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The timecourse of induction of brain-derived neurotrophic factor mRNA and protein in the rat hippocampus following voluntary exercise

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Abstract

In this study we examined the timecourse of induction of brain-derived neurotrophic factor (BDNF) mRNA and protein after 1, 3, 5, 7, 14 and 28 days of exercise in the rat. To measure the expression of mRNA for individual BDNF exons we utilized a semi-quantitative RT-PCR technique, while BDNF protein was assessed using commercial ELISA kits. We demonstrated that the distance run by animals increased significantly ($P < 0.05$) after 4 weeks. BDNF protein was significantly ($P < 0.05$) increased after 4 weeks of exercise, while the mRNA for individual BDNF exons increased significantly ($P < 0.05$) over the timecourse (exon I after 1 and 28 days and exons II and V after 28 days). The Morris water maze was then utilized to demonstrate that 3 weeks of prior exercise enhanced the rate of learning on this task. Exercise, therefore, was shown to modulate BDNF induction in a time-dependent manner, and this may translate to improvements in neurotrophin-mediated tasks within the CNS.

Keywords: Physical activity; Brain-derived neurotrophic factor; Protein; mRNA

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that is distributed throughout different regions of the brain [4,9,11,17,22], and which is found at high levels within the hippocampus [6]. BDNF provides both neurotrophic and neuroprotective support to different subpopulations of neurons throughout development and into adulthood, and is particularly associated with both learning and memory processes within the hippocampus [10,23]. It has previously been reported that voluntary running significantly upregulates the expression of BDNF mRNA [12,13,15,16]. Previous studies, however, utilized an exercise regime that incorporated 'pretraining' exercise prior to the start of the experimental period. In one of these studies the coding exon for BDNF mRNA (exon V) was examined after 0, 2, 4, and 7 nights of running [12] while exons I and II were examined after 2, 7, and 20 days of exercise [16] in the other. As the 'pretraining' period may prime the BDNF response to future running, it is preferable to utilize exercise regimes that do not incorporate the pretraining. Two such studies have been conducted, examining BDNF exons I–V after 6 and 12 h of exercise [13], and exon V after 20 days of exercise [15]. Such studies, therefore, have not completely addressed the timecourse of change in BDNF mRNA with exercise. Perhaps more importantly, particularly given that BDNF mRNA does not always translate to changes at the protein level [20], the timecourse of change in BDNF protein following voluntary exercise has not been examined in the rat. We have undertaken this study, therefore, in order to elucidate the effect of exercise (without a pretraining period) on BDNF mRNA (exons I–V) and protein following different periods of exercise (up to 28 days). We have also utilized the Morris water maze to determine whether exercising animals show faster rates of learning on this spatial memory task.

All animal procedures were approved by the Institutional Animal Care and Use Committee for the University of California at Irvine. Intact male Sprague–Dawley rats (2 months of age) were used to avoid the confounding effect of cycling hormone levels on BDNF expression in females [2]. Animals were individually housed in regular polyethylene cages (35 cm long, 22 cm wide, 18 cm deep) with ad libitum access to food and water in a 12:12 h light/dark vivarium. A subset of animals was housed in a polyethylene cage with a

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running wheel (Nalgene, 48 × 27 × 20 cm) for either 1, 3, 5, 7, 14, or 28 days ($n = 6$ running animals at each timepoint). Continuous recording of the number of wheel revolutions was achieved using computer software (RatRun, Carl Hage) integrated with hardware (magnetic switches) attached to each running wheel. The experiments were staggered so that all running animals were sacrificed within a 3 day period (two running groups sacrificed each day). Sedentary control animals ($n = 12$ in total), that had been housed for the same length of time as the running animals, were also sacrificed during this 3 day period (two sedentary animals were assigned to each group of running animals). All animals were sacrificed between 7 and 9 a.m. to avoid any variation introduced by the circadian rhythm of BDNF [3]. Furthermore, all animals were housed in the same room to avoid variation in vivarium conditions. The hippocampus from each hemisphere of the brain was micro-dissected so that there were two sets of hippocampal tissue from each animal, with one being utilized for protein analysis and the other for RNA analysis. BDNF protein was assessed using the BDNF E-Max ELISA kit (PROMEGA) according to the manufacturer's recommendations, and samples were not acid treated. Total RNA was extracted using Trizol (Life Technologies) following the manufacturer's instructions. RNA quality was assessed on an Agilent bioanalyzer system with RNA chips and only samples with a 28S/18S ratio greater than 1 were used. RT-PCR reactions included oligonucleotides for amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for normalization. Conditions were optimized to produce yields for both the GAPDH and BDNF amplification products, within the same reaction, in the linear range of detection for the Agilent bioanalyzer system using DNA-500 LabChips. Oligonucleotide combinations and specific conditions are detailed in Table 1. Oligonucleotide sequences for BDNF exon amplification (adapted from Ref. [18]) are as follows: BDNF-E1F (5′-CTCAAAGGGAAACGTGTCTCT-3′), BDNF-E2F (5′-CTAGCCACCGGGGTGGTGTAA-3′), BDNF-E3F (5′-TGCGAGTATTACCTCCGCCAT-3′), BDNF-E4F (5′-TTGGGGCAGACGAGAAAGCGC-3′), BDNF-E5R (5′-GAGAAGAGTGATGACCATCCT-3′) and BDNF-E5B (5′-TCACGTGCTCAAAAGTGTCAG-3′). Sequences for amplification of GAPDH are GAPDH1 (5′-TCCATGACAACTTTGGCATCGTGG-3′) and GAPDH2 (5′-GTTGCTGTTGAAGTCACAGGAGAC-3′). RT-PCR amplifications were carried out in a Robocycler (Stratagene) with hot top (50 °C for 30 min; 95 °C for 15 min followed by 25 cycles of 94 °C for 60 s; 57 °C for 60 s; 72 °C for 60 s and then incubation at 70 °C for 10 min). Then 1 μl of each reaction was diluted and analyzed by capillary electrophoresis on a DNA-500 LabChip® (Agilent systems). The corrected area of each amplification product was used to calculate the relative amount of the BDNF exon, expressed as a percentage of the GAPDH amplification product (GAPDH expression did not significantly change across experimental conditions).

For water maze studies, animals were exercised for 3 weeks prior to, as well as during, testing ($n = 7$ per group). A sedentary group was tested concurrently ($n = 8$ per group). The protocol was carried out as previously described [8], except that animals were only given two trials per day for 6 consecutive days. All trials were videotaped using an overhead CCD camera attached to a VCR. Swimming speed was determined by video tape analysis (measurement of relative swimming distance per unit time). BDNF exon and protein analyses were conducted using an analysis of variance (ANOVA) with an alpha level of 0.05 and Bonferroni post-hoc analysis. As there was no significant difference in BDNF expression between any of the sedentary animals, they were combined into one group for analysis. An analysis of covariance was utilized to determine whether the heterogeneity in the running distances affected the BDNF effects observed across the different timepoints. Correlational analyses were used to measure the association between BDNF exon and protein expression and both the running distance and the number of days running. Water maze data were analyzed using a repeated measures ANOVA with Bonferroni post-hoc analysis. All statistical analysis was carried out using StatView 5.0 for Macintosh.

The average distance run by the animals over the timecourse (shown in Fig. 1) showed an overall main effect ($F(5, 30) = 8.96$, $P < 0.0001$). Bonferroni post-hoc analysis revealed that the group given 28 days exposure to the running wheel had significantly higher average running distances compared to all other timepoints ($P < 0.0001$). There were no significant differences between any of the other timepoints.

The induction of BDNF protein across the timecourse (shown in Fig. 2) showed an overall main effect

Table 1
Oligonucleotide combinations and specific conditions for the amplification of each BDNF exon and the size of the amplification product

Exon	Forward oligo	Reverse oligo	Concentration of each BDNF oligo (μM)	Concentration of each GAPDH oligo (μM)	BDNF exon product size	Total RNA per 10 μl reaction (μg)
I	BDNF-E1F	BDNF-E5B	1.2	0.12	516	0.2
II	BDNF-E2F	BDNF-E5B	2.4	0.12	259	0.4
III	BDNF-E2F	BDNF-E5B	1.2	0.12	441	0.2
IV	BDNF-E4F	BDNF-E5B	2.4	0.12	337	0.4
V	BDNF-E5R	BDNF-E5B	1.2	0.12	215	0.2

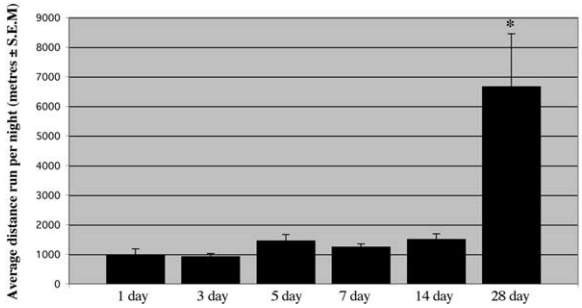

Fig. 1. Average distance (m) run per night ± standard error of the mean (SEM) for animals across the 4 week timecourse. There is a significant (*$P < 0.0001$) increase in running distance after 28 days that is significantly different as compared to all other timepoints. At no other timepoint does the average running distance increase significantly.

($F(6, 41) = 8.25$, $P < 0.0001$). Bonferroni post-hoc analysis revealed that the group given 28 days exposure to the running wheel had a significant upregulation in BDNF protein compared to the sedentary group (271% increase, $P < 0.0001$). This group also had significantly higher BDNF levels compared to all other timepoints ($P < 0.0001$). Further analyses did not indicate any other significant differences in BDNF protein between the running and sedentary groups at different timepoints. There was, however, a trend to increased BDNF protein after 14 days of exercise (166% of sedentary).

Across the timecourse there was a significant correlation when comparing the average protein expression and the average distance run at each timepoint ($R^2 = 0.91$, $P = 0.0013$).

The relative expression of each exon is shown in Fig. 3. Overall main effects are shown for exon I ($F(6, 41) = 8.66$, $P < 0.0001$), exon II ($F(6, 41) = 9.22$, $P < 0.0001$), exon IV ($F(6, 41) = 2.7$, $P = 0.027$) and exon V ($F(6, 41) = 6.83$, $P < 0.0001$).

Bonferroni post-hoc analysis revealed that, as compared to sedentary values, exon I was significantly ($P < 0.0001$) upregulated after both 1 (195% of sedentary) and 28 (230% of sedentary) days of exercise. Across the timecourse, there was a significant difference between 3 and 28 ($P = 0.0003$), 5 and 28 ($P = 0.0002$), 7 and 28 ($P = 0.0002$) and 14 and 28 ($P = 0.0001$) days of exercise.

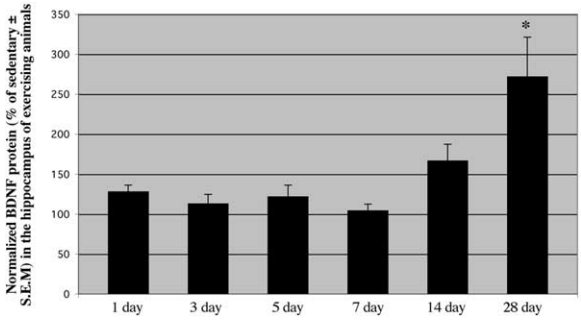

Fig. 2. Normalized BDNF protein (% of sedentary ± SEM) in the hippocampus of exercising animals (the average sedentary value is 100 ± 10%). There is a significant (*$P < 0.0001$) increase in BDNF protein after 28 days of voluntary exercise, as compared to sedentary, and this is also significantly ($P < 0.0001$) different from the exercise-induced BDNF protein levels at all other timepoints. BDNF protein levels after 1, 3, 5, 7, and 14 days of exercise are not significantly different from sedentary values, or from each other.

Exon II was significantly ($P < 0.0001$) upregulated, as compared to sedentary values, after only 28 (152% of sedentary) days of exercise. Across the timecourse, there was a significant difference between 1 and 28 ($P < 0.0001$), 3 and 28 ($P = 0.0008$), 5 and 28 ($P < 0.0001$), 7 and 28 ($P < 0.0001$) and 14 and 28 ($P = 0.0008$) days of exercise.

Exon IV showed only a significant difference across the timecourse, between 3 and 28 ($P = 0.0021$) days of exercise.

Exon V was significantly ($P < 0.0001$) upregulated, as compared to sedentary values, after only 28 (149% of sedentary) days of exercise. Across the timecourse, there was a significant difference between 1 and 28 ($P = 0.0001$), 3 and 28 ($P < 0.0001$), 5 and 28 ($P = 0.0015$), 7 and 28 ($P = 0.0007$) and 14 and 28 ($P = 0.0004$) days of exercise.

Across the entire timecourse there was a significant correlation, when comparing the average exon expression and the average distance run at each timepoint, for exon II ($R^2 = 0.73$, $P = 0.027$) and exon V ($R^2 = 0.96$, $P < 0.0001$). When analyzing exon expression and distance run at each individual timepoint, there were significant correlations for exon I after 7 days of exercise ($R^2 = 0.66$, $P = 0.048$), exon II after 14 days of exercise ($R^2 = 0.81$, $P = 0.01$), exon III after 28 days of exercise ($R^2 = 0.84$, $P = 0.007$) and exon V after 28 days of exercise ($R^2 = 0.83$, $P = 0.008$).

As shown in Fig. 4, exercised animals showed a decreased escape latency, compared to sedentary animals, in the Morris water maze. There was a significant overall effect ($F(1, 13) = 7.79$, $P = 0.015$), with a significant difference shown after 2 days ($P = 0.037$). Examination of the data for the first 2 days demonstrates that the exercised animals maintained their improvement on the task between days better than sedentary animals (Fig. 5). The comparison between trials across each day (i.e. trial 1 on day 1 compared to trial 1 on day 2, etc.) demonstrates that exercised animals significantly ($P < 0.05$) improved their performance between days, whereas the sedentary animals did not. There was no significant difference in swimming speed between the groups, consistent with the literature [21].

We have demonstrated that physical activity results in an increase in BDNF mRNA transcripts and protein, and that the most significant upregulation of both occurs following 28 days of voluntary exercise in the intact male rat. We further demonstrated that exercising animals showed an enhanced rate of learning in the Morris water maze.

The most significant increase in average running distance occurred between 14 and 28 days of exercise, which may reflect an improvement in physical conditioning of the animals. The average distances run by animals in this study, however, are in contrast to other studies where Sprague–Dawley rats have been reported to run more than 18000 m

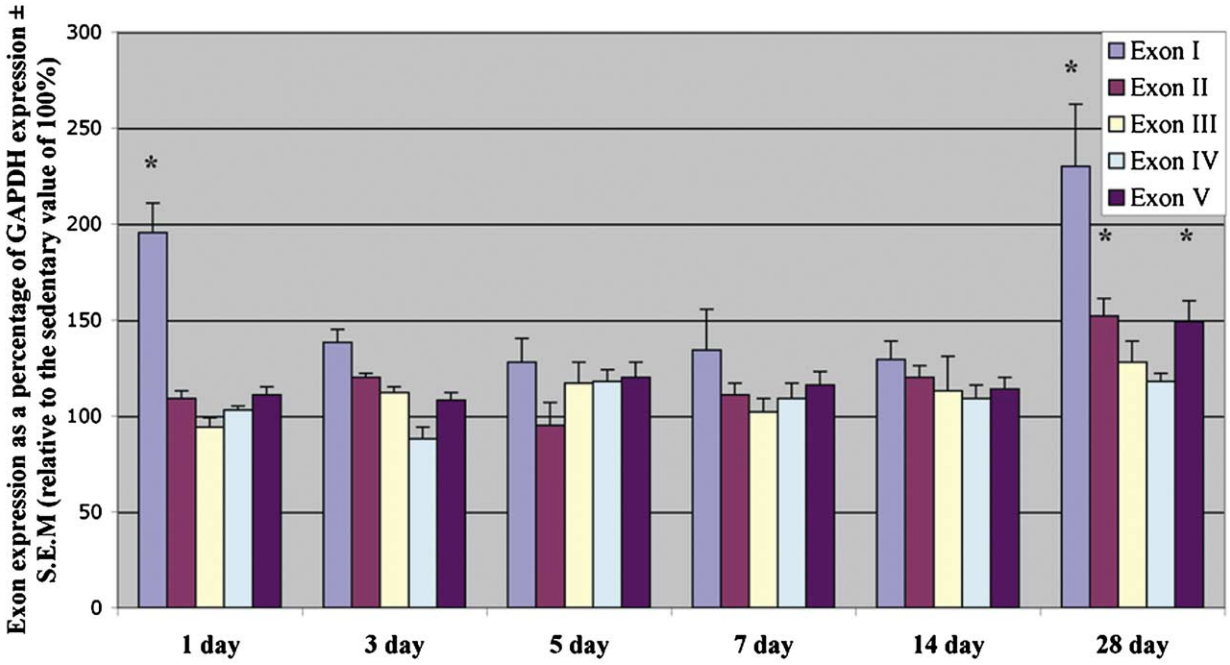

Fig. 3. BDNF exon expression as a percentage of GAPDH ± SEM in the hippocampus of exercising animals (relative to the average sedentary value of 100 ± 7%). Significant (*$P < 0.0001$) increases in exon expression as a result of exercise, as compared to sedentary, are shown for exon I at both 1 and 28 days, exon II at 28 days and exon V (the coding exon) at 28 days. There are also significant differences between individual exons across the timecourse, as shown for exon I (between 3 and 28 day; 5 and 28; 7 and 28; 14 and 28), exon II (all timepoints and 28 day), exon IV (3 and 28 day), and exon V (all timepoints and 28 day).

per night after 4 weeks of training [7]. There is, therefore, a heterogeneity within animal groups with regards their propensity to exercise, and this may have implications for BDNF expression.

The results of this study suggest that BDNF expression may be augmented as a function of both time spent exercising and the amount of exercise activity. The increase in protein observed at 14 days in this investigation, for example, was not associated with a large increase in running distance and may, therefore, be the combined result of the prior low levels of activity. Under periods of much greater activity, however, the induction of BDNF protein may be more directly related to the amount of exercise (such as seen after 28 days), and this may be the result of a close link between BDNF mRNA expression and the level of activity.

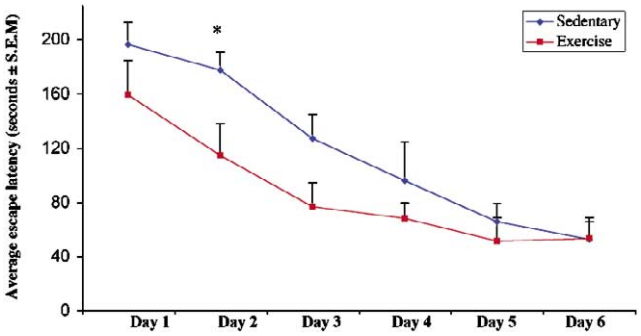

Fig. 4. Average escape latencies (s) ± SEM for the Morris water maze. Exercised animals, as compared to sedentary animals at each timepoint, show a trend to decreased escape latency that is significant (repeated measures ANOVA, *$P = 0.037$) only on day 2. There was no significant difference in the swimming speeds between the two groups.

BDNF protein expression is under complex regulation by multiple different transcript forms of BDNF mRNA, which is comprised of a variable 5′ end (encoded for by exons I–IV) and a common 3′ end (encoded by exon V) in the rat. The differential usage of the 5′ promotors, in response to different stimuli, results in differential patterns of expression of the BDNF transcripts [19]. Therefore, we examined the expression of the different BDNF exons (subfield changes were not delineated, as whole hippocampal homogenates were used to match the protein assessments) across the timecourse to gain insight into the transcriptional mechanisms driving BDNF protein regulation with physical activity. Studies using comparable exercise regimes have only examined BDNF mRNA after 6 and 12 h (exons I–V [13]) and 20 days of exercise (exon V [15]). This study demonstrated that a number of BDNF exons showed the greatest change following 28 days of voluntary exercise, where exon 5 (encoding mature BDNF protein) also correlated to the average running distance. Transcripts beginning at exon I showed the largest upregulation after 28 days, consistent with the hypothesis that transcription from this exon may contribute to the long term maintenance of BDNF expression. It was hypothesized that exon III would increase significantly early in the timecourse, as it is a calcium responsive transcript that has been shown to be upregulated within 60 min of membrane depolarization-induced calcium influx in cortical cultures, and even further

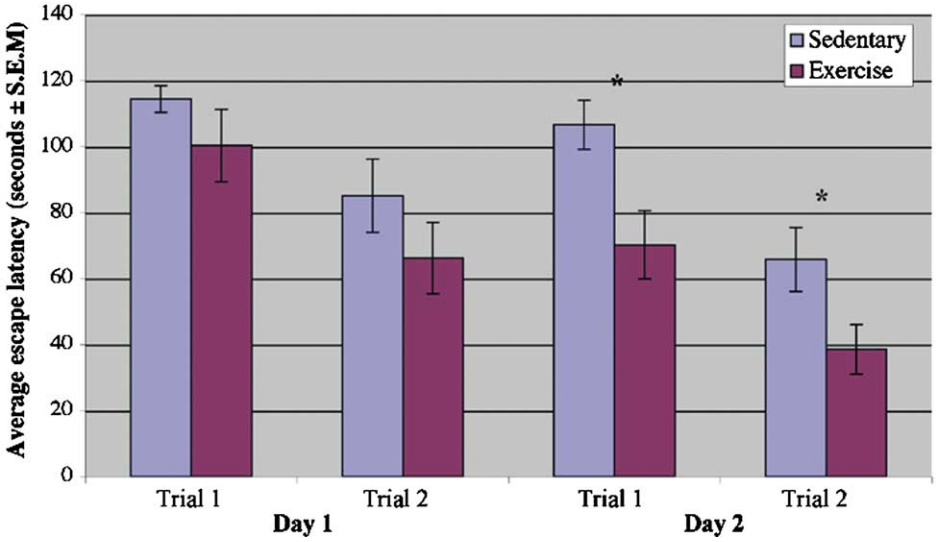

Fig. 5. Average escape latencies (s) ± SEM for the first 2 days of trials in the Morris water maze. There is a significant difference between escape times in the exercise group, as compared to the sedentary group, on both trials of day 2 (*$P < 0.05$). The comparison between trials across each day (i.e. trial 1 on day 1 compared to trial 1 on day 2, etc. within each group) demonstrates that exercised animals significantly ($P < 0.05$) improved their performance between days, whereas the sedentary animals did not.

induced after depolarization for 3–6 h [17]. We did not observe any significant early changes in exon III as a function of exercise, suggesting that changes in this exon are more transient and occur earlier than detectable in this timecourse. In summary, the machinery driving the expression of BDNF protein appears to be sensitive to high activity levels.

The water maze data are consistent with other studies demonstrating that rats exercised prior to testing on an eight arm radial maze required 30% fewer trials to acquire criterion performance than the sedentary animals [1]. Similarly, in a passive avoidance task, exercised rats had significantly better short and long term memory than sedentary control animals [14]. These data are also consistent with those demonstrated in exercised mice tested in the Morris water maze [21]. Exercise, therefore, may facilitate the encoding of new information.

We have shown that exercise training modulates the induction of BDNF mRNA and protein in a time-dependent manner within the hippocampus, and this may contribute to the maintenance of brain health and plasticity [5]. Further studies are required to understand the link between high activity levels and BDNF regulation.

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