Effects of Exercise Following Lateral Fluid Percussion Brain Injury in Rats

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Abstract

Previous studies have suggested that brain-derived neurotrophic factor (BDNF) is involved in memory and learning, and may be neuroprotective following various brain insults. Exercise has been found to increase BDNF mRNA levels in various brain regions, including specific subpopulations of hippocampal neurons. In the present study, we were interested in whether following traumatic brain injury, exercise could increase BDNF mRNA expression, attenuate neuropathology, and improve cognitive and neuromotor performance. We subjected adult male Sprague-Dawley rats to a fluid percussion brain injury, followed by either 18 days of treadmill exercise or handling. Spatial memory was evaluated in a Morris Water Maze (MWM) and motor function was evaluated with a battery of neuromotor tests. Neuropathology was evaluated by measuring the cortical lesion volume and the extent of neuronal loss in the hippocampus. Expression of BDNF mRNA in the hippocampus was assessed with in situ hybridization and densitometry. Hybridization signal for BDNF mRNA was significantly increased bilaterally in the exercise group in hippocampal regions CA1 and CA3 (p < 0.05), but not in the granule cell layer of the dentate gyrus. No significant differences were observed between the groups in neuropathology, spatial memory, or motor performance. This study suggests that after traumatic brain injury, exercise elevates BDNF mRNA in specific regions of the hippocampus.

Keywords: brain-derived neurotrophic factor, cognition, traumatic brain injury

1. Introduction

An important question facing rehabilitation specialists is how to maximize functional recovery after traumatic brain injury (TBI). A variety of functional deficits may be present after TBI, but impairments in information processing, perceptual function, and memory are the most common [27]. Numerous studies have demonstrated that exercise may be important for maintaining cognitive and memory function in humans (for review see [10]). Although the exact physiological mechanisms underlying these improvements are unknown, several molecular events in the brain that are associated with physical activity may be important.

In humans, moderate to high intensity exercise increases regional cerebral blood flow [22,47], as well as plasma levels of noradrenaline and serotonin, neurotransmitters that are thought to be associated with memory storage and retrieval [9]. In animal studies, exercise has been linked to an increase in neuronal activity in the hippocampus, as demonstrated by elevations in extracellular lactate [5]. In aged rats, exercise increases antioxidant enzymes in brain tissue [45]. Exercise prior to an ischemic injury in gerbils, lowered mortality and attenuated damage in the cortex, striatum, and hippocampus [46]. Alterations in neurotrophic factor levels have also been...
associated with exercise. Following 2–7 days of exercise on free-running wheels, brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and basic fibroblast growth factor (bFGF) were increased in various regions of the hippocampus and cortex in rats [15,34,35].

BDNF is the most prevalent neurotrophin in the brain, with especially high levels in the neocortex, cerebellum and hippocampus [19,28]. Although the function of BDNF is incompletely understood, it appears to play a role in long-term potentiation (LTP) and memory formation [8,12,24], and activity-dependent neuroplasticity [13,30,39]. Housing rats in an enriched environment not only improves spatial memory [16,32] and increases synaptic density and branching in the hippocampus [23], it also up-regulates BDNF mRNA [11]. The present study tested whether exercise following a lateral fluid percussion (FP) brain injury could increase BDNF mRNA expression in the hippocampus and attenuate the neuropathology and behavioral deficits that are associated with this model of experimental brain injury in rats [17,43].

2. Materials and methods

2.1. Surgical procedures

Lateral FP brain injury was performed as previously described [31]. Briefly, male Sprague-Dawley rats (360–410 g, n = 20) were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) ten minutes after receiving 0.15 ml of atropine (0.4 mg/ml), and placed in a stereotaxic frame. The scalp and temporal muscles were reflected, and a stainless-steel screw was secured to the skull 1 mm anterior to bregma. A Luer-Lok connector was rigidly fixed with dental cement to a 5 mm diameter craniotomy centered over the left parieto-occipital cortex, midway between bregma and lambda. Sixty minutes after receiving anesthesia, rats were removed from the stereotaxic frame, attached to the FP device via the connector, and given a unilateral brain injury of moderate severity (2.0–2.1 atm). Following the injury, the Luer-Lok connector was removed from the scalp and the skin was sutured. Normothermia was maintained throughout the procedure by placing the animal on a heating pad.

2.2. Treadmill training

Beginning the day after FP injury, the experimental group of rats (n = 10) was given daily incremental exercise on a treadmill during the diurnal part of the light-dark cycle. On the first day, rats were given 5 minutes of exercise, which was increased by 5 minutes per day until they were exercising for 60 minutes. The rate of the treadmill was set at 11.3 meters per minute with a belt inclination of 6°. On days 5–10 (20–45 min of exercise) rats were given one 2 min rest, and on days 11–14 (50–60 min of exercise) they were given two 2 min rests. Animals were not exercised on days that they were undergoing cognitive and neuromotor testing (days 15–18). After the completion of neuromotor testing, animals resumed exercise for 60 min without a rest period (days 18–21). Rats ran a total of 7.8 km over the course of 18 days of exercise.

On the last six days of exercise, the animals reached the maximum period of 60 minutes and were running 0.68 km/day. The control group of rats (n = 9) did not exercise on the treadmill, but were handled 30–60 s daily. All of the animals were housed 2–3/cage with a 12 hr light-dark cycle. Treadmill exercise and handling procedures were given during the light part of the cycle.

2.3. Cognitive and neuromotor function

Spatial learning and memory were evaluated by using a Morris Water Maze (MWM) procedure as described previously [26]. All testing was performed by an observer blinded to each animal’s treatment. The MWM is a 1.15 m diameter circular pool filled with water. All animals were trained to find a hidden platform using external visual cues. Animals were trained on post-surgery days 15–17, performing one block of four acquisition trials per day, for a total of twelve trials. The time required for each animal to find the platform (goal latency) was recorded for each trial. After completing the last acquisition trial, each rat underwent a single probe test to assess their spatial memory. The platform was removed and the swim pattern of each animal was videotaped for 90 seconds. The distance, time, and number of visits to the previous platform location as well as visits to similar regions in the other quadrants were computed by a video motion analyzer (Videomex V, Columbus Instruments). Relative target visits were calculated by dividing visits to the platform location by the sum of the visits to all zones.

The day after the MWM test (day 18), animals underwent a battery of tests for neurologic motor function that were adapted from previously published reports. All testing was performed by two independent observers that were blinded to each animal’s treatment. The motor tests used were the inclined plane test for the right and left side [38], visual limb plac ing [6], vertical righting response [29], and the grip test [1]. The inclined plane test measured the animal’s ability to maintain its body position on an inclined board for 5 seconds. Animals were placed sideways on a board, which is covered with a rubber mat and inclined to a 45° angle. Animals received a score of 2 (able to maintain body position for ≥5 sec), 1 (able to maintain position for 1–4 sec), or 0 (unable to maintain position). Rats were tested for both sides of the body. For visual limb placing, a rat was held 10 cm above a table top and slowly lowered toward it with free hanging forelimbs. Normal rats reach, stretch and place both forepaws on the table top. Animals received a score of 0 (no placing, limb flexion), 1 (incomplete and/or delayed), or 2 (immediate and complete placing). The righting response records the amount of time it takes within a 60 second interval for rats that are placed face down on a vertically oriented wire grid, to assume a head up position. For the grip test, rats were suspended from a wooden dowel (1 cm diameter) that is positioned 40 cm above a foam mat. The length of time the animals held on to the wire within a 30 second interval was measured. Mean scores or latencies for each test were calculated by averaging the values assigned to each rat by the two testers.
2.4. Weight loss

Weight loss or gain was calculated by subtracting the animals weight on day 1 (prior to the FP injury) from the weight on day 18.

2.5. In situ hybridization

Animals were deeply anesthetized with an overdose of sodium pentobarbital and decapitated 3 weeks after the FP injury. Brains from 6 randomly selected rats from each group were rapidly removed and frozen over dry ice. Tissue sections through the hippocampus were cut in the coronal plane at 14 μm in a cryostat, thaw-mounted onto Superfrost Plus (Fisher Scientific) glass slides, and stored at −20 °C until processing for the in situ hybridization localization of BDNF mRNA as previously described [41,42]. The BDNF cRNA probe was prepared by in vitro transcription from a linearized cDNA construct with T3 polymerase in the presence of 35S-UTP. The 540-base rat BDNF probe includes the coding region [14,20]. Hybridization was conducted at 60 °C for 18–24 h with the 35S-labeled cRNA at a concentration of 1x10^6 cpm/50 μl/slide. Following post-hybridization washes and ribonuclease treatment, the sections were air-dried and exposed to β-Max Hyperfilm (Amersham) for 15 days at room temperature for generation of film autoradiograms. After autoradiographic film development, the sections were dipped in NTB2 nuclear track emulsion (Kodak; 1:1 in H2O), air-dried and exposed in light-tight slide boxes at 4 °C for 4–6 weeks. After autoradiographic development of the emulsion, the sections were coverslipped in D.P.X. (Fluka) and analyzed with a Nikon Optiphot-2 microscope equipped with brightfield and darkfield optics. Control sections that had been treated with ribonuclease A before hybridization or processed with an appropriate sense-strand riboprobe (see [14]) were devoid of specific labeling. Film autoradiograms were analyzed with Image 1.57 software (NIH) to compare the density of hybridization for BDNF mRNA in various hippocampal subfields (dentate gyrus, CA1 and CA3) between the exercise and control groups after FP injury. Background hybridization in the corpus callosum was subtracted from the hybridization in the hippocampal subfields to obtain corrected optical density measurements (OD). At least three sections taken from the dorsal hippocampus between bregma –2.80 to –4.30 [37] were analyzed per animal.

2.6. Histological Evaluation

Alternate sections were fixed in formalin for 10 min, taken through xylenes and graded ethanol, stained with hematoxylin and eosin, dehydrated, cleared, and coverslipped with Permount. The areas of the right and left neocortex were measured every 500 μm between bregma –2.56 and –6.04 [37] using an image processor (NIH Image, 1.57). Damaged or necrotic tissue in the neocortex was omitted from the area measurements. Neocortical volumes were calculated by summation of neocortical areas for each animal. The percentage of the tissue that was damaged by the FP injury (% lesion volume) was calculated by dividing the tissue volume on the side ipsilateral to the impact by the volume on the contralateral side and subtracting this number from 100. Previous studies have demonstrated that a moderate lateral FP injury typically damages portions of the posterior parietal, temporal, and occipital cortices [17].

Hippocampal neuropahtology was assessed by scoring neuronal cell loss and injury in the CA3 region by two independent, blinded investigators. The CA3 region was selected because previous studies have demonstrated that after FP injury, hippocampal damage is most visible in this region [4,17]. Neuronal loss in the CA3 region was scored as 0 (normal), 1 (barely visible thinning of the cell layer or a few abnormal appearing cells), 2 (cell loss estimated to be less than 25 %), 3 (cell loss estimated between 25–50 %), and 4 (cell loss estimated to be greater than 50 %).

2.7. Statistical analysis

All measurements were analyzed with a statistical software package (SYSTAT, version 5.2) and expressed as means ± SEM. Percent lesion volume, weight loss, grip test, vertical righting latencies, and MW goal latencies and relative target visits were compared with a t-test. Hippocampal damage and the inclined plane and limb placing scores were compared with the Mann-Whitney U-test. A one-way ANOVA followed by the Newman-Keuls post-hoc test was used to compare right and left neocortical volumes between groups. A two-way ANOVA was used to compare the BDNF mRNA optical density measurements by group and by side. Statistical significance was obtained with p values < 0.05.

3. Results

One animal died shortly after the FP injury, therefore 9 animals were placed in the control (handling) group. In addition, one animal from each group was euthanized 12 days after the FP injury because of severe weight loss and debilitation. In the surviving animals, mean weight loss was not significantly different between the exercise (–2.8 ± 3.6 g) and control animals (–6.9 ± 4.8 g).

Treadmill exercise following FP injury increased the hybridization density of BDNF mRNA in the hippocampus compared to injured animals that were not exercised (Fig. 1). In situ hybridization for BDNF mRNA showed a clear pattern of hybridization in the hippocampal pyramidal cell layers and the granule cell layer of the dentate gyrus, with emulsion grains closely associated with the cell bodies in these layers. No differences were observed between O.D. values for the right and left sides of the hippocampus, so the data were combined. Comparison of group mean corrected O.D. values revealed that significant increases in BDNF mRNA were present in the CA1 (F1 = 7.78, p < 0.02) and CA3 (F1 = 5.11, p < 0.05) pyramidal cell layers in the exercise group (Fig. 2). An small increase was also observed in the granule cell layer of the dentate gyrus, but this did not reach statistical significance.
Histological evaluation revealed that there were no significant differences in the scores for hippocampal damage between the exercise and control groups (Table 1). Nor was the cortical lesion volume significantly different between the exercise and control groups (Table 1). However, there was a trend toward attenuation of damage in the exercise group, in that significant asymmetries between the left (injured) and right neocortical volumes were observed in the control group, but not in the exercise group ($F_{3} = 5.43, p < 0.01$, Table 1).

Analysis of MWM performance (Table 1) revealed that there were no significant differences in goal latencies or relative target visits between the exercise and control injured animals, although both were significantly impaired compared to uninjured (sham) animals (unpublished data). Neuro motor scores and timed tests after FP injury were also unaffected by exercise (Table 1).

**Effects of Exercise on BDNF Expression in the Hippocampus After FP Injury**

![Graphs showing BDNF expression in CA1, CA3, and Dentate Gyrus](image)

Fig. 2: Corrected optical density (O.D.) ± SEM measurements of BDNF hybridization in various regions of the hippocampus 21 days following a FP injury. Statistically significant increases were observed in the CA1 and CA3 regions in animals that received exercise after injury (FPI-exercise) compared to those that were not exercised (FPI-control).
TABLE I. Summary of histological and behavioral data

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Treadmill</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Hippocampal cell loss</td>
<td>1.4 ± 0.5</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>Cortex lesion volume</td>
<td>29.6 ± 7.8</td>
<td>17.4 ± 4.5</td>
</tr>
<tr>
<td>Neocortical volume</td>
<td>45.7 ± 5.8*</td>
<td>54.0 ± 2.2</td>
</tr>
<tr>
<td>Right neocortical</td>
<td>64.1 ± 2.7</td>
<td>63.4 ± 3.2</td>
</tr>
<tr>
<td>Goal latency</td>
<td>22.2 ± 5.5</td>
<td>21.6 ± 7.3</td>
</tr>
<tr>
<td>Relatively target visit</td>
<td>30.2 ± 3.0</td>
<td>34.8 ± 8.1</td>
</tr>
<tr>
<td>Inclined plane – left</td>
<td>1.3 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Inclined plane – right</td>
<td>1.9 ± 0.1</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Limb placing</td>
<td>1.3 ± 0.3</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Righting response</td>
<td>13.4 ± 3.1</td>
<td>11.6 ± 4.4</td>
</tr>
<tr>
<td>Grip test</td>
<td>7.0 ± 2.4</td>
<td>7.0 ± 2.3</td>
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*The volume of the left neocortex (side ipsilateral to the injury) was significantly less ($p < 0.01$) than the volume of the right neocortex in the control group, but not in the treadmill group.

1. Discussion

To our knowledge, this paper is the first investigation of the effects of an exercise program on neural plasticity, neuropathology, and behavior following experimental brain trauma. Our results demonstrate that exercise on a treadmill following FP injury significantly increases hippocampal BDNF mRNA levels compared to injured, unexercised animals, but does not attenuate histological, cognitive, or neuromotor deficits. The increase in BDNF mRNA following exercise is in agreement with a previous study conducted on normal rats [34,35]. Traumatic brain injury by itself can lead to up-regulation of BDNF mRNA in the hippocampus [18,51]. However, we believe that the increases observed in the present study are separate from those associated with FP injury because post-injury elevations in BDNF mRNA are acute, not chronic, and are found in different subregions of the hippocampus [18]. These spatial and temporal differences suggest that FP injury and exercise may involve separate pathways for the up-regulation of BDNF.

Under normal conditions, BDNF appears to be an important factor for neural plasticity and LTP [8,12,24,30,39]. BDNF has also been linked to neuroprotection following injury [2,40,48]. In the present study, the increases in BDNF mRNA were not associated with a significant attenuation of the neuropathology, however there was a trend toward improvement in the injured cortex (Table 1). This trend appears to be related to events in the damaged tissue, rather than to non-specific increases in cortical volume, because the contralateral cortex was not affected by the exercise. Also, it is important to note that the exercise did not worsen the cortical lesion, as has been reported after a forced-use paradigm following cortical ablation [25].

There are several possible explanations for why the treadmill exercise did not enhance recovery of cognitive or neuromotor function after FP injury. The role of exercise on cognitive performance is controversial, with some studies reporting positive correlations, and others negative (see [10] for review). It has been suggested that the duration and intensity of the exercise are important factors, and that low levels of physical activity are ineffective in improving cognitive performance. The graduated exercise program in the present study may not have been intense or long enough to produce effects.

Another possible explanation is that forced, diurnal treadmill training may have induced a stress response [50]. Exposure to stress is associated with impaired spatial memory and neuronal damage in the hippocampus [49]. Stress also significantly decreases BDNF mRNA levels in the hippocampus [44,49]. Thus, while exercise is generally associated with attenuation of the stress response [7], the conditions in which we exercised our rats may have actually contributed to it and interfered with beneficial effects of exercise on memory and learning. A stress response may also have attenuated the increase in BDNF mRNA that we observed in the hippocampus.

It has also been suggested that activities that require motor learning are better than repetitive exercise at enhancing cognitive and neuromotor performance [3]. Exposure to an enriched environment provides animals with an opportunity for motor learning as they explore novel objects placed in their cages. Animals placed in an enriched environment after a midline FP injury had improved spatial memory [16]. After an ischemic injury, rats placed in an enriched social environment or just a social environment (no access to exercise equipment) outperformed isolated rats with access to a free-running wheel on neuromotor tasks [21].

In conclusion, this study demonstrates that even animals that have undergone experimental brain trauma are able to increase neurotrophin levels in the brain in response to exercise. Despite these molecular events, the treadmill exercise program used to rehabilitate the rats after FP injury was not associated with an improvement in cognitive or neuromotor function. Whether the lack of behavioral effects are attributable to specific features of the exercise paradigm employed in this study or to exercise in general should be investigated in future studies.

Acknowledgments

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References


[42] Seroogy, K.B., and Herman, J. (1997). In situ hybridization approaches to the study of the nervous system, in Neurochemistry: A


