Long-term exercise is needed to enhance synaptic plasticity in the hippocampus

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Exercise can have many benefits for the body, but it also benefits the brain by increasing neurogenesis, synaptic plasticity, and performance on learning and memory tasks. The period of exercise needed to realize the structural and functional benefits for the brain have not been well delineated, and previous studies have used periods of exercise exposure that range from as little as 3 d to up to 6 mo. In this study, we systematically evaluated the effects of differential running periods (3, 7, 14, 28, and 56 d) on both structural (cell proliferation and maturation) and functional (in vivo LTP) changes in the dentate gyrus of adult male Sprague–Dawley rats. We found that voluntary access to a running wheel for both short- and long-term periods can increase cell proliferation in the adult DG; however, increases in neurogenesis required longer term exposure to exercise. Increases in immature neurons were not observed until animals had been running for a minimum of 14 d. Similarly, short-term periods of wheel running did not facilitate LTP in the DG of adult animals, and reliable increases in LTP were only observed with 56 d of running. These results provide us with a greater understanding of the time course of wheel running access needed to enhance DG function. Furthermore, the results indicate that the new neurons produced in response to exercise in rats do not contribute significantly to synaptic plasticity until they mature.

The brain is not a static entity; rather it can show both structural and functional changes in response to environmental and experiential demands. One example of brain plasticity is adult neurogenesis. This is the process that produces new neurons in the brain and integrates them into the existing CNS circuitry throughout the life span. All areas of the brain do not exhibit neurogenesis, but it occurs robustly in the young adult hippocampus. Here, progenitor cells create neuroblasts in the subgranular zone (SGZ) of the dentate gyrus (DG) that migrate into the granule cell layer (GCL) and differentiate into excitatory dentate granule cells (Cameron et al. 1993). These new neurons incorporate themselves into existing neuronal circuits (Gheusi and Lledo 2007) and display action potentials and functional synaptic inputs similar to DG granule cells formed early in development (van Praag et al. 2002). Moreover, adult neurogenesis may be important for hippocampal-dependent learning tasks (Gould et al. 1999; Kempermann and Gage 2002) and can facilitate synaptic plasticity (van Praag et al. 1999b).

Synaptic plasticity refers to the process by which neurons alter their ability to communicate with one another. Enhancing synaptic efficacy, also known as long-term potentiation (LTP), is largely dependent upon kinase activation and protein synthesis, and serves as the primary biological mechanism for understanding how learning and memory processes operate in the brain (Bliss and Lomo 1973; Bliss and Collingridge 1993). LTP can be induced in the DG by stimulating the perforant path projections from the entorhinal cortex to the DG granule cells (Bliss and Lomo 1973). It has been shown that adult-generated DG granule cells receive normal excitatory input from afferents of the perforant path (van Praag et al. 2002), indicating that adult neurogenesis produces neurons that function normally and integrate into hippocampal circuitry. Interestingly, adult-born neurons have a lower threshold for the induction of LTP (Snyder et al. 2001), suggesting that this phenomenon may increase plasticity in the DG and enhance the acquisition of new memories (van Praag et al. 1999b; Kempermann 2008).

Voluntary exercise can facilitate both structural and functional plasticity and enhances cell proliferation and neurogenesis (van Praag et al. 1999a,b), synaptic plasticity (van Praag et al. 1999b; Farmer et al. 2004; Kronenberg et al. 2006; Liu et al. 2011; Titterness et al. 2011), and spatial learning (Fordyce and Wehner 1993) in both rats and mice. Because new neurons may play an important role in DG LTP and thus learning and memory (for review, see Deng et al. 2010), it is critical to understand the time course of exercise required to reliably produce benefits for both structural and functional plasticity. The aim of this study was to systematically evaluate the effects of different running periods on both structural (cell proliferation and maturation) and functional (in vivo LTP) changes in the DG of adult male Sprague–Dawley rats.
Exercise, neurogenesis, and synaptic plasticity

Table 1. Weight data for control vs. exercising animals

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<th>7 d</th>
<th>14 d</th>
<th>28 d</th>
<th>56 d</th>
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<td>Control</td>
<td>353.0 ± 2.6</td>
<td>387.6 ± 8.1</td>
<td>468.3 ± 20.8</td>
<td>456.3 ± 12.6</td>
<td>591.0 ± 26.8</td>
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<tr>
<td>Runner</td>
<td>338.6 ± 5.6</td>
<td>378.8 ± 6.5</td>
<td>404.0 ± 7.7</td>
<td>443.6 ± 8.3</td>
<td>520.4 ± 17.8**</td>
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(∗∗) P < 0.01 compared to 56-d control animals.

Results

Weight data

A two-way ANOVA for age and group (control or runner) was used to evaluate the weights of the animals at the time of sacrifice. As expected, there was a significant main effect of age, with older animals weighing considerably more than younger animals (F(4,15) = 74.51, P < 0.0001), see Table 1. There was also a main effect of group (F(1,15) = 16.3, P < 0.001) and an interaction between age and group (F(4,75) = 2.61, P < 0.05). Tukey’s post-hoc analysis revealed that animals given access to a running wheel for 56 d weighed significantly less than control animals that were sedentary for 56 d (P < 0.01).

Running distance

The distance run per day (in kilometers per animal per day) increased considerably the longer the animal was given access to the running wheel (F(2,25) = 5.67, P = 0.001) (Fig. 1). After 28 and 56 d, there was more than a 100% increase in running distance per day compared to animals that ran for only 3 d (28 d, P < 0.01; 56 d, P < 0.05).

Effect of exercise on cell proliferation and immature neuron numbers

To investigate whether exercise affected the actual number of neuronal precursors (and hence volume) in the GCL, we performed cross-sectional area measurements to estimate the total volume of the GCL of the DG using Cavalieri’s principle. No main effects of condition (runner or control) or days were observed (F(3,37), P = 0.719) for total GCL volumes. Consequently, it was not necessary to convert total cell counts to cell densities for further analysis.

Exercise significantly increased cell proliferation after 3 d of running (P < 0.05), 7 d of running (P < 0.05), and 28 d of running (P < 0.01) (Fig. 2). Although a trend toward an increase in proliferation was observed after 14 d of running, this did not reach significance (P = 0.09).

Wheel running access for 3 or 7 d did not significantly increase the number of immature neurons as assessed by NeuroD staining (P = 0.69 and P = 0.24, respectively) (Fig. 3). However, longer periods of access to the running wheel significantly increased the number of immature neurons in the DG, 14 d (P < 0.01) and 28 d (P < 0.05).

Effect of exercise on synaptic plasticity

To determine whether there is a link between the amount of exercise (i.e., number of days of running) and the degree of synaptic plasticity in the DG, we examined LTP in vivo in animals from all running time periods along with their age-matched controls. LTP in the DG of sedentary control animals was 27.3 ± 2.5% 1 h following weak theta stimulation (Fig. 4). A one-way ANOVA revealed a significant main effect of running (F(5,70) = 2.80, P < 0.05) and a one-way Dunnett post-hoc test (with the assumption that runners would have higher LTP than controls) revealed that 56 d of voluntary running wheel access significantly increased LTP compared to control animals (P < 0.05). LTP in the DG of the 56-d running group was 37.1 ± 3.5% 1 h following weak theta burst stimulation (Fig. 4E).

Running for shorter periods (3–28 d) did not significantly increase LTP beyond control levels. It is possible that weak theta burst stimulation (100 Hz) is below the threshold for induction of changes in synaptic efficacy with these shorter time periods of running. To evaluate this hypothesis, strong theta burst stimulation (400 Hz) was used to induce LTP in animals that had access to voluntary running for 14 d. Under control conditions LTP was 30.3 ± 2.8% 1 h after strong theta burst stimulation and 14 d of voluntary exercise did not lead to increased synaptic efficacy in adult rats (LTP = 34.7 ± 1.7%, F(4,14) = 1.44, P = 0.27).

Discussion

Both short- and long-term exercise increase cell proliferation

Exercise increased cell proliferation (Fig. 2), and this was observed after just 3 d of voluntary exercise, and at all time points following. These results are in accordance with previous literature that has shown that the effect of exercise on proliferation begins at 3 d (Ferreira et al. 2011) and also occurs with longer periods of running (Farmer et al. 2004; Eadie et al. 2005).

Exercise increases the number of immature neurons in the dentate gyrus

NeuroD expression was significantly increased after 14 and 28 d of running. These results were expected due to the time course of neuronal differentiation—the progression between proliferating cells and neuronal differentiation takes between 7 and 14 d (Duan et al. 2008), thus explaining why increases in the presence of immature neurons were not observed after just 3 and 7 d of running. These results are comparable to those of Kronenberg et al. (2006) who also saw increases in neuronal differentiation using the marker doublecortin after 10 and 32 d of running, but not after 3 d of running. As with cell proliferation, the percent increase in the number of immature neurons was similar between animals that had access to the running wheel for 14 d and those that had access for 28 d (data not shown).
Figure 2. The effect of running on the number of proliferating cells in the granule cell layer of the DG. (A) Cell proliferation was examined using the endogenous marker Ki-67. Running for as little as 3 d was enough to increase cell proliferation and increases in proliferation were observed with all periods of running examined. (* P < 0.05, ** P < 0.01. Representative micrographs from a control (B, C) and runner (D, E) immunolabeled for Ki-67. Magnification, 4× (B, D) or 40× (C, E). Bars, 100 μm (B, D) or 10 μm (C, E).

Although the positive effect of voluntary running was still observed after 28 d, the overall NeuroD expression observed in both the nonrunners and runners was significantly reduced compared to the amount of NeuroD expression observed at earlier time points. This effect was unexpected and the exact reason for this decrease is unclear, particularly because the same trend was not observed in proliferation levels (Ki-67). Proliferation and neuronal differentiation decrease with age (Gil-Mohapel et al. 2013), with a major decrease occurring at 3 mo of age in mice. The rats that ran for 28 d were 3 mo of age at the time of sacrifice so it is possible that these overall decreases are due to the natural aging process.

Exercise and synaptic plasticity
Voluntary exercise for up to 28 d was unable to increase LTP induction in adult male rats. However, when access to the running wheel was increased to 56 d enhanced LTPs were observed. This is in contrast to other studies, including previous studies from our laboratory, that have shown increases in LTP following voluntary exercise paradigms lasting for as little as 7 d (Farmer et al. 2004; Christie et al. 2008; Liu et al. 2011). A further study has shown that 1 mo of running increases DG LTP, enhances acquisition of the Morris water maze task, and increases proliferation (measured by BrdU labeling) in mice (van Praag et al. 1999b). Although in our study we observed increases in proliferation similar to the BrdU results in the study of van Praag et al. (1999b), we could not replicate the increases in LTP. Farmer et al. (2004) have also shown that exercise can increase synaptic plasticity through enhanced NMDA-dependent LTP in rats, and similar increases in DG LTP have been shown in vitro in mice (van Praag et al. 1999b; Vasuta et al. 2007) and rats (Christie et al. 2005), and in the CA1 region in mice (Duffy et al. 2001), so a species difference could not be the cause of the differences in effects that we observe. However, Farmer et al. (2004) observed no LTP in control animals with 100-Hz stimulation and only observed a 27% increase in LTP in control animals with 400-Hz stimulation. In our study, 100 Hz was used to induce LTP and under these conditions we observed 27.3% LTP in controls (i.e., nonrunners), which is much higher than that reported by Farmer et al. (2004). In the present study, using one time point (14 d of exercise) 400-Hz stimulation was employed and this stimulation paradigm led to only a small additional increase in LTP (30.3% LTP in controls with 400-Hz stimulation, data not shown). One possibility for the lack of additional benefit using this more intense stimulation paradigm is that it may lead to a saturation effect. LTP requires cooperative interaction between afferent fibers, and at very high stimulation intensities maximum potentiation is achieved. This results in a saturated system where increased high-frequency stimulation (HFS) cannot elicit additional LTP (Johnston and Wu 1995). On the flip side, it is also important to be aware that a stimulus threshold must be reached for the induction of LTP and running is thought to enhance LTP by lowering this threshold (Farmer et al. 2004; Titterness et al. 2011). In our study, using 100 Hz to induce LTP, we would not expect this relatively mild intensity to have reached saturation, and thus mask the effects of running. In our study voluntary exercise could have, in fact, lowered the threshold for LTP, but at 100-Hz stimulation, but nonrunners were able to overcome their threshold and reach similar levels of potentiation. However, Titterness et al. (2011) found that exercise enhanced LTP in adolescent male rats, but not in female adolescent rats, regardless of their threshold and reach similar levels of potentiation. However, Titterness et al. (2011) found that exercise enhanced LTP in adolescent male rats, but not in female adolescent rats, regardless of their threshold and reach similar levels of potentiation. However, Titterness et al. (2011) found that exercise enhanced LTP in adolescent male rats, but not in female adolescent rats, regardless of their threshold and reach similar levels of potentiation.

As discussed above, proliferating cells take ~2 wk to differentiate into a neuronal phenotype and it is not until this time that the neurons begin to receive functional inputs from the perforant path (van Praag et al. 2002; Ge et al. 2006). Furthermore, it is between 4 and 8 wk following the birth of new neurons when enhanced synaptic plasticity is observed (Schmidt-Hieber et al. 2004; Ge et al. 2007). If we assume that the new neurons produced

Figure 3. The effect of running on neuronal differentiation in the granule cell layer of the DG. (A) Neuronal differentiation was examined using the endogenous marker NeuroD. Neuronal differentiation increased after 14 and 28 d of running, but increases were not observed at earlier time points. (* P < 0.05, ** P < 0.01. Representative micrographs from a control (B, C) and runner (D, E) immunolabeled for NeuroD. Magnification, 4× (B, D) or 40× (C, E). Bars, 100 μm (B, D) or 10 μm (C, E).
from exercise are contributing to the LTP measured in the DG in this study, then perhaps the reason we do not see any increases in LTP until 56 d of running is because the new neurons are not mature enough to contribute to LTP at the earlier time points. When animals have been running for 56 d (8 wk), the cells that began to proliferate at the beginning of the running period would be fully mature and showing enhanced synaptic plasticity at 56 d, which may explain why increases were only observed at this time point. Another factor that should be considered is dendritic morphology. Exercise is known to enhance the length and complexity of immature neurons in the subgranular zone and may also influence the dendritic arbors of mature neurons in the DG (Eadie et al. 2005; Redila and Christie 2006; Stranahan et al. 2007). Although these changes are observed with as little as 2–3 wk of running (Eadie et al. 2005; Redila and Christie 2006), they persist with 2 mo of running (Stranahan et al. 2007). In future studies, the time course of exercise-induced changes in dendritic morphology would be interesting to examine as these changes may correlate with changes in LTP better than changes in basal neurogenesis, as shown in the current study.

Disparity between the ability of exercise to enhance neurogenesis but not LTP

New neurons may play a role in learning and memory. Learning has been shown to selectively increase hippocampal neurogenesis in a hippocampal-dependent Morris water maze task (Gould et al. 1999; Kempermann and Gage 2002), but not all hippocampal-dependent learning and memory tasks have shown this. Furthermore, ablating neurogenesis using manipulations such as pharmacological agents, radiation, or genetic models have shown varying results as to whether neurogenesis is needed for learning and memory (Shors et al. 2001, 2002). It may be that hippocampal neurogenesis is only involved in some forms of learning and memory. This may explain why we see robust increases in proliferation and neuronal differentiation with short periods of running but no changes in LTP induction in our animals until 56 d of running. While the neurogenesis observed may be important for certain aspects of learning and memory, these increases may not be able to be measured through inducing NMDA-dependent LTP in the DG as we did in this study. Additional behavioral tests should be examined in these animals following different periods of exercise in order to examine the exact learning and memory processes the new neurons could be contributing to, but this is outside the realm of this study.

Concluding remarks

We have demonstrated that both short- and long-term exercise increase cell proliferation and neuronal differentiation in the adult DG. Increases in cell proliferation are significant after only 3 d of running, whereas increases in neuronal differentiation are not observed until at least 2 wk of running. In our hands, exercise did not increase the capacity of LTP induced in the DG until animals had been running for 56 d, which indicates that the new neurons produced in response to exercise are not contributing to synaptic processes in this area until well after they are mature. This disparity could indicate that enhancements in neurogenesis are improving a different aspect of learning and memory that is not measurable by the DG LTP examined in this study.

Figure 4. The effect of running on LTP in the DG measured using in vivo electrophysiology. Periods of exercise ranging from 3 to 28 d did not increase LTP in the DG (A–D). Fifty-six days of exercise was enough to significantly increase the amount of LTP generated in the DG 60-min post-TBS (E). (F) LTP summary graph with averages of the last 5 min of recording (55- to 60-min post-stimulation). LTP is significantly enhanced compared to nonrunning control animals in animals that run for 56 d. (*) P < 0.05.
Materials and Methods

All animal procedures were performed in accordance with the University of Victoria and Canadian Council for Animal Care policies. Male Sprague-Dawley rats were obtained from Charles River Laboratories (St. Constant, Canada) and housed in a colony room at the University of Victoria Animal Care Unit. Animals were ordered to be postnatal day (PND) 42 on the day of arrival. Animals were housed in pairs in clear polycarbonate cages (46 × 24 × 20 cm) with Carefresh contact bedding (Absorption Corp.). The room was maintained on a 12-h light–dark cycle with constant humidity and temperature (22°C). Animals underwent an acclimation period in the unit of 18 d (until they reached PND 60). At PND 60, subjects were assigned to a cage that either contained a running wheel (runners) or a cage that did not (nonrunners). In either case, subjects remained in their respective cages for 3, 7, 14, 28, or 56 d.

Voluntary exercise

When animals reached PND 60, they were assigned to the different experimental groups (runner or nonrunner). Animals assigned to the running group were given free access to a running wheel while the sedentary controls were left undisturbed during the same period. The running wheels were connected to a PC computer and the running distance was constantly recorded at 1-min intervals using VitalView Software (mini Mitter). The total distance ran over the period of access to the running wheel (3, 7, 14, 28, or 56 d) and was calculated and expressed as kilometers per animal per day. Half the animals in each group and at each time point were used for electrophysiological studies and half were used for immunohistochemical studies.

Sacrifice and tissue processing

Animals used for immunohistochemistry were sacrificed immediately after being removed from the running wheels (control animals were sacrificed following the running group) by transcardial perfusion. Animals were deeply anesthetized with urethane (250 mg/mL in water; 1.5 g/kg body weight i.p.) and then perfused with 0.9% NaCl (in Tris-buffered saline [TBS]) followed by 4% paraformaldehyde (PFA) in PBS. The animal was then decapitated and the brain removed and placed in 4% PFA overnight at 4°C. The following day, brains were transferred to a 30% sucrose solution of increasing concentrations (50%, 70%, 95%, 100%) followed by 0.25% Triton X-100 and incubated with a biotin-conjugated horse anti-goat IgG secondary antibody (1:200; BA-9500, Vector Laboratories). The slides were coverslipped immediately after incubation using a Permount medium (Fisher Scientific).

A further series of brain sections was used for evaluation of early neuronal differentiation by examining the NeuroD protein, a helix–loop–helix transcription factor that is expressed during the early stages of neuronal differentiation (Brunet and Ghysen 1999; Miyata et al. 1999). Sections were quenched in 3% hydrogen peroxide for 15 min at room temperature and then washed three times in 0.1 M TBS. Sections were blocked in 5% normal horse serum and incubated with a goat polyclonal primary antibody against NeuroD (1:200; SC-1084, Santa Cruz Biotechnology) for 48 h at 4°C. Sections were rinsed in 0.1 M TBS and further incubated with a biotin-conjugated horse anti-goat IgG secondary antibody (1:200; BA-9500, Vector Laboratories) in 5% blocking solution at room temperature for 2 h. The bound antibodies were visualized using the methods described above.

Quantification of stained cells

All morphological analyses were performed on coded slides, with the experimenter blinded, using an Olympus BX51 microscope equipped with 10 ×, 40 ×, and 100 × objectives. Image Pro-Plus software (version 6.0 for Windows, Media Cybernetic Inc.) and a Cool Snap HQ camera (Photometrics) were used for image capture. The number of Ki-67 and NeuroD-immunopositive cells present in the SGZ of the DG were quantified by manually counting all DAB-positive cells. DAB-positive cells that appeared outside of 2–3 nuclear diameters of the SGZ were not included in analysis.

In vivo electrophysiology

Animals were anesthetized with urethane (1.5 mg/kg) intraperitoneally and placed on a Kopf stereotactic apparatus. Body temperature was maintained at 37 ± 0.5°C throughout the experiment with a grounded homeothermic temperature control unit (Harvard Instruments). Extracellular field potentials were recorded by inserting a 125-μm stainless-steel recording electrode into the hilus of the DG (3.5 mm anterior, 2.0 mm lateral to bregma) and a 125-μm monopolar stimulating electrode into the ipsilateral medial perforant path (7.4 mm anterior, 4.2 mm lateral to bregma) (Paxinos and Watson 2007). A ground electrode was placed posterior to λ, and a reference electrode was placed anterior to bregma. Stimulating and recording electrodes were lowered to elicit a maximal response and the stimulation required to induce a 1–2 mV population spike was determined (dorsal-ventral [DV] coordinates between 2.8 and 3.8 mm). Basal recordings were first obtained by administering a square pulse (0.12-msec duration) at 0.067 Hz. Once a stable baseline was observed for at least 15 min, LTP was induced by applying theta burst stimulation consisting of 10 bursts of five pulses at 100 Hz with an inter-burst interval of 200 msec which was repeated four times at 30-sec intervals. In one cohort of animals strong theta burst stimulation (400 Hz) was applied. The pulse duration was changed to 0.25 msec during stimulation. Following theta burst stimulation, baseline stimulation resumed for 60 min as described previously (Titterness and Christie 2012).

Signals from the DG were collected using custom software (Lee Campbell; Getting Instruments). Signals were amplified (Getting Instruments), filtered (1 Hz–3 kHz), and digitized at 5 kHz.
For analysis the slope of the rising phase of the field EPSP was used to determine alterations in the level of synaptic efficacy. All EPSP slope data are presented as the mean percent change from the pre-conditioning baseline ± standard error of the mean (SEM).

Statistical analysis

Statistical analysis was performed using the Statistica 7.1 analytical software (StatSoft Inc.). All data are presented as mean ± SEM. A two-way ANOVA for time period (3, 7, 14, 28, or 56 d) and condition (control or runner) was used to examine the effect of exercise on weight gain. For neurogenesis data, independent t-tests were conducted for each time point comparing runners to nonrunners. DG area measurements were compared between groups using a factorial ANOVA for condition (runner vs. nonrunner) and time period of exercise.

For electrophysiological data, the control group was combined because the age difference across nonrunners (PND 63–116) did not lead to significant differences in LTP (F(4,140) = 0.545, P = 0.704), and a one-way ANOVA was used to examine the effect of different periods of exercise on LTP. A Dunnett posthoc test was used to compare each running group to the control group. A one-sided hypothesis was used based on previous studies (Christie et al. 2005; Titterness et al. 2011) indicating that exercise increases LTP.

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