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Research report

Cessation of voluntary wheel running increases anxiety-like behavior and impairs adult hippocampal neurogenesis in mice

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HIGHLIGHTS

- Relatively little is known regarding how reduction of physical activity affects brain function.
- Cessation of running performed during the growth period is anxiogenic.
- Cessation of running inhibited the differentiation of new cells to immature neurons.
- Reduction of physical activity is likely a risk factor for impaired hippocampal function.

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ABSTRACT

While increasing evidence demonstrates that physical exercise promotes brain health, little is known on how the reduction of physical activity affects brain function. We investigated whether the cessation of wheel running alters anxiety-like and depression-like behaviors and its impact on adult hippocampal neurogenesis in mice. Male C57BL/6 mice (4 weeks old) were assigned to one of the following groups, and housed until 21 weeks old; (1) no exercise control (noEx), housed in a standard cage; (2) exercise (Ex), housed in a running wheel cage; and (3) exercise–no exercise (Ex–noEx), housed in a running wheel cage for 8 weeks and subsequently in a standard cage. Behavioral evaluations suggested that Ex–noEx mice were more anxious compared to noEx control mice, but no differences were found in depression-like behavior. The number of BrdU-labeled surviving cells in the dentate gyrus was significantly higher in Ex but not in Ex–noEx compared with noEx, indicating that the facilitative effects of exercise on cell survival are reversible. Surprisingly, the ratio of differentiation of BrdU-positive cells to doublecortin-positive immature neurons was significantly lower in Ex–noEx compared to the other groups, suggesting that the cessation of wheel running impairs an important component of hippocampal neurogenesis in mice. These results indicate that hippocampal adaptation to physical inactivity is not simply a return to the conditions present in sedentary mice. As the impaired neurogenesis is predicted to increase a vulnerability to stress-induced mood disorders, the reduction of physical activity may contribute to a greater risk of these disorders.

1. Introduction

Exercise or physical activity is a key factor in promoting brain health and cognitive functions [1–3]. Epidemiological research demonstrates that higher levels of physical activity lessen the severity of anxiety and depression symptoms [4,5], reduce the risk of a number of neurodegenerative diseases, including Alzheimer's disease [6,7], and improve cognitive functions [8,9]. A wealth of data from animal studies reveal that exercise exerts functional and structural changes in the hippocampus, a brain area involved in learning, memory, and emotion, with these changes likely to be

Abbreviations: OFT, open field test; EPM, elevated-plus maze; FST, forced swim test; DG, dentate gyrus; DCX, doublecortin.

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responsible for many of the abovementioned positive effects of physical activity [10].

It has been consistently demonstrated that exercise increases neurogenesis [11–15] and the expression of neurotrophic factors expression [2,16–18] in the hippocampus of rodents. However, while anxiolytic and anti-depressive effects of exercise have been demonstrated [19–22], the issue still remains controversial. Indeed, there is conflicting evidence that exercise is anxiogenic [23–25]. Because the assessed outcomes of exercise intervention vary with experimental procedures, including the duration of the intervention [26,27], form and intensity of the exercise [17,28,29], and housing conditions [30–32], more controlled experiments would be required to further elucidate the effects of exercise on these emotional behaviors. From a different perspective, it is also important to understand how a reduction of physical activity might affect these emotional behaviors and the underlying brain functions.

To the best of our knowledge, there have been few experimental approaches developed specifically to investigate how the reduction of physical activity affects rodent brain function. One reason for this may be that laboratory rodents are confined to "standard" small cages, making it difficult to further decrease their level of physical activity. Forced physical inactivity by hindlimb suspension, an animal model commonly used to investigate disuse muscle atrophy, has been shown to cause anhedonia, a key depressive symptom [33], and to inhibit hippocampal neurogenesis [34]; however, hindlimb suspension is a form of stress which can itself negatively impact brain function.

In an attempt to improve our understanding of the impact of physical activity on brain function, we aimed to examine how a reduction of physical activity affects anxiety-like and depression-like behaviors in mice. We also examined adult hippocampal neurogenesis which is an intriguing feature involved in these emotional behaviors [35–37]. We employed a reverse intervention to control the animal's physical activity. Post-weaned mice were reared in a cage with a running wheel until early adulthood in order to help them acquire the long-term effects of higher physical activity during the growth period, when the hippocampus is highly plastic. Subsequently, the mice were placed in a standard laboratory cage with no running wheel and housed for a further 9 weeks in order to rule out any acute effects that may have been caused by the change of housing environment. With this intervention of interrupting exercise, daily physical activity of the mice was relatively reduced without the use of any physical restraint.

2. Materials and methods

2.1. Animals and experimental design

Forty male C57BL/6 mice (3 weeks old, just after weaning) were purchased from a commercial breeder (Harlan Labs, Barcelona, Spain). Mice were housed at 22 ± 1 °C with a 12/12 h light/dark cycle (light on at 0700), and provided food and water ad libitum. All experimental procedures were performed in accordance with European Community guidelines (directive 86/609/EEC).

After a week of initial acclimatization, mice were randomly assigned to one of the following groups (Fig. 1): no exercise control (noEx, $n = 15$), exercise (Ex, $n = 10$), and exercise–no exercise (Ex–noEx, $n = 15$). noEx mice were housed in standard plastic cages (L × W × H = 26.7 cm × 22.3 cm × 14.5 cm) throughout the experiment. Ex mice were housed in plastic cages (L × W × H = 27.9 cm × 21.6 cm × 15.2 cm) equipped with a running wheel (ENV-3046, Med Associate Inc., Georgia, USA) from the age of 4 weeks until the end of the experiment. Ex–noEx mice were housed in cages with a running wheel from 4 to 11 weeks of age then in standard cages. Consequently, the Ex–noEx mice ceased voluntary wheel running from 12 weeks old.

In all groups, because social isolation is known to increase anxiety-like and depression-like behaviors [32] and to suppress exercise-induced neurogenesis in the hippocampus [30], each cage consisted of five mice, and the groups of cage mates were not changed throughout the experiment. The number of wheel rotations was recorded each morning, and body weight (g) and food intake (g/cage) were measured each week. Behavioral tests were performed at 21 weeks of age.

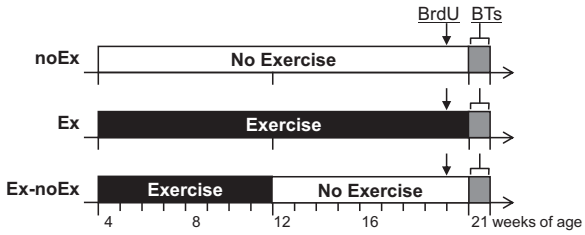

Fig. 1. Timeline of the experiment. Ex mice continued running throughout the experiment, while the Ex–noEx mice stopped running at 12 weeks of age. Mice were subjected to behavioral tests (BTs) at 21 weeks of age. BrdU was injected 2 weeks prior to sacrifice.

2.2. Behavioral tests

Behavioral tests included the open field test (OFT), elevated plus maze (EPM), and forced swim test (FST). Tests were performed in this order, reflecting the relative stressfulness of these tests. Before commencing the tests, mice were acclimated to a behavioral testing room for 2 consecutive days (60 min/day). Mice were transferred to the testing room a minimum of 30 min prior to each test session. All mice performed both the OFT and EPM. Because forced swimming is a severe stressor which could affect hippocampal neurogenesis [38], each group was further divided into two sub-groups. Two-thirds of the mice in each group performed the FST (noEx, $n = 10$; Ex, $n = 6$; Ex–noEx, $n = 10$), while the remaining one-third did not (noEx, $n = 5$; Ex, $n = 4$; Ex–noEx, $n = 5$). All tests were performed between 0900 and 1300, with a 24-h interval in between.

2.2.1. Open field test (OFT)

The OFT was performed in order to assess general locomotor activity and unconditioned anxiety-like behavior in the mice using the VersaMax Animal Activity Monitor (Accuscan Instruments, Ohio, USA). This system was equipped with an open field arena (20 cm × 20 cm) made of clear Plexiglas and horizontal and vertical beam sensors. Disruptions of the beam were recorded as activity counts. Mice were placed individually in the center of the arena, and allowed to explore for 5 min. The following behavioral parameters were analyzed: (1) horizontal activity (counts), (2) vertical activity (counts), and (3) the total time spent in the center of the arena (seconds). After each test, the arena was thoroughly cleaned with ethanol and dried to avoid olfactory cues.

2.2.2. Elevated plus maze (EPM)

Next, the EPM, a well-established paradigm for assessing unconditioned anxiety-like behavior of rodents, was performed. The apparatus was equipped with two open arms (W × L = 5 cm × 30 cm), two closed arms (W × L = 5 cm × 30 cm, with 15-cm-height walls), and a connecting central platform, which was elevated 40 cm over the floor level. Mice were individually introduced on the center facing an open arm and allowed to explore for 5 min. The behavior of the mice was video recorded, and manually evaluated on a computer screen by a blinded experimenter. The following parameters were analyzed manually: (1) open, closed, and total arm entries (times), (2) percentage of open arm entries (open arm entries/total arm entries × 100), and (3) time spent in open arm (seconds). An arm entry was defined as all four paws entering into the arm.

2.2.3. Forced swim test (FST)

Lastly, the FST, a test commonly used to assess depression-like behavior in rodents, was performed. The mice were introduced into a clear Plexiglas cylinder (12 cm in diameter, 29 cm in high) filled with water (23 ± 1 °C), for 6 min on 2 consecutive days. The behavior of the mice was video recorded, and evaluated manually on a computer screen, as has been previously described in detail [38]. Briefly, the animal's behavior was assigned to one of four categories: climbing, swimming, staying afloat, and immobile. Staying afloat was considered to be the small movements necessary to equilibrate the posture without displacement. The data are presented as time spent in immobile plus staying afloat during the last 5 min on the second day of the test. After removal from the water, mice were wiped with dry paper towels.

2.3. Histology for adult hippocampal neurogenesis

Two weeks before sacrifice, 5-bromo-2-deoxy uridine (BrdU, 50 mg/kg body weight) dissolved in saline was injected intraperitoneally three times at 3-h intervals (1000, 1300, and 1600) in order to label dividing cells. Two hours after the FST, mice were deeply anesthetized with pentobarbital and transcardially perfused with cold saline. The brain was quickly removed and post-fixed in 4% paraformaldehyde in phosphate buffer overnight. Coronal brain sections (50 μm) were obtained through the whole hippocampus using a vibratome (VT-1000s, Leica, Bensheim, Germany).

One-in-eight series of sections of each hemisphere were randomly selected for Nissl staining to estimate the total volume of the dentate granule cell layer (GCL) using the Cavalieri method [38]. Other series were used for labeling phospho-histone