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VOLUNTARY EXERCISE INCREASES OLIGODENDROGENESIS IN SPINAL CORD

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Abstract

Exercise has been shown to increase hippocampal neurogenesis, but the effects of exercise on oligodendrocyte generation have not yet been reported. In this study, we evaluated the hypothesis that voluntary exercise may affect neurogenesis, and more in particular, oligodendrogenesis, in the thoracic segment of the intact spinal cord of adult nestin-GFP transgenic mice. Voluntary exercise for 7 and 14 days increased nestin-GFP expression around the ependymal area. In addition, voluntary exercise for 7 days significantly increased nestin-GFP expression in both the white and gray matter of the thoracic segment of the intact spinal cord, whereas, 14 days-exercise decreased nestin-GFP expression. Markers for immature oligodendrocytes (Transferrin and CNPase) were significantly increased after 7 days of voluntary exercise. These results suggest that voluntary exercise positively influences oligodendrogenesis in the intact spinal cord, emphasizing the beneficial effect of voluntary exercise as a possible co-treatment for spinal cord injury.

Keywords

exercise; nestin; neurogenesis; oligodendrocyte

INTRODUCTION

It is now widely established that the adult mammalian central nervous system (CNS) retains the ability of producing neural progenitor cells (NPC), an important feature in view of the continuous neural plasticity. The adult brain can display regenerative potential (Rao, 1999, Kulbatski et al., 2007). Proliferation, differentiation and survival of these NPC are regulated by many kinds of neurotrophic factors, which in turn, can be modulated by exercise (Reynolds and Weiss, 1992; Morshead et al., 1994; Craig et al., 1996; Weiss et al., 1996; Kuhn et al., 1997; Tropepe et al., 1997).

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The beneficial effects of exercise on the brain and spinal cord have been extensively investigated and recognized (for review see: Ang and Gomez-Pinilla, 2007). Voluntary exercise increases neurogenesis in the adult rodent brain (van Praag et al., 1999a, b; Munehiro et al., 2006). It was also established that the beneficial effects of exercise are mediated by increased levels of the trophic factors brain derived neurotrophic factor (BDNF), insulin-like growth factor-I (IGF-I) and vascular endothelial growth factor (VEGF) (Gomez-Pinilla et al., 2001, 2002, 2007; Skup et al., 2002; Ying et al., 2003, 2005). Trophic factors, such as BDNF or IGF-I, drive and support NPC as well as oligodendrocytes (OL) development. In the adult spinal cord, NPC may preferentially give rise to OL and radial glia (Cotman and Gerchtold, 2002; Perreau et al., 2005; Kublatski et al., 2007).

OL are glial cells that intermingle with neurons in the CNS and form myelin sheaths (de Castro and Brian, 2005). In the adult spinal cords, oligodendrocyte progenitors (OLP) can still be generated without differences in the rate of division or the persistence of dividing cells in the dorsal, lateral and ventral regions (Horner et al., 2000). Undetectable baseline levels of neurogenesis and oligodendrogenesis were reported in the intact adult spinal cord (Engesser-Cesar et al., 2007), suggesting the spinal cord has a very limited regenerative ability. The goal of the present study was to examine how voluntary exercise may influence neurogenesis in the intact spinal cord with a particular focus on the OLP lineage. In our work, we used voluntary exercise as a paradigm that closely applies to the human behavior and benefit (Dunn et al., 1996; Droste et al., 2003; Ghiani et al., 2007). We now show that voluntary exercise increases neurogenesis around the ependymal area in a time-dependent manner, and in particular increased immature OL in the intact spinal cord.

MATERIALS AND METHODS

Experimental Groups

Six month-old nestin-GFP transgenic mice were used in this study with an average weight of 27.53 ± 3.39 g. The nestin-GFP transgenic mice (Yamaguchi et al., 2000) are bred at UCLA in a restricted access, temperature-controlled vivarium on a 12 h light/dark cycle, food and water *ad libitum*. The animals were randomly assigned to three groups Sedentary (Sed, $n=9$), Exercise for 7 days (Ex 7 days, $n=9$) and Exercise for 14 days (Ex 14 days, $n=9$). They were used because NPCs can be easily identified, since nestin is a marker for NPCs and had been shown to be up-regulated after exercise in the hippocampus. Exercised mice were placed in cages equipped with running wheel (diameter 11.5 cm). On average, the minimum distance run by each animal was at least 3 km per day. The sedentary mice were left undisturbed in standard cages. The number of wheel revolutions per hour was recorded using VitalViewer Data Acquisition System software (Mini Mitter Inc., Sunriver, OR).

Immunohistochemistry

Deeply anesthetized animals were perfused with 4% paraformaldehyde and the spinal cords were rapidly removed. After post fixing overnight in 4% paraformaldehyde, tissue was cryopreserved in 30% sucrose. The thoracic levels (T12) of the spinal cord were sectioned (20 μ m) on a cryostat (Micron) places on the glass slides and dried for 1 h on a warmer.

Double immunofluorescence was performed as previously described (Espinosa et al., 2006). Briefly, after blocking for 1 h at room temperature with 10% NGS in PBS, primary antibodies (Table 1) were incubated overnight at 4 °C. After rinsing, appropriate secondary antibodies (Table 1) were incubated for 1 h at room temperature to visualize the four primary antibodies mentioned above. Serial images stained sections were examined and photographed using the LSM-510 META confocal microscope (Zeiss)

Cell Counts

For cell counting, cross-sections at the thoracic (T12) level of the spinal cord were divided in 6 areas as shown in figure 1A: A, dorsal funiculus; B and C, left and right sides of lateral funiculus, respectively; D, ventral funiculus; E, dorsal horn; F, ventral horn. The number of cells positive for Tf, CNPase, nestin-GFP expressing cells or both double labeled nestin-GFP/Tf and nestin-GFP/CNPase were counted from each of the six areas shown in figure 1A from four consecutive sections.

Assessment of Nestin expression

We examined the co-localization of nestin-GFP/nestin antibody and the effects of exercise on nestin-GFP expression around the ependymal area by using an indirect quantitative method recently devised in our laboratory. We use this method when cell counting is not possible because the cell markers do not clearly define the cell body or cells are in clusters, where individual cells cannot be distinguished. Images were acquired by a Zeiss LSM-510 META confocal microscope. We used the specific program from confocal microscope to present the total intensity (pixel) of each image (101250 pixels per image). The intensity (pixel) of nestin-GFP around the ependymal area from each image was detected and converted to percent intensity compare to the total intensity.

The intensity of nestin-GFP and nestin antibody was detected by confocal microscope. The intensity of nestin-GFP was switched to percent co-expression compare to the nestin antibody. Ten images of the ependymal canal were taken every two slices; whilst for analysis of co-localization for nestin-GFP/nestin, image for six different fields (A to F; Fig 1A) from four consecutive sections per animal were analyzed.

Western blot Analysis

Western blot analysis of NG2 (1:500), Nestin (1:1000), MBP (1:1000), GFAP (1:1000) and β III-tubulin (1:10000) expression was performed as previously described (Ghiani et al., 2007). Appropriate secondary antibodies (HRP-conjugated goat anti-mouse and goat anti-rabbit; Cell Signaling Technology, Denver, MA) were used at a dilution of 1:3000. See table 1 for detailed information on the primary antibodies.

Statistical Analysis

Mean values of percent nestin-GFP/nestin co-localization were presented as bar graphs. Results from cells count, protein level and quantitative nestin-GFP expression were presented as bar graphs. The data were expressed as mean $\pm$ standard deviation (SD). Statistical analysis for cells count and nestin-GFP expression was performed by One-way analysis of variance (ANOVA) following by Tukey's test using Statpage software (*StatPage.net*). Protein levels were analyzed by One-way ANOVA, followed by Tukey's test using Prism 5 Program (GraphPad Software, Inc. San Diego, CA). The level of significance was chosen as $p < 0.05$.

RESULTS

The nestin-GFP transgenic mice express GFP under the control of the nestin promoter, since nestin is a marker for NPC, this model allowed us to clearly identify changes in the NPC population. As expected, nestin-GFP expressing cells in the spinal cord were co-labeled by a nestin specific antibody in both sedentary and exercised animals (Fig 1B and 3A). Hence, the nestin-GFP mouse is a good model to evaluate neurogenesis/oligodendrogenesis.

Voluntary Exercise Induces a Time-Dependent Increase in Nestin-GFP Expressing Cells in the Ependymal Area

Nestin-GFP expressing cells were found around the spinal cord ependymal layer (Fig 1C–E) in sedentary animals. The expression of nestin-GFP in this area was significantly increased by exercise in a time-dependent fashion (Fig 3B). Furthermore, the total number of nestin-GFP expressing cells was significantly increased by 7 days-exercise in other areas of the spinal cord compared to sedentary mice. However, opposite to what was seen in the ependymal canal, the total number of nestin-GFP expressing cells in the remaining areas of the spinal cord was decreased by 14 days-exercise (Fig 2A–C and 3C). The highest number of nestin-GFP expressing cells was found in the dorsal funiculus and dorsal horn after 7 days-exercise, but, no significant difference were found between sedentary and animals exercised for 14 days (Fig 3D). In addition, we found that the protein levels of nestin displayed non-statistically significant increase after exercise for 7 and 14 days (24% and 37.5 % over sedentary animals, respectively) (data not shown). These results suggest that voluntary exercise promotes generation of NPCs in the adult intact spinal cord.

Voluntary Exercise Increased Immature OL Generation in the Intact Spinal Cord

To evaluate how voluntary exercise may impact the OL population, we investigated the expression levels of NG2, a marker for OLP, in the spinal cord of both sedentary and exercised-animals. By Western Blot, NG2 levels were significantly increased (124% over sedentary animals) in the thoracic segment of the spinal cord after 7 days-exercise (Fig 4A–B) but not after 14 days.

To further characterize this neural cell population, we determined the number of cells positive for two markers for immature OL, transferrin (Tf) and CNPase, as well as the number of cells co-expressing nestin-GFP and Tf, or nestin-GFP and CNPase. The total number of nestin-GFP/Tf co-expressing cells was significantly increased by exercise in a time-dependent fashion (Fig 2D–F and 3E). This effect was prominent in the white matter areas. No significant differences in the number of nestin-GFP/Tf positive cells were found in the dorsal funiculus, ventral funiculus and dorsal horn of the thoracic segment of the intact spinal cord between sedentary and 7 days-exercise animals, whereas a significant increase was observed at 14 days. Only the ventral part of the gray matter displayed a time-dependent increase in the expression of nestin-GFP/Tf positive cells (data not shown).

The total number of Tf positive cells was significantly increased by exercise in a time-dependent fashion (Fig 3C). The Tf positive cells were mostly localized in the white matter. The lateral funiculus areas displayed the highest increase in the number of Tf positive cells, followed by the dorsal funiculus and ventral funiculus areas, respectively (data not shown). Furthermore, the cells co-expressing nestin-GFP/CNPase displayed an increase after 7 days-exercise followed by a decrease at 14 days-exercise (Fig 1F–H and 3F). Interestingly, in the ventral horn and ventral funiculus areas, the number of nestin-GFP/CNPase positive cells was increased also after 14 days-exercise (data not shown).

The total number of cells that stained positive for CNPase was significantly increased in the thoracic segment of the spinal cord of animals that exercise for 7 days compared to sedentary animals (Fig 3C). On the other hand, the number of CNPase positive cells in the spinal cord of animals that exercised for 14 days was not significantly different from sedentary animal. When we compared different areas within the white matter, the highest number of CNPase positive cells after 7 days-exercise was found in the lateral funiculus areas, followed by the ventral and dorsal funiculus areas, respectively. Since voluntary exercise increased the expression of markers for OLP and immature OL, we sought to determine if also the expression of mature OL markers such as myelin basic protein (MBP) was modified. As shown in Fig 4,

no differences were seen in MBP protein levels in the spinal cord of sedentary and exercised mice. These findings suggest that exercise positively modulates oligodendrogenesis.

Voluntary Exercise Increased Neuronal and Astrocytic Markers

To further characterize the effects of exercise on neural cell populations, we analysed by Western Blot the expression levels of β III-tubulin, and GFAP, markers for neurons and astrocytes, respectively. The protein levels of both markers (Fig 4A–B) were significantly increase by 14 days-exercise in the thoracic segment of the spinal cord as compared to sedentary animals.

DISCUSSION

In this study, we took advantage of the nestin-GFP transgenic mice to investigate the effects of exercise on neurogenesis in the adult spinal cord, an area in which no detectable baseline levels of neurogenesis or oligodendrogenesis have been reported (Engesser-Cesar et al., 2007). Our data provide evidence that voluntary exercise promotes the generation of cells that belong to the OL lineage in the adult intact spinal cord. We showed that voluntary exercise increased nestin-GFP expression in the areas surrounding the ependymal canal as well as the expression of markers for immature OL in the intact spinal cord in a time-dependent manner.

Regulation of Neurogenesis by Voluntary Exercise in the Adult Spinal Cord

The intact spinal cord has potential for neurogenesis as suggested by the presence of nestin-GFP expressing cells around the ependymal area of the spinal cord of sedentary animals. The co-expression of nestin-GFP and nestin protein (Fig 1B), detected by a specific antibody, confirmed that the nestin-GFP expressing cells in these animals are indeed nestin positive cells, which represent NPC/OLP.

Here we report that voluntary exercise significantly increased nestin-GFP expression in the intact spinal cord after exercise for 7 days but not after 14 days, suggesting that 7 days-exercise is a suitable time point to drive cell differentiation in the spinal cord. In agreement, previous studies reported that long-term voluntary running for 28 days reduced cell proliferation in the subgranular zone of the dentate gyrus (Kronenberg et al., 2003, 2006; Naylor et al., 2005). We evaluated the effects of voluntary exercise on neurogenesis at two time points (7 and 14 days) showing that voluntary exercise for 14 days decreased nestin-GFP expressing cells in the spinal cord to levels lower than in sedentary mice.

Currently, it is not yet clear which molecular mechanisms underlie the effects of voluntary exercise on adult neurogenesis in the intact spinal cord. However it could be speculated that exercise effects are modulated by the reported increase in trophic factors, such as, BDNF or FGF-2, known to be involved in regulating neurogenesis in the brain (Neeper et al., 1995, 1996; Aberg et al., 2000; Gomez-Pinilla et al., 1997, 2001, 2002, 2007; Engesser-Cesar et al., 2007; Ghiani et al., 2007). Another possible mechanism could be increased survival of neural cells that would, otherwise, be eliminated by apoptotic cell death (Biebl et al., 2000; Kronenberg et al., 2003). Exercise-elicited pro-survival effects on proliferating precursor cells in the CNS could be explained by the well described increase in trophic factors, such as BDNF and IGF-I, which accompanies exercise effects (Ghiani et al., 2007). For instance, it was shown that IGF-I and VEGF combine both mitogenic and survival-promoting effects *in vivo* (Aberg et al., 2003; Carro et al., 2000, 2001; Jin et al., 2002; Kronenberg et al., 2006). Therefore, it could be possible that increased neurotrophic support elicited by voluntary exercise, plays a crucial role in promoting and supporting the described increase in NPC in the spinal cord.

The effect of these neurotrophic factors on neural cell development have been extensively described (McMorris and Dubois-Dalcq, 1988; Richardson et al., 1988; McKinnon et al., 1990; Baron-Van et al., 1991; Yeh et al., 1991; Carson et al., 1993; Gomez-Pinilla et al., 1997, ²⁰⁰⁷; Calver et al., 1998; Fruttiger et al., 1999; Aberg et al., 2000; Carro et al., 2001; Skup et al., 2002; Bansal et al., 2003; Hsieh et al., 2004; Gibney and McDermott, 2007). For instance it is well established that IGF-I, as well as other neurotrophic factors increased by exercise, play key roles in OL development. Hence, it is possible that the exercise-induced increase in trophic factors plays a role in promoting oligodendrogenesis also in the spinal cord (McTigue et al., 1998; Scarisbrick et al., 2000; Skup et al., 2002).

To our knowledge, this is the first study to report that the ependymal area of the adult spinal cord can be stimulated to produce NPCs by voluntary wheel running, previously reported to promote neurogenesis in the hippocampus (van Praag et al., 1999a, b; Engesser-Cesar et al., 2007). Interestingly, the ependymal area responded to voluntary exercise differently from the other areas (Fig 1A) of the intact spinal cord that we examined. We showed a consistent significant increase in the nestin-GFP expression in the ependymal area after both 7 days and 14 days of exercise. The different effects of voluntary exercise on the ependymal area compared to the other areas could be explained by the fact that nestin positive cells do not constitute a homogenous population *in vivo* (Filippov et al., 2003). They in fact can give rise to different cells types of named type 1, type 2a and type 2b cells. Among these cells, type 2a and type 2b were reported to be the most sensitive to exercise effect (Kronenberg et al., 2003).

It may be possible that NPCs in the ependymal area divide asymmetrically and exercise selectively increases cell proliferation of type 2a and 2b cells. Presumably, these cells would migrated out of the ependymal area and populate the gray and white matter of the spinal cord, which could explain the increase found in the first 7 days of exercise. Once these cells reach the gray and white matter, they begin to differentiate into one of the neural cell types and down-regulate nestin expression.

Regulation of Oligodendrogenesis by Voluntary Exercise in the Adult Spinal Cord

Our data provide evidence for an effect of voluntary exercise on oligodendrogenesis. It has been reported that glial progenitors are located throughout the adult spinal cord (Horner et al., 2000). In the present study, we used specific markers to identify cells developing along the OL lineage. CNPase is a marker for immature and mature OLs and is expressed in the cell body, processes and in the myelin sheath. Here we show that CNPase was highly expressed in the thoracic segment of the adult spinal cord after exercise for 7 days, and decreased by 14 days of exercise. Concurrently, Tf positive cells were also increased in a time-dependent fashion. Furthermore, the cells co-expressing nestin-GFP/CNPase or nestin-GFP/Tf were mostly found in the dorsal and ventral funiculus areas as well as in the ventral horn. Nestin-GFP/Tf co-expressing cells were also found to be increased in a time-dependent way in the lateral funiculus areas.

Our results strongly suggest that the adult intact spinal cord has the potential to generate OLs and this process can be enhanced by voluntary exercise. It has been demonstrated that the outer border of the spinal cord is a common area for cell division, which leads to the formation of new OLs in normal adult spinal cords (Horner et al., 2000). In agreement, here we reported that immature OL were present both dorsally and ventrally in the outer circumference of the spinal cord in exercised-mice compared to sedentary mice. It is possible that immature OL migrating out of their site of origin follow motor fibers to reach the ventro-dorsal regions (Vick et al., 1992; Miller, 1996). Voluntary exercise, by stimulating the motor fibers and circuits in the spinal cord, may be the cause of the increase in immature OL in the border of the ventro-dorsal areas. However, the mechanism of these results is still unclear.

Regulation of Astrogliosis by Voluntary Exercise in the Adult Spinal Cord

We also reported that exercise promoted an increase in GFAP protein levels in the intact spinal cord. This increase could be also mediated by the increase in neurotrophic factor such as IGF-I and VEGF which were shown to affect astrocytes differentiation in the spinal cord (Ding et al., 2004; Li et al, 2005).

In conclusion, our data strongly suggest that voluntary exercise increases nestin-GFP expressing cells in a time-dependent way. In addition, voluntary exercise appears to drive the commitment of NPCs to the OL lineage, as shown by the increased number of cells co-expressing nestin-GFP/Tf or nestin-GFP/CNPase. These effects are likely mediated by the exercise-induced increase in neurotrophic factors. Our findings have a therapeutic potential as they will contribute to unravel the cellular mechanisms by which exercise may help to enhance the intrinsic potential for regeneration of the adult spinal cord.

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References

- Aberg MA, Aberg ND, Hedbacker H, Oscarsson J, Eriksson PS. Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. *J Neurosci* 2000;20(8):2896–2903. [PubMed: 10751442]
- Aberg MA, Aberg ND, Palmer TD, Alborn AM, Carlsson-Skewir C, Bang P, Rosengren LE, Olsson T, Gage FH, Eriksson PS. IGF-I has a direct proliferative effect in adult hippocampal progenitor cells. *Mol Cell Neurosci* 2003;24(1):23–40. [PubMed: 14550766]
- Ang ET, Gomez-Pinilla F. Potential therapeutic effects of exercise to the brain. *Curr Med Chem* 2007;14(24):2564–2571. [PubMed: 17979709]
- Bansal R, Magge S, Winkler S. Specific inhibitor of FGF receptor signaling: FGF-2-mediated effects on proliferation, differentiation, and MAPK activation are inhibited by PD173074 in oligodendrocyte-lineage cells. *J Neurosci Res* 2003;74(4):486–493. [PubMed: 14598292]
- Baron-Van EA, Olichon-Berthe C, Kowalski A, Visciano G, Van Obberghen E. Expression of IGF-I and insulin receptor genes in the rat central nervous system: a developmental, regional and cellular analysis. *J Neurosci Res* 1991;28(2):244–253. [PubMed: 1851850]
- Biebl M, Copper CM, Winkler J, Kuhn HG. Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain. *Neurosci Lett* 2000;291(1):333–340.
- Calver AR, Hall AC, Yu WP, Walsh FS, Heath JK, Betsholtz C, Richardson WD. Oligodendrocyte population dynamics and the role of PDGF *in vivo*. *Neuron* 1998;20(5):869–882. [PubMed: 9620692]
- Carro E, Nunez A, Busiguina S, Torres-Aleman I. Circulating insulin-like growth factor I mediates effects of exercise on the brain. *J Neurosci* 2000;20(8):2926–2933. [PubMed: 10751445]
- Carro E, Trejo JL, Busiguina S, Torres-Aleman I. Circulation insulin-like growth factor I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy. *J Neurosci* 2001;21(15):5678–5684. [PubMed: 11466439]
- Carson MJ, Behringer RR, Brinster RL, McMorris FA. Insulin-like growth factor I increases brain growth and central nervous system myelination in transgenic mice. *Neuron* 1993;10(4):729–740. [PubMed: 8386530]
- Cotman CW, Gerchtold NC. Exercise: a behavioral intervention to enhance brain health and plasticity. *Trends Neurosci* 2002;25(6):295–301. [PubMed: 12086747]

- Craig CG, Tropepe V, Morshead CM, Reynolds BA, Weiss S, van der Kooy D. In vivo growth factor expansion of endogenous subependymal neural precursor cell populations in the adult mouse brain. *J Neurosci* 1996;16(8):2649–2658. [PubMed: 8786441]
- de Castro F, Brian A. The molecular orchestra of the migration of oligodendrocyte precursors during development. *J Brain Res Rev* 2005;49(2):227–241.
- Ding YH, Luan X, Li J, Rafols JA, Guthikonda M, Diaz FG, Ding Y. Exercise-induced overexpression of angiogenic factors and reduction of ischemia/reperfusion injury in stroke. *Curr Neurovasc Res* 2004;1(5):411–420. [PubMed: 16181089]
- Droste SK, Gesing A, Ulbricht S, Muller MB, Linthorst AC, Teul JM. Effects of long-term voluntary exercise on the mouse hypothalamic-pituitary-adrenocortical axis. *Endocrinology* 2003;144(7):3012–3023. [PubMed: 12810557]
- Dunn AL, Reigle TG, Youngstedt SD, Armstrong RB, Dishman RK. Brain norepinephrine and metabolites after treadmill training and wheel running in rats. *Med Sci Sports Exerc* 1996;28(2):204–209.
- Engesser-Cesar C, Anderson AJ, Cotman CW. Wheel running and fluoxetine antidepressant treatment have differential effects in the hippocampus and the spinal cord. *Neuroscience* 2007;144(3):1033–1044. [PubMed: 17137724]
- Espinosa-Jeffrey A, Zhao P, Awosika W, Wo N, Macias F, Cepeda C, Levine M, de Vellis J. Activation, proliferation and commitment of endogenous stem/progenitor cells to the oligodendrocyte lineage by TS1 in a rat model of dysmyelination. *Dev Neurosci* 2006;28(6):488–498. [PubMed: 17028426]
- Filippov V, Kronenberg G, Pivneva T, Reuter K, Steiner B, Wang LP, Yamaguchi M, Kettenmann H, Kempermann G. Subpopulation of nestin-expressing progenitor cells in the adult murine hippocampus shows electrophysiological and morphological characteristics of astrocytes. *Mol Cell Neurosci* 2003;23(3):373–382. [PubMed: 12837622]
- Fruttiger M, Karlsson L, Hall AC, Abramsson A, Calver AR, Bostrom H, Willetts K, Bertold CH, Heath JK, Betsholtz C, Richardson WD. Defective oligodendrocyte development and severe hypomyelination in PDGF-A knockout mice. *Development* 1999;126(3):457–467. [PubMed: 9876175]
- Ghiani CA, Ying Z, de Vellis J, Gomez-Pinilla F. Exercise decreases myelin-associated glycoprotein expression in the spinal cord and positively modulates neuronal growth. *Glia* 2007;55(9):966–975. [PubMed: 17497667]
- Gibney SM, McDermott KW. Differentiation of oligodendrocytes in neurospheres derived from embryonic rat brain using growth and differentiation factors. *J Neurosci Res* 2007;85(9):1912–1920. [PubMed: 17526011]
- Gomez-Pinilla F, Daw L, So V. Physical exercise induces FGF-2 and its mRNA in the hippocampus. *Brain Research* 1997;764(1–2):1–8. [PubMed: 9295187]
- Gomez-Pinilla F, Ying Z, Opaza P, Roy RR, Edgerton VR. Differential regulation by exercise of BDNF and NT-3 in rat spinal cord and skeletal muscle. *Eur J Neurosci* 2001;13(6):1078–1084. [PubMed: 11285004]
- Gomez-Pinilla F, Ying Z, Roy RR, Molteni R, Edgerton VR. Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. *J Neurophysiol* 2002;88(5):2187–2195. [PubMed: 12424260]
- Gomez-Pinilla F, Huie JR, Ying Z, Ferguson AR, Crown ED, Baumbauer KM, Edgerton VR, Grau JW. BDNF and learning: evidence that instrumental training promotes learning within the spinal cord by up-regulating BDNF expression. *Neuroscience* 2007;148(4):893–906. [PubMed: 17719180]
- Horner PJ, Power AE, Kempermann G, Kuhn HG, Palmer TD, Winkler J, Thal LJ, Gage FH. Proliferation and differentiation of progenitor cells throughout the intact adult rat spinal cord. *J Neurosci* 2000;20(6):2218–2228. [PubMed: 10704497]
- Hsieh J, Bimone JB, Kaspar BK, Kuwabara T, Nakashima K, Gage FH. IGF-I instructs multipotent adult neural progenitor cells to become oligodendrocytes. *J Cell Biol* 2004;164(1):111–122. [PubMed: 14709544]
- Jin K, Zhu Y, Sun Y, Mao XO, Xie L, Greenberg DA. Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. *Proc Natl Acad Sci U S A* 2002;99(18):11946–11950.

- Kronenberg G, Reuter K, Steiner B, Brandt MD, Jessbetger S, Yamaguchi M, Kempermann G. Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. *J Comp Neurol* 2003;467(4):455–463. [PubMed: 14624480]
- Kronenberg G, Bick-Sander A, Bunk E, Wolf C, Ehninger D, Kempermann G. Physical exercise prevents age-related decline in precursor cell activity in the mouse dentate gyrus. *Neurobiol Aging* 2006;27(10):1505–1513.
- Kuhn HG, Winkler J, Kempermann G, Thal LJ, Gage FH. Epidermal growth factor and fibroblast growth factor-2 have different effects on neural progenitors in the adult rat brain. *J Neurosci* 1997;17(15):5820–5829. [PubMed: 9221780]
- Kulbatski I, Mothe AJ, Keating A, Hakamata Y, Kobayashi E, Tator CH. Oligodendrocytes and radial glia derived from adult rat spinal cord progenitors: Morphological and immunocytochemical characterization. *J Histochem Cytochem* 2007;55(3):209–222. [PubMed: 17101728]
- Li J, Ding YH, Rafols JA, Lai Q, McAllister JP II, Ding Y. Increased astrocyte proliferation in rats after running exercise. *Neurosci Lett* 2005;386(3):160–164. [PubMed: 16024173]
- McKinnon RD, Matsui T, Dubois-Dalcq M, Aaronson SA. FGF modulates the PDGF-driven pathway of oligodendrocyte development. *Neuron* 1990;5(5):603–614. [PubMed: 2171589]
- McMorris FA, Dubois-Dalcq M. Insulin-like growth factor I promotes cell proliferation and oligodendroglial commitment in rat glial progenitor cells developing in vitro. *J Neurosci Res* 1998;21(2–4):199–209. [PubMed: 3216421]
- McTigue DM, Horner PJ, Stokes BT, Gage FH. Neurotrophin-3 and brain-derived neurotrophic factor induce oligodendrocyte proliferation and myelination of regenerating axons in the contused adult rat spinal cord. *J Neurosci* 1998;18(14):5354–5365. [PubMed: 9651218]
- Miller RH. Oligodendrocyte origins. *Trends Neurosci* 1996;19(3):92–96. [PubMed: 9054062]
- Morshead CM, Reynolds BA, Craig CG, McBurney MW, Staines WA, Morassutti D, Weiss S, van der Kooy D. Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron* 1994;13(5):1071–1082. [PubMed: 7946346]
- Munehiro U, Minenori I, Katsuya K, Mitsuhiro M. Effects of chronic treadmill running on neurogenesis in the dentate gyrus of the hippocampus of adult rat. *J Brain Res* 2006;1104(1):64–72.
- Naylor AS, Persson AI, Eriksson PS, Jonsdottir IH, Thorlin T. Extended voluntary running inhibits exercise-induced adult hippocampal progenitor proliferation in spontaneously hypertensive rat. *J Neurophysiol* 2005;93(5):2406–2414. [PubMed: 15615829]
- Neeper SA, Gomez-Pinilla F, Choi J, Cotman CW. Exercise and brain neurotrophins [letter]. *Nature* 1995;373:109. [PubMed: 7816089]
- Neeper SA, Gomez-Pinilla F, Choi J, Cotman CW. Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. *Brain Res* 1996;726(1–2):49–56. [PubMed: 8836544]
- Perreau WM, Paul AA, Anderson AJ, Cotman CW. Exercise-induced gene expression changes in the rat spinal cord. *Gene Express* 2005;12(2):107–121.
- Rao MA. Multipotent and restricted precursors in the central nervous system. *Anat Rec* 1999;257(4):137–148. [PubMed: 10467245]
- Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 1992;255(5052):1707–1710. [PubMed: 1553558]
- Richardson WE, Pringle N, Mosely MJ, Westermarck B, Dubois-Dalcq M. A role of platelet-derived growth factor in normal gliogenesis in the central nervous system. *Cell* 1988;53(2):309–319. [PubMed: 2834067]
- Scarisbrick IA, Asakura K, Rodriguez M. Neurotrophin-4/5 promotes proliferation of oligodendrocyte-type-2 astrocytes (O-2A). *Brain Res Dev Brain Res* 2000;123(1):87–90.
- Skup M, Dwornik A, Macias M, Sulejczak D, Wiater M, Czarkowska-Bauch J. Long-term locomotor training up-regulates TrkB receptor-like proteins, brain-derived neurotrophic factor, and neurotrophin 4 with different topographies of expression in oligodendroglia and neurons in the spinal cord. *Exp Neuro* 2002;176(2):289–307.
- Tropepe V, Craig CG, Morshead CM, van der Kooy D. Transforming growth factor- α null and senescent mice show decreased neural progenitor cell proliferation in the forebrain subependyma. *J Neurosci* 1997;17(20):7850–7859. [PubMed: 9315905]

- van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 1999a;2(3):266–270. [PubMed: 10195220]
- van Praag H, Christie BR, Sejnowski TJ, Gage FH. Running enhances neurogenesis, learning and long-term potentiation in mice. *Proc Natl Acad Sci U S A* 1999b;96(23):13427–13431. [PubMed: 10557337]
- Vick RS, Collins JM, de Vries GH. Cell cycle and proliferation dynamics of adult rat oligodendrocytes. *J Neurosci Res* 1992;33(1):75–81. [PubMed: 1453485]
- Weiss S, Dunne C, Hewson J, Wohl C, Wheatley M, Peterson AC, Reynolds BA. Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. *J Neurosci* 1996;16(23):7599–7609. [PubMed: 8922416]
- Yamaguchi M, Saito H, Suzuki M, Mori K. Visualization of neurogenesis in the central nervous system using nestin promoter-GFP transgenic mice. *Neuroreport* 2000;11(9):1991–1996. [PubMed: 10884058]
- Yeh HJ, Ruit KG, Wang YX, Parks WC, Snider WD, Deuel TF. PDGF A-chain gene is expressed by mammalian neurons during development and in maturity. *Cell* 1991;64(1):201–216. [PubMed: 1986866]
- Ying Z, Roy RR, Edgerton VR, Gomez-Pinilla F. Voluntary exercise increases neurotrophin-3 and its receptor TrkC in spinal cord. *Brain Res* 2003;987(1):93–99. [PubMed: 14499950]
- Ying Z, Roy RR, Edgerton VR, Gomez-Pinilla F. Exercise restores levels of neurotrophins and synaptic plasticity following spinal cord injury. *Exp Neurol* 2005;193(2):411–419. [PubMed: 15869943]

Figure 1.

Comparative views of nestin-GFP expressing cells in spinal cord cross-section at the thoracic level (T12) from sedentary, and mice that exercised for 7 days and 14 days. **A:** Diagram of a transverse section of the spinal cord showing the six different areas used to examine nestin-GFP, nestin, Transferrin (Tf), CNPase, and co-expression of these markers. **B:** The nestin-GFP expressing cells highly co-localized with an anti-nestin specific marker. (Quantification shown in Fig 3A). **C–D:** Photomicrographs were taken at the thoracic level of the adult spinal cord of sedentary mice (C), exercised mice for 7 days (D) and 14 days (E). Exercised mice showed increased nestin-GFP expression around the ependymal area in a time-dependent way (Quantification shown in Fig 3B). **F–H:** The nestin-GFP expressing cells (green), CNPase positive cells (red) and the co-expression cells were increased after exercise for 7 days (G) and they were decreased after exercise for 14 days (H) (Quantification shown in Fig 3C and F).

Figure 2.

Nestin-GFP expressing cells in 6 different areas (Fig. 1A) in cross-section of the thoracic (T12) of the adult intact spinal cord from sedentary mice (A), exercised mice for 7 days (B) and 14 days (C). **A–C:** Nestin-GFP expression cells were increased by voluntary exercise. The 7 days exercised mice showed the highest increase of nestin-GFP expression (Quantification shown in Fig 3D). **D–F:** Nestin-GFP expressing cells (green), Tf positive cells (red) and the co-expressing cells in the dorsal funiculus area from the thoracic level of 3 groups increased in the dorsal funiculus area from the thoracic level (T12) of the adult of these 3 groups. The more exercise the more expression of Tf positive cells.

Figure 3.

A: Quantification of co-expression of nestin-GFP/nestin specific marker performed in the nestin-GFP transgenic mice from the thoracic level (T12) of adult intact spinal cord from sedentary mice and mice exercised for 7 and 14 days (see Fig. 1B). The percent co-localization in these animals were higher than 90% **B:** Quantification of nestin-GFP expressing cells in the endymal area, exercised mice showed increased nestin-GFP expressing levels in the endymal area ($p < 0.01$, see Fig. 1C–E). Bars represent means $\pm$ SD. **C:** Quantification of total number of nestin-GFP expressing cells, CNPase positive cells and Tf positive cells. The total cell counts performed in 6 different areas (Fig. 1A) of the total cross-section of spinal cord from the thoracic level (T12) of the adult spinal cord from sedentary, exercise for 7 days and 14 days. The 7 days exercised mice showed increased in the total number of nestin-GFP, CNPase and Tf expressing cells. The nestin-GFP and CNPase expressing cells were decreased after exercise for 14 days. However, Tf positive cells were increased after exercise for 14 days. (Bars represent means $\pm$ SD; $p < 0.01$). **D:** Quantification of nestin-GFP expressing cells in 6

different areas (Fig 1A) in the cross-section of the thoracic level (T12) of the adult spinal cord in 3 different groups. The dorsal funiculus showed highest expressing of nestin-GFP expressing cells after exercise for the white matter areas. The dorsal horn showed the highest expression of nestin-GFP expressing cells for the gray matter areas. **E:** Quantification of total number of co-expressed nestin-GFP and Tf, performed in 6 different areas. The co-expressed nestin-GFP and Tf cells were increased after exercise for 7 days and 14 days (Bars represent means $\pm$ SD; $p < 0.01$). **F:** Quantification of total number of co-expressed nestin-GFP and CNPase, performed in 6 different areas. The 7 days exercised mice showed an increase in co-expressed nestin-GFP and CNPase expressing cells. The nestin-GFP and CNPase co-expressing cells were decreased after exercise for 14 days. However, it was higher than sedentary mice. (Bars represent means $\pm$ SD; $p < 0.01$).

Figure 4.

Panel A Western blot analysis of neural cell proteins in the thoracic segment of the spinal cord of sedentary mice and mice exercised for 7 days and 14 days. Twenty to twenty-five μg of total protein were loaded on the gel for each sample. Two animals per group are shown. **Panel B** Histograms represent relative of NG2, GFAP, β III-tubulin and MBP determined by densitometric analysis of autoradiographs from Western blots. Values are expressed as ratios of α -tubulin and mean $\pm$ SEM of three animals. (* $p < 0.05$, ANOVA test)

Table 1

Primary and secondary antibodies.

Primary Antibodies		
1.	Anti-Nestin	Amersham Pharmacia Biotech, Piscataway, NJ
2.	Anti-Transferrin (Tf)	INRA-Physiologie de la Reproduction des Mammifères Domestiques
3.	2', 3'-cyclic nucleotide 3' phosphodiesterase (CNPase)	Chemicon International Inc., Temecula, CA
4.	Anti-NG2	
5.	Anti- β III-tubulin	Chemicon International Inc., Temecula, CA
6.	Anti-Myelin Basic Protein (MBP)	Covance, Richmond, CA DakoCytomation
7.	Anti-Glia Fibrillary Acidic Protein (GFAP)	Chemicon International Inc., Temecula, CA
Secondary Antibodies		
1.	Texas Red	Jackson Immunological Research Laboratories Inc., West Grove, PA
2.	Alexa Fluor 635\	Invitrogen, Carlsbad, CA
3.	Cy3	Jackson Immunological Research Laboratories Inc., West Grove, PA