Habituation Training Improves Locomotor Performance in a Forced Running Wheel System in Rats

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Increasing evidence supports that physical activity promotes mental health; and regular exercise may confer positive effects in neurological disorders. There is growing number of reports that requires the analysis of the impact of physical activity in animal models. Exercise in rodents can be performed under voluntary or forced conditions. The former presents the disadvantage that the volume and intensity of exercise varies from subject to subject. On the other hand, a major challenge of the forced training protocol is the low level of performance typically achieved within a given session. Thus, the aim of the present study was to evaluate the effectiveness of gradual increasing of the volume and intensity (training habituation protocol) to improve the locomotor performance in a forced running-wheel system in rats. Sprague-Dawley rats were randomly assigned to either a group that received an exercise training habituation protocol, or a control group. The locomotor performance during forced running was assessed by an incremental exercise test. The experimental results reveal that the total running time and the distance covered by habituated rats was significantly higher than in control ones. We conclude that the exercise habituation protocol improves the locomotor performance in forced running wheels.

Keywords: physical activity, exercise, rodents, familiarization protocols, acclimation protocols

INTRODUCTION

According to the World Health Organization (2010), the lack of physical activity is the fourth leading risk factor for global human mortality. For instance, reduced physical activity is associated with higher risks of developing obesity, type 2 diabetes, osteoporosis, depression and cardiovascular diseases (World Health Organization, 2010; Dishman et al., 2013; Morán-Rodríguez et al., 2016). On the other hand, there is growing evidence supporting a positive impact of increasing regular levels of physical activity on public health (Dishman et al., 2006; Hillman et al., 2008; van Praag, 2009; Vivar et al., 2012). These studies suggest that motor skill training and regular exercise are beneficial to sustaining proper executive functions of cognition and learning (e.g., motor learning in the spinal cord; Edgerton et al., 2004; Hillman et al., 2008), and in some cases it may confer protective effects against the onset of neurological disorders including Parkinson’s disease (Smith and Zigmond, 2003), Alzheimer’s dementia (Cotman and Berchtold, 2002) and stroke (Stummer et al., 1994). However, the neurobiological
Mechanisms associated with physical activity are not entirely known, partly due to a lack of uniformity and parameterization in experimental protocols employed to assess the impact of exercise in animal models. For example, rodent studies using running-wheels often employ protocols that allow ad libitum access to the wheel. While such an approach has its advantages, both the intensity and volume varies significantly from subject to subject due to the “voluntary” nature of the experimental design (van Praag et al., 2005; Kregel et al., 2006; Leasure and Jones, 2008; Creer et al., 2010; Kobilo et al., 2011; Marlett et al., 2012). One way to overcome these challenges is to implement a forced running-wheel protocol in which the same training load is applied to all subjects (Auriat et al., 2006; Shimizu and Yamanouchi, 2011; Wang et al., 2013; Chen et al., 2014). It becomes clear from these studies that the inclusion of a pretraining stage of habituation prior to the testing phase is a crucial step to achieving better performances in response to increasing running demands (Dick, 2007). Thus, the aim of the present study is to develop and evaluate a protocol of habituation training to enhance the locomotor performance of young adult rats subjected to a progressive incremental running load test in a forced running-wheel system (Bentley et al., 2007).

**MATERIALS AND METHODS**

All experimental procedures were approved by the University of Murcia’s animal care and use committee according to the Spanish regulation (Royal Decree 1201/2005) and European Union Directive 2003/65/EC of the European Parliament (Amending Council Directive 86/609/EEC) guide for care and use of laboratory animals.

**Animals and Experimental Groups**

Young adult male Sprague-Dawley rats (Laboratory Animals Facilities at the University of Murcia) were group housed (2–3 rats/cage) and kept in a 12:12 h light/dark cycle room (dark period from 8 AM to 8 PM) at 21–23°C and 55 ± 5% of relative humidity, with food and water available ad libitum. Rats were randomly assigned to receive either the protocol of habitation training (habituated) or not (non-habituated) for eight consecutive days. The locomotor performance to incremental intensities of forced running was assessed at 1, 3, 31 and 33 days post-last habituation session.

**Running-Wheel Training**

Six polycarbonate motor running-wheels were purchased from Lafayette-Campden system (80805A model, dimensions 129.54 × 45.47 × 42.93 cm). The internal surface of the running-wheel was covered with custom-made denim fabric to provide a smooth flatten running surface (Figures 1A–C). The habituation phase is comprised of 10 sessions distributed across 8 days of training. During these sessions, both intensity (speed) and volume (time) were increased following an upward progressive pattern as summarized in Figures 1D,F. Once the habituation phase is completed, rats were subjected to the first incremental exercise test 24 h after the last habituation session. In order to determine whether the habituation sessions exert an enduring impact on forced running performance, rats were tested again at 3, 31 and 33 days from the last session of habituation training. During the testing phase of incremental forced running, a defined speed of 9 m/min is introduced at the beginning of each test followed by increments of 0.9 m/min every 5 min until a failure to maintain a running pattern becomes apparent (Figure 1E). Criteria to stop the incremental test include jumping, crawling and/or rolling within the running-wheel. Typically, rats are removed from the running-wheel and the test stopped if two or more consecutive uncontrolled laps are detected. The time spent in the running wheel was determined from the beginning of the test to its termination when the animal fails to maintain a running pattern.

**Statistical Analysis**

All data were presented as mean ± standard error of the mean. A two-tailed student’s t-test was used for two-group comparison involving a single continuous variable and a one-way repeated measures analysis of variance (ANOVA) test was used for intra-subjects multiple comparison. Differences between the experimental groups were considered statistically significant at \( P < 0.05 \) (StatSoft, Tulsa, OK, USA).

**RESULTS**

Commerially available running-wheels were modified to include a custom-made denim fabric into the internal surface of the wheel to provide a smooth flatten running surface (Figures 1A–C). In order to assess the impact of habituation training, a cohort of young adult male Sprague-Dawley rats were subjected to a protocol of habituation comprised of 10 sessions in 8 days during which the intensity (speed) and the volume (time) of the training sessions were increased following an upward progressive pattern (Figures 1D–F). We observed a 100% success rate response, that is, all animals (\( n = 23 \)) tested with this training protocol managed to complete the entire 8 days of habituation running.

We next developed a progressive incremental running load test (Figure 1E) to assess the impact of habituation training on locomotor performance. Relative to the non-habituated group (\( n = 12 \)), rats that underwent the habituation training phase exhibited a six fold increase in performance to the incremental running load test as revealed by the time and distance spent in the wheel (Figure 2). While most of the animals from the non-habituated group failed to pass the first 5 min step of the incremental test, habituated rats run an average of ∼32 min covering a mean distance of ∼370 m when tested 24 h after the last habituation session (Figures 2A,B).

Finally, the incremental test was repeated to evaluate long term effects of the habituation. The locomotor performance at 3 days remains similar; however it is significantly decreased.
FIGURE 1 | (A) Forced motor wheel with aluminum bars in the running surface. (B) Running surface of the wheel covered by denim fabric. (C) Forced motor wheel system during a running session. (D) The schedule shows the exercise program developed during the habituation exercise protocol. The speed, time and number of sessions by day are described. The incremental test is developed after 24 h finished the habituation, and repeated at 3, 31 and 33 days later. (E) This graphic is a representation of time variation (X axis) in relation to the speed variation (Y axis) during the development of the incremental exercise test. Notice that every 5 min the speed changes increasing in 0.9 m/min giving the aspect of steps. (F) Bars and lines graph which represents the training load in each day (X axis) of the habituation protocol. Bars indicate time of running (Y axis, left). Line indicates speed (Y axis, right). The training load, speed and time followed an upward progressive pattern. WU, warming up phase.

both at 31 and 33 days (habituated rats run an average of ∼16/18 min covering a mean distance of ∼162/194 m; respectively; **Figures 3A,B**).

These results support that the habituation period improves the locomotor performance in forced running wheels; but this effect is progressively decreased without a permanent training.

DISCUSSION

The present study was developed to evaluate the impact of an habituation training in the locomotor performance, using a forced running wheel system in young adult rats. It is known that wild rats cover long distances during the night, running bursts of short periods at high speeds (Tchernichovski and Benjamini, 1998). A similar pattern of running is detected in voluntary exercise laboratory paradigm, that allow rats to reach higher intensities and cover longer distances than in forced exercise; but restricting the possibility to manage intensity and volume of running (training load; Leasure and Jones, 2008). This situation highlights the difficulty of the voluntary running to do precise correlations between training load and the observed effects (van Praag et al., 2005; Leasure and Jones, 2008; Creer et al., 2010; Kobilo et al., 2011; Marllatt et al., 2012). On the other hand, in forced conditions, rats run during larger periods of time but at lower speed than observed during voluntary running (Narath et al., 2001; Leasure and Jones, 2008). Although forced models are consistent with the uniformity of the physical activity carried out for the group of rats, running on a treadmill or a motorized wheel can be a challenge for the animals. In fact, as many as 10% of the rats refuse to walk or run on a treadmill; and these animals must be removed from exercise studies (Jasperse and Laughlin, 1999; Koch and Britton, 2001; Kregel et al., 2006). Our results support that
habituation exercise training are key to get better locomotor performances to develop successfully training programs in forced conditions.

Only a few of the current works in forced running paradigm consider the implementation of an habituation phase as a good strategy to reduce the number of rats classified as nonrunners (Kregel et al., 2006; O'Dell et al., 2007; Chen et al., 2014). For this aim the animals are introduced in treadmills or motor wheels with a gradual increase in training load. This condition may be relevant to improve locomotor performance and minimize potential injuries that can occur in this new environment for the rodents. In our study a 100% of rats finished the whole running habituation period. However, the habituation protocol is absent in some studies using forced motor wheel; which maintain a constant intensity and volume from the beginning and throughout all the sessions during their programs (Clement et al., 1993; Ji et al., 2014). The intensity of the exercise developed in these works during the training protocol was between 1.22 m/min and 12 m/min; that is lower in comparison with other forced protocols that includes a progressive increment in the training load, and reaches a maximum speed of 30 m/min (Clement et al., 1993; Leasure and Jones, 2008; Chen et al., 2014; Ji et al., 2014). Some works developed habituation-like protocols, that consist in an increase of training load pattern during the entire training period. Under this conditions the highest speed reached during the training protocol was between 12 m/min and 14 m/min (Sandrow-Feinberg et al., 2009; Caton et al., 2012; Griesbach et al., 2013). However, others works that implement pretraining sessions do not report the training load details of the protocol employed, such us duration, speed or number of sessions; but reported a maximum speed of 21 m/min (O’Dell et al., 2007; Hu et al., 2010; Kennard and Woodruff-Pak, 2012). Finally, only few studies indicate all the detailed features of the habituation protocol developed; reaching the highest speed (30 m/min; Auriat et al., 2006; Shimizu and Yamanouchi, 2011; Wang et al., 2013; Chen et al., 2014). According to Chen et al. (2014), running behavior in motor wheels for rodents is more laborious than the linear motion of the treadmill, in particular in the absence of an adaptive learning stage. Our results demonstrated that habituated rats can sustain a higher forced running speed when compared to the non-habituated group. Interestingly, such locomotor improvement is transient as the forced running speed decreases progressively over time within a period of 30 days.

Several mechanisms could contribute to sustaining a higher running speed following a progressive habituation protocol to the running wheel. It is well known that physical activity produces biochemical changes that improves the muscular aerobic metabolism (Terjung and Hood, 1986). While data on how a forced running wheel affects muscular endurance are lacking, evidences from a number of studies using treadmill suggest that a 60 min/day/1 week of running at 25 m/min is needed to induce muscular endurance as determined by changes in cytochrome c, citrate synthase, 3-Ketoadic-CoA transferase (Booth and Holloszy, 1977; Terjung, 1979; Dudley et al., 1982), and myoglobin concentration (Lawrie, 1953; Pattengale and Holloszy, 1967; Terjung and Hood, 1986). In this regard, it is unlikely that 7 days of progressive augmentation in duration and intensity reaching a maximal speed of 9 m/min for 1 h during the last day is sufficient to elicit adaptive changes in muscular endurance. Another contributing factor is stress. Stress hormones are known to increase during forced exercise (Saito and Soya, 2004; Chen et al., 2016), which in turn are increased during
acute running according to exercise intensity and duration (Chennouei et al., 2002; Kawashima et al., 2004; Saito and Soya, 2004; Chen et al., 2016). Acute stress conditions promote fight-flight responses producing elevation of peripheral blood pressure and heart rate; and facilitates energy utilization (Maier et al., 1998; Moraska et al., 2000; Norris and Carr, 2013). However, our habituation protocol reaches a maximal speed of 9 m/min only during the last day for 1 h; and the evidence from a number of studies using treadmill suggest that a running speed of at least 25 m/min (just above the lactate threshold) is required to induce changes in blood lactate, plasma ACTH, plasma glucose and adrenaline (Timofeeva et al., 2003; Saito and Soya, 2004; Soya et al., 2007; Chen et al., 2016). One study showed that activation of CRH neurons in the paraventricular nucleus emerges following 1 h of acute forced running wheel (Yanagita et al., 2007), yet data showing changes in stress hormones are currently lacking.

Based on currently available literature, it is possible that a change in the dopaminergic system may contribute to enhance the forced running-wheel performance observed following the habituation training. It has been shown that pharmacological activation of the dopaminergic system is sufficient to improve motor coordination and its endurance (Tamsay et al., 1981; Freed and Yamamoto, 1985; Boldry et al., 1991; Meeusen and De Meirleir, 1995; Sutoo and Akiyama, 1996; Chen et al., 2016). Future studies are warranted to determine the role of dopamine and related catecholamines in sustaining better locomotor performance in a forced running-wheel system.

Further studies using metabolic chambers or implanted chip systems will be essential to develop the best training condition and standardized exercise training programs in rodents. A rigorous analysis of physiological variations such as VO2max, lactate threshold, heart rate or body composition, a set of data that is currently used in elite sports training in humans, is necessary to adapt the training program to the specific features of each experimental subject (Copp et al., 2009; Zhou et al., 2010).

**AUTHOR CONTRIBUTIONS**

AT and JLF: study conception and design, data collection and analysis, interpretation, drafting and revising the manuscript. RB: data collection and analysis, interpretation, drafting and revising the manuscript. EDC: study conception and design, data collection and analysis, interpretation, and revising the manuscript. NM-D: data collection and analysis, revision of the manuscript. JGP: study conception and design, data collection and analysis, revision of the manuscript. AA: data analysis, interpretation, revision of the manuscript. KYT: study conception and design, data analysis, interpretation, revision of the manuscript. All authors have approved the final manuscript version.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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