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Exercise, antidepressant treatment, and BDNF mRNA expression in the aging brain

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Abstract

Principal mental disorders affecting the geriatric population include dementia and depression. A lack of trophic support is thought to contribute to the pathology of these disorders. Physical activity and antidepressant treatment increase the expression of brain-derived neurotrophic factor (BDNF) in the young rat hippocampus. Herein, we investigated the responsiveness of the aging rat hippocampus to antidepressant treatment and voluntary exercise. In situ hybridization revealed that, in young animals, exercise, antidepressant treatment, or their combination elevated BDNF mRNA levels in several hippocampal regions, most notably in the CA3, CA4, and dentate gyrus (DG). This effect was rapid (detectable at 2 days) and sustainable to 20 days. In aged (22-month-old) rats, hippocampal responsiveness to antidepressant treatment and exercise was also rapid and sustainable, but evident mostly in the CA1 and CA2. Daily swimming also revealed that small amounts of activity led to marked elevations in hippocampal BDNF mRNA. The differences in regional patterns of BDNF mRNA elevations between young and aged animals observed with running were maintained with this different exercise modality. Our results indicate that the aged brain is responsive to exercise and antidepressant treatment, and changes in regional response patterns may reflect shifts in hippocampal physiology during the lifespan.

Keywords: Hippocampus; Physical activity; In situ hybridization; Sprague–Dawley; Fischer-344; Neurotrophin; Elderly; Tranylcypromine

1. Introduction

The principal mental disorders affecting late life are dementias, such as Alzheimer's disease (AD) and the primary mood disorder, depression. Emotional disturbances, such as depressed mood, apathy, anxiety, and aggression, are among the most common and problematic symptoms in neurodegenerative disorders (Reisberg et al., 1987). Many have theorized that neuronal atrophy and death in neurodegenerative disorders result, in part, from a lack of trophic support (Appel, 1981). In fact, a key member of the neurotrophin family, brain-derived neurotrophic factor (BDNF), is diminished in the hippocampi of AD patients (Murray et al., 1994; Phillips et al., 1991). BDNF, the most widely distributed neurotrophin within the adult central nervous system (CNS), is present in highest concentrations within the hippocampus, a brain area with vital functions in learning and memory as well as in behavioral regulation (Hofer et al., 1990).

Previous studies have shown that BDNF promotes the growth and survival of several neuronal classes, including mesencephalic dopaminergic neurons (Knüsel et al., 1991), septal and basal forebrain cholinergic neurons (Nonner et al., 1996), and striatal GABAergic neurons (Ventimiglia et al., 1995). BDNF also serves as a neurotransmitter modulator (Frerking et al., 1998; Kafitz et al., 1999) and participates in use-dependent plasticity mechanisms, such as long-term potentiation and learning (Figurov et al., 1996; Levine et al., 1995; Lindsay et al., 1994). After binding to its tyrosine kinase receptor, trkB, BDNF activates a number of growth and survival-promoting intracellular signaling pathways, including the Ras/MAP kinase

Abbreviations: 5-HT, serotonin; AD, Alzheimer's disease; BDNF, brain-derived neurotrophic factor; CA1–4, cornus ammonis subregions 1–4; CNS, central nervous system; CREB, cyclic AMP response element-binding protein; DG, dentate gyrus; F-344, Fischer-344; ip, intraperitoneal; NE, norepinephrine; PB, phosphate buffer; PSLD, post-hoc least significant difference; SD, Sprague–Dawley; SPB, Sorenson's phosphate buffer; SSC, saline sodium citrate buffer; ss DNA; salmon sperm DNA.

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and phosphotidylinositol-3 kinase/Akt cascades (Yuan and Yankner, 2000).

Hippocampal BDNF is down-regulated in response to stress (Smith et al., 1995). Both clinical and animal studies have indicated that prolonged and severe stress may play an important role in the pathophysiology of depression and other psychiatric disorders. Neuronal atrophy and cell death have been observed in the hippocampi of animals exposed to chronic stress (Sapolsky, 2000; Watanabe et al., 1992), and animals subjected to stress also show behavioral changes associated with a depressive state (Benelli et al., 1999; Redrobe and Bourin, 1999). In addition, humans with a history of chronic, recurrent depression, or posttraumatic stress disorder have shown significant hippocampal atrophy in imaging studies (Bremner et al., 1995; Sheline et al., 1996).

One measure taken to treat emotional symptoms associated with mental disorders of aging is the administration of antidepressant medications. In recent years, evidence has been gathering that BDNF expression may be a downstream target of monoamine-enhancing, mood-stabilizing antidepressant treatments, and could be an important agent for therapeutic recovery from depression and the protection against stress-induced neuronal damage (D'Sa and Duman, 2002). The direct infusion of BDNF into the midbrain has been shown to produce an antidepressant-like effect in behavioral models, such as the learned helplessness and forced swim paradigms (Shirayama et al., 2002; Siuciak et al., 1997). Moreover, chronic treatment with a variety of antidepressant medications has been shown to up-regulate the cAMP–CREB (cyclic AMP response element-binding protein) cascade and expression of BDNF mRNA levels in the rat hippocampus (Nibuya et al., 1996; Thome et al., 2000).

Recent evidence also suggests that our daily behaviors and lifestyle influence the level of BDNF expression in the brain. Experiences often associated with enhanced emotional health, such as exercise and environmental enrichment, increase transcription of this important neurotrophin (Ickes et al., 2000; Neeper et al., 1995; Schoups et al., 1995) and also regulate adult neurogenesis within the hippocampus (Duman et al., 2001; van Praag et al., 1999). The ability of exercise to improve the psychiatric status of young and middle-aged depressed patients has been suggested for some time now (Hill et al., 1993; Labbe et al., 1988). In addition, Singh et al. (2001) have shown that unsupervised exercise in elderly subjects maintained an antidepressant effect over a 20-week period. Studies from our laboratory suggest that physical activity both enhances and accelerates BDNF mRNA up-regulation brought about by antidepressant treatment in the rat (Russo-Neustadt et al., 2000). These studies also suggest that the interventions of exercise and antidepressant treatment enhance BDNF expression via similar intracellular pathways. Taken together, current evidence suggests that a BDNF deficit may play an important role in the pathophysiology of mental illness, and its enhancement may play an important role in treatment.

Pharmacological treatment in geriatric patients is challenging, as the aging brain is thought to be less plastic and more beset by complications, such as oxidative damage and metabolic changes (Navarro et al., 2002; Stewart, 1982; Toescu et al., 2000). In our study, we have assessed the responsiveness of the aging rat hippocampus to antidepressant treatment and exercise. We have employed the use of commercially available, aged Fischer-344 (F-344) rats in our experiments. To ensure continuity and comparability with our previous studies of hippocampal BDNF expression, we have also used Sprague–Dawley (SD) rats as animal subjects. We have asked whether antidepressant treatment may enhance BDNF expression in the aging hippocampus, and whether this response could be accelerated or augmented by voluntary physical exercise, as is evident in the young animal.

2. Materials and methods

2.1. Animal subjects

Three-month-old male SD rats (Charles River), 3-month-old male F-344 rats (National Institute of Aging/Harlan), and 22-month-old male F-344 rats (National Institute of Aging/Harlan) were housed singly with food and water ad libitum in a vivarium with controlled temperature and humidity and a 12:12-h (06:00–18:00 h) light/dark cycle. All rats were allowed to acclimate to the vivarium for 1 week prior to the start of experiments. For the voluntary wheel-running experiment, animals were randomly assigned to one of four groups: sedentary, saline (SS); activity, saline (AS); sedentary, tranylcypromine (ST); or activity, tranylcypromine (AT). Young SD rats underwent these interventions at all three time intervals tested (see below), as did aged F-344 animals. In addition, young F-344 animals received antidepressant and/or exercise for 14 days to assess any possible strain differences in response to these interventions. Additional experimental animals underwent daily swimming, an alternate (nonvoluntary) form of physical activity with fixed intervals. Young and aged rats were assigned to a control/sedentary group or to a swimming group. All animal use procedures, described below, were in strict accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals (1996), and all efforts were made to minimize the number of animals used and potential pain and distress.

2.2. Drug treatments

Young (3-month-old) SD rats and aged (22-month-old) F-344 rats received daily (09:00 h) injections of tranylcypromine, a monoamine oxidase inhibitor, at a dose of 7.5 mg/kg ip for 2, 14, or 20 days. Young F-344 rats received daily injections of 7.5 mg/kg ip tranylcypromine

for 14 days. Control animals received saline (vehicle) injections. All animal treatment groups are outlined in Table 1.

2.3. Exercise

2.3.1. Voluntary physical activity

Exercising animals engaged in voluntary physical activity through free access to running wheels throughout the duration of the experiment. After a week of initial acclimation to the vivarium, the animals were placed in polyethylene cages equipped with 34.5-cm-diameter running wheels (Nalgene, OR). Distance run per 24 h was recorded by computer using Ratrun software (C. Hage Associates, CA). Animals in the control groups remained sedentary in their cages for the duration of the experiments.

2.3.2. Swimming

Young and aged rats underwent physical activity in the form of swimming in a Morris water tank. The water tank was 2.4 m in diameter and 0.61 m in height and it was filled up to 0.46 m with water at room temperature. Each rat from either young or aged groups was allowed to swim for two 2-min trials, twice a day (12:00 and 17:00 h) for 2 or 14 days. Animals in the control group remained sedentary in their cages for the duration of the experiment.

2.3.3. Summary

Table 1 summarizes the age (young vs. aged), strain (SD vs. F-344), treatment groups (SS, ST, AS, or AT), sample size (*n*), and the mean running distances for each time interval (2, 14, or 20 days).

Table 1
Summary of the animal groups, sample size, age, strain, and running distance for each experimental time point

Running

Time point (days)	Age and strain	Group/*n*	Running distance (km/24 hr) (mean ± S.E.M.)
2	young SD	SS/7	NA
		ST/7	NA
		AS/7	1.8 ± 0.34
		AT/7	1.1 ± 0.19
	aged F-344	SS/5	NA
		ST/5	NA
		AS/7	0.195 ± 0.015
		AT/4	0.287 ± 0.05
14	young SD	SS/7	NA
		ST/7	NA
		AS/7	3.8 ± 0.49
		AT/7	3.2 ± 0.39
	young F-344	SS/7	NA
		ST/4	NA
		AS/7	0.423 ± 0.067
		AT/NA	NA
	aged F-344	SS/5	NA
		ST/4	NA
		AS/7	0.375 ± 0.036
		AT/6	0.45 ± 0.031
20	young SD	ST/8	NA
		SS/8	NA
		AS/8	2.5 ± 0.38
		AT/5	2.1 ± 0.43
	aged F-344	SS/6	NA
		ST/7	NA
		AS/7	0.333 ± 0.026
		AT/7	0.486 ± 0.038

Swimming

Time point (days)	Age and strain	Group/*n*
2	young SD	SS/6 swim/saline/5
	aged F-344	SS/7 swim/saline/4
14	young SD	SS/6 swim/saline/7
	aged F-344	SS/5 swim/saline/5

2.4. Tissue preparation

All experimental animals were sacrificed at 06:00 h following the last treatment day, a time previously reported for peak diurnal baseline expression of BDNF (Berchtold et al., 1999). Brains were extracted from both young and aged rats in less than 30 s and were quick-frozen in a dry ice/2-methylbutane bath (~ −60 °C) and stored at −80 °C. Coronal brain sections (12 μm thick) were thaw-mounted onto Vectabond-pretreated (Vector labs) slides, postfixed in 4% paraformaldehyde, rinsed in 0.1 M phosphate buffer (PB), and stored at −20 °C with desiccant until use for in situ hybridization.

2.5. Preparation of cRNA probes

The BDNF riboprobe was prepared from a cDNA template coding for the full-length rat BDNF gene (kindly provided by Dr. Paul Isackson). A pBluescript KS 700 basepair fragment, linearized with *Xba*I, and transcribed with T3 RNA polymerase, generated antisense cRNA. Linearization with *Hin*dIII and transcription with T7 RNA polymerase generated sense cRNA.

2.6. In situ hybridization

2.6.1. Prehybridization

Slides were washed at room temperature 2 × 3 min in 0.1 M glycine in PB, 1 × 15 min in PB, 10 min in 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8), followed by 2 × 15 min rinses in 2 × saline sodium citrate buffer (SSC). Tissue was dehydrated and defatted by transferring through 50% ethanol (1 min), 70% ethanol (1 min), 95% ethanol (1 min), 100% ethanol (1 min), chloroform (5 min), 100% ethanol (1 min), 95% ethanol (1 min), then air-dried.

2.6.2. Hybridization

One hundred twenty to 180 μl hybridization solution was applied to each slide (1×10^6 cpm/100 μl hybridization solution, 35 × Denhardts, 1 mM EDTA, 0.3 M NaCl, 0.15 mg/ml tRNA, 300 μg/ml salmon sperm DNA, 40 mM dithiothreitol), coverslipped with mounting medium (DEPEX), and incubated at 55–60 °C for 18–20 h.

2.6.3. Posthybridization

Slides were rinsed in 4 × SSC buffer at 60 °C, treated with 20 μg/ml ribonuclease A for 30 min at 45 °C, and washed through descending concentrations of SSC to a final wash in 0.1 × SSC at 60 °C. Tissue and ^{14}C-labeled standards were exposed to Amersham β-max film (Amersham Labs) for 5 days.

2.7. Analysis

Hybridization densities were measured from film autoradiograms using computer densitometry (MCID image processing system; St. Catherines, Ontario, Canada) and calibrated relative to film images of ^{14}C-labeled standards. For each animal, the mean labeling density was calculated as averages of 7×0.03 mm^2 areas randomly selected from each hippocampal area from two different tissue sections, minus background levels (measured from film adjacent to a section). Specificity of hybridization was confirmed by demonstrating no significant hybridization with ^{35}S-labeled sense BDNF riboprobe. Significance of effects of treatment were evaluated by one-way analysis of variance using Statview software (Abacus Concepts, CA), with a significance level of $P < .05$, and Fisher's post hoc least significant difference test.

3. Results

3.1. BDNF mRNA levels with acute treatment (2 days)

In young SD rats, the combination of tranylcypromine and physical activity led to small but significant elevations in hippocampal BDNF mRNA levels after 2 days of treatment in all subregions examined, except in the CA2 [CA1, $F(3,24) = 5.130$, $P < .01$, 16% above controls; CA3, $F(3,24) = 3.772$, $P < .05$, 66% above controls; CA4, $F(3,23) = 5.080$, $P < .01$, 81% above controls; and dentate gyrus (DG), $F(3,24) = 2.655$, $P < .05$, 37% above controls] (Fig 1A). Individually, tranylcypromine elevated BDNF mRNA levels in the CA1 and DG: 13% and 35%, respectively. It should also be noted that the combination treatment led to significantly higher BDNF levels than either intervention alone in the CA4 ($P < .05$). SD rats from the saline-treated group ran an average of 1.8 ± 0.34 km per 24-h period, and the antidepressant group ran an average of 1.1 ± 0.19 km per 24-h period. The difference in running distances between the groups was not statistically significant. Overall, the combination of exercise and antidepressant treatment, over a short period, led to a significant increase in BDNF mRNA, particularly in the CA3 and CA4 regions of the young SD rat hippocampus.

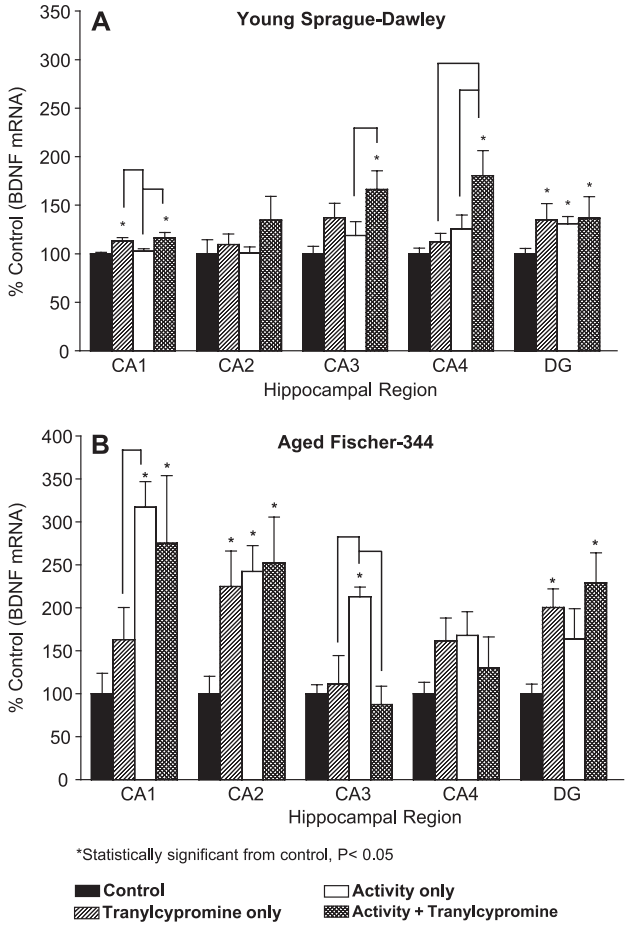

Fig. 1. BDNF mRNA levels with acute treatment (2 days). Hippocampal regional differences in BDNF mRNA levels in response to the four treatment conditions in (A) young (3-month) and (B) aged (22-month) rats at 2 days. (A) Quantitative in situ hybridization reveals that the combination of antidepressant treatment and voluntary physical exercise increased hippocampal BDNF mRNA in the CA1, CA3, CA4, and DG regions, with highest levels evident in CA3 and CA4. (B) In aged animals, short-term interventions were also effective in elevating hippocampal BDNF mRNA levels in the CA1, CA3, and DG regions. Results are displayed as the percentage of control and represent the mean ± S.E.M. Asterisks denote statistically significant differences from the control group (SS), $P < .05$. Bridges between bars denote statistical significance ($P < .05$) between the indicated groups.

In the older F-344 rats, short-term voluntary exercise led to marked increases in BDNF mRNA in the CA1 [$F(3,17) = 6.261$, $P < .01$, 217% above controls], CA2 [$F(3,17) = 3.808$, $P < .01$, 142% above controls], and CA3 [$F(3,17) = 9.651$, $P < .001$, 113% above controls] subregions. Acute tranylcypromine treatment led to significant elevations in BDNF mRNA within the CA2 (125% above controls) and the DG [$F(3,17) = 3.140$, $P < .05$, 101% above controls] (Fig. 1B). The activity–antidepressant combination also led to significant BDNF mRNA increases in the CA1 (175% above controls),

CA2 (152% above controls), and DG [$F(3,17)=3.140$, $P<.05$, 129% above controls] regions of the aging rat hippocampus (Fig 1B). During this 2-day period, animals from the saline-treated group ran an average of 195.3 ± 14.8 m per 24-h period and the antidepressant group ran an average of 287.9 ± 49.8 m per 24-h period. The difference in running distances between the groups was not statistically significant. Our results demonstrate that voluntary exercise and antidepressant treatment increase BDNF transcription within 2 days in the aging hippocampus, and that these changes appear to be most marked in the CA1.

3.2. BDNF mRNA levels with subchronic (14-day) treatment

Two weeks of voluntary wheel running in young SD rats led to robust BDNF mRNA increases in all subregions of the hippocampus (Fig 2A). Similarly, drug treatment enhanced BDNF mRNA levels in all (except CA2 and CA4) subregions. Physical activity combined with antidepressant treatment also led to significant BDNF mRNA increases within the CA2 [$F(3,24)=7.210$, $P<.001$, 53% above controls], CA3 [$F(3,24)=9.357$, $P<.001$, 113% above controls], CA4 [$F(3,24)=7.541$, $P<.001$, 106% above controls], and DG [$F(3,24)=9.951$, $P<.001$, 98% above controls]. Animals from the saline-treated group ran an average of 3.8 ± 0.49 km per 24-h period and the antidepressant group ran an average of 3.2 ± 0.39 km per 24-h period. The difference in running distances between the groups was not statistically significant.

In young F-344 rats, 14 days of voluntary running similarly led to significant increases in hippocampal BDNF mRNA in all subregions (Fig. 2B) [CA1: $F(2,15)=4.762$, $P<.05$, 45% above controls; CA2: $F(2,15)=3.773$, $P<.05$, 41% above controls; CA3, $F(2,15)=13.783$, $P<.001$, 36% above controls; CA4, $F(2,15)=9.966$, $P<.05$, 32% above controls; DG, $F(2,15)=11.747$, $P<.001$, 25% above controls]. Tranylcypromine treatment led to significant BDNF mRNA elevations in the CA3 (23% above controls), CA4 (21% above controls), and DG regions (24% above controls). All but one of the young F-344 rats receiving the combination treatment of exercise-plus-tranylcypromine died during the course of the 14-day treatment. Thus, this group was excluded from the analyses. The young F-344 rats receiving exercise ran an average of 423 ± 67 m per 24-hr period.

Descriptive statistics comparing the percent increases in BDNF mRNA levels in the various hippocampal subregions of the young animals of both SD and F-344 strains are displayed in Table 2. Inspection of Table 2 reveals that increases in hippocampal BDNF mRNA levels were more

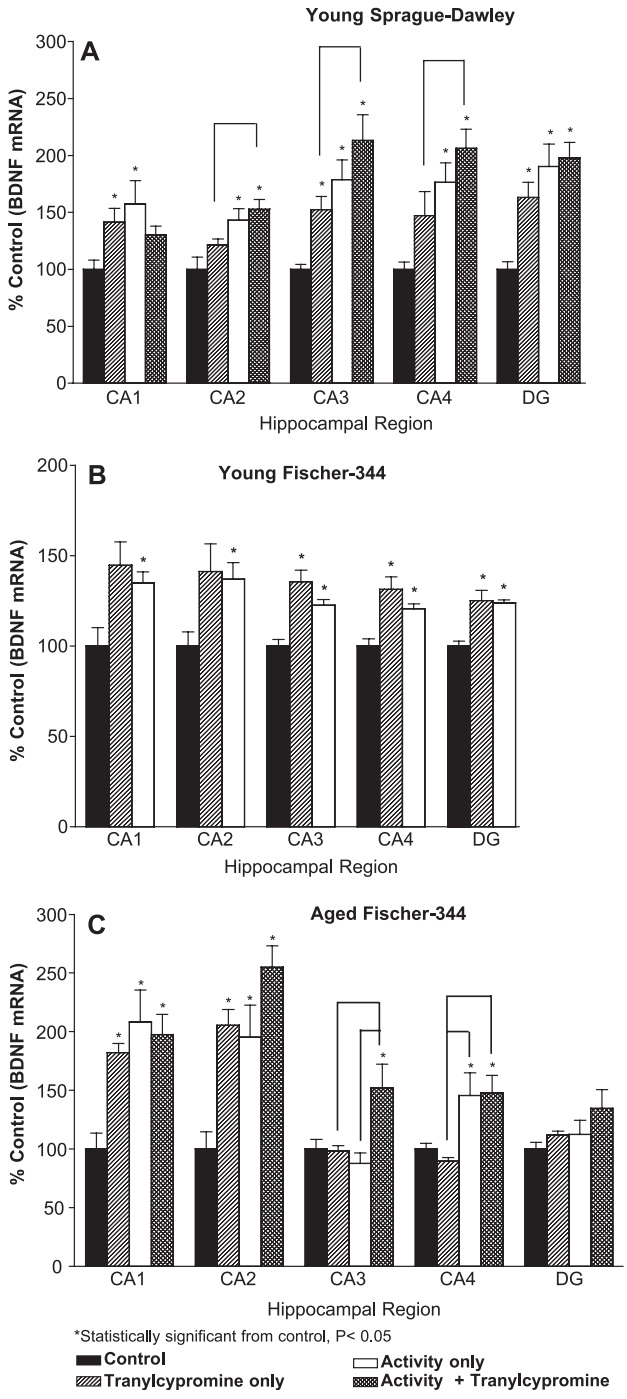

Fig. 2. BDNF mRNA levels with subchronic (14-day) treatment. Hippocampal regional differences in BDNF mRNA levels in response to the four treatment conditions in (A) young SD (3-month), (B) Young F-344 (3-month) and (C) aged F-344 (22-month) rats at 14 days. (A) In situ hybridization reveals the majority of interventions led to marked increases in BDNF transcription in several hippocampal cell fields. (B) Voluntary wheel running increased the expression of BDNF MRNA in all hippocampal areas examined in young F-344 rats. Tranylcypromine alone increased the expression of BDNF MRNA in the CA3/CA4/DG loop. (C) In aged animals, voluntary wheel running and tranylcypromine treatment alone significantly increased hippocampal BDNF mRNA levels in the CA1 and CA2 subregions. The combination of tranylcypromine treatment with activity led to significant increases in all hippocampal areas, with the most striking increases evident in the CA1 and CA2. Results are displayed as the percentage of control and represent the mean ± S.E.M. Asterisks denote statistically significant differences from the control group (SS), $P<.05$. Bridges between bars denote statistical significance ($P<.05$) between the indicated groups.

Table 2
Region-by-region and treatment-by-treatment comparison of mean BDNF mRNA levels between young SD and young F-344 rats during the 14-day treatment regimen

Strain	Treatment	CA1	CA2	CA3	CA4	DG
SD	tranylcypromine	141.36 ± 12* (+41%)	121.2 ± 5.4 (+21%)	152.2 ± 11.7* (+52%)	147 ± 21.2 (+47%)	163 ± 13.48** (+63%)
	exercise	157.37 ± 21** (+57%)	143.1 ± 10** (+43%)	178.56 ± 18** (+79%)	176.2 ± 17.4** (+76%)	190.3 ± 20**** (+90%)
	combination	129.9 ± 7.8 (+30%)	152.9 ± 8.3*** (+53%)	213.3 ± 23**** (+113%)	206 ± 17.1*** (+106%)	197.8 ± 14**** (+98%)
F-344	tranylcypromine	135 ± 6.2 (+35%)	137 ± 9.2 (+38%)	122.6 ± 3.1* (+23%)	120.5 ± 2.9* (+21%)	123.8 ± 1.8** (+24%)
	exercise	144.8 ± 13** (+45%)	141.2 ± 15* (+41%)	135.5 ± 7**** (+36%)	131.5 ± 6.8*** (+32%)	125.2 ± 5.5*** (+25%)
	combination	NA	NA	NA	NA	NA

Values are the mean ± S.E.M. with percent increase (+) from controls appearing in parentheses below each entry.
* Statistically significant differences above control levels at $P \leq .05$.
** Statistically significant differences above control levels at $P \leq .01$.
*** Statistically significant differences above control levels at $P \leq .001$.
**** Statistically significant differences above control levels at $P \leq .0001$.

profound in the SD rats (with a range of 41–113% above control levels) than in the F-344 rats (21–45% above control levels). This appeared to be in proportion to the relative activity levels of these two strains (shown in Table 1). Regional patterns of enhanced BDNF expression (most marked in the CA3, CA4, and DG) were maintained in both strains of young animals.

In the aging F-344 rat brain, the most robust BDNF responses to 2 weeks of wheel-running exercise, antidepressant treatment, and their combination were once again evident in the CA1 [$F(3,18) = 5.218$, $P < .05$, 82% above control, as was observed after 2 days] and in the CA2 [$F(3,18) = 8.482$, $P = .001$, 106% above control levels] (Fig 2C). Specifically, tranylcypromine treatment on its own significantly elevated BDNF mRNA levels in these areas. In addition, the combination treatment over this 2-week period enhanced BDNF transcription in the CA1 (98% above controls) and CA2 (155% above controls), and to a lesser, but significant, extent in the CA3, [$F(3,18) = 5.308$, $P < .01$, 52% above controls] and CA4 [$F(3,18) = 3.743$, $P < .05$, 48% above controls]. Overall, the combination of antidepressant treatment with concomitant voluntary exercise in aged F-344 rats led to significant increases in BDNF mRNA in several regions, including the CA3 where no significant change was evident with either intervention alone (Fig. 2C). In this experiment, aged F-344 rats from the saline-treated group ran an average of 375.49 ± 36.1 m per 24-h period and the antidepressant group ran an average of 449.5 ± 31.3 m per 24-h period. The difference in running distances between these two groups was not statistically significant.

3.3. BDNF mRNA levels with chronic (20-day) treatment

After the 20-day experimental period, physical exercise combined with antidepressant treatment significantly elevated BDNF mRNA in the CA1 [$F(3,25) = 2.195$, $P < .05$, 34% above controls], CA4 [$F(3,25) = 4.603$, $P < .05$, 87% above controls], and DG [$F(3,25) = 2.415$, $P < .05$, 64% above controls] regions of the young SD rat hippocampus (Fig. 3A). In contrast, tranylcypromine or activity, applied individually, led to little significant change; exercise alone increased BDNF mRNA in the CA4 (45% above controls). In this experiment, the young SD rats from the saline-treated group ran an average of 2.5 ± 0.38 km per 24-h period and the antidepressant group ran an average of 2.1 ± 0.43 km per 24-h period. The difference in running distances between the groups was not statistically significant.

Long-term interventions in the aged F-344 rats led to strikingly different regional responses compared to those of young animals. No enhancement of BDNF transcription was evident following any interventions in the CA3, CA4, or DG. In contrast, robust responses to both antidepressant treatment (193% above controls) and the antidepressant–activity combination (137% above controls) were evident in the CA1 [$F(3,25) = 7.041$, $P < .01$] (Fig 3B). In addition, a smaller, significant elevation in BDNF mRNA was evident in the CA2 [$F(3,23) = 3.863$, $P < .05$, 54% above controls] following 20 days of tranylcypromine treatment. During this experimental period, rats from the saline-treated group ran an average of 332.6 ± 26.2 m per 24-h period and the antidepressant group ran an average of 486.13 ± 37.6 m per 24-h period. In these rats, there was a small but significant effect of increased activity in the tranylcypromine group.

3.4. BDNF mRNA levels with 2-day swim activity

Two days of swimming activity in young SD rats led to significant elevations of BDNF mRNA in the CA3 [$F(1,9) = 6.176$, $P < .05$, 46% above controls] and DG

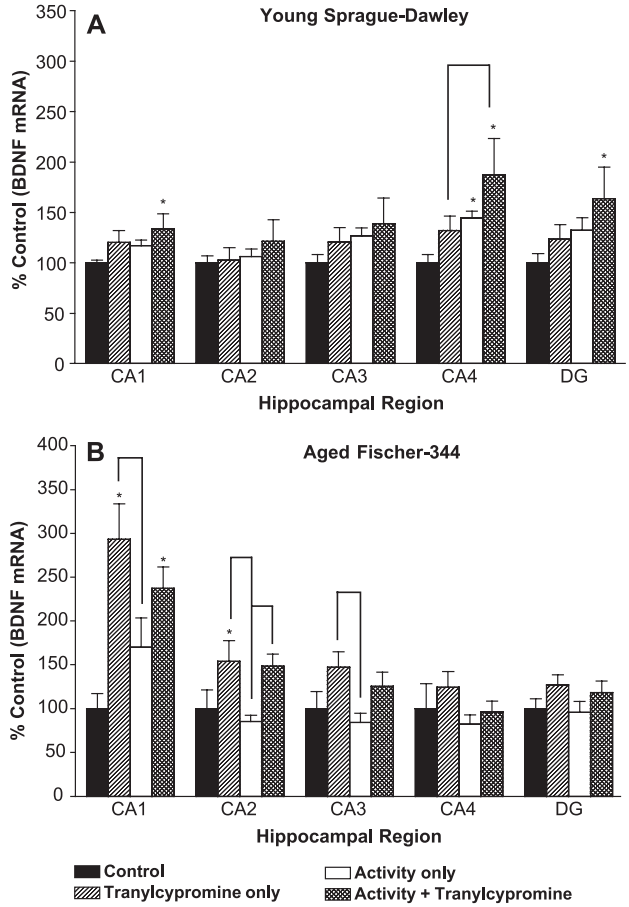

Fig. 3. BDNF mRNA levels with chronic (20-day) treatment. Hippocampal regional differences in BDNF mRNA levels in response to the four treatment conditions in (A) young (3-month) and (B) aged (22-month) rats at 20 days. (A) Quantitative in situ hybridization reveals the combination of voluntary physical activity with antidepressant treatment significantly elevated BDNF mRNA levels in the CA1, CA4, and DG regions. (B) In aged animals, marked increases in BDNF mRNA were evident in the CA1 following antidepressant and antidepressant/activity, and significant increases were also evident in the CA2 following tranylcypromine treatment. Results are displayed as the percentage of control and represent the mean ± S.E.M. Asterisks denote statistically significant differences from the control group (SS), $P<.05$. Bridges between bars denote statistical significance ($P<.05$) between the indicated groups.

[$F(1,9)=22.557$, $P=.001$, 92% above controls] (Fig 4A). A small but significant decrease in BDNF mRNA was observed in the CA1 region [$F(1,9)=5.93$, $P<.05$, 8% below controls]. Except for the slight decrease observed in the CA1 region of rats that swam, the regional pattern of BDNF expression was similar to animals running on wheels for 2 days (Fig 1A). In older F-344 animals, 2 days of swimming markedly increased BDNF mRNA levels in all (but the CA2) hippocampal subregions. Fig. 4B reveals elevations of up to 179% 165%, 103%, and 105% in the CA1, CA3, CA4, and DG, respectively [CA1: $F(1,9)=21.195$, $P=.001$; CA3: $F(1,9)=25.488$, $P<.001$; CA4, $F(1,9)=30.1$, $P<.001$; DG, $F(1,9)=21.747$, $P=.001$].

3.5. BDNF mRNA levels with 14-day swim activity

Two weeks of daily swimming activity led to no significant increases in hippocampal BDNF transcription in young SD rats (Fig 5A). On the other hand, significant BDNF mRNA increases were observed with aged F-344 rats in the CA1 [$F(1,8)=8.121$, $P<.05$, 59% above controls] and CA2 [$F(1,8)=16.85$, $P<.005$, 66% above controls) (Fig 5B). This pattern was similar to that observed in aged animals after 14 days of voluntary running (Fig 2B); however, a significant decrease in BDNF mRNA was

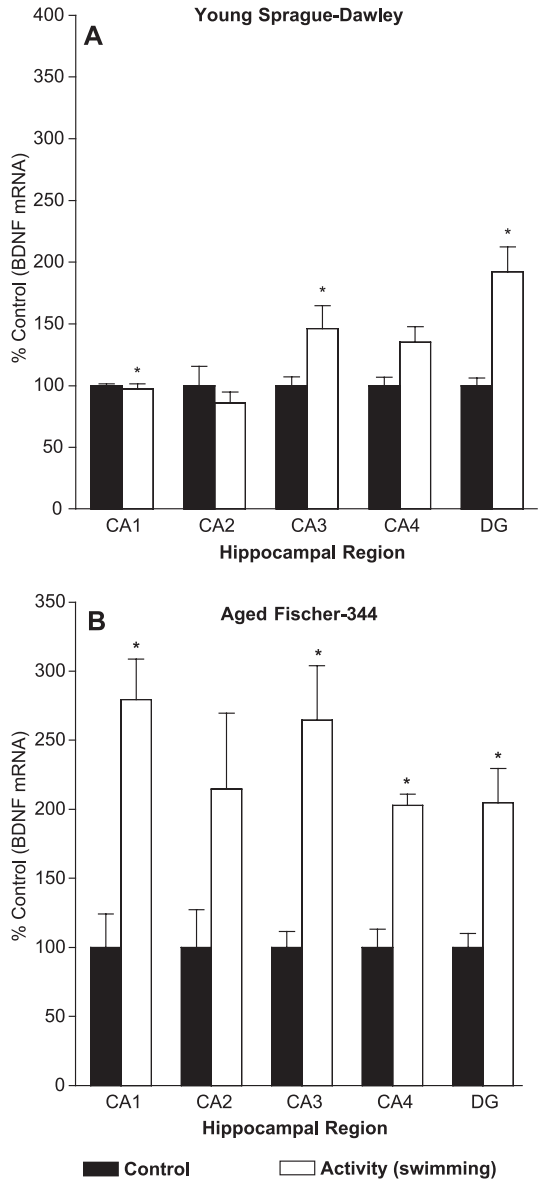

Fig. 4. Swimming activity (8 min/day total) for 2 days significantly increased BDNF mRNA levels in the CA3 and DG subregions of the young (A) rat hippocampus. In older animals (B) swimming activity led to marked increases in BDNF mRNA in all hippocampal subregions compared to sedentary controls. Results are displayed as the percentage of control and represent the mean ± S.E.M. Asterisks denote statistically significant differences from the control group (SS), $P<.05$.

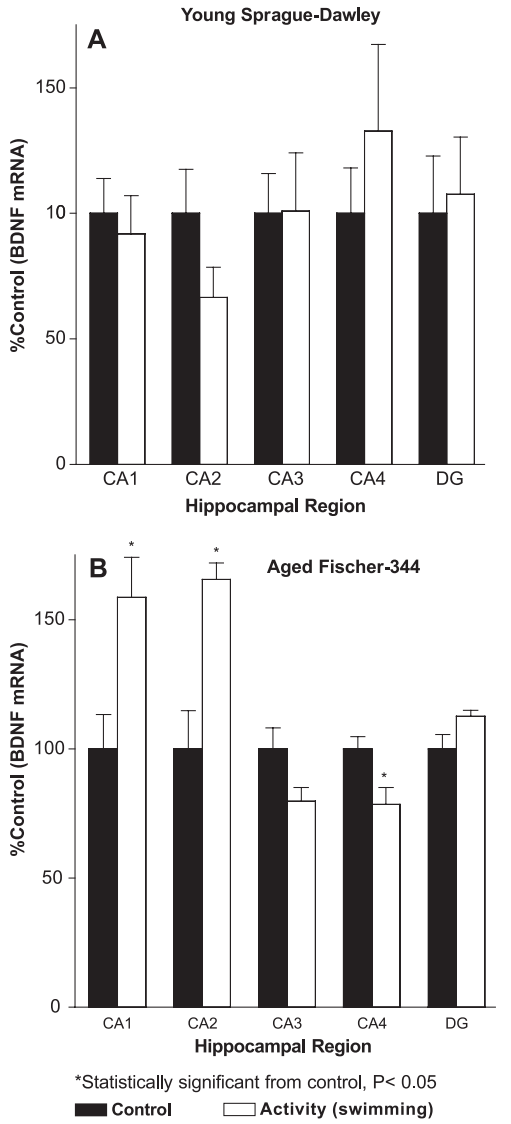

Fig. 5. Swimming activity (8 min/day total) in young (A) animals for 14 days did not significantly increase BDNF mRNA levels in any of the hippocampal subregions. In older animals (B) swimming activity led to significant increases in BDNF mRNA in the CA1 and CA2 hippocampal subregions compared to sedentary controls. Results are displayed as the percentage of control and represent the mean ± S.E.M. Asterisks denote statistically significant differences from the control group (SS), $P < .05$.

observed in the CA4 region [$F(1,8) = 7.315$, $P < .05$, 21% below controls] of rats in the swimming group, compared to that of controls (Fig 5B).

4. Discussion

Our previous studies revealed that in young adult rats, antidepressant treatment, voluntary physical exercise, or the two interventions combined resulted in significant increases in hippocampal BDNF mRNA expression after 2, 7 (Russo-Neustadt et al., 2000), or 20 (Russo-Neustadt et al., 1999) days. The largest BDNF mRNA increases were observed in the CA3, CA4, and DG subfields. To ensure continuity and comparability with these previous studies, we used young rats of the same strain (SD rats) for our current experiments. In addition, we also employed the use of F-344 animals, as only this strain was available to us commercially aged. Therefore, to account for any possible strain differences in the pattern of hippocampal responses to exercise/antidepressant, we also studied young F-344 rats. As expected, the same general pattern of BDNF mRNA expression observed previously was noted in young adult SD rats: BDNF mRNA was elevated most markedly in the CA3/CA4/DG region. Similarly, 2 weeks of activity or antidepressant treatment in young F-344 rats yielded a similar expression profile: the most marked changes in hippocampal BDNF transcription were observed in the CA3/CA4/DG loop in both rat strains.

The distribution of BDNF mRNA increases following exercise or antidepressant interventions contrasted strikingly in older rats. In these 22-month-old animals, hippocampal BDNF mRNA expression was significantly increased in the CA1 in response to exercise and the combination of exercise-plus-antidepressant treatment at all time intervals measured. Also, significant increases in BDNF mRNA were measured in both CA1 and CA2 in response to 14 and 20 days of treatment. It should be noted, in addition, that tranylcypromine significantly increased running behavior in aged animals at the longest time interval measured (20 days). Our findings of a CA3/CA4/DG response pattern with young Fischer rats (above) and a tendency for greatest responses in the CA1/CA2 with older animals undergoing daily swimming activity (Figs. 4B and 5B) support the idea that these patterns of response are particular to older animals, and not strain- or exercise modality-specific.

Contrary to what we expected, hippocampal responsiveness to antidepressant treatment was not attenuated in older rats. Instead, this ability to respond to treatment appears to have been shifted from the CA3/CA4/DG loop to the CA1 and CA2 fields. For example, older animals showed BDNF mRNA increases of up to 180% in the CA1 after antidepressant or exercise, even after brief treatment intervals (Figs. 1B and 4B). It is also noteworthy that older animals experienced an up-regulation in hippocampal BDNF mRNA after relatively small amounts of exercise (approximately 300–450 m of voluntary running on average in contrast to 2000–3800 m in younger animals, or 8 min/day total swimming). Taken together, these observations indicate that significant hippocampal responses can occur in the older brain following relatively small amounts of activity. As shown in one of our previous studies (Russo-Neustadt et al., 2001), as well as in several studies from other laboratories, forced swimming in cool water without known escape induces stress (indicated by increased corticosterone levels) and decreases hippocampal BDNF expression, possibly through activation of glutamate- and/or glucocorticoid-based mechanisms (Smith, 1996). On the other hand, time-limited bouts of swimming in warmer

water enhances hippocampal BDNF expression. This phenomenon is particularly marked when the swimming is coupled with a learning task (Kesslak et al., 1998) in which, an exercise-only (yoked swimming) component is evident. We did not monitor corticosterone levels in our rats, but did observe a coincident increase in hippocampal BDNF mRNA with swimming exercise. In most cases, BDNF levels were surprisingly comparable to those observed following wheel running.

Our results have led to several observations: (1) Exercise- and/or antidepressant treatment-induced transcription of BDNF is rapid, robust, and sustainable in both young and old rats. (2) Significant elevations in BDNF transcription were observed in the aged rat brain after relatively small amounts of activity. (3) A significant shift in hippocampal regional BDNF responsiveness across the life span is suggested.

The most noteworthy finding from our current study was the young-to-aged transitional shift in the hippocampal BDNF response profile at all three time points. As noted above, we observed the largest increases in BDNF mRNA expression in the CA3–CA4–DG loop in young rats of both F-344 and SD strains. Throughout the time course of our study, the most robust BDNF mRNA increases in the aged brains in response to running exercise, antidepressant, or the combination were observed in the CA1 and CA2. Although regional, baseline hippocampal BDNF mRNA levels are not diminished throughout the lifespan of a normal, healthy rat (Lapchak et al., 1993), it appears that BDNF mRNA up-regulation was diminished in one area of the hippocampus and increased in another (current study). Evidence supports the possibility of functional shifts between hippocampal subregions as these animals age. Most noteworthy is increased dendritic sprouting in the CA1 and CA2 subfields observed in the aging rat (Turner and Deupree, 1991; Pyapali and Turner, 1996). Such sprouting may represent a compensatory reaction to normal, age-related loss of CA3 pyramidal neurons (Kadar et al., 1990) and DG neurons (West, 1993), as well as dramatically decreased rate of neurogenesis in the DG (Gould et al., 1999; Seki and Arai, 1995; Kuhn et al., 1996). In addition, Barnes has shown that in aged rats, there is a decrease in synaptic input to the DG from the entorhinal cortex and concomitant increases in the strength of existing connections (Barnes, 1994, 1999). Finally, compared to their younger counterparts, aged rats have displayed a 27% decrease in perforant path–DG synaptic contacts (Geinisman and Bondareff, 1976) and consistently, the aged hippocampus has exhibited a greater reduction in AMPA binding sites in the CA3 and DG than in the CA1, compared with AMPA binding in younger rats (Clark et al., 1992), suggesting decreased glutamate receptor expression and functioning (Pagliusi et al., 1994).

In the rat brain, dentate granule cells send mossy fiber terminals to the CA3 and the hilus, which receive extrinsic noradrenergic, serotonergic, and cholinergic afferents from the locus coeruleus, median and dorsal raphe, and the medial septal area, respectively. Chronically exercised animals show increased levels of norepinephrine (NE) and serotonin (5-HT) in several brain areas, as compared to sedentary controls (Brown et al., 1979; Dey, 1994; Samorajski et al., 1987). Conceivably, then, because antidepressant treatment, exercise, and the combination treatment can each increase the levels of circulating neurotransmitters, these interventions may amplify hippocampal NE and/or 5-HT signaling, which may enhance BDNF expression.

To our knowledge, ours is the first report of a developmental shift in hippocampal BDNF transcription following activating interventions. The forgoing discussion suggested possible anatomical and/or pharmacological bases for this shift; however, the precise mechanism underlying this change is currently unknown. Collectively, investigations aimed at exploring the structural and chemical changes in the aging hippocampus are fraught with inconsistent results (Geinisman, 1999; Smith, 1996). Whatever these changes are, they underscore the need for more studies to be performed on the functional interaction between the various individual hippocampal subfields, throughout the lifespan (Geinisman, 1999).

The incidence of depression is known to be higher in the elderly population (Blazer and Williams, 1980). Successful antidepressant treatment in the elderly is difficult, in part because brain noradrenergic (Bickford-Wimer et al., 1988) and serotonergic (Levkovitz et al., 1994) levels and function often decline with age. Because of these issues, the use of alternative or supplementary interventions might be valuable in the treatment of depression for this age group (Gareri et al., 2000). Administration of antidepressants may begin to offset decreases in NE and 5-HT. In addition, exercise is known to increase the levels of hippocampal 5-HT and circulating NE from sympathetic nerve endings (Dey, 1994; Dey et al., 1992; Meeusen and De Meirleir, 1995; Wilson and Marsden, 1996). Singh et al. (2001) found that elders undergoing a 20-week exercise regimen were significantly less depressed than their nonexercising counterparts. The increase in BDNF as a result of one or both of these interventions reveals possible convergent intracellular mechanisms taking place. The ultimate and net effect, therefore, of antidepressant (or exercise) treatment is an alteration in gene expression of the receptors themselves (Dey, 1994; Yau et al., 1999), their transporters (Kakiuchi et al., 2001), or their downstream effectors, such as CREB (Nibuya et al., 1996) and BDNF (Gomez-Pinilla et al., 2001), which, in turn, can stabilize (Pelleymounter et al., 1996), or increase (Siuciak et al., 1996) 5-HT levels.

Given the important role of BDNF in neuroplasticity, development (Narisawa-Saito and Nawa, 1996), and repair (Lo, 1995; McAllister et al., 1995, 1999), an increase in

hippocampal BDNF (mRNA) levels in response to antidepressant and/or exercise underscores the ameliorative, protective effects these interventions may have for hippocampal function. Just as depression can be viewed as a form of sustained chemically induced lesion (Leonard, 2000), exercise can be viewed as an inducer of hippocampal neural plasticity (Cotman and Berchtold, 2002; Molteni et al., 2002). Indeed, continued exercise has recently been shown to result in enhanced expression of plasticity-related genes in the hippocampus (Molteni et al., 2002). It is important to note that hippocampal BDNF protein levels were significantly increased in response to exercise and exercise/antidepressant treatment. BDNF protein was significantly increased after 7 days of treatment and maintained at 14 days (unpublished data from our laboratory). This apparently follows BDNF mRNA enhancements, as increases in mRNA were evident in as little as 2 days (current study).

Our data may thus be interpreted as a dynamic process occurring with age in response to exercise and/or antidepressant treatment. Older rats showed lessened responsiveness, in terms of BDNF mRNA increases, in the CA3–CA4–DG loop over time (moderate responses at 2 days and no significant response by 20 days, see Figs. 1B and 3B). Nevertheless, these same animals showed BDNF mRNA responses in the CA1 that were enhanced as compared to young rats and were maintained over time (among 2, 14, and 20 days, see Fig. 1B, 2B, and 3B). The results of the current study support the possibility of functional shifts between hippocampal subregions as these animals age. Age-related enhanced dendritic sprouting in the CA1 and CA2 may compensate for the normal age-related loss of neurons and synaptic contacts, decreased rate of neurogenesis, and attendant loss of excitatory input due to decreased excitatory neurotransmitter receptor functioning in the CA3 and DG regions. Our current results underscore the importance of a regular exercise regimen for all age groups, as an enhancer of neuronal (and emotional) health.

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References

Appel SH. A unifying hypothesis for the cause of amyotrophic lateral sclerosis, parkinsonism, and Alzheimer disease. Ann Neurol 1981;10:499–505.

Barnes CA. Normal aging: regionally specific changes in hippocampal synaptic transmission. Trends Neurosci 1994;17:13–8.

Barnes CA. Do synaptic markers provide a window on synaptic effectiveness in the aged hippocampus? Neurobiol Aging 1999;20:349–51 [discussion 359–360].

Benelli A, Filaferro M, Bertolini A, Genedani S. Influence of *S*-adenosyl-L-methionine on chronic mild stress-induced anhedonia in castrated rats. Br J Pharmacol 1999;127:645–54.

Berchtold NC, Oliff HS, Isackson P, Cotman CW. Hippocampal BDNF mRNA shows a diurnal regulation, primarily in the exon III transcript. Brain Res Mol Brain Res 1999;71:11–22.

Bickford-Wimer PC, Miller JA, Freedman R, Rose GM. Age-related reduction in responses of rat hippocampal neurons to locally applied monoamines. Neurobiol Aging 1998;9:173–9.

Blazer D, Williams CD. Epidemiology of dysphoria and depression in an elderly population. Am J Psychiatry 1980;137:439–44.

Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, et al. MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry 1995;152:973–81.

Brown BS, Payne T, Kim C, Moore G, Krebs P, Martin W. Chronic response of rat brain norepinephrine and serotonin levels to endurance training. J Appl Physiol 1979;46:19–23.

Clark AS, Magnusson KR, Cotman CW. In vitro autoradiography of hippocampal excitatory amino acid binding in aged Fischer 344 rats: relationship to performance on the Morris water maze. Behav Neurosci 1992;106:324–35.

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

Dey S. Physical exercise as a novel antidepressant agent: possible role of serotonin receptor subtypes. Physiol Behav 1994;55:323–9.

Dey S, Singh RH, Dey PK. Exercise training: significance of regional alterations in serotonin metabolism of rat brain in relation to antidepressant effect of exercise. Physiol Behav 1992;52:1095–9.

D'Sa C, Duman RS. Antidepressants and neuroplasticity. Bipolar Disord 2002;4:183–94.

Duman RS, Nakagawa S, Malberg J. Regulation of adult neurogenesis by antidepressant treatment. Neuropsychopharmacology 2001;25:836–44.

Figurov A, Pozzo-Miller LD, Olafsson P, Wang T, Lu B. Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. Nature 1996;381:706–9.

Frerking M, Malenka RC, Nicoll RA. Brain-derived neurotrophic factor (BDNF) modulates inhibitory, but not excitatory, transmission in the CA1 region of the hippocampus. J Neurophysiol 1998;80:3383–6.

Gareri P, Falconi U, De Fazio P, De Sarro G. Conventional and new antidepressant drugs in the elderly. Prog Neurobiol 2000;61:353–96.

Geinisman Y. Age-related decline in memory function: is it associated with a loss of synapses? Neurobiol Aging 1999;20:353–6 [discussion 359–360].

Geinisman Y, Bondareff W. Decrease in the number of synapses in the senescent brain: a quantitative electron microscopic analysis of the dentate gyrus molecular layer in the rat. Mech Ageing Dev 1976;5:11–23.

Gomez-Pinilla F, Ying Z, Opazo P, Roy RR, Edgerton VR. Differential regulation by exercise of BDNF and NT-3 in rat spinal cord and skeletal muscle. Eur J Neurosci 2001;13:1078–84.

Gould E, Reeves AJ, Fallah M, Tanapat P, Gross CG, Fuchs E. Hippocampal neurogenesis in adult old world primates. Proc Natl Acad Sci USA 1999;96:5263–7.

Hill RD, Storandt M, Malley M. The impact of long-term exercise training on psychological function in older adults. J Gerontol 1993;48:12–7.

Hofer M, Pagliusi SR, Hohn A, Leibrock J, Barde YA. Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. EMBO J 1990;9:2459–64.

Ickes BR, Pham TM, Sanders LA, Albeck DS, Mohammed AH, Granholm AC. Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain. Exp Neurol 2000;164:45–52.

Kadar T, Silbermann M, Brandeis R, Levy A. Age-related structural changes in the rat hippocampus: correlation with working memory deficiency. Brain Res 1990;512:113–20.

Kafitz K, Rose CR, Thoenen H, Konnerth A. Neurotrophin-evoked rapid excitation through TrkB receptors. Nature 1999;401:918–21.
Kakiuchi T, Tsukada H, Fukumoto D, Nishiyama S. Effects of aging on serotonin transporter availability and its response to fluvoxamine in the living brain: PET study with. Synapse 2001;40:170–9.
Kesslak JP, So V, Choi J, Cotman CW, Gomez-Pinilla F. Learning upregulates brain-derived neurotrophic factor messenger ribonucleic acid: a mechanism to facilitate encoding and circuit maintenance? Behav Neurosci 1998;112:1012–9.
Knüsel B, Winslow J, Rosenthal A, Burton L, Seid D, Nikolics K, et al. Promotion of central cholinergic and dopaminergic neuron differentiation by brain-derived neurotrophic factor but not neurotrophin 3. Proc Natl Acad Sci USA 1991;88:961–5.
Kuhn HG, Dickinson-Anson H, Gage FH. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci 1996;16:2027–33.
Labbe EE, Welsh MC, Delaney D. Effects of consistent aerobic exercise on the psychological functioning of women. Percept Mot Skills 1988;67: 919–25.
Lapchak PA, Araujo DM, Beck KD, Finch CE, Johnson SA, Hefti F. BDNF and trkB mRNA expression in the hippocampal formation of aging rats. Neurobiol Aging 1993;14:121–6.
Leonard BE. Evidence for a biochemical lesion in depression. J Clin Psychiatry 2000;61(Suppl. 6):12–7.
Levine ES, Dreyfus CF, Black IB, Plummer MR. Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. Proc Natl Acad Sci USA 1995;92:8074–7.
Levkovitz Y, Richter-Levin G, Segal M. Effect of 5-hydroxytryptophane on behavior and hippocampal physiology in young and old rats. Neurobiol Aging 1994;15:635–41.
Lindsay RM, Wiegand SJ, Altar CA, DiStefano PS. Neurotrophic factors: from molecule to man. Trends Neurosci 1994;17:182–90.
Lo DC. Neurotrophic factors and synaptic plasticity. Neuron 1995;15: 979–81.
McAllister AK, Lo DC, Katz LC. Neurotrophins regulate dendritic growth in developing visual cortex. Neuron 1995;4:791–803.
McAllister AK, Katz LC, Lo DC. Neurotrophins and synaptic plasticity. Annu Rev Neurosci 1999;22:295–318.
Meeusen R, De Meirleir K. Exercise and brain neurotransmission. Sports Med 1995;20:160–88.
Molteni R, Ying Z, Gomez-Pinilla F. Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. Eur J Neurosci 2002;16:1107–16.
Murray KD, Gall CM, Jones EG, Isackson PJ. Differential regulation of brain-derived neurotrophic factor and type II calcium/calmodulin-dependent protein kinase messenger RNA expression in Alzheimer's disease. Neuroscience 1994;60:37–48.
Narisawa-Saito M, Nawa H. Differential regulation of hippocampal neurotrophins during aging in rats. J Neurochem 1996;67:1124–31.
Navarro A, Sanchez Del Pino MJ, Gomez C, Peralta JL, Boveris A. Behavioral dysfunction, brain oxidative stress, and impaired mitochondrial electron transfer in aging mice. Am J Physiol Regul Integr Comp Physiol 2002;282:R985–992.
Neeper SA, Gomez-Pinilla F, Choi J, Cotman C. Exercise and brain neurotrophins. Nature 1995;373:109.
Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 1996;16:2365–72.
Nonner D, Barrett E, Barrett J. Neurotrophin effects on survival and expression of cholinergic properties in cultured rat septal neurons under normal and stress conditions. J Neurosci 1996;16:6665–75.
Pagliusi SR, Gerrard P, Abdallah M, Talabot D, Catsicas S. Age-related changes in expression of AMPA-selective glutamate receptor subunits: is calcium-permeability altered in hippocampal neurons? Neuroscience 1994;61:429–33.
Pelleymounter MA, Cullen MJ, Baker MB, Gollub M, Wellman C. The effects of intrahippocampal BDNF and NGF on spatial learning in aged Long Evans rats. Mol Chem Neuropathol 1996;29:211–26.
Phillips HS, Hains JM, Armanini M, Laramee GR, Johnson SA, Winslow JW. BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. Neuron 1991;7:695–702.
Pyapali GK, Turner DA. Increased dendritic extent in hippocampal CA1 neurons from aged F344 rats. Neurobiol Aging 1996;17:601–11.
Redrobe JP, Bourin M. The effect of lithium administration in animal models of depression: a short review. Fundam Clin Pharmacol 1999; 13:293–9.
Reisberg B, Borenstein J, Salob SP, Ferris SH, Franssen E, Georgotas A. Behavioral symptoms in Alzheimer's disease: phenomenology and treatment. J Clin Psychiatry 1987;48:9–15 [Suppl].
Russo-Neustadt A, Beard RC, Cotman CW. Exercise, antidepressant medications, and enhanced brain derived neurotrophic factor expression. Neuropsychopharmacology 1999;21:679–82.
Russo-Neustadt AA, Beard RC, Huang YM, Cotman CW. Physical activity and antidepressant treatment potentiate the expression of specific brain-derived neurotrophic factor transcripts in the rat hippocampus. Neuroscience 2000;101:305–12.
Russo-Neustadt A, Ha T, Ramirez R, Kesslak SP. Physical activity-antidepressant treatment combination: impact on brain-derived neurotrophic factor and behaviour in animal model. Behav Brain Res 2001; 120:87–95.
Samorajski T, Rolsten C, Przykorska A, Davis CM. Voluntary wheel running exercise and monoamine levels in brain, heart and adrenal glands of aging mice. Exp Gerontol 1987;22:421–31.
Sapolsky RM. The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. Biol Psychiatry 2000;48: 755–65.
Schoups AA, Elliott RC, Friedman WJ, Black IB. NGF and BDNF are differentially modulated by visual experience in the developing geniculocortical pathway. Brain Res Dev Brain Res 1995;86:326–34.
Seki T, Arai Y. Age-related production of new granule cells in the adult dentate gyrus. NeuroReport 1995;6:2479–82.
Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci USA 1996;93:3908–13.
Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 2002;22:3251–61.
Singh NA, Clements KM, Singh MA. The efficacy of exercise as a long-term antidepressant in elderly subjects: a randomized, controlled trial. J Gerontol A Biol Sci Med Sci 2001;56:M497–504.
Siuciak JA, Boylan C, Fritsche M, Altar CA, Lindsay RM. BDNF increases monoaminergic activity in rat brain following intracerebroventricular or intraparenchymal administration. Brain Res 1996;710:11–20.
Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM. Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacol Biochem Behav 1997;56:131–7.
Smith MA. Hippocampal vulnerability to stress and aging: possible role of neurotrophic factors. Behav Brain Res 1996;78:25–36.
Smith MA, Makino S, Kvetnansky R, Post RM. Effects of stress on neurotrophic factor expression in the rat brain. Ann NY Acad Sci 1995;771: 234–9.
Stewart CM. Age-related changes in the nervous system. J Neurosurg Nurs 1982;14:69–73.
Thome J, Sakai N, Shin K, Steffen C, Zhang YJ, Impey S, et al. cAMP response element-mediated gene transcription is upregulated by chronic antidepressant treatment. J Neurosci 2000;20:4030–6.
Toescu EC, Myronova N, Verkhratsky A. Age-related structural and functional changes of brain mitochondria. Cell Calcium 2000;28:329–38.
Turner DA, Deupree DL. Functional elongation of CA1 hippocampal neurons with aging in Fischer 344 rats. Neurobiol Aging 991;12: 201–10.
van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci USA 1999;96:13427–31.

Ventimiglia R, Mathe RP, Jones B, Lindsay R. The neurotrophins BDNF, NT-3 and NT-4/5 promote survival and morphological and biochemical differentiation of striatal neurons in vitro. Eur J Neurosci 1995;7:213–22.

Watanabe Y, Gould E, McEwen BS. Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res 1992;588: 341–5.

West MJ. Regionally specific loss of neurons in the aging human hippocampus. Neurobiol Aging 1993;14:287–93.

Wilson WM, Marsden CA. In vivo measurement of extracellular serotonin in the ventral hippocampus during treadmill running. Behav Pharmacol 1996;7:101–4.

Yau JL, Olsson T, Noble J, Seckl JR. Serotonin receptor subtype gene expression in the hippocampus of aged rats following chronic amitriptyline treatment. Brain Res Mol Brain Res 1999;70:282–7.

Yuan J, Yankner B. Apoptosis in the nervous system. Nature 2000;407: 802–9.