Learning & Behavior (2019) 47:131–140
https://doi.org/10.3758/s13420-018-0348-8

Motivation to run measured by progressive ratio tests: Failure to support the addiction hypothesis for rats

Maximilian B. L. Cordony[1] · Julie Y. L. Chow[1] · Robert A. Boakes[1]

Published online: 21 August 2018

Abstract

When laboratory rats are given repeated access to an activity wheel, the amount that they run steadily increases. This suggests an analogy with drug dependency in animals and humans, in that this is marked by both increasing intakes of the drug and increasing motivation to obtain the drug (craving). This analogy was examined by measuring motivation to obtain an opportunity to run using a progressive ratio (PR) schedule, whereby the number of lever presses required to release a brake on an activity wheel was increased progressively. Each of two experiments included two groups of rats that differed in running experience. In Experiment 1, both groups were given 17 wheel-running sessions before they were given the PR test, with sessions for the *short* group lasting only 30 min, while those for the *long* group lasted 4.5 hrs. In Experiment 2, both groups were given 3-hr wheel sessions, with the *short* group given only four such sessions and the *medium* groups given 12 such sessions prior to their PR test. In both experiments, the PR tests revealed that motivation to run was greater when the rats had not had an opportunity to run for at least 24 hrs prior to the test than when they had run the previous day. However, neither experiment produced evidence that motivation to run increased with the amount of previous running. Given only limited support for the analogy between running and drug addiction, steady increases in running may instead reflect circadian adaptation and/or increases in fitness.

Keywords Running · Activity wheel · Motivation · Progressive ratio schedule · Addiction · Rats

Spontaneous running in an activity wheel has been reported for a wide range of species. This is not a product of laboratory conditions; when an activity wheel was placed in a natural environment, video recordings revealed that a variety of free-living species came to spend time running in this wheel (Meijer & Robbers, 2014). In several species, the amount of spontaneous running has been found to increase with increased experience of wheel running (Sherwin, 1998).

Several findings have suggested that wheel running may in some sense become addictive. For example, after rats have run more and more each day, they develop tolerance to the endogenous opioids released during running (Lett, Grant, Koh, & Flynn, 2002). Indirect evidence for running-produced opioid release comes from studies showing that increased preference for a flavor (Hughes & Boakes, 2008) or for one side of a place preference chamber (Basso & Morrell, 2015; Greenwood et al., 2011) can be obtained when these are paired with the aftereffects of running; however, it should be noted that the aftereffects of addictive drugs are often opposite to the effects of the drugs themselves. Stronger support for the suggestion that wheel running can become addictive comes from the finding that rats can display withdrawal symptoms like shakes and writhing when denied further wheel access (Kanarek, D'Anci, Jurdak, & Mathes, 2009; M. A. Smith & Yancey, 2003).

An important defining feature of addiction is that, compared with recreational users, addicted drug users spend more time and effort in working to obtain and take drugs when the drug is not easily available (Everitt, Dickinson, & Robbins, 2001). Despite becoming increasingly motivated to obtain drugs, long-term drug users enjoy the drugs less (Kalivas & Volkow, 2005; Koob & Le Moal, 1997). Similarly, long-term wheel running seems to reduce the pleasure of running relative to a rat's motivation to run; thus, opioid antagonists, which are claimed to block the pleasurable aftereffects of running, prevent wheel-naïve rats from adopting wheel running, but do nothing to stop running in long-term runners (Vargas-Pérez, Borrelli, & Díaz, 2004).

These lines of evidence suggest that an animal's desire to run might increase with its experience of running. As

✉ Robert A. Boakes
bob.boakes@sydney.edu.au

[1] School of Psychology (A18), University of Sydney, Camperdown, NSW 2006, Australia

already noted, wheel running by rats escalates rapidly with long daily wheel access (Eikelboom & Lattanzio, 2003). These researchers also found that rats began to run more "intensely," as judged by the distance run during the first 30 min of wheel access. Since intensity has been found to correlate positively with lever pressing for opportunities to run on fixed ratio (FR) schedules, more intense running may indicate greater motivation to run (Belke, 1997; Iversen, 1993; see also Poucet, Durup, & Thinus-Blanc, 1988). Finally, a long-term (e.g., 6 week) running routine led to accumulation of FosB gene transcription factors in rats' nACC and striatum (Greenwood et al., 2011; Werme et al., 2002). It has been suggested that FoSB indicates increased drug motivation and the transition to "end-state" addiction (Kalivas & Volkow, 2005).

The measure that is generally accepted to provide the best method for assessing an animal's motivation to obtain some outcome is performance on a progressive ratio (PR) schedule. Furthermore, PR schedules are especially suited for testing motivation in the context of addiction, because they involve working hard for a reward that is not freely available (Arnold & Roberts, 1997). Working for reward is more analogous to real-world addiction (Everitt, Dickinson, & Robbins, 2001) and depends on neural systems distinct from those used to consume reward (Salamone, Correa, Mingote, &Weber, 2005). Whereas on an FR schedule, each reinforcement (outcome) is contingent upon a fixed number of responses, in a PR schedule the number of lever presses required for reinforcement increases with each reinforcement (e.g., 2 presses: 2, 4, 6 . . . 24 . . .) within a session. The maximum number of lever presses for a single reinforcement within a session is called the "breakpoint." This indicates how much an animal is willing to work in order to obtain the reinforcer (e.g., Roberts, Morgan, & Liu, 2007).

PR schedules have been used to test how much rats want drugs of abuse, natural rewards, and intracranial self-stimulation (Richardson & Roberts, 1996). They have also been used to measure motivation for an opportunity to run in an activity wheel. One such study compared food-deprived and well-fed rats: Well-fed rats reached breakpoints averaging 40, whereas food-deprived rats reached higher breakpoints (Pierce, Epling, & Boer, 1986). In the only other study to use a PR schedule to measure motivation to gain access to an activity wheel, the breakpoints for obese rats were found to be lower than those for lean rats (S. L. Smith & Rasmussen, 2010). No experiment to date appears to have used a PR schedule to compare motivation to run in rats with a history of excessive running and those with limited experience of running. This was the primary aim of the present study.

The animals used in these two experiments were female rats. Females were chosen because they run more than males of the same age (e.g., Boakes, Boot, Clarke, & Carver, 2000; Boakes, Mills, & Single, 1999; Eikelboom & Mills, 1988). Whether this choice produced complications due to the estrus cycle is discussed in the General Discussion.

Experiment 1

As shown in Table 1a, the first part of this experiment contained three stages. In Stage 1, all rats were trained to press a lever in order to release a brake and so provide them with the opportunity to run. The lever-press response was first maintained on an FR schedule, with the number of responses required to release the brake progressively increased up to FR12, and then a PR schedule was introduced. In Stage 2, rats that had successfully completed training in Stage 1 were divided into two matched groups. The *long* group was given seventeen 4.5-hr sessions of free running, while the *short* group was given the same number of sessions, but these lasted only 30 min. In Stage 3, rat's motivation to run was assessed using the same PR schedule as that introduced at the end of Stage 1.

An unexpected finding in the first part of the experiment was that in Stage 3, rats tended to display greater motivation to run after they had had a day without wheel access than when they had run the previous day. To provide stronger evidence for this effect, a second part was added to the experiment. What became Stage 4 consisted of eight sessions of free running, followed by a Stage 5 that gave each rat two PR tests, one following a nonrunning day and the other following a running day.

The procedures used in this and the following experiment were approved by the University of Sydney Animal Ethics Committee, Project No. 5786.

Method

Subjects

Twenty-four female Sprague Dawley rats had previously served in a classical conditioning experiment, in which visual and auditory stimuli had been variously paired with delivery of food pellets. They had no previous experience of activity wheels. Throughout the experiment, rats were housed in groups of four in large plastic cages with raised wire lids, measuring 60 × 37 × 26 cm, where they had unrestricted access to lab chow and water throughout the experiment. The home cages were kept in a temperature-controlled and humidity-controlled room on a reversed 12-hr light/12-hr dark cycle, with lights off at 0900 hrs. The rats had been kept under these conditions for several weeks beforehand. At the start of the experiment, the rats were 4 mos. old and weighed an average of 306 g (range: 258–343 g). They were weighed at least once per week to monitor health.

Table 1 Procedural outline of (**a**) Experiment 1 and (**b**) Experiment 2

a. Experiment 1

Groups	Stage 1 Preliminary training (25 sessions)	Stage 2 Free running 6 days/week (17 sessions)	Stage 3 PR tests (4 sessions)	Stage 4 Free running 6 days/week (8 sessions)	Stage 5 PR tests Deprive first vs, no dep first (2 sessions/rat)
Long	Habituation, Pavlovian conditioning, instrumental training, PR tests (3 sessions)	4.5 hr/session	PR tests	4.5 hr/session	Deprive day first No dep first
Short		0.5 hr/session		0.5 hr/session	Deprive day first No dep first

b. Experiment 2

Groups	Stage 1 Preliminary training (23 sessions)	Stage 2 Free running 6 days/week (3 hr/session)	Stage 3 PR tests (2 sessions)
Medium	Habituation, Pavlovian conditioning, instrumental training, PR tests (4 sessions)	12 days × 3-hr sessions	No dep and 1-day dep (counterbalanced) No dep and 2-day dep (counterbalanced)
Short		4 days × 3-hr sessions	No dep and 1-day dep (counterbalanced) No dep and 2-day dep (counterbalanced)

Apparatus

Each of 12 activity wheels, with diameter of 36 cm and width of 10c m, could be entered from a side chamber, 20 × 30 × 20 cm (Med Associates "Activity Wheel With Chamber" ENV-042A W/ENV-042OE). Each complete wheel turn equaled a distance of 1.13m. The 10 × 5 cm doorway between the wheel and side chamber was always open, so all running was voluntary. The floor of the wheels and of the side chambers consisted of evenly spaced cylindrical metal bars of 0.5 cm diameter. The wheels were modified to include two custom-made brakes: a rubber pad applied to the side of the wheel to stop the wheel and a solenoid-operated plastic bolt inserted through the upper metal bars of the wheel to lock the wheel. The side chamber contained a retractable lever with surface dimensions 5 × 2 cm (Med Associates "Retractable Lever," ENV-112CM) and a light, both mounted to the chamber wall, respectively, 6 and 3 cm above the floor. The lever and light were, respectively, 20 and 15 cm from the door to the wheel.

Custom software, written in LabVIEW®, controlled events within each wheel, and recorded wheel turns and the timing of events.

Procedure

All sessions were conducted 6 days per week during the dark phase of the dark/light cycle. Except during wheel-habituation sessions, all brake-off periods were accompanied by a light flashing at 10 Hz. The rats were always run in two successive 12-rat squads. Unless otherwise specified, sessions lasted 40 min.

Stage 1 (preliminary training and baseline PR test: 25 sessions) began with four habituation sessions, in which rats were placed in the side chambers and given unrestricted access to the wheels. These were followed by four Pavlovian sessions, in which 3-min periods of brake on (i.e., no opportunity to run) alternated with brake-off periods of varying duration, starting with 8 min and gradually decreasing to 30 s; the light was flashing throughout all brake-off periods. During the final two sessions the brake-off time was held constant at 30 s. The aim of this Pavlovian training was for rats to associate the flashing light with the opportunity to run. During subsequent instrumental training sessions, a lever was inserted into each chamber for the first time. For the first seven sessions, a single lever press (FR1) initiated a 30-s brake-off period signaled by the light. At the end of 180 s without a lever press (maximum brake-on period), rats received a 30-s brake-off period signaled by the light, to maintain the light–brake-off association. By the third FR1 session, six rats failed to lever press more than eight times; these rats' levers had to be baited before they pressed regularly. Following completion of FR1, all rats received instrumental training on larger FR schedules: one session on FR2, one on FR4, three on FR8, and two on FR12.

This stage ended with three sessions with the following PR schedule. Two lever presses were required to release the wheel brake and initiate the first 30-s run period. From then on within each 40-min session the number of lever presses required to release the brake increased incrementally by a fixed amount of two presses (i.e., 2, 4, 6, 8, etc.). The break point was defined as the final ratio that the animals reached within a session. The three-session average was taken as the baseline break point; this was used to rank the rats and to allocate them into two matched groups.

In Stage 2 (free running; 17 sessions), rats received uninterrupted wheel access for 6 days per week. The *short* group

received 30-min daily wheel access from 1200–1230 hrs, whereas the *long* group received 4.5-hr daily wheel access from 1300–1730 hrs. Both periods were still within the dark phase. While rats in one group were placed in the wheels, the other rats were singly housed during this time to control for potential isolation-related stress.

In Stage 3 (PR testing; 4 × 40-min sessions), the PR schedule used at the end of Stage 1 was reintroduced. Because of interest in whether performance could be influenced by the starting ratio, on Days 1 and 4, the ratio started at 2 (i.e., 2, 4, 6 . . .) and on Days 2 and 3, the ratio started at 6 (i.e., 6, 8, 10 . . .).

In Stage 4, all rats were given eight further sessions of free running. As previously, these lasted 30 min for the *short* group and 4.5 h for the *long* group.

Stage 5 consisted of two PR tests for each rat, arranged so that this stage provided both a further test for a difference between the short and long groups and a test of the effect of a day without placement in a wheel, a "deprivation" day (Mueller, Herman, & Eikelboom, 1999). Thus, "deprivation" was a within-subject variable, whereby each rat was tested both after 48 hrs since most recent wheel access and after no such deprivation (i.e., 24 hrs since most recent wheel access), in counterbalanced sequence, while "group" compared rats in the long versus short condition. Within each group, half of the rats were given the deprivation condition first and the other half given the no-deprivation condition first.

Results

In analyzing data from Stages 1–3, we specified an inclusion criterion whereby rats needed to reach a breakpoint of 6 in at least one of the three baseline PR tests at the end of Stage 1. Two rats failed this criterion, and their data were excluded from analyses of data from the first three stages. However, these rats continued to be trained, and they eventually lever pressed well above the criterion in the PR tests given in Stage 3; consequently, their data were included in analysis of data from Stages 4 and 5.

We observed during Stage 1 of preliminary training that lever-pressing rates declined within each session. Figure 1a shows this for Session 7, as an example, when all rats still had to make a single lever press to release the brake (FR1 schedule). Lever presses were counted in successive 5-min bins. It may be seen that that lever pressing was greatest during the first 5-min bin of the 40-min session and that on average rats continued responding throughout this training session. A mixed ANOVA (Group × Bin) found a main effect of bins, $F(7, 154) = 13.73$, $p < .001$, but no main effect of group ($F < 1$). A linear trend indicated that lever pressing decreased across 5-min bins, averaged across group, $F(1, 22) = 38.51$, $p < .001$, and at a decreasing rate across the session, as indicated by a quadratic trend, $F(1, 22) = 11.52$, $p = .003$. No Group × Bin interactions were detected ($F < 1$). Informal observations suggested that lever pressing on FR2, FR4, FR8, and FR12 schedules produced similar functions.

Over the three PR tests at the end of Stage 1, rats reached mean break points ranging from 4 to 42. The long ($M = 17.6$, $SD = 10.1$) and short groups ($M = 17.2$, $SD = 10.0$) were matched for break point, $t(20) < 1$, and for amount of running during these tests, operationalized as total number of wheel turns ($M_{long} = 131.6$, $SD_{long} = 96.9$; $M_{short} = 124.8$, $SD_{short} = 94.4$), $t(20) < 1$.

Figure 2a shows mean daily wheel turns in Stage 2 of free running for the long and short groups; 4.5 hrs/session and 0.5 hrs/session, respectively. A mixed ANOVA with group as a between-subjects factor and days as a within-subjects factor revealed an overall group effect, whereby rats in the long group ($M = 2{,}131$, $SD = 1{,}561$) ran more than in the short group ($M = 532.7$, $SD = 183.6$), $F(1, 20) = 14.63$, $p = .001$. There was also a significant overall linear trend across days of running, suggesting that daily wheel turns increased across these 17 days, $F(1, 20) = 12.20$, $p = .002$. Importantly, there was a significant interaction between group and days, $F(1, 22) = 6.055$, $p < .001$. Teasing apart the interaction, the linear trend across days was significant for the long group, $F(1, 10) = 11.2$, $p = .007$, but not for the short group, $F(1, 10) = 3.442$, $p = .093$, suggesting that only rats in the long group were running more with increased consecutive exposure to the wheel, as predicted.

Because of mechanical failures in the first of the four PR tests in Stage 3, complete data were collected only for Tests 2, 3, and 4. Performance, in terms of break points, during these three tests is shown in Fig. 3. A mixed ANOVA with group (long vs. short) as a between-subjects factor and session (2, 3, and 4) as a within-subjects factor revealed a significant main effect of session, $F(2, 44) = 8.28$, $p = .002$, with a linear trend, $F(1, 22) = 12.05$, $p = .002$, confirming a decrease in break points across these three tests. This trend did not differ as a function of group ($F < 1$), and there was also no main effect of group ($F < 1$).

In Stage 4, when rats were returned to unrestricted wheel access, performance of the two groups was similar to that seen in Stage 2 (see Fig. 2b). A mixed ANOVA (Group × Day) revealed a main effect of group, such that long rats ($M = 2{,}613$, $SD = 1{,}623$) ran significantly more than short rats ($M = 489.3$, $SD = 174.6$), $F(1, 22) = 23.60$, $p < .001$. There was an overall positive linear trend across days, $F(1, 22) = 13.77$, $p = .001$, and a Group × Days interaction, $F(3.04, 66.9) = 3.48$, $p = .002$. Further analysis revealed that the linear trend across days was present for the long group, $F(1, 11) = 12.6$, $p = .005$, but not for the short group, $F(1,11) = 1.25$, $p = .287$. These analyses suggest that daily wheel turns by the long group continued to increase, whereas those by the short group remained fairly stable across these further eight sessions of unrestricted wheel access.

Figure 4 illustrates the results obtained from the final PR tests in Stage 5. In order to determine if there were differences in break point as a function of time spent running and whether

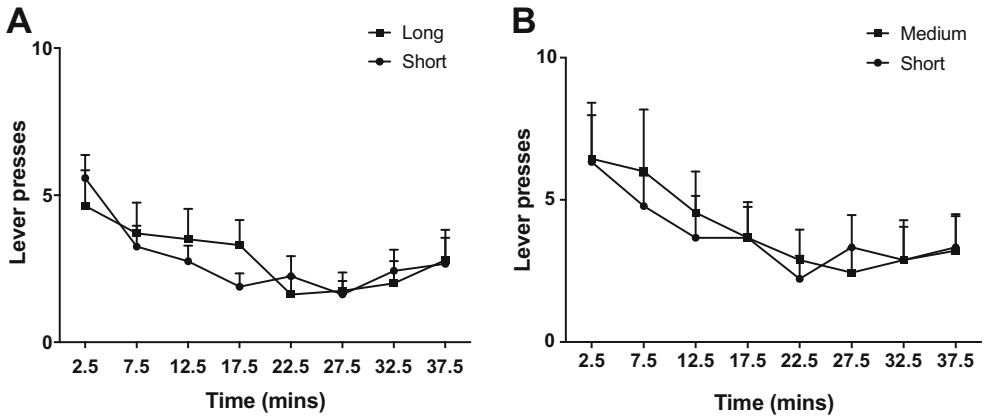

Fig. 1 Mean lever presses (+ *SEM*) to earn 30-s wheel access per 5-min bin across a 40-min session, using Day 7 of training on FR1 as the exemplar in (**a**) Experiment 1 and (**b**) Experiment 2

or not rats were tested the day after a running day or 2 days later (1-day deprivation), a three-way mixed ANOVA was applied, with group (long vs. short) and order (deprivation first vs. no-deprivation first) as between-subjects factors and deprivation (1-day deprivation vs. no deprivation) as the within-subjects factor. This revealed a main effect of deprivation, $F(1, 20) = 10.36$, $p = .004$, such that rats reached higher break points when tested 48 hrs since running ($M = 23.9$, $SD = 5.79$) than after 24 hrs since running ($M = 21.3$, $SD = 6.04$). As suggested by Fig. 4a, there was no indication of the predicted main effect of group ($F < 1$).

Figure 4b shows performance in the final PR tests in terms of postreinforcement pauses (PRPs); the PRP was defined for each rat as the time between the brake onset and the first subsequent lever press, averaged across the session. A mixed-effects ANOVA, with group and order as between-subjects factors and deprivation as the within-subjects factor found an interaction between group and deprivation, suggesting that the impact of a day without running on PRP differed between the groups, $F(1, 20) = 7.55$, $p = .012$. Paired-sample t tests revealed a significant difference between deprivation and no deprivation on PRP for rats in the long group, $t(11) = 2.84$, $p = .016$, but not for rats in the short group, $t(11) = 1.16$, $p = .269$. No other effects were found (Fs < 1).

Discussion

The main, and unexpected, outcome from this first experiment was that rats with very extensive experience of unrestricted wheel running—totaling 112.5 hrs by the time of the final PR tests—were no more strongly motivated to run than rats that had had only 12.5 hrs of free running prior to these tests. No differences between the long and short group were detected in terms of the break-point measure either in the first or the final PR tests (see Figs. 3 and 4a). The only indication of a difference between the groups came from analysis of PRPs in the final PR tests. An interaction effect (see Fig. 4b) indicated that the long group paused for a shorter time before initiating another 30-s bout of running after being deprived of running for a day than when these rats had run the previous day; in contrast, no such effect was detected in the short group. How to interpret this result is discussed later.

Data reported by Ferreira et al. (2006) suggest that there could be large individual differences in rats' proneness to become "dependent" on wheel running. Thus, a possible reason for the failure to find a significant group effect in the present experiment could be that the long group contained an insufficient number of dependence-prone individuals. However, this seems unlikely in that the mean

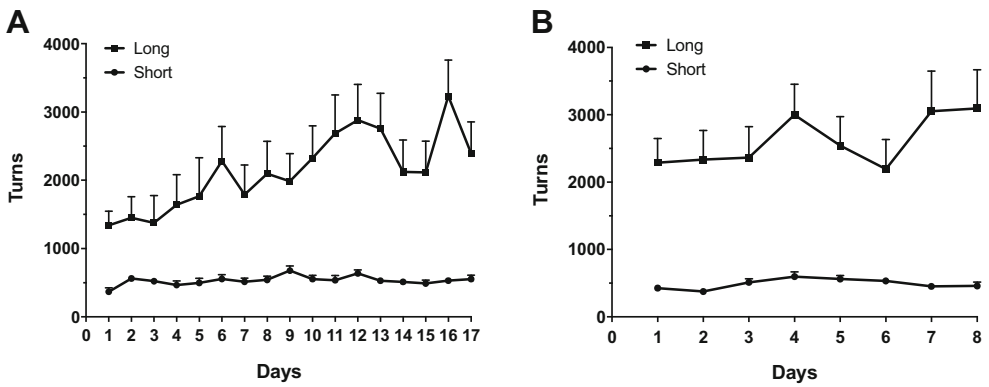

Fig. 2 Experiment 1. Mean daily wheel turns (+ *SEM*s) by the long (4.5-hr access) and short (0.5-hr access) groups during (**a**) Stage 2 and (**b**) Stage 4. These stages were separated by four progressive ratio tests

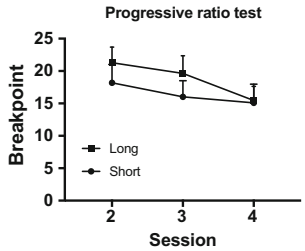

Fig. 3 Experiment 1. Mean break points (+ *SEM*s) in the last three progressive ratio tests in Stage 3

break-point values were almost identical in the two groups (see Fig. 4a); that is, the failure to find a group difference does not appear to be due to a high degree of individual variability in the long group.

The finding that introducing a day without running prior to a PR test produced higher break points extends the finding of Mueller et al. (1999) that, following up to 3 days of such deprivation, rats run more than when they have had access to the wheel on the previous day.

Experiment 2

Human dependency on a drug usually develops as a result of repeated exposure to the drug. Therefore, it was of interest to test whether varying the overall *number* of sessions in which rats were given an opportunity to run would produce differences in motivation to run. In Experiment 1, the two groups of rats differed in terms of total amount of running by the end of Stage 1, but not in terms of the number of sessions: Both groups received 17 free-running sessions in Stage 2 and eight sessions in Stage 4. In this second experiment, the two groups differed in terms of how many sessions in the activity wheel they were given—four versus twelve—but the session durations were the same for both groups, namely, 3 hrs.

The procedures used in this experiment were very similar to those in Experiment 1. However, one change was to extend the length of the PR tests from 40 min in Experiment 1 to 80 min in the present experiment. This change was introduced because we suspected that 40-min tests had been too short for highly motivated rats in Experiment 1, in that they would have reached even higher break points if tests had been longer.

In attempting to extend the evidence from Experiment 1 that a deprivation period increases rats' motivation to run, in Experiment 2 PR tests were conducted after deprivation periods of 1 or 2 days and compared with PR tests a day after a session of running. As the PRP measure used in Experiment 1 had detected differences between the groups, this was again included as a measure of performance in the PR tests, together with break point.

Method

Subjects

Twenty-four female Sprague Dawley rats were from the same source and had the same prior experience as rats in Experiment 1. Housing, handling, and feeding conditions were also identical to those in Experiment 1. At the start of the present experiment, rats were 5 mos. old with a mean weight of 296 g (range: 260–347 g).

Apparatus

Apparatus was the same as in Experiment 1.

Procedure

As shown in Table 1b, there were three stages to this experiment. Stage 1 consisted of preliminary training followed by four PR sessions. In Stage 2, rats were divided into two

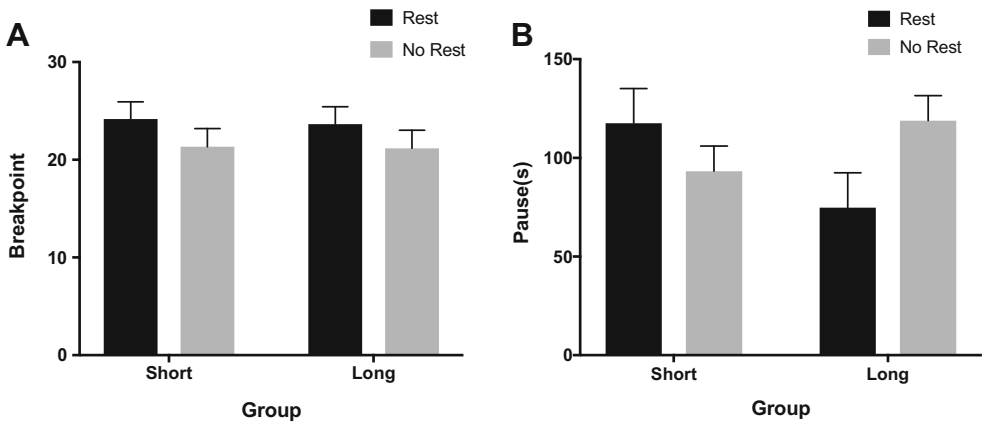

Fig. 4 **a** Performance on the PR tests indexed by mean breakpoint (i.e., maximum ratio of lever presses to reinforcement), **b** Mean postreinforcement pauses (i.e., time between the brake-on and next first lever press) as a function of group (short vs. long) and testing day (dep day vs. no-dep day), average across order of test (dep day first vs. no-dep first). Error bars + *SEM*. Dep = deprivation of wheel access

groups, given free running for either 4 consecutive days (short group) or 12 consecutive days (medium group). Stage 3 consisted of final PR tests.

In Stage 1, training was similar to that in Experiment 1, but with the following minor differences. Rats first received three sessions of wheel habituation and Pavlovian training instead of four. Each Pavlovian session started with 8-min periods of brake off (i.e., opportunity to run) paired with light, with these progressively decreasing to 30-s intervals. Because some rats failed to press the lever in the first two instrumental training session, the levers were smeared with a 10% fructose solution from Session 3 to 7. Once they were pressing consistently, rats received seven sessions on FR1 and two on each of FR2, FR4, and FR8.

The first PR session lasted 40 min, whereas the following three PR sessions lasted 80 min. Rats were then allocated to two groups matched on the basis of their mean break point over the last three sessions, with each group divided evenly into two groups (medium vs. short).

In Stage 2, all rats were provided with unrestricted wheel access that lasted for 3 hrs in each session. The morning squad ran from 0900 hrs to 1200 hrs, and the midday squad ran from 1300 hrs to 1600 hrs. The medium group received 12 consecutive sessions after remaining in the home cages for the first 8 days, the short group received four consecutive sessions on the same days as the medium group were given their final four sessions.

Stage 3 started the day after the final free-running session. All rats received two PR tests: one directly after an unrestricted-running day and the other after a number of days without wheel access. In the 1-day condition, rats were tested after 1 deprivation day (i.e., 48 hrs since most recent run). In the 2-day condition, rats were tested after 2 deprivation days (i.e., 72 hrs since most recent run). Half of the rats in each group were first tested without a deprivation period, and the other half were tested after either 1 or 2 days of deprivation. However, since in Experiment 1 no effect of test order (dep first vs. no-dep first) was detected on any measure, data analysis for PR performance in Experiment 2 was collapsed across the order conditions.

The PR schedule started at 2 with an increment of 2 (i.e., 2, 4 ,6 . . .), and all PR tests lasted 80 min.

Results

At the end of Stage 1, six of the 24 rats failed to reach the criterion of a break point of at least six in one of the final three PR tests. Only data from the remaining rats were included in the analyses and results reported here.

During instrumental training, rates of lever pressing declined within each session, as found in Experiment 1; see Fig. 1b for performance by the present rats on an FR1 schedule in Session 7 in terms of lever presses in successive 5-min bins. A mixed ANOVA with group as the between-subject factor and bin as the within-subject factor found a significant effect of bins, with a linear trend, $F(1, 16) = 15.2$, $p = .001$, and a quadratic trend, $F(1, 16) = 14.03$, $p = .002$, suggesting that lever pressing decreased over time, and the rate of decline was slower toward the end of the session. There was no effect of group, nor an interaction between group and bins (Fs < 1).

Averaging for each rat over the last three 80-min PR tests at the end of Stage 1, the overall mean breakpoint was 21.8 (SEM = 2), range: 6–37. Rats were allocated to two groups matched for breakpoint, short (M = 21.8, SD = 9.09) and medium (M = 21.9, SD = 21.9), $t < 1$, and additionally for wheel turns per PR session (M_{short} = 173.2, SD_{short} = 116.8; M_{medium} = 193.5, SD_{medium} = 144.9), $t < 1$.

Mean daily wheel turns by rats in Stage 2, running for either 4 days (short group) or 12 days (medium group), are shown in Fig. 5. Trend analysis suggests medium rats marginally increased their running over 12 days, $F(1, 8) = 5.28$, $p = .051$, but this was not found for rats in the short group ($F < 1$). Contrary to expectation, a comparison of mean wheel turns across the last 4 days failed to detect a difference between groups, $F(1,16) = 1.27$, $p = .28$.

The more important data are those obtained from the Stage 3 PR tests. Break points from these tests are shown in Fig. 6a, and PRPs are shown in Fig. 6b. A mixed ANOVA was first applied to the break-point data, with group (short vs. medium) as a between-subjects factor and deprivation (deprivation vs. no deprivation) as a within-subjects factor, in order to detect potential differences in breakpoint averages as a function of wheel deprivation. This analysis revealed a main effect of deprivation, $F(1, 16) = 4.55$, $p = .049$, with slightly higher break points when rats were tested after a deprivation period, average across both 1-day and 2-days (M = 23.8, SD = 11.9), than on the day after a session in the wheels (M = 20.4, SD = 9.14). Subsequent planned comparisons revealed that this difference between the deprivation and no-deprivation conditions was significant only in rats given the 2-day condition, $F(1, 7) = 11.07$, $p = .013$, but not in rats tested given the 1-day condition ($F < 1$). Importantly,

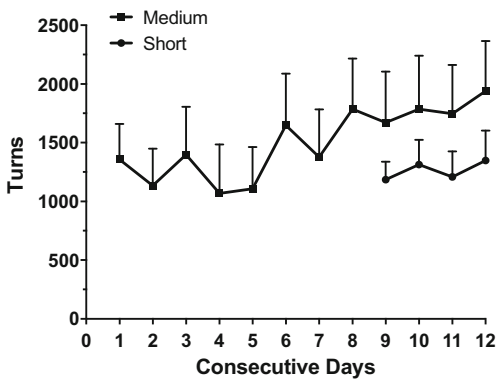

Fig. 5 Experiment 2. Mean wheel turns (+ SEM) over consecutive 3-hr free running sessions by the medium (12 days) and short (4 days) groups

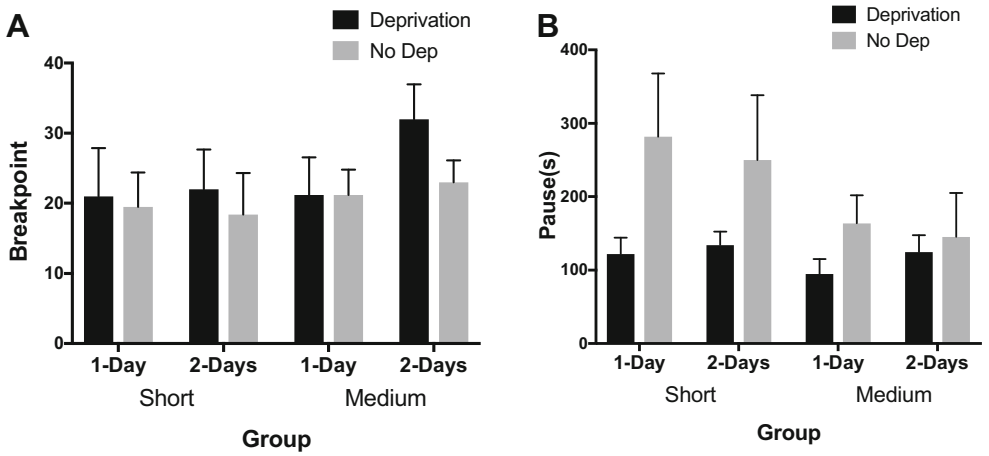

Fig. 6 **a** Breakpoints. **b** Postreinforcement pauses in PR test (+ *SEM*) as a function of group (short vs. medium), deprivation, and amount of deprivation (1 vs. 2 days)

neither a main effect of the group factor nor an interaction involving this factor were found (*F*s < 1).

A similar mixed ANOVA with group as a between-subjects factor and deprivation as a within-subjects factor was conducted on PRPs; these data are shown in Fig. 6b, with one rat excluded as its mean PRP was two standard deviations above the mean. This analysis revealed a main effect of deprivation, $F(1, 15) = 8.41$, $p = .011$, with the no-deprivation condition ($M = 211$, $SD = 144$) producing longer pauses between brake onset and lever press relative to the deprivation condition ($M = 118$, $SD = 42.8$). There was no main effect of group, $F(1, 15) = 3.01$, $p = .103$, nor an interaction between the two factors, $F(1, 15) = 1.99$, $p = .179$.

Discussion

As in Experiment 1, the PR tests failed to detect any difference between rats that had previously run a great deal—twelve 3-hr sessions in the medium group—and those that had very limited experience of free running, the short group that was given only four 3-hr sessions. This experiment also confirmed the finding from Experiment 1 that break points were higher and pauses after a reinforcement (a bout of running) were shorter when rats had had at least 1 day without access to the wheels before being tested.

General discussion

In neither experiment did we detect a main effect of prior amount of running on either mean break points or PRPs during the PR tests. This suggests that extended periods of running, operationalized either as total length of time spent running (Experiment 1) or number of sessions (Experiment 2) did not increase these rats' motivation to run relative to rats with very limited experience of free running. Some of the rats in the short group of Experiment 2 were reaching break points in the range 25 to 30 in the PR tests (cf. Fig. 6), which was in the same range as break points reached by the long group in Experiment 1 (cf. Fig. 4). These are remarkably high, given that the outcome of making this large number of lever presses was a mere 30-s opportunity to run. Therefore, these experiments provided no support for the suggestion that rats become "addicted" to running in terms of the present behavioral measure of motivation to run. Given that this is a null result, it is possible that future research using some variation in the present procedure might obtain support for the addiction hypothesis. However, it is not at all obvious how such a different method could improve on the one used in the present study.

One alternative account for the steady increase in running that rats display as they gain increasing experience of wheel running is that this results from gradual adaptation to a new daily regime. When rats' access to food is limited to, say, 90 min at a fixed time each day, how much they eat gradually increases over a period of up to 12 days or more (e.g., Boakes et al., 1999; Dwyer & Boakes, 1997; Mistlberger, 1994). To our knowledge in all studies, in which rats have been given daily access to activity wheels over many days, this access has been provided at around the same time of day, as in the present study. It is possible, then, that daily increases in running results from the slow development of a new circadian rhythm rather than an increase in "wanting" (i.e., motivation) to run. Even when rats have unrestricted access to an activity wheel, it seems likely that the typical steady increase in running over 1 to 2 weeks reflects the development of a new circadian rhythm, whereby running progressively fits into an increasingly fixed daily pattern, alongside eating, drinking, sleeping, and other behaviors.

Such a development may involve a process known as allostasis, the process of achieving stability through physiological or behavioral change (Sterling, 2004). In the present case, this

could involve the development of a range of physiological responses that anticipate periods of running and function to manage the stress involved in running (McEwen & Wingfield, 2010).

Another—and compatible—possibility is that rats become progressively fitter with time spent in an activity wheel (cf. Mueller et al., 1999).

It has long been known that how much a female rat runs in an activity wheel can vary across its estrus cycle (Wang, 1923). Consequently, the choice of female rats for these two experiments raises the possibility that estrus-based variability in their performance contributed to the failure to detect higher break points in rats with greater experience of running. This seems unlikely for two main reasons. First, the mean break points for the two groups in Experiment 1 and for the two groups in Experiment 2 were very similar, with no indication that group differences would have become detectable with increased statistical power. Second, in both experiments. significant group differences in break points were produced by varying the amount of deprivation—time without access to the wheels—prior to a test; these effects were admittedly obtained from within-subjects comparisons, but this means that the animals were likely to have been tested at different phases in their estrus cycle and thus, if the latter were an important factor, this would have contributed to the variability of their performance in the PR tests. Finally, we note that in a related study that also involved female rats, there was no sign of any systematic 4-day cycle in wheel-running rats that could indicate an effect of an estrus state (Belke & Pierce, 2016, p. 9).

Differences in brain histochemistry, especially in the mesolimbic reward pathway, have been reported between male rats given 6 weeks of 24-h access to running wheels and sedentary controls (Greenwood et al., 2011). This study did not test whether the same results could have been obtained with rats given only, say, 2 weeks of wheel access. Thus, it does not provide strong grounds for believing that, if our female rats had been given much more extensive experience of wheel running, then they might have reached even higher break points. Furthermore, especially given the variety of differences between the exercised rats and the sedentary controls in Greenwood et al. (2011), the reported changes in histochemistry do not necessarily indicate the development of an addiction.

A novel result from these experiments was that, when given a PR test, the rats reached higher break points after a day or 2 without wheel access. It has long been known that rats run more after being denied access to a wheel for 1 to 2 days; Shirley was the first to demonstrate this deprivation effect and suggested that "rest breeds restlessness" (Shirley, 1928, p. 184). However, the present data appear to be the first to show that *motivation* to run, as measured by performance on a PR schedule, is increased by such deprivation. In doing so, it supplements the finding that short-term deprivation, just 45-min with a rat's wheel locked at the start of a session, can increase motivation to run, as measured indirectly via a matching-law analysis of performance on variable-interval schedules (Belke & Heyman, 1994).

To investigate the involvement of different brain areas in what they termed "running rebound"—in their case, increased running after a 72-hr deprivation period—Basso and Morrell (2015) found that this was reduced by temporary inactivation of the medial prefrontal cortex (mPFC) and nucleus accumbens (NA); they argued that "running rebound" was similar to drug bingeing after withdrawal from the rewarding effect of running ("wheel withdrawal"). However, they did not compare rats with differing experience of wheel running and, as discussed above, the present experiments failed to confirm the analogy with drug taking, in that motivation to run did not increase with experience. Furthermore, unlike drug withdrawal, running rebounds are reversed when rats are denied access to a wheel for longer than 3 days (Mueller et al., 1999; Shirley, 1928), although this may be mainly due to loss of fitness (Mueller et al., 1999).

An unusual measure used in the present PR tests was the length of the pause between the end of a 30-s bout of running and the first lever press a rat made on the PR schedule that followed. For the long group in Experiment 1 (see Fig. 4b) and for both groups in Experiment 2 (see Fig. 6b), this measure also turned out to be sensitive to deprivation: Rats paused for a shorter time before initiating a run of lever presses if they had not been given any access to the wheels for 48 hrs or more than if they had run the previous day. On the other hand, in general the groups did not differ in terms of PRPs. Thus, the pattern of results from this measure was similar to that obtained using the traditional break-point measure. Although this measure is rarely reported for studies using PR schedules (but see Olarte-Sánchez, Valencia-Torres, Cassady, & Bradshaw, 2015), a mathematical model of behavior on PR schedules predicts a reduction in PRP with an increase in the incentive value of a reinforcer (Bradshaw & Killeen, 2012).

The effect of denying access to the wheels is clearly not a result of recovery from physical fatigue in that the rebound effect in both the present experiments was as large for the rats that ran very little, especially the short group in Experiment 1 that were given only 30 min of daily wheel access, as for those that ran a great distance each day.

In summary, the present study sought to extend the evidence suggesting that rats become addicted to spontaneous running by assessing how motivation to run changes with extended running experience. Motivation to run was defined in terms of performance on a PR schedule that measures instrumental behavior to obtain an otherwise unavailable reinforcer. However, the addiction hypothesis was not supported by the present results, in that no evidence was found that rats with extensive experience of running become more motivated to run. Therefore, addiction is a less likely explanation for rats' impressive increases in spontaneous wheel running. Rather, rats may be entraining their bodies to a new circadian rhythm or becoming fitter.

Acknowledgements The authors are very grateful to Nenad Petrovski for the major technical support he provided and to the School of Psychology, University of Sydney, for financial support. We also thank Kelly Clemens for her advice on progressive ratio schedules.

References

Arnold, J. M., & Roberts, D. C. S. (1997). A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. *Pharmacology Biochemistry & Behavior, 57*, 441–447.

Basso, J. C., & Morrell, J. I. (2015). The medial prefrontal cortex and nucleus accumbens mediate the motivation for voluntary wheel running in the rat. *Behavioral Neuroscience, 129*, 457–472.

Belke, T.W. (1997). Running and responding reinforced by the opportunity to run: Effect of reinforce duration. *Journal of the Experimental Analysis of Behavior, 67*, 337–351.

Belke, T. W., & Heyman, G. M. (1994). A matching law analysis of the reinforcing efficacy of wheel running in rats. *Animal Learning & Behavior, 22*, 267–274.

Belke, T. W., & Pierce, W. D. (2016). Wheel-running reinforcement in free feeding and food-deprived rats. *Behavioural Processes, 124*, 1–9.

Boakes, R. A., Boot, B., Clarke, J. V., & Carver, A. (2000). Comparing albino and hood Wistar rats of both sees on a range of behavioral and learning tasks. *Psychobiology, 28*, 339–359.

Boakes, R. A., Mills, K. J., & Single, J. P. (1999). Sex differences in the relationship between activity and weight loss in the rat. *Behavioral Neuroscience, 113*, 1080–1089.

Bradshaw, C. M., & Killeen, P. R. (2012). A theory of behavior on progressive ratio schedules, with applications in behavioural pharmacology, *Psychopharmacology, 222*, 549–564.

Dwyer, D. M., & Boakes, R. A. (1997). Activity-based anorexia in rats as failure to adapt to a feeding schedule. *Behavioral Neuroscience, 111*, 195–205.

Eikelboom, R., & Lattanzio, S. B. (2003). Wheel access duration in rats: II. Day–night and within-session changes. *Behavioral Neuroscience, 117*, 825–832.

Eikelboom, R., & Mills, R. (1988). A microanalysis of wheel running in male and female rats. *Physiology & Behavior, 43*, 625–630.

Everitt, B. J., Dickinson, A., & Robbins, T. W. (2001). The neurophysiological basis of addictive behaviour. *Brain Research Reviews, 36*, 129–138.

Ferreira, A., Lamarque, S., Boyer, P., Perez-Diaz, F., Jouvent, R., & Cohen-Salmon, C. (2006). Spontaneous appetence for wheel running: A model of dependency on physical activity in rat. *European Psychiatry, 21*, 580–588.

Greenwood, B. N., Foley, T. E., Le, T. V., Strong, P. V., Loughridge, A. B., Day, H. E. W., & Fleshner, M. (2011). Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. *Behavioral Brain Research, 217*, 354–362. https://doi.org/10.1016/j.bbr.2010.11.005

Hughes, S., & Boakes, R. A. (2008). Flavor preferences produced by backward pairing with wheel running. *Journal of Experimental Psychology: Animal Behavior Processes, 34,* 283–293.

Iversen, I. H. (1993). Techniques for establishing schedules with wheel running as reinforcement in rats. *Journal of the Experimental Analysis of Behavior, 60*, 219–238.

Kalivas, P. W., & Volkow, N. D. (2005). The neural basis of addiction: A pathology of motivation and choice. *American Journal of Psychiatry, 162*, 1403–1413.

Kanarek, R. B., D'Anci, K. E., Jurdak, N., & Mathes, W. F. (2009). Running and addiction: Precipitated withdrawal in a rat model of activity-based anorexia. *Behavioral Neuroscience, 123*, 905–912.

Koob, G. F., & Le Moal, M. (1997). Drug abuse: Hedonic homeostatic dysregulation. *Science, 278*, 52–58.

Lett, B. T., Grant, V. L., Koh, M. T., & Flynn, G. (2002). Prior experience of wheel running produces cross-tolerance to the rewarding effect of morphine. *Pharmacology Biochemistry and Behavior, 72*, 101–105.

McEwen, B. S., & Wingfield, J. C. (2010). What is in a name? Integrating homeostasis, allostasis and stress, *Hormones and Behavior, 57*, 105–111.

Meijer, J. H., & Robbers, Y. (2014). Wheel running in the wild. *Proceedings of the Royal Society, B, 281*. https://doi.org/10.1098/rspb.2014.0210

Mistlberger, R. E. (1994). Circadian food-anticipatory activity: Formal models and physiological mechanisms. *Neuroscience and Biobehavioral Reviews, 18*, 171–195.

Mueller, D.T., Herman, G., & Eikelboom, R. (1999). Effects of short- and long-term deprivation on wheel running, *Physiology & Behavior, 66*, 101–107.

Olarte-Sánchez, C. M., Valencia-Torres, L., Cassady, H. J., & Bradshaw, C. M. (2015). Quantitative analysis of performance on a progressive-ratio schedule: Effects of reinforcer type, food deprivation and acute treatment with Delta^9-tetrahydrocannabinol (THC). *Behavioural Processes, 113*, 122–131.

Pierce, W. D., Epling, W. F., & Boer, D. P. (1986). Deprivation and satiation: The interrelations between food and wheel running. *Journal of the Experimental Analysis of Behavior, 46*, 199–210.

Poucet, B., Durup, M., & Thinus-Blanc, C. (1988). Short-term and long-term habituation of exploration in rats, hamsters and gerbils, *Behavioural Processes, 16*, 203–211.

Richardson, N. R., & Roberts, D. C. S. (1996). Progressive ratio schedules in drug self-administration studies in rats: A method to evaluate reinforcing efficacy. *Journal of Neuroscience Methods, 66*, 1–11.

Roberts, D. C. S., Morgan, D., & Liu, Y. (2007). How to make a rat addicted to cocaine, *Progress in Neuropsychopharmacology and Biological Psychiatry, 31*, 1614–1624.

Salamone, J. D., Correa, M., Mingote, S. M., & Weber, S. M. (2005). Beyond the reward hypothesis: Alternative functions of nucleus accumbens dopamine. *Current Opinion in Pharmacology, 5*, 34–41.

Sherwin, C. M. (1998). Voluntary wheel running: A review and novel interpretation. *Animal Behaviour, 56*, 11–27.

Shirley, M. (1928). Studies in activity: II. Activity rhythms; age and activity; activity after rest. *Journal of Comparative Psychology, 8*, 159–168.

Smith, M. A., & Yancey, D. L. (2003). Sensitivity to the effects of opioids in rats with free access to exercise wheels: Opioid tolerance and physical dependence. *Psychopharmacology, 168*, 426–434.

Smith, S. L., & Rasmussen, E. B. (2010). Effects of 2-AG on the reinforcing properties of wheel activity in obese and lean Zucker rats. *Behavioural Pharmacology, 21*, 292–300.

Sterling, P. (2004). Principles of allostasis: optimal design, predictive regulation, pathophysiology and rational therapeutics. In J. Shulkin (Ed.), Allostasis, homeostasis, and the costs of adaptation. (pp. 17–63) Cambridge: Cambridge University Press.

Vargas-Pérez, H., Borrelli, E., & Díaz, J.L. (2004). Wheel running use in dopamine D2L receptor knockout mice. *Neuroscience Letters, 366*, 172–175.

Wang, G. H. (1923). The relation between spontaneous activity and the oestrous cycle in the white rat. *Comparative Psychology Monographs, 2*(6), 27.

Werme, M., Messer, C., Olson, L., Golden, L., Thorén, P., Nestler, E. J., & Brené, S. (2002). Delta FosB regulates wheel running. *Journal of Neuroscience, 22*, 8133–8138.