RAPID COMMUNICATION

Emotional Consequences of Wheel Running in Mice: Which Is the Appropriate Control?

Sarah Dubreucq, Giovanni Marsicano, and Francis Chaouloff*

ABSTRACT: An overview of the literature on the emotional impacts of wheel running reveals contradictory findings. Among the hypotheses underlying such a discrepancy, that related to the different housing conditions of the controls, i.e., standard housing without any object or housing with blocked running wheels, merits attention. We addressed this point in C57Bl/6N mice by examining the consequences of chronic wheel running on anxiety, context fear recall, and behavioral despair compared either to standard control housing or to housing with blocked wheels. Compared to standard housing, wheel running proved anxiolytic while facilitating fear memory. On the other hand, wheel running increased behavioral despair but influenced neither anxiety nor fear memory when compared to housing with blocked wheels. This study suggests that investigations aimed at measuring the emotional consequences of wheel running should take into consideration the housing conditions of the controls to which are compared the runners. © **2010 Wiley-Liss, Inc.**

KEY WORDS: wheel running; housing conditions; anxiety; context fear; behavioral despair

An abundant literature indicates that chronic physical exercise bears a positive influence on cognitive and emotional processes (for reviews: Salmon, 2001; Hillman et al., 2008). Indeed, depending on the severity of the illnesses, physical exercise has been proposed as an antidepressant and anxiolytic treatment or as an effective adjuvant to antidepressant and anxiolytic therapies in psychiatric patients (Salmon, 2001). On the basis of this clinical evidence, it is now decades since animal models have been developed to define the mechanisms underlying these positive effects of physical exercise. Among these animal models, wheel running and to a lower extent treadmill running have been the most common models used so far.

Running wheels have been used since the end of the 19th century (for a review: Sherwin, 1998), and these last 15 years have seen an exponential number of publications related to the physiological and behavioral effects of wheel running. There are numerous reasons for such a success, among which the ability to model voluntary/spontaneous exercise (but see Sherwin, 1998). It is widely accepted that chronic wheel running increases adult neurogenesis in the hippocampus, alters expression of neurotrophins, modifies the thresholds for synaptic plasticity, and induces morphological rearrangements (for a review: van Praag, 2009). These effects are thought to play a major role in the improvement of cognitive capacities induced by wheel running (van Praag, 2009). In addition to its positive consequences on neuroplasticity, wheel running has been claimed to possess antidepressant and anxiolytic properties but, as opposed to its neurogenic impact, such mood-elevating properties are still a matter of debate due to contradictory findings. This is true for the consequences of wheel running on anxiety, as assessed by rodent approach/avoidance tests (e.g., elevated plus-maze or light/dark box: Binder et al., 2004; Burghardt et al., 2004; Duman et al., 2008; Fuss et al., in press). Similar discrepancies are found when considering the impact of wheel running on so-called depression-like behaviors in despair tests (forced swim, tail suspension) or in the learned helplessness test (Yoo et al., 2000; Greenwood et al., 2003; Duman et al., 2008; Fuss et al., in press).

It is likely that this lack of consensus on the anxiolytic and antidepressant effects of wheel running is accounted for by differences in experimental setups between studies. Among these, the issue related to the housing of the control (i.e., nonexercising) animals is noteworthy. An overview of running wheel studies reveals that the experimental control animals to which are compared the wheel runners undergoes two types of housing, i.e., they are either left in standard cages or housed in cages containing running wheels locked on purpose. There are three reasons why a high number of studies use control animals housed in standard cages. The first one is economical (running wheels are quite expensive and the use of half of them in the locked mode may be a financial issue), the second is practical (the wheel running controls do not differ from the controls used for other scientific purposes, and thus they can be compared with respect to several variables of interest), and the third is scientific (locked wheels promote behaviors which could bias the results of the study). As concerns this last point, Koteja et al. (1999) reported that mice housed with locked wheels spend as much as a third of their time climbing and hanging on the wheels during the active (dark) phase of the light-dark cycle. In keeping with this result, it

NeuroCentre INSERM U862, University of Bordeaux 2, Bordeaux, France

Grant sponsors: NARSAD, la Fédération pour la Recherche sur le Cerveau, la Délégation Générale pour l'Armement du Ministère de la Défense, the AVENIR/INSERM Program (Fondation Bettencourt-Schueller), and the Aquitaine Region.

*Correspondence to: Francis Chaouloff, NeuroCentre INSERM U862, Equipe AVENIR ''Endocannabinoïdes et NeuroAdaptation,'' 146 rue Léo Saignat, Bordeaux 33077, France. E-mail: francis.chaouloff@inserm.fr

Accepted for publication 4 January 2010
DOI 10.1002/hipo.20778
Published online in Wiley InterScience (www.interscience.wiley.com).

is argued that a locked wheel is not the most appropriate control for wheel running because it increases physical activity per se. However, this argument may be contradicted by several observations. First, free running wheel behavior has been shown to compete with, rather than add to, regular home cage activity (de Visser et al., 2005), and it is likely that this also holds true for animals left with locked wheels. Second, lid hanging, whether horizontal or vertical, belongs to the normal behavioral repertoire of mice housed in standard cages (Steele et al., 2007). Third, hanging onto a rotating wheel may also represent as much as a third of the wheel rotations performed by mice housed with free wheels (Koteja et al., 1999), thus indicating that animals housed with free wheels, as opposed to blocked ones, mostly differ by running activity. This is confirmed by the significant difference in body weight measured after several weeks of wheel running, compared to controls housed with blocked wheels (Koteja et al., 1999; Swallow et al., 1999; Burghardt et al., 2006).

We believe that the issue related to the housing conditions of control animals deserves attention, especially when addressing the emotional impact of wheel running. Unfortunately, to the best of our knowledge, there is no report on the emotional effects of wheel running when compared simultaneously to the two control conditions defined earlier. This issue was thus addressed by investigating how control mice housed either under standard criteria (no object in the cage) or with blocked wheels score with respect to wheel runners in an anxiety (light/dark) test, in a behavioral despair (forced swim) test and in a contextual fear memory test (Burghardt et al., 2006; Greenwood et al., 2009). To this end, 8-week-old male C57Bl6/N mice (Janvier, Le Genest Saint Isle, France) were immediately housed singly without any object, i.e., without running wheel (no RW group; $n = 6$) or with a running wheel (Intellibio, Seichamps, France) set free (free RW group; $n = 5$) or blocked permanently (blocked RW group; $n = 5$). The cages were located in a temperature-controlled room with food and water ad libitum and maintained under a regular 12-h light/dark cycle (07:00 h/19:00 h). All wheels were connected to a computer, which allowed the online recording of all running variables (speed, distance covered, number of running episodes). Twenty-four days after their arrival, each mouse was exposed (between 14:00 h and 17:00 h) for 5 min to a light/dark (340 lux/30 lux) apparatus. Twenty-four hours later, all mice were submitted to a classical contextual fear conditioning test (3 min of free exploration of a shock chamber followed by one single 0.5-mA/1-s footshock administration and another min of free exploration), and reexposed for 3 min to the same context a day later. This retrieval session was followed 24 h later by a 6-min forced swim session in a 5-l glass cylinder filled with 3 l of water (height: 14 cm; temperature: 26°C ± 1°C). After each of these tests, the mice were returned back to their respective housing cages. Pain and discomfort of the animals were reduced at minimum in strict compliance with European directives and French laws on animal experimentation (autorisation no. 06369).

Figure 1A shows that at the time of testing, wheel runners had stabilized their running performance, covering 6–7 km daily. In the light/dark box, the percent time spent in the lit side was increased in the free RW group, as compared to the no RW group ($z = 2.1$, $P < 0.05$ by Mann–Whitney *U*-test; Fig. 1B). This suggests that the former group displayed less anxiety than the latter group (Bourin and Hascoët, 2003). On the other hand, the free RW group did not differ from the blocked RW in this test (Fig. 1B). When assessed for contextual fear memory, mice from the free RW group displayed higher conditioned freezing scores than the no RW group, whether expressed as the total percent time spent freezing during the 3-min recall (37% ± 8% and 16% ± 3%, respectively; $z = 2$ and $P < 0.05$ by Mann–Whitney *U*-test) or expressed as percent freezing during each of the three 1-min blocks ($F_{1,9} = 6.37$ and $P < 0.05$ for the influence of the housing condition, as indicated by a repeated ANOVA; Fig. 1C). At first glance, this difference in freezing indicates that free wheel running facilitated contextual fear memory, compared to the no RW group. However, because the free RW and blocked RW groups did not differ, these results rather suggest that the presence of a wheel, per se, may have had some stimulatory impact on the freezing response. Lastly, when tested in the forced swim test, the free RW group displayed more immobility than the blocked RW group ($z = 2.4$, $P < 0.05$ according to Mann–Whitney *U*-test) but its performance did not differ from the no RW group (Fig. 1D. Although care should be taken with regard to the behavioral meaning of immobility scores (Holmes, 2003), the classical interpretation of these results would be that wheel running, per se, increased behavioral despair (Porsolt et al., 1977).

Taken together, the results suggest that the conclusions regarding the behavioral effects of wheel running may depend on the control group to which wheel runners are compared. As an illustration, the exclusive use of control mice housed in standard cages would have led us to conclude that wheel running decreases anxiety, facilitates fear memories, and does not affect behavioral despair. Conversely, the exclusive use of control mice housed with blocked wheels would have led us to conclude that wheel running does not affect anxiety and fear memory but increases behavioral despair. It is of course too premature to build firm conclusions on the basis of this short study. As indicated earlier, the use of locked wheels in running wheel studies should allow to observe the consequences of exercise per se. However, locked wheels could also be viewed as novel objects with different environmental consequences than free wheels which are used for exercise purposes. If so, our results could then indicate that the permanent presence of an object in the cage and allowing mice to run on wheels both trigger identical emotional changes. Further, the extent to which the presence of locked wheels altered quantitatively and/or qualitatively mouse activity, compared to control mice (i.e., housed without wheels) and how these changes, if any, affected emotional behaviors are unknown. In keeping with past evidence that emotional and mnemonic profiles of mice housed with either a free wheel or a locked wheel depend on the enrichment of the house cage (Pietropaolo et al., 2006), the results presented here suggest that great attention should be

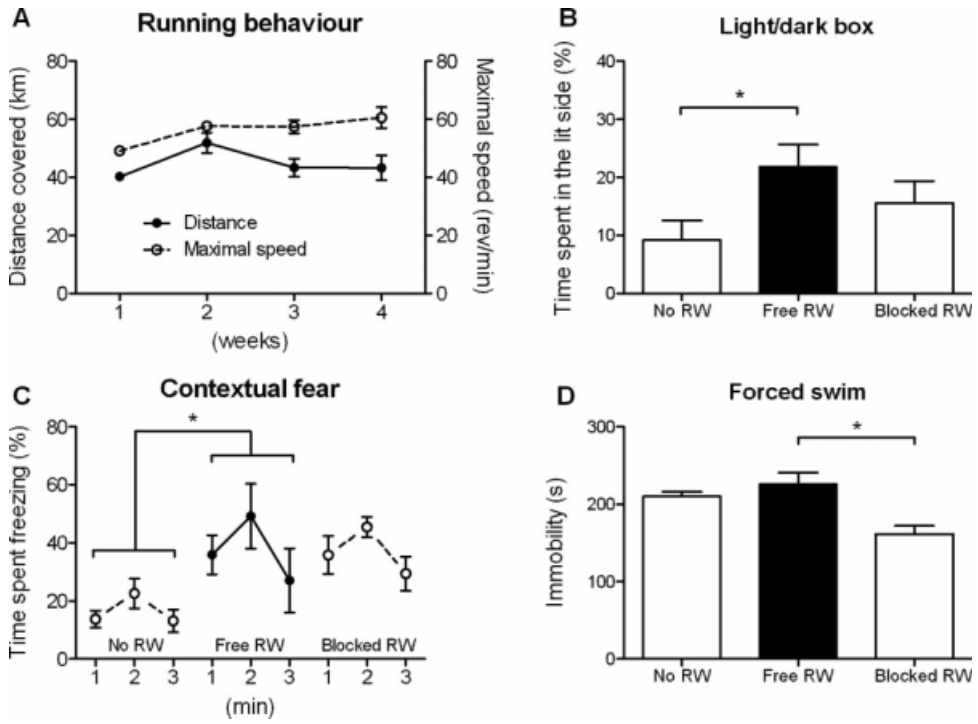

FIGURE 1. Emotional behaviors of free wheel runners (free RW) compared respectively to controls without wheels (no RW) or to controls with blocked wheels (blocked RW). (A) Running behavior over 4 weeks in the free RW group. (B) The time spent in the lit side over the time spent in both sides of a light/dark box was higher in the free RW group compared to the no RW group. (C) The free RW group freezed more than the no RW group in a contextual fear recall test. A similar difference was found when assessing freezing over the 3 min of recall (see text). (D) The free RW group displayed more immobility than the blocked RW group in the forced swimming test. There were five to six animals/group, and statistical comparisons were achieved by comparing the free RW group to either of the two control groups (no RW, blocked RW). * indicates significance at $P < 0.05$ (see text for statistics).

paid to the housing conditions of the control animals to which the wheel runners are compared before drawing firm conclusions on the emotional consequences of voluntary wheel running. Lastly, we do not know whether these observations extend first to other mouse strains and then to other species such as the rat. Indeed, a recent report has shown that rats and mice differ with respect to adult neurogenesis, including in the hippocampus, and that such differences underlie the species-dependent functional contribution of neurogenesis to contextual fear memory processes (Snyder et al., 2009).

REFERENCES

Binder E, Droste SK, Ohl F, Reul JMHM. 2004. Regular voluntary exercise reduces anxiety-related behaviour and impulsiveness in mice. Behav Brain Res 155:197–206.

Bourin M, Hascoët M. 2003. The mouse light/dark box test. Eur J Pharmacol 463:55–65.

Burghardt PR, Fulk LJ, Hand GA, Wilson MA. 2004. The effects of chronic treadmill and wheel running on behavior in rats. Brain Res 1019:84–96.

Burghardt PR, Pasumarthi RK, Wilson MA, Fadel J. 2006. Alterations in fear conditioning and amygdalar activation following chronic wheel running. Pharmacol Biochem Behav 84:306–312.

de Visser L, van den Bos R, Spruijt BM. 2005. Automated home cage observations as a tool to measure the effects of wheel running on cage floor locomotion. Behav Brain Res 160:382–388.

Duman C, Schlesinger L, Russell DS, Duman RS. 2008. Voluntary exercise produces antidepressant and anxiolytic behavioral effects in mice. Brain Res 199:148–158.

Fuss J, Ben Abdallah NMB, Vogt MA, Touma C, Pacifici PG, Palme R, Witzemann V, Hellweg R, Gass P. Voluntary exercise induces anxiety-like behavior in adult C57BL/6J mice correlating with hippocampal neurogenesis. Hippocampus 10.1002/hipo.20634.

Greenwood BN, Foley TE, Day HEW, Campisi J, Hammack SH, Campeau S, Maier SF, Fleshner M. 2003. Freewheel running prevents learned helplessness/behavioral depression: Role of dorsal raphe serotonergic neurons. J Neurosci 23:2889–2898.

Greenwood BN, Strong PV, Foley TE, Fleshner M. 2009. A behavioral analysis of the impact of voluntary physical activity on hippocampus-dependent contextual conditioning. Hippocampus 19:988–1001.

Hillman CH, Erickson KI, Kramer AF. 2008. Be smart, exercise your heart: Exercise effects on brain and cognition. Nat Rev Neurosci 9: 58–65.

Holmes PV. 2003. Rodent models of depression: Reexamining validity without anthropomorphic inference. Crit Rev Neurobiol 15:143–174.

Koteja P, Garland T Jr, Sax JK, Swallow JG, Carter PA. 1999. Behaviour of house mice artificially selected for high levels of voluntary wheel running. Anim Behav 58:1307–1318.

Pietropaolo S, Feldon J, Alleva E, Cirulli F, Yee BK. 2006. The role of voluntary exercise in enriched rearing: A behavioral analysis. Behav Neurosci 120:787–803.

Porsolt RD, Bertin A, Jalfre M. 1977. Behavioral despair in mice: A primary screening tests for antidepressants. Arch Int Pharmacodyn Ther 229:327–336.

Salmon P. 2001. Effects of physical exercise on anxiety, depression, and sensitivity to stress: A unifying theory. Clin Psychol Rev 21: 33–61.

Sherwin CM. 1998. Voluntary wheel running: A review and novel interpretation. Anim Behav 56:11–27.

Snyder JS, Choe JS, Clifford MA, Jeurling SI, Hurley P, Brown A, Kamhi JF, Cameron HA. 2009. Adult-born hippocampal neurons are more numerous, faster maturing, and more involved in behavior in rats than in mice. J Neurosci 29: 14484–14495.

Steele AD, Jackson WS, King OD, Lindquist S. 2007. The power of automated high-resolution behavior analysis revealed by its application to mouse models of Huntington's and prion diseases. Proc Natl Acad Sci USA 104:1983–1988.

Swallow JG, Koteja P, Carter PA, Garland T Jr. 1999. Artificial selection for increased wheel running activity in house mice results in decreased body mass at maturity. J Exp Biol 202:2513–2520.

van Praag H. 2009. Exercise and the brain: Something to chew on. Trends Neurosci 32:283–290.

Yoo HS, Bunnell BN, Crabbe JB, Kalish LR, Dishman RK. 2000. Failure of neonatal clomipramine treatment to alter forced swim immobility: Chronic treadmill or activity-wheel running and imipramine. Physiol Behav 70:407–411.