Effect of Cage Enrichment on the Daily Use of Running Wheels by Syrian Hamsters

Stéphan G. Reebs* and Dominique Maillet

Département de Biologie, Université de Moncton, Moncton, NB, Canada

ABSTRACT

Institutional animal care committees may one day require for the welfare of captive hamsters more floor space and the introduction of tunnels and toys. As hamsters are popular animal subjects in chronobiological research, and as clock phase is usually measured through running wheel activity, it is important to determine what effect cage enrichment might have on daily wheel use. Here the daily number of wheel revolutions, the daily duration of the running activity phase, the phase relationship between lights-off and onset of running activity, and the free-running period of circadian activity rhythms were measured in Syrian hamsters, Mesocricetus auratus, housed in single cages or in multiple cages linked by tunnels and supplied with commercial wooden toys. Free-running periodicity was not affected by cage enrichment. In multiple-cage systems, there were fewer daily revolutions, shorter wheel-running activity phases, and delayed running activity onsets. These effects, however, were small as compared to interindividual and week-to-week variation. They were statistically significant only under a light-dark cycle, not in constant darkness, and only when interindividual variation was eliminated through a paired design or when the number of cages was increased to five (the maximum tested). Daily wheel use is thus affected by cage enrichment, but only slightly.

Key Words: Animal welfare; Circadian; Daily activity; Enrichment; Hamster; Wheel running.

*Correspondence: Stéphan Reebs, Département de Biologie, Université de Moncton, Moncton, NB, E1A 3E9, Canada; Fax: 506-858-4541; E-mail: reebss@umoncton.ca.
INTRODUCTION

Animal care committees may one day require improvements to the conditions that are currently imposed for the housing of laboratory rodents. These improvements could include additional space and the introduction of toys and novel objects in the cage, as these factors have already been shown to matter to some animals (Sherwin, 1996; Sherwin and Nicol, 1996; 1997). If and when improvements of this kind are imposed, chronobiologists might worry that such changes would disrupt the way laboratory rodents use their running wheel. Running wheel activity is the main “hand” of the clock measured in rodent-based chronobiological research. Although any putative change in daily wheel use would not matter in studies where such changes would remain constant every day for all experimental groups, it would still pose problems for the results comparison with those from past studies conducted under different standards of animal care.

The goal of the present research was to determine the extent to which enriched cage conditions might affect daily wheel use in Syrian hamsters, *Mesocricetus auratus*. Hamsters are popular subjects in chronobiological research because of their precise daily pattern of wheel running activity. One possible way to enrich their environment—the one we used in this study—is to give them access to more cages linked by tunnels and to provide wooden toys in these additional cages. Hamsters like crawling in tunnels and gnawing on wooden objects (Morin, 1978), and these accessories are commonly sold in pet shops for the specific purpose of improving captivity conditions for hamsters. Following such enrichment, hamsters could conceivably include more patrolling and gnawing in their daily routine. If such activities compete with wheel running in a hamster’s daily time budget, then one might see fewer daily revolutions in the wheel, delayed onset of wheel use relative to dark onset, and shorter duration of the running activity phase. In constant darkness (DD), one might see fewer revolutions and shorter activity phases, as well as changes in the expressed free-running periodicity (tau, τ). Previous studies have reported tau alterations in rodents caused by differences in wheel type (Mrosovsky, 1999), wheel size (Deboer and Tobler, 2000), and wheel use (Aschoff et al., 1973; Edgar et al., 1991; Shioiri et al., 1991; Weisberger et al., 1997).

GENERAL MATERIAL AND METHODS

Hamsters were obtained from Charles River Canada. All were male, 60-d old at the beginning of each experiment. They were kept in windowless rooms under artificial photoperiods provided by incandescent lights, about 200 lux at cage level as measured with a Gossen Lunasix 3 photometer. Ambient temperature was maintained at 21.5 ± 1.5°C. Each cage was 48 × 27 × 20 cm and made of white polypropylene. Litter (Beta Chip) was present in each cage. Food pellets for laboratory rodents (Hagen item # H-1052) and water were available ad libitum.

Single-cage systems consisted simply of one cage with a running wheel. Multiple-cage systems were made by linking a linear series of additional cages to the wheel cage (Fig. 1). Plastic tunnels between cages were 5 cm in diameter, with ascending sections 15 cm high and transverse sections 25 cm long. Each additional cage contained a toy. Toys were made of hardwood; they were either pierced hollow balls, 12 cm in diameter, through
Figure 1. Schematic side view of a five-cage system. The number of cages in the system could be varied by removing some of the end cages and blocking their corresponding tunnels. W = running wheel; T = tunnel; L = wooden toy labyrinth; B = wooden toy bridge.

which the hamsters could crawl (labyrinth, Hagen item # H-1404), or raised platform, 21 × 5 × 7 cm (bridge, Hagen item # H-1412). The wheel cage did not contain toys, but it had the only water bottle in the system and was also where the food pellets were originally put on the cage floor (the hamsters could, and usually did, move the pellets to other cages, though they had to come back to the wheel cage to drink and run in the wheel).

The wheel was 34.5 cm in diameter and its outer surface was wrapped in a Vexar plastic mesh (5 mm mesh size) to provide a better running surface and minimize the possibility of leg injury (Mrosovsky et al., 1998). Each wheel was connected to a computer that tallied the number of wheel revolutions every 6 min (program: Dataquest III, obtained from Mini-Mitter Co., Bend, OR). These data could be downloaded to another computer for the creation of actograms (program: TAU, from Mini-Mitter Co.).

The following variables were obtained from the computer or measured directly on the actograms: REV = the total number of wheel revolutions per day; psi (ψ) = the time interval between the time of lights-off and the daily onset of wheel running (with negative values representing activity onset occurring after lights-off); and alpha (α) = the duration of the daily wheel-running activity phase, i.e., the interval between daily activity onset and offset. Activity onset was defined as the beginning of two consecutive 6-min bins with at least 20 wheel revolutions in each, close to the time of lights-on (or, in DD, close to the previous daily onset time) and preceded by 1 h without such bins. Activity offset was defined as the end of two consecutive 6-min bins with at least 10 wheel revolutions in each, close to the time of lights-off (or, in DD, close to the previous daily offset time) and followed by 1 h without such bins. Figure 2 shows representative examples.

Each variable was measured for each hamster every day, and individual averages were calculated over 7-d or 10-d periods. For the experiments in DD, ψ did not exist; τ was obtained instead by fitting a least-square regression line through 7–10 daily activity onsets and calculating its slope.

EXPERIMENT I: ONE VS. TWO VS. THREE CAGES, LD, UNPAIRED DESIGN

Methods

Hamsters were individually assigned to One-, Two-, and Three-cage systems (n = 9 per system). They were allowed five days to habituate to their surroundings, after which
time data collection ran for three weeks, all under LD 14:10 h. The position of the sleeping hamsters, their food, and their feces were noted each day during the day to verify that all cages were used.

REV, \( \psi \), and \( \alpha \) were averaged every week for each hamster. These data were analyzed with a repeated-measure ANOVA (SPSS 7.5 for Windows), with time (weeks) as the within-group factor and cage system as the between-group factor. Significance level was set at \( P = 0.05 \).

Results

Most hamsters in the two- and three-cage systems were found sleeping in cages other than the wheel cage. A few were sometimes found sleeping in the tunnels. There was intraindividual variation in sleeping place, but in the three-cage system there was a preference (60% of all checks) for the cage farthest away from the wheel cage. Feces were present in all cages, and signs of gnawing appeared on most toys. It seems therefore that the hamsters used all cages at their disposal.

There was no time effect for \( \alpha(F = 0.4, p = 0.67) \), but there was one for REV \( (F = 6.1, p = 0.004) \) and \( \psi(F = 28.5, p < 0.001) \), with REV increasing from week to week, and \( \psi \) decreasing (left side of Fig. 3). The influence of time was similar for all cage groups, as none of the interaction terms were significant \( (F \text{ between 0.1 and 0.5, } p \text{ between 0.75 and 0.97}) \).

No significant difference was detected between the one-, two-, and three-cage groups for either REV \( (F = 2.59, p = 0.096) \), \( \psi(F = 1.03, p = 0.371) \), or \( \alpha(F = 2.67, p = 0.090) \). In cases where a trend was present \( (0.10 > p > 0.05, \text{ REV and } \alpha) \), the difference between the one- and two-cage groups went in the expected direction.
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![Graphs showing running wheel use and phase relationship](image)

Figure 3. Parameters (mean ± SD, n = 9) of running wheel use by hamsters housed in one-, two-, or three-cage systems. SDs have been omitted for some data points, for the sake of clarity (the size of the omitted SDs was within the range of those that are shown). Note that the y-axis for phase relationship has been inverted so that the largest delays of activity onset relative to the time of lights-off are shown as being higher up on the graph. LD = light : dark 14:10h.

(fewer REV and shorter α for the group with more cages), but the difference between the two- and three-cage groups did not; instead, the three-cage group was often intermediate between the one- and two-cage groups (top and bottom panels on the left side of Fig. 3).

Discussion

No significant effect of cage enrichment was detected in this experiment. It can be argued that the sample size (n = 9) was too small in the face of a large within-group (interindividually) variability. Yet even if the sample size had been larger, it would remain difficult to explain why the comparisons between the one- and two-cage groups on the one hand and between the two- and three-cage groups on the other hand did not yield changes in
the same direction (Fig. 3). A paired design would be more appropriate, as it would eliminate
the noise introduced by interindividual variation. This was done in Experiment II.

EXPERIMENT II: ONE VS. THREE CAGES, LD, PAIRED DESIGN

Methods

Nine new hamsters were placed individually in three-cage systems. They were given
day to habituate, after which time data collection ran for six weeks, all under LD
14:10 h. At the end of week 1, all tunnels were removed, leaving the hamsters in one-cage
systems. At the end of week 2, the tunnels were put back in place. This went on every week
until each hamster had spent a total of three weeks in three-cage systems alternating with a
total of three weeks in one-cage systems. Cage systems were inspected every day as in
Experiment I to check for hamster position.

REV, ψ and, α were plotted against time (weeks) for each hamster. Temporal trends
were removed by fitting a curve (Sigma-Plot 4.0 for Windows) through the six weekly data
points for each hamster. Residuals were calculated for each weekly data point. These
residuals were entered in a repeated-measure ANOVA with week (first pair of weeks,
second pair of weeks, third pair of weeks) as the within-group factor, and cage system as
the between-group factor.

Results

When in the three-cage system, hamsters were found sleeping in the cage next to the
wheel cage about one-third of the time, and in the cage furthest away from the wheel cage
about two-thirds of the time. This was true at the inter- as well as intraindividual level.
Hamsters almost never slept in the wheel cage. Feces were present in all the cages.

REV and α clearly increased from week to week, whereas ψ decreased (right side of
Fig. 3), justifying the need for temporal trend removal. The lines for REV and α on the
right side of Fig. 3 show a shallow but consistent sawtooth pattern (the individual curves
from which the average curves on Fig. 3 are derived also showed a sawtooth pattern), with
three-cage weeks consistently occupying the valleys, indicating that the larger cage system
led to fewer daily revolutions and shorter running activity phases. The ANOVA showed
a significant effect of cage systems for REV (F = 10.6, p = 0.005) and
α(F = 19.33, p < 0.001). There was no significant cage effect for ψ(F = 0.09, p =
0.769), consistent with the fact that the lines for phase relationship did not show a
sawtooth pattern (middle panel on the right side of Fig. 3). There was no more time effect
after temporal trend removal, for any of the three variables (F between 1.03 and 1.64, p
between 0.21 and 0.37).

Discussion

Significant effects of cage enrichment were found in this experiment. Though phase
relationship was not affected, the number of daily revolutions and the duration of running
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phase were less in the larger cage systems. The paired design was therefore useful, though
the demonstrated effect remains a small one. Consider for example how shallow the
sawtooth patterns are on the right side of Fig. 3 relative to the overall temporal trend.

Paired designs are not as common as unpaired ones in chronobiological studies. The
question arises as to what level of cage enrichment would be necessary for effects to
appear even in an unpaired design. The next experiment addressed this question by
introducing five-cage systems. It also looks at the situation in DD and effects on \( \tau \).

EXPERIMENT III: ONE VS. FIVE CAGES, LD AND DD

Methods

Eleven and 10 new hamsters were individually placed in one- and five-cage systems,
respectively. They were given five days to habituate. Data collection then ran for three
weeks under LD 14:10 h, and then for a further 33 days in DD. Two dim red lights
remained on in DD to facilitate the task of cage maintenance. Light intensity was about
1 lux (Gossen Lunasix 3 photometer) in the two cages directly below the red bulbs, and
less than 1 lux in the others.

Data analysis was as in Experiment I. Separate ANOVAs were run for the LD and DD
portions of the experiment. In the DD part of this experiment, \( \tau \) replaced \( \psi \) as a variable,
and three blocks of 10 days each replaced the three weeks of LD. This was done to
accommodate the schedule of litter change, which took place after each 10-day block (and
was not included in the next 10-day block).

Results

In the 5-cage systems, in both LD and DD, the preferred sleeping places were the two
cages farthest away from the wheel cage. Hamsters were infrequently (less than 15% of the
time) found sleeping in the other cages or in the tunnels. Feces and signs of gnawing on
toys were present in all cages.

In LD, differences between the one- and five-cage groups were significant for
\( \psi (F = 5.44, p = 0.03) \) and \( \alpha (F = 9.71, p = 0.006) \), and nearly significant for REV
\( (F = 4.01, p = 0.06) \). As expected, the larger cage system resulted in fewer daily
revolutions, later daily starts, and shorter running activity phases (left side of Fig. 4).
There was also a significant time \( (F = 5.49, p = 0.008) \) and interaction \( (F =
3.41, p = 0.043) \) effect for REV, reflecting the fact that REV tended to stay stable
for the one-cage group but to increase from week to week for the five-cage group
(top-left panel of Fig. 4).

In DD, the significant differences between cage groups disappeared (left side of
Fig. 4, REV: \( F = 0.87, p = 0.36; \alpha : F = 2.04, p = 0.17 \)). Neither were there any
significant differences between cage groups for \( \tau (F = 2.28, p = 0.15) \). There were
significant time effects for all three variables \( (F \) between 3.25 and 9.27, \( p \) between 0.001
and 0.05), but this effect was the same in both cage groups (interaction terms: \( F \) between
0.05 and 1.69, \( p \) between 0.20 and 0.95; left side of Fig. 4).
Figure 4. Parameters (mean ± SD, n = 9–12) of running wheel use by hamsters housed in one- or five-cage systems. LD = light : dark 14:10 h; DD = constant darkness.

Discussion

The increase to five cages yielded significant effects in LD, even with this unpaired design. However, the effects disappeared in DD. It is not clear whether this is as a consequence of DD per se or of the total time spent by the animals in the cages irrespective of lighting conditions. For example, the left side of Fig. 4 shows that the between-group
difference for the number of wheel revolutions, and to some extent for activity phase duration as well, tended to decrease as the LD experiment went on (this decrease was in fact significant for wheel revolutions as shown by the interaction term). So the situation in DD may have been the conclusion of a trend initiated in LD. The next experiment addressed this possibility by comparing one- and five-cage systems in DD imposed from the start of the experiment.

**EXPERIMENT IV: ONE VS. FIVE CAGES, DD FROM START**

**Methods**

Twelve and nine new hamsters were individually placed in one- and five-cage systems, respectively, in DD (two dim red lightbulbs remained on all the time, as in Experiment III). The experiment ran for 33 days, but only the first three weeks were retained for analysis because many hamsters in both groups started to show signs of colliding (Mrosovsky and Hallonquist, 1986) after 21 days. Data collection and analysis were as in Experiment III, except that the experiment was divided in three 7-d periods rather than three 10-d periods.

**Results**

No significant differences between the cage groups could be detected (right side of Fig. 4; REV: $F = 2.29, p = 0.12$; $\alpha : F = 1.56, p = 0.21$; $\tau : F = 0.68, p = 0.48$). $\alpha$ increased from week to week ($F = 116.2, p < 0.001$) and so did $\tau(F = 22.36, p < 0.001$), but the changes were the same for both cage groups (interaction terms: $F$ between 0.18 and 3.00, $p$ between 0.08 and 0.82).

**Discussion**

These results confirm those of Experiment III: the effect of five-cage systems cannot be detected in DD. In the DD part of Experiment III, the differences between the one- and five-cage groups were not significant, but at least they went in the expected direction of fewer revolutions and shorter running activity phase in the multiple-cage system. In Experiment IV, however, the differences were not consistent from one week to the next and did not always go in the expected direction (see in particular running phase duration on the right side of Fig. 4). Even if our sample sizes had been higher and our tests therefore more powerful, the lack of consistent differences from week to week would have been hard to interpret. It seems more likely that there is simply no effect of cage enrichment in DD.

We had no a priori expectation about the effect of cage enrichment on $\tau$, as we could not formulate a rationale linking these two parameters. In both this experiment and the DD part of experiment III, $\tau$ on average was slightly, but not significantly, shorter in the five-cage group. In mice, access to a wheel as opposed to the same environment without it (Edgar et al., 1991) or access to a preferred wheel size as opposed to a wheel too small in
which the mice run less (Deboer and Tobler, 2000) also shortens $\tau$. The same is true of rats (Shioiri et al., 1991; Yamada et al., 1988). In hamsters, Aschoff et al. (1973) found longer $\tau$ expressed in cages supplied with wheels as opposed to without. However, Refinetti et al. (1994) found no difference between these two conditions. Likewise, Pratt and Goldman (1986) found no difference in $\tau$ expressed in wheel systems versus underground burrow and open space (no wheel) systems. In two experiments, Mrosovsky (1999) did not detect significant $\tau$ differences in preferred versus less preferred wheels (both types had running surfaces made of rods, but the preferred wheels also had plastic mesh wrapped around them—as in the present study—resulting in more running); however, in a third replicate (with experienced animals of intermediate age), $\tau$ was significantly shorter in the preferred wheels. The lack of consistent results, at least in the hamster work, makes it difficult to reach a firm conclusion yet about $\tau$ and its relationship to environmental conditions.

Within cage systems (as opposed to the comparisons between cage systems in the previous paragraph), the bulk of the evidence for rodents is that individuals that run more tend to exhibit shorter $\tau$ (Aschoff et al., 1973; Edgar et al., 1991; Mrosovsky, 1999; Shioiri et al., 1991; Weisberger et al., 1997; Yamada et al., 1990). Activity and/or one of its correlates may influence the circadian pacemaker through as-yet undefined mechanisms that possibly involve the intergeniculate leaflet (Kuroda et al., 1997) or a phase-response curve to nonphotic stimuli (Mrosovsky, 1996; 1999). The present study unfortunately cannot throw light on this topic, because we did not quantify activity outside the wheel. Any conclusion about a possible link between wheel use and $\tau$ in our study would be hampered by our ignorance of the extent to which less intense running was compensated by more climbing and crawling in tunnels or more gnawing on toys, and of whether such alternate activities can have effects of their own on $\tau$.

**GENERAL DISCUSSION**

Cage enrichment of the type used here had some effects on the daily pattern of wheel-running activity in our hamsters. Access to more cages, tunnels, and toys led to fewer daily wheel revolutions, shorter running activity phases, and sometimes a later daily start in the wheel relative to the time of lights-off. These effects, however, were small. They were too small, relative to interindividual variability, to be detected in an unpaired design when the number of cages was raised to three; it took an increase to five cages for effects to be detected in an unpaired design with sample sizes of 9–12 per group (a not-uncommon sample size in chronobiological studies), and even then the differences between groups could only be detected under LD, not under DD. And it took a paired design, eliminating interindividual variation, for an increase to three cages to yield significant effects, though even then these effects appeared smaller than the week-to-week variation. We consider the effects of cage enrichment small enough that the results of any further chronobiological research done under enriched conditions of the type used here could still be reasonably compared with past studies conducted under different standards of animal welfare.

Of the four variables measured here, only $\tau$ is truly circadian. The others could certainly be influenced by changes in the circadian system but also—and perhaps more simply—by a change in the animal’s routine between wake-up time and sleep onset. Hamsters do not jump into their wheel as soon as they wake up; instead, they
spend considerable time grooming and eating (personal observation; see also Korenman et al., 1988). An increase in cage space and complexity may introduce the need for territory inspection, and if the animal decides to do this very early in its daily routine, then the onset of wheel running could be delayed, leading to a more negative phase relationship with dark onset. A similar change in routine at the end of the activity phase could similarly lead to a shorter $\alpha$ (as defined relative to wheel use). Yet all this would not necessarily reflect a fundamental change in the organization and workings of the internal oscillator(s). The number of daily wheel revolutions is also likely to be somewhat independent of internal clocks, and more related to a motivation to run. These considerations are a reminder that wheel running is not as proximate to the output of the internal clock as other events such as wake-up time or sleep initiation time, and that it depends on how hamsters organize their daily routine (Enright, 1975).

On the other hand, the present study indicates that it is not easy to affect daily wheel use in hamsters. Wheel running seems to be a highly rewarding activity for these animals and for rodents in general (Sherwin, 1996; 1998a,b; Sherwin and Nicol, 1996), and the present results show that distractions introduced by a more complex cage environment do not alter greatly the times and levels at which hamsters use their wheel. Wheel-running activity therefore remains a robust way of measuring clock phase in hamsters, even in enriched cage systems of the type studied here.

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