: a Ca-45 autoradiographic study. Brain Res 624:94–102.

Finkbeiner S (2000) Calcium regulation of the brain-derived neurotrophic factor gene. Cell Mol Life Sci 57:394–401.

Finkbeiner S, Tavazoie SF, Maloratsky A, Jacobs KM, Harris KM, Greenberg ME (1997) CREB: a major mediator of neuronal neurotrophin responses. Neuron 19:1031–1047.

Friedland RP, Fritsch T, et al (2001) Patients with Alzheimer's disease have reduced activities in midlife compared with healthy control-group members. Proc Natl Acad Sci USA 98:3440–3445.

Fordyce DE, Wehner JM (1993) Physical activity enhances spatial learning performance with an associated alteration in hippocampal protein kinase C activity in C57BL/6 and DBA/2 mice. Brain Res 619:111–119.

Ginsberg MD, Zhao W, Alonso OF, Loorestades JY, Dietrich WD, Busto R (1997) Uncoupling of local cerebral glucose metabolism and blood flow after acute fluid-percussion injury in rats. Am J Physiol Heart Circ Physiol 41:H2859–H2868.

Gomez-Pinilla F, So V, Kesslak JP (2001) Spatial learning induces neurotrophin receptor and synapsin I in the hippocampus. Brain Res 904:13–19.

Gottschalk WA, Jiang H, Tartaglia N, Feng L, Figurov A, Lu B (1999) Signaling mechanisms mediating BDNF modulation of synaptic plasticity in the hippocampus. Learn Mem 6:243–256.

Grealy MA, Johnson DA, et al (1999) Improving cognitive function after brain injury: the use of exercise and virtual reality. Arch Phys Med Rehabil 80:661–667.

Greengard P, Valtorta F, Czernik AJ, Benfenati F (1993) Synaptic vesicle phosphoproteins and regulation of synaptic function. Science 259:780–785.

Griesbach GS, Hovda DA, Molteni R, Gomez-Pinilla F (2002) Alterations in BDNF and synapsin i within the occipital cortex and hippocampus after mild traumatic brain injury in the developing rat: reflections of injury-induced neuroplasticity. J Neurotrauma 19: 803–814.

Gross PM, Marcus ML, Heistad DD (1980) Regional distribution of cerebral blood flow during exercise in dogs. J Appl Physiol 48:213–217.

Hicks A, Davis S, Rodger J, Helme-Guizon A, Laroche S, Mallet J (1997) Synapsin I and syntaxin 1B: key elements in the control of neurotransmitter release are regulated by neuronal activation and long-term potentiation in vivo. Neuroscience 79:329–340.

Hovda DA (1996) Metabolic dysfunction. In: Neurotrauma (Narayan

RK, Wilberger JE, Povlishock JT, eds), pp 896–918. New York: McGraw-Hill, Inc.
Humm JL, Kozlowski DA, Bland ST, James DC, Schallert T (1999) Use-dependent exaggeration of brain injury: is glutamate involved? Exp Neurol 157:349–358.
Ip EY, Giza CC, Griesbach GS, Hovda DA (2002) Effects of enriched environment and fluid percussion injury on dendritic arborization within the cerebral cortex of the developing rat. J Neurotrauma 19:573–585.
Ip EY, Roncati Zanier E, Moore AH, Lee SM, Hovda DA (2003) Metabolic, neurochemical and histological responses to vibrissa motor cortex stimulation following traumatic brain injury. J Cereb Blood Flow Metab 23:900–910.
Ivanco TL, Greenough WT (2000) Physiological consequences of morphologically detectable synaptic plasticity: potential uses for examining recovery following damage. Neuropharmacology 39: 765–776.
Jansen EM, Low WC (1996) Quantitative analysis of contralateral hemisphere hypertrophy and sensorimotor performance in adult rats following unilateral neonatal ischemic-hypoxic brain injury. Brain Res 708:93–99.
Jovanovic JN, Czernik AJ, Fienberg AA, Greengard P, Sihra TS (2000) Synapsins as mediators of BDNF-enhanced neurotransmitter release. Nat Neurosci 3:323–329.
Kawamata T, Katayama Y, Hovda DA, Yoshino A, Becker DP (1995) Lactate accumulation following concussive brain injury: the role of ionic fluxes induced by excitatory amino acids. Brain Res 674:196–204.
Kolb B (1999) Synaptic plasticity and the organization of behaviour after early and late brain injury. Can J Exp Psychol 53:62–76.
Kozlowski DA, James DC, Schallert T (1996) Use-dependent exaggeration of neuronal injury after unilateral sensorimotor cortex lesions. J Neurosci 16:4776–4786.
Kozlowski DA, Schallert T (1998) Relationship between dendritic pruning and behavioral recovery following sensorimotor cortex lesions. Behav Brain Res 97:89–98.
Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K (2001) Physical activity and risk of cognitive impairment and dementia in elderly persons. Arch Neurol 58:498–504.
Lee SM, Wong MD, Samii A, Hovda DA (1999) Evidence for energy failure following irreversible traumatic brain injury. Ann NY Acad Sci 893:337–340.
Li L, Chin LS, Shupliakov O, Brodin L, Sihra TS, Hvalby O, Jensen V, Zheng D, McNamara JO, Greengard P, et al (1995) Impairment of synaptic vesicle clustering and of synaptic transmission, and increased seizure propensity, in synapsin I-deficient mice. Proc Natl Acad Sci USA 92:9235–9239.
Lifshitz J, Friberg T, Neumar RW, Raghupathi R, Welsh FA, Janmey P, Saatman KE, Wieloch T, Grady MS, McIntosh TK (2003) Structural and functional damage sustained by mitochondria after traumatic brain injury in the rat: evidence for differentially sensitive populations in the cortex and hippocampus. J Cereb Blood Flow Metab 23:219–231.
Llinas RG, Gruner JA, Sugimori M, McGuinness TL, Greengard P (1991) Regulation by synapsin I and Ca-calmodulin-dependent protein kinase II of the transmitter release in squid giant synapse. J Physiol (Lond) 436:257–282.
Melloni RH, Apostolides PJ, Hamos JE, DeGennaro LJ (1994) Dynamics of synapsin I gene expression during the establishment and restoration of functional synapses in the hippocampus. Neuroscience 58:683–703.
Mizuno M, Yamada K, He J, Nakajima A, Nabeshima T (2003) Involvement of BDNF receptor TrkB in spatial memory formation. Learn Mem 10:108–115.
Molteni R, Ying Z, Gomez-Pinilla F (2002) Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. Eur J Neurosci 16:1107–1116.
Moore TH, Osteen TL, Chatziioannou TF, Hovda DA, Cherry TR (2000) Quantitative assessment of longitudinal metabolic changes in vivo after traumatic brain injury in the adult rat using FDG-microPET. J Cereb Blood Flow Metab 20:1492–1501.
Neeper SA, Gomez-Pinilla F, Choi J, Cotman C (1995) Exercise and brain neurotrophins [letter]. Nature 373:109.
Orgogozo JM, Larsen B (1979) Activation of the supplementary motor area during voluntary movement in man suggests it works as a supramotor area. Science 206:847–850.
Pieribone VA, Shupliakov O, Brodin L, Hilfiker-Rothenfluh S, Czernik AJ, Greengard P (1995) Distinct pools of synaptic vesicles in neurotransmitter release. Nature 375:493–497.
Samorajski T, Delaney C, Durham L, Ordy JM, Johnson JA, Dunlap WP (1985) Effect of exercise on longevity, body weight, locomotor performance, and passive-avoidance memory of C57BL/6J mice. Neurobiol Aging 6:17–24.
Schabitz WR, Schwab S, Spranger M, Hacke W (1997) Intraventricular brain-derived neurotrophic factor reduces infarct size after focal cerebral ischemia in rats. J Cereb Blood Flow Metab 17:500–506.
Shen H, Tong L, Balazs R, Cotman CW (2001) Physical activity elicits sustained activation of the cyclic AMP response element-binding protein and mitogen-activated protein kinase in the rat hippocampus. Neuroscience 107:219–229.
Schmued LC, Hopkins KJ (2000) Fluoro-Jade B: a high affinity fluorescent marker for the localization of neuronal degeneration. Brain Res 874:123–130.
Signoretti S, Marmarou A, Tavazzi B, Lazzarino G, Beaumont A, Vagnozzi R (2001) *N*-acetylaspartate reduction as a measure of injury severity and mitochondrial dysfunction following diffuse traumatic brain injury. J Neurotrauma 18:977–991.
Silva AJ, Kogan JH, Frankland PW, Kida S (1998) CREB and memory. Annu Rev Neurosci 21:127–148.
Stummer W, Weber K, Tranmer B, Baethmann A, Kempski O (1994) Reduced mortality and brain damage after locomotor activity in gerbil forebrain ischemia. Stroke 25:1862–1869.
Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD (2002) From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning [review]. Learn Mem 9:224–237.
van Praag H, Christie BR, Sejnowski TJ, Gage FH (1999) Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci USA 96:13427–13431.
Vissing J, Andersen M, Diemer NH (1996) Exercise-induced changes in local cerebral glucose utilization in the rat. J Cereb Blood Flow Metab 16:729–736.
Vitale AE, Sullivan SJ, Jankowski LW, Fleury J, Lefrancois C, Lebouthillier E (1996) Screening of health risk factors prior to exercise or a fitness evaluation of adults with traumatic brain injury: a consensus by rehabilitation professionals. Brain Injury 10:367–375.
Yamashita K, Wiessner C, Lindholm D, Thoenen H, Hossmann KA (1997) Post-occlusion treatment with BDNF reduces infarct size in a model of permanent occlusion of the middle cerebral artery in rat. Metab Brain Dis 12:271–280.
Yoshino A, Hovda DA, Kawamata T, Katayama Y, Becker DP (1991) Dynamic changes in local cerebral glucose utilization following cerebral conclusion in rats: evidence of a hyper- and subsequent hypometabolic state. Brain Res 561:106–119.
Zanier ER, Lee SM, Vespa PM, Giza CC, Hovda DA (2003) Increased hippocampal CA3 vulnerability to low-level kainic acid following lateral fluid percussion injury. J Neurotrauma 20:409–420.

(Accepted 25 January 2004)