Behavioural Brain Research 203 (2009) 165–172

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Effects of voluntary running on spatial memory and mature brain-derived neurotrophic factor expression in mice hippocampus after status epilepticus

César Renato Sartori[a,*,1], Fernanda Campos Pelágio[a,1], Simone Aparecida Teixeira[b,2], Verónica Sandra Valentinuzzi[a,1], André Luiz Nascimento[a,1], Fábio Rogério[a,1], Marcelo Nicolás Muscará[b,2], Elenice Aparecida de Moraes Ferrari[a,1], Francesco Langone[a,3]

[a] Department of Physiology & Biophysics, Institute of Biology, State University of Campinas (UNICAMP), P.O. Box 6109, 13083-970, Campinas, SP, Brazil
[b] Department of Pharmacology, Institute of Biomedical Sciences 1 (ICB 1), University of São Paulo (USP), 05508-900, São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 16 December 2008
Received in revised form 2 April 2009
Accepted 5 April 2009
Available online 3 May 2009

Keywords:
Physical activity
Exercise
Spatial memory
Water maze
Mice
Epilepsy
BDNF
Pilocarpine

ABSTRACT

Voluntary physical activity improves memory and learning ability in rodents, whereas status epilepticus has been associated with memory impairment. Physical activity and seizures have been associated with enhanced hippocampal expression of BDNF, indicating that this protein may have a dual role in epilepsy. The influence of voluntary physical activity on memory and BDNF expression has been poorly studied in experimental models of epilepsy. In this paper, we have investigated the effect of voluntary physical activity on memory and BDNF expression in mice with pilocarpine-induced epilepsy. Male Swiss mice were assigned to four experimental groups: pilocarpine sedentary (PS), pilocarpine runners (PRs), saline sedentary (SS) and saline runners (SRs). Two days after pilocarpine-induced status epilepticus, the affected mice (PR) and their running controls (SR) were housed with access to a running wheel for 28 days. After that, the spatial memory and the expression of the precursor and mature forms of hippocampal BDNF were assessed. PR mice performed better than PS mice in the water maze test. In addition, PR mice had a higher amount of mature BDNF (14 kDa) relative to the total BDNF (14 kDa + 28 kDa + 32 kDa forms) content when compared with PS mice. These results show that voluntary physical activity improved the spatial memory and increased the hippocampal content of mature BDNF of mice with pilocarpine-induced status epilepticus.

1. Introduction

Clinical and experimental data demonstrate that status epilepticus (SE) induced by pilocarpine leads to brain damage [34,51]. The hippocampal formation is especially vulnerable to injury following SE, as indicated by loss of CA1, CA3, hilar cells [13,14]. Such loss has been associated with memory impairment in human mesial temporal sclerosis and in animal models of epilepsy induced by pharmacological agents such as pilocarpine and kainic acid [2,9,13,14,25,33,36]. However, other brain areas important for cognitive functions, like the septal area, the olfactory tubercle, the amygdala, piriform cortex, neocortex and several thalamic nuclei may also be damaged as well [13].

The beneficial effects of regular physical activity have been observed in humans, where it leads to a reduction in the rate of epileptic seizures and the depressive symptoms usually experienced by these patients [42,49]. Various studies in rodents have also reported beneficial effects of physical activity on plastic processes related to hippocampal-dependent memory. These include processes such as an increase in neurotrophic factor expression, the strengthening of synaptic long-term potentiation, and the enhancement of hippocampal neurogenesis [4,12,17,24,26,38,43,59,62–65,70]. In rats with SE induced by pilocarpine or kainic acid, such activity, whether voluntary or forced, reduces the rate of seizures and hippocampal cell loss, as well as enhancing performance in the water maze task [5,20,70]. However, few studies have investigated the mechanisms mediating this beneficial influence of physical activity on epilepsy [70].

For rats performing voluntary physical activity on running wheels, there was a positive correlation between the up-regulation of BDNF expression in the hippocampus and an improved performance in the water maze task [1,65]. Such an increase in the

* Corresponding author. Tel.: +55 19 35216207; fax: +55 19 35216185.
E-mail addresses: cesarsartori@yahoo.com.br (C.R. Sartori), ferpelagio@yahoo.com (F.C. Pelágio), mone@usp.br (S.A. Teixeira), veroval@hotmail.com (V.S. Valentinuzzi), andreluiz.nexp@epm.br (A.L. Nascimento), fabio@fcm.unicamp.br (F. Rogério), muscara@usp.br (M.N. Muscará), elenice@unicamp.br (E.A.d.M. Ferrari).
[1] Tel.: +55 19 35216207.
[2] Tel.: +55 11 30917741.
[3] Francesco Langone deceased on May 1st, 2009.

0166-4328/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2009.04.022

expression of BDNF and its receptors in the hippocampus have also been reported for rats suffering from epileptic seizures [7,50,56], although there is some controversy as to whether this increase in BDNF has beneficial or deleterious effects on the epileptic state [7,29,54,56].

Voluntary physical activity is naturally triggered in rodents that have free access to a running wheel [55]. This behavior thus provides an excellent opportunity for analyses of the effect of physical activity without the stress associated with forced physical activity [17]. However, no studies have reported the effects of voluntary physical activity on epilepsy in animal models. We have thus investigated the influence of such activity on BDNF protein expression and neuronal loss in the hippocampus, as well as spatial memory, a typical hippocampal-dependent mnemonic task [57,48,61], for mice with pilocarpine-induced SE.

2. Materials and methods

2.1. Animals and experimental groups

Male Swiss mice (35–40 g, 10–12 weeks old) obtained from the Multidisciplinary Center for Biological Investigation (CEMIB) at State University of Campinas (UNICAMP) were housed at 21 °C in a 12 h light/dark cycle (lights on at 7 a.m.), with access to food and water *ad libitum*. The mice were randomly allocated to one of four groups ($n = 8$ per group): pilocarpine sedentary (PS), pilocarpine runner (PR), saline sedentary (SS) and saline runner (SR). The body weight of all mice was registered before the SE induction or saline injection, and before the sacrifice. All mice were handled identically throughout the experiments. The experimental procedures were approved by the institutional Committee for Ethics in Animal Experimentation at UNICAMP (CEEA/IB-UNICAMP, 1136-1).

2.2. Pilocarpine-induced status epilepticus

Status epilepticus was induced in PS and PR mice. Seventy-two mice were initially treated with methylscopalamine (1 mg/kg; s.c.) in order to limit peripheral cholinergic effects. This was followed 30 min later by a single injection of pilocarpine (340 mg/kg; i.p.). SE describes an enduring epileptic state during which seizures are unremitting and are characterized by generalized tonic–clonic seizures [13,14]. The mice with clearly established SE were observed for 6 h, and only these mice were randomly assigned to the PS and PR groups. The remaining mice either died or did not in some way meet the criteria to SE [13,14]. The control mice (SS and SR) were injected with a saline solution (0.9% NaCl). The pilocarpine-induced SE mice were observed two times a day during maintenance of the cages (1–2 h), and 2 h immediately before and after the behavioral test to detect the occurrence of spontaneous seizures.

2.3. Housing and physical activity

After the induction of SE or saline administration, the mice were housed individually in standard cages. The sedentary mice (PS and SS groups) were maintained in these cages, while the runner mice (PR and SR groups) were housed individually in cages where they had free access to a stainless steel running wheel (12 cm diameter; 60 g) for 28 days. The PR mice were transferred to these cages 48 h after the induction of SE. The activity on the running wheel was recorded daily for 24 h by use of an electronic counter connected to a computer for data storage [62]. The distance run by each mouse was reported in km per day and was considered to correspond to the amount of physical activity.

2.4. Water maze test

Water maze tests to assess spatial memory were conduct during the five days immediately following the 28 days of physical activity. On the days of testing, the mice were transferred from the animal facility to the room beside the test room, where they were observed for 2 h. For these tests, the mice were allowed to swim freely in a circular pool (120 cm in diameter, 50 cm high) filled with water (26 ± 1 °C) that was made opaque with nontoxic, white paint, and a movable, transparent circular plastic platform 9 cm in diameter, mounted on a plastic column, was placed inside the pool approximately 1 cm below the water surface. The pool was located in a room (3 m × 3 m) with several geometric figures hanging from each of the walls. These figures served as external spatial cues for the mice. The sessions were recorded with a video camera positioned 2 m above the center of the pool and connected to a recording system located in an adjacent room. The platform was fixed in the northwest quadrant, and the animal was placed in the water from a different starting point for each of the trials. Four training sessions of four trials each (with 10 min intervals between trials) were carried out on four consecutive days. Each trial lasted for a maximum of 2 min, or until the hidden platform was found by the animal. The latency or time required to find the platform was recorded for each trial. On the fifth day of testing, a 1 min probe test was performed to evaluate spatial memory retention. The platform was removed from the pool and the animal was then placed in the quadrant opposite that from which the platform had been removed and allowed to swim freely. For the probe test, a circular area (three times the platform diameter) surrounding the former location of the platform was delimited and used as the counting zone for establishing successful memory retention. The number of times that an animal swam into the counting zone (frequency of entrance) was recorded during the test. Moreover, the swim speed and path-length were recorded. All behavioral data were analyzed using Ethovision XT software (Noldus, The Netherlands).

2.5. BDNF expression assessment by Western blotting

After the behavioral tests, four mice were randomly selected from each group and decapitated. The brain was quickly removed and the hippocampus was dissected, frozen in liquid nitrogen, and stored at −80 °C until use for Western blotting. Aliquots from hippocampal homogenates (60 μg of protein/lane) were separated by SDS-PAGE on 15% polyacrylamide gels [30] and transferred electrophoretically to a nitrocellulose membrane (0.22 μm; 40 V, 30 min). After blocking non-specific binding sites with 0.2% casein, the membranes were incubated overnight at 18 °C with primary anti-BDNF antibody (rabbit, polyclonal, 1:1000; [N-20] sc-546, Santa Cruz Biotechnology, USA) that detects precursor and mature forms of BDNF. The membranes were washed with Tris-buffered saline containing 0.1% Tween 20 and then incubated with the secondary antibody (alkaline phosphatase-conjugated goat anti-rabbit). The blots were stripped with glycine–HCl (pH 2.4) and probed again with anti-α-tubulin antibody (mouse, monoclonal, 1:1000; sc-5286, Santa Cruz Biotechnology). A chemiluminescent assay (Lumi Phos WB; Pierce, USA) was used to identify the immunoreactive bands, and intensities of the bands were estimated by densitometry. The density of the α-tubulin bands was used as an internal control for the correction of unequal protein loading. The results were expressed as the ratio of the optical densities of each BDNF form to that of α-tubulin. For total BDNF, the results were expressed as the ratio of the optical densities of total BDNF (14 kDa + 28 kDa + 32 kDa forms) to α-tubulin. The ratio of the optical density of mature BDNF (14 kDa) to that of the total BDNF (14 kDa + 28 kDa + 32 kDa) was also determined.

2.6. Histological analysis

Twenty-four hours after the behavioral tests, four other mice from each group were anesthetized with 3% sodium pentobarbital (60 mg/kg; i.p.) and perfused transcardially with 100 ml of heparinized 0.9% NaCl (1000 UI heparin/500 ml of 0.9% NaCl) followed by 120 ml of 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.40). The brain was removed from the skull 24 h after the end of the perfusion and was post-fixed in the same paraformaldehyde fixative solution for 12 h before embedding in paraffin. Coronal sections (8 μm thick) extending from 1.0 to 3.0 mm posterior to the bregma [21] were obtained and stained with cresyl violet.

Neuronal loss was quantified in the CA1 and CA3 hippocampal areas, as well as in the hilus of the dentate gyrus of the dorsal hippocampal formation. Only large neurons with noticeable nucleoli were counted [10]. The number of neurons in the CA1 and CA3 was counted in three sections in the range of 1.6–2.4 mm posterior to the bregma for each mouse [21]. Counting was done using a magnification of 400× and a counting grid (0.2 mm square divided into four hundred 0.01 mm squares) superimposed over three sectors of the CA1 (medial, middle and lateral, 10,000 μm^2 each) and one of the CA3 (15,000 μm^2), a modification of a previously described procedure [15,23]. The counts for the left and right hemispheres were combined and expressed as the number of neurons per 10,000 μm^2. The number of hilar neurons in the hippocampus was counted in 5–7 sections in the range of 2.1–2.4 mm posterior to the bregma for each mouse [10,21]. Counting was done in the whole hilar region in both hemispheres, using a magnification of 400×. The counts were expressed as the number of neurons per section.

2.7. Statistical analysis

A two-way ANOVA with repeated measures followed by a Newman–Keuls multiple comparisons test was used to analyze the data of latency in reaching the platform, path-length during training sessions and the swim speed in the water maze test, as well as the amount of physical activity. A one-way ANOVA followed by a Bonferroni test was used to analyze the frequency of entrance into the counting zone during the probe test in the water maze, to analyze BDNF Western blots data, and the neuronal counts in the hippocampus.

The results were expressed as mean ± S.E.M. and the analyses were made using Statistical Analysis Software (SAS), version 6.1, and the level of significance was set at $p < 0.05$.

3. Results

3.1. Behavioral effects of pilocarpine

Behavioral changes were observed within 5–10 min after the administration of pilocarpine and were similar in all pilocarpine-

induced SE mice. Akinesia was followed by wet dog shakes with facial automatisms, salivation, and masticatory jaw movements. These manifestations progressively developed into whole-body tremor, forelimb and hindlimb clonus, short-lived motor seizures, rearing and falling until the mice developed the generalized, long-term clonus characteristic of SE. The onset of SE in the PR and PS groups occurred 39.5 ± 4.0 min after the administration of the pilocarpine, and the symptoms continued for at least 6 h. No spontaneous recurrent seizures were observed in runner and sedentary pilocarpine-induced SE mice during the daily maintenance of the cages.

3.2. Amount of physical activity and body weight

The PR mice and SR mice ran an average distance of 1.54 ± 0.09 km/day and 5.37 ± 0.22 km (mean ± S.E.M.), respectively ($F_{1,14} = 13.75$; $p < 0.01$). The voluntary running activity of PR and SR mice took place mainly at night (7 p.m.–7 a.m.). PR mice had a peak of physical activity at the third day (2.90 ± 0.97 km/day) and after that showed a decrease to steady values around 1.50 km/day. In contrast, SR mice showed a gradual increase in physical activity beginning with 3.28 ± 0.57 km/day and reached steady values around 6 km/day after the 13th day. These variations were confirmed as an effect of time ($F_{1,27} = 2.0$; $p < 0.01$) and significant interaction between group and time ($F_{1,27} = 5.15$; $p < 0.001$).

At the beginning of the experiments, the body weights of all mice were similar (SS = 38.31 ± 0.68 g; SR = 37.05 ± 0.76 g; PS = 38.81 ± 1.26 g; PR = 37.89 ± 0.90 g). At the time of sacrifice the control groups exhibit an equivalent increase of body weight when compared with that registered at the beginning of the experiments (SS = 42.16 ± 0.63 g, $p < 0.01$; SR = 41.28 ± 0.78 g; $p < 0.001$) mice. However, PS showed a significant body weight reduction (34.68 ± 0.92 g; $p < 0.001$). On the other hand, no significant changes were observed in PR mice (37.40 ± 2.4 g; $p = 0.79$).

3.3. Spatial memory

No spontaneous recurrent seizures were observed in runner and sedentary pilocarpine-induced SE mice during the water maze test, as well as 2 h before and after the test sessions.

Mice with pilocarpine-induced SE showed no evident disability in swimming during the water maze test. Furthermore, the swim speed of these mice was similar to that registered in saline-treated control mice (SR: 24.97 ± 1.74 cm/s; SS: 18.58 ± 2.34 cm/s; PR: 20.03 ± 2.5 and PS: 20.45 ± 1.83 cm/s), and no statistically significant differences were observed among groups ($F_{3,28} = 2.66$; $p > 0.05$) as well as among sessions ($F_{3,28} = 2.04$; $p > 0.05$). PR mice, however, performed better than PS mice in the spatial memory task, as shown by their lower latency in finding the platform during the training sessions, as well as the decreases in the latency in finding the platform across the four successive sessions. When compared to these PR and PS mice, SS and SR mice had lower latencies and a more pronounced decrease in latency during the fourth session in comparison with that of the first session (PR: first = 108.3 ± 5.8 s, fourth = 77.5 ± 13.2 s; PS: first = 117 ± 3 s, fourth = 117.4 ± 2.6 s; SS: first = 76.3 ± 4.7 s, fourth = 35.8 ± 5.1 s; SR: first = 97.4 ± 9.6 s, fourth = 24.6 ± 6.6 s) and in the path-length to the platform, which are indicative that those animals learned to localize and reach the platform. Statistical analysis with ANOVA confirmed that the latency values in finding the platform had a significant effect of group ($F_{3,28} = 22.76$; $p < 0.0001$), of session ($F_{3,28} = 20.12$; $p < 0.0001$) and a significant interaction between group and session ($F_{3,9} = 3.56$; $p < 0.001$). Multiple *post hoc* comparisons showed that PR mice had latency values significantly different from PS, SR and SS mice ($p < 0.05$) and that latency values in the first session were significantly different from the second, third, and fourth sessions. Comparisons between the first and fourth sessions showed that SR, SS and PR mice had significant decreases in latency values ($p < 0.05$), while PS mice had not ($p > 0.05$). The mice with the best performance also showed a greater frequency of entrance into the counting zone during the probe test. PR mice entered this zone more frequently than did PS mice (PR: 3.9 ± 1.3; PS: 1.5 ± 0.7). A significant effect of group was confirmed with the test one-way ANOVA ($F_{3,28} = 6.88$; $p < 0.001$), which was related with the differences observed between PS mice and both SR and SS mice, as well as between PR mice and SR mice ($p < 0.05$).

The path-length of PR mice decreased from the first to the fourth session, in contrast to behavior of PS mice, which showed no differences in the path-length (PR: first = 2303 ± 110.5 cm, fourth = 1381 ± 359.6 cm; PS: first = 2088 ± 138.1 cm, fourth = 1966 ± 298.6 cm). SS and SR mice also showed a decrease in path-length during the sessions (SR: first = 2462 ± 205.3 cm, fourth = 668.4 ± 214.7 cm; SS: first = 1592 ± 117.3 cm, fourth = 533.2 ± 81.07 cm). Statistical comparisons of the path-length values, conducted with ANOVA, indicated a significant effect of group ($F_{3,28} = 6.87$; $p < 0.001$), of session ($F_{3,28} = 26.52$; $p < 0.0001$) and a significant interaction between group and session ($F_{3,9} = 3.49$; $p < 0.01$). Multiple *post hoc* comparisons showed that the path-length values of SS mice were significantly different from those of SR, PS and PR mice ($p < 0.05$), which can be related to the across session decreases in path-length and particularly to the significant differences between the first and fourth sessions ($p < 0.05$) that were not observed only for PS ($p < 0.05$) (Fig. 1).

3.4. BDNF protein expression

Western blot analysis revealed differences in the ratio of mature BDNF (mBDNF; 14 kDa) to total BDNF (14 kDa + 28 kDa + 32 kDa) among the groups ($F_{3,12} = 103.68$; $p < 0.001$). The *post hoc* analysis indicated that SR and PR mice expressed a statistically significant larger ratio of mature BDNF (14 kDa) to total BDNF (14 kDa + 28 kDa + 32 kDa) than did the respective sedentary groups, SS and PS ($p < 0.05$). Sedentary and runner pilocarpine-induced SE mice expressed a larger ratio of this mBDNF than did the respective saline-treated control groups ($p < 0.05$) (Fig. 2A and B).

3.5. Neuronal loss

Histological analysis revealed neuronal loss in the hippocampal area CA3 and hilus of the dentate gyrus of mice with pilocarpine-induced SE (SR and PR). This observation was confirmed by statistical analyses as significant effect of group for cell counts in CA3 ($F_{3,12} = 10.75$; $p < 0.01$) and hilus ($F_{3,12} = 30.02$; $p < 0.001$), but not in CA1 ($F_{3,12} = 2.76$; $p = 0.09$). *Post hoc* analyses indicated that PR mice had more cells in CA3 as compared to PS mice ($p < 0.05$), but did not differ from SS and SR mice ($p > 0.05$). However, PR mice showed a lower number of cells in the hilus than SS and SR mice ($p < 0.05$). PS mice exhibited a marked reduction in the number of cells in CA3 and hilus when compared with SS and SR mice ($p < 0.05$). There were no significant differences between PR and PS mice in the number of cells in CA1 and the hilus (Figs. 3 and 4).

4. Discussion

In the present study, we observed a positive effect of voluntary physical activity on the spatial memory of mice with pilocarpine-induced SE, which is consistent with the fact that for healthy rodents voluntary physical activity on a running wheel is the most important aspect of an enriched environment for promoting positive effects on spatial memory [26,63]. Our data are in agreement with previous reports that rats with lithium–pilocarpine-induced

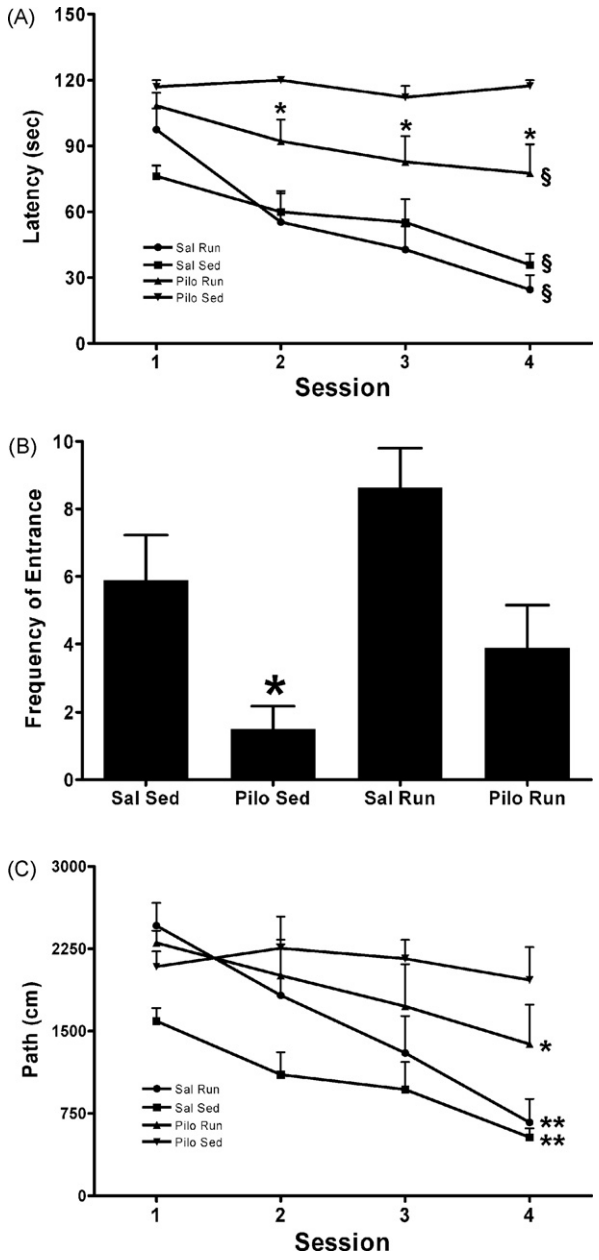

Fig. 1. Effect of voluntary physical activity on spatial memory. (A) PR, SS and SR mice showed a progressive decrease in the latency for finding the platform in the water maze test from the first to the fourth session ($^{\S}p < 0.05$). During the second, third and fourth sessions, the latency for PR mice was significantly lower than that of PS mice and higher than that of SS and SR mice ($^{*}p < 0.05$). (B) In the probe test, PS mice showed a lower frequency of entrance into the counting zone than did PR, SS and SR mice ($^{*}p < 0.05$). This frequency was similar to that observed for SS mice ($p > 0.05$). (C) The path-length of the PR, SS and SR mice decreased from the first to the fourth session ($^{*}p < 0.05$; $^{**}p < 0.01$), in contrast to that of the PS mice, which showed no decrease in path-length ($p > 0.05$). Values correspond to mean ± S.E.M.; $n = 8$ for each group.

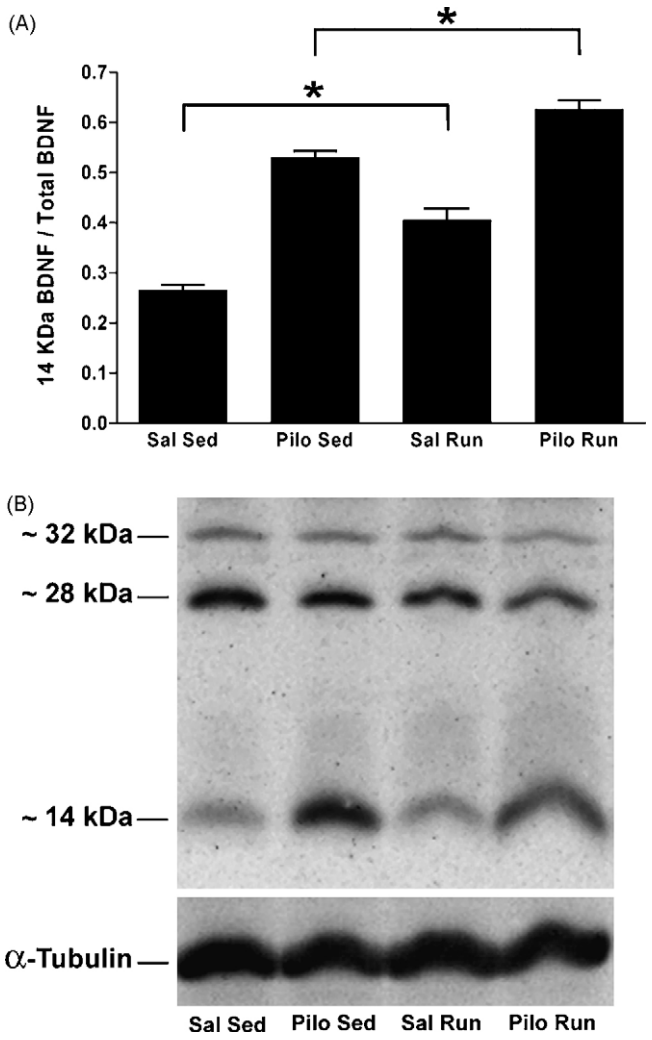

Fig. 2. BDNF expression in the hippocampus. (A) Ratio between mature BDNF (mBDNF; 14 kDa) and total BDNF (14 kDa + 28 kDa + 32 kDa). SR and PR mice showed a significant increase in mature BDNF in contrast to the SS and PS mice, respectively ($^{*}p < 0.05$). (B) Typical Western blot for BDNF precursors (28 and 32 kDa) and mature BDNF (14 kDa). Columns correspond to mean ± S.E.M.; $n = 4$ for each group.

SE that had access to a running wheel in an enriched environment showed improved performance in the water maze task [20].

Recent evidence has linked physical activity to an increase in neurotrophic factor expression, as well as transcription of early response genes and neurogenesis in the hippocampal formation [3,4,11,12,17,24,43,59,63,65]. Specifically, rats undertaking voluntary physical activity on running wheels showed enhanced expression of BDNF in the hippocampus and better performance in the water maze test than did sedentary animals [1,43,65]. However, the expression of pro and mature BDNF forms was not evaluated in these studies. We have found that PR mice showed a higher ratio of mature BDNF (14 kDa) to total BDNF than did PS mice, which suggests that such an increase underlies the better performance of PR mice in the water maze test.

In the mammalian brain, BDNF occurs either as a precursor protein (proBDNF, 32 and 28 kDa) or as mature BDNF (14 kDa), with the latter being derived from the precursors by proteolytic cleavage [8,41]. After synthesis in the endoplasmic reticulum, proBDNF is transferred to the Golgi apparatus, where it is submitted to (1) intracellular cleavage followed by secretion, (2) secretion followed by extracellular cleavage or (3) secretion without subsequent cleavage [35]. The mechanisms that determine these pathways for BDNF are currently unknown, but they can be modified by physiological and pathological conditions [35].

It has, for example, been observed that the seizure activity induced by kainic acid or pilocarpine generates a coordinated increase in messenger RNA for BDNF and the prohormone convertase PC1 in the rodent hippocampus [37,39].

Various studies have suggested that the precursor and mature forms of neurotrophins interact with different receptor/signaling systems, resulting in the induction of opposing biological effects [31,35]. mBDNF is considered to be more effective than proBDNF in activating the TrkB signaling pathways associated with neuronal

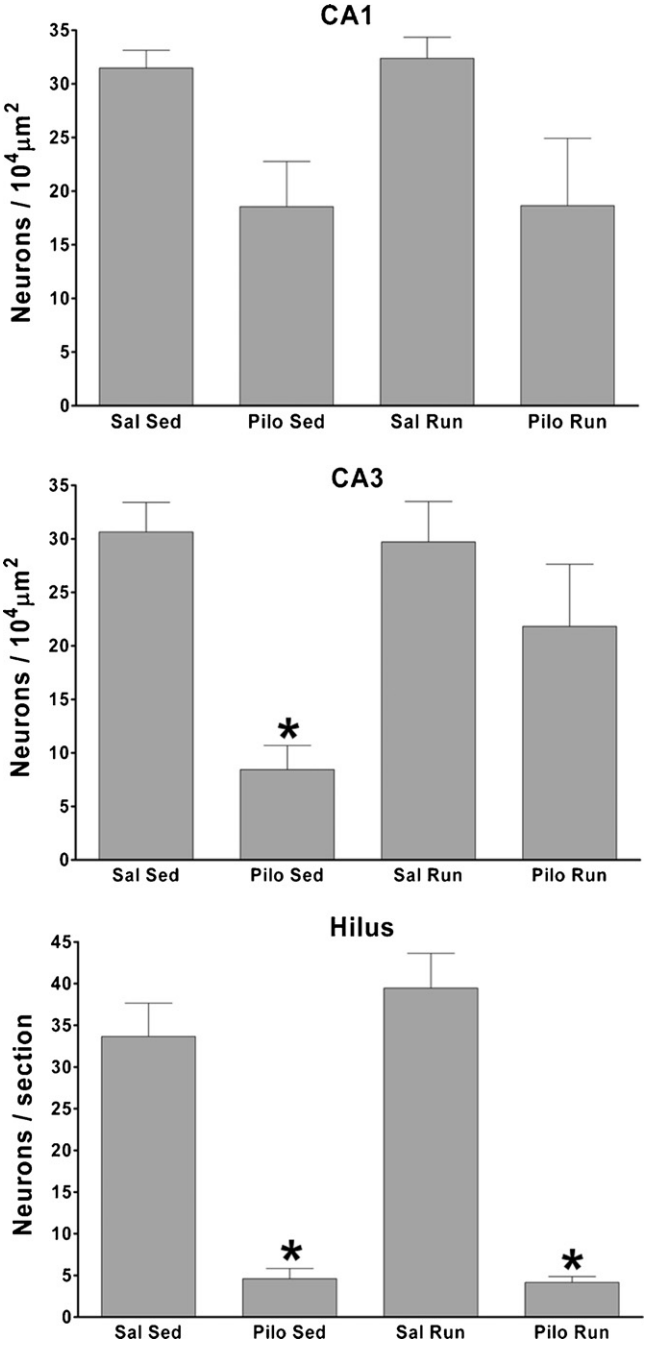

Fig. 3. Number of healthy neurons in the hippocampal areas CA1 and CA3, and in the hilus of dentate gyrus. PS mice had less healthy neurons in the CA3 than did PR, SS and SR mice (*$p < 0.05$). PS and PR mice had fewer healthy neurons in the hilus than did SS and SR mice (*$p < 0.05$). Columns correspond to mean ± S.E.M.; $n = 5$ for each group.

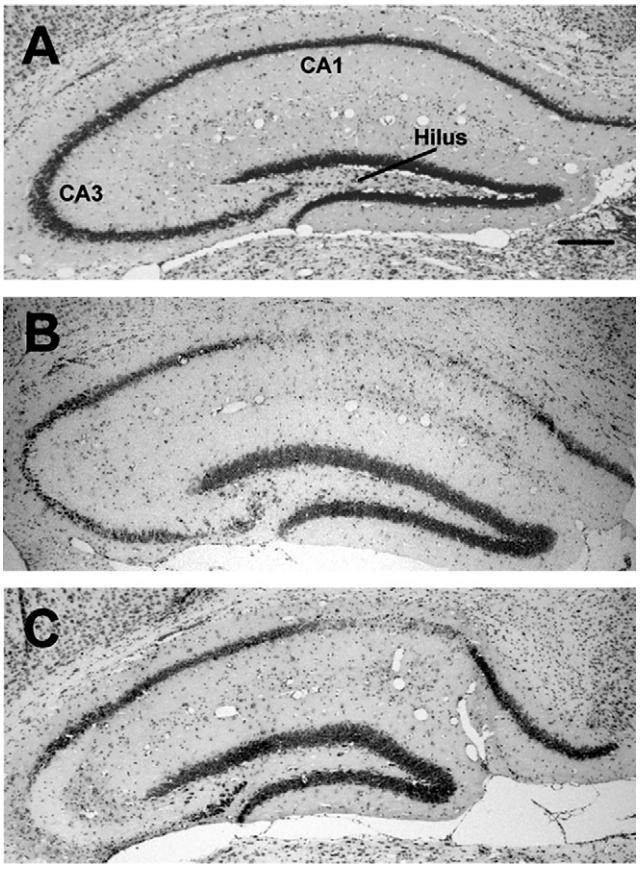

Fig. 4. Typical images of histological lesions in mice with pilocarpine-induced status epilepticus. (A) Coronal section from SS mice showing intact hippocampal formation. (B) Coronal section from PR mice showing neuronal loss in the hilus and CA1 but little loss in the CA3. (C) Coronal section from PS mice showing marked neuronal loss in the CA1, CA3 and the hilus. Scale bar = 50 μm.

survival, synaptic plasticity, and memory [8,35,58]. On the other hand, proBDNF binds to p75 with high affinity and activates signaling pathways in neuronal apoptosis [31,58]. Seizures increase the expression of BDNF and its TrkB and p75 receptors [7,50,56], but the relevance of these increases in epilepsy remains unclear [7,29,54,56].

No spontaneous recurrent seizures were observed in runner or sedentary pilocarpine-induced SE mice during the daily maintenance of the cages, during the water maze test, as well as 2 h before and after the test sessions. These observations do not exclude the possibility that seizures occurred during other periods. However, it was demonstrated that rats with epilepsy induced by pilocarpine exhibited reduction in seizure frequency and in epilepsy-induced hippocampal electrophysiological abnormalities when subjected to physical exercise in the treadmill as compared to rats that had not been trained [5,6]. These data support the possibility that, in our experiments, the frequency of seizures during the non-monitored period was the same or lower in pilocarpine-induced SE runner mice when compared to sedentary mice, leading to the notion that the higher expression of mBDNF in PR mice when compared with PS mice was not promoted by seizure-driven increased brain activity, but was related primarily to physical exercise.

The higher ratio of mBDNF to total BDNF seen in PR and SR mice, in comparison with PS and in SS mice, respectively, suggests that the proteolytic pathways responsible for the conversion of proBDNF to the mature form is stimulated by physical activity. In support of this hypothesis, it can be seen that voluntary physical activity on a running wheel increased the expression of mRNA for the TrkB receptor and BDNF in the rodent hippocampus, but not for the p75 receptor [65,67]. Moreover, BDNF and TrkB receptor expression is downregulated in the hippocampus of rats deprived of habitual running [67]. Vaynman et al. [65] have observed that the positive effect of exercise on learning and memory in rats was prevented by blocking the binding of BDNF to the TrkB receptor in the hippocampus. Other studies also support a central role for the BDNF/TrkB signaling system in the neuroplasticity events underlying hippocampal-dependent learning and memory [45,61,68]. Pang et al. [45] report that the conversion of proBDNF to mBDNF was fundamental for the late phase of long-term potentiation, a phe-

nomenon characterized by morphological and functional synaptic alterations related to long-term memory processes. Together, these findings are in agreement with our results regarding the better performance of PR mice in the water maze test than that of the PS mice.

Our results in the water maze test have revealed a tendency to lower latency values for runner controls when compared to sedentary controls. Although these differences are not significant, the results are in agreement with the observation that SR mice exhibit a greater expression of mBDNF than to SS mice. The absence of significant differences in the latency values for SR and SS mice may be attributed to the water maze protocol used. Van Praag et al. [62] describe similar results in a water maze protocol consisting of four trials per session, as done here. However, when these authors [62] used a protocol consisting of two trials per session, the exercising mice performed better than the sedentary mice. The two-trial protocol is considered to be more challenging and more sensitive than the four-trial protocol for detecting differences in learning between sedentary and exercising animals [62,65].

Previous studies have shown that susceptibility to seizure-induced cell damage depends on the animal strain used [52,53]. Outbred Swiss mice are widely used in the well-characterized model of pilocarpine-induced epilepsy and the histological alterations seen here were similar to those described by others [14,60]. Neuronal loss was noticeable in the hippocampal formation and was most severe in the CA1, CA3 and the hilus. Although we did not employ a stereological method for cell counting, the procedure we used was efficient to detect a higher number of neurons in the CA3 of PR mice in comparison to PS mice. In contrast, there were no differences in neuronal loss in the CA1 and the hilus for PR and PS mice. The CA3 has already been linked to an increase in the expression of BDNF mRNA after exercise on a running wheel, although no such increase was observed in the CA1 and dentate gyrus [67]. Moreover, it has been reported that rats exposed to a running wheel in an enriched environment before kainate induction of SE show a reduced neuronal loss in the CA3 and the hilus of the dentate gyrus [70]. Our results suggest that there is an increase in mBDNF expression in the hippocampus brought on by physical activity, and thus provides neuroprotection to the CA3 of the PR mice. Furthermore, the increase in mBDNF may have enhanced the synaptic strength between surviving neurons, which would lead to a functional improvement in the hippocampal circuitry .

The mossy fiber pathway in the hippocampal circuitry contains a high concentration of BDNF [16,69]. It has been demonstrated that the status epilepticus leads to a dramatic increase in BDNF immunoreactivity in the pool of BDNF-expressing granule cell presynaptic terminals contacting CA3 pyramidal cells [18]. Our data show that PR and SR mice expressed a significantly larger ratio of mature BDNF than did PS and SS mice, respectively. We also saw a preservation of the CA3 region in PR animals. Our favored interpretation is that running increased mBDNF, which in turn helped preserve the CA3 region in pilocarpine-treated animals. Alternatively, running may have contributed to preserving the mossy fiber pathway in mice exhibiting status epilepticus, which would be the reason why the BDNF content is higher in these animals, but this possibility would not account for the increases in mBDNF in SR mice, as compared to SS mice, since these animals have not been treated with pilocarpine.

Considering that the BDNF neuroprotective action is credited to mBDNF [35], our data on the reduced neuronal loss in the CA3 of PR mice in comparison with PS mice is in conformity with this hypothesis.

Since body weight loss might be indicative of the consequences of the SE on the general condition, as showed by Klitgaard et al. [27], the voluntary running wheel activity seems to have beneficial effects on the PR mice. At the end of the experimental period PR mice did not exhibit body weight loss in contrast with PS mice.

The amount of voluntary physical activity on the running wheel recorded for SR mice was similar to the one previously reported for isogenic and non-isogenic mouse strains [19,28,32,62]. Such activity by PR mice was lower than that of SR mice, but it was sufficient to increase the mBDNF:total BDNF ratio when compared to PS mice. To our knowledge, this is the first study to report a pattern of voluntary physical activity on a running wheel in the mouse model of epilepsy. Previous studies examining the relationship between animal models of epilepsy and an enriched environment, including free access to a running wheel, did not assess locomotory behavior [20,70]. There is evidence that motor behavior in rodents is modulated by the hippocampus, and that this affects qualitative aspects of movement [40,44,47,60]. Rodents with hippocampal lesions were able to move but they could not produce the intense movements associated with certain motor activities such as high jumps or fast wheel running [47]. These findings support the assumption that the limited physical activity on the running wheel revealed by PR mice may be explained by the hippocampal damage observed in these mice. On the other hand, these data show that the mice in all four groups swam at a similar speed in the water maze, suggesting that the mice with pilocarpine-induced SE did not reveal motor deficit under the specific circumstances of the test. There was, however, a cognitive advantage for the PR mice over the PS mice, which suggests that the voluntary physical activity on the running wheel may have contributed to the reduced latency in their performance in the water maze.

Various studies have suggested that seizure-mediated enhancement in hippocampal BDNF production can contribute to epileptogenesis [7,54,56], although a definite association between BDNF and epileptogenesis has yet to be established. The potential epileptogenic activity of BDNF is based on its ability to increase neuronal excitability in the hippocampal circuitry [7,54,56]. An epileptic crisis may lead to increased BDNF expression, which would in turn potentiate glutamatergic transmission and result in a positive feedback mechanism leading to additional seizures and excitotoxicity [7,54,56]. Moreover, the alterations mediated by BDNF, such as ectopic neurogenesis and axon/dendritic sprouting in the hippocampal formation, would contribute to neuronal hyperactivity in this region [29,54]. On the other hand, BDNF expression in the hippocampus has been correlated with the expression of neuropeptide Y [66], which inhibits hippocampal excitability [22]. In view of this apparent contradiction, Reibel et al. [46] proposed that BDNF has a biphasic effect on epileptogenesis, with the seizure-induced increase in BDNF expression potentiating hippocampal excitatory synaptic transmission, which in turn, may be attenuated by neuropeptide Y [46]. However, many studies that have investigated this question have used either the administration of exogenous BDNF or genetic interventions to alter BDNF expression. Little is known about the changes in endogenous BDNF expression under physiological conditions, or about the equilibrium between proBDNF and mBDNF and their potentially different effects on epileptogenesis [54]. In our study the finding that PR mice had an increased expression of mBDNF and that they also revealed a better performance in the water maze test than the PS mice would suggest that mBDNF would be implicated in neuroprotective events.

In conclusion, physical activity had a definite beneficial effect on the spatial memory of mice with pilocarpine-induced SE in the water maze test. This effect was associated with a greater content of mBDNF in the hippocampus of these mice when they had access to the running wheel (PR) than when they had no such access (PS). Thus, our data highlight the importance of the ratio of mBDNF to proBDNF when considering the role of BDNF in epilepsy. Further studies are necessary to understand the intracellular signal-

ing mechanisms involved in mBDNF-promoted neuronal protection and memory formation associated with physical activity in experimental models of epilepsy.

Acknowledgements

The authors thank Dr. Henriette Van Praag for the specifications of the running wheel setup and Eduardo Veiga for building the running wheel and providing the data acquisition setup. We also thank Dr. Linda Gentry El-Dash and Dr. Fabio Papes for reviewing the English. César Renato Sartori and Fernanda Campos Pelágio were supported by fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq – 132464/2003-9). This work was supported by Fundo de Apoio ao Ensino e Pesquisa – UNICAMP (919/03). Francesco Langone, Marcelo N. Muscará and Elenice A.M. Ferrari are recipients of research fellowships and grants from CNPq.

References

[1] Adlard PA, Perreau VM, Engesser-Cesar C, Cotman CW. The time-course of induction of brain-derived neurotrophic factor mRNA and protein in the rat hippocampus following voluntary exercise. Neurosci Lett 2004;363:43–8.
[2] Aldenkamp AP, Bodde N. Behaviour, cognition and epilepsy. Acta Neurol Scand 2005;182:19–25.
[3] Anderson MF, Aberg MA, Nilsson M, Eriksson PS. Insulin-like growth factor and neurogenesis in the adult mammalian brain. Dev Brain Res 2002;134:115–22.
[4] Ang ET, Wong PTH, Moochhala S, Ng YK. Neuroprotection associated with running: is it a result of increased endogenous neurotrophic factors? Neuroscience 2003;118:335–45.
[5] Arida RM, Scorza FA, Santos NF, Peres CA, Cavalheiro EA. Effect of physical exercise on seizure occurrence in a model of temporal lobe epilepsy in rats. Epilepsy Res 1999;37:45–52.
[6] Arida RM, Scorza FA, Scorza CA, Cavalheiro EA. Is physical activity beneficial for recovery in temporal lobe epilepsy? Evidences from animal studies. Neurosci Biobehav Rev 2009;33:422–31.
[7] Binder DK, Croll SD, Gall CM, Sharfman HE. BDNF and epilepsy: too much of a good thing? Trends Neurosci 2001;24:47–53.
[8] Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth Factors 2004;22:123–31.
[9] Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation of hippocampus. Nature 1993;361:31–9.
[10] Borges K, Gearing M, McDermott DL, Smith AB, Almont AG, Wainer BH, et al. Neuronal and glial pathological changes during epileptogenesis in the mouse pilocarpine model. Exp Neurol 2003;182:21–34.
[11] Carro E, Nuñez A, Busiguina S, Torres-Alemán I. Circulating insulin-like growth factor I mediates effects of exercise on the brain. J Neurosci 2000;20:2926–33.
[12] Carro E, Trejo JL, Busiguina S, Torres-Alemán I. Circulating insulin-like growth factor I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy. J Neurosci 2001;21:5678–84.
[13] Cavalheiro EA. The pilocarpine model of epilepsy. Ital J Neurol Sci 1995;16:33–7.
[14] Cavalheiro EA, Santos NF, Priel MR. The pilocarpine model of epilepsy in mice. Epilepsia 1996;37:1015–9.
[15] Colbourne F, Corbett D. Delayed postischemic hypothermia: a six month survival study using behavioral and histological assessments of neuroprotection. J Neurosci 1995;15:7250–60.
[16] Conner JM, Lauterborn JC, Yan Q, Gall CM, Varon S. Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. J Neurosci 1997;17:2295–313.
[17] Cotman CW, Berchtold NC. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci 2002;35:295–301.
[18] Danzer SC, McNamara JO. Localization of brain-derived neurotrophic factor to distinct terminals of mossy fiber axons implies regulation of both excitation and feedforward inhibition of CA3 pyramidal cells. J Neurosci 2004;24(50):11346–55.
[19] Dohm MR, Richardson CS, Garland T. Exercise physiology of wild and random-bred laboratory house mice and their reciprocal hybrids. Am J Physiol Regul Integr Comp Physiol 1994;267:R1098–108.
[20] Faverjon S, Silveira DC, Fu DD, Cha BH, Akman C, Hu Y, et al. Beneficial effects of enriched environment following status epilepticus in immature rats. Neurology 2002;59:1356–64.
[21] Franklin KBJ, Paxinos G. The mouse brain in stereotaxic coordinates. San Diego: Academic Press; 1997.
[22] Gall C, Lauterborn J, Bundman M, Murray K, Isackson P. Seizures and the regulation of neurotrophic factor and neuropeptide gene expression in brain. Epilepsy Res Suppl 1991;4:225–45.
[23] Gobbo OL, O'Mara SM. Exercise, but not environmental enrichment, improves learning after kainic acid-induced hippocampal neurodegeneration in association with an increase in brain-derived neurotrophic factor. Behav Brain Res 2005;159:21–6.
[24] Gómez-Pinilla F, So V, Kesslak JP. Spatial learning and physical activity contribute to the induction of fibroblast growth factor: neural substrates for increased cognition associated with exercise. Neuroscience 1998;85:53–61.
[25] Helmstaedter C, Kurthen M. Memory and epilepsy: characteristics, course, and influence of drugs and surgery. Curr Opin Neurol 2001;14:211–6.
[26] Kempermann G, Gast D, Gage FH. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann Neurol 2002;52:135–43.
[27] Klitgaard H, Matagne A, Vanneste-Goemaere J, Margineanu DG. Pilocarpine-induced epileptogenesis in the rat: impact of initial duration of status epilepticus on electrophysiological and neuropathological alterations. Epilepsy Res 2002;51(1–2):93–107.
[28] Koteja P, Swallow JG, Carter PA, Garland T. Energy cost of wheel running in house mice: implications for coadaptation of locomotion and energy budgets. Physiol Biochem Zool 1999;72:238–49.
[29] Koyama R, Ikegaya Y. To BDNF or not to BDNF: that is the epileptic hippocampus. Neuroscientist 2005;11:282–7.
[30] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
[31] Lee R, Kermani P, Teng KK, Hempstead BL. Regulation of cell survival by secreted proneurorophins. Science 2001;294:1945–8.
[32] Lightfoot JT, Turner MJ, Daves M, Vordermark A, Kleeberger SR. Genetic influence on daily wheel running activity level. Physiol Genomics 2004;19:270–6.
[33] Liu Z, Mikati M, Holmes GL. Mesial temporal sclerosis: pathogenesis and significance. Pediatr Neurol 1995;12:5–16.
[34] Lowenstein DH, Alldredge BK. Status epilepticus. N Engl J Med 1998;338:970–6.
[35] Lu B, Pang PT, Woo NH. The yin and yang of neurotrophin action. Nat Neurosci 2005;6:603–14.
[36] Lynch M, Sayin U, Bownds J, Janumpalli S, Sutula T. Long-term consequences of early postnatal seizures on hippocampal learning and plasticity. Eur J Neurosci 2000;12:2252–64.
[37] Marcinkiewicz M, Nagao T, Day R, Seidah NG, Chrétien M, Avoli M. Pilocarpine-induced seizures are accompanied by a transient elevation in the messenger RNA expression of the prohormone convertase PC1 in rat hippocampus: comparison with nerve growth factor and brain-derived neurotrophic factor expression. Neuroscience 1997;76(2):425–39.
[38] Mattson MP. Neuroprotective signaling and the aging brain: take away my food and let me run. Brain Res 2000;886:47–53.
[39] Meyer A, Chrétien P, Massicotte G, Sargent C, Chrétien M, Marcinkiewicz M. Kainic acid increases the expression of the prohormone convertases furin and PC1 in the mouse hippocampus. Brain Res 1996;732(1–2):121–32.
[40] Morris RGM, Hagan JJ. Hippocampal electrical activity and ballistic movement. In: Seifert W, editor. Neurobiology of the hippocampus. London: Academic Press; 1983.
[41] Mowla SJ, Farhadi HF, Pareek S, Atwal JK, Morris SJ, Seidah NG, et al. Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. J Biol Chem 2001;16:12660–6.
[42] Nakken KO, Bjorholt PG, Johannessen SI, Loyning T, Lind E. Effect of physical training on aerobic capacity, seizure occurrence, and serum level of antiepileptic drugs in adults with epilepsy. Epilepsia 1990;31:88–94.
[43] Neeper SA, Gomez-Pinilla F, Choi J, Cotman CW. Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. Brain Res 1996;726:49–56.
[44] Oddie SD, Bland BH. Hippocampal formation theta activity and movement selection. Neurosci Biobehav Rev 1998;22:221–31.
[45] Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, et al. Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science 2004;306:487–91.
[46] Reibel S, Depaulis A, Larmet Y. BDNF and epilepsy: the bad could turn out to be good. Trends Neurosci 2001;24:318–9.
[47] Rhodes JS, Garland T, Gammie SC. Patterns of brain activity associated with variation in voluntary wheel-running behavior. Behav Neurosci 2003;17:1243–56.
[48] Riedel G, Micheau J. Function of the hippocampus in memory formation: desperately seeking resolution. Prog Neuropsychopharmacol Biol Psychiatry 2001;25(4):835–53.
[49] Roth LD, Goode KT, Willians VL, Faught E. Physical exercise, stressful life experience, and depression in adults with epilepsy. Epilepsia 1994;35:1248–55.
[50] Roux PP, Colicos MA, Barker PA, Kennedy TE. p75 neurotrophin receptor expression is induced in apoptotic neurons after seizure. J Neurosci 1999;19:6887–96.
[51] Sankar R, Shin D, Mazarati AM, Liu H, Katsumore H, Lezama R. Epileptogenesis after status epilepticus reflects age- and model-dependent plasticity. Ann Neurol 2000;48:580–9.
[52] Schauwecker PE. Complications associated with genetic background effects in models of experimental epilepsy. Prog Brain Res 2002;135:139–48.
[53] Schauwecker PE. Modulation of cell death by mouse genotype: differential vulnerability to excitatory amino acid-induced lesions. Exp Neurol 2002;178:219–35.
[54] Sharfman HE. Brain-derived neurotrophic factor—a missing link? Epilepsy Curr 2005;5:83–8.
[55] Sherwin CM. Voluntary running wheel: a review and novel interpretation. Anim Behav 1998;56:11–27.
[56] Simonato M, Tongiorgi E, Kokaia M. Angels and demons: neurotrophic factors and epilepsy. Trends Pharmacol Sci 2006;27(12):631–8.
[57] Squire LR. Memory and the hippocampus: a synthesis from findings with rats, monkeys and humans. Psychol Rev 1992;99:195–231.

[58] Teng HK, Teng KK, Lee R, Wright S, Tevar S, Almeida RD, et al. ProBDNF induces neuronal apoptosis via activation of a receptor complex of $p75^{NTR}$ and sortilin. J Neurosci 2005;25:5455–63.
[59] Trejo JL, Carro E, Torres-Alemán I. Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus. J Neurosci 2001;21:1628–34.
[60] Turski WA, Cavalheiro EA, Bortolotto ZA, Mello LM, Schwarz M, Turski L. Seizures produced by pilocarpine in mice: electroencephalographic and morphological analysis. Brain Res 1984;321:237–53.
[61] Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD. From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. Learn Mem 2002;9: 224–37.
[62] Van Praag H, Christie RB, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning and long-term potentiation in mice. Proc Natl Acad Sci U S A 1999;96:13427–31.
[63] Van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Neurosci 2000;1:191–8.
[64] Van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 1999;2:266–70.
[65] Vaynman S, Ying Z, Gomez-Pinilla F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci 2004;20:2580–90.
[66] Vezzani A, Ravizza T, Moneta D, Conti M, Borroni A, Rizzi M. Brain-derived neurotrophic factor immunoreactivity in the limbic system of rats after acute seizures and during spontaneous convulsions: temporal evolution of changes as compared to neuropeptide Y. Neuroscience 1999;90:1445–61.
[67] Widenfalk J, Olson L, Thorén P. Deprived of habitual running, rats downregulates BDNF and TrkB messages in the brain. Neurosci Res 1999;34:125–32.
[68] Yamada K, Nabeshima T. Brain-derived neurotrophic factor/TrkB signaling in memory process. J Pharmacol Sci 2003;91:267–70.
[69] Yan Q, Rosenfeld RD, Matheson CR, Hawkins N, Lopez OT, Bennett L, et al. Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. Neuroscience 1997;78:431–48.
[70] Young D, Lawlor PA, Leone P, Dragunow M, During MJ. Environmental enrichment inhibits spontaneous apoptosis, prevents seizure and is neuroprotective. Nat Med 1995;5:448–53.