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Molecular Brain Research 61 (1998) 147–153

MOLECULAR BRAIN RESEARCH

Research report

# Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus

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Accepted 11 August 1998

**Abstract**

Previous results from our laboratory indicate that two nights of voluntary wheel running upregulates brain-derived neurotrophic factor (BDNF) mRNA expression in the hippocampus. In order to investigate the time-course of the BDNF response and to examine how physical activity preferentially activates particular transcriptional pathways, the effects of 6 and 12 h of voluntary wheel running on BDNF and exons I–IV mRNA expression were investigated in rats. Hippocampal full-length BDNF mRNA expression was rapidly influenced by physical activity, showing significant increases in expression levels as soon as 6 h of voluntary wheel running. Moreover, there was a strong positive correlation between distance run and BDNF mRNA expression. Exon I mRNA expression was significantly upregulated after 6 h of running and was maintained or enhanced by 12 h of voluntary running. Exon II had a slower time-course and was significantly upregulated after 12 h, selectively in the CA1 hippocampal region. Exon III and Exon IV showed no significant increase in expression level after 6 or 12 h of running in the paradigm studied. It is significant that the rapid neurotrophin response is demonstrated for a physiologically relevant stimulus, as opposed to the extreme conditions of seizure paradigms. Furthermore, exercise-induced upregulation of BDNF may help increase the brain's resistance to damage and neurodegeneration that occurs with aging. 

*Keywords:* Brain-derived neurotrophic factor; Exon; Exercise; Rat; Hippocampus

## 1. Introduction

Neurotrophins, including brain-derived neurotrophic factor (BDNF), can enhance the survival and differentiation of neurons. If these neurotrophic factors fall below a certain level it is generally believed that neurons are more vulnerable to damage, or conversely, if levels are maintained or enhanced this may provide neurons with a margin of protection. Moreover, BDNF has been shown to protect against neuronal degeneration due to ischemia and other neurodegenerative disorders [12]. Because the effects of neurotrophins may produce both rapid and enduring changes in synaptic efficacy, changes in neurotrophins and their receptors have been implicated in memory and learning (long-term potentiation, LTP) [17].

Exercise (i.e., voluntary running), is a natural activity which can regulate BDNF expression [14,15] and has been proposed to be a factor in reducing age-related decline in memory and cognition [4,6,19]. Previously it has been reported that there is decreased mortality and brain damage following cerebral ischemia in rats that have been exercised for two weeks prior to the ischemic insult [22]. One possible mechanism for this protection may be that exercise increases BDNF expression, which increases the resistance to brain damage. Thus, exercise-induced upregulation of BDNF may have a significant role in normal and neuroprotective processes in the brain.

The rat BDNF gene consists of four short 5′-exons (exons I–IV) linked to separate promoters and one 3′-exon (full-length BDNF) encoding the mature BDNF protein

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PII: S0169-328X(98)00222-8

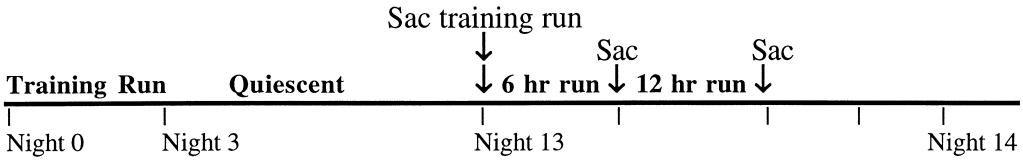


Fig. 1. Timeline demonstrating the experimental design (sac = sacrifice).

[24]. BDNF mRNAs constituting exons I, II, and III are expressed predominantly in the brain, while exon IV transcripts are found primarily in the lung and heart [24]. There is a differential regulation of the individual BDNF transcripts via multiple promoters. In the brain, activation of the different BDNF promoters is region-specific and dependent on the type of brain insult (epileptic seizures vs. cerebral ischemia vs. hypoglycemic coma) [7]. Hippocampal kindling (a model of epilepsy) potentiates exon I and III expression [8]; forebrain ischemia potentiates exon III expression [7]; and adrenalectomy potentiates exon I and II expression [9,10]. Furthermore, the regional distribution of these BDNF transcripts differs following kainic acid administration [24]. Exon I and exon II mRNA expression is regulated, at least in part, by calcium/calmodulin-dependent protein kinases following kainic acid induced seizures [5], whereas exon III and exon IV share properties with immediate early genes [11,20,23]. Additional experiments with these exons will give further insight into the mechanisms of BDNF gene regulation.

Previous results from our laboratory indicate that two nights of voluntary wheel running upregulates BDNF mRNA expression in the hippocampus. Given the rapidity of response seen under seizure conditions, it is likely that an effect of physical activity on BDNF mRNA levels may be occurring at an earlier timepoint than after two nights. In order to investigate the time-course of the BDNF response to physical activity, the effect of 6 and 12 h of wheel running were investigated in young male rats. In addition, it is possible that physical activity preferentially activates particular transcriptional pathways to upregulate BDNF mRNA. This would be reflected by a differential effect of physical activity on the expression of the various exons. Hence, mRNA expression of exons I–IV was also examined after 6 and 12 h of wheel running.

## 2. Materials and methods

Male Fisher-344 rats ($n = 7–8$/group; 3–4 months of age; Harlan Sprague–Dawley/National Institute on Aging, Indianapolis, IN) were exposed to a 0600/1800 h light/dark cycle and allowed access to food and water ad lib. During exercise periods the rats were housed individually in cages with running wheels (Nalgene, OR). A magnet attached to each running wheel triggered a magnetic reed switch connected to a computer that monitored the number of wheel revolutions (Ratrun software, C. Hage Associates, CA). To reduce the effect of novelty, rats were trained in running wheels for three nights, followed by 10 nights without the wheel to attenuate any effect of running. After the 10 nights without running wheels, one

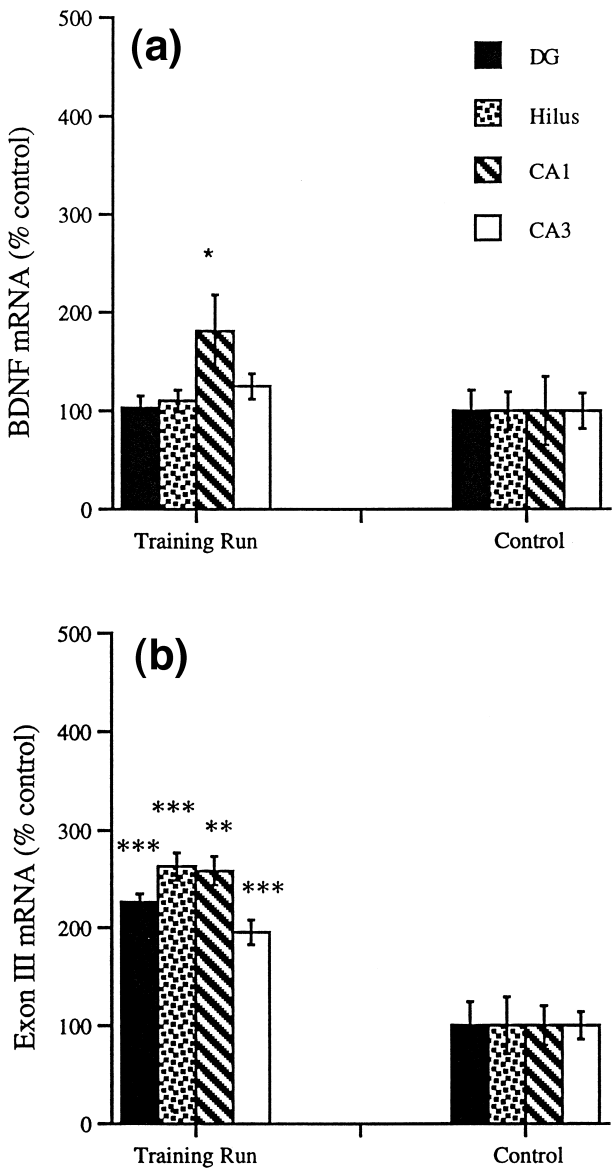


Fig. 2. Effect of training run on (a) BDNF and (b) exon III mRNA expression in hippocampal regions. Exon I, II and IV mRNA expression was not significantly different from control values. $* p < 0.05$, $** p < 0.01$, $*** p < 0.001$.

group of animals was sacrificed without further running. For the remaining animals, running wheels were replaced, and following 6 or 12 h of voluntarily running the animals were sacrificed. Control rats were sacrificed at the same time points but were never exposed to running wheels. This paradigm is similar to our previous study [15] examining 2 days of running on growth factor expression.

All rats were decapitated and the brains rapidly removed. The brains were submerged into freezing cold isopentane for 5 min and then stored at −70°C. The fresh frozen tissue was cut into 14 μm sections on a cryostat and collected on vectabond-coated (Vector Labs) slides. The mounted sections were post-fixed in 4% paraformaldehyde, air dried and stored with desiccant at −20°C.

### 2.1. Preparation of riboprobes

BDNF cRNA probes were prepared from cDNA templates encoding the rat BDNF gene (kindly provided by Dr. Paul Isackson). A pBSks plasmid was linearized with *xba* and transcribed using T3 RNA polymerase, to produce a 780-base probe containing a 750-base region complementary to BDNF mRNA. Linearizing with *Hin*dIII and transcribing with T7 polymerase yielded the equivalent size sense strand.

cDNA probes specific for each of the four BDNF transcripts (kindly provided by Dr. Paul Isackson) containing distinct 5′ ends were obtained by PCR amplification of mouse genomic DNA with 20 base oligonucleotides designed from the rat sequences of Timmusk et al. [24]. PCR amplified fragments were gel purified, blunt-ended with T4 DNA polymerase and ligated to *Hin*dII-digested, phosphatase-treated pBS (Stratagene). The identity of isolated recombinant plasmids was confirmed by DNA sequence analysis. The exon I-specific clone contains a 387 bp cDNA fragment corresponding to bases 787–1165 (genomic fragment A, [24]). The exon II-specific clone contains a 468 bp fragment corresponding to bases 1761–2229 (fragment A, [24]) which spans exons 2a, b and c identified by Nakayama et al. [13]. The exon III-specific clone contains a 391 bp fragment corresponding to bases 636–1027 (genomic fragment B, [24]). The exon IV-specific clone contains a 350 bp fragment corresponding to bases 1730–2080 (genomic fragment B). Antisense RNA probes were synthesized from *Pst*I-linearized exon I and *Hin*dIII-linearized exon II and exon III with T7 RNA polymerase, while *xba*I-linearized exon IV was transcribed with T3 RNA polymerase. Sense strand probes were transcribed from *Bam*H1-linearized exons I–III with T3 RNA polymerase. Transcription reactions were per-

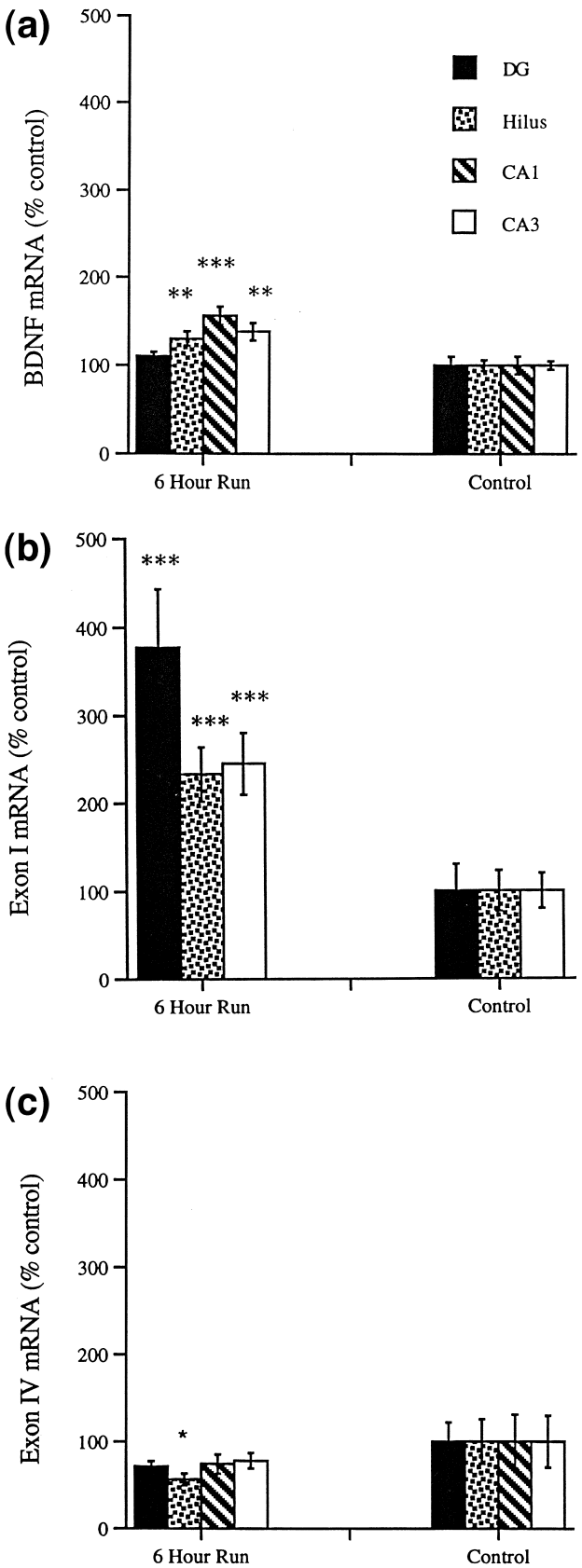


Fig. 3. Effect of 6 h of voluntary wheel running on (a) BDNF, (b) exon I and (c) exon IV mRNA expression in hippocampal regions. Exon II and III mRNA expression was not significantly different from control values. $* p < 0.05$, $** p < 0.01$, $*** p < 0.001$.

formed using a Promega transcription kit (Promega, WI) and $^{35}$S-UTP (Dupont, MA; 1000–1500 Ci/mmol).

### 2.2. In situ hybridization and image analysis

Coronal brain sections (14 μm) were treated with a series of washes: 0.1 M glycine in phosphate buffer at room temperature (RT) twice, phosphate buffer for 15 min at RT, 0.1 M triethanolamine (TEA) with 0.25% acetic anhydride for 10 min at RT, and 2 × saline-sodium citrate (SSC) for 15 min at RT twice. Sections were subsequently dehydrated and defatted with graded alcohol and chloroform, followed by overnight incubation at 60°C with hybridization buffer (0.7% ficcoll, 0.7% polyvinylpyrrolidone, 0.7% bovine serum albumin, 10% dextran sulfate, 50% formamide, 1 mM EDTA, 0.3 M NaCl, 0.15 mg/ml tRNA, 300 μg/ml salmon sperm DNA, 40 mM dithiothreitol) containing $^{35}$S-labeled cRNA probe at a final concentration of $1 \times 10^6$ cpm/100 μl. As a control for hybridization specificity, additional tissue sections were hybridized with sense riboprobes for the same growth factor or treated with ribonuclease A (20 μg/ml, 45°C, 30 min) prior to normal hybridization.

After hybridization, the sections were washed with 4 × SSC twice for 30 min at 60°C. Then BDNF hybridized sections were incubated with 20 μg/ml RNAse A, or the exon hybridized sections were incubated with 10 μg/ml RNAse A for 30 min at 45°C. Next the sections were washed four times with 2 × SSC for 30 min at RT, two times with 0.5 × SSC for 30 min at 60°C, two times with 0.1 × SSC for 30 min at 60°C and lastly one time with 0.1 × SSC for 30 min at RT. Finally, slides were rinsed in deionized water for 1 min.

Slides and $^{14}$C-labeled standards were placed against β-max film (Amersham Labs, IL) for 5–21 days depending upon the probe. Film relative optical density (ROD) readings were taken and converted to equivalent mCi$^{35}$S/g tissue using the Microcomputer Imaging Device (MCID) (Imaging, St. Catherines, Ontario, Canada) and a $^{14}$C–$^{35}$S conversion table established by the supplier. Multiple random fields from the dentate gyrus (DG), hilus and CA1 and CA3 hippocampal regions were selected for quantification. Data from three tissue sections were pooled to obtain the mean value for each animal. To control for slight differences in background hybridization, average ROD levels from the area of the thalamus directly below the hippocampus were subtracted from ROD values of the regions of interest. The thalamic regions showed no specific hybridization patterns and thus were considered background. Evaluation of differences in mRNA levels between groups was performed using analysis of variance (ANOVA) and Fisher's PLSD post-hoc tests.

## 3. Results

Similar to our previously published paradigm [15], rats were initially provided with a 3-day exposure to running wheels in order to acclimate them to the wheels, and then to reduce the practice effects, this was followed by a 10 day period of quiescence. After the 10 days of quiescence, rats were sacrificed following 0, 6, or 12 h of additional running (Fig. 1).

### 3.1. Training run

Following the 3 days running/10 days quiescence, rats in this group were sacrificed with no subsequent running activity, and were compared to controls sacrificed at the same time. BDNF mRNA was significantly increased in the CA1 (81% over control values, $p = 0.03$) (Fig. 2a). In addition, exon III mRNA displayed increased expression in the DG (126%, $p = 0.008$), CA1 (158%, $p = 0.010$), CA3 (95%, $p = 0.002$) and hilus (162%, $p = 0.002$) over time-matched controls (Fig. 2b). No other exon was significantly altered by the training run.

### 3.2. Six hours running

In this series of experiments, after the initial three nights of training followed by 10 nights of quiescence, animals were sacrificed after 6 h of voluntary wheel

Table 1
Correlation between distance run and BDNF mRNA expression in hippocampal areas of rats ($n = 7$) sacrificed after 6 h of voluntary running

| BDNF | Dentate | Hilus | CA1 | CA3 |
|---|---|---|---|---|
| 1 h dist. | NS | NS | NS | NS |
| 2 h dist. | NS | S $r = 0.747$, $p < 0.031$ | S $r = 0.880$, $p < 0.002$ | T $r = 0.690$, $p < 0.060$ |
| 3 h dist. | NS | S $r = 0.814$, $p < 0.023$ | S $r = 0.731$, $p < 0.037$ | NS |
| Total dist. | NS | S $r = 0.866$, $p < 0.003$ | S $r = 0.894$, $p < 0.001$ | S $r = 0.704$, $p < 0.050$ |

1 h dist., distance the rat ran the hour prior to sacrifice.
2 h dist., total distance the rats ran the last 2 h prior to sacrifice.
3 h dist., total distance the rats ran 3 h before sacrifice.
Total dist., total distance the rat ran up until sacrifice (6 h).
NS, not significant.
S, significant correlation.
T, trend toward significance (positive linear relationship).

running and compared to time-matched controls. BDNF was significantly upregulated as compared to non-running rats (Fig. 3) after 6 h of voluntary wheel running. BDNF mRNA expression increased 56% over control values ($p = 0.005$) in the CA1 region of the hippocampus, increased 38% ($p = 0.008$) in the CA3 and increased 30% ($p = 0.015$) in the hilus. Six hours of running further increased the BDNF mRNA expression in all hippocampal regions including the CA1 region where it increased 23.4% over the increased BDNF expression following the training period. This is not readily apparent in Figs. 1 and 2, since, in addition to the exercise effect there is an endogenous change in BDNF/transcripts expression [1], and 'training run' vs. '6 h run' animals were sacrifice at different timepoints in the 24 h period. Moreover, there were positive correlations between distance run and BDNF expression (Table 1). The CA1 and hilar regions of the hippocampus showed significant positive correlations between BDNF expression and (1) the total distance run, (2) the distance run 2 h prior to sacrifice, and (3) the distance run 3 h prior to sacrifice. The CA3 region exhibited a significant positive correlation between the total distance run and BDNF expression, while the distance run 2 h before sacrifice showed a positive trend toward significance, and there was no correlation at 3 h. There was no correlation between the distance run the hour just prior to sacrifice and BDNF expression in any area of the hippocampus.

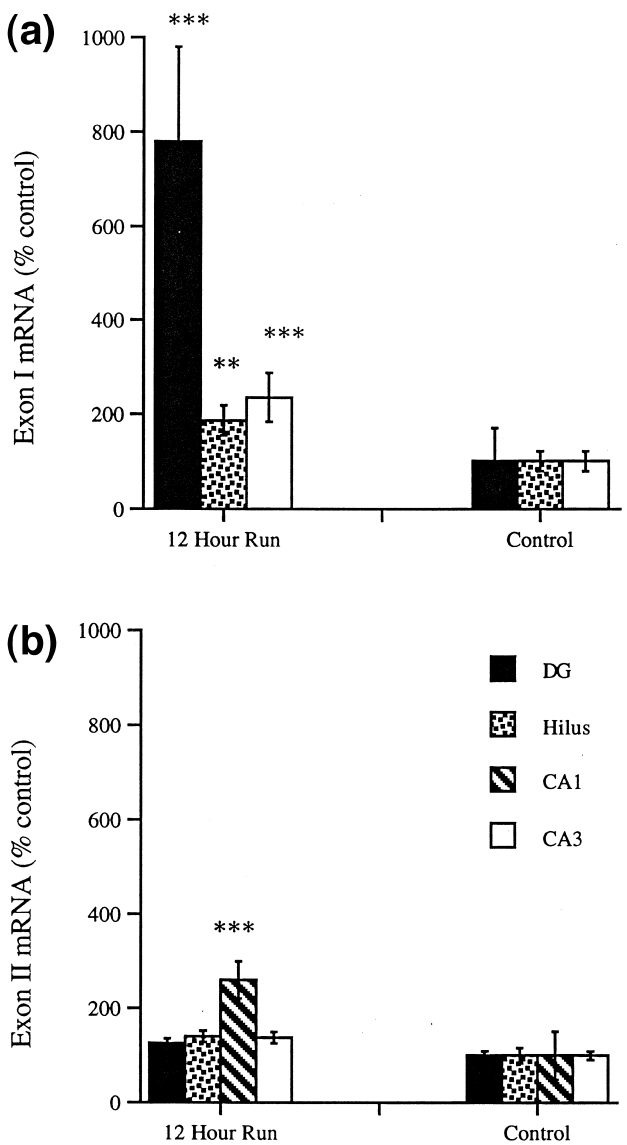


Fig. 4. Effect of 12 h of voluntary wheel running on (a) exon I and (b) exon II mRNA expression in hippocampal regions. BDNF, exon III and IV mRNA expression was not significantly different from control values. $** p < 0.01$, $*** p < 0.001$.

Six hours of running had the most dramatic effect on exon I expression (Fig. 3b). Exon I expression increased in the DG (277% over controls values, $p = 0.001$), hilus (133%, $p = 0.001$), and CA3 (145%, $p = 0.002$). Expression of exon I was below detection in the CA1. Exon II and Exon III mRNA expression were not significantly altered by 6 h of running. In contrast, exon IV mRNA expression was decreased by 56% ($p = 0.04$) in the hilus as compared to non-running rats (Fig. 3c). Regression analysis revealed no significant correlation between distance run and mRNA expression level of any of the exons, in any hippocampal subfield.

### 3.3. Twelve hours (one night) running

After 12 h of running (Fig. 4), exon I expression was further increased in the DG to 680% ($p = 0.001$), and remained elevated to 86% ($p = 0.02$) in the hilus and 135% ($p = 0.002$) in the CA3 above time-matched control levels. Exon II expression was significantly increased in the CA1 (160%, $p = 0.002$) region, and the increased expression in the CA3 (38%) and hilar regions (40%) approached significance ($p = 0.057$ and $p = 0.057$, respectively). There was no significant difference in expression of BDNF or exons III and IV from control levels after 12 h of running.

## 4. Discussion

Since BDNF can promote the survival of neurons and enhance LTP, the ability to regulate its expression may aid in the maintenance of plastic functions in the brain. In order to gain insight into the regulatory mechanisms of BDNF mRNA expression, mRNA expression levels of the four transcript forms of BDNF, each putatively linked to a unique promoter and transcription mechanism, were examined in parallel with BDNF expression. The exercise paradigm involved a training period in which the rats had voluntary access to running wheels for three nights. This was followed by 10 nights without access to the wheels after which some rats where sacrificed immediately, while others were once again allowed access to the running

wheels. These rats were sacrificed after 6 or 12 h of additional voluntary running. Previously we have used this paradigm because it demonstrated BDNF mRNA induction after two nights of running [14,15]. The training period was initially chosen to minimize any effect of novelty and emphasize the physical activity. The goal of the present study was to use a similar paradigm to see how BDNF mRNA expression is effected by shorter running times and whether the effect is exon-specific.

Rats which were sacrificed immediately after the 10 nights of quiescence with no additional running showed significant upregulation (2 to 2.5-fold) of exon III mRNA expression (Fig. 2). Thus, the elevated exon III expression persisted after 10 nights of 'rest'. This finding was unexpected because in animals subjected to kindling stimulation, the elevation of exon III mRNA has been shown to decline markedly by four h and to return to baseline by 24 h [7]. This indicates that the activity induced by kindling and by voluntary exercise each have a profoundly different impact on BDNF exon regulation and expression.

After training, 10 nights quiescence and an additional 6 h of running, the responses of BDNF and the various exons differed in magnitude, regional distribution and sensitivity. There was rapid induction of the BDNF message in the hilus, CA1, and CA3 subfields (1.3 to 1.5-fold increase), with the increase in all subfields, including the CA1 region, surpassing the increase following the training period. Exon I was the most responsive to the running stimulus, showing a strong upregulation (2 to 4-fold increase) in all hippocampal subfields, with exception to the CA1 region where expression was below detection (Fig. 3). None of the other exons showed changes in expression level of similar magnitude to exon I, and indeed, exons III and IV showed virtually no change in expression level after 6 h of running. These findings are in agreement with other studies which showed differential responses of exons I–IV to various stimuli or manipulations [7,9,24].

Exons I–IV were also differentially regulated by 12 h of running activity. The increased exon I mRNA expression seen at 6 h continued to rise with further running (12 h) especially in the DG, where expression levels were increased 8-fold over time-matched control expression (Fig. 4). In contrast, the induction of exon II expression, which was not significantly increased after 6 h of running, was significantly increased (1.5 to 2.5-fold of control) after 12 h of running, in all subfields excluding the DG (Fig. 4). Although elevated levels of exon III mRNA expression persisted for 10 days after a 3-day running period (Fig. 2), the expression was barely above control levels after 6 or 12 h of running (graph not shown). It is possible that had the animals run for longer than 6 or 12 h (for example 3 days), exon III mRNA expression would have been further upregulated. This sustained elevation requires further investigation. Exon IV showed virtually no change in expression level after 12 h of running. Interestingly, not only voluntary running but also kindling-seizures, cerebral ischemia and insulin-induced hypoglycemic coma [7] exhibited a lack of effect on the level of exon IV mRNA in the brain, suggesting that exon IV is relatively insensitive to regulation in the brain. Clearly, the exons are differentially responsive to the running stimulus, indicating that only particular transcriptional mechanisms are being activated by wheel running.

In agreement with a prior study [15], the neurotrophin response to running appears to be a graded response. In the present study, there was a positive correlation between the total distance run after 6 h and changes in BDNF expression in the hilus, CA1 and CA3 hippocampal subfields (Table 1). The rats ran almost every hour during the first 6 h of running indicating the presence of a constant and apparently accumulating stimulus. There may also be a threshold of activity which must be reached since animals running minimal distances had no induction of BDNF/exons mRNA expression. Thus, it is likely that there is a threshold level of activity beyond which gene expression is potentiated, though we cannot exclude the possibility that the mRNA expression levels did indeed increase, but could not be reliably detected due to limitations in sensitivity of the assay. While in this study there was a strong correlation between BDNF mRNA expression and total running activity, there was no similar correlation between running activity and mRNA expression levels of any of the exons.

It is likely that there is physiologically functional relevance to our finding that voluntary physical activity regulates expression of BDNF and exons I–IV in regions of the hippocampus. Exercise has beneficial effects on the brain [3,4,6,16,18,19], and perhaps some of these effects are mediated by altering BDNF expression. For instance, increased availability of BDNF may provide vulnerable cell types with a margin of protection. Indeed, it has already been shown that there is a decrease in neuronal loss and marked improvement in survival in rodents that were allowed to engage in spontaneous wheel running for 2 weeks prior to a cerebral ischemic insult [22]. Also along this line, stress and adrenal hormone have been shown to reduce BDNF expression [2,10,21] and this may make neurons more vulnerable to the exacerbation of excitotoxic injury [21]. Since exercise has been shown to reduce stress in both adults and adolescents [3,16,18], the effect of exercise on circulating adrenal hormones levels may provide one potential mechanism underlying the observed exercise-induced upregulation of BDNF. Our findings, that exercise can induce and regulate expression of BDNF and its splice variants, combined with the fact that BDNF enhances the survival and functioning of neurons, suggest a potential value in promoting health and maintaining cognition during aging. Most importantly, exercise (such as wheel running) is a physiologically relevant behavior which provides neuronal stimulation, and in part may help enhance memory and cognition, as well as decrease vulnerability to neuronal insult and the deleterious effects of stress.

## Acknowledgements

Many thanks to Dr. Elizabeth Head for her feedback on the statistics. This work was supported by NIA grant #AG 13411.

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