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Research report

Effects of genetic background and environmental novelty on wheel running as a rewarding behaviour in mice

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Abstract

Recent studies suggest running wheel activity to be naturally rewarding and reinforcing; considering the shared neuro-behavioural characteristics with drug-induced reward situations, wheel running behaviour gains interest as a tool to study mechanisms underlying reward-sensitivity. Previously, we showed that wheel running has the potential to disrupt the daily organization of home cage behaviour in female C57BL/6 [de Visser L, van den Bos R, Spruijt BM. Automated home cage observations as a tool to measure the effects of wheel running on cage floor locomotion. Behav Brain Res 2005;160:382–8]. In the present study, we investigated the effects of novelty-induced stress on wheel running and its impact on home cage behaviour in male C57BL/6 and DBA/2 mice. Our aim was to determine whether wheel running may be used as a tool to study both genetic and environmentally induced differences in sensitivity to rewarding behaviour in mice. One group of male mice was placed in an automated home cage observation system for 2 weeks with a wheel integrated in the cage. A second group of mice was allowed to habituate to this cage for 1 week before a running wheel was introduced. Results showed a pronounced sensitising effect of novelty on the level of wheel running in C57Bl/6 mice but not in DBA mice. Overall levels of wheel running were higher in DBA/2 mice. Furthermore, wheel running affected circadian rhythmicity in DBA/2 mice but not in C57BL/6 mice.

From these findings we tentatively suggest that wheel running behaviour could serve as a tool to study the interaction between genetic and environmental factors in sensitivity to rewarding behaviour in mice. As it is displayed spontaneously and easy to monitor, wheel running may be well suitable to be included in high-throughput phenotyping assays.

Keywords: Wheel running activity; Mice; Reward-sensitivity; Inbred strains; Home cage observations

1. Introduction

Wheel running activity has since long been used as a read out parameter for locomotor and circadian activity in rodents. However, evidence of the rewarding and potentially addictive properties of running wheels is currently accumulating [2]. In mice, wheel running behaviour is performed at the expense of other behaviours, such as resting and cage floor locomotion, and has the potential to disrupt the daily organization of activity [13,29]. Behavioural studies investigating the rewarding properties of wheel running have unequivocally shown that rodents are highly motivated to gain access to running wheels and display conditioned place preference to an environment associated with wheel running [17,23].

Neuronal substrates for the rewarding and reinforcing aspects of wheel running behaviour are suggested to be related to the mesolimbic dopamine-opioid system [18,26,30,32] which is also involved in the addictive properties of drugs of abuse [16,21]. Similar to drugs of abuse, wheel running increases neuronal activation in the nucleus accumbens [27,31], which suggests a common neurobiological background. The involvement of dopamine in the acquisition and maintenance of high levels of wheel running is confirmed by several studies using mutant mice with impaired dopamine functioning, such as the D2L receptor-deficient [26] and Nurr1-deficient mice [32]. Considering the shared behavioural and neurological characteristics with drug-induced reward situations, wheel running behaviour

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gains interest as a tool to study mechanisms underlying reward-sensitivity. The present study aims to determine whether wheel running could be used as a tool to study interactions between genetic and environmentally induced differences in sensitivity to rewarding behaviours in mice.

Previously, we showed that wheel running markedly affected home cage behaviour in female C57BL/6 mice [29]. In this study three aspects of wheel running were described: (1) the level of wheel running activity in terms of the time spent in the running wheel, (2) the effect of access to a running wheel on other home cage behaviour, such as cage floor movement and (3) the effect of the running wheel on circadian rhythmicity of cage floor movement. In the present study, we further investigated these three aspects of wheel running.

Both animal and human studies report gender differences in the responses to a rewarding substance and a possible influence of the menstrual cycle on the enhanced response in females [10,15]. First aim of the present study was therefore to investigate wheel running in male C57BL/6 mice to detect a possible gender difference when comparing the results of the present study with male C57BL/6 mice to our earlier findings in female C57BL/6. We expected a lower level of wheel running in male C57BL/6 mice.

C57BL/6 and DBA/2 mice are known to differ in their response to psychostimulants, such as amphetamine [4,22], and provide the opportunity to investigate the genetic basis of sensitivity to rewards. A second aim of the present study therefore was to compare wheel running between male C57BL/6 and DBA/2 mice. As C57BL/6 are characterized as being more sensitive to the locomotor-activity enhancing effects of rewarding substances such as amphetamine [22], we expected C57BL/6 mice to display higher levels of wheel running than DBA/2 mice.

In our previous study in female C57BL/6 mice, animals were exposed to the running wheel immediately upon introduction to a novel home cage [29]. It is known that stress may enhance behavioural responses to rewarding stimuli [20] that is often reflected by increased locomotor activity [22]. This stress-induced sensitisation is caused by an increase in glucocorticoid hormones that appear to exert their effects on the mesolimbic dopamine system via the glucocorticoid receptor [8]. Exposure to a novel environment is known to increase glucocorticoid levels in mice [13,14] and is considered a mild psychological stressor [1]. In addition, increased familiarity with the environment attenuates the locomotor response to a dopamine-agonist (apomorphine; [12]) and stimulants such as cocaine [5]. Therefore, a third aim of the present study was to investigate whether exposure to a novel environment has a sensitising effect on the level of wheel running and an impact on home cage behaviour. Mice were either provided with a running wheel immediately upon introduction to a novel home cage or after 1 week of habituation to the novel cage. We hypothesized increased levels of wheel running in mice that were exposed to the wheel under novelty conditions.

In sum then, the present study aims at investigating effects of genetic and environmental factors on wheel running in mice.

2. Methods

2.1. Animals and husbandry

Male mice of the C57BL/6JOlaHsd (C57, $n = 24$) and DBA/2JOlaHsd (DBA, $n = 24$) inbred strain were obtained from Harlan (Germany) at 8 weeks of age; experiments started when the animals were 10–11-weeks old. Prior to the experiments, mice were individually housed in Macrolon type II cages and maintained under a reversed light/dark cycle (white light: 19.00–07.00 h) with food and water available ad libitum. All animals were provided with a shelter, tissues and paper shreds as environmental enrichment. Humidity was kept at a constant level and room temperature was maintained at $21.0 \pm 2.0\,^{\circ}C$. The Animal Ethical Committee of Utrecht University approved all experiments.

2.2. Home cage and running wheel activity

During the experiment, home cage behaviour and running wheel activity was automatically recorded by videotracking in specially designed home cages (PhenoTyper® and EthoVision 3.0, Noldus Information Technology, Wageningen, The Netherlands). de Visser et al. [29] provide a detailed description of this system. In short, each cage contained a feeding station, water bottle and a shelter (10 cm × 10 cm × 5 cm) and nesting material (tissues and paper shreds) as environmental enrichment. A running wheel was attached to one of the walls of the cage, either prior to introduction of the mice to the new home cage, or after 1 week of habituation (see Section 2.3). The running wheels had a perimeter of 38 cm and a circular running surface consisting of steel rods. Running wheel revolutions were automatically recorded by a sensor connected to a X-keys device that converted the signal into keyboard input. EthoVision integrated this keyboard input to count the number of revolutions per cage during the experiment.

Several parameters were calculated which were subjected to further analysis: 'sheltertime' (time spent inside the shelter in s), 'cage floor movement' (time spent on moving on the cage floor in s), 'velocity' (speed of moving in cm/s), 'feeding' (time spent at the feeding station in s) and 'RWtime' (time spent in the running wheel in s). All parameters were calculated in 1-h bins and subsequently averaged for 12-h fragments to distinguish dark/light periods. For circadian rhythmicity, hourly values of the parameter 'cage floor movement' were used.

Table 1
Experimental design

Group	Strain	n	Week 0 (7 days)	Week 1 (7 days)	Week 2 (7 days)	Week 3 (7 days)	Week 4 (1 day)
NO-HAB	C57	12	n.a.	RW	RW	no RW	RW
HAB	C57	12	no RW	RW	RW	no RW	RW
NO-HAB	DBA	12	n.a.	RW	RW	no RW	RW
HAB	DBA	12	no RW	RW	RW	no RW	RW

Mice of both C57BL/6 (C57) and DBA/2 (DBA) strains were randomly assigned to either a group that was provided with a running wheel immediately upon introduction to the new home cage (NO-HAB) or to a group that was allowed to habituate for 1 week (week 0) before they were provided with a running wheel (HAB). n.a., mice were not yet introduced to the new cage (NO-HAB group only); no RW, no running wheel present in the cage; RW, ad libitum access to a running wheel.

2.3. Experimental design

Mice of both strains were randomly assigned to either of two groups (Table 1): one group was introduced to the PhenoTyper-cage with a running wheel integrated in the cage (NO-HAB, $n = 12$ per strain), while the other group was allowed to habituate to the cage for 1 week before a running wheel was introduced (HAB, $n = 12$ per strain). The habituation period of 1 week was determined in earlier experiments with male mice of C57 and DBA strains (unpublished data). Introduction of the wheel in the HAB-group was performed during cage cleaning while the mice were removed from the cage for body weight measurement. Each group had ad libitum access to the running wheels for 2 weeks to study acquisition and maintenance of wheel running. After 2 weeks, the running wheels were removed and reintroduced after 1 week to measure a possible rebound effect. During the experimental period, behavioural recordings were only interrupted for cage cleaning, which occurred on the first day of every week.

2.4. Statistical analysis

Statistical analyses were conducted using SPSS 10.0 for Windows. Analysis was done separately for the dark and light period of the day. To detect strain differences in the influence of novelty on wheel running activity, repeated measures ANOVAs were performed (within-subjects factor: 'week' and between-subjects factors: 'group' and 'strain'). Post hoc analysis was performed separately for each strain with one-way ANOVA with factor 'group' to detect differences between NO-HAB and HAB groups within each strain in wheel running activity. A possible rebound in wheel running activity after removal of the running wheel, was tested by comparing mean RWtime of week 2 with mean RWtime of week 4 in a repeated measures ANOVA (within-subjects factor 'week' for weeks 2 and 4 and between-subjects factors 'group' and 'strain'). Note that week 4 consisted only of 1 day.

To determine differences between strains (C57 and DBA), groups (NO-HAB and HAB) or weeks (week 0, week 1, week 2 and week 3), one-way ANOVAs were performed using means per week. Note that for week 0, mean of day 7 only was used for analysis as this day represents a baseline in home cage behaviour prior to introduction of the running wheel. Furthermore, baseline values found in the HAB group during week 0 were also used as baseline values for the NO-HAB group. Post hoc comparisons between weeks were carried out using the Scheffé test.

Strain differences in the effect of the running wheel on circadian rhythmicity was calculated with repeated measures ANOVA using the within-subjects factors 'hour' for hour 13–24, i.e. the dark period of the day, and 'week' (i.e. weeks 0 and 2) and the between-subjects factors 'group' and 'strain'. Post hoc analysis was performed separately for each strain with repeated measures ANOVA with within-subjects factors 'hour' and 'week'. Statistical significance was assigned at $p \leq 0.05$.

3. Results

3.1. General remark

As activity during the light period of the day was minimal and did not show any differences between strains or groups, it was decided to present data only of the dark period for both running wheel activity and other home cage behaviour. A series of power failures in the surroundings of the animal facility resulted in data loss. The number of animals used in analysis therefore varies between weeks and groups, but for each test n was between 8 and 12 animals. However, for week 3 of the C57 (NO-HAB) group, data of only four animals could be rescued.

Regarding the description of the results, there were three factors that could differ between sets of data: (1) the between-subjects factor 'strain' for comparisons between C57 and DBA mice, (2) the within-subjects factor 'group' for comparisons between mice that were either provided with a running wheel immediately upon introduction to the novel home cage (NO-HAB) and mice that were allowed to habituate (HAB) and (3) the within-subjects factor 'week' for comparisons between data from the different weeks of the experiment. Notably, for the effects of wheel running on circadian rhythmicity, a third within-subject factor 'hour' was used that reflected the hourly distribution of cage floor movement over the time span of a dark period.

3.2. Level of running wheel activity

Running wheel activity was measured as both the distance run based on the number of revolutions and the time spent in the running wheel. A significant, positive, correlation was found between distance run and time spent in the running wheel ($r = 0.710$, $p < 0.001$). Therefore it was decided to use a single measure of running wheel activity for further analysis. Time spent in the running wheel ('RWtime') was chosen because it could be directly compared to time spent on other home cage behaviour, such as sheltertime and cage floor movement.

Fig. 1 shows differences between NO-HAB and HAB groups in RWtime for both C57 (Fig. 1A) and DBA mice (Fig. 1B). Novelty differentially affected the level of wheel running in C57 and DBA mice, reflected by a significant two-way interaction (between-subjects factors group × strain interac-

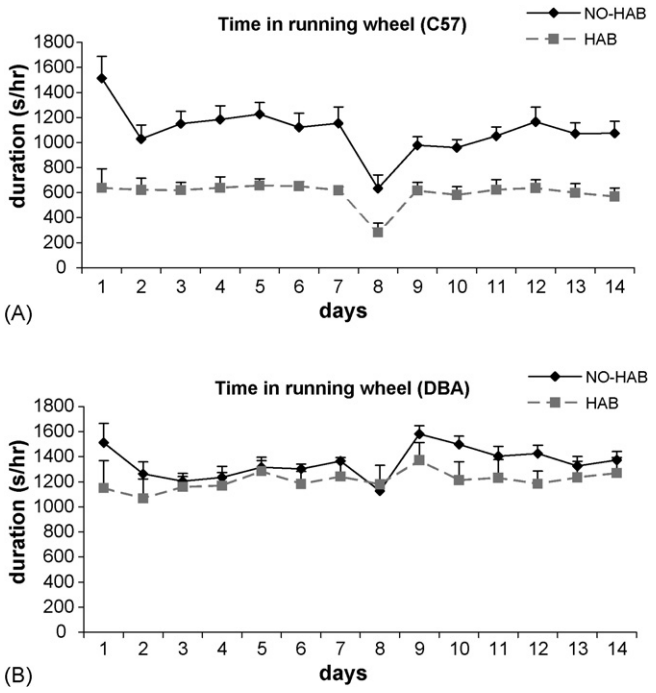

Fig. 1. Time spent in the running wheel during the dark period in week 1 (days 1–7) and week 2 (days 8–14) for mice provided with running wheel immediately upon introduction to the new home cage (NO-HAB) and mice that were allowed to habituate to the new cage for 1 week before a running wheel was provided (HAB). Results are presented separately for C57 mice (panel A) and DBA mice (panel B). Means and S.E.M. of 12-h bins are used. In C57 mice, a strong increase is found in wheel running in mice of the NO-HAB group compared to the HAB group, whereas no difference is seen between HAB and NO-HAB groups in DBA mice.

Table 2
Mean values per week for weeks 0–3 of home cage behaviours and running wheel activity

Parameter	Strain	Group	Week 0	*Week*1	*Week*2	Week 3
Cage floor movement	C57	NO-HAB	n.a.	154.6 (14.2)	125.3 (9.6)	163.6 (14.8)$^{\&}$
		HAB	109.5 (10.5)	116.5 (7.9)$^{\#}$	81.3 (11.3)$^{\#}$	63.6 (11.2)
	DBA	NO-HAB	n.a.	182.2 (19.4)	148.8 (15.9)	164.2 (12.3)
		HAB	143.8 (11.4)*	161.8 (11.5)*	142.3 (13.0)*	141.3 (6.3)*
Velocity	C57	NO-HAB	n.a.	9.7 (0.2)	10.6 (0.6)	9.3 (0.3)
		HAB	10.2 (0.4)	10.0 (0.2)	10.8 (0.3)	11.3 (0.5)
	DBA	NO-HAB	n.a.	10.4 (0.3)*	11.8 (0.6)	9.9 (0.5)
		HAB	10.5 (0.3)	9.9 (0.2)	11.3 (0.9)	9.8 (0.1)*
Feeding	C57	NO-HAB	n.a.	55.5 (19.6)$^{\&}$	143.3 (47.8)	263.2 (73.6)
		HAB	450.2 (133.6)	152.9 (36.4)$^{\#}$	150.2 (39.1)	658.4 (82.9)
	DBA	NO-HAB	n.a.	371.7 (89.8)*	369.7 (77.0)*	738.1 (87.4)*
		HAB	498.5 (68.8)	215.2 (36.1)$^{\&}$	251.1 (51.8)$^{\&}$	799.0 (93.0)$^{\&}$
Sheltertime	C57	NO-HAB	n.a.	1456.2 (169.5)$^{\&}$	1666.6 (136.1)$^{\&}$	2231.1 (84.2)
		HAB	2185.4 (114.0)	1879.0 (172.4)$^{\#}$	2095.8 (126.4)$^{\#}$	2435.8 (116.9)
	DBA	NO-HAB	n.a.	1098.2 (112.6)$^{\&}$	1183.6 (145.7)$^{\&}$	1859.2 (137.3)$^{\&}$
		HAB	2349.5 (104.7)	1327.7 (110.1)$^{\&}$	1337.5 (131.9)$^{\&}$	1941.5 (79.9)$^{\&}$
RWtime	C57	NO-HAB	n.a.	1197.7 (98.2)	991.4 (83.8)	n.a.
		HAB	n.a.	635.8 (63.1)$^{\#}$	558.5 (70.2)$^{\#}$	n.a.
	DBA	NO-HAB	n.a.	1314.1 (110.0)	1390.4 (87.4)*	n.a.
		HAB	n.a.	1179.5 (104.5)*	1240.4 (144.9)*	n.a.

Definition of behaviours are stated in Section 2. Symbols in bold refer to statistical significance: $^{*}p < 0.05$ compared to C57 of the same group; $^{\&}p < 0.05$ compared to week 0; $^{\#}p < 0.05$ compared to NO-HAB group.

tion, $F_{(1,89)} = 7.867$, $p = 0.006$). Post hoc analysis revealed that within the C57 strain, mice that were allowed to habituate to the new home cage before introduction of the running wheel spent only half as much time in the wheels as mice that were provided with a running wheel immediately upon introduction to the new cage (one-way ANOVA with between-subjects factor 'group'; $F_{(1,43)} = 42.831$, $p < 0.001$). In contrast, in DBA mice, no differences were found between mice provided with running wheel upon introduction to the novel cage (NO-HAB group) and mice that were allowed to habituate (HAB group) (one-way ANOVA, between-subjects factor 'group': $F_{(1,44)} = 1.932$, $p = 0.172$). Overall, level of wheel running was higher in DBA mice compared to C57 mice, when factor 'group' (NO-HAB or HAB) was ignored (repeated measures ANOVA, between-subjects factor 'strain', $F_{(1,89)} = 25.528$, $p < 0.001$).

Notably, running wheel activity was highly stable over the course of the experiment in both strains. This was reflected by a non-significant effect of the within-subject factor 'week', irrespective of strain ($F_{(1,89)} = 0.334$, $p = 0.565$). However, on day 1 of the second week a decrease in time spent in the running wheel was seen in all groups (see Fig. 1), because of cage cleaning that was performed at the beginning of the dark period. During this time period mice normally ran the largest part of their daily distance (data not shown).

To determine whether a rebound effect existed after removal of the running wheel, RWtime during the day before removal (day 7 of week 2) was compared to the first day of reintroduction of the wheel (day 1 of week 4). No significant differences were found, irrespective of strain or group (repeated measures ANOVA, within-subjects factor 'week', $F_{(1,35)} = 0.647$, $p = 0.427$), indicating that mice did not increase their RWtime after a period of deprivation of the wheel.

3.3. Impact wheel running on other home cage behaviours

Table 2 shows the means per week for all home cage measurements and significant differences between groups, strains and weeks. The impact of wheel running on other home cage behaviours consisted of two aspects; the short-term effect during exposure to the running wheel (means of weeks 1 and 2 compared to baseline) and the long-term effects after removal of the wheel (means of week 3 compared to baseline). Furthermore, differences between NO-HAB and HAB groups for each strain were determined to account for the effect of novelty on the impact of the running wheel.

The results in Table 2 shows that access to a running wheel differentially affected other home cage behaviours in male C57 and DBA mice. In C57 mice, time spent at the feeder and inside the shelter decreased during exposure to the running wheel for the NO-HAB group only. In DBA mice, however, time spent at the feeder and inside the shelter decreased both in the NO-HAB and HAB group. Moreover, during exposure to the wheel, differences between HAB and NO-HAB groups were found in C57 mice, reflected by higher activity levels (in terms of cage floor movement, sheltertime and RWtime) under novelty conditions. By contrast, no differences were found between NON-HAB and HAB groups in DBA mice.

Long-term effects of the running wheel were seen after removal of the wheel. Time at feeder increased markedly in the NON-HAB group in both strains and in the HAB group in DBA. Interestingly, time at feeder in this period did not differ

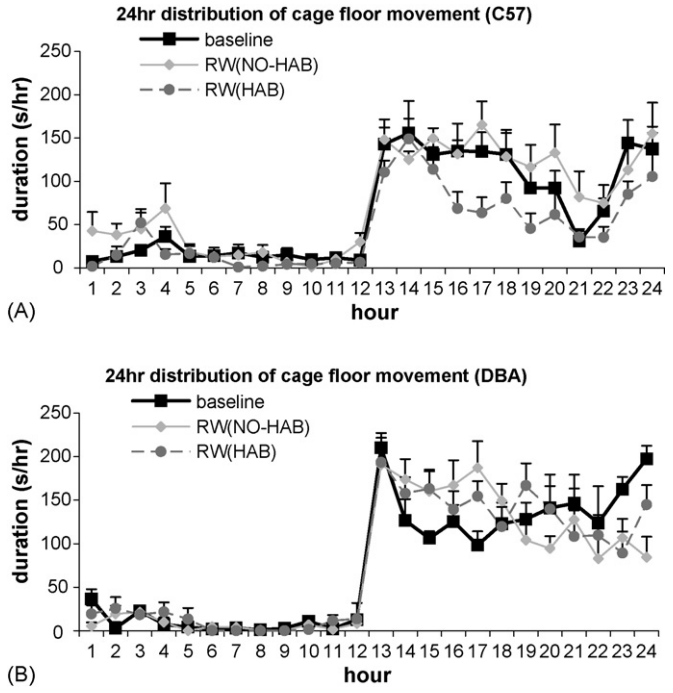

Fig. 2. Circadian rhythmicity expressed as the 24-h distribution of cage floor movement prior to introduction to the running wheel (baseline; day 7 of week 0) or after 2 weeks of running in both NO-HAB and HAB groups (RW(NO-HAB) and RW(HAB); day 7 of week 2). Results are presented separately for C57 mice (panel A) and DBA mice (panel B). Means and S.E.M. of 1-h bins are used. The white bar represents the light period of the day (hours 1–12), whereas the black bar represents the dark period of the day (hours 13–24). In DBA mice there was a significant difference in the pattern of cage floor movement during baseline compared to after 2 weeks of running RW(NO-HAB) and RW(HAB). This effect was not found in C57 mice.

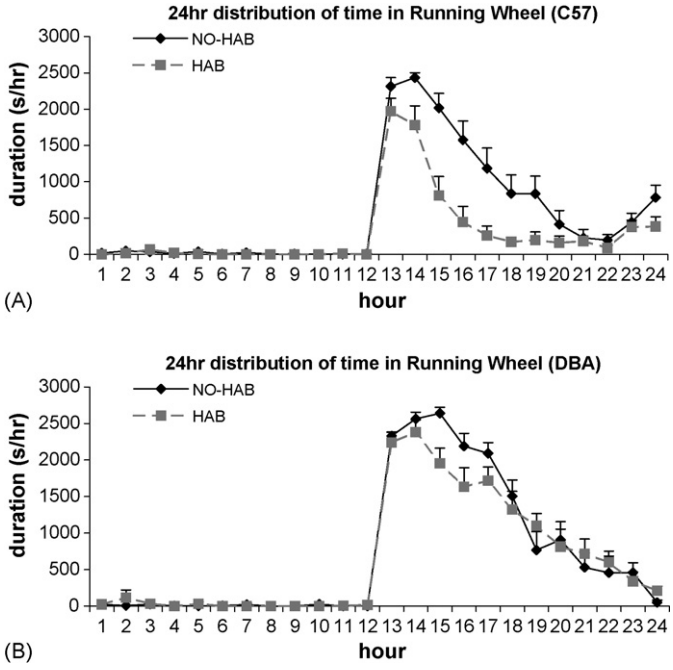

Fig. 3. Circadian rhythmicity of time spent in the running wheel after 2 weeks of running in NO-HAB and HAB groups (day 7 of week 2). Results are presented separately for C57 mice (panel A) and DBA mice (panel B). Means and S.E.M. of 1-h bins are used. The white bar represents the light period of the day (hours 1–12), whereas the black bar represents the dark period of the day (hours 13–24).

from baseline levels in C57, but did so in DBA (higher in week 3 compared to week 0). Also, time in shelter increased in week 3 with respect to baseline in DBA mice, whereas in C57 mice there was an increase in cage floor movement in week 3 compared to baseline.

3.4. Impact wheel running on circadian rhythmicity

Access to a running wheel differentially affected circadian rhythmicity of cage floor movement in C57 and DBA mice (Fig. 2). This was reflected by a significant three-way interaction between the within-subjects factors 'hour' and 'week' and between-subjects factor 'strain' in a repeated measures ANOVA ($F_{(1,330)} = 3.097$, $p = 0.007$). A post hoc analysis revealed a significant within-subjects 'hour' × 'week' interaction in DBA mice ($F_{(11,154)} = 3.883$, $p = 0.002$), indicating that the hourly distribution of cage floor movement in this strain was affected when a running wheel was present, irrespective of group (HAB versus NO-HAB). At baseline, the pattern consisted of two distinct activity peaks, at the start and the end of the dark phase. When a running wheel was present, the second peak was less pronounced (HAB group) or absent (NO-HAB group). The pattern of cage floor movement during exposure to the running wheel was similar to that of running wheel activity, which also consisted of one marked peak at the beginning of the dark phase and subsequently a gradual decrease (Fig. 3). In C57 mice, no influence of the running wheel on circadian rhythmicity could be detected (repeated measures ANOVA, within-subjects factor 'hour' × 'week' interaction, $F_{(11,176)} = 0.742$, $p = 0.580$).

4. Discussion

4.1. General

In the present study, we investigated whether wheel running could be used as a tool to study both genetic and environmentally induced differences in sensitivity to rewarding behaviours in mice. Wheel running under novelty-induced and habituated conditions was found to differentiate between male C57 and DBA mice. DBA mice showed higher levels of running wheel activity, but were apparently unaffected by environmental novelty. In contrast, exposure to a novel environment profoundly increased wheel running in C57 mice. In addition, home cage behaviour in DBA mice seemed more disrupted by the running wheel as reflected by both the circadian pattern of cage floor movement and the inability to adjust behaviour after removal of the running wheel. The results of the present study combined with findings from our earlier study on wheel running in female C57BL/6 mice [29] are summarized in Table 3. Here, relative scores are assigned to each test group for all three aspects of wheel running (level of wheel running, impact on home cage behaviours and impact on circadian rhythmicity).

Table 3
Summary of findings from the present study and an earlier study on wheel running in female C57BL/6 mice [29]

	C57			DBA	
	NO-HAB		HAB Male	NO-HAB Male	HAB Male
	Female[a]	Male			
Level of wheel running	***	**	*	***	***
Home cage behaviour	+	+	−	+	+
Circadian rhythmicity	+	−	−	+	+

Relative scores are assigned to each group for three aspects of wheel running: (1) the level of wheel running, (2) impact of wheel running on home cage behaviour and (3) impact of wheel running on circadian rhythmicity. A relative score for the level of wheel running is indicated with asterisks (* = low, ** = intermediate and *** = high). Impact of wheel running on home cage behaviour and circadian rhythmicity is indicated as + (impact) or − (no impact).

[a] Results from de Visser et al. [29].

4.2. Gender difference in wheel running

The disruptive properties of wheel running were previously described in female C57 mice [29]. In the latter study, a group of female mice was presented with a running wheel immediately upon introduction to the novel home cage (similar to the NO-HAB group in the present study) and compared to a control group that did not have access to a running wheel. Mice with a running wheel showed a severely disturbed pattern of cage floor movement during the dark phase, an increase in overall activity levels and in velocity of cage floor movement indicating an elevated state of arousal. If we compare these data to the present findings of the male C57 mice, a remarkable gender difference within the C57 strain appears in all three aspects of wheel running. Females display higher levels of wheel running and are more affected by the disruptive properties of wheel running on other home cage behaviours and circadian rhythmicity. These findings are in line with our hypothesis and supported by evidence from studies that found an influence of hormonal fluctuations during the menstrual cycle on the enhanced behavioural response to rewarding substances in female rats [10] and humans [15].

4.3. Inbred strain differences in wheel running

Male DBA mice but not male C57 mice showed a disrupted circadian pattern of cage floor activity and were predominantly affected after removal of the wheel, indicating an increased sensitivity to both the disruptive and long-term effects of wheel running that was in contrast to our hypothesis. This is also reflected in the overall higher levels of wheel running in DBA mice compared to C57 mice. The high levels of running wheel activity of DBA compared to C57 are supported by a study that compared wheel running in 21 inbred strains of mice [19]. However, in drug-induced reward situations such as amphetamine administration [4], C57 mice most often display a stronger increase in locomotor behaviour than DBA mice, which was also confirmed in our lab (data not shown). These data indeed indicate a higher sensitivity in C57 mice to rewarding substances. A possible explanation for these apparently contradictory results may be that baseline activity prior to introduction of the running wheel was also higher in DBA mice compared to C57 mice. However, time spent on wheel running after 2 weeks of exposure to the wheel and cage floor movement at baseline were not correlated (12-h average, Pearson correlation: $r = 0.230$, $p = 0.317$). Apparently, wheel running cannot be directly compared to other forms of locomotion, as was also concluded by others [24]. Thus, the higher baseline activity in DBA mice cannot explain why DBA mice were more sensitive to wheel running in the present study. Alternatively, an explanation might be found in one of the properties of wheel running. Voluntary wheel running causes an increase in plasma corticosterone levels in female house mice [11] and male C57 mice [9]. We performed a pilot study aimed at investigating the relation between wheel running and corticosterone in DBA and C57 mice and found that already after 90 min of wheel exposure there was an increase in plasma corticosterone. Interestingly, this increase was only significant in DBA mice (178% relative to baseline, measured in mice habituated to the cage for 1 week, one-sample t-test: $p = 0.036$, $n = 6$ per group). Following lines of evidence that suggest a facilitating role of glucocorticoids on sensitivity to several rewarding substances, modulation of the HPA-axis by chronic wheel running may underlie the high levels of running seen in DBA mice. This hypothesis is supported by evidence of high vulnerability to develop stress-induced behavioural sensitisation in DBA mice [4]. While DBA mice show only a moderate increase in locomotor activity following amphetamine administration, a reversal is seen under stressfull conditions, such as food deprivation. In addition, DBA mice react to chronic or repeated stressors with enhanced locomotor activity and stereotypic behaviour, while stressed C57 mice show a reduced locomotor response. Thus, the chronic increase in corticosterone levels caused by wheel running combined with high stress-vulnerability might result in a 'feed-forward' mechanism that boosts levels of wheel running in DBA mice and increases their sensitivity to the impact of wheel running on other home cage behaviours and circadian rhythmicity.

4.4. Environmental novelty increases level of wheel running in C57

Environmental novelty resulted in a two-fold increase in wheel running activity in male C57 mice. Interestingly, this increase in wheel running remained constant over the course of the experiment, even though mice are known to habituate to the novel cage within 4–5 days [28]. These pronounced effects

of environmental novelty were not seen in DBA mice. A possible 'ceiling' effect, a physical maximum due to e.g. exhaustion, in DBA mice may have masked the effects of novelty. But also when other home cage behaviours are considered, novelty does not seem to have an effect in DBA mice, whereas in C57 mice environmental conditions did affect the impact of wheel running on activity measures (sheltertime and cage floor movement). The pronounced effect of a novel environment on wheel running in C57 mice might be explained by the high reactivity to novel environments that is characteristic for this strain [7]. Furthermore, it was found that C57 mice only showed behavioural sensitisation to a rewarding substance, such as amphetamine, in a novel environment and not in the home cage [3]. The reinforcing properties of the running wheel itself may have resulted in a continuation of the initial high levels of wheel running that were a direct response to the novel environment.

4.5. Wheel running as a tool to investigate reward-sensitivity

The findings summarized in Table 3 suggest future directions for further research to find underlying neuronal mechanisms of sensitivity to the rewarding properties of wheel running. Both the level of wheel running and the disruptive effects of wheel running on other behaviours and the circadian pattern of activity may model behavioural responses to rewarding substances in general.

The role of novelty-induced stress in the enhanced wheel running response may be studied using antagonists of the glucocorticoid-receptor that is thought to be involved in stress-induced sensitisation regulated by the mesolimbic dopamine system [8,20] Thus, GR-antagonist should block the increase in wheel running activity seen in C57 mice under novelty conditions.

The disruption of home cage behaviours and circadian rhythmicity seen in male DBA mice as well as female C57 mice may be behavioural indicators of the addictive properties of wheel running suggested by others [2,30,32]. One of the approaches to determine whether wheel running can be regarded as a compulsive behaviour may be to present an aversive conditioned stimulus that should then not be able to suppress wheel running. This approach has already been proven to distinguish compulsive from casual drug seeking in rats [25].

The running wheel can be used as a tool to investigate aspects of reward-sensitivity in more detail using the appropriate conditions. Within the C57 strain, the observed gender differences in disruption of home cage behaviour by wheel running under novelty conditions can be used to investigate the hormonal control of sensitivity to disruptive and maybe even addictive properties of rewarding behaviours. Within male C57 mice, wheel running allows investigation of the modulation of the response to rewarding stimuli by environmental conditions or stressors. Genetic factors underlying sensitivity to disruptive properties of rewarding behaviours can be studied using differences in wheel running between C57 and DBA mice. Interestingly, these inbred strains are also the parental lines for the genetic reference population of recombinant BxD inbred lines which allows a comparison of phenotypic data with genetic information to find QTLs for specific phenotypic traits [6] (http://www.genenetwork.org).

5. Concluding remarks

Wheel running can be used to study both environmental and genetic factors underlying reward-sensitivity in mice. Together, the level of wheel running and the disruptive effects on other behaviours and circadian rhythmicity are indicators of sensitivity to the rewarding properties of wheel running and may model aspects of sensitivity to rewarding substances. Both genetic background and environmental novelty were found to affect wheel running.

Integration of running wheels in an automated home cage observation system allows simultaneous measurements of wheel running activity and its impact on other home cage behaviours. As it is displayed spontaneously and easy to monitor, wheel running may be well suitable to be included in high-throughput phenotyping assays.

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References

[1] Adriani W, Laviola G. A unique hormonal and behavioral hyporesponsivity to both forced novelty and d-amphetamine in periadolescent mice. Neuropharmacology 2000;39:334–46.
[2] Belke W. Investigating the reinforcing properties of running: or, running is its own reward. In: Epling WF, Pierce WD, editors. Activity anorexia: theory, research, and treatment. Mahwah, NJ: Erlbaum; 1996. p. 45–55.
[3] Cabib S. Strain-dependent behavioural sensitization to amphetamine: role of environmental influences. Behav Pharmacol 1993;4:367–74.
[4] Cabib S, Bonaventura N. Parallel strain-dependent susceptibility to environmentally induced stereotypies and stress-induced behavioral sensitization in mice. Phys Behav 1997;61:499–506.
[5] Carey RJ, DePalma G, Damianopoulos E. Acute and chronic cocaine behavioral effects in novel versus familiar environments: open-field familiarity differentiates cocaine locomotor stimulant effects from cocaine emotional behavioral effects. Behav Brain Res 2005;158:321–30.
[6] Chesler EJ, Wang J, Lu L, Qu Y, Manly KF, Williams RW. Genetic correlates of gene expression in recombinant inbred strains: a relational model to explore for neurobehavioral phenotypes. Neuroinformatics 2003;1:343–57.
[7] Crawley JN, Belknap JK, Collins A, Crabbe JC, Frankel W, Henderson N, et al. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology 1997;132:107–24.
[8] DeJong IEM, DeKloet ER. Glucocorticoids and vulnerability to psychostimulant drugs: toward substrate and mechanism. Ann NY Acad Sci 2004;1018:192–8.
[9] Droste SK, Gesing A, Ulbricht S, Muller MB, Linthorst ACE, Reul JMHM. Effects of long-term voluntary exercise on the mouse hypothalamic–pituitary–adrenocortical axis. Endocrinology 2003;144:3012–23.
[10] Festa ED, Quinones-Jenab V. Gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine. Horm Behav 2004;46:509–19.

[11] Girard I, Garland Jr T. Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. J Appl Physiol 2002;92:1553–61.
[12] Harkin A, Kelly JP, Frawley J, O'Donnell JM, Leonard BE. Test conditions influence the response to a drug challenge in rodents. Pharmacol Biochem Behav 2000;65:389–98.
[13] Harri M, Lindblom J, Malinen H, Hyttinen M, Lapvetelainen T, Eskola S, et al. Effect of access to a running wheel on behavior of C57BL/6J mice. Lab Anim Sci 1999;49:401–5.
[14] Hennessy MB, Foy T. Non-edible material elicits chewing and reduces the plasma corticosterone response during novelty exposure in mice. Behav Neurosci 1987;101:237–45.
[15] Justice AJH, De Wit H. Acute effects of D-amphetamine during the follicular and luteal phases of the menstral cycle in women. Psychopharmacology 1999;145:67–75.
[16] Koob GF. Neural mechanisms of drug reinforcement. Ann NY Acad Sci 1992;654:171–91.
[17] Lett BT, Grant VL, Byrne MJ, Koh MT. Pairings of a distinctive chamber with the after effect of wheel running produce conditioned place preference. Appetite 2000;34:87–94.
[18] Lett BT, Grant VL, Koh MT, Flynn G. Prior experience with wheel running produces cross-tolerance to the rewarding effect of morphine. Pharmacol Biochem Behav 2002;72:101–5.
[19] Lightfoot JT, Turner MJ, Daves M, Vordermark A, Kleeberger SR. Genetic influence on daily wheel running activity level. Physiol Genomics 2004;19:270–6.
[20] Marinelli M, Piazza PV. Interaction between glucocorticoid hormones, stress and psychostimulant drugs. Eur J Neurosc 2002;16:387–94.
[21] Nestler E. Molecular mechanisms of drug addiction. J Neurosci 1992;12:2439–50.
[22] Phillips TJ, Dickinson S, Burkhart-Kasch S. Behavioral sensitization to drug stimulant effects in C57BL/6J and DBA/2J inbred mice. Behav Neurosci 1994;108:789–803.
[23] Sherwin CM. The use and perceived importance of three resources which provide caged laboratory mice the opportunity for extended locomotion. Appl Anim Behav Sci 1998;55:353–67.
[24] Sherwin CM. Voluntary wheel running: a review and novel interpretation. Anim Behav 1998;56:11–27.
[25] Vanderschuren LJMJ, Everitt BJ. Drug seeking becomes compulsive after prolonged cocaine self-administration. Science 2004;305:1017–9.
[26] Vargas-Perez H, Borrelli E, Diaz J-L. Wheel running use in dopamine D2L receptor knockout mice. Neurosci Lett 2004;366:172–5.
[27] Vargas-Perez H, Mena-Segovia J, Giordano M, Diaz J-L. Induction of c-fos in nucleus accumbens in naive male Balb/c mice after wheel running. Neurosci Lett 2003;352:81–4.
[28] de Visser L, van den Bos R, Kuurman WW, Kas MJH, Spruijt BM. Novel approach to the behavioural characterization of inbred mice: automated home cage observations. Genes Brain Behav 2006;5:458–68.
[29] de Visser L, van den Bos R, Spruijt BM. Automated home cage observations as a tool to measure the effects of wheel running on cage floor locomotion. Behav Brain Res 2005;160:382–8.
[30] Werme M, Hermanson E, Carmine A, Buervenich S, Zetterstrom RH, Thoren P, et al. Decreased ethanol preference and wheel running in Nurr1-deficient mice. Eur J Neurosci 2003;17:2418–24.
[31] Werme M, Messer C, Olson L, Gilden L, Thoren P, Nestler EJ, et al. Delta FosB regulates wheel running. J Neurosci 2002;22:8133–8.
[32] Werme M, Thoren P, Olson L, Brene S. Running and cocaine both upregulate dynorphin mRNA in medial caudate putamen. Eur J Neurosci 2000;12:2967–74.