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Blockade of Insulin-Like Growth Factor-I Has Complex Effects on Structural Plasticity in the Hippocampus

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ABSTRACT: Physical exercise enhances adult neurogenesis in the hippocampus. Running induces the uptake of blood insulin-like growth factor-I (IGF-I) into the brain. A causal link between these two phenomena has been reported; running-induced increases in adult neurogenesis can be blocked by peripheral infusion of anti-IGF-I. Running also alters other aspects of hippocampal structure, including dendritic spine density. It remains unclear, however, whether these effects are also mediated through an IGF-I mechanism. To examine this possibility, we blocked peripheral IGF-I and examined adult neurogenesis and dendritic spine density in treadmill running mice. Two weeks of running resulted in an increase in cell proliferation in the dentate gyrus (DG) as well as an increase in dendritic spine density on DG granule cells and basal dendrites of CA1 pyramidal neurons, while having no effect on apical or basal dendritic spine density of CA3 pyramidal neurons. IGF-I blockade reduced cell proliferation in both sedentary and running mice, but by contrast, this treatment had no effect on granule cell or CA3 pyramidal cell dendritic spine density in sedentary or running mice. However, IGF-I antibody treatment seemed to prevent the running-induced increase in spine density on basal dendrites of CA1 pyramidal cells. These results suggest that IGF-I exerts a complex influence over hippocampal structure and that its effects are not restricted to those induced by running. © **2009 Wiley-Liss, Inc.**

KEY WORDS: dentate gyrus; CA1 pyramidal neuron; CA3 pyramidal neuron; physical exercise; insulin-like growth factor-I

INTRODUCTION

The effects of running on structural plasticity in the dentate gyrus (DG) of the hippocampus have been well investigated. Running enhances adult neurogenesis in both rats and mice (van Praag et al., 1999, 2007; Trejo et al., 2001; Fabel et al., 2003; Stranahan et al., 2006), as well as increases dendritic spine density of granule cells in the DG (Eadie et al., 2005; Redila and Christie, 2006; Zhao et al., 2006; Stranahan et al., 2007) and pyramidal cells of the CA1 region (Stranahan et al., 2007). Running-induced enhancements in adult neurogenesis have been linked to insulin-like growth factor-I (IGF-I). Peripheral IGF-I is elevated in running animals—IGF-I crosses the blood–brain barrier where it interacts with brain receptors, including those located in the hippocampus. Moreover, peripheral infusion of IGF-I antibodies prevents running-induced increases in adult neurogenesis in rats (Trejo et al., 2001).

Recent studies have shown that mutant mice deficient in IGF-I exhibit reduced adult neurogenesis and alterations in hippocampal function—these abnormalities are not reversed by running but can be reversed by exogenous application of IGF-I (Trejo et al., 2008). Since running is known to alter learning and memory and anxiety functions in control animals (van Praag et al., 1999; Lambert et al., 2005; Clark et al., 2008; Duman et al., 2008; Thomas et al., 2008), it is possible that IGF-I blockade might be a useful tool for investigating the relative contribution of adult neurogenesis to running-induced changes in hippocampal function (for review, see Llorens-Martin et al., 2009). To be useful, however, its specificity as an inhibitor of adult neurogenesis must be ascertained. To date, no studies have examined whether other types of structural plasticity, such as changes in dendritic spine density, are influenced by IGF-I antibody infusion.

Here, we examined the effects of exercise on cell proliferation and dendritic spine density in the hippocampus of adult mice infused with either vehicle or anti-IGF-I. Running for 2 weeks increased the number of proliferating cells in the DG, as well as increased dendritic spine density on granule cells and basal dendrites of CA1 pyramidal neurons. However, 2 weeks of treadmill running did not alter the density of dendritic spines on CA3 pyramidal neurons. The effects of IGF-I antibody infusions were complex: inhibition of cell proliferation in both sedentary and running mice; no change in granule cell and CA3

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pyramidal cell dendritic spine density and a prevention of the running-induced increase in spine density on basal dendrites of CA1 pyramidal neurons. Taken together, these results provide evidence that IGF-I may influence cell proliferation under baseline conditions and running-induced dendritic spine density in a cell-type-specific manner.

MATERIALS AND METHODS

Animals

Forty adult male C57/BL6 mice (Cajal Institute) were used in this experiment. All mice were 3 months of age, housed at 22 ± 1°C with a light/dark cycle of 12/12, and had ad libitum access to food and water. Mice were housed under standard laboratory conditions in accordance with guidelines established by the European Community Council (directive 86/609/EEC).

Experimental Design

Four groups of 10 mice were used in this experiment. Twenty mice were trained in a treadmill, while 20 mice were used as sedentary controls. Ten mice of each group received an anti-IGF-I treatment, while 10 others received a nonimmune normal rabbit serum (NRS) vehicle treatment, during the exercise/sedentary period.

Exercise Protocol

Mice were habituated to the treadmill apparatus for 15 min (Cibertec, Madrid, Spain). The training of exercised mice included gradual adaptation to the running schedule (Trejo et al., 2008). The mice ran at 0.2 m/s, 7 days/week, for 40 min/day over 2 weeks. Sedentary mice remained on the treadmill without running for the same period of time.

Bromodeoxyuridine

Bromodeoxyuridine (BrdU; 50 mg/kg) was administered by intraperitoneal injection 2 h after the last exercise session, and mice were then sacrificed 24 h after BrdU injection.

Blocking Anti-IGF-I Treatments

To inhibit brain uptake of IGF-I, a blocking anti-IGF-I antiserum (20% in saline; Carro et al., 2000) was chronically infused subcutaneously through an osmotic minipump (Alzet 1002 in mice; Alza, Palo Alto, CA). Infusion was maintained throughout the exercise protocol. This procedure blocks the exercise-stimulated entrance of serum IGF-I into the brain, because anti-IGF-I antiserum blocks binding of IGF-I to its receptor (Trejo et al., 2001). The anti-IGF-I antiserum has <1% crossreactivity with either insulin or IGF-II, as determined by competition with ^{125}I-IGF-I. All control groups received an infusion of nonimmune NRS (20% in saline), which does not impede the entrance of serum IGF-I into the brain (Trejo et al., 2001).

BrdU Immunohistochemistry and Analysis

Following the various experimental protocols, mice were deeply anesthetized with pentobarbital and transcardially perfused with 0.9% saline and 4% paraformaldehyde in phosphate buffer (PB). Brains were removed and postfixed overnight at 4°C with 4% paraformaldehyde in PB. Coronal sections (50 μm thick) were collected individually in 96-multiwell culture plates. One-in-eight random series was collected for immunohistochemistry and slices were preincubated in PB with 1.0% Triton X-100 and 0.1% bovine serum albumin.

BrdU immunohistochemistry was performed as previously described (Trejo and Pons, 2001). Briefly, DNA was denatured for 30 min in 2 N HCl at room temperature followed by incubation of slices with a mouse anti-BrdU antibody (Developmental Studies Hybridoma Bank, Iowa City, IA, 1:15,000) for 3 days at 4°C, followed by incubation with a biotin-conjugated goat anti-mouse secondary antibody (Pierce, Rockford, IL, 1:1,000) for 24 h at 4°C, followed by a peroxidase-based ABC system (Vector Laboratories, Burlingame, CA).

Slides were coded prior to data collection and the code was broken after the completion of the analysis. Estimates of total numbers of BrdU-labeled cells were determined using a modified stereology protocol (Leuner et al., 2009). Labeled cells on every sixth unilateral section throughout the entire rostrocaudal extent of the dentate gyrus (granule cell layer, subgranular zone, and hilus) were counted at 1000× on an Olympus BX-50 light microscope. Counts were multiplied by 16 to obtain estimates of BrdU-labeled cells per brain. For BrdU-labeled cell counts in the dentate gyrus, 9-10 brains per group were averaged to obtain group means. Differences in the number of mice included in the analysis were a result of missed injections.

Golgi-impregnation and Analysis

Single-section Golgi impregnation was carried out on brain sections throughout the rostrocaudal extent of the hippocampus, as previously described (Kozorovitskiy et al., 2005). Briefly, small blocks of tissue containing the hippocampus (trimmed to ~1.5 cm^2 by 3 mm thick) were incubated in 4% potassium dichromate: dH_2O in glass scintillation vials. Vials were placed on a shaker at room temperature in the dark. Potassium dichromate solution was changed daily for 3 days. Sections were then washed in increasing concentrations of silver nitrate (0.25, 0.5, 0.75, and 1%:dH_2O) for 5 min each, followed by 1 week of incubation in 1% silver nitrate in the dark on a shaker. Brains were cut at 100 μm on a vibratome and quickly rinsed in increasing concentrations of ethanol (75, 95, and 100%:dH_2O) for 5 min each. Sections were then cleared in Citrisolv for 10 min, mounted, and immediately coverslipped under Permount.

Granule cells of the DG and apical and basal pyramidal neurons of area CA1 and CA3 were analyzed at 1000×. Golgi-impregnated neurons were included in the analysis if they were

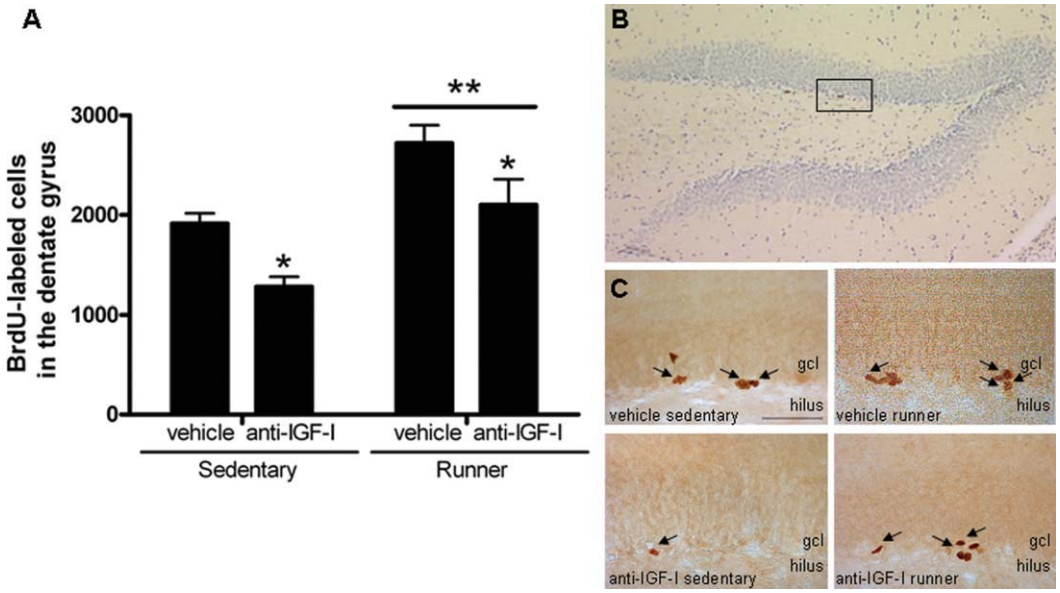

FIGURE 1. Anti-insulin-like growth factor (IGF)-I and treadmill running alter the number of BrdU-labeled cells in the dentate gyrus (DG). (A) Treadmill running significantly increased the number of bromodeoxyuridine (BrdU)-labeled cells in the DG of vehicle-treated runners compared to vehicle-treated sedentary mice. Treatment with an anti-IGF-I blocking serum significantly reduced the number of BrdU-labeled cells in the DG of sedentary and treadmill running mice; however, anti-IGF-I treatment did not prevent an exercise-induced increase in the number of BrdU-labeled cells. (B) Low-magnificaiton Nissl-stained section of the DG. Box displays field magnified in (C). (C) Representative photomicrographs of BrdU-labeled cells (arrow); scale bar, 50 µm. *Significant difference from vehicle. **Significant difference from sedentary. [Color figure can be viewed in the online issue, which is available at www. interscience.wiley.com.]

completely stained without truncated primary dendrites and located in sufficient isolation from neighboring stained cells to trace the dendritic segments back to their parent dendrite. Dendritic spines were sampled from secondary and tertiary dendrites of DG granule cells, as well as apical and basal trees of CA1 and CA3 pyramidal cells. For dendritic spine density, five cells of each type per animal (n = 5–10 mice per cell type, five dendritic segments per cell) were averaged to give the mean spine density for each mouse. Differences in the number of mice included in the analysis for each neuron type were a result of staining variability.

Statistical Analysis

Data from BrdU-labeled cells and spine density counts were analyzed via two-way ANOVA with activity (sedentary or running) and treatment (vehicle or anti-IGF-I) as independent variables followed by Bonferroni text for post hoc analysis.

RESULTS

Bromodeoxyuridine

Significant main effects of exercise [$F(1,34) = 21.56$, $P < 0.0001$] and treatment [$F(1, 34) = 12.77$, $P = 0.001$], but no interaction between exercise and treatment [$F(1,34) = 0.00$, $P = 0.97$], were observed on BrdU-labeled cells in the DG (Fig. 1). Overall, exercise significantly increased the number of BrdU-labeled cells, while treatment with the anti-IGF-I blocking serum significantly reduced cell proliferation. Among sedentary mice, a significant reduction in the number of BrdU-labeled cells was observed with anti-IGF-I treatment, compared to vehicle-treated mice ($P < 0.05$, for post hoc comparison). Among runners, anti-IGF-I treatment significantly reduced cell proliferation compared to vehicle-treated mice ($P < 0.05$, for post hoc comparison).

Granule Cell Dendritic Spines

A main effect of exercise [$F(1,23) = 4.46$, $P = 0.05$], no main effect of treatment [$F(1,23) = 0.00$, $P = 0.97$], and no interaction between exercise and treatment [$F(1,23) = 0.01$, $P = 0.92$] was observed on dendritic spines of granule cells in the DG (Fig. 2). Overall, treadmill running significantly increased the density of dendritic spines of granule cells in the DG. Treatment with the anti-IGF-I blocking serum did not alter dendritic spine density of either sedentary or treadmill running mice.

CA1 Pyramidal Cell Dendritic Spines

No main effect of exercise [$F(1,30) = 0.89$, $P = 0.35$], treatment [$F(1,30) = 0.04$, $P = 0.85$], or an interaction between exercise and treatment [$F(1,30) = 0.42$, $P = 0.52$] was observed on dendritic spines of apical dentrites on CA1 pyramidal neurons. Neither treadmill running nor the anti-IGF-I blocking serum altered spine density of apical dendrites on pyramidal neurons in area CA1 of the hippocampus (Figs. 3A,D).

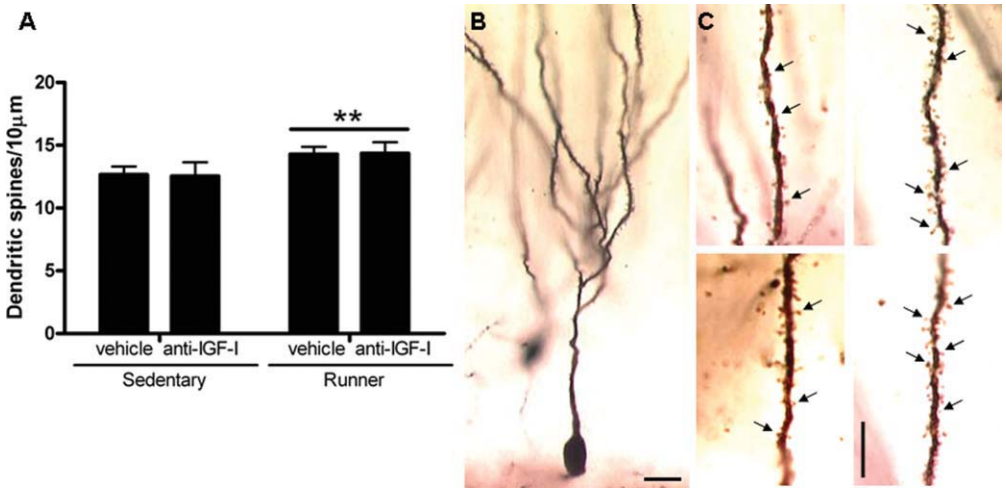

FIGURE 2. Running, but not anti-IGF-I, alters spine density of dentate gyrus (DG) granule cells. (A) Treadmill running significantly increased the density of dendritic spines of granule cells in the DG; however, treatment with the anti-IGF-I blocking serum did not alter dendritic spine density. (B) Representative photomicrograph of a fully impregnated Golgi-stained DG granule cell (scale bar, 25-µm). (C) Golgi-stained dendritic spine segments of granule cells in the DG (arrows; scale bar, 25-µm cells, 5-µm segments)—top left, sedentary vehicle; top right, runner vehicle; bottom left, sedentary anti-IGF-I; bottom right, runner anti-IGF-I. **Significant difference from sedentary. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

A main effect of exercise [$F(1,30) = 8.51$, $P = 0.01$], no main effect of treatment [$F(1,30) = 0.56$, $P = 0.46$], and no interaction between exercise and treatment [$F(1,30) = 2.26$, $P = 0.14$] was observed on dendritic spines of basal dendrites on CA1 pyramidal neurons. Overall, exercise significantly increased dendritic spine density of basal dendrites on CA1

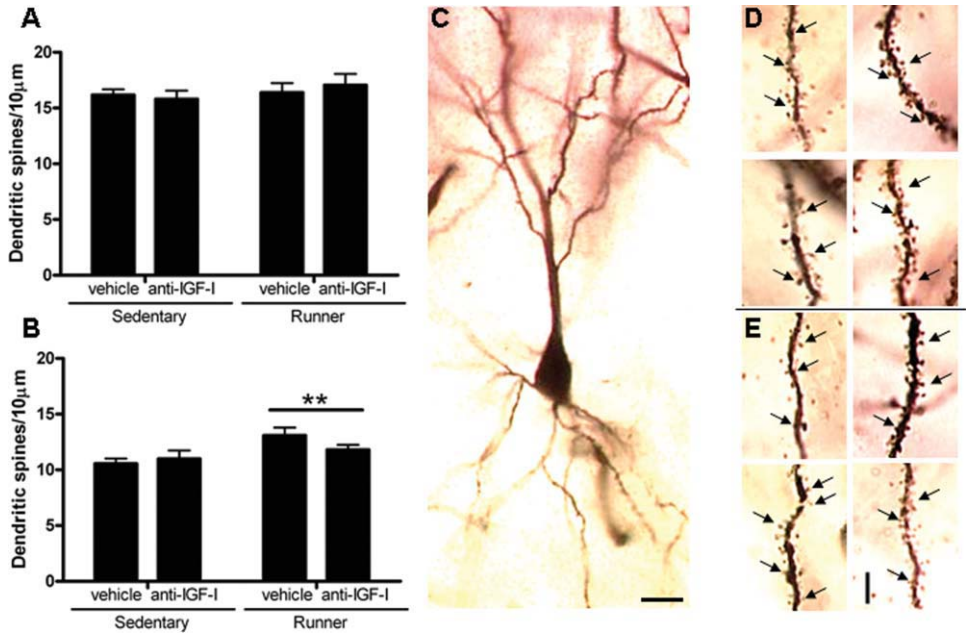

FIGURE 3. Running and anti-IGF-I differentially alter spine density of apical and basal CA1 pyramidal cell dendrites. (A) Treadmill running did not alter dendritic spine density of apical dendrites in area CA1 of the hippocampus. (B) Treadmill running significantly increased the density of dendritic spines of basal dendrites in area CA1 of vehicle-treated mice. However, treatment with the anti-IGF-I blocking serum prevented this running-induced increase in dendritic spine density (E, bottom right). (C) Representative photomicrograph of a fully impregnated Golgi-stained CA1 pyramidal cell (scale bar, 10-µm). (D, E) Golgi-stained dendritic spine segments of pyramidal cells in area CA1 of the hippocampus (arrows; scale bar, 5-µm segments)—top left, sedentary vehicle; top right, runner vehicle; bottom left, sedentary anti-IGF-I; bottom right, runner anti-IGF-I. **Significant difference from sedentary. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

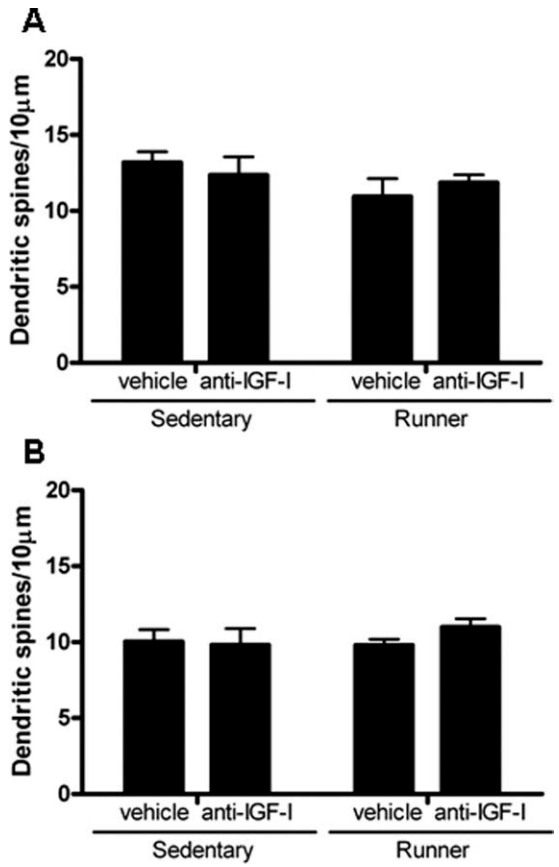

FIGURE 4. Neither running nor anti-IGF-I alters spine density of apical and basal CA3 pyramidal cell dendrites. Treadmill running and anti-IGF-I treatment did not alter dendritic spine density of (A) apical or (B) basal dendrites in area CA3 of the hippocampus.

pyramidal cells in the hippocampus. Treatment with the anti-IGF-I blocking serum prevented the running-induced increase in spine density of basal dendrites on CA1 pyramidal cells (Figs. 3B,E).

CA3 Pyramidal Cell Dendritic Spines

No main effect of exercise [apical: $F(1,22) = 1.96$, $P = 0.17$; basal: $F(1,18) = 0.43$, $P = 0.52$] or treatment [apical: $F(1,22) = 0.00$, $P = 0.97$; basal: $F(1,18) = 0.46$, $P = 0.51$], and no interaction between exercise and treatment [apical: $F(1,22) = 0.80$, $P = 0.38$; basal: $F(1,18) = 0.93$, $P = 0.35$] was observed in the density of spines of CA3 apical and basal pyramidal cell dendrites. Neither 2 weeks of treadmill running nor treatment with anti-IGF-I blocking serum altered spine density on CA3 apical or basal pyramidal cell dendrites in the hippocampus (Fig. 4).

DISCUSSION

The present results provide evidence that IGF-I increases the rate of cell proliferation under baseline conditions—infusion of IGF-I antibodies lowered the number of BrdU-labeled cells in the sedentary group. Although this treatment also diminished cell proliferation in the running group, there remained a running-induced increase in the IGF-I antibody mice. This suggests that although IGF-I can influence cell proliferation, it is not necessary for running-induced enhancements in cell proliferation in mice. Regarding other forms of structural plasticity, IGF-I antibodies exert a complicated influence—having no effect on dendritic spine density of granule cells, apical and basal dendrites on CA3 pyramidal cells, or apical dendrites on CA1 pyramidal cells, but preventing the running-induced increase in spine density of basal dendrites of CA1 pyramidal cells.

The enhancement of hippocampal neurogenesis by exercise is a robust phenomenon that has been repeatedly demonstrated in both rats and mice (van Praag et al., 1999, 2007; Trejo et al., 2001; Fabel et al., 2003; Stranahan et al., 2006). Running enhances IGF gene expression (Ding et al., 2006) and protein levels in the hippocampus (Carro et al., 2000). In addition, treadmill running increases serum levels of IGF (Carro et al., 2000). Previous studies have shown that the stimulatory effect of exercise on the number of newly born cells in the rat DG is inhibited by infusion of an anti-IGF-I blocking serum (Trejo et al., 2001).

Our current findings provide further evidence of a relationship between cell proliferation and exercise, as well as provide more information regarding the complicated nature of the relationship between cell proliferation and IGF-I. Anti-IGF-I reduced the baseline level of cell proliferation and, from this new baseline, running elicited a significant increase in cell proliferation among anti-IGF-I treated mice, therefore not preventing the running-induced increase in cell proliferation observed previously in the rat (Trejo et al., 2001). While anti-IGF-I did not completely prevent a running-induced enhancement in cell proliferation, IGF-I blockade significantly reduced cell proliferation among exercising mice. Running increased cell proliferation among vehicle-treated mice, while also increasing cell proliferation among the anti-IGF-I treated mice. This change in basal levels of cell proliferation by anti-IGF-I blocking serum was not reported in the initial studies performed in the rat (Trejo et al., 2001), raising the possibility that species differences account for this discrepancy. In a previous study, treatment with anti-IGF-I blocking serum resulted in a reduction in basal levels of cell survival 3 weeks post-BrdU injection in the rat (Trejo et al., 2001). Given the apparent discrepancies between the effects of IGF-I on basal levels of cell proliferation in rats and mice, cell survival differences between the two species warrants further investigation. Although our current study did not investigate whether IGF-I blockade could alter cell survival in the wild-type mouse, recent findings suggest that IGF-I may play a significant role in cell survival, as liver-IGF-I deficient mice, with low serum levels of IGF-I, had reduced cell survival 7 days and 1 month following BrdU administration. Adult neurogenesis was restored to a rate similar to controls following subcutaneous administration of IGF-I (Trejo et al., 2008).

Running also enhances other types of hippocampal growth. Running-induced enhancements of dendritic spine density on

granule cells of the DG (Eadie et al., 2005; Redila and Christie, 2006; Zhao et al., 2006; Stranahan et al., 2007) and apical and basal dendrites of CA1 pyramidal cells (Stranahan et al., 2007) have been observed. Here, we show that just 2 weeks of treadmill running enhances dendritic spine density of granule cells in the DG and basal, but not apical, dendritic spines of CA1 pyramidal cells in mice. A longer duration of exercise may be required to observe an increase in the density of dendritic spines of apical dendrites on CA1 pyramidal cells (Stranahan et al., 2006). Alternatively, the stress of treadmill running in combination with elevated glucocorticoids that accompany exercise (Coleman et al., 1998) may have differentially affected the formation of new dendritic spines of apical and basal dendrites on pyramidal neurons in area CA1 of the hippocampus. Two weeks of treadmill running was unable to alter dendritic spine density of apical or basal dendrites on CA3 pyramidal neurons. Chronic stress has been reported to decrease CA3 apical dendritic morphology (Magarinos et al., 1996; reviewed in McEwen and Magarinos, 1997; Magarinos et al., 1998; McKittrick et al., 2000), including activity stress (Lambert et al., 1998). Although treadmill running is associated with an elevation in glucocorticoids (Coleman et al., 1998), 2 weeks of running may not have been sufficient to produce changes in CA3 pyramidal cell morphology.

There is mounting evidence to support both neurogenesis-dependent and -independent mechanisms in the effects of physical exercise on both brain and behavior (Scharfman and Hen, 2007). Running-induced alterations in adult neurogenesis, as well as dendritic spine density, may present the possibility that these changes accompany alterations in hippocampal function (van Praag et al., 1999). It is difficult to attribute changes in hippocampal function to either adult neurogenesis or other forms of hippocampal structural plasticity. Blocking IGF-I diminishes hippocampal cell proliferation, but not the density of dendritic spines on granule cells in the DG, dendritic spines on apical dendrites of CA1 pyramidal cells, or dendritic spines of apical and basal dendrites on CA3 pyramidal cells. Anti-IGF-I did not significantly reduce the number of dendritic spines on basal dendrites of pyramidal cells in area CA1 of treadmill runners. Treadmill running increased dendritic spine density among the vehicle-treated mice; however, when comparing the sedentary mice treated with anti-IGF-I to treadmill running mice treated with anti-IGF-I, no differences in dendritic spine density were revealed. This may indicate that blocking the uptake of IGF-I can prevent an exercise-induced increase in basal spine density. Furthermore, no change in dendritic spine density was observed in either apical or basal trees of CA3 pyramidal neurons with IGF-I antibody infusion.

Exercise-induced effects on hippocampal mediated behaviors, such as spatial learning and anxiety, may be a result of the combined effects on enhanced neurogenesis and changes in other types of hippocampal structural plasticity, mediated by IGF-I-dependent mechanisms (Trejo et al., 2008). It should be noted that improved water maze performance was observed following environmental enrichment when neurogenesis was ablated by radiation (Meshi et al., 2006). However, the water maze task is not selective for the function of the DG (Kesner, 2007; McHugh et al., 2007); therefore, the relationship between enhanced neurogenesis and improvements in other types of learning following an enrichment paradigm is still a possibility.

Other neurotrophic factors, such as fibroblast growth factor-2 (FGF-2), nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF), have been proposed to participate in exercise-induced enhancements in adult neurogenesis (van Praag et al., 1999). Running induces FGF-2 expression in the hippocampus (Gomez-Pinilla et al., 1998), but does not increase hippocampal neurogenesis when infused into the adult brain (Kuhn et al., 1997; Wagner et al., 1999). Running increases NGF (Neeper et al., 1996) and BDNF (Neeper et al., 1996; Oliff et al., 1998; Vaynman et al., 2003; Farmer et al., 2004) expression in the hippocampus; however, in contrast to FGF-2, BDNF infusions into the adult DG enhance neurogenesis (Scharfman et al., 2005). BDNF has also been shown to increase dendritic spine density of CA1 pyramidal neurons (Tyler and Pozzo-Miller, 2003; Alonso et al., 2004; Chapleau et al., 2008); therefore, the ability of either of these neurotrophic factors to dissociate the effects of adult hippocampal neurogenesis from other enhancements in hippocampal structural plasticity is unlikely.

Although extensive research into adult neurogenesis has been carried out, its functional significance remains unknown. The ability to selectively reduce newly generated cells via an anti-IGF-I blocking serum, in combination with behavioral tasks that preferentially test the function of the DG, might provide a tool to elucidate whether adult neurogenesis plays a significant functional role. However, the results of the present study suggest that this approach may produce results that are difficult to interpret, because IGF-I antibody infusion alters baseline cell proliferation, as well as some running-induced changes in dendritic spines.

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