Behavioural Brain Research 282 (2015) 1–5

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

Duration- and environment-dependent effects of repeated voluntary exercise on anxiety and cued fear in mice

Sarah Dubreucq[a,b], Giovanni Marsicano[a,b], Francis Chaouloff[a,b,*]

[a] Endocannabinoids & NeuroAdaptation, NeuroCentre INSERM U862, 33077 Bordeaux, France
[b] University of Bordeaux, 33077 Bordeaux, France

HIGHLIGHTS

- Wheel running anxiolysis is accounted for by home cage housing with the wheel.
- Wheel running effects on anxiety depend on the test used to assess that behavior.
- Unlimited, but not limited, wheel running increases the duration of social contacts.
- Housing with a wheel underlies wheel-running-induced decreases in cued fear memory.

ARTICLE INFO

Article history:
Received 17 November 2014
Received in revised form
12 December 2014
Accepted 18 December 2014
Available online 26 December 2014

Keywords:
Running wheel
Running duration
Elevated plus-maze
Light/dark box
Social interaction
Freezing

ABSTRACT

Several studies have indicated that animal models of exercise, such as voluntary wheel running, might be endowed with anxiolytic properties. Using the light/dark test of unconditioned anxiety, we have reported that one confounding factor in the estimation of wheel running impacts on anxiety might be the housing condition of the sedentary controls. The present mouse study analyzed whether the aforementioned observation in the light/dark test (i) could be repeated in the elevated plus-maze and social interaction tests of unconditioned anxiety, (ii) extended to conditioned anxiety, as assessed during cued fear recall tests, and (iii) required unlimited daily access to the running wheel. Housing with a locked wheel or with a free wheel that allowed limited or unlimited running activity triggered anxiolysis in the light/dark test, but not in the elevated plus-maze test, compared to standard housing. In the social interaction test, the duration, but not the number, of social contacts was increased in mice provided unlimited (but not limited) access to a wheel, compared to standard housing or housing with a locked wheel. Lastly, freezing responses to a cue during fear recall tests indicated that the reduction in freezing observed in mice provided limited or unlimited access to the wheels was fully accounted for by housing with a wheel. Besides confirming that the housing condition of the sedentary controls might bias the estimation of the effects of wheel running on anxiety, this study further shows that this estimation is dependent on the test used to assess anxiety.

Regular physical activity bears both preventive and treatment properties on mood and cognition pathologies [1,2]. This indication has proved of major interest for neuroscientists as physical exercise might provide a useful model to uncover the biological bases of mental health disorders. Accordingly, several animal models of physical exercise have been developed, among which the running wheel model, which has gained most interest due to its volitional aspect (but see [3,4]). By this means, several studies have indicated that wheel running bears positive behavioral impacts, including anxiolysis [5,6]. At first glance, this observation supports the translational usefulness of wheel running to elucidate the central mechanisms through which human exercise bears anxiolytic properties. However, the latter proposal suffers several limits, the first being the inability of several laboratories to observe running-induced anxiolysis [5,6]. Actually, wheel-running has even been reported to promote anxiety in two different studies from the same group [7,8]. In keeping with the aforementioned translational interest for this animal model of exercise, there is a crucial need to understand why wheel running might bear differential effects on anxiety. Actually, such a divergence may lie on several bases, among which the species, strain and housing conditions (isolated, collective), the frequency and duration of wheel running, and the

* Corresponding author at: Endocannabinoids & NeuroAdaptation, NeuroCentre INSERM U862, 146 rue Léo Saignat, 33077 Bordeaux, France. Tel.: +33 557573755; fax: +33 557573669.
E-mail address: francis.chaouloff@inserm.fr (F. Chaouloff).

http://dx.doi.org/10.1016/j.bbr.2014.12.042
0166-4328/

nature of the tests that were performed to evaluate anxiety. An additional variable that might have a major impact is the level of environmental enrichment under which the sedentary controls are housed. Despite its potential importance, this issue has been however only poorly addressed [9]. Thus, a majority of studies have used sedentary groups of animals that were housed in cages devoid of any enrichment material, as opposed to studies wherein sedentary animals were provided running wheels that were locked as to prevent running activity. Besides providing the most appropriate paradigm to study the intrinsic impact of running, it is worthy of mention that the presence of a wheel, even locked, enriches the living environment with noticeable consequences. Thus, the running-induced increase in proliferation during the hippocampal neo-neurogenic process was found to be accounted for by the presence of the running wheel rather than by running activity per se [10]. In this context, we have reported one series of observations in the light/dark test of unconditioned anxiety suggesting that mice housed with running wheels, albeit displaying anxiolysis compared to mice housed in standard cages, did not differ from mice housed with locked wheels [9]. This result suggested that the presence of the wheel, rather than wheel-running per se, was responsible for anxiolysis. Interestingly, these observations extended to conditioned anxiety, as assessed by freezing behavior during recall sessions of contextual fear [9]. However, the possibility remains that the housing condition might have not impacted on anxiety if other tests of unconditioned/conditioned anxiety had been used. Furthermore, this study, as do most studies using the running wheel model of exercise, allowed animals to run throughout their daily period of activity. This observation raises in turn the question of the impact of wheel running on anxiety if animals are provided shorter access (i.e. few hours) to the wheels as to further approach, if possible, exercise conditions in humans.

The aim of this study was thus to investigate the respective impacts of the above mentioned sedentary housing conditions and daily wheel running activity (unlimited or limited to 3 h during the active period) on three tests of unconditioned anxiety, namely the elevated plus-maze, the light/dark test, and the social interaction test [11,12]. In keeping with the aforementioned control group-dependent effects of wheel running on contextual fear [9], we further investigated whether this differential impact of wheel-running extended to cued (auditory) fear.

This study involved 4 series of experiments with 8-weeks old C57BL/6NCrl mice purchased from Janvier (Le Genest Saint-Isle, France). For each series of experiments, mice were housed individually without (standard cages) or with a running wheel either locked permanently, unlocked for 3 h/day (see below) or unlocked permanently ($n = 4$–6 mice/housing condition/series of experiments). The cages, including the standard ones, were 36.5 cm long × 20.7 cm wide × 14 cm high. All wheels (23 cm diameter; Intellibio, Nomeny, France) were connected to a computer that recorded the running distances and durations, and which allowed to block the wheels whenever needed [9]. The mice were provided food and water ad libitum under a 12 h–12 h light/dark cycle with lights on from 07:00 AM to 07:00 PM. Mice that were provided limited access to the wheels could run for a 3-h daily period that began 5 h after lights turned off and ended at 03:00 AM (i.e. in the middle of the active period). These specific time and duration intervals were chosen based on prior evidence for its positive influence on hippocampal neurogenesis [13], one hallmark of physical exercise [14]. All experiments were conducted in strict compliance with the European directive 2010/63/EU and French laws on animal experimentation (authorization number 06369 to F.C.).

The animals were left under their respective housing conditions for 3 weeks before being tested successively for their unconditioned anxiety levels in an elevated plus-maze, a light/dark box, and an open field allowing social interaction measurements. These tests were respectively performed 22, 23 and 26 days after the initial assignment to each housing condition (see above). The day after social interaction tests, mice were then cued fear conditioned before being tested in a fear recall session the following day. All tests were performed between 01:00 PM and 05:00 PM in rooms adjacent to the housing room. For unconditioned anxiety tests, all behaviors were recorded using a video-camera placed above the respective apparatus. Anxiety and fear-related behaviors were analyzed using a customized EVENTLOG program.

The elevated plus-maze, made of black Perspex, consisted in four elevated arms (height: 66 cm) 45-cm long and 10-cm wide (Letica, Barcelona, Spain). The arms were arranged in a cross-like disposition, with two opposite arms being enclosed by 50-cm high walls made of gray Perspex, and the two other arms being open. The four arms were connected by a squared central platform (10 cm × 10 cm). Both the central platform and the open arms were under bright illumination (100–120 lx) whilst the closed arms were under weak illumination (30 lx). Each mouse was placed on the central platform, facing an open arm. The number of visits to, and the time spent on, the open arms and the closed arms were recorded for 5 min.

The light/dark box consisted in two compartments connected to each other by a small opening (width, 7 cm; height, 7 cm). The first compartment was made of white Perspex (length, width and height: 27 cm), illuminated by a white bulb delivering 340 lx. The second compartment, smaller than the first one (length: 18 cm; width and height: 27 cm), was made of black Perspex and illuminated by a red bulb delivering 30 lx. Both white and red bulbs were located 37 cm above the apparatus floor. Each mouse was placed in the center of the white compartment facing the small opening. When the mouse entered into the black compartment, the number of transitions into the white compartment and the relative time spent therein were measured for 5 min.

For social interaction tests, mice were placed in pairs at two opposite corners of a white square arena made of wood (60 × 60 cm; height 19 cm) under a 100-lx illumination. For each pair of mice, the two individuals had always the same group origin (i.e. housing condition; see above). By means of a video-camera located above the apparatus, the number of voluntary interactions and their duration were quantified for each animal.

Cued fear conditioning sessions were performed using a conditioning box, made of gray Perspex (length: 26 cm; width: 18 cm; height: 25 cm) with a metal grid floor that was located in a sound-proof chamber (length: 55 cm; width: 60 cm; height: 50 cm; Imetronic, Pessac, France). The ceiling had a video-camera allowing the recording of all behaviors. On the conditioning day, each mouse was placed in the conditioning box and left free to explore for 3 min. A sound (1.5 kHz, 60 dB) was then emitted for 20 s with the last second of tone emission being coupled to one single foot-shock (0.5 mA). The animal was left in the fear conditioning box for another min without any stimulus before being removed from the apparatus, and housed back in its home cage. The following day, the top of each home cage was removed to be covered by a grid allowing full observation of the mouse in its cage. The home cage was then placed into the sound-proof chamber. After a 3-min pre-tone period, the tone used for conditioning was presented again for a 3-min period. The mouse was then left for another min in the chamber before removal of the home cage which was returned back to the housing facility room. The presence of freezing (i.e. lack of movements excepted those associated with breathing) was monitored during the 3-min exposure to sound.

All statistical analyses were performed with the GB-Stat software (v10; Dynamic Microsystems Inc., Silver Spring, MD, USA).