Behavioural Brain Research 337 (2018) 8–16

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Exercise pattern and distance differentially affect hippocampal and cerebellar expression of FLK-1 and FLT-1 receptors in astrocytes and blood vessels

Morgan E. Stevenson, Vienna K. Behnke, Rodney A. Swain*

Department of Psychology, University of Wisconsin Milwaukee, Milwaukee, WI, USA

ARTICLE INFO

Keywords:
Angiogenesis
Astrocyte
Exercise
VEGF
Flk-1
Flt-1

ABSTRACT

Aerobic exercise benefits the body and brain. In the brain, benefits include neuroprotection and improved cognition. These exercise-induced changes are attributed in part to angiogenesis: the growth of new capillaries from preexisting vessels. One critical factor involved in the regulation of angiogenesis is VEGF and its receptors Flk-1 and Flt-1. Although exercise is generally found to be beneficial, there are wide variations in exercise regimens across experiments. This study standardized some of these variations. Rats were assigned to a voluntary or a forced wheel running exercise condition. Within each condition, animals ran for either a long (1000 m) or short distance (500 m) for up to 24 h. Additionally, one voluntary group had unrestricted access to the wheels for the full 24 h. Exercising animals were then compared to inactive controls, based on unbiased stereological quantification of Flk-1 and Flt-1 immunohistochemical labeling in the hippocampus and cerebellum. Findings indicated that voluntary exercise, but not forced exercise, could significantly increase Flk-1 and Flt-1 expression in the hippocampus. Interestingly, Flk-1 expression was elevated in astrocytes and Flt-1 in vessels. In the cerebellum long distance forced exercise resulted in the least Flk-1 expression compared to other conditions, and Flt-1 expression in exercising animals either did not change or was suppressed relative to inactive controls.

1. Introduction

Aerobic exercise produces neurovascular changes in the brain, which can be neuroprotective and support recovery from brain injuries, including ischemic stroke [1,2]. Exercise may exert these protective effects, at least in part, through increased angiogenesis: the growth of new capillaries from preexisting blood vessels [3–5]. Brain regions in which angiogenesis has been detected following exercise include the hippocampus, and motor regions such as the cerebellum, motor cortex, and basal ganglia [6–8,4]. One potent angiogenic factor is vascular endothelial growth factor (VEGF), which regulates angiogenesis in the brain and periphery throughout the lifespan, and has a critical role in exercise-induced plasticity in the brain [1,9–16,3,8].

VEGF would not exert these effects without signaling through its two high-affinity receptors: FMS-like tyrosine kinase receptor (Flt-1, also termed VEGFR1) and fetal liver kinase 1 receptor (Flk-1, also termed KDR or VEGFR2). Flt-1 regulates the initial organization of vasculature during development and may also serve as a negative regulator of the angiogenic actions of VEGF, preventing vascular overgrowth [17–19]. Flk-1 is responsible for the mitogenic effects of VEGF and has a leading role in the development of new endothelial cells [20,21]. Like VEGF, Flk-1 and Flt-1 are elevated in both the brain and periphery in response to aerobic exercise [22–24]. It is important to note that foremost, VEGF has been identified as an angiogenic factor. However, some evidence suggests that, in the brain, VEGF does not solely regulate angiogenesis, but also has more wide-ranging functions in glial cells and neurons. For example, following brain injury, VEGF signaling increases astroglial proliferation [25], and VEGF has been implicated in regulating neurogenesis in the hippocampus following brain injury and aerobic exercise [26–28].

Numerous studies report exercise promotes plasticity in the neurovascular system and provides behavioral benefits, including improved learning and memory performance and recovery of motor function following cerebral ischemia [29–31]. However, the characteristics of the exercise paradigms used across studies vary. There are two exercise patterns commonly used within the laboratory: voluntary and forced. Voluntary exercise involves unrestricted access to a running wheel located in the rodent's home cage. Forced exercise typically involves the animal being removed from the home cage and placed on a treadmill with a shock-grid to prevent the animal from stopping. Although both

* Corresponding author at: Department of Psychology, University of Wisconsin Milwaukee, 2441 East Hartford Ave., Garland 224, Milwaukee, WI 53211, USA.
E-mail address: rswain@uwm.edu (R.A. Swain).

http://dx.doi.org/10.1016/j.bbr.2017.09.037
Received 5 May 2017; Received in revised form 19 September 2017; Accepted 23 September 2017
Available online 25 September 2017
0166-4328/

patterns generate neurovascular changes to some degree, findings differ when directly compared. Some studies have found that voluntary exercise increases levels of brain-derived neurotrophic factor (BDNF), enhances the survival of newly birthed neurons in the dentate gyrus, and produces enhanced motor function recovery from cerebral ischemia compared to forced exercise and inactive controls [31,32]. In contrast, others have found that forced exercise, but not voluntary exercise, is neuroprotective and results in a smaller infarct volume [33]. Hypoxia inducible factor-1α (HIF-1α; a transcription factor for VEGF) mRNA and protein has been found to be significantly elevated in animals that engaged in forced exercise, compared to voluntary exercise and control groups [34]. In regards to neurogenesis, both voluntary and forced exercise groups displayed increased percentages of surviving neuronal progenitor cells, but the pool was larger in the forced exercise group [30]. In sum, studies investigating which pattern of exercise promotes the greatest neurovascular changes have come to differing conclusions, and the exercise paradigms used employ an array of parameters [30–35].

This study aimed to compare voluntary and forced exercise by manipulating exercise distance. Because the animal, not the experimenter, primarily regulates voluntary exercise, it is difficult to control parameters like speed and duration. Others have found it is not possible for animals to reach the rapid speeds reported during voluntary exercise in a forced exercise paradigm, and the duration of voluntary exercise sessions can greatly vary as animals take breaks at their leisure [30,36]. However, distance is one parameter that can be controlled in both voluntary and forced exercise patterns. The primary aim of this study was to manipulate the distance run by voluntary and forced exercise animals, and compare its effects on the expression of VEGF receptors Flk-1 and Flt-1 in regions of the hippocampus and cerebellar paramedian lobule. The paramedian lobule is involved in controlling limb movement [4]. Furthermore, this study aimed to quantify these differences after an acute bout of exercise. Most previous experiments have had animals run for longer durations, ranging from 1 to 8 weeks, before quantifying neurovascular changes [37,30]. However, some evidence suggests these angiogenic changes begin within hours of exercise onset [3]. Lastly, after noticing Flk-1 appeared to also display expression in non-endothelial cells, a final aim was to determine whether changes in expression of Flk-1 in exercising animals was driven by endothelial cells, astrocytes, or neurons.

VEGF and its receptors have extensive roles in neuroprotection following cerebral ischemia [38]. Understanding how different exercise parameters influence Flk-1 and Flt-1 expression could improve exercise rehabilitation programs following ischemic stroke. Presumably, the exercise that produces the most robust effects early in training would be most beneficial for patients recovering from an ischemic insult.

2. Method

2.1. Experiment 1

2.1.1. Animals

Sixty male, Long Evans hooded rats (175–200 g) were divided into six equal groups: Voluntary Exercise- Unrestricted (VX-U; $n = 10$), Voluntary Exercise- Long Distance (VX-L; $n = 10$), Voluntary Exercise- Short Distance (VX-S; $n = 10$), Forced Exercise- Long Distance (FX-L; $n = 10$), Forced Exercise- Short Distance (FX-S; $n = 10$), and Inactive Control (IC, $n = 10$). All animals were housed in standard shoebox cages ($47.82 \times 20.32 \times 22.86$ cm^3) for a one-week acclimation period prior to beginning the experiment. Animals had access to food and water ad libitum throughout the experiment. The Institutional Animal Care and Use Committee (IACUC) at the University of Wisconsin-Milwaukee approved all procedures.

2.1.2. Pre-exposure

After the one-week acclimation, all animals were pre-exposed to voluntary wheel running. For two days, animals were placed individually in cages with voluntary exercise wheels (Lab Products Inc., no longer sold; 35 cm in diameter, each revolution equates to ~1 m) for 10 min per day. After the 10 min pre-exposure, wheel revolutions were recorded and animals were returned to their home cages. The cages used for pre-exposure were the same cages used in the voluntary exercise conditions. This procedure familiarized the FX groups with wheel running, prior to engaging in forced exercise on the motorized wheel. VX and IC animals also engaged in the pre-exposure training to control for the effects of this exercise on Flk-1 and Flt-1 expression.

2.1.3. Exercise

After the two days of pre-exposure, all animals began their exercise conditions, except IC groups, which remained inactive in their home cages for 24 h. VX-U rats were moved to cages with attached wheels and allowed unrestricted access to the running wheels for the full 24 h. At the end of the 24 h period, the distance completed was recorded. VX-L and VX-S rats were also moved to the same cages with attached running wheels, but they were returned to their home cages after reaching 1000 m (long distance) and 500 m (short distance), respectively. FX-L and FX-S animals were required to run on a motorized wheel at ~9 m/min. The motorized wheel was designed in-house by attaching a belt (to the hub of the wheel) and motor to the same wheels used by VX animals. Experimenters were able to control the speed, and animals were monitored throughout the duration of the exercise. A Plexiglas door was attached at the entry of the wheel to prevent animals from escaping the wheel. FX-L and FX-S rats were returned to their home cages after reaching 1000 m and 500 m, respectively; see Table 1 for a summary of the exercise conditions.

2.1.4. Tissue preparation

Twenty-four hours after the start of exercise, all animals were sacrificed by immersion in a carbon dioxide chamber. Animals were decapitated, and brains were removed and snap frozen in chilled isopentane. Brains were stored at −80 °C until prepared for immunohistochemistry (IHC). One hemisphere per animal was randomly selected for IHC. Brains were hemisected and sectioned at 12 μm using a Leica CM 3050S cryostat (Wetzlar, Germany). The cerebrum was sectioned coronally, through the entire dorsal hippocampus starting −1.90 mm from Bregma and ending at −3.90 mm [39]. Sixteen sections per animal were randomly selected, and these sixteen sections were placed on two different slides (eight sections for Flk-1 IHC and eight sections for Flt-1 IHC). The cerebella were sectioned sagittally, through the paramedian lobule from 3.90 mm medial-lateral to 1.90 mm [39], and the collection of sections was identical to that of the hippocampi.

Before beginning IHC targeting Flk-1 (Santa Cruz Biotechnology, Dallas, TX) and Flt-1 (Santa Cruz Biotechnology, Dallas, TX), tissue was

Table 1
Summary of all exercise conditions. Forced exercise used a motorized wheel and voluntary exercise used a standard in-cage wheel. Animals in short distance groups ran 500 m and those in the long distance groups ran 1000 m. Inactive controls remained sedentary.

Conditions	Exercise Wheel	Exercise Distance (m)
Forced Exercise Short- Distance (FX-S)	Motorized	1000
Forced Exercise Long- Distance (FX-L)	Motorized	500
Voluntary Exercise Short- Distance (VX-S)	Standard, In-cage Wheel	1000
Voluntary Exercise Long-Distance (VX-L)	Standard, In-cage Wheel	500
Voluntary Exercise Unrestricted (VX-U)	Standard, In-cage Wheel	Unlimited
Inactive Control (IC)	No Wheel	0