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# Compulsive exercise acutely upregulates rat hippocampal brain-derived neurotrophic factor

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**Summary.** This study was to examine the effects of treadmill exercise on the expression of brain-derived neurotrophic factor (BDNF) in rat hippocampus. After 1-wk treadmill familiarization, animals in exercise groups received a 4-wk exercise training or an acute exercise. They were sacrificed 2 h or 2 d after exercise and their hippocampal BDNF mRNA and protein levels were determined. We demonstrated that 1) hippocampal BDNF mRNA and protein levels were both elevated in response to exercise training at 2 h after the last run but not after 2 d; 2) an acute moderate exercise (1 or 3 d) increased BDNF protein levels; 3) acute severe exercise increased BDNF protein and mRNA levels in animals under a familiarization regimen, while suppressed the BDNF mRNA level in rats without treadmill familiarization, paralleling the stress effect of immobilization/water exposure. We conclude that compulsive treadmill exercise with pre-familiarization acutely upregulates rat hippocampal BDNF gene expression.

**Keywords:** Treadmill exercise, neural plasticity, stress, BDNF.

## Introduction

Brain-derived neurotrophic factor (BDNF), broadly and abundantly expressed in mammalian brain, is a member of the neurotrophin family. It plays an important role in various aspects of neural plasticity, such as neurogenesis, long-term potentiation (LTP), learning and memory, and even mood changes (Yamada et al., 2002). The mutation or deletion of BDNF gene in mice results in learning deficits and LTP impairment, whereas re-expression of BDNF restores LTP (Korte et al., 1996; Gorski et al., 2003). In patients with Alzheimer's disease or major depression, the brain or serum levels of BDNF are decreased (Murer et al., 2001; Karege et al., 2002). Accrued evidence has demonstrated that stress or corticosterone administration reduces BDNF mRNA expression in the brain (Smith et al., 1995; Schaaf et al., 1997; Ueyama et al., 1997).

Epidemiological studies suggest that physical activity is associated with a low incidence of cognitive impairment and Alzheimer's disease (Byrne and Byrne, 1993). Furthermore, some studies suggest that voluntary exercise

enhances neural plasticity and improves learning and memory (Fordyce and Wehner, 1993; Cotman and Berchtold, 2002). Taken together, these reports imply that exercise has beneficial effects on the brain function even though the underlying mechanisms deserve a full spectrum of understanding. Several studies suggest that exercise leads to changes in the expression of a number of genes that are involved in synaptic function and neural plasticity. For instance, voluntary wheel running exercise increases the BDNF gene expression in rat hippocampus (Neeper et al., 1995; Oliff et al., 1998; Adlard et al., 2004). Thus, it has been thought that exercise may exert beneficial effects on the brain function by regulating BDNF levels and its related neural plasticity.

Running wheel exercise is commonly used in previous studies, as treadmill running is considered as a stressor to rodents. Because stress and glucocorticoids have been reported to suppress the BDNF expression in hippocampus (Smith et al., 1995), the possible adverse effect of compulsive exercise needs to be avoided. Nonetheless, results obtained from voluntary wheel running studies could not completely rule out possible confounding effects of non-exercise training factors. For example, there might be genetic differences between good runners and poor runners. Likewise, voluntary wheel running designs could not rule out the effects of environment enrichment. Moreover, the temporal relationship between the end of running wheel exercise and the effect measurement was ill-defined, as evidenced by animal sacrifice with little information about the duration, intensity, and cessation time of their last run. Therefore, the purpose of this study was to study whether the treadmill exercise protocols with controlled exercise duration, intensity and cessation time affect rat hippocampal BDNF gene expression, and to further characterize whether such effects were acute or chronic. Since compulsive treadmill exercise was thought to have stress effects, additional experiments were performed using rats subjected to two different stressors, i.e., acute severe treadmill exercise without familiarization or exposure to immobilization/water, as a comparison. Our results indicated that compulsive exercise, when executed properly, induced transient BDNF gene expression in rat hippocampus, and that this effect depended on the intensity/previous experience of exercise.

## Materials and methods

### *Animals and exercise/stress protocols*

Male Wistar rats were used in this study. All animals were housed in an environmentally controlled room (temperature $25 \pm 1^{\circ}C$; 12 h light/12 h dark cycle) in groups of three or four per cage with rat chow and water ad libitum. This study was conducted with approval of the National Cheng Kung University Animal Care and Use Committee and was carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996. All efforts were made to minimize the number of animals used and their suffering.

For the exercise-training study, 4-wk-old male Wistar rats were allowed to run on the treadmill at a low speed of 9 m/min for 10 min each day for 5 days to get familiar with the treadmill running. They were then randomly divided into control and exercise groups. The animals in exercise groups were trained for 4 weeks on a motor-driven leveled treadmill (Model T408E, Diagnostic & Research Instruments Co., Taoyuan, Taiwan) at an intensity of ~70% of maximal oxygen consumption for 60 min/d, 5 d/wk for 4 weeks (Chu et al., 2000). The training speeds began at 12 m/min and reached 15 m/min by the end of the experiments. The sedentary control groups were placed in the treadmill without running for 10 min/d. After training, animals either received Morris water maze tests or were sacrificed 2 h or 2 d after the last run for sample collections.

For the acute-exercise study, 8-wk-old male Wistar rats were assigned to either control or acute exercise groups after 1-wk familiarization. The exercised animals performed moderate exercise (15 m/min for 30 min/d, for 1 or 3 consecutive days) or a single bout of severe exercise until exhaustion (beginning with 12 m/min followed by 3 m/min increments every

3 min up to 24 m/min until exhaustion). Because our preliminary time-course study (0, 1, 2 and 4 h) showed that an elevated BDNF protein level only occurred at 2 or 4 h after a single bout of exercise (data not shown), the time point for animal sacrifice in the present study was chosen at 2 h after exercise cessation.

To study the stress effects, some rats (8-wk-old) were forced to run on the treadmill until exhaustion without familiarization, or were immobilized by mesh and exposed to 1.5 cm-depth water ($20 \pm 2$°C) for 1 h (Morimoto et al., 2000). These animals were sacrificed 2 h after stress intervention.

The electric shock was never applied to animals during treadmill running, and these animals were killed under carbon dioxide euthanasia at specified time points after exercise/stress treatments. In order to avoid circadian differences in BDNF gene expression, all animals were sacrificed around 2 p.m.

### *Determination of citrate synthase activity in soleus muscles*

An increase in citrate synthase activity is commonly used to confirm the exercise training effect. In the present study, citrate synthase activity in soleus muscles was measured using a method reported in details previously (Yang et al., 2003).

### *Morris water maze task*

A separate group of animals received the water maze test at the end of 4-wk treadmill running. A plastic circular pool, 1.8 m in diameter, and 0.6 m in height, were filled with water ($20 \pm 2$°C) 1.5 cm above a hidden plexiglass platform, which was placed at a specific location away from the edge of the pool. Animals were subjected to four trials a session, two sessions a day, with one given in the morning and the other in the afternoon. A total of four sessions were completed in two days. In each trial, animals were placed at four different starting positions equally spaced around the perimeter of the pool in a random order. Animals were allowed to find the platform in 120 s. If an animal could not find the platform within this period of time, it would be guided to the platform. After mounting the platform, animals were allowed to stay there for 20 s. The time each animal spent to reach the platform was recorded as the escape latency. The average escape latencies between control and exercise groups were compared. To rule out the effects of differential motor activities/motivation between control and exercise animals, the visible platform experiments were performed following the water maze test.

### *Measurement of hippocampal BDNF protein levels*

The hippocampus of control or experimental (exercise or stress) animals was obtained after $CO_2$ euthanasia. The BDNF protein concentration in the hippocampal homogenate was determined in duplicate using an enzyme-linked immunosorbent assay (ELISA) kit (Promega, Madison, WI, USA) following the manufacturer's instructions.

### *Measurement of the hippocampal BDNF mRNA levels*

Total RNA was extracted from the hippocampus of individual rat using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) after homogenizing the hippocampal tissues by a homogenizer (Tissue Tearor, Model 985370, Biospec Products). DNase I (Promega, Madison, WI, USA) treatment was used to remove DNA contamination in the total RNA. The BDNF mRNA was measured by real-time RT-PCR (LightCycler System, Roche, Mannheim, Germany), and was normalized by the mRNA level of β-tubulin. Briefly, 1 μg of hippocampal total RNA from each rat was reversely transcribed by the reverse transcriptase SuperScript™ II (Invitrogen) in a 20 μl reaction mixture. The RT products were diluted 10 times. Five μl of the diluents were mixed with 15 μl of the amplification mixture containing 1× LightCycler FastStart Reaction Mix SYBR Green I, FastStart Enzyme and 1 μM each of the specific primers. The mixture was subjected to initial denaturation at 95°C for 7 min followed by a 45-cycle amplification program consisting of denaturation at 95°C for 5 s, annealing at 60°C for 5 s, and elongation at 72°C for 10 s. The temperature transition rate for the amplification program was 20°C/s. The melting curve analysis was performed immediately after amplification to confirm the specificity of PCR products, and consisted of 1 cycle of heating at 20°C/s to 95°C with a 0-s hold, cooling at 20°C/s to 65°C with a 15-s hold, and heating at 0.1°C/s to 98°C with a 0-s hold. For the establishment of standard curves, 5 μl of BDNF or tubulin cDNA diluted in series were added into the amplification mixture. The primer sequences were: forward 5′-GGACATATCCATGACCAGAAAGAAA-3′ and reverse 5′-GCAACAAACCACAACATTATCGAG-3′ for BDNF exon V (Korte et al., 1996); forward 5′-CCAGATCGGTGCTAAGT-3′ and reverse 5′-CGGAGTCCATAGTCCC-3′ for β-tubulin. The amplicon size is 89 bp for BDNF gene, and 185 bp for β-tubulin gene. Fluorescence signals of each capillary were continuously acquired once per cycle for quantification analysis during the melting curve analysis.

The values of fluorescence signals and crossing points were used to establish standard curves. Data analyses were performed using the Lightcycler software Version 3.5.

### *Determination of serum corticosterone levels*

To evaluate the possible stress levels associated with our exercise protocols, serum corticosterone levels were measured by ELISA (IDS, Bolodn, UK).

### *Statistical analysis*

Data were expressed as mean ± SEM. Sample sizes were indicated by n. Unpaired Student's *t* test was used to compare the differences between the exercise and control groups. One-way analysis of variance was employed to analyze the differences, followed by Tukey's multiple comparison if appropriate. Significance was established at $P<0.05$.

## Results

### *Citrate synthase activity*

The enzyme activity of citrate synthase, an index of oxidative metabolism, was significantly increased after 4-wk exercise training ($1.26\pm0.03$ vs. $1.18\pm0.03$ μmole/min/g wet weight for exercise training and sedentary control groups, respectively; $P<0.05$), indicating that the exercise training protocol was effective.

### *Effects of exercise on BDNF mRNA and protein expressions*

After 4 weeks of treadmill exercise training, an increase in the expression of BDNF mRNA and protein was found 2 h after the last run, but not 2 days later (Fig. 1). These results imply that the effect of exercise training on BDNF expression is an acute rather than a chronic effect. As for acute exercise experiments, we also found that 2 h after single bouts of either moderate or severe exercise significantly elevated BDNF protein expression (Fig. 2B). Interestingly, acute exercise with high intensity dramatically increased BDNF mRNA expression, whereas acute exercise with moderate intensity was ineffective in this regard (Fig. 2A).

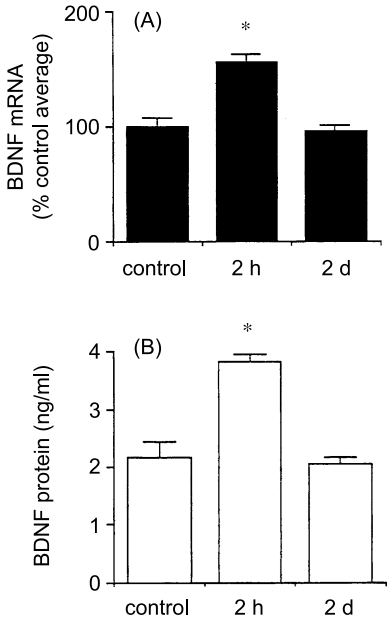


**Fig. 1.** Effects of 4-wk treadmill running on the expression of BDNF mRNA (**A**) and protein levels (**B**). Increases in BDNF mRNA and protein expression occurred 2 h ($^*P<0.05$, comparing with sedentary control), but not 2 d, after the last run (n = 7–10 for each group). *h* hour; *d* day

### *Effects of acute stress on BDNF mRNA expression*

Figure 3 shows that stress from immobilization plus water exposure for 1 h led to a decrease in BDNF mRNA and protein expression in animals sacrificed 2 h post-stress. Similarly, an acute severe exercise without familiarization also resulted in a reduction of BDNF gene expression (Fig. 4).

### *Effects of exercise training and restraint stress on spatial learning*

Animals after 4-wk exercise training had lower mean escape latency than the controls ($37.6\pm2.2$ and $44.3\pm3.1$ s, respectively; $P<0.05$, n = 24). This result was not due to

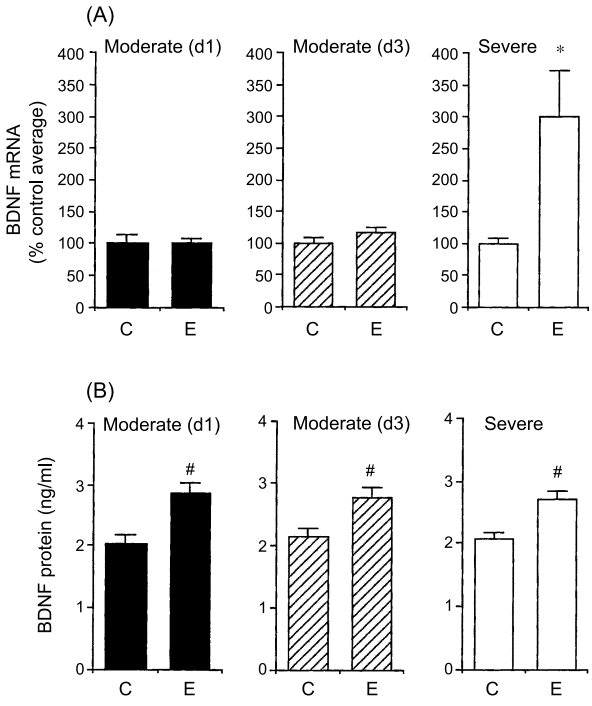


**Fig. 2.** Effects of acute treadmill exercise with previous familiarization on the BDNF mRNA expression (**A**) and protein levels (**B**). BDNF protein levels in rat hippocampus significantly increased 2 h after a single bout of severe/moderate exercise, or three repetitive moderate exercises ($^{\#}P<0.01$, comparing with resting control). However, BDNF mRNA was significantly upregulated 2 h after a single bout of severe exercise ($^{*}P<0.05$, comparing with resting control), but not after one or three repetitive moderate exercise (n = 9–11 for each group). *C* control; *E* exercise

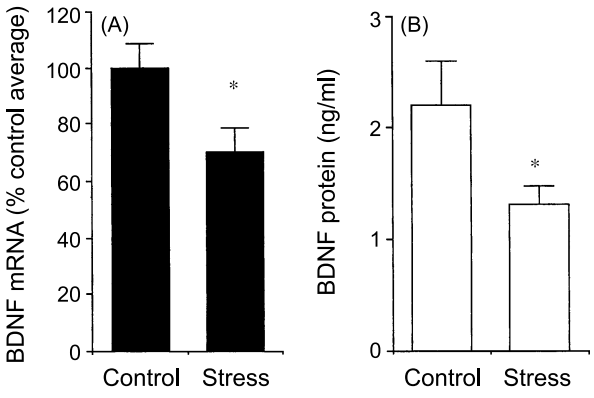


**Fig. 3.** Effects of immobilization stress on the expression of BDNF mRNA (**A**) and protein (**B**). BDNF mRNA expression and protein levels were significantly suppressed 2 h after immobilization stress intervention ($^{*}P<0.05$, comparing with unstressed control; n = 4 for each group)

Immediately after acute exercise, the serum corticosterone level drastically increased (Fig. 5), an expected normal response to acute exercise. One hour later, serum corticosterone levels returned back to resting values. We also observed that exercise training did not alter resting serum corticosterone level measured two days after the last running. These results supported that the treadmill exercise with a familiarization protocol used in the present study did not cause significant stress to the animals.

differences in swimming capability or motivation between control and exercise groups since both groups performed similarly in visible platform experiments ($3.2 \pm 0.1$ vs. $3.0 \pm 0.1$ s for control and exercise groups, respectively, $P>0.1$). In contrast, animals in the immobilization/water exposure stress group tended to spend more time to find the hidden platform ($50.2 \pm 4.0$ vs. $45.6 \pm 4.7$ s for the stress and control groups, respectively, n = 12–13). These results suggested that 4 weeks of treadmill exercise training significantly improved the spatial learning capability, whereas acute stress seemed to impair it.


**Fig. 4.** Effects of acute severe exercise stress without familiarization on the expression of BDNF mRNA (**A**) and protein (**B**). BDNF mRNA expression and protein levels were significantly suppressed 2 h after this intervention ($^{*}P<0.05$, comparing with unstressed control; n = 4 for each group)

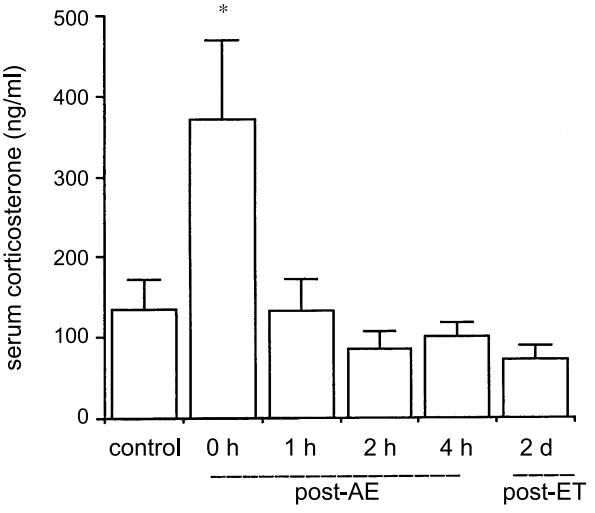


**Fig. 5.** Effects of exercise on serum corticosterone levels. The serum corticosterone levels increased immediately after acute moderate exercise (i.e., 0 h; $^{*}P < 0.05$, comparing with resting control), and returned to basal levels after 1 h (n = 4 for each group). In addition, treadmill exercise training did not significantly change resting serum corticosterone levels, as shown in 2 d. *AE* acute exercise; *ET* exercise training

## Discussion

Compulsive treadmill exercise and voluntary wheel running are two totally different exercise paradigms. Previous reports using voluntary wheel running paradigm as the exercise protocol have demonstrated the beneficial effect of exercise on the brain. This study is the first to reveal that treadmill exercise with careful animal handling in Wistar rats can also be a good paradigm to study the effects of exercise on the brain function. Our results indicate that a single bout of compulsive treadmill exercise or repetitive moderate exercise training with previous familiarization acutely induces hippocampal BDNF gene expression. In addition, we have demonstrated that the BDNF mRNA expression is reduced not only by immobilization plus water exposure but also by strenuous exercise without familiarization.

In addition to enhancing the hippocampal BDNF gene expression, treadmill exercise also improves spatial learning capability, in confirming with a previous study done by Fordyce and Wehner (1993). The fact that treadmill exercise in the present study has hippocampal BDNF upregulating effects provides noteworthy implication. First, treadmill running not only offers a well-controlled exercise paradigm but also is suitable for studying the exercise effects on brain functions, if the animals are handled carefully to avoid confounding stress effects. Second, as a compulsive exercise has beneficial effects in terms of BDNF upregulation and learning/memory improvement in rats, a similar mechanism may explain why human exercise with efforts is effective in improving cognitive functions (Byrne and Byrne, 1993). Several previous reports have indicated that voluntary wheel running for various days increases BDNF gene expression in the hippocampus of either male or female Sprague-Dawley rats (Neeper et al., 1995; Oliff et al., 1998; Berchtold et al., 2001; Adlard et al., 2004). Similar results have been confirmed in a study comparing various rat strains (Sprague-Dawley, Brown Norway, Dark Aguouti, and PVG), indicating that BDNF levels increase with voluntary exercise regardless the strain differences in spontaneous activity (Johnson and Mitchell, 2003).

When comparing running wheels with treadmills, differential exercise effects on brain monoamine levels and some behavioral tests have been reported (Dishman, 1997). Assuming that the compulsive treadmill running is stressful to rodents, many investigators prefer to use running wheel as an exercise mode. The strength of running wheel protocol is that animals exercise voluntarily and thus avoiding possible stress effects. In our hands, the possible stress associated with treadmill exercise was minimal if animals were familiarized with the treadmill running for one week before the experiments. If this familiarization step was omitted, acute severe exercise would impair the gene expression of BDNF, similar to that exposed to the immobilization stress. These

results are consistent with previous studies reporting that stress and glucocorticoids suppress hippocampal BDNF mRNA expression in normal or adrenalectomized rats (Smith et al., 1995; Schaaf et al., 1997; Ueyama et al., 1997).

It is interesting to note that repetitive exercise increases BDNF protein levels to a greater extent than a single bout of exercise (Figs. 1 and 2); possibly due to the priming effect of repetitive exercise (Berchtold et al., 2005). Moreover, the exercise-induced BDNF upregulation is an acute effect in the current study. That is, BDNF gene expression was upregulated 2 h, but not 2 d, after the last run of 4-wk treadmill running. In fact, BDNF has been reported to act as a brain-activity dependent immediate-early gene (Lautherborn et al., 1996; Castren et al., 1998; Hall et al., 2000). To our knowledge, rats have higher activity in the dark phase than in the light phase. Animals accessing the running wheels in previous studies were likely to be sacrificed within a few hours after their last run, i.e., in the early morning of the following day. Therefore, it would be difficult to rule out the possibility that the effect of voluntary wheel running on BDNF gene expression may be an acute effect.

Furthermore, the exercise-evoked BDNF mRNA expression was absent either after acute moderate exercise or 2 d after 4-wk exercise training, but it occurred 2 h after acute strenuous exercise or 2 h after the last run of exercise training. These observations suggest that the upregulation of BDNF mRNA expression depends on exercise intensity or duration. However, both acute moderate and severe exercise elevated BDNF protein contents in the hippocampus. We therefore speculate that acute treadmill exercise, regardless of its intensity, may alter BDNF expression at the translational level or posttranslational level, whereas only high intensity or long-term exercise can affect BDNF gene expression at the transcriptional level. In general, the real-time PCR analysis is rather confined to steady state mRNA levels, and mRNA abundance dose not necessarily reflect the levels of corresponding proteins (Pradet-Balade et al., 2001). Protein levels are regulated not only by transcriptional mechanism but also by several posttranscriptional mechanisms including transcript localization and stability, translational regulation and protein degradation (Sonenberg et al., 2000). Further study is needed to explore the underlying mechanisms of exercise-evoked BDNF expression.

Although the underlying mechanisms of treadmill exercise-induced upregulation of BDNF gene expression are still unknown, several possible explanations are enlisted in the following. First, an elevation of catecholamine, such as norepinephrine (NE), during exercise may activate BDNF gene expression. It has been suggested that the released NE may act upon its G-protein coupled receptor, which in turn activates downstream intracellular signaling pathways. One possibility is the cAMP/protein kinase A/CREB pathway. Another possibility is that the transactivation of tropomyosin related kinase B by NE receptor binding activates the phosphatidylinositol 3-kinase/glycon synthase kinase-3/CREB pathway. The phosphorylated CREB then binds to the cAMP response element on the promoter region of the BDNF gene and increases the BDNF mRNA and protein levels (Chen and Russo-Neustadt, 2005). In contrast, NE blockade has been reported to inhibit BDNF mRNA activation following exercise in an animal study (Ivy et al., 2003). Second, exercise-evoked neuronal activity may increase BDNF gene expression. As indicated in previous animal studies, an increase in the neuronal activity enhances BDNF transcription (Castren et al., 1998; Murer et al., 2001). Third, an elevated calcium level in the brain during exercise (Akiyama and Sutto, 1999) may upregulate BDNF gene expression via the activation of cyclic AMP response element-binding protein (Shen et al., 2001; West et al., 2001).

In this study, we found that 4-wk treadmill exercise running not only increased hippocampal BDNF gene expression but also improved spatial learning. Whether the increased BDNF expression contributes to the better spatial learning after treadmill exercise training is still unknown. An indirect association between hippocampal BDNF level and learning/memory is likely. Elevated BDNF levels may facilitate neurogenesis and increase synaptic connections, which in turn help learning and memory functions (Yamada et al., 2002). A recent study illustrates that inhibiting BDNF action blocks the beneficial effects of running wheel exercise on the performance in the Morris water maze task (Vaynman et al., 2004). Therefore, treadmill exercise training in the present study could improve spatial learning by elevating BDNF gene expression. To clarify the crucial role of BDNF in treadmill exercise-improved spatial learning, further studies by using BDNF knockout mice will be needed in the future.

The rat BDNF gene consists of four 5′-exons (exons I–IV) and one 3′-exon (exon V) encoding the mature protein (Timmusk et al., 1993). In the present study, we measured the total mRNA expression of BDNF by designing the primers for exon V. Previous studies have shown that neuronal activity induced by stimulation of a single paroxysmal afterdischarge mainly increases the expression of BDNF exon III- and IV-containing transcripts in 2 h (Lautherborn et al., 1996), and that 3 days of voluntary wheel running significantly upregulates BDNF exon III mRNA expression in hippocampal regions (Oliff et al., 1998). Whether the treadmill exercise upregulates BDNF exon III or other transcripts remains to be answered.

In conclusion, our results demonstrate that treadmill exercise training in rats improves spatial learning, and that compulsive treadmill exercise with familiarization and careful animal handling increases hippocampal BDNF gene expression transiently.

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## References

Adlard PA, Perreau VM, Engesser-Cesar C, Cotman CW (2004) The timecourse of induction of brain-derived neurotrophic factor mRNA and protein in the rat hippocampus following voluntary exercise. Neurosci Lett 363: 43–48

Akiyama K, Sutto D (1999) Rectifying effect of exercise on hypertension in spontaneously hypertensive rats via a calcium-dependent dopamine synthesizing system in the brain. Brain Res 823: 154–160

Berchtold NC, Kesslak JP, Pike CJ, Adlard PA, Cotman CW (2001) Estrogen and exercise interact to regulate brain-derived neurotrophic factor mRNA and protein expression in the hippocampus. Eur J Neurosci 14: 1992–2002

Berchtold NC, Chinn G, Chou M, Kesslak JP, Cotman CW (2005) Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. Neuroscience 133: 853–861

Byrne A, Byrne DG (1993) The effect of exercise on depression, anxiety and other mood states: a review. J Psychosom Res 37: 565–574

Castren E, Berninger B, Leingartner A, Lindholm D (1998) Regulation of brain-derived neurotrophic factor mRNA levels in hippocampus by neuronal activity. Prog Brain Res 117: 57–64

Chen MJ, Russo-Neustadt AA (2005) Exercise activates the phosphatidylinositol 3-kinase pathway. Mol Brain Res 135: 181–193

Chu TF, Huang TY, Jen CJ, Chen HI (2000) Effects of chronic exercise on calcium signaling in rat vascular endothelium. Am J Physiol 279: H1441–H1446

Cotman CW, Berchtold NC (2002) Exercise: a behavioral intervention to enhance brain health and plasticity. Trends Neurosci 25: 295–301

Dishman RK (1997) Brain monoamines, exercise, and behavioral stress: animal models. Med Sci Sports Exerc 29: 63–74

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

Gorski JA, Balogh SA, Wehner JM, Jones KR (2003) Learning deficits in forebrain-restricted brain-

derived neurotrophic factor mutant mice. Neuroscience 121: 341–354

Hall J, Thomas KL, Everitt BJ (2000) Rapid and selective induction of BDNF expression in the hippocampus during contextual learning. Nat Neurosci 3: 533–535

Ivy AS, Rodriguez FG, Garcia C, Chen MJ, Russo-Neustadt AA (2003) Noradrenergic and serotonergic blockade inhibits BDNF mRNA activation following exercise and antidepressant. Pharmacol Biochem Behavior 75: 81–88

Johnson RA, Mitchell GS (2003) Exercise-induced changes in hippocampal brain-derived neurotrophic factor and neurotrophin-3: effects of rat strain. Brain Res 983: 108–114

Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry J-M (2002) Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res 109: 143–148

Korte M, Griesbeck O, Gravel C, Carroll P, Staiger V, Thoenen H, Bonhoeffer T (1996) Virus-mediated gene transfer into hippocampal CA1 region restores long-term potentiation in brain-derived neurotrophic factor mutant mice. Proc Natl Acad Sci USA 93: 12547–12552

Lautherborn JC, Rivera S, Stinis CT, Hayes VY, Isackson PJ, Gall CM (1996) Differential effects of protein synthesis inhibition on the activity-dependent expression of BDNF transcripts: evidence for immediate-early gene responses from specific promoters. J Neurosci 16: 7428–7436

Morimoto K, Tan N, Nishiyasu T, Sone R, Murakami N (2000) Spontaneous wheel running attenuates cardiovascular responses to stress in rats. Pflugers Arch Eur J Physiol 440: 216–222

Murer MG, Yan Q, Raisman-Vozari R (2001) Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. Prog Neurobiol 63: 71–124

Neeper SA, Gomez-Pinilla F, Choi J, Cotman C (1995) Exercise and brain neurotrophins. Nature 373: 109

Oliff HS, Berchtold NC, Isackson P, Cotman CW (1998) Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus. Mol Brain Res 61: 147–153

Pradet-Balade B, Boulme F, Beug H, Mullner EW, Garcia-Sanz JA (2001) Translation control: bridging the gap between genomics and proteomics? Trends Biochem Sci 26: 225–229

Schaaf MJM, Hoetelmans RWM, de Kloet ER, Vreugdenhil E (1997) Corticosterone regulates expression of BDNF and trkB but not NT-3 and trkC mRNA in the rat hippocampus. J Neurosci Res 48: 334–341

Shen H, Tong L, Balazs R, Cotman CW (2001) Physical activity elicits sustained activation of the cyclic AMP response element-binding protein and mitogen-activated protein kinase in the rat hippocampus. Neuroscience 107: 219–229

Smith MA, Makino S, Kvetnansky R, Post RM (1995) Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J Neurosci 15: 1768–1777

Sonenberg N, Hershey JWB, Mathews MB (2000) Translational control of gene expression. Cold Spring Harbor Laboratory Press, Plainview, NY (Cold Spring Harbor Monograph Series)

Timmusk T, Palm K, Metsis M, Reintam T, Paalme V, Saarma M, Persson H (1993) Multiple promoters direct tissue-specific expression of the rat BDNF gene. Neuron 10: 475–489

Ueyama T, Kawai Y, Nemoto K, Sekimoto M, Tone S, Senba E (1997) Immobilization stress reduces the expression of neurotrophins and their receptors in the rat brain. Neurosci Res 28: 103–110

van Praag H, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nat Neurosci 2: 266–270

Vaynman S, Ying Z, Gomez-Pinilla F (2004) Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci 20: 2580–2590

West AE, Chen WG, Dalve MB, Dolmetsch RE, Kornhauser JM, Shaywitz AJ, Takasu MA, Tao X, Greenberg ME (2001) Calcium regulation of neuronal gene expression. Proc Natl Acad Sci USA 98: 11024–11031

Yamada K, Mizuno M, Nabeshima T (2002) Role of brain-derived neurotrophic factor in learning and memory. Life Sci 70: 735–744

Yang AL, Jen CJ, Chen HI (2003) Effects of high-cholesterol diet and parallel exercise training on the vascular function of rabbit aortas: a time course study. J Appl Physiol 95: 1194–1200

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