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WHEEL RUNNING AND FLUOXETINE ANTIDEPRESSANT TREATMENT HAVE DIFFERENTIAL EFFECTS IN THE HIPPOCAMPUS AND THE SPINAL CORD

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Abstract—Exercise and antidepressants used independently have been shown to increase hippocampal brain-derived neurotrophic factor (BDNF) and neurogenesis. Despite the fact that patients with depression are often prescribed both, the effects of the exercise and fluoxetine antidepressant treatment used in combination are unknown. Using C57Bl/10 female mice, BDNF protein, insulin-like growth factor 1 (IGF-1) protein and neurogenesis were measured in the hippocampus after 21 days of wheel running, 21 days of fluoxetine antidepressant therapy (daily i.p. injections of 5 mg/kg, 10 mg/kg or 25 mg/kg) and the combination of the two. BDNF protein and cytogenesis/neurogenesis increased in the hippocampus with fluoxetine (high dose), but not wheel running. Hippocampal IGF-1 protein did not change with either treatment. There were no synergistic effects of combining exercise and fluoxetine treatment. Recent reports have also shown that exercise induces molecular mechanisms that benefit the spinal cord and can improve recovery after spinal cord injury (SCI); therefore, we repeated the assays in the spinal cord. Results showed that BDNF, IGF-1 and neurogenesis behave independently in the hippocampus and spinal cord. BDNF protein did not change in the spinal cord with either wheel running or fluoxetine treatment. Spinal cord IGF-1 protein did not change with wheel running, but it decreased with fluoxetine (high dose). Furthermore, spinal cord cytogenesis decreased with fluoxetine treatment. The combined wheel running and fluoxetine groups did not show synergistic results. Thus, the hippocampus and the spinal cord respond in distinct ways to wheel running and fluoxetine, and a prior induction of BDNF, IGF-1 or cytogenesis is unlikely to be the mechanism for wheel running providing a margin of protection against SCI.
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Key words: exercise, BDNF, IGF, neurogenesis.

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Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BrdU, bromodeoxyuridine; DCX, doublecortin; ELISA, enzyme-linked immunosorbent assay; IGF-1, insulin-like growth factor 1; PLSD, protected least significant difference; SCI, spinal cord injury.

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Exercise is an intervention that improves various neurological conditions: age-related neurodegeneration, depression, Alzheimer's disease, traumatic brain injury and spinal cord injury (SCI), for example (Scully et al., 1998; Babyak et al., 2000; Carro et al., 2001; Will et al., 2004; Adlard et al., 2005b; Engesser-Cesar et al., 2005). Other than brain-derived neurotrophic factor (BDNF) the underlying mechanisms have not been well investigated. Furthermore, the possibility that combining exercise with a pharmacological treatment may potentiate one of these mechanisms has not been explored.

Exercise, antidepressants and the hippocampus

The use of exercise in combination with antidepressant therapy is a common recommendation for individuals with depression. Patients who use exercise as a part of their treatment plan are less likely to relapse (Babyak et al., 2000). Indeed, BDNF, which is thought to underlie the effectiveness of antidepressants, is activated more rapidly when used in combination with exercise and the increase in BDNF mRNA is potentiated compared with either intervention alone (Russo-Neustadt et al., 1999, 2000, 2001). It is unknown however, whether this increase in gene expression is paralleled by an increase in BDNF protein.

A second mechanism that may be shared by exercise and antidepressants is the induction of neurogenesis. Both antidepressants and exercise increase cell proliferation (cytogenesis) and cell survival (neurogenesis) in the hippocampus (van Praag et al., 1999a,b; Malberg et al., 2000), but the effect of combining these interventions is unknown.

In addition to BDNF and neurogenesis, a third potential mechanism underlying improvements in depression and injury, which may be shared by exercise and antidepressants is the induction of insulin-like growth factor 1 (IGF-1). In fact, increases in IGF-1 have been linked to increased hippocampal neurogenesis, exercise-mediated recovery from traumatic brain injury (Carro et al., 2000; Trejo et al., 2001), and fluoxetine administration (Khawaja et al., 2004). Previous research has shown exercise increases IGF-1 protein in whole brain homogenates and correlated this result to increased hippocampal cell proliferation and cell survival (Carro et al., 2000; Trejo et al., 2001). It is unknown if the exercise induction of BDNF is directly mediated by IGF-1 but data suggest that IGF-1 and BDNF could act synergistically (Carro et al., 2000; Trejo et al., 2001). Antidepressant treatment, specifically fluoxetine hy-

drochloride, may also induce hippocampal IGF-1 (Khawaja et al., 2004).

Exercise, antidepressants and the spinal cord

In the brain, increases in BDNF mRNA and protein, IGF-1 protein and neurogenesis have been identified as histological correlates of exercise (Carro et al., 2001). Only BDNF mRNA and protein have been identified as potential correlates in the spinal cord despite recent reports that physical activity improves recovery after SCI (Van Meeteren et al., 2003; Hutchinson et al., 2004; Engesser-Cesar et al., 2005). Therefore, one aim of this experiment was to identify mechanisms through which pre-injury exercise might benefit recovery from SCI. Currently, there are conflicting reports about the effect of exercise on BDNF in the spinal cord (Gomez-Pinilla et al., 2001).

While the role of exercise in neurogenesis in the spinal cord has not been investigated, it is known that there is no baseline level of neurogenesis in the spinal cord (Horner et al., 2000). Furthermore, although neurogenesis increases after brain injury, there is no neurogenesis after SCI (Yamamoto et al., 2001; Couillard-Despres et al., 2005). The lack of spinal cord neurogenesis has raised questions of whether these observations are due to a non-neurogenic environment or whether spinal cord neural progenitor cells lack the ability to form new neurons (Shihabuddin et al., 2000). Identifying ways to modulate the spinal cord environment, for instance, with exercise or antidepressant treatment, is of interest.

If the spinal cord responds similarly to hippocampus, it is possible that antidepressants, or the combination of exercise and antidepressants, could induce BDNF or IGF-1 protein, and neurogenesis in the spinal cord. Both BDNF and IGF-1 have already been shown to improve recovery after SCI (Jakeman et al., 1998; McTigue et al., 1998; Namiki et al., 2000; Novikova et al., 2000, 2002; Ikeda et al., 2001, 2002; Nakao et al., 2001; Widenfalk et al., 2001; Zhou and Shine, 2003). Because antidepressants are already in wide use for post-SCI depression (Kemp et al., 2004), which is high after SCI (Dryden et al., 2005), an alternative therapeutic use of antidepressant drugs after injury would be easy to implement.

In this experiment, exercise and the antidepressant fluoxetine were used to test the hypothesis that the combination of the two would potentiate the effects of either intervention alone on BDNF and IGF-1 protein levels and neurogenesis in both the hippocampus and the spinal cord.

EXPERIMENTAL PROCEDURES

All procedures involving animals were approved by the University of California, Irvine, Institutional Animal Care and Use Committee in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments were conducted to minimize the number of animals used and their suffering.

Animals

Young adult female (2 month) C57 Bl/10 mice (National Cancer Institute, Bethesda, MD, USA) were acclimatized to the vivarium conditions for approximately one week prior to the start of the experimental protocol, including 7 days of antibiotic administration (Baytril, Western Medical Supply, Arcadia, CA, USA; 2.5 μg/g body weight). A pre-experiment assessment of locomotor behavior was used to exclude any animals that may have had problems running in the wheels. All animals were individually housed with *ad libitum* access to food and water in a 12-h light/dark vivarium. Animals were randomly assigned to either a sedentary cage (standard size cage, no running wheel) or to an exercise cage (a 48×27×20 cm polyethylene cage equipped with a running wheel; Mini Mitter, Bend, OR, USA). Running wheels were covered in a flat surface as described previously for SCI running wheel experiments (Engesser-Cesar et al., 2005). Within these two groups, animals were randomly assigned to saline control, 5 mg/kg dose, 10 mg/kg dose or 25 mg/kg dose groups (n=14/group). Mice received daily i.p. injections of fluoxetine hydrochloride (Sigma, St. Louis, MO, USA), at the appropriate doses, dissolved in 0.2 mL of 0.9% sterile saline for 21 days. Beginning at 14 days, a subset of the animals (n=6/group) received 7 days of once daily 50 mg/kg i.p. injections of bromodeoxyuridine (BrdU) (Sigma) dissolved in 0.1 mL of 0.9% sterile saline. All animals were killed 24 h after the last injection of fluoxetine or fluoxetine and BrdU. To minimize any differences in vivarium conditions, all animals were housed in the same room, and the experiments were staggered so that animals were killed within a four day period.

Tissue collection

At the end of the experiment, animals used for enzyme-linked immunosorbent assays (ELISAs) (n=8/group) were decapitated and trunk blood collected. Brains were rapidly removed and the two hemispheres separated along the midline. Hippocampus and three segments of spinal cord, rostral (brain–T6), thoracic (T7–T12), and lumbar (T13–L4), were rapidly dissected, frozen in dry ice and subsequently stored at −80 °C until analysis. Animals injected with BrdU (n=6/group) were perfused with 4% paraformaldehyde and the spinal cords blocked into the three segments as above. The brains and cords were cryoprotected overnight in 20% sucrose in 4% paraformaldehyde at 4 °C and the following day, snap frozen in isopentane at −65 °C and stored at −80 °C until processing for histology.

ELISAs

BDNF was measured following kit directions (Promega, Madison, WI, USA). Samples were weighed and homogenized in lysis buffer (hippocampus 1:6, rostral cord 1:7, thoracic cord 1:6, and lumbar cord 1:5). A sample of the homogenate was removed (50–75 μl) and stored at 4 °C overnight and the remainder stored at −80 °C for use in IGF-1 ELISAs. The following day the 60 μl was diluted in Dulbecco's PBS (0.2 g KCl, 8.0 g NaCl, 0.2 g KH_2PO_4, 1.15 g Na_2HPO_4, 133 mg $CaCl_2 \cdot 2H_2O$, 100 mg $MgCl_2 \cdot 6H_2O$ in one liter H_2O, pH 7.35) (hippocampus 1:4; rostral cord 1:4; thoracic cord 1:4; lumbar 1:3) acid treated and spun at 14,000×g for 3 min. The following volumes of supernatant were loaded onto the 96-well plate in triplicate, hippocampus 60 μl; rostral cord 75 μl; and in duplicate, thoracic cord 100 μl; lumbar cord 75 μl. Using homogenates stored from the BDNF experiment, IGF-1 protein was assayed using an ELISA (R&D Systems, Minneapolis, MN, USA). Samples were centrifuged at 14,000×g for 3 min and diluted in Calibrator Diluent provided in the kit (serum samples 1:500; hippocampus and spinal cord samples 1:10). Samples were run in duplicate following kit instructions. Protein concentration was assayed using a BCA Assay (Pierce, Rockford, IL, USA; #23235). Absorbance readings were averaged and then corrected by the

protein concentration and dilution factors. For the figures, data were expressed as a percent of the control by dividing each group mean by the control group mean and multiplying by 100.

Immunohistochemistry

Both brain and spinal cord sections were cut on a sliding microtome at 40 μm and collected free floating into PBS in 0.1% sodium azide and stored at 4 °C until staining. All brain sections that included hippocampus, and every other lumbar section, were saved for immunohistochemistry. A one in six series through the extent of hippocampus was stained for BrdU (1:100, Roche, Mannheim, Germany), and doublecortin (DCX) (1:50, Santa Cruz Biotechnology, Santa Cruz, CA, USA) using biotin-conjugated anti-mouse or goat secondary (1:500; 1:250 respectively, Jackson ImmunoResearch, West Grove, PA, USA) and visualized with diaminobenzidine (DAB) and SG Blue (DCX) (Vector Laboratories, Carpinteria, CA, USA). A one in eight series of lumbar coronal sections was stained free floating as above. For the figures, data were expressed as a percent of the control by dividing each group mean by the control group mean and multiplying by 100.

Quantification

Cell counts of BrdU and estimates of DCX positive cells were made using Stereoinvestigator (Microbrightfield, VT, USA). Contours were traced around areas of interest, either the dentate granule cell layer in the hippocampus or the entire lumbar coronal section. Using the Optical Fractionator probe with a counting frame (240 μm$\times$180 μm for hippocampus and 500 μm$\times$325 μm for lumbar) and grid of equal size, every BrdU positive cell was counted at 40$\times$ (hippocampus) or 20$\times$ (lumbar) magnification. After marking BrdU positive cells, any double-labeled cells were counted with a different marker. DCX positive cells were counted using the Optical Fractionator probe with a counting frame of 40 μm$\times$180 μm and a grid size of 120 μm$\times$180 μm. Tissue thickness was measured at each site with positive cells, thus the tissue-weighted section thickness estimate was used for a more precise estimate of total cells. The equation is as follows: cell number=Q$\times$1/asf$\times$1/ssf$\times$1/hsf; where Q=total counts, asf=area sampling fraction, ssf=section sampling fraction, and hsf=height sampling fraction (West et al., 1991). The average coefficients of error (Gunderson m=1) for the above parameters were hippocampal BrdU 0.05, hippocampal DCX 0.04, and lumbar spinal cord BrdU 0.062. Planimetric volume estimates were gathered from the traced contours. Numerical density of both BrdU and DCX was calculated by dividing the optical fractionator estimate by the total planimetric volume estimate.

Statistics

All statistics were calculated with StatView. Running distance during week three was compared with a one-way analysis of variance (ANOVA) and analyzed using a post hoc Fisher's protected least significant difference (PLSD) with the P value set at 0.05. Animal weights, ELISA data from BDNF and IGF-1, and cytogenesis and neurogenesis counts were compared with a two-way ANOVA ($P<0.05$), using exercise and fluoxetine dose as the main factors. Where $P<0.05$, a post hoc Fisher's PLSD ($P<0.05$) was used. If there was a significant effect for one factor alone, either exercise or fluoxetine dose, a post hoc Fisher's PLSD ($P<0.05$) was used to compare groups.

RESULTS

Animal behavior changes with the fluoxetine dose

The effects of 3 weeks of fluoxetine administration on BDNF levels, IGF-1 levels, and neurogenesis were measured using the design in Fig. 1. Fluoxetine was administered in doses of 5 mg/kg (low dose), 10 mg/kg (middle dose) and 25 mg/kg (high dose) with and without voluntary running for 21 days (n=28 per dose, 14 running and 14 sedentary). The average running per day across the 3 weeks for the vehicle control group, 2543 m/day, was lower than previous experiments from our laboratory (Adlard and Cotman, 2004; Adlard et al., 2004a, 2005a; Engesser-Cesar et al., 2005). However, this decrease was predicted because the rungs on the wheels were covered with a flat-surface to replicated conditions shown to be successful in improving locomotor recovery after SCI (Engesser-Cesar et al., 2005). Fluoxetine treatment, however, was not predicted to affect running distance. The treated groups averaged 2171 m/day (low dose), 2202 m/day (middle dose) and 911 m/day (high dose). Additionally, the high dose group decreased in running activity as the experiment progressed (Fig. 2A). At the end of the experiment, the high dose group ran significantly less than the other groups (week 3 running distance one-way ANOVA, F=5.230, $P<0.01$; post hoc Fisher's versus control, $P<0.001$; versus low dose, $P<0.01$; versus middle dose, $P<0.01$). Along with decreasing activity, fluoxetine significantly increased the weight gain during week three of the experiment (two-way ANOVA, F=9.592, $P<0.0001$, post hoc Fisher's, high dose compared with all other doses $P<0.0001$) (Fig. 2B). Furthermore, blinded technicians noted that the high dose group was larger in appearance and more difficult to capture and inject compared with the other groups.

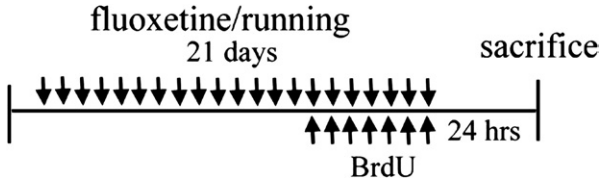

Fig. 1. C57Bl 10 female mice (n=112) were separated randomly into one of eight groups for 21 days of saline or fluoxetine hydrochloride injections with and without voluntary wheel running. Sedentary and wheel running groups included a saline control, and doses of 5 mg/kg, 10 mg/kg and 25 mg/kg. A subset of each group (n=6) received BrdU injections for the last 7 days. Animals were killed 24 h after the last injections.

Exercise and fluoxetine increase BDNF in the hippocampus

Although previous research reported increases in hippocampal BDNF protein with 3 weeks of running (Adlard et al., 2005a), here, hippocampal BDNF protein was not induced by exercise. The previous experiments used young male C57Bl/6 mice which ran greater amounts than the female C57Bl/10 mice used here, which, again, were chosen in attempts to identify potential mechanisms that might explain how exercise prior to a SCI could contribute to improved locomotor recovery (Engesser-Cesar et al., 2005). Fluoxetine treatment in the absence of exercise significantly increased hippocampal BDNF protein levels (two-way ANOVA, F=39.942, $P<0.0001$) (Fig. 3A). BDNF

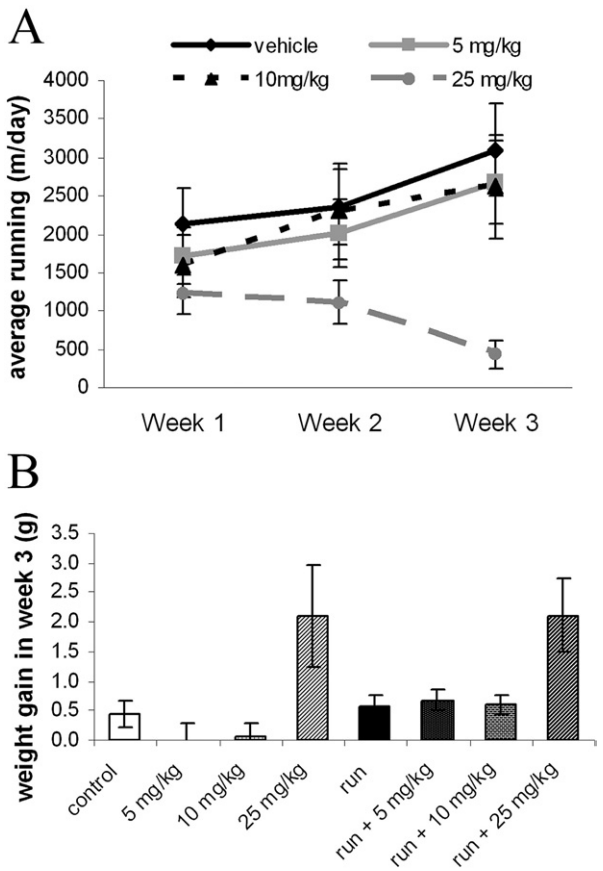

Fig. 2. Animal behavior during the 21 day experiment changed with the 25 mg/kg dose. (A) Animals in the 25 mg/kg dose group ran significantly less than the other three groups during week 3 (one-way ANOVA, $P<0.001$ (vehicle), $P<0.01$ (5 mg/kg and 10 mg/kg)) with the largest change during the last week of the experiment. The numbers reported are less than previous experiments, due to the use of a covered running wheel as used in previous SCI experiments. (B) Animals in the 25 mg/kg groups gained significantly more weight in the last week of the experiment compared with the other groups (two-way ANOVA, $F=9.592$, $P<0.0001$, post hoc Fisher's, 25 mg/kg dose compared with all other doses $P<0.0001$). Error bars are ±S.E.M.

expression in the high dose group was 496% of control and was significantly elevated compared with the vehicle control and the low and middle dose groups (post hoc Fisher's $P<0.001$). There was no interaction between exercise and fluoxetine treatment (two-way ANOVA, $F=1.69$, $P=0.18$), however, the results with the high dose of fluoxetine were at the maximal part of the sensitivity range of the BDNF assay, so there may have been a synergistic effect that was not detectable with the conditions used.

Exercise and fluoxetine do not change IGF-1 in the hippocampus

Hippocampal levels of IGF-1 protein were not significantly changed by fluoxetine treatment, exercise or the combination (Fig. 3B). Although there was a strong trend suggesting that fluoxetine decreased IGF-1 protein (two-way ANOVA, $F=2.691$, $P=0.059$). Likewise, serum levels of IGF-1 were not changed by exercise, fluoxetine treatment or the combination of the two (Fig. 3C).

Exercise and fluoxetine increase cytogenesis in the hippocampus

Both cytogenesis and neurogenesis in the hippocampus have been investigated with exercise and antidepressant treatment alone; however, the combination of the two has not been measured. Representative hippocampal sections stained with both BrdU and DCX are shown in Fig. 4A and B. Analysis with a two-way ANOVA showed a significant effect of treatment with fluoxetine on cytogenesis, BrdU expression ($F=6.111$, $P<0.01$; post hoc Fisher's high dose versus vehicle control, low and middle doses $P<0.05$) and DCX expression, neurogenesis, ($F=15.197$, $P<0.001$; post hoc Fisher's high dose versus vehicle control, low and middle doses, $P<0.001$) in the hippocampus (Fig. 4C and D). There was, however, no synergistic effect of combining exercise and fluoxetine.

The volume of the dentate granule cell layer increased with fluoxetine, specifically the high dose group (two-way ANOVA, $F=9.115$, $P<0.001$) (Fig. 4E). Comparisons of numerical density calculations showed DCX, but not BrdU increased with fluoxetine treatment (two-way ANOVA, $F=4.254$, $P<0.05$) (Fig. 4F and G).

Spinal cord BDNF and IGF-1 change with exercise and fluoxetine

The three regions of the spinal cord showed neither consistent changes nor trends in levels of BDNF protein with exercise (Fig. 5A, B, C). These results support previous data from this laboratory, and others, showing that wheel running does not change BDNF protein in the spinal cord (Johnson et al., 2003; Perreau et al., 2005). However, in the thoracic cord, there was an effect of fluoxetine treatment on BDNF expression (two-way ANOVA, $F=4.774$, $P<0.01$) and a significant interaction of exercise and fluoxetine treatment (two-way ANOVA, $F=3.280$, $P<0.05$). Conversely, there was no effect of fluoxetine or any interactions in the rostral or the lumbar spinal cord.

In contrast to the hippocampal data, IGF-1 protein levels did change in the spinal cord (Fig. 5D, E, F). Although exercise alone did not change IGF-1 protein level (lumbar cord: two-way ANOVA, $F=3.451$, $P=0.07$), fluoxetine decreased IGF-1 protein in all three regions of the spinal cord (two-way ANOVAs: rostral $F=9.155$, $P<0.0001$; thoracic ANOVA $F=9.396$, $P<0.0001$; lumbar $F=12.7$, $P<0.0001$). Similar to the hippocampus, there were no interactions of exercise and fluoxetine treatment on IGF-1 in the spinal cord.

Exercise and fluoxetine decrease cytogenesis in the spinal cord

Again, the spinal cord data contrasted with the hippocampal data. Because the central pattern generator responsible for locomotion is located in the lumbar spinal cord and BDNF levels had been reported by others to increase in this region with exercise, the lumbar region was selected for

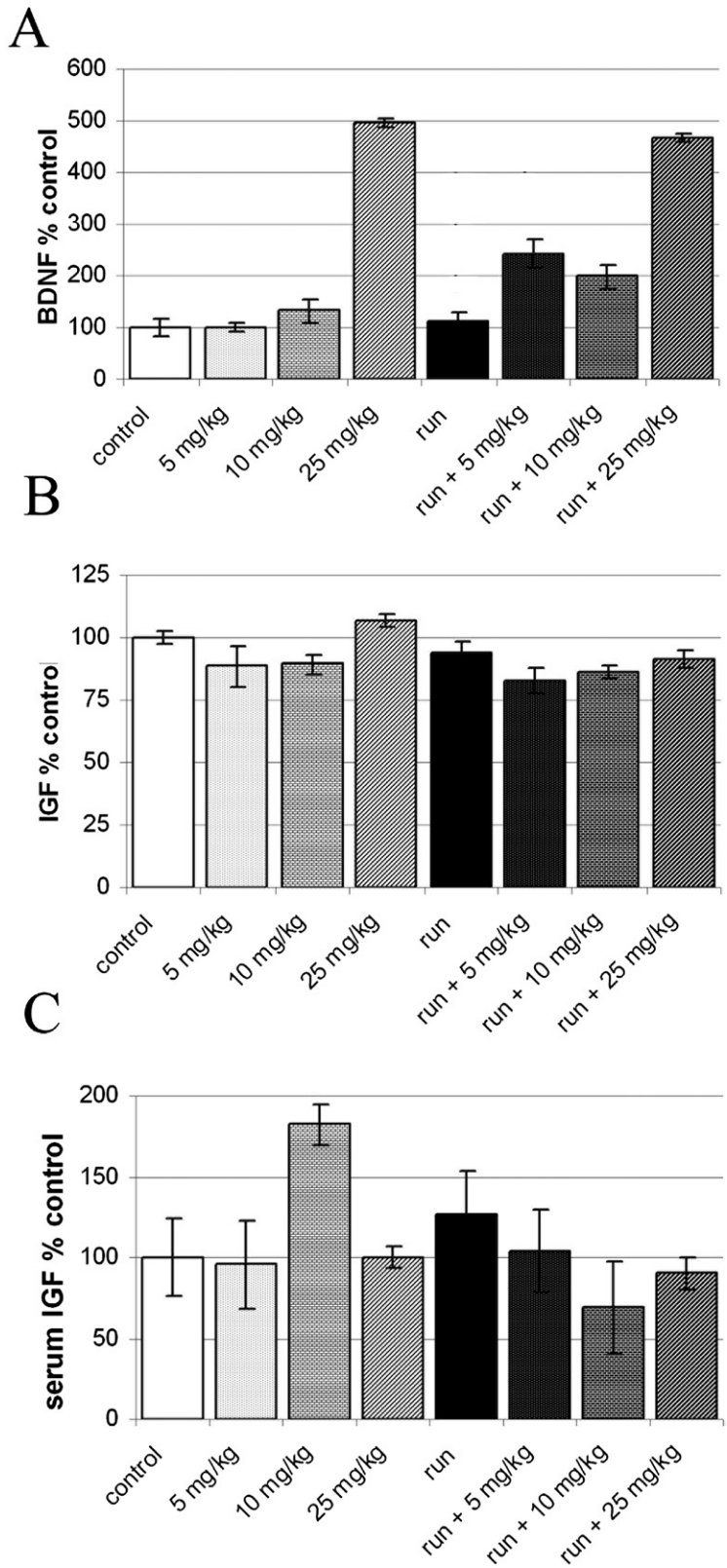

Fig. 3. BDNF and IGF-1 protein was assayed using ELISA kits. Values are expressed as a percent of control for hippocampal BDNF (A), hippocampal IGF-1 (B) and serum IGF-1 (C). Groups were compared using two-way ANOVAs. There was a significant effect of dose on BDNF levels ($F=39.942$, $P<0.0001$). The 25 mg/kg dose group was significantly elevated compared with all other doses (post hoc Fisher's $P<0.001$). There was no interaction between exercise and fluoxetine treatment ($F=1.69$, $P=0.18$). There were no significant effects of exercise or fluoxetine on hippocampal IGF-1 (fluoxetine, $F=2.691$, $P=0.059$) or serum IGF-1. Error bars are ±S.E.M.

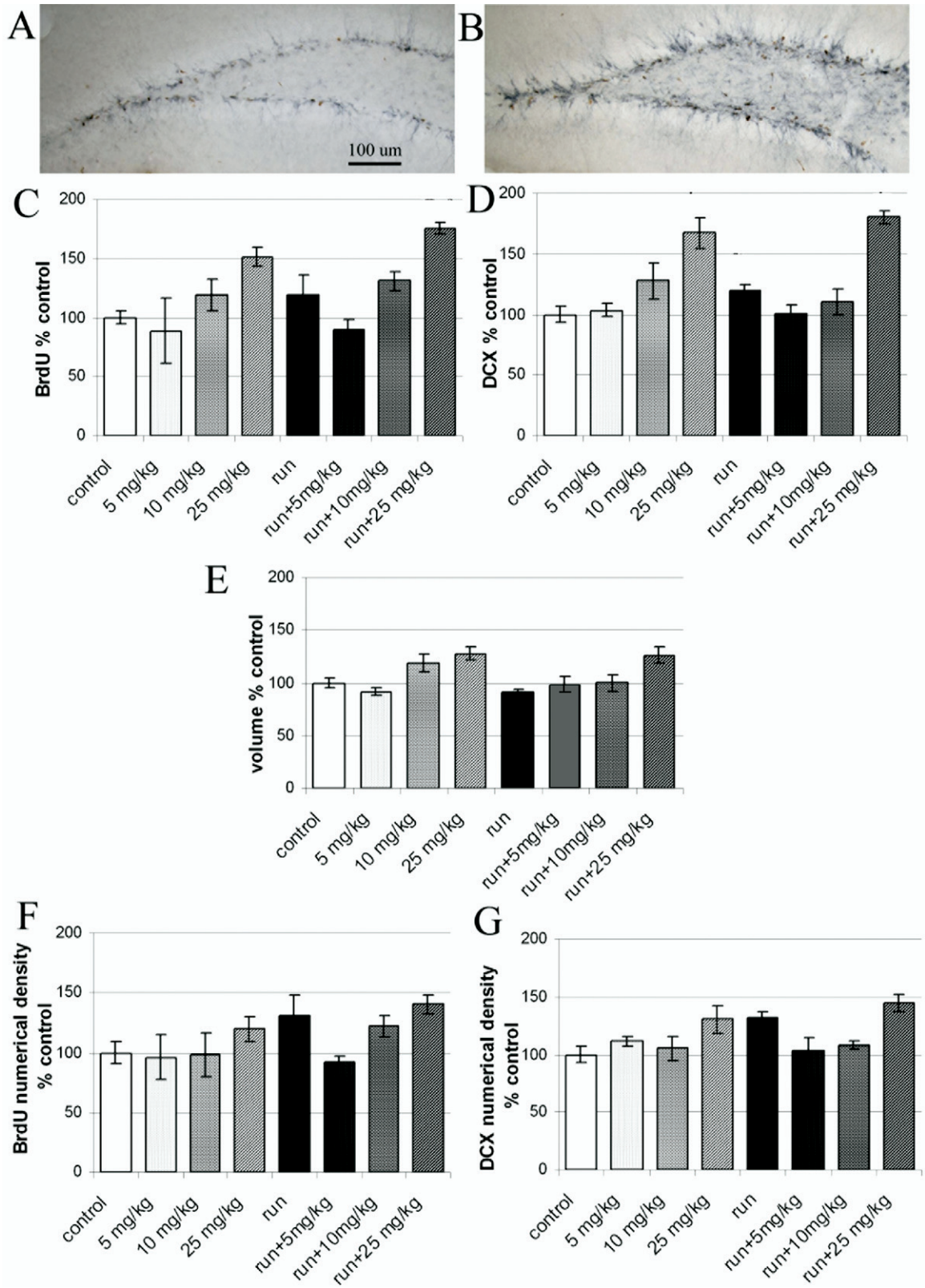

Fig. 4. Hippocampal cytogenesis and neurogenesis were estimated using modified stereology. Representative staining shown at 100× from a control (A) and a run+25 mg/kg high dose animal (B). Groups were compared with two-way ANOVAs. Total cell numbers were estimated using the Optical Fractionator after counting BrdU positive cells (C) and DCX positive cells (D). There was a significant increase with fluoxetine in BrdU expression ($F=6.111$, $P<0.01$; post hoc Fisher's 25 mg/kg dose versus all other doses $P<0.05$) and DCX expression ($F=15.197$, $P<0.001$; post hoc Fisher's 25 mg/kg dose versus all other doses, $P<0.001$). Total volume of the dentate granule cell layer was estimated from contour traces in Stereoinvestigator (E) and increased with fluoxetine ($F=9.115$, $P<0.001$). Cell numerical density was calculated by dividing the total cell number by the total volume (F, BrdU and G, DCX). DCX, but not BrdU increased with fluoxetine treatment ($F=4.254$, $P<0.05$). Error bars are ±S.E.M.

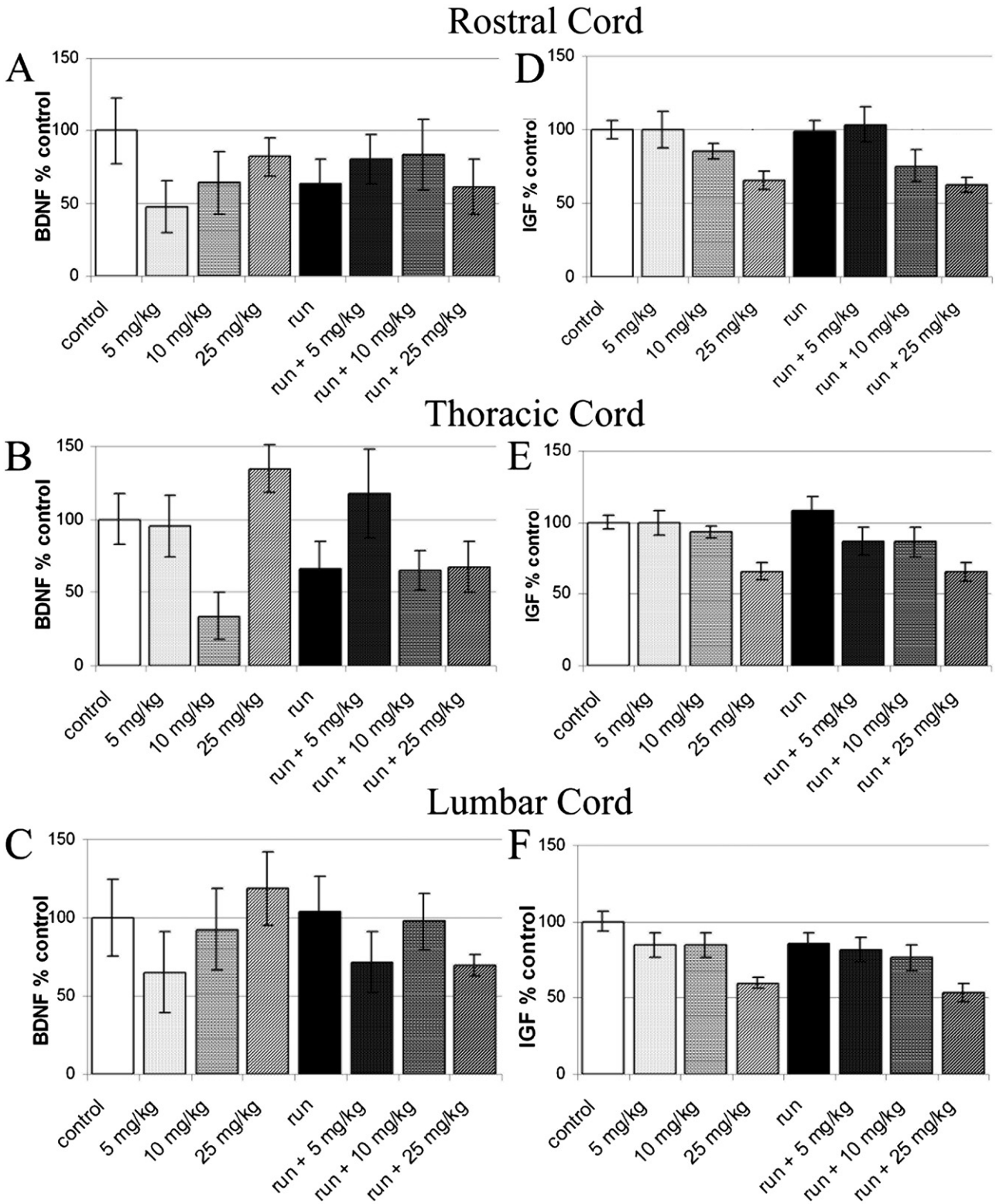

Fig. 5. BDNF and IGF-1 protein was measured using ELISA kits. Values are expressed as a percent of control for BDNF (A, B, C) and IGF-1 (D, E, F). In the thoracic cord (B), there was a significant effect of fluoxetine treatment on BDNF expression ($F=4.774$, $P<0.01$) and a significant interaction of exercise and fluoxetine treatment ($F=3.280$, $P<0.05$). Fluoxetine significantly decreased IGF-1 protein in all three regions of the spinal cord (rostral $F=9.155$, $P<0.0001$; thoracic ANOVA $F=9.396$, $P<0.0001$; lumbar $F=12.7$, $P<0.0001$). Error bars are ±S.E.M.

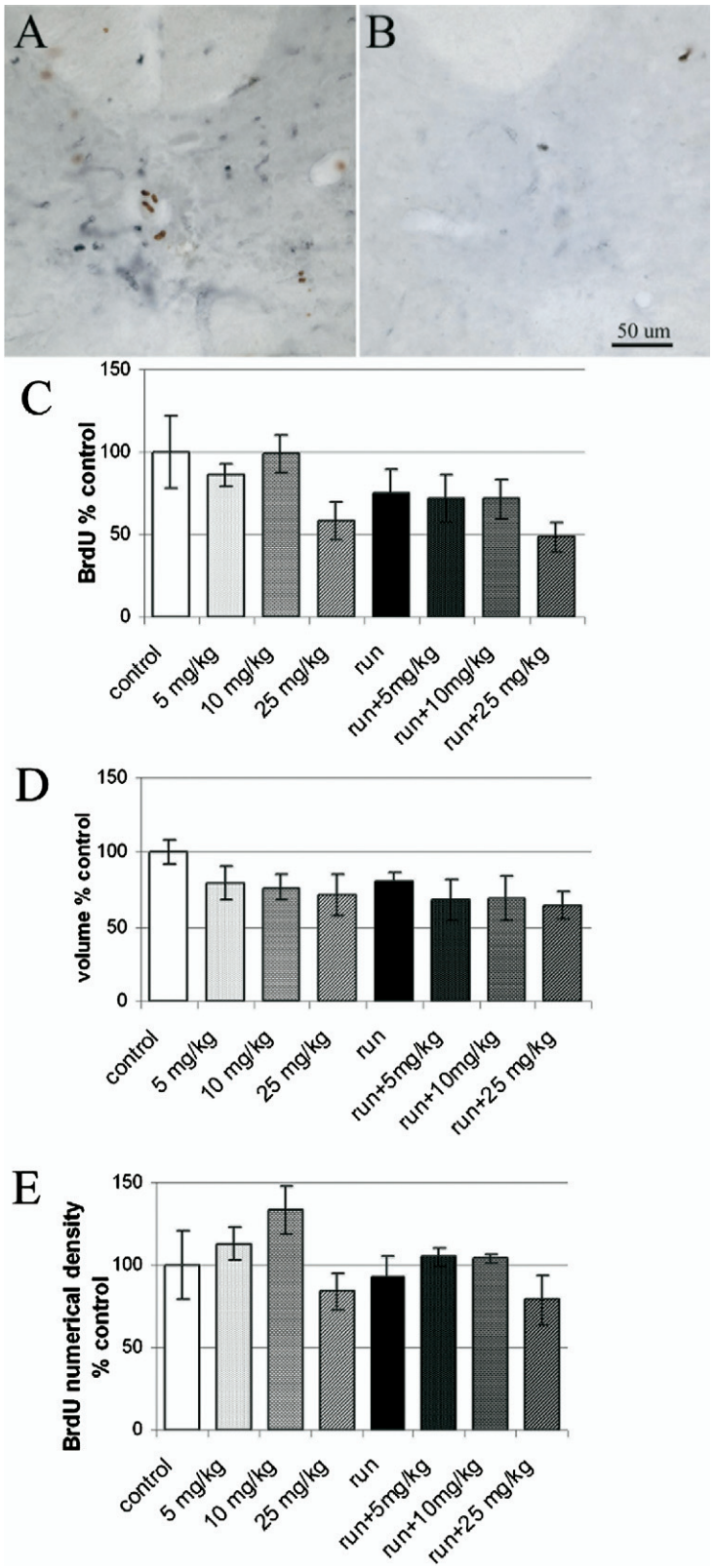

Fig. 6. Lumbar cord cytogenesis was measured using modified stereology, by counting BrdU positive cells. No evidence of neurogenesis (DCX positive cells) was found in lumbar cord. Representative staining shown at 200× from control (A) and run+25 mg/kg high dose (B). Groups were compared using two-way ANOVAs. Total BrdU cell numbers were estimated using the Optical Fractionator (C). Fluoxetine treatment significantly decreased BrdU expression ($F=3.261$, $P<0.05$). Total volume was estimated from contour traces in Stereoinvestigator (D). Cell density was calculated by dividing the total cell number by the total volume (E). Fluoxetine decreased BrdU cell density ($F=3.763$, $P<0.05$). See Experimental Procedures for specifics. Error bars are ±S.E.M.

analysis of cytogenesis. Representative lumbar sections stained with both BrdU and DCX are shown in Fig. 6A and B. There was a trend for the exercise animals to have decreased BrdU positive cells (two-way ANOVA $F=3.604$, $P=0.067$) (Fig. 6) and fluoxetine treatment significantly decreased BrdU expression (two-way ANOVA, $F=3.261$, $P<0.05$). Thus, cytogenesis decreases with fluoxetine treatment in the lumbar spinal cord compared with the increases in the hippocampus. There was no synergistic effect of combining fluoxetine and exercise. Furthermore, there was no evidence of neurogenesis in the lumbar spinal cord based on a lack of DCX staining (data not shown). Volume of the lumbar spinal cord did not significantly differ with exercise or fluoxetine treatment. After correcting for volume, there was still a significant effect of fluoxetine treatment on cytogenesis (BrdU numerical density $F=3.763$, $P<0.05$).

DISCUSSION

The use of antidepressants and exercise in combination as treatment for depression is an accepted protocol in the clinic, however, the effects of the two used in combination have not been investigated. Here, the data demonstrate that the combination of the antidepressant fluoxetine hydrochloride and wheel running has differential effects on BDNF, IGF-1 and neurogenesis. Furthermore, the data show that in this model, exercise or antidepressant treatment does not increase the growth factor IGF-1 as predicted from previous research. Lastly, the data show that the spinal cord and the hippocampus have differing responses to both exercise and antidepressants.

Exercise, fluoxetine and hippocampal mechanisms associated with depression

Both exercise and antidepressants have been shown to increase hippocampal BDNF mRNA and neurogenesis (Neeper et al., 1995, 1996; Malberg et al., 2000; Namestkova et al., 2005). These two endpoints have thus been targeted as potential mechanisms underlying the effectiveness of both exercise and antidepressants in treating depression. Furthermore, because it has been shown that exercise and antidepressants used in combination potentiate the BDNF response, it has been hypothesized that they may share mechanisms.

Recent reports have shown increases in hippocampal BDNF protein after exercise in female rats and male mice (Berchtold et al., 2001; Adlard et al., 2004b, 2005a) and fluoxetine treatment in male rats (De Foubert et al., 2004). In contrast, the data reported here using female mice, showed no change in BDNF protein with wheel running. However, important differences exist between the experiments. In the current experiments, we used the wheel surface from the SCI experiment (Engesser-Cesar et al., 2005), resulting in the mice running only 25% of the distance the mice ran in the study by Adlard et al. (2005a). Interestingly, aged male mice in the prior experiment (Adlard et al., 2005a), which ran the same as the young female mice in this experiment, also did not show an exercise-induced increase in hippocampal BDNF protein, suggesting there may be threshold in running distance needed to induce an increase in BDNF protein. Thus, gender, strain and/or differences in the amount of exercise could contribute to the differing results (Chen et al., 2005; Pollak et al., 2005).

The effects of antidepressants on BDNF have largely measured changes in mRNA levels (Russo-Neustadt et al., 1999, 2001), but one report assessing protein BDNF protein levels after treatment with 10 mg/kg of fluoxetine showed non-significant hippocampal increases with an ELISA (120% of control) and CA2 and CA3 specific increases in BDNF positive cells using immunohistochemistry (De Foubert et al., 2004). Here, after 21 days of fluoxetine administration results were similar to this report; hippocampal BDNF protein increased non-significantly with the middle dose and increased significantly with a higher dose. These previous studies were conducted in male rats, suggesting gender and species/strain are important variables to consider. Furthermore, previous reports have shown that the combination of exercise and antidepressant treatment potentiates the BDNF response (Russo-Neustadt et al., 2000), but the data here do not show the same effect. This may be due to several factors. First, the animals did not exhibit increases in BDNF with exercise alone, suggesting that wheel running was not contributing to the BDNF levels in the hippocampus even in the control animals, which ran significantly more than the high dose animals. Second, the animals receiving the high dose of fluoxetine had a ceiling effect with the BDNF assay, so a further increase could not have been detected. It appears that a threshold for both exercise and fluoxetine dose must be met to see results in the hippocampus. However, because the combination of running and the low/middle doses of fluoxetine do not show an interaction, it may be that the two interventions are acting via different mechanisms to induce BDNF and/or neurogenesis. If they shared a mechanism it would be predicted that sub-threshold doses of the two used in combination would produce a change in BDNF and/or neurogenesis.

Contrary to our hypothesis, there were no differences in IGF-1 protein in the hippocampus. Previous reports have shown exercise-induced increases in total brain IGF-1 (Carro et al., 2000), fluoxetine-induced increases in hippocampal IGF-1 protein (Khawaja et al., 2004), as well as antidepressant-like behavioral effects with IGF-1 administration (Carro et al., 2000; Khawaja et al., 2004; Hoshaw et al., 2005). IGF-1 has also been linked to increased neurogenesis and angiogenesis in the hippocampus (Trejo et al., 2001; Lopez-Lopez et al., 2004), however, exercise induction of IGF-1 protein has not been directly measured in the hippocampus. Our data suggest increases in hippocampal IGF-1 are not necessary for hippocampal changes in BDNF and neurogenesis.

As predicted, fluoxetine (high dose) increased both cytogenesis and neurogenesis in the hippocampus. However, increases with running alone were not significant as predicted, which could be explained by strain differences (Kempermann et al., 1997), gender differences (Perfilieva

et al., 2001), decreased running distances, but may also may suggest that exercise- and antidepressant-induced increases in neurogenesis are dependent on BDNF. Prior research showed similar results in mice with a deficient BDNF system after antidepressant treatment. That is, there was no antidepressant induced increase in neurogenesis in BDNF± or trkB-impaired mice (Sairanen et al., 2005) Again, there was no interaction on cytogenesis or neurogenesis when exercise and fluoxetine treatment were combined. Fluoxetine treatment also increased the volume of the dentate granule cell layer. An increase in hippocampal volume is one of the hypothesized effects of antidepressant treatment, as hippocampal volume has been reported to be decreased in depressed patients (Malberg and Schechter, 2005). The increased dentate granule cell layer volume may be result in part, from a change in the dendritic field of the dentate granule cells and thus an increase in volume of the dentate granule cells to support the increased dendritic field.

The spinal cord responds differently to exercise and fluoxetine than the hippocampus

In agreement with previous results from our laboratory and with the hippocampal data, no region of the spinal cord showed an increase in BDNF protein after wheel running. Indeed, the thoracic cord showed decreased BDNF protein with exercise. In contrast to hippocampal effects, fluoxetine treatment did not increase BDNF protein in the spinal cord. Additionally, whereas IGF-1 was not affected by exercise or fluoxetine treatment in the hippocampus, the high dose of fluoxetine significantly decreased IGF-1 protein in the spinal cord. Finally, exercise, fluoxetine antidepressant treatment, or the combination, did not show potential as an intervention capable of altering the spinal cord environment to be permissive for cytogenesis and/or neurogenesis. There was no evidence of neurogenesis in the spinal cord in any condition, and more interestingly, there was a significant decrease in cytogenesis in the lumbar region with the high dose and a strong trend for an exercise-induced decrease in cytogenesis.

The uninjured spinal cord does not show protective changes in BDNF, IGF-1 or neurogenesis with exercise or fluoxetine treatment

Post-injury exercise has been shown to improve recovery from both brain and SCI (Humm et al., 1998; Bland et al., 2000; Carro et al., 2001; Van Meeteren et al., 2003; Engesser-Cesar et al., 2005). One aim of study was to identify mechanisms through which pre-injury exercise might benefit recovery from SCI. Induction of BDNF expression and/or neurogenesis in the spinal cord with wheel running are potentials mechanisms for improved recovery, but there are conflicting reports in the literature (Gomez-Pinilla et al., 2001; Perreau et al., 2005). Here none of the mechanisms tested showed changes in a direction that would suggest involvement in a pre-injury-exercise-induced mechanism that could benefit SCI. However, it is possible that the effects of both exercise and fluoxetine on any of the markers measured could be different in an animal after a SCI.

Exercise and fluoxetine, might still affect these mechanisms after SCI, such that prophylactic exercise and/or fluoxetine may be protective against SCI or promote either trophic factor or cytogenesis as a recovery mechanism after SCI. Indeed, the consequences of fluoxetine treatment and exercise prior to an injury are unknown. Because proliferating cells in the spinal cord differentiate into glial cells and not neurons (Horner et al., 2000), a decrease in BrdU in the spinal cord likely reflects a decrease in glial progenitors. The consequences of a decrease in the glial population prior to an injury are unclear. For instance, if such a decrease in the preexisting glial population were to result in a decrease in glial scarring after SCI, it could result in a more regenerative environment and increased fiber sprouting, which could be beneficial to recovery. Conversely, it could also result in an inability to adequately or quickly reestablish the blood–brain/spinal barrier, which would likely result in an expansion of the area of primary damage and exacerbation of locomotor deficits (Faulkner et al., 2004).

CONCLUSION

This work extends previous research investigating the effects of exercise and antidepressants on the hippocampus and extends the assays to the spinal cord. Specifically, it shows the effects of a commonly prescribed antidepressant, fluoxetine, in combination with exercise. In the hippocampus, fluoxetine increased BDNF, cytogenesis and neurogenesis. In the spinal cord, fluoxetine decreased cytogenesis and IGF-1. Animals receiving the high dose of fluoxetine showed the greatest changes in both the hippocampus and the spinal cord. The data suggest that strain differences, gender differences and possibly differences in running distance may be important variables in the effects of the two interventions. These data also show the hippocampus and the spinal cord respond differently to exercise and fluoxetine treatment and finally, that exercise prior to SCI does not benefit locomotor recovery through an induction of BDNF, IGF-1 or neurogenesis in the spinal cord.

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