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BDNF-Exercise Interactions in the Recovery of Symmetrical Stepping After a Cervical Hemisection In Rats

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Abstract

Clinical evidence indicates that motor training facilitates functional recovery after a spinal cord injury (SCI). Brain-derived neurotrophic factor (BDNF) is a powerful synaptic facilitator and likely plays a key role on motor and sensory functions. Spinal cord hemisection decreases the levels of BDNF below the injury site, and exercise can counteract this decrease (Ying et al., 2005). It is not clear, however, whether the exercise-induced increases in BDNF play a role in mediating the recovery of locomotion after a SCI. We performed a lateral cervical (~C4) hemisection in adult rats. Seven days after hemisection, the BDNF inhibitor trkB IgG was injected into the cervical spinal cord below the lesion (~C5-C6). Half of the rats were exposed to voluntary running wheels for 14 days. Locomotor ability was assessed by determining the symmetry between the contralateral (unaffected) vs. the ipsilateral (affected) forelimb at the most optimum treadmill speed for each rat. Sedentary and exercised rats with BDNF inhibition showed a higher level of asymmetry during the treadmill locomotion test than rats not treated with the BDNF inhibitor. In hemisected rats, exercise normalized the levels of molecules important for synaptic function, such as cyclic AMP response element binding protein (CREB) and synapsin I, in the ipsilateral cervical enlargement, whereas the BDNF blocker lessened these exercise-associated effects. The results indicate that BDNF levels play an important role in shaping the synaptic plasticity and in defining the level of recovery of locomotor performance after a SCI.

Keywords

spinal cord injury; locomotion; TrkB IgG; synaptic plasticity; neurotrophin

Although neurotrophins have been identified as molecular systems with the potential to enhance spinal cord repair, most of the strategies to induce motor recovery after a spinal cord injury (SCI) have involved the addition of exogenous neurotrophins into the CNS. These strategies, however, do not address the intrinsic potential of the neural system to produce neurotrophins that could have an important effect on the recovery of stepping after a SCI. Given

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the capacity of voluntary exercise to induce endogenous neurotrophins in the spinal cord (Ying et al., 2003), we hypothesized that neurotrophins play a crucial role in the effects of exercise on the recovery of motor function after a SCI.

A large number of studies have shown the potential of motor training to promote functional recovery after a SCI (Edgerton et al., 2004, Frigon and Rossignol, 2006), yet the specific mechanisms and molecular systems involved remain largely unidentified. It is known that physical activity increases the expression of select neurotrophins, such as brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3), in the intact and injured spinal cord (Gomez-Pinilla et al., 2002, Ying et al., 2003). The role of these neurotrophins in promoting synaptic plasticity or functional recovery after a SCI, however, remains unclear. In particular, BDNF is a powerful modulator of neuronal excitability and synaptic transmission (Lu and Figurov, 1997, Kafitz et al., 1999), two processes that are crucial for functional recovery after an injury. The well-described involvement of BDNF in promoting neuronal excitability and synaptic modification suggests that it could play a supporting role in determining the level of functional recovery after a SCI. For example, in addition to promoting axonal growth (Bregman et al., 1997), BDNF delivered to the injured spinal cord can facilitate step-like oscillations in adult rats after a mid-thoracic contusion or complete spinal cord transection when the hindlimbs are suspended. In addition, when BDNF was administered continuously over a 4-week period, an open field motor score in complete spinal rats was significantly higher in treated than non-treated rats during weekly testing (Jakeman et al., 1998).

A further question is “through what mechanisms might BDNF operate to alter the course of functional recovery after a SCI”? Several of the downstream molecular systems that can mediate the action of BDNF on synaptic plasticity have been identified. BDNF affects the synthesis (Wang et al., 1995) and phosphorylation (Jovanovic et al., 1996) of synapsin I. Synapsin I is a member of a family of nerve terminal-specific phosphoproteins involved in neurotransmitter release (Jovanovic et al., 2000), axonal elongation, and maintenance of synaptic contacts (Brock and O'Callaghan, 1987). The transcription factor cyclic AMP response element binding protein (CREB) is required for various forms of memory including spatial learning (Silva et al., 1998) and appears to play a role in neuronal resistance to insult in conjunction with BDNF (Walton et al., 1999). CREB is characterized by its ability to modulate gene expression encoding BDNF and cell survival in the CNS (Tao et al., 1998, Ying et al., 2002). GAP-43 is present in growing axon terminals and has an important role in axonal growth, neurotransmitter release (Oestreicher et al., 1997), and learning and memory (Routtenberg et al., 2000).

In the present study, we investigated the role of BDNF and some of its downstream molecular regulators in mediating the effects of exercise on the recovery of motor function after a SCI. We used a specific molecule, i.e., trkB IgG, to block the function of BDNF during voluntary exercise in rats hemisected at a cervical level. We hypothesized that BDNF modulation induced by exercise plays a critical role in facilitating the recovery of motor function following a SCI. The results, in general, support this hypothesis.

Exprimental Procedures

Animals and General Procedures

Male Sprague-Dawley rats at 2 months of age (n=34, Charles River, San Diego, CA) were housed singly in standard polyethylene cages. After one week of acclimation, the animals were assigned randomly to either a sedentary control group (Con, n=5) or one of four hemisected groups, i.e., sedentary (Sed) or exercised (Ex) with saline (Sal) injection, (Sed/Sal, n=8, Ex/Sal, n=8), Sed or Ex with BDNF-inhibitor (IgG) injection (Sed/IgG, n=8, Ex/IgG, n=5). All rats were housed in standard polycarbonate cages (10.25″ × 18.75″ × 8″) individually during

the experimental period. Rats in the exercise groups were housed in standard cages equipped with running wheels beginning one week after the cervical hemisection. The vivarium room was maintained at 26 ± 1°C, with 40% humidity and a 12:12 h light: dark cycle. All rats were supplied with rat chow and water ad libitum. The studies were approved by the UCLA Chancellor's Animal Research Committee and followed the American Physiological Society Animal Care Guidelines.

Cervical Hemisection Procedures

All surgeries and injections were performed under aseptic conditions. The rats received an analgesic (Buprenex, 1.0 mg/kg, s.c.) 45 min prior to surgery. Surgery was performed with the rats anesthetized deeply with isoflurane gas (1.0 to 2.5%) via facemask and placed on a water-circulating heating pad maintained at 37°C to prevent hypothermia. A longitudinal dorsal midline skin incision was made over the spinal column from ~C2 to C6 and the muscles overlying the vertebral column were reflected. A partial laminectomy was performed approximately between vertebral levels C4 and C6. The dura mater was incised longitudinally and lidocaine hydrochloride (1%; 2 or 3 drops) was applied at the hemisection site (~C4). The right one-half of the spinal cord was isolated using a specifically designed probe and then transected using microscissors. Small cotton balls were used to separate the cut ends of the hemicord to assure a complete hemisection. Gelfoam was inserted between the cut ends of the hemicord. The paravertebral muscles and fascia surrounding the spinal column were sutured using 4-0 Dexon and the skin incision was sutured using 4-0 Ethilon. The rats were allowed to fully recover from anesthesia in an incubator (27°C) and were given lactated Ringers solution (5 ml, s.c.). PolyFlex (G.C. Hanford Manufacturing Co., Syracuse, NY), a general antibiotic, was administered (100 mg/kg, s.c., twice daily) during the first 3 days of recovery.

Inhibitor Preparation and Cervical Spinal Cord Injection Procedures

Recombinant Human TrkB/Fc Chimera (TrkB IgG) was used to block the BDNF action by subtracting it from the media. It was in powder form when purchased from R&D systems, Inc., Mineapolis, MN. Sterile PBS containing 0.1% of bovine serum albumin (BSA) was added to the vial to prepare a stock solution of 100 ug/mL. Saline was used as a standard control for microspheres injection. Microspheres were used as the vehicle for drug insertion to spinal cord. We prepared microspheres (Lumaflour, New York, NY) using the methods described by (Riddle et al., 1997) and used in our laboratory (Vaynman et al., 2004). These procedures consisted of coating the microspheres with each solution via passive absorbency by incubating overnight at 4°C with a 1:5 mix of microspheres to trkB IgG or saline. The morning after coating the microspheres, the solution was centrifuged at 14,000g for 30 min and the microspheres were resuspended in sterile water at a 10% concentration. After one week of recovery from the surgery, the hemisected rats were assigned randomly into either a sedentary or an exercise group. Under the same surgical conditions as described above, a partial laminectomy was performed between C5 and C7 to expose the dorsal spinal cord. One half of the rats in each group were injected with trkB IgG (Sed/IgG and Ex/IgG groups) and the other one-half with the same volume of a standard control saline solution (Sed/Sal and Ex/Sal groups). For these injections, the vertebral column was stabilized on a stereotaxic apparatus using clamps on the spinal processes at C2 and C9. TrkB IgG (5 ug/ul) or saline coated with microspheres were injected bilaterally at C5-C6 (0.6 mm lateral to midline, 1.5 mm below the duramater for the first injection, and 0.8 mm below the duramater for the second injection) using a Hamilton syringe in a volume of 2 μl over a 15 min period. For the next 14 days, the rats in the exercised groups were housed in cages with voluntary running wheels, whereas the non-exercised rats were housed in same type of cages but without running wheels. A fifth group of rats served as a sedentary control group, i.e., no surgery, no exercise, and no injections. TrkB IgG fusion proteins mimic the TrkB receptor and abolish BDNF function by sequestering free

BDNF. We have successfully used TrkB IgG embedded in microspeheres to block BDNF action for up to 14 days after the injection (Vaynman et al., 2004).

Voluntary Exercise Procedures

The rats in the exercise groups (Ex/Sal and Ex/IgG) were placed in standard cages equipped with running wheels that rotated against a resistance of 100 g (Ying et al., 2005). Sedentary rats (Con, Sed/Sal, and Sed/IgG) were left undisturbed in their home cages. The numbers of wheel revolutions were monitored and recorded by computer).

Biochemical Analyses

The day after the last exercise session (14 days after the BDNF inhibitor or saline treatment), all rats were decapitated in the early morning. The cervical spinal cord enlargement (C3-C6) ipsilateral to the lesion was dissected rapidly, frozen on dry ice, and stored at −70° C until processed. We used the RNA STAT-60 kit (TEL-TEST, Friendswood, TX) and the manufacturer's protocol for total RNA isolation. The mRNAs for BDNF, NT-3, Synapsin I, GAP-43, and CREB were measured in the total RNA obtained from individual animals by TaqMan real time quantitative RT-PCR using the ABI PRISM 7700 Sequence detection system (Applied Biosystems), and TaqMan EZ RT-PCR Core reagents (Applied Biosystems). This system directly detects the increase in fluorescence of a dye-labeled DNA probe specific for each factor under study plus a probe specific for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene used as an endogenous control. The sequences of probes, forward and reverse primers (Integrated DNA Technologies, Coralville, IA) were: BDNF:(5′ AGTCATTTGCGCACAACTTTAAAAGTCTGCATT-3′), forward: (5′ GGACATATCCATGACCAGAAAGAAA-3′), reverse: (5′-GCAACAAACCACAACATTATC GAG-3′); NT-3: (5′-TGACCGACAAGTCCTCAGCCATTGAC-3′); forward: (5′-TGTGACAGTGAGAGCCTGTGG-3′), reverse: (5′-TGTAACCTGGTGTCCCCGAA-3′); synapsin I (5′CATGGCACGTAATGGAGACTACCGCA3′), forward: (5′-CCGCCAGCTGCCTTC-3), reverse: (5′-TGCAGCCCAATGACCAAA-3′); GAP-43:(5′–CTCATAAGGCTGCAACCAAAATTCAGGCT-3′), forward: (5′-GATGGTGTCAAACCGGAGGAT-3′), reverse: (5′-CTTGTTATGTGTCCACGGAAGC-3′); CREB: (5′-CATGGCACGTAATGGAGACTACC GCA-3′); forward: (5′-CCGCCAGCATGCCTTC-3′), reverse: (5′-TGCAGCCCAATGACCAAA-3′). The RT-PCR reaction conditions were for 2 min at 50°C as an initial step to activate uracil glycosylase (UNG), followed by 30 min at 60°C as reverse transcription and completed by UNG-deactivation at 95°C for 5 min. The 40 cycles of the two-step PCR-reaction conditions were 20 s at 94°C and 1 min at 62°C.

Assessment of Quadrupedal Locomotor Ability

Prior to the hemisection surgery all rats were acclimated to running on a treadmill at speeds ranging from 6 to 30 cm sec^{-1} for 10-15 min on two occasions. Thirteen days after treatment with the inhibitor or saline injections, all rats were tested for quadrupedal locomotor capability. The SIMI Motion Analysis System (zFlo; Quincy, Massachusetts) was used to collect video from 4 synchronized cameras at 100frames/sec. The forelimbs were shaved bilaterally and retroreflective markers were placed on bony landmarks at the shoulder, elbow, wrist, and tip of the 5th metacarpal-phalange. During the testing, the rats were stepped on a treadmill beginning at 3 cm/sec and the speed was increased by ~3.5 cm/sec until the rat could no longer maintain that speed. The maximum speed of stepping was considered that speed at which the rat could successfully complete 10 consecutive steps. All other measures related to the kinematics of stepping of the forelimbs were determined from the steps performed at the maximum speed and normalized to a distance of 100 cm on the treadmill. Each step was

considered weight-bearing or oscillating based on visual inspection of the video, i.e., a step was considered oscillating when the forelimb was actively moved forward and backward regardless of the level of weight-bearing. The evaluators were blind to the group assignment. The percent time in stance for each step cycle was determined as the time from toe-on to toe-off the treadmill. The number of weight-bearing steps and oscillating steps, and the stance duration relative to step period are reported as the ratio between the right (non-hemisected) and left (hemisected) sides. In addition, the proportion of steps that were weight-bearing on the hemisected side is reported. The angle of the wrist relative to the movement forward at a plane perpendicular to the treadmill through the shoulder marker was used to indicate the maximum forward reaching position of both forelimbs.

Statistical Analyses

For quantification of RT-PCR results, fluorescent signal intensities were plotted against the number of PCR cycles on a semi logarithmic scale. The amplification cycle at which the first significant increase of fluorescence occurred was designed as threshold cycle (Ct). The process was automatically carried out by ABI sequence detector software version 1.6.3. GAPDH was used as an endogenous control to standardize the amount of sample RNA. Measurements of BDNF, synapsin I, CREB, NT-3, Gap-43 mRNA for individual animals were normalized with corresponding GAPDH values. Statistical analysis was performed using GraphPad Prism 4.01 software (GraphPad Software, San Diego, CA). Overall differences in the biochemical and functional data were determined using analysis of variance (ANOVA) and individual group differences were determined using Tukey's post-hoc tests. Statistical differences were considered significant at $p < 0.05$. The functional measures are presented as right (hemisected side)/left (contralateral side) ratios to show the degree of symmetry between the two forelimbs, whereas the biochemical data are expressed as percent of sedentary control values for graphic clarity. Values are reported as mean ± standard error of the mean (SEM) for 5 to 8 rats per group.

Results

The gait patterns of the forelimbs changed dramatically immediately after the cervical hemisection. Although the locomotor capability improved with time, observable deficits in forelimb function remained throughout the experimental period. Multiple measures of the kinematics of the forelimbs were examined to gain a reasonably comprehensive understanding of the level and nature of the recovery that had occurred among the different groups of rats.

Effects of BDNF Blocking on Locomotor Performance

The trajectory of the forelimbs ipsilateral (right) and contralateral (left) to the lesion was assessed to critically evaluate the stepping performance. The level of impairment in the step trajectories was greatest in the ipsilateral limbs of the rats treated with the BDNF blocker. This impairment was reflected in the differences in the shape and the consistency of the trajectory of the wrist during approximately 5 consecutive steps between the ipsilateral and contralateral forelimbs. Examples of the trajectories of the forelimb during the best stepping observed in each group are illustrated in Figure 1. In general the symmetry of the Ex/Sal rats was more similar to that observed in control rats compared to the IgG-treated groups. Clearly, the most marked asymmetry in the trajectories was observed in the two groups receiving IgG. The swing phase of one step cycle from the left and right forelimbs from one rat for each group is illustrated as a stick diagram in the middle of Figure 1. The specific step cycle illustrated as a stick diagram is shown in each trajectory plot as a bold line. These illustrations provide a readily visible perception of the movement deficiencies that typically characterized each group of rats. As shown in Figure 2F, the amount of forward region of the forelimb was compromised the most in the right forelimb (lesioned side) of animals treated with the inhibitor.

The maximum speed at which a rat can generate successful and consistent stepping serves as one general measure of the neural control of forelimb function. There were no significant differences in the maximum speed of quadrupedal locomotion among the groups, although the Sed/IgG group had the slowest mean maximum speed of locomotion (Fig. 2A). The level of symmetry in the movement of the two forelimbs expressed as the ratio of the right limb (ipsilateral to the hemisection)/left limb (contralateral to the hemisection) was assessed using a series of parameters. There were no group differences in the ratio of the duration of the stance phase of a step relative to the cycle period duration (Fig. 2B). However, the ratio of the number of backward and forward step-like motions (ratio of oscillating steps) during the locomotor test was closer to unity in the rats not receiving the inhibitor compared to the IgG treated rats, with the value being significantly lower in the Ex/IgG and in the Sed/IgG than in the Sed/Sal and in the Ex/Sal groups ($p < 0.05$) (Fig. 2C). The Ex/Sal group showed a trend ($p = 0.19$) to have the best performance. This measure included all oscillating movements independent of whether there was any weight bearing in the limbs.

The number of weight-bearing steps provided similar results, with the only significant difference being a lower value in the Ex/IgG than the Ex/Sal group (Fig. 2D). The Ex/Sal group showed a trend to have a better performance compared to the Sed/Sal group ($p = 0.29$). Figure 2E shows the proportion of weight-bearing steps on the hemisected side (right side), the Ex/IgG group showed a marginally significant decrease compare to Sed/sal group ($P<0.055$). The most discriminating feature of the forelimb movement was the distance that the wrist was extended beyond an imaginary vertical line through the shoulder during the swing phase of the step cycle. This distance was shorter on the lesioned than the non-lesioned side in both groups treated with the inhibitor(Fig. 1F). Again, the Ex/Sal group showed a tendency ($p=0.13$) for improvement compared to the Sed/Sal group on the lesioned side, and there was a significantly lower value on the lesioned side in the Ex/IgG compared to the Ex/Sal group.

The general conclusion from these results on stepping symmetry is that the lesion limited the ability of the affected limb to execute alternating swing-stance phases, to bear weight, and the degree to which the forelimb could reach forward before initiating the next stance phase. In addition, the level of impairment of these features, in general, was greatest in the rats treated with the BDNF blocker.

Effects of Exercise and BDNF Blocking on BDNF-Mediated Plasticity

BDNF mRNA levels in the cervical hemicord ipsilateral to the cervical hemisection in Sed/Sal rats decreased to 82% of Con (intact group) values ($p<0.05$), (Fig. 3A). After two weeks of exercise, the BDNF mRNA levels in the Ex/Sal rats were increased to Con values (99% of Con values). In the rats injected with the BDNF inhibitor, the levels of BDNF mRNA were reduced to 72% (Sed/IgG, $p<0.05$) and 81% (Ex/IgG, $p<0,05$) of Con values: these values also were lower than in the Ex/Sal group. Synapsin I is a downstream effector for the action of BDNF on synaptic plasticity. We found that synapsin I mRNA levels were significantly lower in Sed/Sal than Con rats (71% of Con, $p<0.05$)), but similar (91% of Con) to Con in the Ex/Sal rats (Fig. 3B). In rats receiving the inhibitor, the levels of synapsin I mRNA were reduced to 80% (Sal/IgG, $p<0.05$) and 70% (Ex/IgG, $p<0.05$) of Con values. In addition, the levels in the Ex/IgG group were significantly ($P<0.05$) lower than in the Ex/Sal group. The effects of exercise were abolished by the BDNF inhibitor. We assessed the levels of BDNF inhibition on another member of the neurotrophin family, i.e., NT-3, that has an important role in spinal cord plasticity. NT-3 mRNA levels after the hemisection (Sed/Sal) were reduced to 67% of Con values ($p<0.05$) (Fig. 3C). With exercise (Ex/Sal) the levels of NT-3 mRNA remained at Con levels (91% of Con). NT-3 mRNA levels in sedentary with inhibitor rats (Sed/IgG) were at the control levels (92% of Con), whereas these levels in the Ex/IgG group were reduced to 72% of Con value.

CREB mRNA levels were determined based on the involvement of CREB in BDNF-mediated neuronal plasticity (Barde, 1994, Acheson et al., 1995, Hardingham and Bading, 2002, Hardingham et al., 2002). The levels of CREB mRNA in the Sed/Sal rats were reduced to 77% of Con values ($p<0.05$) (Fig. 3D). In contrast, CREB mRNA levels in the Ex/Sal rats (107% of Con) were maintained at Con values, however, the values were elevated relative to the injured group without exercise (Sed/Sal). The CREB mRNA levels in Sed/IgG and Ex/IgG groups were 85% and 71% ($P<0.05$) of Con, respectively. Levels of GAP-43 were measured to have an estimate of the interventions on axonal growth events. GAP-43 mRNA levels in Sed/Sal rats were 91% of Con values (Fig. 3E). In contrast, the GAP-43 mRNA levels in Ex/Sal rats (115%) were significantly ($P<0.05$) higher than Con values. The levels of GAP-43 in both the sedentary and exercised rats treated with the inhibitor were similar to Con. The GAP-43 values were significantly higher in the Ex/Sal than the Sed/Sal and Ex/IgG groups.

Relationships Between Motor Performance and the Levels of the Markers of Plasticity

There was a positive relationship between the number of total wheel revolutions and the BDNF mRNA levels in the Ex/Sal group (Fig. 4A, $r = 0.71$, $p<0.05$). Treatment using the BDNF inhibitor trkB IgG reduced the correlation coefficient to $r= 0.51$, $p=0.3772$ (Fig. 4A). There also was a positive relationship between the ratio (R/L) of weight-bearing steps (the most sensitive measure of stepping ability) and the levels of Synapsin I (Fig. 4B, $r = 0.56$, $p<0.05$), and GAP-43 (Fig. 4D, $r = 0.40$, $p<0.05$) across all groups. Interestingly, the correlation between the ratio of weight-bearing steps and either CREB mRNA (Fig. 4C, $r = 0.34$), BDNF mRNA (Fig. 4E, $r= 0.29$), or NT-3 mRNA (Fig. 4F, $r= 0.06$) were not significant.

Discussion

Given the actions of BDNF on neuronal excitability and synaptic function and findings that the level of physical activity is an important modulator of BDNF in the spinal cord, we examined whether the activity-mediated modulation of BDNF plays a central role in determining the level of recovery of motor function following a SCI. We examined the effects of cervical lateral hemisection on plasticity-associated molecules that are modulated as a function of BDNF, in the periphery of the injured area. In addition, adult rats were injected with a BDNF blocker and exposed to voluntary running wheel exercise for 14 days. Blocking BDNF abrogated the counteractive effects of exercise on the locomotor deficits. BDNF blocking also limited the effects of exercise in counteracting the decrease in synaptic plasticity-related molecules observed after the hemisection. These results indicate that BDNF plays an important role in mediating the effects of exercise on the recovery of locomotion after a SCI.

Exercise and Locomotor Performance in the Injured Spinal Cord

The interlimb coordination of the forelimbs of sedentary and exercised, hemisected rats had some degree of asymmetry, and the level of asymmetry was greater in the inhibitor-treated rats. The high level of asymmetry in both the Sed/IgG and Ex/IgG groups suggests that BDNF inhibition was sufficient to limit spontaneous and exercise-induced motor recovery. Based on the number of wheel revolutions, it appears that the level of exercise was similar in the Ex/Sal and Ex/IgG groups. Some caution is warranted in the interpretation of these data because the number of wheel revolutions does not necessarily represent the use of the affected forelimb: e.g., the rats could run effectively in the wheel by running tripedally, i.e., not using the affected forelimb. However, it has been observed previously that wheel running after a SCI improves locomotor recovery and stimulates serotonergic fiber growth (Engesser-Cesar et al., 2007). Accordingly, it is possible that the effects of exercise on spinal cord injured rats can be enhanced using a more severe training paradigm, i.e., treadmill training.

Mechanisms of Action

The ability of exercise to up-regulate BDNF in the spinal cord based on the amount of wheel running (Fig. 4A) is consistent with exercise promoting CNS plasticity. The reduction in BDNF levels observed after hemisection was normalized when the rats were allowed to run in a wheel. It is clear that the level of wheel running was sufficient to increase the levels of BDNF and NT-3 mRNA relative to sedentary rats (Fig. 3A and 3C). This exercise effect also was evident for several molecules that are modulated by BDNF and linked to synaptic plasticity such as synapsin I (Fig. 3B), CREB (Fig. 3D), and GAP-43 (Fig. 3E). In contrast, the inhibitor abolished the increase in the markers of synaptic plasticity and, in most cases, these marker levels were somewhat lower in Ex/IgG than Sed/IgG rats. For example, blocking of BDNF action abrogated the ability of exercise to maintain normal levels of synapsin I mRNA after injury. BDNF phosphorylates synapsin I primarily through the trkB receptor to induce the mitogen-activated protein kinase signaling pathway that modulates neurotransmitter release (Jovanovic et al., 2000). In addition to the effects of TrkB IgGs on blocking BDNF unction, we cannot discard the possibility that the TrkB IgG intervention may have some detrimental effects on the recovery from stepping not directly associated with BDNF blocking. Exercise normalized the depressed levels of CREB mRNA after the hemisection, and the inhibition of BDNF abolished the effects of exercise on CREB mRNA levels. CREB is one of the best-characterized transcription factors in the CNS and can be modulated by BDNF (Finkbeiner et al., 1997) CREB is phosphorylated by BDNF at the transcription regulatory site, and CREB can feed-back on BDNF by regulating its gene transcription via a calcium-dependent mechanism (Finkbeiner et al., 1997). CREB is required for several forms of memory (Silva et al., 1998) and appears to play a role in neuronal resistance to insult (Walton et al., 1999) these are likely two basic components of functional recovery after a SCI. BDNF blocking in the hippocampus using a similar trkB-IgG treatment has been shown to reduce the hippocampal increase in synapsin I mRNA and CREB mRNA associated with exercise (Vaynman et al., 2004), consistent with the interpretation that BDNF may modulate synapsin I and CREB during exercise.

We have previously shown that exercise attenuates the decrease in GAP-43 observed after a spinal cord hemisection (Ying et al., 2005). The present results provide new evidence that BDNF may be involved in the effects of exercise on GAP-43 as the BDNF blocker abolished the effects of exercise on GAP-43. Given the role of GAP-43 on axonal growth, neurotransmitter release, and learning and memory, it is likely that exercise-related increases in GAP-43 can be associated with maintaining synaptic function (Oestreicher et al., 1997). Indeed, a potential action of BDNF on synaptic plasticity associated with exercise would likely involve mechanisms that support neurite outgrowth. BDNF also could facilitate locomotor recovery through other mechanisms. It can stimulate locomotor-like activity in adult rats by increasing the excitability of spinal locomotor networks (Jakeman et al., 1998). BDNF also can affect muscle excitability. For example, it has been reported that BDNF stimulates the release of acetylcholine at the neuromuscular synapse of cultured myocytes resulting in the potentiation of spontaneous twitching (Kleiman et al., 2000).

It is notable that the biochemical markers of synaptic plasticity in the Ex/IgG rats were somewhat lower than those of Sed/IgG rats (Fig. 3B, D, and E) and that this apparent exercise effect on biochemical markers corresponds to consistently poorer stepping performance in the Ex/IgG rats (Fig. 1C and D). This relationship can be seen more clearly by the correlations between the behavioral performance and the levels of the biochemical markers (Fig. 4). The correlation ratios suggest that there is a significant link between the levels of several mRNA's that mediate plasticity and the recovery in weight-bearing stepping after a cervical hemisection. Although several of these correlations are significant, it is apparent that there are other modulatory factors that affect recovery in these hemisected rats. One possible explanation is

that the Ex/IgG rats indeed not only had less potential for mediating changes because of reduced levels of BDNF (and NT-3), but when exercised there also was a reinforcement of an already existing asymmetry by practicing asymmetric stepping.

BDNF and NT-3 Interaction

Spinal cord hemisection also reduced levels of NT-3 mRNA while exercise returned NT-3 mRNA to nearly normal levels. We have previously shown that a thoracic spinal cord hemisection does not affect levels of NT-3 in the lumbar enlargement (Ying et al., 2005). These results appear to indicate that the rostrocaudal level of the injury or distance from the injury site are important factors for the NT-3 modulation. It is interesting that exercise elevated the expression of NT-3 in both of these studies, suggesting that exercise can affect injured tissue as well as tissue distant from the injury site. It is intriguing that blocking BDNF maintained NT-3 mRNA to near control levels in spinal cord hemisected rats. It is even more intriguing that blocking BDNF abolished the effects of exercise on NT-3 elevation. These observations may reflect a compensatory mechanism, as it is known that BDNF interacts with NT-3 for their own expression regulation (Ullal et al., 2007). It is known that in spite of the high affinity of trkB receptors for BDNF, these receptors can also be activated by NT-3. It is also significant that trkB signalling can contribute to NT-3 activation. A study performed on axotomized adult rat rubrospinal neurons showed that application of BDNF in conjunction with NT-3 had a more potent effect on neuronal survival than administration of either one alone. (Novikova et al., 2000).

Conclusions

Our results show that a spinal cord hemisection lesion reduced locomotor performance in rats, and that voluntary exercise counteracted this impairment in locomotor ability. In addition, blocking the BDNF action with a specific inhibitor abrogated the action of exercise in ameliorating the behavioral deficit. These results are consistent with BDNF playing an active role in mediating the improvement in stepping that occurs with appropriate rehabilitative training after a SCI. It is, however, crucial to determine how the action of BDNF interfaces with other factors and molecular signaling mechanisms activated by exercise to have a comprehensive outlook of the effects of exercise-induced BDNF on functional recovery.

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Abbreviations

BDNF	brain-derived neurotrophic factor
CNS	central nervous system
CREB	cyclic AMP response element binding protein
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
SCI	spinal cord injury
UNG	uracil glycosylase

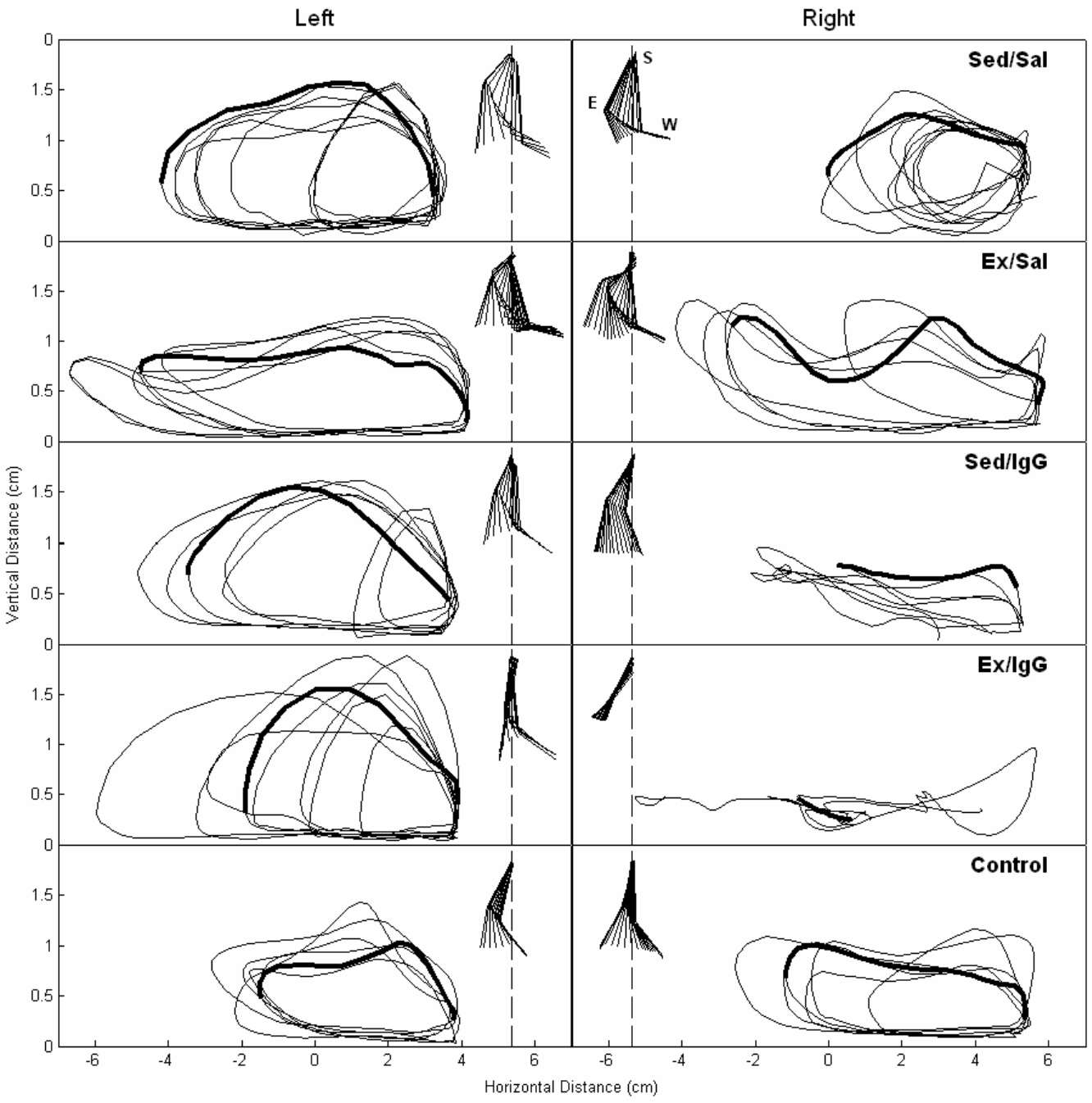

Figure 1.

Examples of the trajectory of the ipsilateral (right) and contralateral (left) forelimbs during stepping on a treadmill at the maximum speed for a representative rat in each group are shown. Note that the highest level of asymmetry is present in the rats in the two groups receiving the BDNF blocker. Stick figures of the swing phase of the shoulder (S), elbow (E) and wrist (W) of the left and right forelimb for one-step cycle for one rat from each group is illustrated in the middle columns of the figure. The step cycle represented in the stick figure from the multiple steps illustrated as wrist trajectories is marked with a bold line. The displacement values represent absolute positions in space, but the multiple trajectories were transposed so that the beginning of the stance phase was positioned consistently relative to the other step cycles.

Therefore, the absolute vertical distance of the trajectory only approximates the relative distance of the wrist to the surface of the treadmill belt. Group abbreviations are the same as in Fig. 1.

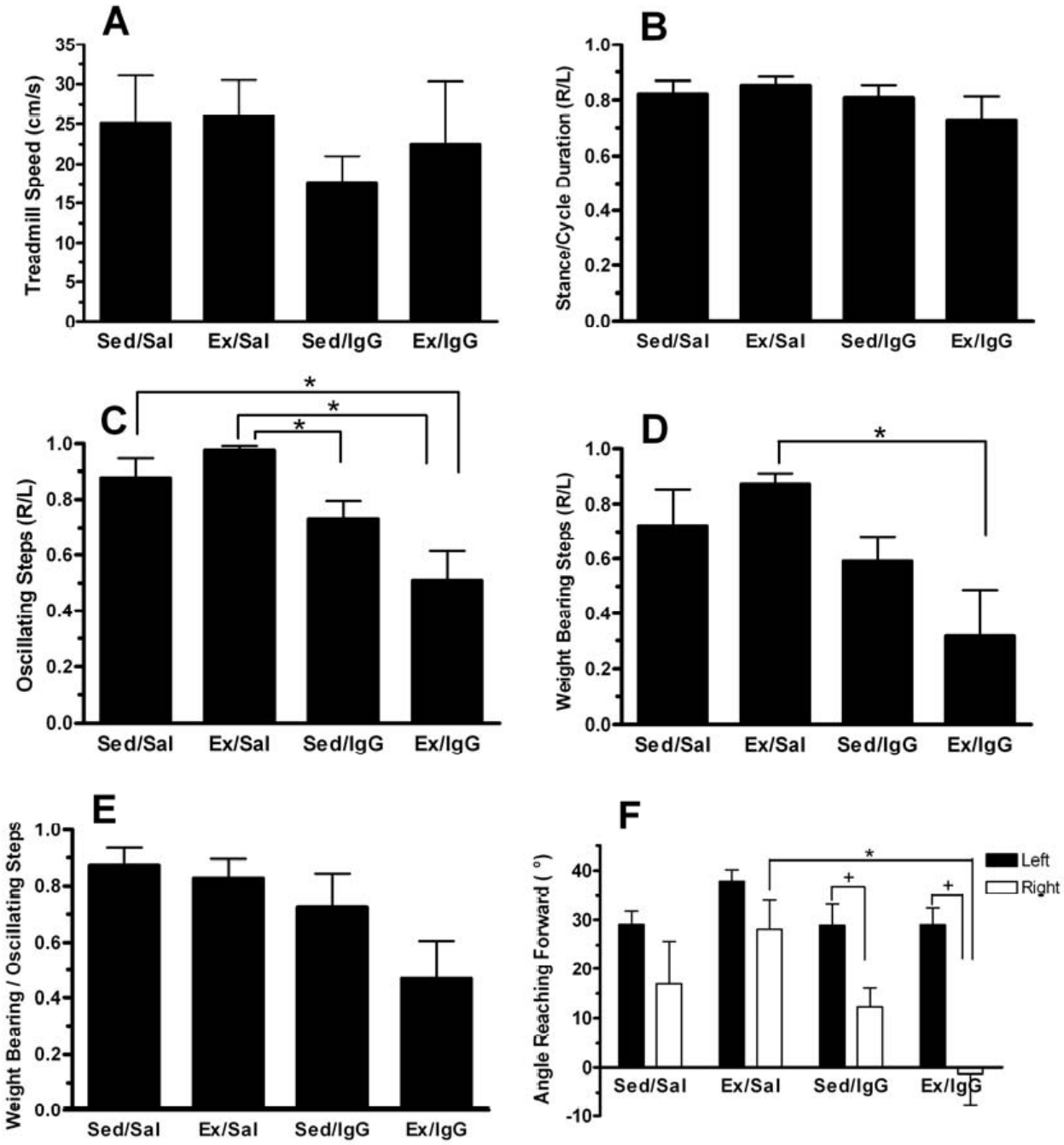

Figure 2.

The measures of locomotor performance are shown for each hemisected group: (A) maximum speed of treadmill locomotion; (B) ratio of percent stance per step cycle; (C) ratio of oscillating steps (weight-bearing plus non-weight bearing steps); (D) ratio of weight-bearing steps; (E) proportion of weight-bearing steps on the hemisected side (right side); and (F) the distance that the wrist was extended beyond the vertical line through the shoulder during the swing phase of the step cycle for both the right and left forelimbs. All ratios are expressed as right limb (R, ipsilateral to the hemisection)/left limb (L, contralateral to the hemisection). Sed, sedentary; Sal, saline, Ex, exercise: IgG, BDNF blocker. Values are mean ± SEM. * and +, significant group difference and right-left difference, respectively, at $P < 0.05$.

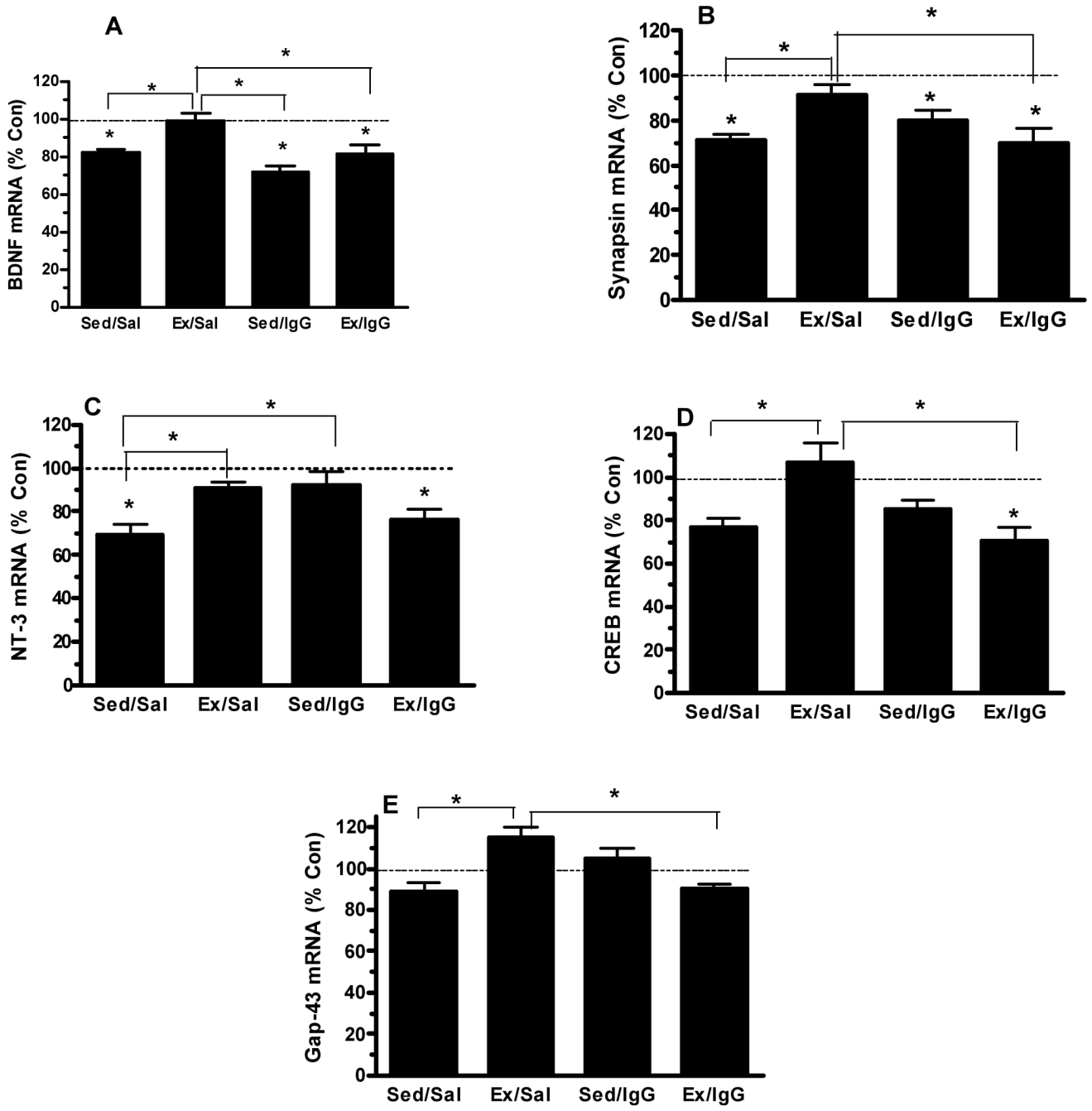

Figure 3.

Relative levels of mRNAs for BDNF (A), synapsin I (B), NT-3 (C), CREB (D), and GAP-43 (E) in the cervical region of the ipsilateral hemicord in sedentary (Sed/Sal, Sed/IgG) and exercised (Ex/Sal, Ex/IgG) hemisected rats that received saline or trkB IgG treatment. Data are presented as a percent of sedentary intact control rats. mRNA levels were measured using realtime RT-PCR and corrected for equivalent levels of total mRNA using a GAPDH mRNA probe in the same assay solution. Values are means ± SEM for 5-8 rats/group. * $P < 0.05$.

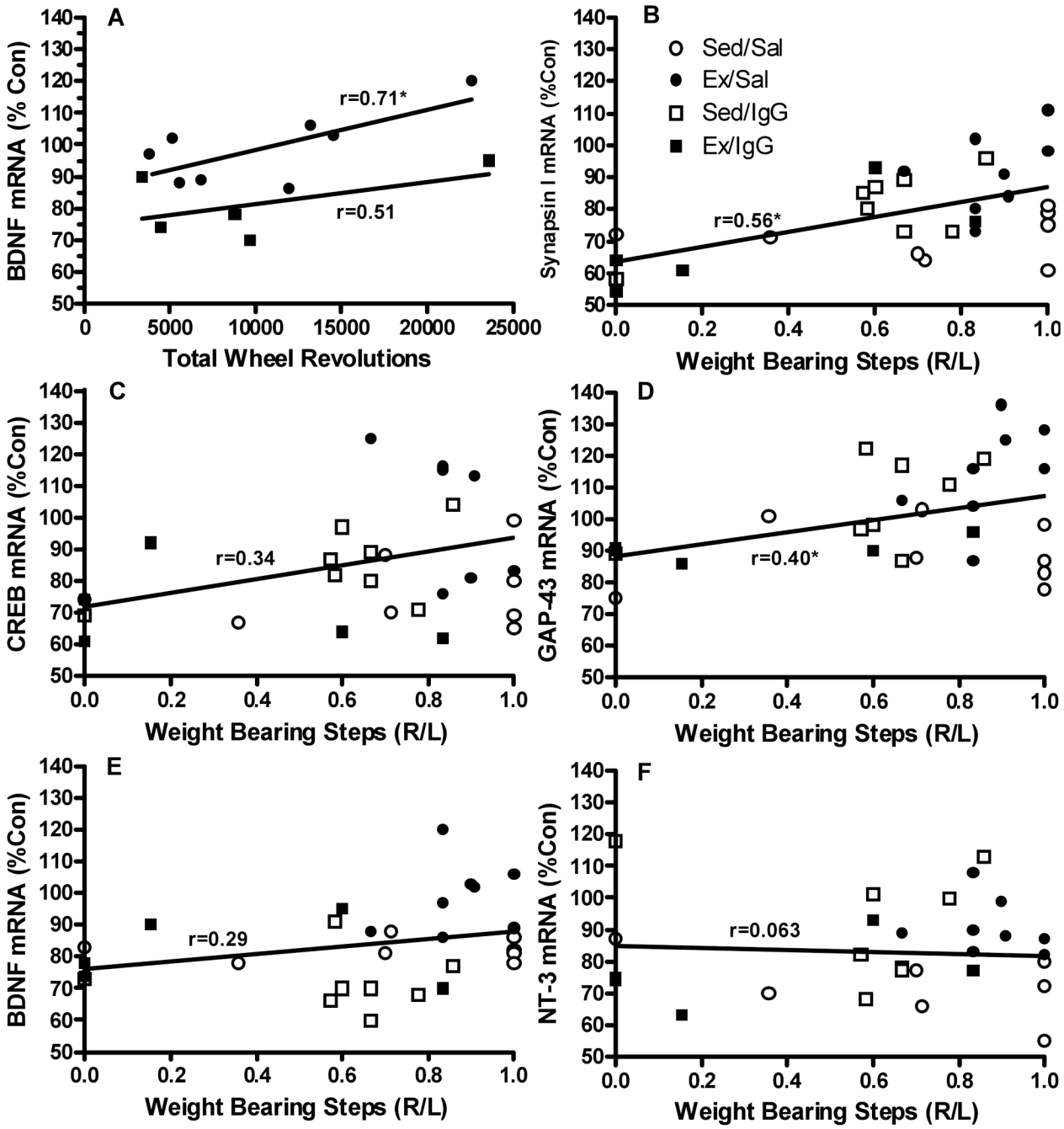

Figure 4.

The relationship between BDNF mRNA levels and total wheel revolutions for each rat in the Ex/Sal and Ex/IgG groups is shown in (**A**). The relationships between the levels of Synapsin I, CREB, GAP-43, BDNF and NT-3 mRNA levels and the ratio (ipsilateral/contralateral to the hemisection) of weight-bearing steps are shown in (B), (C), (D), (E), and (F), respectively. Group abbreviations are the same as in Fig. 1. * $p < 0.05$.