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EXERCISE PRIMES A MOLECULAR MEMORY FOR BRAIN-DERIVED NEUROTROPHIC FACTOR PROTEIN INDUCTION IN THE RAT HIPPOCAMPUS

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Abstract—Exercise is an important facet of behavior that enhances brain health and function. Increased expression of the plasticity molecule brain-derived neurotrophic factor (BDNF) as a response to exercise may be a central factor in exercise-derived benefits to brain function. In rodents, daily wheel-running exercise increases BDNF gene and protein levels in the hippocampus. However, in humans, exercise patterns are generally less rigorous, and rarely follow a daily consistency. The benefit to the brain of intermittent exercise is unknown, and the duration that exercise benefits endure after exercise has ended is unexplored. In this study, BDNF protein expression was used as an index of the hippocampal response to exercise. Both daily exercise and alternating days of exercise increased BDNF protein, and levels progressively increased with longer running duration, even after 3 months of daily exercise. Exercise on alternating days was as effective as daily exercise, even though exercise took place only on half as many days as in the daily regimen. In addition, BDNF protein remained elevated for several days after exercise ceased. Further, after prior exercise experience, a brief second exercise re-exposure insufficient to cause a BDNF change in naïve animals, rapidly reinduced BDNF protein to levels normally requiring several weeks of exercise for induction. The protein reinduction occurred with an intervening "rest" period as long as 2 weeks. The rapid reinduction of BDNF by an exercise stimulation protocol that is normally subthreshold in naïve animals suggests that exercise primes a molecular memory for BDNF induction. These findings are clinically important because they provide guidelines for optimizing the design of exercise and rehabilitation programs, in order to promote hippocampal function. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: physical activity, plasticity, brain, enrichment.

The neurotrophin brain-derived neurotrophic factor (BDNF) has been a focus of intense interest for its key role in activity-dependent neuronal plasticity in the adult brain. Synaptic activity upregulates BDNF gene expression and stimulates BDNF protein release into synapses, enhancing synaptic transmission and neuronal excitability (Kang and Schuman, 1995; Levine et al., 1995; Figurov et al., 1996; Griesbeck et al., 1996; Rutherford et al., 1998; Hartmann et al., 2001). BDNF modulates synaptic change, including hippocampal long-term potentiation (LTP), a cellular model of learning. Mice deficient in BDNF have severely impaired hippocampal LTP, and show deficits in hippocampal-dependent learning (Poo, 2001; Tyler et al., 2002). Conversely, restoring BDNF by adenoviral-mediated re-expression (Korte et al., 1996) or exogenous BDNF administration (Patterson et al., 1996) reverses both the electrophysiological and learning deficits. A recent study using an inducible knockout system to delete BDNF selectively in the forebrain of adult animals further supports a critical function for forebrain BDNF in hippocampal-dependent learning and LTP (Monteggia et al., 2004). In line with a role in modulating long term plasticity, extracellular release of BDNF from dendrites can stimulate dendritic branching (McAllister et al., 1995), while synaptic BDNF release is thought to increase spine formation (Tyler and Pozzo-Miller, 2001). Finally, BDNF is an essential survival factor for some cell types, and can provide neuroprotection from a variety of insults.

In parallel with animal studies, a role for BDNF in human cognition has recently been established by genetic studies highlighting polymorphisms in the BDNF gene. A single amino acid substitution in the coding region of the BDNF gene (val/met codon 66 in the pro-region) results in abnormal intracellular trafficking and impaired activity-regulated release of BDNF. This impaired trafficking and release is associated with impaired memory function and abnormal hippocampal activation in young adult (cognitively intact) humans (Egan et al., 2003). Additional studies demonstrate that a number of different BDNF polymorphisms are risk factors for Alzheimer's disease (AD) (Kunugi et al., 2001; Riemenschneider et al., 2002; Ventriglia et al., 2002; Egan et al., 2003) as well as mood and eating disorders (Neves-Pereira et al., 2002; Sen et al., 2003; Ribases et al., 2004). The Egan et al. (2003) study in particular, in combination with the animal studies on reduced BDNF signaling, underscore the emerging understanding that decreased availability of BDNF can compromise brain function and plasticity. Elucidating the variables that regulate BDNF availability in the brain is thus an important goal for identifying interventions that support/promote brain health and function.

One such intervention is exercise, or physical activity (Cotman and Berchtold, 2002). In rodents, it is well established that daily wheel-running exercise increases BDNF

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Abbreviations: AD, Alzheimer's disease; BDNF, brain-derived neurotrophic factor; LTP, long-term potentiation; TBST, 20 mM Tris–HCl, 150 mM NaCl, 0.05% Tween 20.

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gene and protein expression in the hippocampus (Berchtold et al., 2001, 2002; Gomez-Pinilla et al., 2002; Farmer et al., 2004; Russo-Neustadt et al., 2004). Functional benefits of exercise that may be related in part to increased BDNF availability include increased neuronal survival and resistance to brain insult (Stummer et al., 1994; Carro et al., 2001; Ding et al., 2004), enhanced hippocampal neurogenesis (van Praag et al., 1999) increased resistance to depression (Moraska and Fleshner, 2001; Greenwood et al., 2003) and facilitated learning (van Praag et al., 1999; Young et al., 1999; Adlard et al., 2004). Similar benefits of exercise have been demonstrated in human studies, where exercise participation predicts better cognitive function (Blomquist and Danner, 1987; Rogers et al., 1990; Berkman et al., 1993; Hill et al., 1993; Colcombe and Kramer, 2003), lower risks of AD and lower risks of dementia in general (Friedland et al., 2001; Laurin et al., 2001).

While animal studies have focused on the benefits of daily activity, exercise patterns are generally less rigorous in humans, and rarely follow a sustained daily regularity. The benefit to the brain of intermittent exercise is unknown, and the duration that exercise benefits endure after exercise has ended is unexplored. There are three main questions to investigate: 1) How do hippocampal BDNF protein levels respond to intermittent exercise, such as exercise on alternating days? 2) After exercise has ended, do BDNF protein levels remain elevated or do they decline rapidly back to baseline? 3) How does a second exercise exposure regulate BDNF protein levels, after levels have decayed back to baseline? These questions have relevance for understanding mechanisms by which an experience is encoded, the stability of this encoding, and how previous experience allows the nervous system to respond later to the same experience. In addition, these questions are relevant to the field of human physiology and CNS health.

EXPERIMENTAL PROCEDURES

Animals

Adult male Sprague–Dawley rats (Charles-River Inc., MA, USA), 7–8 weeks of age (150–200 g) at experimental onset, were individually housed with *ad libitum* access to food and water in a 12-h light/dark vivarium. Each cage of the exercising animals was individually equipped with a running wheel (Minimitter, OR, USA) that occupied 1/2 of the cage. Running activity was voluntary, and the nightly distance run was monitored by computer software (VitalView, Minimitter Co., OR, USA). For the "intermittent-run" animals, wheels were locked on alternating days, so that animals could only exercise on alternating days. Sedentary animals were housed in standard cages with no running wheel. To control for the presence of the wheel, one group of sedentary animals was housed with a locked running wheel. Initially when animals first have access to running wheels, they run approximately 1 km per night, and progressively increase their running distance, to an average of 4 km per night by about the fifth night of running. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) for the University of California at Irvine, CA, USA and were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the suffering and the number of animals used.

Running paradigms

The following exercise paradigms were used. Daily vs. Intermittent running: To investigate the effect of different training paradigms on BDNF protein levels, rats were allowed to exercise under two conditions of voluntary wheel-running, either continuous daily access for 2, 4, 7, 14, 28, or 90 days (Fig. 1A) or intermittent access on alternating days for 7, 14, 21 or 28 days (Fig. 1B). Time-matched sedentary animals were killed in parallel with exercised animals. Group sizes were $n=7–8$/timepoint. Decay timecourse: The stability of elevated BDNF was examined after the completion of a 4-week program of daily running. At the end of the 4-week running period, exercise wheels were locked and animals were killed 0, 1, 3, 7, and 14 days following the end of the exercise period (see Fig. 1C). Time-matched sedentary animals were killed in parallel with exercised animals. Group sizes were $n=7–8$/timepoint. Reinduction: The reinduction of BDNF protein levels by a second short "test" exercise period (2 days) was examined after 7 or 14 days of quiescence. The initial running period ("priming run") was a total of 14 days' running, taking place over either 14 days of daily running (Fig. 1D, E), or 28 days of alternating-day running (Fig. 1F, G). The test exposure consisted of 2 days of daily activity, in all paradigms. During the period of inactivity, the running wheel was locked but remained in the cage. Time-matched sedentary animals were killed in parallel with exercised animals. Group sizes were $n=5–6$/timepoint. Animals were killed by decapitation in the morning (7 AM) immediately after the end of the dark (active) period without anesthesia. Brains were rapidly removed and both hippocampi were dissected, pooled, and frozen on dry ice. Hippocampi from each animal were processed as separate samples. Hippocampal tissue was stored at −70 °C until processing for protein by enzyme-linked immunosorbent assay (ELISA).

BDNF ELISA

BDNF protein was assessed in the hippocampus using the E-Max ELISA kit (Promega, WI, USA) according to manufacturer's recommendations. Dissected hippocampus was homogenized in lysis buffer (18 μl/mg tissue) containing 137 mM NaCl, 20 mM Tris–HCl (pH 8.0), 1% NP40, 10% glycerol, 1 mM PMSF, leupeptin (1 μg/ml), sodium vanadate (0.5 mM), AEBSF (100 mg/ml). Homogenized samples were diluted in two volumes DPBS buffer (0.2 g KCl, 8.0 g NaCl, 0.2 g KH_2PO_4, 1.15 g Na_2HPO_4, 654 μl 1 M $MgCl_2$, 905 μl 1 M $CaCl_2$) and centrifuged 3 min at 14,000 r.p.m. at 4 °C. Supernatant was collected and diluted 1:2 in block and sample buffer (B&S buffer: supplied with kit). For the ELISA, 96-well flat-bottomed Immulon-2 plates (DYNEX Technologies) were incubated overnight at 4 °C with carbonate-coating buffer containing anti-BDNF monoclonal antibody. Plates were blocked for 1 h with B&S buffer, followed by incubation with samples in triplicate and BDNF standards in duplicate for 2 h at room temperature with shaking. A standard curve was established using serial dilutions of known amounts of BDNF ranging from 0 to 500 pg/ml, diluted in B&S buffer. Plates were washed 5× with TBST (20 mM Tris–HCl, 150 mM NaCl, 0.05% Tween 20), followed by incubation with anti-human BDNF polyclonal antibody, five washes with TBST, and 1 h incubation with horseradish peroxidase. Enzyme solution (1:1 TMB and peroxidase substrate) was prepared 1 h in advance and subsequently incubated on the plate for 10 min. After samples turned blue, the reaction was stopped with phosphoric acid and absorbance was read at 450 nm using a plate reader. The R value for the standard curve was consistently ≥ 0.99. All sample values were in the linear region of the standard curve, with values from sedentary animals falling in the range of 25–45 pg/ml (which translates to 3.46 ± 0.9 ng/mg tissue).

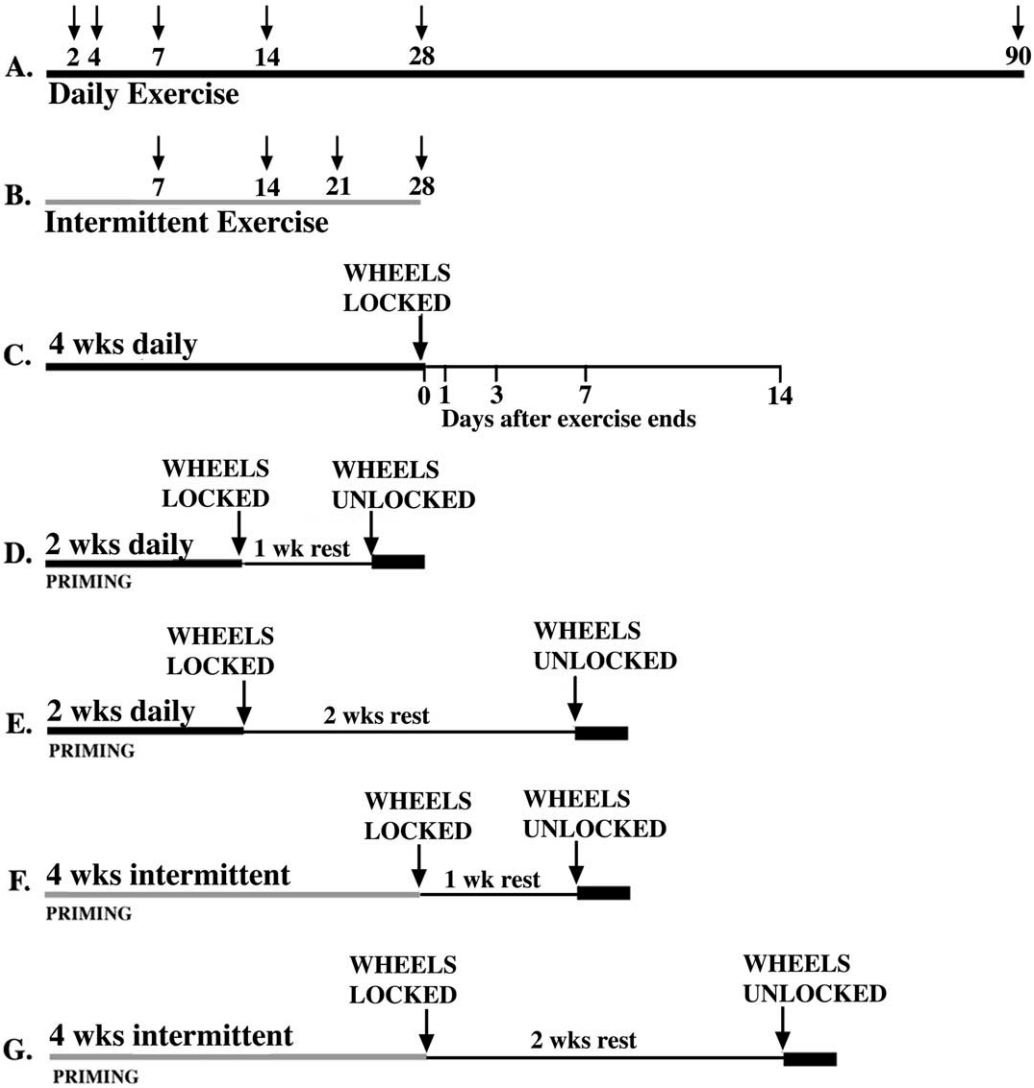

Fig. 1. Experimental design illustrating the exercise paradigms. To investigate BDNF induction by daily versus intermittent exercise, animals were allowed daily access to running wheels for 2, 4, 7, 14, 28, or 90 days (A), or alternating-day access to running wheels for 7, 14, 21, or 28 days, with wheels locked every other day (B). Arrows indicate sacrifice timepoints. Running onset was staggered so that all animals were killed on 2 consecutive days, along with sedentary controls. In the decay paradigm (C), animals exercised daily for 28 days. Wheels were locked the morning after the end of the exercise period (day 0), and animals were killed at 0, 1, 3, 7, or 14 days after the end of exercise, along with time-matched sedentary controls. In the priming/reinduction experiments (D–G), there was an initial priming period of 14 days of daily activity (D, E), or 28 days of alternating days activity (F, G). Thus, each priming condition consisted of 14 days of exercise. After priming, wheels were locked for 7 days (D, F) or 14 days (E, G), followed by a reinduction test period of exercise, consisting of 2 days of daily activity for all groups. Additional animals were killed at each arrowhead to assess the BDNF response to the priming treatments alone, as well as BDNF levels at 7 and 14 days after exercise had ceased.

Data analysis

BDNF ELISA data were obtained in triplicate for each sample, and triplicates were averaged to obtain one value per sample. Data were normalized relative to sedentary controls and analyzed by one-way ANOVA, followed by Fisher test for post hoc analysis (Statview 4.5). Data were considered significant if $P<0.05$.

RESULTS

BDNF induction by daily vs intermittent exercise

To investigate the effect of different training paradigms on BDNF regulation, rats were allowed to exercise under two conditions of voluntary wheel-running: either daily access for 2, 4, 7, 14, or 28 days (Fig. 1A), or exercise on alternating-days over a period of 7, 14, 21, or 28 days (Fig. 1B). To assess the effect of long-term running on BDNF protein levels, an additional group of animals had daily wheel access for 3 months.

BDNF protein levels increased in response to both daily activity ($F(7,78)=6.71$, $P<0.0001$) and intermittent exercise ($F(4,54)=4.54$, $P<0.0001$). Baseline levels of BDNF protein in sedentary animals were 3.46 ± 0.9 ng/mg tissue. Daily access to running wheels produced the most rapid increase in BDNF protein levels (Fig. 2a). In re-

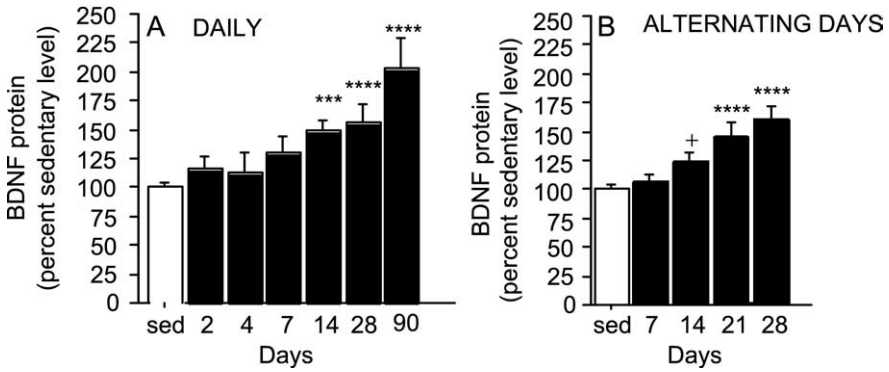

Fig. 2. BDNF protein levels in the hippocampus are increased by different exercise regimens. BDNF protein levels increase progressively with increasing days of running, in response to both daily exercise (A) or alternating days exercise (B). While daily activity appears to elevate BDNF protein levels more rapidly, BDNF protein levels attain equivalent levels in response to daily or alternating days activity after both 14 and 28 days, even though animals only exercise on half as many days in the alternating-day paradigm. Note that in the intermittent exercise paradigm, "7, 14, 21, 28 days" is respectively "4, 7, 11, and 14" days of total exercise days. Sedentary (sed), Sedentary locked-wheel controls (sed-lock). All values are mean±S.E.M., normalized to sedentary levels. **** $P<0.0001$, *** $P<0.005$, $^{+}$ trend $P=0.057$.

sponse to daily running, BDNF protein levels increased gradually with sustained daily running, and were significantly elevated over sedentary levels after 14 days (150%, $P<0.005$) and 28 days (174%, $P<0.0001$). BDNF protein levels continued to rise even after 90 days (222%, $P<0.0001$) of daily activity, indicating that enhancement of hippocampal BDNF protein is a remarkably sustained response to exercise.

A similar pattern of increased BDNF expression occurred in response to intermittent exercise, but at a slower rate (Fig. 2b). A small increase in BDNF protein levels was seen after 14 days in this paradigm (124%, $P=0.057$), with greater increases attained after 21 days (145%, $P<0.0001$) and 28 days (159%, $P<0.0001$). Interestingly, BDNF protein levels attained equivalent levels after 28 days of daily exercise or 28 days of intermittent exercise, even though animals only ran on half as many days in the alternating-days exercise paradigm. This suggests that the exercise-induction of BDNF protein has a time component that interacts with the exercise stimulus. Thus, while continuous training can produce more rapid elevation in BDNF levels, intermittent training appears to ultimately be as effective as daily training to elevate BDNF protein levels in the hippocampus.

BDNF protein remains elevated for several days after exercise ends

To assess how long BDNF protein remains elevated once induced by exercise, BDNF protein levels were quantified at 0, 1, 3, 7, and 14 days following a 28 day period of daily

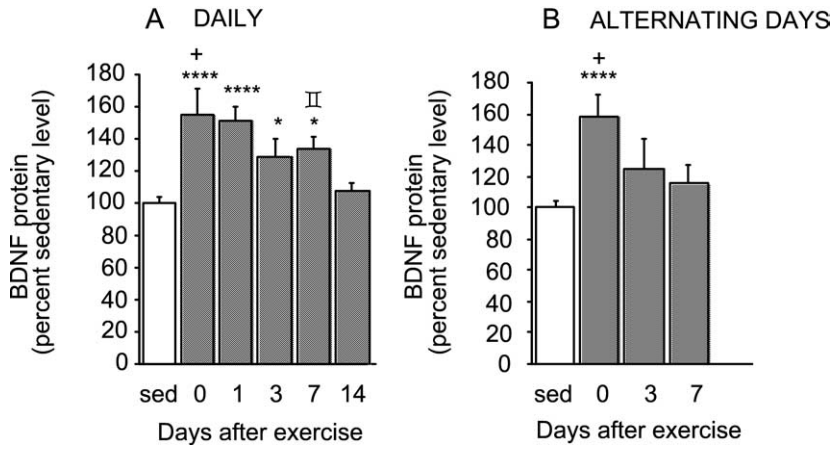

Fig. 3. BDNF protein levels remain elevated for several days after exercise has ceased. After 28 days of daily exercise (A), hippocampal BDNF protein levels are significantly elevated over sedentary levels and remain elevated over sedentary levels for at least 7 days after exercise has ended. BDNF protein levels remain at peak levels for at least 1 day after exercise has ceased. By 3 days, BDNF protein levels are significantly reduced compared with peak levels, however are still significantly elevated over baseline. In contrast, after 28 days of alternating-days exercise (B), BDNF levels are not significantly elevated over baseline at 3 or 7 days after exercise has ceased, even though BDNF levels were increased to the same level at the end of exercise in both paradigms. There is a trend for elevated BDNF protein levels at the 3 day timepoint after alternating days exercise, relative to baseline ($P=0.086$). Values are mean±S.E.M., normalized to sedentary levels. **** $P<0.0001$ vs sed, * $P<0.05$ vs sed, $^{+}$ $P<0.05$ vs day 3 and day 7, ^{II} $P<0.05$ vs day 14.

exercise, or at 0, 3, and 7 days after a 28 day period of alternating-days exercise (Fig. 3).

At the end of the 28 day period of daily exercise (day 0), BDNF protein levels were significantly elevated over sedentary levels ($F(5,80)=8.84$, $P<0.0001$) and remained significantly elevated after 1 day (151%, $P<0.0001$), 3 days (128.5%, $P=0.011$), and 7 days (133%, $P=0.024$) over baseline levels (Fig. 3a). While BDNF levels gradually declined from the peak observed at day 0 (exercise 160%, sedentary 100%, $P<0.0001$), the protein levels were stably maintained at the peak level for at least 1 day after exercise had ceased and were significantly elevated above baseline even after 7 days of inactivity. Relative to peak levels detected on day 0, BDNF protein levels were significantly lower after 3 days of inactivity ($P<0.05$, relative to day 0), and gradually declined to approximately baseline levels after 14 days of inactivity (107%, $P=$N.S. relative to sedentary). Thus, following a paradigm of 28 days of daily exercise, BDNF protein levels remained significantly elevated over sedentary levels even after 1 week of inactivity.

To assess if intermittent exercise paradigms result in a similar stability of BDNF, protein levels were assessed at 0, 3, or 7 days following 28 days of intermittent exercise. As shown in Fig. 2, BDNF protein levels were similarly increased after 28 days of exercise in the two paradigms (160% and 159%, daily and alternating-day levels respectively, $P<0.0001$ for both). However, in contrast to the decay timecourse that followed daily exercise, in the intermittent paradigm, BDNF protein levels were no longer significantly elevated after 3 days (124%, $P=0.086$) or 7 days (115%, $P>0.1$) of quiescence (Fig. 3B). Thus, while BDNF protein levels were initially elevated to the same level in the two paradigms despite that fact that animals exercised on only half as many days in the alternating-day paradigm, the protein levels decline faster when exercise occurs only on alternating days.

Timecourse of decay corresponds to number of running days, rather than the exercise paradigm

In the intermittent exercise paradigm, animals ran on only half as many days as animals in the daily activity paradigm. Thus it is possible that the faster rate of decline seen after the alternating-day treatment may be related to the absolute number of running days in a given time period, rather than the pattern of exercise (daily vs. alternating). To address this possibility, BDNF protein levels were compared after 7 days of quiescence following two exercise conditions that involved the same amount of absolute running days (14 days). In one paradigm, exercise occurred daily over 14 days, while in the other paradigm, exercise took place on alternating days over 28 days. In these conditions, BDNF protein levels were significantly elevated at the end of the running period to 150% and 159% in the daily and intermittent paradigms respectively ($P<0.0001$ for both). However, 7 days after cessation of exercise, BDNF protein levels were no longer significantly elevated in either the daily or intermittent exercisers (108% and 115%, respectively). These results suggest that BDNF protein levels decline similarly regardless of the exercise paradigm (daily versus alternating-days), but that the rate of decline is dependent on the absolute amount of exercise days that preceded.

Interestingly, data from the preceding section demonstrated that exercising on alternating days is as effective as daily exercise to elevate BDNF protein. However, data in this section clarify that the greater stimulus exposure of daily running (over a given duration of time) has benefits over an intermittent exercise paradigm. The more sustained effect of daily exercise on BDNF protein levels after exercise has ceased may be due to a change in translation rate, or to post-translational changes that maintain the stability of the protein once induced.

BDNF protein is rapidly reinduced by a brief second exercise session

Once BDNF protein levels return to baseline, it is important to determine how rapidly BDNF can be reinduced. There are two possibilities: (1) the induction may follow the same timecourse as observed in naïve animals (Fig. 2), requiring ~14 days of exercise exposure before rising significantly, or (2), the induction may be more rapid in animals that have previously exercised, compared with the naïve animal response. To evaluate these two hypotheses, the effect of a brief second exercise period on BDNF protein level was assessed. The exercise duration selected for the second exercise period was 2 days of daily running, which is on its own insufficient to increase BDNF protein in naïve animals. As shown in Fig. 1, the second exercise period was undertaken after two durations of inactivity (7 or 14 days), following an initial running paradigm of either (a) 14 days of daily running (Fig. 1D, E) or (b) 28 days of intermittent activity (Fig. 1F, G). Thus, the initial priming paradigms each contained 14 days of exercise, and the subsequent test period consisted of 2 days of daily activity, in both priming paradigms.

In both exercise paradigms, the second exercise exposure significantly increased BDNF protein levels above sedentary control levels, at both timepoints examined ($F(4,35)=8.881$, $P<0.0001$). As an initial stimulus, without any prior exercise exposure, 2 days of exercise is insufficient to significantly increase BDNF protein levels (117% $P=0.17$). However, as a second exercise stimulus, 2 days of exercise is sufficient to increase BDNF protein levels over sedentary control levels, even after 7 or 14 days of inactivity (Fig. 4).

Thus, while the two exercise paradigms differed in the subsequent rate of decline in BDNF after exercise ended (Fig. 3), they are equivalent in priming the hippocampus ($F(3,15)=0.659$; $P=0.59$). In the two paradigms, after 7 days of inactivity, the second exercise exposure elevated BDNF protein levels to 137% ($P<0.001$, daily exercise priming) or 129% ($P<0.01$, alternating-days priming) of sedentary control levels. Similarly, after 14 days of inactivity, the second exercise exposure elevated BDNF protein levels to136% ($P<0.005$, daily exercise priming) and 149% ($P<0.0001$, alternating-days priming) of sedentary control levels (Fig. 4). These results suggest that once animals have been exposed to the exercise stimulus, a

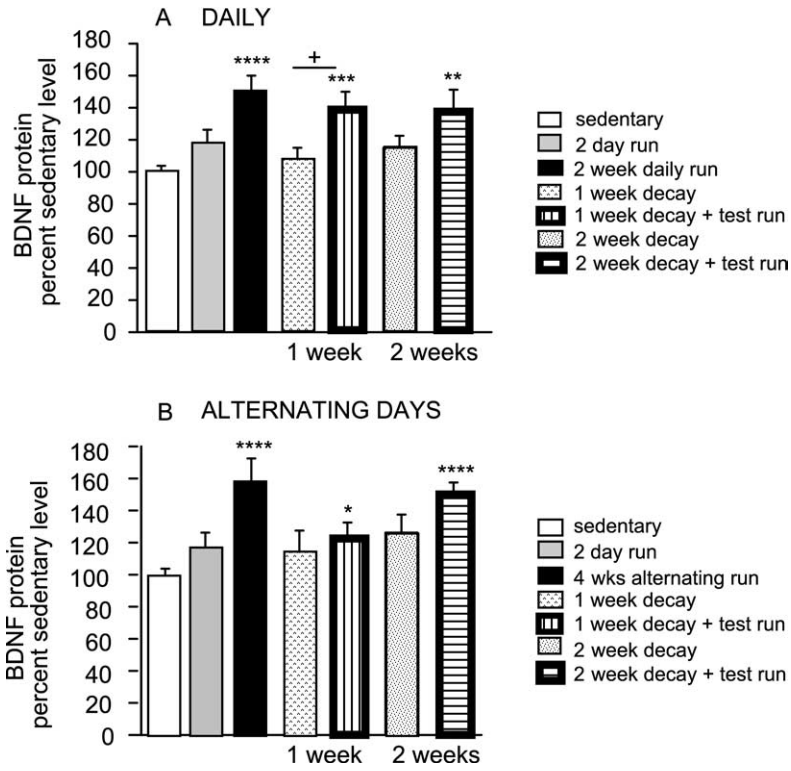

Fig. 4. BDNF protein levels are rapidly reinduced by a brief second running exposure. Animals initially ran daily for 14 days (A) or on alternating days for 28 days (B) after which wheels were locked for 7 or 14 days, in both paradigms. To assess the BDNF response to subsequent exercise, animals were allowed daily access to running wheels for 2 days following the lock-out period, in both paradigms. On its own, 2 days of exercise is not sufficient to increase BDNF protein levels above baseline. However, after prior exercise exposure, 2 days of exercise reinduces BDNF protein above sedentary levels even if the running experiences are separated by a delay of 7–14 days. The effect appears to be more robust if the first "priming" exercise consists of daily exercise, rather than intermittent exercise. Values are mean±S.E.M., normalized to sedentary levels. Relative to sedentary control levels: **** $P<0.0001$, *** $P<0.005$, ** $P<0.01$, * $P<0.05$; relative to matched-decay control timepoint: $^{+}$ trend $P=0.07$.

lower threshold of exercise exposure is subsequently needed to significantly increase BDNF protein levels, in either protocol. This "memory" for BDNF induction by exercise is robust and long-lasting. Even though BDNF levels after intermittent running were not significantly elevated above baseline after 3 days of inactivity (Fig. 3B), the priming effect was intact even after 14 days of inactivity (Fig. 4B).

DISCUSSION

Several novel concepts emerge from this study. In particular, (1) intermittent exercise is as effective as daily exercise to increase BDNF protein levels, (2) BDNF protein remains elevated for several days after exercise has ceased, and (3) BDNF protein is rapidly reinduced to peak levels by a normally-subthreshold exercise re-exposure, even after 14 days of quiescence. Thus, the experience of BDNF induction by exercise is robust and long-lasting. Further, exercise experience increases the readiness for a future BDNF response in the hippocampus. In other words, there is a "molecular memory" of the experience of exercise.

Intermittent exercise is as effective as daily exercise to increase BDNF protein

One objective of this study was to determine how different exercise schedules, daily or intermittent access to running wheels, affect BDNF regulation in the hippocampus. Many exercise studies have assessed the effects of daily activity, which does not reflect typical human exercise schedules where physical activity tends to be more intermittent. Thus, as a first part of this study, animals were trained with varying schedules of activity, to fill in the missing gap on BDNF regulation with respect to continuity and time course of physical activity.

Expanding on our previous findings (Berchtold et al., 2001, 2002; Adlard et al., 2004), BDNF protein levels progressively increase with longer running duration, in response to both daily exercise and alternating-days exercise. Interestingly, while daily training produced more rapid elevation in BDNF levels, intermittent exercise was ultimately as effective as daily exercise, even though exercise took place on only half as many days. This suggests that the induction of BDNF protein has a time component that interacts with the exercise stimulus. In addition, BDNF protein levels continued to rise even after 3 months of daily exercise. To our knowledge,

the effect of 3 months of exercise is the longest timepoint thus far examined in the exercise literature, with respect to BDNF regulation.

BDNF protein remains elevated after exercise end, and the duration of running determines the timecourse of decay

The dynamics of BDNF protein levels after exercise has ceased have until now been unexplored. We report here that BDNF protein remains elevated for several days after exercise has ceased, in some cases returning to baseline levels after 7–14 days of inactivity. The absolute amount of exercise days is an important factor regulating the rate of decline back to baseline. For example, the decay rate is similar after 14 days of exercise regardless if the exercise occurred daily (over 14 days), or on alternating days (over 28 days). Interestingly, the decay is faster after 28 days of intermittent exercise compared with 28 days of daily exercise, even though BDNF protein levels were initially elevated to exactly the same levels. These data suggest that the added stimulus of daily running may instruct regulation of the protein degradation rate, and/or may influence the rate of translation. The stability and decay of BDNF protein levels has interesting implications for determining how long the benefits from a bout of exercise might endure.

Exercise primes a memory for BDNF protein induction

A third aim of this study was to elucidate if exercise exposure can increase the readiness for a future BDNF response to exercise. We have reported that BDNF protein drops back to approximately sedentary levels after 7–14 days of inactivity. Once exercise is revisited, BDNF reinduction does not follow the same timecourse as occurs in naïve animals. Instead, the increase in protein is rapid, and occurs in response to a normally-subthreshold exercise exposure (e.g. 2 days of exercise).

The observation that exercise can increase the readiness for a future BDNF response to exercise effect is an example of "molecular memory." Learning and memory can be defined as a process whereby an experience is encoded such that when re-exposed to the relevant stimulus, the nervous system generates an altered response as a result of the initial experience (Thompson, 2004). Placing the current observations in this context, the exercise stimulus is "remembered" in terms of the BDNF endpoint, with a BDNF protein response subsequently occurring to a previously subthreshold stimulus. The observation that exercise primes a molecular memory for BDNF protein induction is a novel effect for both BDNF as well as for the exercise field.

The mechanisms mediating the priming effect are likely to be quite diverse. Both increased gene transcription and faster turnover of BDNF mRNA to protein may be occurring. However, recent evidence suggests that the exercise-induced increase in BDNF protein is not paralleled by a straightforward increase in BDNF gene expression (Adlard et al., 2004). As another potential mechanism, because BDNF is synthesized as a precursor molecule (pro-BDNF), which is proteolytically cleaved to generate mature BDNF, exercise may increase the conversion of pro-BDNF to the mature form measured here. A further effect of exercise may be to increase the reserve pool of pro-BDNF, allowing for more rapid production of cleaved mature BDNF by circumventing or reducing the need for translation from mRNA to protein. These hypotheses are currently being tested.

Exercise primes mechanisms for learning and memory

One function of exercise may be to generally enhance the machinery important in learning in memory. For example, many of the same genes that are induced during learning and memory are induced in the hippocampus by exercise. These include key molecules such as BDNF, CREB and the NMDA receptor subunit NR2b, among others (Shen et al., 2001; Tong et al., 2001; Molteni et al., 2002; Vaynman et al., 2003; Farmer et al., 2004). Further, exercise primes the induction of perforant path LTP, resulting in a lower threshold for induction by theta-patterned stimulation, as well as enhanced EPSP amplitude (van Praag et al., 1999; Farmer et al., 2004). Exercise thus appears to induce a series of adaptive responses that could translate to facilitated encoding, and improved learning and memory.

Using BDNF induction as an endpoint, this study revealed that exercise effects on the hippocampus endure for some time after exercise has ceased, that intermittent exercise shares some features but is not identical to the effects of daily exercise, and that exercise after a longer period still generates molecular changes that can facilitate encoding and ultimately improve behavior. In addition, exercise induces a molecular mechanism that can be rapidly reactivated when exercise is revisited, even after a long delay. It is possible that learning and memory may be similarly primed by exercise. Such a priming mechanism provides predictions for future animal experiments as well as direction for better understanding the effects of exercise on human cognition/learning and memory.

Clinical relevance of findings

The clinical literature on cognitive health benefits of exercise has tended to compare exercisers versus non-exercising individuals, often without taking into consideration variables such as frequency, duration, and intensity of exercise. As a result, cross-sectional, interventional, and longitudinal studies on the effects of exercise on cognitive decline in humans have been inconsistent. The results of this study demonstrate that a range of variables within exercise can affect the outcome measure, including variables such as exercise frequency, regularity, and renewal effect. Recent studies have begun to quantify the role of intensity and duration in assessing how exercise can affect cognitive function in humans (van Gelder et al., 2004). Our study indicates that while daily exercise is probably better than intermittent exercise, significant benefits can also be obtained from intermittent exercise schedules. The potential translatability of this study is high, as it will help generate hypotheses to assess the relationship of different aspects of exercise to cognitive benefits gained. This, in turn, will provide guidelines for optimizing the design of

exercise and rehabilitation programs, in order to promote optimal hippocampal function as well as a healthy neural environment.

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