J Comp Physiol A (1999) 184: 429–437

ORIGINAL PAPER

U. Redlin · N. Mrosovsky

Masking of locomotor activity in hamsters

Accepted: 8 February 1999

Abstract The inhibition of locomotion by light (masking) was investigated in Syrian hamsters. When 1-h pulses of light were presented in the early night, activity was strongly suppressed by irradiances of about 1 lx or greater. Ultradian light-dark cycles were used as another way to study masking. Hamsters were unable to entrain to 3.5:3.5-h light-dark cycles, thus permitting the masking and the entraining effects of light to be distinguished. Light had greater suppressive effects on activity in home cages than on activity in novel running wheels. Moreover, in home cages activity remained very low for about 30 min after lights were turned off. Post-pulse suppression of activity was not simply a consequence of reduced running, as shown by experiments in which running was temporarily prevented by locking the wheels. A phase response curve for masking was obtained by placing hamsters in novel wheels for 3-h periods at various times throughout their circadian cycles, and then superimposing a 30-min light pulse. The suppressive effect of light was maximal around the onset of activity, which normally coincides with dusk in hamsters. This may have adaptive value in limiting foraging to the hours of darkness.

Key words Circadian · Hamster · Light · Locomotor activity · Masking

Abbreviations *CT* circadian time · *LD* light-dark · *ZT* zeitgeber time

U. Redlin · N. Mrosovsky (✉)
Departments of Zoology, Psychology and Physiology,
University of Toronto, Toronto,
Ontario M5S 3G5, Canada
e-mail: mro@zoo.utoronto.ca
Tel.: +1-416-978-8506; Fax: +1-416-978-8532

N. Mrosovsky
Department of Zoology,
University of Toronto,
Department of Zoology, Toronto,
Ontario M5S 3G5, Canada

Introduction

The Syrian hamster, *Mesocricetus auratus*, has precise and predictable rhythms of locomotor activity, and is easy to keep in captivity. For these reasons it is one of the most commonly studied species in research on circadian rhythms. Landmark papers on rhythms using hamsters include those by Pittendrigh and Daan (1976), Elliott (1976), and Ralph et al. (1990).

Nevertheless, it is generally accepted that a light-entrainable endogenous clock is not the only mechanism controlling overt behaviour and confining activity to appropriate times of day or night. The acute or masking effects of light complement the endogenously controlled output from the clock (Aschoff 1960: Erkert and Gröber 1986; Reebs 1994). Yet, despite the innumerable papers on rhythmicity in hamsters, virtually no attention has been given to masking in this species, and how it might relate to endogenous mechanisms. In this paper we ask a number of specific questions about masking in hamsters.

Experiment 1 tests the sensitivity of the masking to photic stimuli of different irradiance. Pulses of light are given in the early night when the hamsters are spontaneously active.

Experiment 2 addresses the question of how masking by light can be quantified. Just as in studies of effects of light on clock control it is necessary to exclude the acute or masking effects of light on the behavioural variable being measured, so in studies of masking it is necessary to exclude the influence of the endogenous clock. The use of ultradian light-dark (LD) cycles to which hamsters cannot entrain is one approach to this problem (Borbély and Huston 1974).

Experiment 3 investigates what types of activity can be inhibited by light. It is known that hamsters can be induced to be active, even at times when they would normally not be active, by confining them to a novel wheel. Is such induced activity susceptible to masking, or does masking affect only clock-controlled activity? This experiment also takes a closer look at the time-

course of the response after a light pulse is given in the early subjective night, when hamsters are normally active. How fast do the hamsters respond to light, and what happens after lights are switched off?

Experiment 4 asks whether there is a circadian rhythm of masking to light pulses, and what the functional significance of such a rhythm might be.

Behind these specific aims is the more general aim of providing some basic descriptions of masking in this species and possible relationships between masking and circadian rhythms.

Materials and methods

General

Male Syrian hamsters (age 40 days) were obtained from Harlan Sprague-Dawley (Indianapolis, Ind., USA). Hamsters were individually housed in polypropylene cages ($44 \times 23 \times 20$ cm) equipped with a 17.5-cm diameter running wheel. The outside of all wheels was surrounded with a plastic mesh to provide a better footing for the hamsters (Mrosovsky et al. 1998). Wheel running was continuously monitored by a computerized data acquisition system (Dataquest III, Minimitter Inc., Sunriver, Ore., USA). All hamsters were initially entrained to an LD 14:10-h cycle. Illumination was measured with an ISO-TECH ILM350 meter. For experiments involving novel wheels, separate wheels, also surrounded with plastic mesh, were placed next to each cage. Procedures in the dark were carried out with the aid of an infrared viewer (FJW Optical Systems, Palatino, Ill., USA). Hamsters had continuous access to food (rodent chow 5001; PMI, St. Louis, Mo., USA) and water, except during confinement to novel wheels. Temperatures ranged from 18 to 23 °C. Data were analysed by one-way repeated measures ANOVA and Tukey-Kramer post-hoc tests, or two-tailed *t*-tests. Level of significance was set at $P < 0.05$. Data are shown as means ± standard error.

Experiment 1: masking threshold

Hamsters ($n = 24$; 7 months old) were kept in LD 14:10 h and wheel running was continuously monitored. All data from two of these hamsters were discarded because of erratic and low levels of activity. The animals were in a room in which light pulses of variable irradiances could be given. The details of the light sources and setup for altering the illumination are described elsewhere (Mrosovsky et al. 1999). The test procedure required 3 days for each light level. On the first day (maintenance day) filters were changed and other maintenance work was carried out. On day 2 (baseline day) the animals were left undisturbed and home cage wheel running between zeitgeber time (ZT) 13.5 and 14.5 was quantified. On day 3 (test day) the hamsters were given a light pulse from ZT 13.5 to ZT 14.5. Wheel running during the light pulse was compared to the baseline wheel counts of the previous day. Masking was scored as a percentage of baseline wheel counts. Tests with 11 different irradiances (given in stop values of the neutral-density filters) were carried out. Because some hamsters did not show masking in the 0-stop condition with no neutral-density filters, an additional test was run in which the light was made stronger by the addition of two extra light bulbs; this test was labeled as "superbright". The approximate lux values (measured with an ISO TECH ILM350 meter) were: superbright, 1800 lx; 0 stops, 500 lx; 3 stops, 55 lx; 6 stops, 9 lx; 9 stops, 2 lx; 12 stops and higher, < 1 lx. An additional sham test in which an opaque sheet of cardboard blocked the light passage was used to control for possible non-photic factors from the light apparatus. The order in which the tests were run was: 0, 3, 9, 12, 15, 18, 21, 6, 24, 27 stops, sham pulse, 23 stops, superbright. To assess whether masking scores were different from 100%, one-sample two-tailed Wilcoxin signed rank tests were used.

Experiment 2: wheel running during ultradian light-dark cycles

In mice, LD 1:1-h cycles do not entrain circadian activity rhythms (Mrosovsky 1994), permitting masking effects to be distinguished from entraining effects of light. Initially, we tried the LD 1:1-h cycle with hamsters. Possibly because some hamsters have free-running periods close to 24 h, they appeared to entrain to this cycle. Furthermore, for various reasons to be discussed below, this schedule proved suboptimal for demonstrating masking in hamsters. In a second part of the experiment we therefore used an LD cycle of 3.5:3.5 h, to which hamsters are unlikely to entrain. If this schedule is kept going for a week, all phases of the circadian cycle coincide with approximately the same amount of light and dark (Fig. 2).

Hamsters ($n = 24$) were entrained to an LD 14:10-h cycle (fluorescent lighting, illumination about 400 lx) for 26 days. Then, using the same lights, the schedule was switched to LD 1:1 h for 1 week, followed by LD 3.5:3.5 h for another 10 days. Temperature during the LD cycles was recorded; temperature cycles associated with the ultradian LD cycles had amplitudes of < 1 °C; these changes were smaller than the overall temperature fluctuations in the room during this experiment (19–22 °C). Masking was quantified as follows: the total number of wheel revolutions during the dark and light phases of the two LD cycles was calculated for 1-week intervals; masking scores are given as the amount of activity in the dark expressed as a percentage of the total activity.

Experiment 3: comparison of masking in home cages and in novel wheels

In this experiment we tested the effect of a 30-min light pulse on home-cage wheel running and novelty-induced wheel running in the early subjective night, at ZT 14. Before use in this experiment the hamsters had been kept first in LD 14:10 h for 9 days, then in LD 1:1 h for 24 days after which they were kept in LD 14:10 h for the duration of the present experiment. Two groups of hamsters ($n = 10$ per group) were kept in adjacent similar rooms. Incandescent bulbs provided illumination of about 100 lx at cage floor level during the L phase of the cycle. Illumination during the light pulses was about 150 lx on the floor of the home cages and about 140 lx on the base of the novel wheels. Wheel running was recorded in 1-min bins but usually displayed in 5-min bins.

After 47 days in LD 14:10 h, the two groups of hamsters (age 120 days at this stage) were tested on four occasions, with 4 days between each test. In the first two tests, the animals were confined to novel wheels from ZT 13 to ZT 16 and either given a light pulse from ZT 14 to ZT 14.5 or left in the dark. In the subsequent two tests, hamsters remained in their home cages and were either given a light pulse from ZT 14 to ZT 14.5 or left undisturbed.

A subsidiary experiment was added to test whether persistent suppression of wheel running of hamsters after lights were switched off is a specific feature of masking by light or merely results from interruption of wheel running. Twenty-four hamsters (age 4 months; previously used in Experiment 1) were kept in LD 14:10 h; wheel running was recorded in 10-min bins. Hamsters remaining in their home cages were tested on two occasions. On the first occasion, wheels were locked (by sliding in a metal rod through the wheel spokes) from ZT 14 to ZT 14.5. This was done by entering the room with an infrared-viewer 5 min before ZT 14. From ZT 14 to ZT 14.5 all wheels were locked. Starting at ZT 14.5 the room was entered again and the wheels were unlocked. On the second occasion, 3 days after the first, wheels were again locked from ZT 14 to ZT 14.5, using the same procedure as for the first test. In addition, the hamsters were given a light pulse (fluorescent light source, about 400 lx) from ZT 14 to ZT 14.5.

Experiment 4: phase response curve for masking by light

Because light inhibits locomotion in hamsters (negative masking), it is not possible to study masking by light at times of the cycle when the animals are inactive. Therefore, to produce a complete phase response curve for masking of activity by light, it is necessary to induce hamsters to be active during their normal rest hours. This was done by confining them to a novel wheel.

After the initial entrainment to LD 14:10 h (incandescent light source, 100 lx at cage floor level), the hamsters ($n = 24$; age 40 days) were kept in constant darkness (DD) for the remainder of the experiment. Wheel running was recorded in 5-min collection bins. Starting on day 6 of DD, the hamsters were repeatedly confined to the novel wheels for 3 h. On a given test day, all hamsters were confined to the novel wheels at the same clock time, but because rhythms free-ran, various phases of their circadian cycle were screened simultaneously. After 1 h of confinement the hamsters were given a 30-min light pulse (incandescent light source, 140 lx at the base of the novel wheels). After each test the hamsters were left undisturbed for at least 4 days before they were re-tested. To obtain control data, at the end of the experiment (after 74 days of DD), hamsters were confined to novel wheels on four occasions without any light pulses.

Masking by light was quantified as follows: wheel running during the 30-min light pulse was compared to the wheel revolutions made during a 30-min baseline immediately prior to the light pulse. However, some hamsters do not run much when confined to novel wheels; such individuals have been called sluggards (Weisgerber et al. 1997). Those hamsters that were inactive during the baseline were excluded. Only hamsters with activity in all 5-min data collection bins of the 30-min baseline and having made at least 500 wheel turns in this period were included. This criterion selected hamsters which were likely to continue running in the novel wheels. Masking was quantified as wheel revolutions during the light pulse expressed as a percentage of those during the 30-min baseline.

The circadian phase of the light pulse was estimated from activity onsets immediately before the masking day. To count as an activity onset, the activity had to reach at least 81 counts during a 10-min period, followed by at least another 10-min bin with this level of activity within the next 40 min. Individual hamsters contributed between 2 and 9 masking tests to the data set (mean 6.0 ± 0.43 tests).

Results

Experiment 1: masking threshold

Hamsters showed an irradiance-dependent reduction in their spontaneous wheel running when they were tested from ZT 13.5 to ZT 14.5 (Fig. 1). At the 0-stop level (about 500 lx) 17 hamsters completely suppressed their activity (masking scores $< 1\%$). Two hamsters failed to reduce their wheel running during this light pulse, having masking scores of $> 90\%$. These two hamsters also failed to exhibit masking in further tests with decreasing irradiance but reduced their wheel running in response to the superbright pulse to 44% and 67% of the respective baseline activity. On average, masking gradually decreased until the 9-stop test, after which masking rapidly declined with further lowering of irradiance. At the 15-stop level (< 1 lx) hamsters did not reduce their wheel running at all. A consistent increase in wheel running activity at low lighting levels (positive masking), as can be seen in mice (Mrosovsky et al. 1999), was not found. In the range of stop levels from 15–27, only at 23 and 24 stops did the hamsters run significantly more

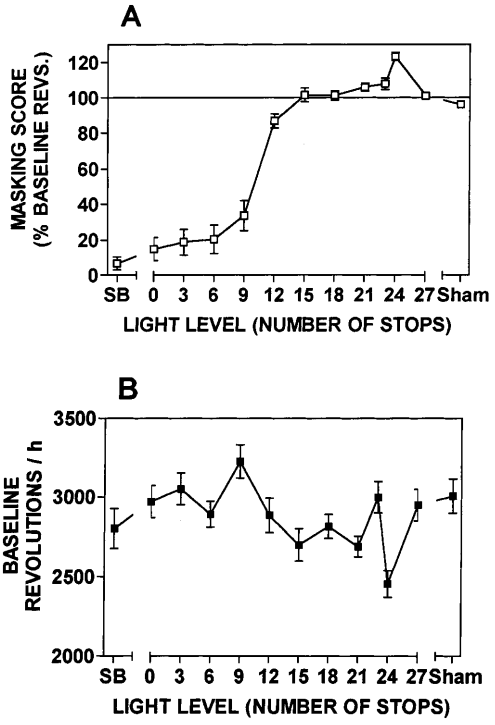


Fig. 1 **A** Masking (as a percentage of baseline; means ± SE) of hamsters as a function of irradiance (shown as stops of the neutral-density filters; *SB* superbright pulse; *Sham* pulse with cardboard blocking light passage; see text for details). Light pulses were given in the early part of the night, from ZT 13.5 to 14.5. **B** Wheel revolutions (means ± SE) during the baseline periods used to calculate masking scores shown in **A**. For details, see text

than during the baseline periods ($P < 0.05$, one-sample Wilcoxin signed rank test, corrected for multiple comparisons). However, at the 24-stop level the high masking score was associated with a drop in baseline activity compared to other days (Fig. 1), which may indicate a measurement problem rather than positive masking.

Experiment 2: wheel running during ultradian light-dark cycles

With an LD 1:1-h cycle, 78.8% of the wheel running took place during the dark phases. This demonstrates

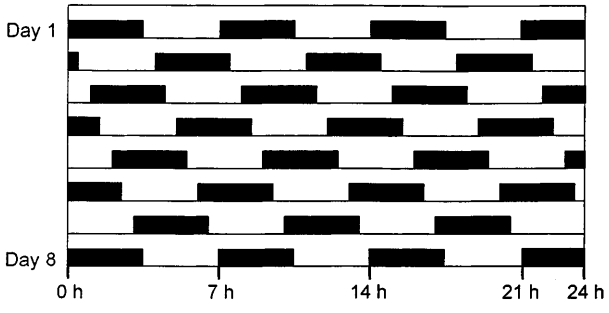


Fig. 2 Diagram of an LD 3.5:3.5-h cycle. *Dark bars* indicate dark phases. Note that the initial phase relationships are regained after 1 week

that, on average, this cycle leads to considerable masking (Fig. 3). However, not all hamsters masked well under this cycle: 6 out of the 24 hamsters had masking scores between 50% and 60%. With an LD 3.5:3.5-h cycle, 90.0% of the running took place during the dark; this was significantly more than was found with the same hamsters in LD 1:1 h (78.8%; paired *t*-test, $P < 0.0001$; Fig. 3). Masking scores in LD 1:1 h correlated with those in LD 3.5:3.5 h ($r = 0.83$, $P < 0.0001$); however, only 1 hamster had a masking score between 50% and 60%, compared to 6 in LD 1:1 h. The mean number of revolutions made in 1 week was $74{,}985 \pm 6558$ in LD 1:1 h. This was not significantly different from the revolutions made in LD 3.5:3.5 h ($86{,}854 \pm 4076$; $P = 0.087$). The 1-week interval was too short to unambiguously exclude entrainment in every case, but some free-running circadian rhythms were observed under both cycles.

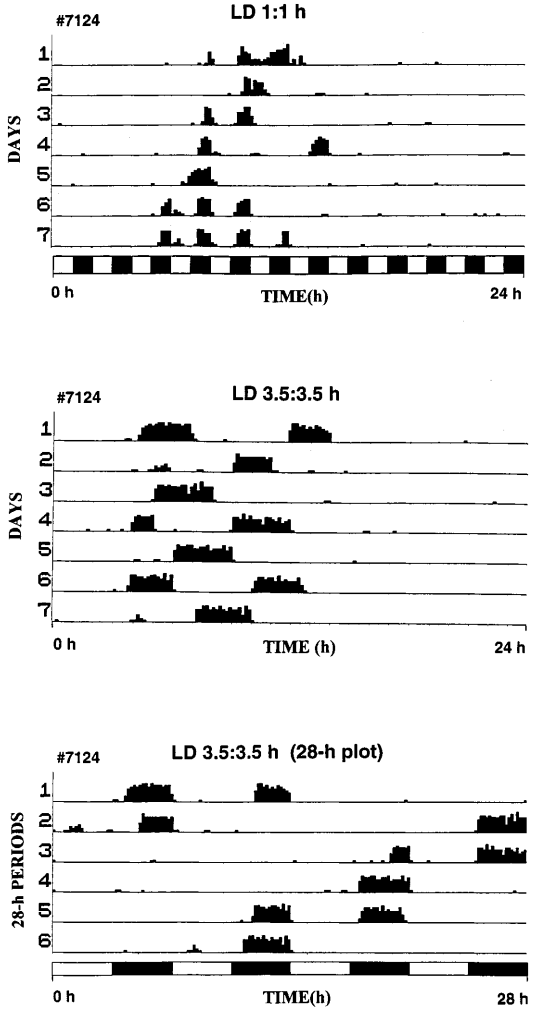


Fig. 3 Actograms for a hamster kept in LD 1:1 h and LD 3.5:3.5 h. Activity is expressed in 15 quantiles, with the first including 1–80 wheel revolutions, the second 81–160 revolutions, etc

Experiment 3: comparison of masking in home cages and in novel wheels

Hamsters in their home cages rapidly reduced their wheel running between ZT 14 and ZT 14.5 from an average of 1149 ± 76 revolutions on the control day to an average of 10 ± 2 revolutions during the light pulse (Fig. 4; $P < 0.001$, Tukey-Kramer post-hoc); this is a reduction of 99%. After lights were switched off again at ZT 14.5, the hamsters did not resume running right away. After 30 min without running they gradually started to use their wheels again (Fig. 4). Associated with the reduction of wheel running during the pulse and after the pulse, there was a significant reduction in the total number of wheel revolutions on the day of the light pulse (control day: $11{,}204 \pm 840$ revolutions; pulse day: 8345 ± 824; $P < 0.01$, Tukey-Kramer post-hoc).

When hamsters were confined to novel wheels from ZT 13 to ZT 16, a light pulse at ZT 14 resulted in a rapid reduction in wheel running within the 1st minute (Fig. 5). Wheel counts from ZT 14 to 14.5 without light on the control day were 1412 ± 60; the light pulse led to a 65% reduction to 498 ± 102 revolutions ($P < 0.001$, Tukey-Kramer post-hoc). Although hamsters reduced their wheel running significantly, they did not stop running in the wheels entirely, as the hamsters in the home cages did (Fig. 4). When the lights were switched off the hamsters quickly resumed running in the wheels, again in contrast to the home cage controls (Fig. 4). The 30-min light pulse did not lead to a significant reduction in the daily wheel revolutions (control day: $12{,}830 \pm 451$ revolutions; pulse day: $10{,}850 \pm 600$ revolutions; $P > 0.05$, Tukey-Kramer post-hoc). Confinement of the hamsters to novel wheels for 3 h starting at ZT 13 without a light pulse resulted in a significant increase in wheel revolutions during this period (novel wheels: 8393 ± 269 revolutions; home cage: 6656 ± 408 revolutions for the same 3-h interval; $P < 0.05$ Tukey-Kramer post-hoc).

When hamsters were prevented from running between ZT 14 and ZT 14.5 by blocking the wheels, wheel running resumed within 10 min after the wheels were unblocked (Fig. 6A). After 30 min, wheel running had completely recovered to pre-blockage level. In contrast, when hamsters were prevented from running in their wheels and in addition were given a light pulse from ZT 14 to ZT 14.5, wheel running remained suppressed completely for 20 min after the pulse. Wheel running slowly began to recover starting 30 min after the light pulse and wheel blockage (Fig. 6B). The post-pulse suppression of wheel running therefore was a result of the exposure to light, and not simply of the reduced running during that period caused by the light.

Experiment 4: phase response curve for masking by light

Hamsters were confined to the novel wheels a total of 329 times. Data for 150 of those occasions were excluded

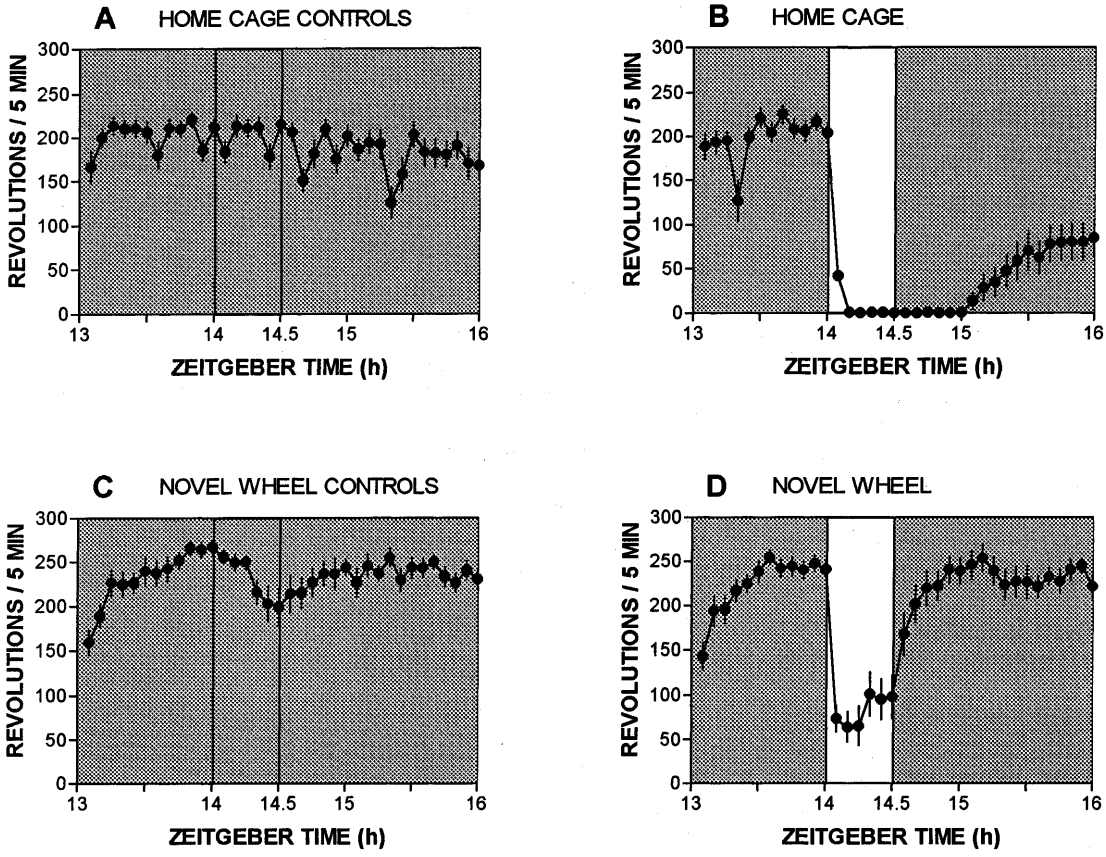
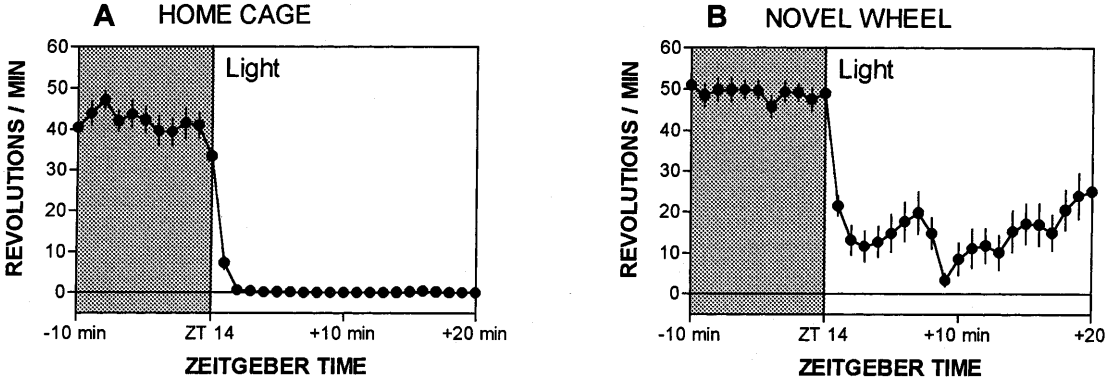


Fig. 4 Wheel running (mean ± SE; $n = 20$) for hamsters in Experiment 3: **A** in their home cages without a light pulse; **B** in their home cages with a light pulse; **C** after confinement to novel wheels without a light pulse; and **D** after confinement to novel wheels with a light pulse. Light pulses are indicated by *white background*; the *lines* in **A** and **C** indicate when the light pulses were given to animals in **B** and **D**. Each data point is plotted at the end of the respective data bin time

from further data analysis because the hamsters were considered sluggards according to the criteria established. The remaining 179 occasions contributed to the data set reported; 144 of those were tests with exposure to light, and 35 yielded control data for induced running without exposure to light.

Masking by light of induced wheel running was strongest from circadian time (CT) 12–14, when running was consistently inhibited in all hamsters tested at that time (Fig. 7B). At other times, masking was more variable, with gradually increasing masking in the hours before CT 12 and decreasing masking in the hours after activity onset (Fig. 7C). The phase response curve for masking to light pulses varied significantly with CT (one-way ANOVA; $P < 0.0001$). Wheel running during the 30-min baseline in the novel wheels immediately prior to the light pulse also varied significantly with CT (one-way ANOVA, $P < 0.0001$; Fig. 7D), but there was no significant correlation between 2-h bin baseline values and masking scores ($r = 0.20$, $P = 0.53$).

Fig. 5 Wheel running of hamsters in Experiment 3 in 1-min bins (mean ± SE; $n = 20$) shortly before and after lights are turned on. **A** Hamsters in their home cages. **B** Hamsters confined to novel wheels. Other conventions as in Fig. 3



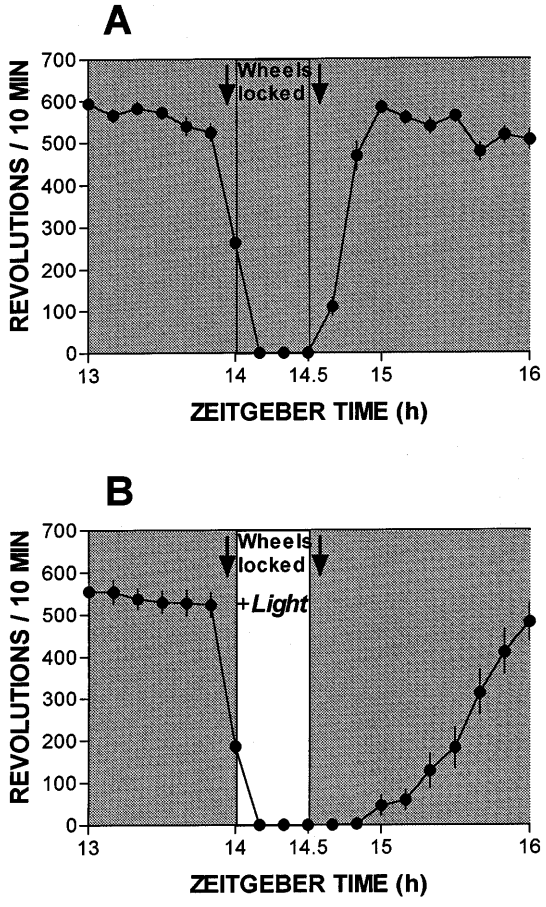


Fig. 6 Wheel running of hamsters in their home cages (mean ± SE; $n = 24$). **A** Wheels locked from ZT 14 to ZT 14.5, no light pulse. **B** Wheels locked from ZT 14 to ZT 14.5, with light pulse from ZT 14 to ZT 14.5

The 35 control tests demonstrate that, on average, wheel running without a light pulse at ZT 14 was not reduced compared to running in the previous half hour of baseline (Fig. 7A). However, in three cases without light there were substantial reductions in activity (at about CT 7, CT 15, and CT 20.5). Nevertheless, the average score as a percentage of baseline activity was 103 ± 6; this was significantly different from the average score for the revolutions made in the novel wheels with a light pulse (50.3 ± 4%; *t*-test: $P < 0.0001$).

Discussion

Comparison of masking in hamsters and mice

Masking of locomotor activity was readily demonstrated in hamsters, but there were several differences compared to mice. First, hamsters appeared to be more sensitive to the suppressive effects of light on activity than mice. Even with lights as dim as 1 lx, hamsters showed considerable masking (Fig. 1 here; also Redlin et al. 1999), whereas at these levels of illumination mice of various strains did not show much masking (Mrosovsky et al. 1999).

Second, in hamsters the suppression of activity often lasted beyond the end of the light pulse. In mice, post-pulse depression of activity is not as pronounced (Mrosovsky 1994, Mrosovsky et al. 1999). The persistence of suppressed activity in hamsters after the light had been turned off is a further indication that light exerts stronger effects in this species.

Third, hamsters do not consistently increase their wheel running at low light levels (positive masking), as has been found in certain strains of mice (Mrosovsky et al. 1999). Positive masking in mice is assumed to be mediated by the classical visual system (Mrosovsky et al. 1999). In hamsters, the classical visual system may therefore be of less importance in the regulation of wheel running than in mice.

Fourth, with mice in an LD 1:1-h cycle, activity reappears quickly in the dark phases (Mrosovsky 1994). With hamsters in LD 1:1 h, however, a period of illumination in the early part of the subjective night sometimes suppressed wheel running almost completely, with this suppression often lasting through much of the next hour when it was dark. At the end of this hour of darkness, the reappearance of light kept activity at a low level. Post-pulse masking in hamsters, as apparent under LD 1:1 h, has implications for devising procedures to study masking; in particular, locomotion of hamsters may not be able to track short LD cycles as neatly as that of mice.

Continued suppression of activity after light pulses

The persistence of activity depression after the light pulses ended enlarges our view of masking effects. These have generally been considered to be acute effects of light, confined to the time when the light is present. As Aschoff (1988) wrote, "masking effects may last for the full duration of a signal such as a pulse of light or dark ...." There has been no suggestion that masking of locomotor activity may last beyond the time of light pulses, as shown in this study. The effect is particularly easily seen in Fig. 4, but it has been equally pronounced in other experiments (N. Mrosovsky, unpublished observations). Post-pulse masking is not a mere consequence of suspended wheel running during the light pulse, as was demonstrated by blocking the wheels in the absence of light (Fig. 6). Given the close relationship between locomotor activity and melatonin (Elliott and Tamarkin 1994), it is worth mentioning that melatonin levels in rats and Syrian hamsters remain low long after a light pulse ends (Illnerova et al. 1979; Brainard et al. 1984; Kennaway and Rowe 1994). In human beings (Petterborg et al. 1991; Czeisler et al. 1995) and in rams (Arendt and Ravault 1988) melatonin returns rapidly to high levels after a light pulse in the night.

Post-pulse masking was less a problem with an LD 3.5:3.5-h cycle. The longer dark periods provided more

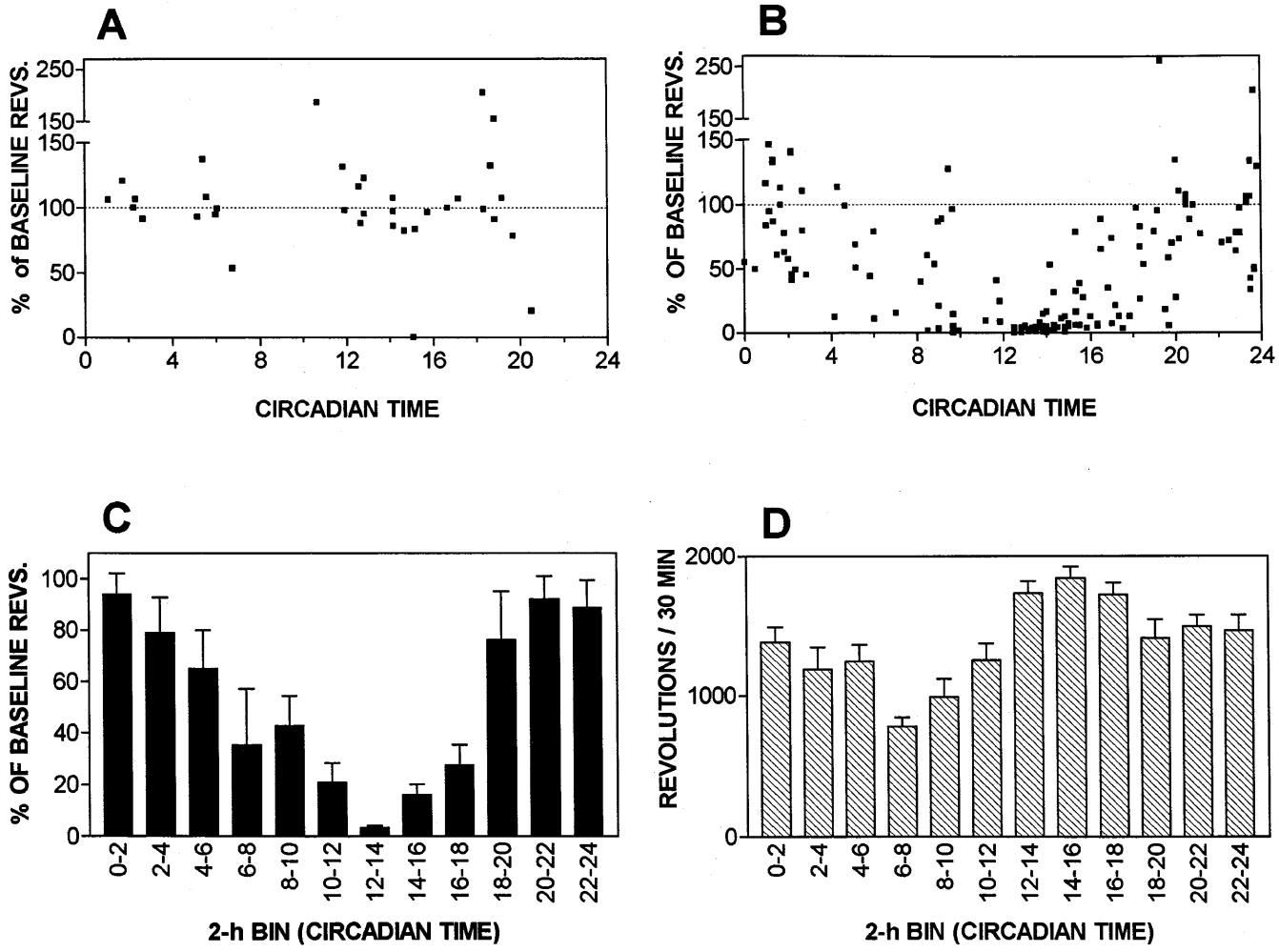


Fig. 7 Masking of induced activity as a function of cycle phase. Scores are wheel revolutions during a 30-min light pulse expressed as a percentage of wheel revolutions in the previous 30 min in the dark (for details of the procedure, see text). **A** Hamsters confined to novel wheels but not given light pulses. **B** Hamsters confined to novel wheels and given light pulses. **C** Mean (±SE) masking scores for 2-h bins for the data shown in **B**. **D** Mean wheel counts for the 30-min baseline used to calculate masking scores in **B** and **C**

time for post-pulse masking to dissipate. Probably this was the reason that total running over 24 h tended to be higher in LD 3.5:3.5-h than in LD 1:1-h cycles. Certainly, masking scores were higher in LD 3.5:3.5 h cycles. Furthermore, it is less likely that hamsters can entrain to an LD 3.5:3.5-h cycle than to an LD 1:1-h cycle. Collectively, these points make LD 3.5:3.5 h the better ultradian cycle for investigating masking by light in hamsters.

Comparison of measuring masking with ultradian LD cycles and with single light pulses

There are, however, some potential drawbacks to the use of cycles such as LD 3.5:3.5 h to study masking. It cannot be incontrovertibly ruled out that the presence of more activity in the dark phases reflects entrainment by the ultradian LD cycles of several endogenous rhythms rather than masking (Vilaplana et al. 1997). However, this seems unlikely in our experiments because the circadian activity seemed to remain intact, with masking effects superimposed (Fig. 3).

A more tangible disadvantage of the present way of using LD 3.5:3.5 h to study masking is that it takes a week for the LD cycle to regain its initial relationship to the circadian cycle. This makes it logical to base masking scores on data for a week or multiples of a week. The ultradian LD cycle method provides a pretty demonstration of masking (Fig. 3), but a quicker method may be preferred for some studies. One such method is to give a single light pulse at a time when the animals are likely to be active. Allowing a day or two before giving the next pulses gives ample time for the baseline to recover from the post-pulse activity suppression.

However, before using the single pulse method, one more potential objection should be considered – an objection that does not apply to circadian rhythms free running in ultradian LD cycles. It is conceivable that when an animal stops running in the early subjective night during a light pulse, it is not because of masking but because of phase shifting. A light pulse in the early

subjective night falls in the delay portion of the photic phase-response curve, and if a phase delay took place rapidly, then the hamster's clock could be set back to before CT 12, its activity onset. If the clock were reset to CT 11 for example, the output would be telling the animal to desist from wheel running.

The design of some of the present experiments makes this possibility implausible. The phase delays obtainable from hamsters to light are about 1 h (Takahashi et al. 1984; Reebs and Doucet 1997). In the present experiments, the light pulses were deliberately scheduled to start 1.5 h after dark onset, so that even if there were an immediate phase delay of up to 1.5 h, the clock would still be at a time when activity normally occurred. In another study we measured the phase shifts of a group of 24 hamsters following a 1-h light pulse (about 500 lx) at ZT 13.8. After the light pulse, hamsters were released into DD (Aschoff Type II method; Mrosovsky 1996). Immediate (-0.47 ± 0.06 h) and steady-state (-0.57 ± 0.07 h) phase delays were not large enough to explain the suppressed activity during the light pulse; this remains true even when phase shifts due to the release into DD without light are considered. Moreover, in the tests with recording in 1-min bins, suppression of activity occurred in the first min (Fig. 5). It seems unlikely that less than 1 min was saturating at this light intensity (Meijer et al. 1992). For these reasons it seems improbable that inactivity during the light pulse is the result of rapid clock resetting. We therefore felt it was safe to use the single-pulse method to study masking.

Comparison of masking in home cages and in novel wheels

The single-pulse method was also used to study whether light suppresses spontaneous home–cage running more than it suppresses running in a novel wheel. Although the decreases in wheel running were similar in absolute terms between home cages (1138 ± 77 fewer revolutions) and novel wheels (914 ± 116 fewer revolutions), we think that hamsters are more susceptible to masking in the home cage than in the novel wheel, for a number of reasons. First, in terms of decrements from baseline, the light reduced running more in the home cages. Second, the post-pulse masking was more pronounced in the home cages.

A third argument might be advanced in support of greater susceptibility of home-cage running to suppression by light: the greatest masking in Experiment 4 was obtained at CT 12–14 and this is just the time when spontaneous activity in hamsters is most likely to occur. In this interpretation, although the tests for masking at different phases of the cycle all took place in the novel wheel, it is assumed the nature of the running in that wheel was not uniform. At phases of the cycle when the animal would normally be resting, placing it in the novel wheel induced activity. At phases when the animal is active, placing it in the novel wheel only slightly increased the amount of activity (Fig. 4); most of the running at such times can be considered as spontaneous, even though the hamster was confined to the novel wheel. Thus, spontaneous activity may be more susceptible to masking than activity induced by other means. It is true that the animals were still relatively sensitive to masking lights even before CT 12, but perhaps the increased masking in the hours leading up to CT 12 reflects a growing inclination for endogenous activity which reaches a threshold at CT 12.

Circadian rhythm in masking

An alternative interpretation of the greater masking at CT 12 is that there is a circadian rhythm in sensitivity to masking. If so, this needs to be related to the study by Aschoff and von Goetz (1988) of positive masking by dark pulses in hamsters as a function of circadian phase. If dark pulses are considered the opposite of light pulses, by simply demasking the effects of light, then both dark and light pulses would be expected to have their greatest effects at the same cycle phase. Aschoff and von Goetz (1988) found that dark pulses increased activity most around the time of the two peaks of spontaneous activity, that is around CT 12 and CT 20–22. In the present study we also found increased masking at CT 12 but no evidence of a second peak of susceptibility to masking. However, comparison and interpretation of phase-dependent masking is made difficult by differences in methods and quantification of masking effects.

From a functional point of view, whether the increased susceptibility to inhibition of activity by light at CT 12 results from that being a particular time on a phase response curve for masking, or from a greater spontaneous component to the running at this time, does not alter the fact that masking is most pronounced when the hamster is most likely to be active anyway, namely at the start of the subjective night. This may have adaptive value. Presumably the hamster's physiology and behaviour have evolved to make the start of the night the best time for it to be active and start foraging. Any direct information about whether it is light or dark at that time might be expected to affect activity, making up for any minor inaccuracies in the clock control (Reebs 1994). In contrast, activity induced at times when the hamster is not usually active (and this includes the end of the subjective night) is not part of the circadian programme, and may be related to coping with new situations or avoiding predators. It would be inappropriate to make such activity especially susceptible to changes in illumination.

Concluding remarks

In conclusion, the present experiments support and extend Aschoff's point that masking complements the circadian system. Masking by light does not indiscriminately affect locomotor activity– the response is tailored

to the situation and the circadian phase. Taken together, these results demonstrate a strong but adjustable masking response to light in the hamster, aiding in restricting the endogenous activity to an appropriate time, which for a hamster is the beginning of the night. Certainly, although it is a system with the power to override circadian control, masking by light has long been under appreciated.

Acknowledgements This work was supported by grants from the R.P. Research Foundation – Fighting Blindness and the Medical Research Council of Canada to N. Mrosovsky. U. Redlin was supported by a grant from the Fight for Sight,, NY, Research Division of Prevent Blindness America in memory of Alexander P. and Mary E. Hirsch. We thank Peggy Salmon and Natalie Chung for their technical assistance. These experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Welfare.

References

Arendt J, Ravault J-P (1988) Suppression of melatonin secretion in Île-de-France rams by different light intensities. J Pineal Res 5: 245–250

Aschoff J (1960) Exogenous and endogenous components in circadian rhythms. Cold Spring Harbor Symp Quant Biol 25: 11–28

Aschoff J (1988) Masking of circadian rhythms by zeitgebers as opposed to entrainment. In: Hekkens WThJM, Kerkhof GA, Rietveld WJ (eds) Trends in chronobiology. Pergamon Press, Oxford, pp 149–161

Aschoff J, Goetz C von (1988) Masking of circadian activity rhythms in hamsters by darkness. J Comp Physiol A 162: 559–562

Borbély AA, Huston JP (1974) Effects of two-hour light-dark cycles on feeding, drinking and motor activity of the rat. Physiol Behav 13: 795–802

Brainard GC, Richardson BA, Hurlbut EC, Steinlechner S, Matthews SA, Reiter RJ (1984) The influence of various irradiances of artificial light, twilight, and moonlight on the suppression of pineal melatonin content in the Syrian hamster. J Pineal Res 1: 105–119

Czeisler CA, Shanahan TL, Klerman EB, Martens H, Brotman DJ, Emens JS, Klein T, Rizzo JF III (1995) Suppression of melatonin secretion in some blind patients by exposure to bright light. N Eng J Med 332: 6–11

Elliott JA (1976) Circadian rhythms and photoperiodic time measurement in mammals. Fed Proc 35: 2339–2346

Elliott JA, Tamarkin L (1994) Complex circadian regulation of pineal melatonin and wheel-running in Syrian hamsters. J Comp Physiol A 174: 469–484

Note added in proof Persistence of inhibition of activity of hamsters after a light pulse has ended has been reported recently by Mistlberger and Antle (1998).

Mistlberger RE, Antle MC (1998) Behavioral inhibition of light-induced circadian phase resetting is phase and serotonin dependent. Brain Res 786: 31–38

Erkert HG, Gröber J (1986) Direct modulation of activity and body temperature of owl monkeys (*Aotus lemurinus griseimembra*) by low light intensities. Folia Primatol 47: 171–188

Illnerová H, Vaněček J, Křeček J, Wetterberg L, Sääf J (1979) Effect of one minute exposure to light at night on rat pineal serotonin *N*-acetyltransferase and melatonin. J Neurochem 32: 673–675

Kennaway DJ, Rowe SA (1994) Impact of light pulses on 6-sulphatoxymelatonin rhythms in rats. J Pineal Res 16: 65–72

Meijer JH, Rusak B, Gänshirt G (1992) The relation between light-induced discharge in the suprachiasmatic nucleus and phase shifts of hamster circadian rhythms. Brain Res 598: 257–263

Mrosovsky N (1994) In praise of masking: behavioural responses of retinally degenerate mice to dim light. Chronobiol Int 11: 343–348

Mrosovsky N (1996) Methods of measuring phase shifts: why I continue to use an Aschoff type II procedure despite the skepticism of referees. Chronobiol Int 13: 387–392

Mrosovsky N, Salmon PA, Vrang N (1998) Revolutionary science: an improved running wheel for hamsters. Chronobiol Int 15: 147–158

Mrosovsky N, Foster RG, Salmon PA (1999) Thresholds for masking responses to light in three strains of retinally degenerate mice. J Comp Physiol A 184: 423–428

Petterborg LJ, Kjellman BF, Thalén BE, Wetterberg L (1991) Effect of a 15-minute light pulse on nocturnal serum melatonin levels in human volunteers. J Pineal Res 10: 9–13

Pittendrigh CS, Daan S (1976) A functional analysis of circadian pacemakers in nocturnal rodents. J Comp Physiol A 106: 223–355

Ralph MR, Foster RG, Davis FC, Menaker M (1990) Transplanted suprachiasmatic nucleus determines circadian period. Science 247: 975–978

Redlin U, Vrang N, Mrosovsky N (1999) Enhanced masking response to light in hamsters with IGL lesions. J Comp Physiol A 184: 449–456

Reebs SG (1994) The anticipation of night by fry-retrieving convict cichlids. Anim Behav 48: 89–95

Reebs SG, Doucet P (1997) Relationship between circadian period and size of phase shifts in Syrian hamsters. Physiol Behav 61: 661–666

Takahashi JS, DeCoursey PJ, Bauman L, Menaker M (1984) Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. Nature (Lond) 308: 186–188

Vilaplana J, Cambras T, Diez-Noguera A (1997) Dissociation of motor activity circadian rhythm in rats after exposure to LD cycles of 4-h periods. Am J Physiol 272: R95–R102

Weisgerber D, Redlin U, Mrosovsky N (1997) Lengthening of circadian period in hamsters by novelty-induced wheel running. Physiol Behav 62: 759–765