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HIPPOCAMPAL BRAIN-DERIVED NEUROTROPHIC FACTOR BUT NOT NEUROTROPHIN-3 INCREASES MORE IN MICE SELECTED FOR INCREASED VOLUNTARY WHEEL RUNNING

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Abstract—Voluntary wheel running in rats increases hippocampal brain-derived neurotrophic factor (BDNF) expression, a neurochemical important for neuronal survival, differentiation, connectivity and synaptic plasticity. Here, we report the effects of wheel running on BDNF and neurotrophin-3 (NT-3) protein levels in normal control mice, and in mice selectively bred (25 generations) for increased voluntary wheel running. We hypothesized that increased voluntary wheel running in selected (S) mice would increase CNS BDNF and NT-3 protein levels more than in control (C) mice. Baseline hippocampal BDNF levels (mice housed without running wheels) were similar in S and C mice. Following seven nights of running, hippocampal BDNF increased significantly more in S versus C mice, and levels were correlated with distance run (considering C and S mice together). Spinal and cerebellar BDNF and hippocampal NT-3 levels were not significantly affected by wheel running in any group, but there was a small, positive correlation between spinal C3–C6 BDNF levels and distance run (considering C and S mice together). This is the first study to demonstrate that mice which choose to run more have greater elevations in hippocampal BDNF, suggesting enhanced potential for exercise-induced hippocampal neuroplasticity.

Key words: BDNF, exercise, genetics, hippocampus, neurotrophin, spinal cord.

Brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are structurally related members of the nerve growth factor family of neurotrophins, and are found in many areas of the brain and spinal cord (Kawamoto et al., 1996; Yan et al., 1997; Friedman et al., 1998; Dreyfus et al., 1999). Neurotrophins play key roles in neuronal survival, differentiation, connectivity and plasticity (for reviews see: Lewin and Barde, 1996; Lu and Chow, 1999; McAllister et al., 1999; Schinder and Poo, 2000; Huang and Reichardt, 2001; Poo, 2001). Neuronal activity regulates neurotrophin expression in the hippocampus (Zafra et al., 1990; Patterson et al., 1992; Nanda and Mack, 2000) and spinal cord (Scarisbrick et al., 1999; Widenfalk et al., 2001). Voluntary exercise also affects neurotrophin expression (for review see: Cotman and Berchtold, 2002). Specifically, running wheel activity increases BDNF mRNA in the hippocampus, cerebellum and spinal cord of rats (Neeper et al., 1995, 1996; Gómez-Pinilla et al., 2001). This mRNA is translated into BDNF protein in the hippocampus and spinal cord (Berchtold et al., 2001; Gómez-Pinilla et al., 2001). Although physical activity increases hippocampal BDNF protein levels in rats, hippocampal NT-3 protein concentrations appear to be differentially regulated, actually decreasing following running-wheel activity (Johnson and Mitchell, 2003).

The importance of exercise as a behavioral intervention to enhance neuroplasticity has recently come to light (for review see: Cotman and Berchtold, 2002). For example, voluntary wheel running is positively correlated with increased neuronal survival and resistance to neural injury (Stummer et al., 1994; Carro et al., 2001), and with increased hippocampal neurogenesis and maze performance in mice (van Praag et al., 1999b). Because hippocampal BDNF is up-regulated following physical activity, this neurotrophin may underlie exercise-induced neuroplasticity. Since exercise-induced changes in hippocampal BDNF and NT-3 levels in rats appear to be correlated with distance run (Neeper et al., 1995; Johnson and Mitchell, 2003), exercise may alter neurotrophins to a greater extent in animals that choose to be more active. Therefore, we studied mice selectively bred (25 generations) for high voluntary running-wheel behavior (S mice) and compared them with non-selected (random bred) controls (C mice; Swallow et al., 1998). Our hypotheses were that 1) voluntary running-wheel activity increases hippocampal, spinal and cerebellar neurotrophin levels (BDNF and NT-3) in mice and 2) neurotrophins increase more in S versus C mice when both are given access to running wheels. Use of a novel genetic model that voluntarily exercises at high running speeds (Swallow et al., 1998; Garland, 2003) should increase statistical power in detecting relationships between voluntary exercise and neurotrophins, and may yield novel insights concerning genetic determinants of neurotrophin regulation. Differences in neurotrophin levels may directly result from specific genetic factors associated

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Abbreviations: ANCOVA, one-way analysis of covariance; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; C mice, randomly bred control mice; ELISA, enzyme-linked immunosorbant assay; IGF-1, insulin-like growth factor-1; NT-3, neurotrophin-3; S mice, mice selectively bred (25 generations) for high voluntary running-wheel behavior.

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with neurotrophin regulation or may be indirectly affected by an increased propensity to undertake physical exercise.

EXPERIMENTAL PROCEDURES

Animals

Male mice from generation 25 of an artificial selection experiment for high voluntary wheel running behavior (Swallow et al., 1998; Garland, 2003) were studied. The original progenitors were outbred, genetically variable laboratory house mice (*Mus domesticus*) of the Hsd:ICR strain. After two generations of random mating, mice were randomly paired and assigned to eight closed lines (10 pairs in each). In each subsequent generation, when the offspring of these pairs were 6–8 weeks old, they were housed individually with access to a running wheel for 6 days. Daily wheel running activity was monitored by an automated system (Lafayette Instruments, Lafayette, IN, USA).

In the four "selected" lines, the highest-running male and female from each family were selected as breeders to propagate the lines to the next generation. Wheel running was quantified as the total number of revolutions run on days 5 and 6 of the 6-day test. In the four "non-selected" lines, a male and female were randomly chosen from each family. Within all lines, the chosen breeders were randomly paired except that sibling matings were not allowed.

To supply animals for the experiments presented here, generation 24 parents were allowed to produce a second litter. Male animals were housed four to a cage before being assigned to experimental groups when they were housed individually. Selected (S) and non-selected (C) lines were randomly divided into four groups: 1) one-night sedentary, 2) one night with access to running wheels, 3) seven-night sedentary, and 4) seven nights of running wheel access. Each group of runners and sedentary mice consisted of 16 and eight mice, respectively. Individuals ranged in mass from 22 to 36 g (30±3 g, mean±S.D.) and were approximately 8 weeks old. All procedures were approved by the School of Veterinary Medicine's Animal Care and Use Committee at the University of Wisconsin. In addition, all efforts were made to minimize the number of animals used and their suffering.

Neurotrophin measurements (enzyme-linked immunosorbant assay [ELISA])

Mice were killed with an overdose of isoflurane followed by dissection of the entire hippocampus, cerebellum and two spinal cord segments (C3–C6 and C7–T2). Tissues were stored on dry ice until they could be stored at −80 °C for future analysis of neurotrophin protein concentrations.

Tissue samples were thawed, weighed and homogenized in cold extraction buffer (Tris-buffered saline, pH 8.0, with 1% NP-40, 10% glycerol, 0.61 mg/ml sodium metavanadate, 0.75 mg/ml phenylmethylsulfonylfluoride, 0.1 mg/ml aprotinin and 9.4 g/ml leupeptin). The homogenates were acidified to pH approximately 3.0 with 1 N HCl, incubated at room temperature for 15 min, and neutralized with 1 N NaOH to pH approximately 7.6. The pH was determined by measuring a drop of the homogenates on pH paper. After acid treatment, the homogenates were microfuged at 3500×*g* for 10 min, and the supernatants were assayed by sandwich ELISAs (BDNF: R & D Systems, Minneapolis, MN, USA; NT-3: Promega Corporation, Madison, WI, USA). BDNF and NT-3 were both analyzed using the same samples and were normalized per gram of wet tissue weight. NT-3 was only measured in the hippocampus.

Statistical methods

Data are presented as mean values±S.E.M. Significance was established at $P<0.05$. SAS (SAS Institute Inc., Cary, NC, USA) PROC MIXED (which employs restricted maximum likelihood) was used to analyze these data. Mouse line was always entered as a random effect nested within the fixed effect, line-type (selected or control). As in previous studies of these mouse lines (Swallow et al., 1998; Girard et al., 2001; Girard and Garland, 2002; Garland, 2003), statistical analyses must employ a nested design because the replicate lines have been genetically closed, independent entities for multiple generations. The replication of lines allows for the possibility that any two lines (i.e. one selected and one non-selected) might diverge by random genetic processes, such as genetic drift. Therefore, in order to attribute a difference in any particular phenotypic trait to the effects of selective breeding per se, it must appear consistently across all replicate lines.

Wheel running was analyzed using a one-way analysis of covariance (ANCOVA), with wheel freeness (an inverse measure of how resistant the wheel is to rotation) entered as a covariate, line-type entered as the fixed factor, and variance estimated separately for the S and C lines.

Neurotrophin concentrations were analyzed using a two-way nested analysis of variance (ANOVA) with the fixed factors line-type, wheel treatment (with or without access to a running wheel), and the interaction between wheel treatment and line-type, followed by Tukey post hoc analyses for multiple comparisons. Data from the one-night and seven-night experiments were analyzed separately except where specified in the text. Degrees of freedom for testing the line-type effect were always 1 and 6; degrees of freedom in the denominator for wheel treatment and the interaction, wheel treatment-by-line-type, depended on the number of individual mice (split plot design; see: Littell et al., 1996). Prior to analysis, BDNF and NT-3 concentrations were logarithmically transformed to stabilize variance between treatments for BDNF and to improve normality of residuals for NT-3 (residuals were positively skewed otherwise for NT-3). Data were also analyzed as a function of running distance (i.e. terms in the linear model included running distance and line as random effects).

RESULTS

Wheel running

All four lines of S mice displayed increased voluntary wheel running behavior as compared with four C mice (Fig. 1). S mice exposed to running wheels for one night ran significantly more than C mice (3.4±0.5 versus 1.5±0.5 km/night, respectively; $P=0.04$; least-square adjusted means±S.E. from the nested analysis). At seven nights, the average distance run was 8.1±0.5 km/night versus 2.8±0.6 km/night in S mice and C mice, respectively ($P=0.0004$).

Hippocampus

BDNF Seven nights of wheel running increased BDNF concentration by a large amount in S mice (171% relative to baseline, sedentary mice; $P<0.0001$) and by a small, non-significant amount in C mice (20%; $P=0.81$; Fig. 2A). The two-way nested ANOVA of log-transformed BDNF reflected this difference by showing a significant interaction between treatment (seven-night runners versus seven-night sedentary mice) and line-type (S versus C; $P=0.002$); main effects of line-type ($P=0.046$) and treatment ($P<0.0001$) were also significant. S mice had 86% higher BDNF concentrations than C mice after seven nights of running ($P=0.001$), but levels were much lower and similar between S and C mice under baseline, sedentary conditions ($P=0.081$; see also Fig. 2A).

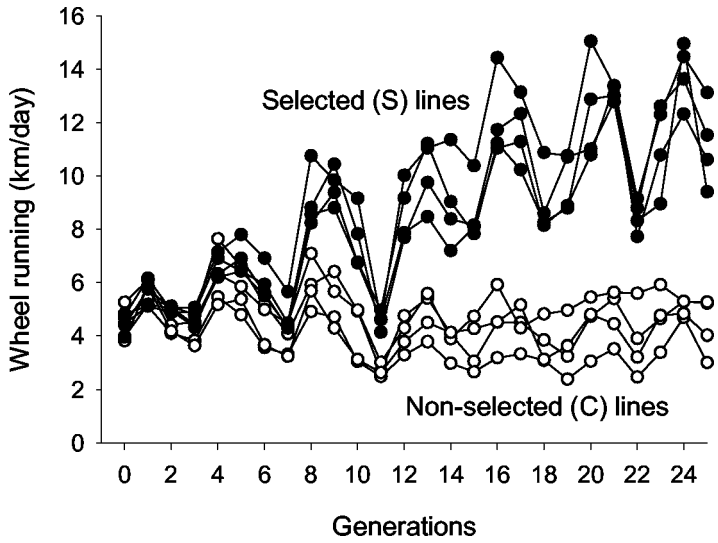

Fig. 1. Mean wheel running (represented as total revolutions per day on days 5 and 6 of a 6-day test) of male mice from four replicate S lines and four replicate C lines across generations. Wheel running increased in each of the selected lines (filled circles), but showed little change in the C lines (open circles). Mice used in this study were from generation 25.

Overall, BDNF concentrations were higher after seven nights of running than after one night of running ($P=0.02$). One night of running did not elevate BDNF levels significantly above sedentary levels in S nor in C mice, and no differences between S and C mice occurred after one night of running [as indicated by no main effect of treatment ($P=0.78$), line-type ($P=0.65$) or interaction ($P=0.35$) in the two-way ANOVA; see Fig. 2A]. For unknown reasons, BDNF concentration averaged 22% lower in the seven-night sedentary group as compared with the one-night sedentary group ($P=0.03$).

When C and S mice were grouped together, hippocampal BDNF levels (log transformed) were positively correlated with average distance run following seven nights of wheel access. In a linear model of log transformed BDNF concentration with mean distance run over the seven nights entered as a covariate, line-type entered as a categorical factor, and the interaction between the running distance and line-type entered as a factor, the correlation with running distance was significant ($P=0.0004$), but neither line-type ($P=0.29$) nor the line-type-by-running distance interaction were significant ($P=0.21$; see Fig. 2B). Thus, irrespective of line-type, mice that ran on average 1 km/night had approximately 20,000 pg/gm tissue of BDNF, whereas mice that ran 11 km/night had approximately 50,000 pg/gm tissue of BDNF. When S mice or C mice were considered alone, log-transformed hippocampal BDNF levels were not correlated with average distance run over seven nights (C mice alone: $P=0.08$; S mice alone: $P=0.66$; Fig. 2B). Similar results were obtained when distance run on the previous night was used in place of average distance run over seven nights. The simplest statistical explanation of the data is that wheel running for seven nights significantly increases BDNF levels, irrespective of line-type.

NT-3

There were no significant differences in log hippocampal NT-3 concentration between any groups ($P>0.05$; Fig. 3A), nor was there any correlation between NT-3 concentration and running distance ($P>0.05$; Fig. 3B).

Spinal cord

Segments C3–C6. One data point was removed from the seven-night C runners because it had an inordinately high concentration of BDNF (16,250 pg/g tissue). There were no significant differences between any groups ($P>0.05$; Table 1). However, there was a marginally non-significant positive correlation between average distance run per night and spinal BDNF concentration considering seven-night C and S runners together. In a linear model of log-transformed BDNF concentration with mean distance run over the seven nights entered as a covariate, line-type entered as a categorical factor, and the interaction between the running distance and line-type entered as a factor, running distance effects were marginally non-significant ($P=0.07$), line-type was not significant ($P=0.57$) and the line-type-by-running distance interaction was not significant ($P=0.72$; data not shown). Animals that ran 1 km/night had approximately 9,000 pg/g tissue of BDNF whereas animals that ran an average of 11 km/night had approximately 10,000 pg/g tissue of BDNF at C3–C6. If the distance run on the previous night is used rather than average distance run, this relationship becomes significant; mice running 1 km on the previous night had approximately 9,000 pg/g tissue and mice running 17 km/night had approximately 11,000 pg/g tissue BDNF. The effect of the running distance was significant ($P=0.03$), but neither line-type ($P=0.32$) nor line-type-by-running distance interaction ($P=0.57$) was significant

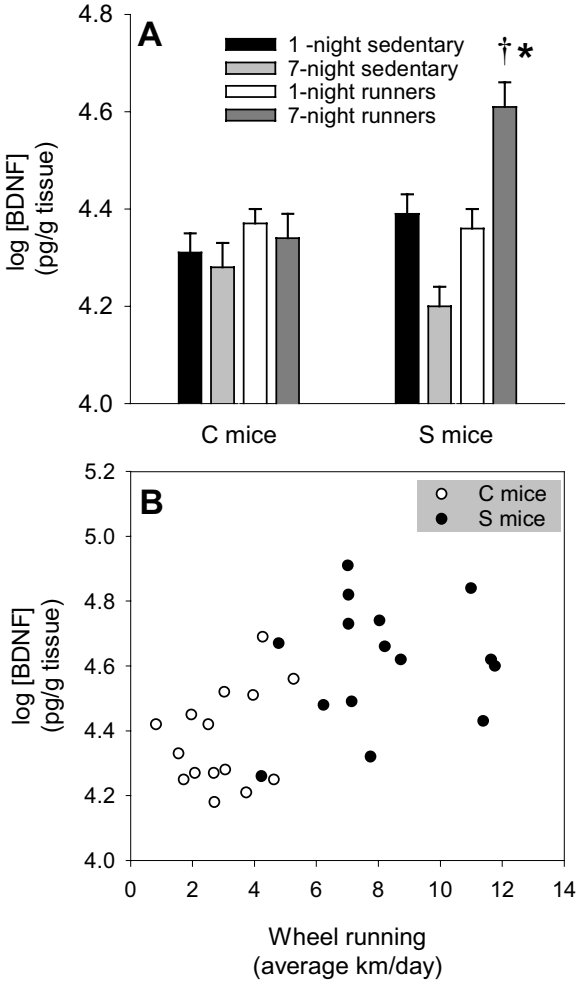

Fig. 2. A. Log hippocampal BDNF concentrations (means±S.E.M.) in sedentary mice and mice following one or seven nights on running wheels. Baseline, sedentary levels of BDNF were similar in S and C mice. One night of running did not increase BDNF in S or C mice, and no differences between S and C mice occurred at this time point. However, seven nights of running significantly increased BDNF in S mice († $P<0.0001$) but not in C mice ($P=0.81$), and after seven nights of running, BDNF levels were higher in S than C mice (* $P=0.001$). For unknown reasons, BDNF levels were lower in seven-night sedentary mice as compared with one-night sedentary mice ($P=0.03$). One-night sedentary mice, black bars; seven-night sedentary mice, light gray bars; one-night runners, white bars; seven-night runners, dark gray bars. 2B. Hippocampal BDNF concentrations in S (filled circles) and C (open circles) mice following seven nights on running wheels correlated with average wheel running. When both S and C mice were grouped following seven nights of wheel access, there was a statistically significant, positive correlation between average distance run per night and hippocampal BDNF levels ($P<0.05$).

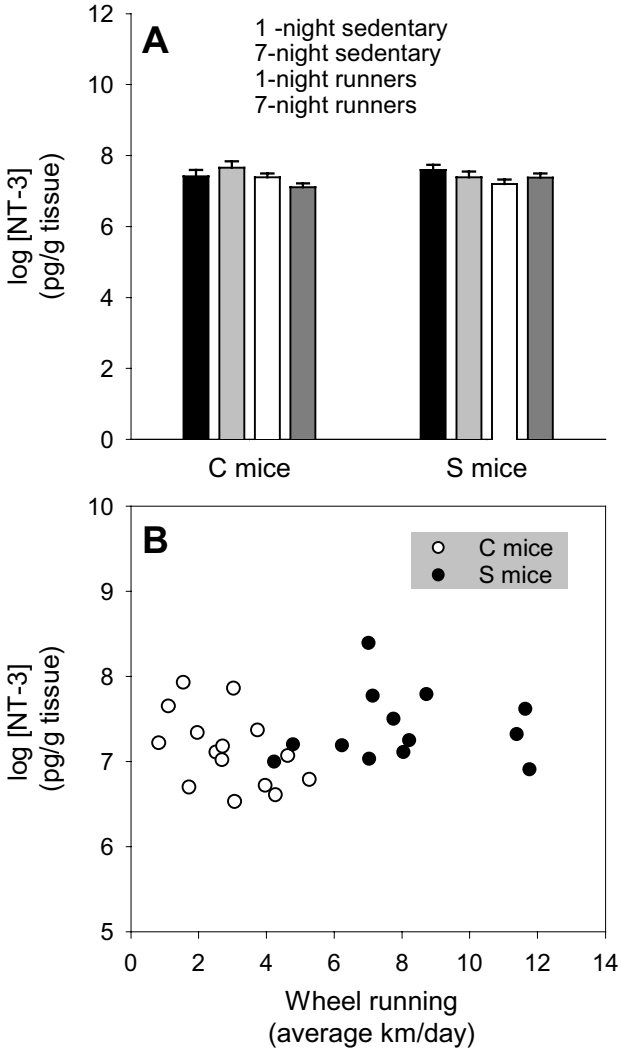

Fig. 3. A. Log hippocampal NT-3 concentrations (means±S.E.M.) in sedentary mice and mice following one or seven nights on running wheels. There were no significant differences in log hippocampal NT-3 concentrations between treatments or between S and C lines. One-night sedentary mice, black bars; seven-night sedentary mice, light gray bars; one-night runners, white bars; seven-night runners, dark gray bars. 3B. Log hippocampal NT-3 concentrations in S (filled circles) and C (open circles) mice following seven nights on running wheels correlated with average wheel running. There was no correlation between average distance run per night and log hippocampal NT-3 for animals with running wheels for seven nights ($P>0.05$, S and C mice combined).

(data not shown). Thus, there is evidence for a positive effect of seven nights' wheel running on cervical spinal BDNF levels; this effect is the same in S and C mice, although quantitatively more in the former. There was no correlation with wheel running distance in the one-night runners ($P>0.05$; data not shown).

Segments C7–T2. There were no significant differences in spinal BDNF levels between any groups ($P>0.05$; Table 1), nor was there any correlation with running distance (all $P>0.05$; data not shown).

Cerebellum. There were no significant differences in cerebellar BDNF concentrations between any groups ($P>0.05$; Table 1), nor was there any correlation with running distance (all $P>0.05$; data not shown).

DISCUSSION

Consistent with previous studies on rats (Neeper et al., 1995, 1996; Berchtold et al., 2001; Gómez-Pinilla et al.,

Table 1. Cerebellar and spinal BDNF concentrations (pg/g tissue) do not change significantly in S or C mice following exercise[a]

	C mice	S mice
Spinal segments C3–C6		
One-night sedentary	9,869±921	10,297±663
Seven-night sedentary	9,664±1,039	9,764±719
One-night runners	8,690±363	9,235±608
Seven-night runners	9,588±541	9,854±495
Spinal Segments C7–T2		
One-night sedentary	21,618±1,684	21,920±1,620
Seven-night sedentary	22,699±2,813	19,691±1152
One-night runners	17,884±1,031	20,383±1,167
Seven-night runners	19,024±1,257	18,137±855
Cerebellum		
One-night sedentary	2,353±182	3,094±390
Seven-night sedentary	2,217±406	2,481±376
One-night runners	2,320±179	2,594±152
Seven-night runners	2,782±247	2,934±229

[a] There were no significant differences in spinal (C3–C6, C7–T2) or cerebellar BDNF levels (pg/g tissue) between treatment groups or between S and C lines.

2001), hippocampal BDNF concentration increased following voluntary wheel running activity in male mice. However, this effect was observed following seven nights, but not one night of running wheel access, and was restricted to S mice. Although hippocampal BDNF levels in C mice tended to increase following seven nights of wheel running, this increase did not reach statistical significance. When all animals were considered together, hippocampal BDNF was significantly correlated with distance run, suggesting that BDNF increases more in S mice because they run more. We hypothesize that high-exercising mice may thus exhibit a greater degree of exercise-induced neuroplasticity (e.g. increased hippocampal neurogenesis; van Praag et al., 1999a,b; Rhodes et al., 2003).

Exercise-induced increases in hippocampal BDNF

Neurotrophins are expressed in an activity-dependent manner (Patterson et al., 1992; Thoenen, 1995). For example, neuronal activation required for hippocampal long-term potentiation increases BDNF synthesis and release (Patterson et al., 1992; Korte et al., 1995), as does hippocampal and spinal activation by kainic acid (Scarisbrick et al., 1999; Nanda and Mack, 2000; Widenfalk et al., 2001). During locomotion, the firing rate of hippocampal neurons is increased (Czurkó et al., 1999). In addition, hippocampal theta activity is proportionally increased (McFarland et al., 1975; Oddie and Bland, 1998; Slawinska and Kasicki, 1998). Thus, increased hippocampal BDNF concentrations following physical activity may result from increased neural activation or altered activity patterns during exercise.

Mice selectively bred for increased voluntary wheel running run faster rather than longer (Girard et al., 2001). Female S mice run in more frequent and shorter bouts than do C mice (Girard et al., 2001). This pattern of locomotor activity may repeatedly increase hippocampal neural activity (Czurkó et al., 1999), thus giving rise to greater hippocampal BDNF concentrations. Transcriptional activity via the promoter 1 region of the BDNF gene is stimulated more effectively by spaced versus massed neuronal activation (Mitchell et al., 2001; S. A. Nanda and K. J. Mack, unpublished observation). Thus, in S mice, the potential for exercise-induced hippocampal neuroplasticity may be maximized.

In addition to centrally mediated, activity-dependent increases in BDNF following exercise, peripheral mechanisms may play a role. For example, circulating insulin-like growth factor-1 (IGF-1) levels increase following physical activity (Schwarz et al., 1996; Carro et al., 2000), increasing hippocampal BDNF mRNA levels (Carro et al., 2000). Other neurochemicals such as corticosteriods (Schaaf et al., 2000; Russo-Neustadt et al., 2001) and estrogen (Berchtold et al., 2001) affect hippocampal BDNF levels and may also mediate changes in neurotrophins following exercise.

The present study of male mice confirms that exercise-induced increases in hippocampal BDNF levels are positively correlated with distance run (Neeper et al., 1995; Johnson and Mitchell, 2003). However, no such correlation exists in female S and C mice after 40 days of wheel access (Rhodes et al., 2003). Whether these differences are attributable to differences between the sexes or to the greater duration of wheel access is unknown. Possibly, BDNF levels may increase more in S mice only in short (e.g. 7 days) time domains, and this effect may disappear by 40 days. On the other hand, estrogen may be a permissive factor necessary for exercise-induced up-regulation of hippocampal BDNF (Berchtold et al., 2001), so possible differences in estrogen levels in S versus C mice may have affected the results.

Lack of exercise-induced increases in hippocampal NT-3

We measured no significant changes in hippocampal NT-3 levels following exercise in mice. This finding contrasts with similar studies on rats from our laboratory, where hippocampal NT-3 was affected by exercise in a complex manner, first (non-significantly) increasing at one night and then decreasing from baseline by seven nights (Johnson and Mitchell, 2003). Thus, in both mice and rats, hippocampal BDNF and NT-3 may be differentially regulated by exercise, as in other neural systems. For example, kainic acid-induced seizures increase BDNF but decrease hippocampal NT-3 protein levels in rats (Katoh-Semba et al., 1999). Furthermore, GABA(B) receptor antagonists increase BDNF but decrease NT-3 concentrations in the rat hippocampus (Heese et al., 2000). NT-3 can offset excitatory BDNF signaling pathways in cultured hippocampal neurons (Paul et al., 2001). Thus, decreased or unchanged NT-3 concentrations post-exercise may support the facilitatory effects of BDNF on hippocampal synaptic function.

Lack of exercise-induced increases in cerebellar and spinal BDNF

Forced treadmill exercise (5 days) increases spinal (lumbar) BDNF protein and mRNA levels in rats (Gómez-Pinilla

et al., 2001). Although we did not find statistically significant exercise-induced increases in spinal BDNF levels in voluntarily exercising mice, a small but significant positive correlation of spinal (C3–C6) BDNF levels with distance run on the previous night was observed. Therefore, exercise may exert a small positive effect on spinal BDNF concentration in mice, an effect consistent with previous studies of rats (Gómez-Pinilla et al., 2001). Our results may vary somewhat from previous rat investigations (Gómez-Pinilla et al., 2001) since we used different experimental methods (i.e. lumbar versus cervical spinal cord, mouse versus rat, relative exercise intensity, etc.).

Cerebellar BDNF mRNA increases in rats following four nights of running wheel access, but is decreased again by seven nights (Neeper et al., 1996). Although we did not observe changes in cerebellar BDNF protein concentrations in either S or C mice, different durations of wheel access may explain this apparent difference.

Our data demonstrate a genetic difference in BDNF expression associated with selection for increased voluntary wheel running. The genetic basis of this difference between strains may relate to mutations in genes directly regulating BDNF expression or, more likely, may result indirectly from non-specific factors associated with increased running activity. For example, exercise-associated increases in hippocampal neural activity (Czurkó et al., 1999), plasma corticosterone levels (Girard and Garland, 2002), or circulating IGF-1 (Schwarz et al., 1996; Carro et al., 2000) may influence BDNF expression.

We speculate that greater hippocampal neuronal activation associated with high wheel running speeds explains the greater increase in hippocampal BDNF in S versus C mice. Increased BDNF concentrations may support enhanced neuroplasticity, such as the increased neurogenesis observed in S versus C mice (Rhodes et al., 2003). Although the present study strengthens the argument that exercise promotes brain plasticity, future studies are necessary to identify both the mechanism underlying this effect and the functional significance of exercise-induced hippocampal neuroplasticity.

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