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Rest-activity rhythm of the blind mole rat *Spalax ehrenbergi* under different lighting conditions

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Abstract

The mole rat is a solitary, subterranean and photoperiodic rodent. We investigated its rest activity behavior under several lighting conditions, complemented our observations with light-induced c-fos expression, and compared the activity behavior of two chromosomal forms (2n = 58 and 60). The 26 mole rats had a clear overall preference for activity in the light or dark period, but prolonged recordings in five individuals showed that the initial preference was not stable in the nocturnal animals, they became diurnal. A 6-h advance of the light-dark (LD) cycle induced a shift of activity and the previous LD preference was reestablished. The large daily variability of activity onset did not allow this study to determine whether the animals were entrained to the LD cycle upon release into constant darkness (DD) or whether activity had been masked by light. The period of the motor activity rhythm in DD free ran in more than 50% of the animals. No differences in activity were observed between the two karyotypes. Immunohistochemistry for c-fos expression in the nucleus suprachiasmaticus at different circadian times showed that c-fos was induced only in animals exposed to a 1-h light pulse during the subjective night, but not during the subjective day or in control animals in the absence of a light pulse. The large intra- and inter-individual variability in daily motor activity both in LD and in DD suggest only a weak photic entrainment of the circadian clock to light of approximately 100 lux, and possibly a weak regulation of behavior by the circadian clock.

Keywords: Blind mole rat; *c-fos*; Circadian rhythm; Light–dark cycle; Motor activity; Running wheel; SCN; Subterranean

1. Introduction

The regulation of mammalian circadian behavior is under the control of a circadian pacemaker located in the suprachiasmatic nuclei (SCN) in the hypothalamus [16,31,32]. A specific retino-hypothalamic tract conveys light information from the eyes to the SCN and allows the entrainment of the pacemaker to the daily light–dark (LD) cycle. The mechanisms involved in this entrainment pathway include the induction of *c-fos* in the SCN [29].

Specialized animals which are not obviously exposed to the light–dark cycle of the environment may serve to understand the role of the circadian system under conditions where the timing of behavior may be less important for survival. The habitat of subterranean animals often exhibits only small fluctuations which may lack the capacity to serve as entraining stimuli. Although the exposure of subterranean species to light is limited (it is estimated that many species spend approximately 99% of their time in complete darkness), most do have specific activity rhythms (e.g. European mole, *Talpa europea*, [9,34]; pocket gopher, *Thomomys bottae*, [8];

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Scalopus aquaticus, [10]; solitary Cape mole rat, *Georychus capensis* [14]; *Cryptomys hottentotus* [1,11,13]; and *Cryptomys damarensis* [15]), though behavioral sleep based on scoring of video recordings of two colonies of the eusocial naked mole rat *Heterocephalus glaber (*Bathyergidae) was not entrained to the LD cycle [7].

The mole rat (*Spalax ehrenbergi*; Spalaxidae) is a solitary species which mates in the winter and exhibits many adaptations to the fossorial habitat [19,21]. Recently its optical system was described in detail. The structures which serve to detect shapes and movements are regressed, the number of ganglion cells is reduced, and a hypertrophy of the SCN was found [3,5,6,28,30]. Thus, although *Spalax* is totally blind, it does have light perception. Laboratory studies have suggested entrainment to an LD cycle, though many individuals were arrhythmic [2,20,27], and in continuous dim white or red light approximately 50% of the animals free ran with a period differing from 24 h [27] indicating that the circadian system may be intact despite the reduced light perception.

Though the locomotor-activity rhythm of *Spalax* has been investigated in the natural habitat [26] no measures of light exposure have been performed in wild populations. When mole rats excavate their tunnel system, they must bring the earth to the surface and thereby may be exposed to light. It is unknown whether this suffices to entrain the circadian system and to detect photoperiodic changes in the course of the year. Photoperiod perception was demonstrated in female mole rats in an LD 16:8 cycle [23]. Photoperiodic synchronization may be necessary for a successful reproduction.

The mole rat *Spalax ehrenbergi* is distributed from Southeast Turkey through the near east to North Africa and is subdivided into four morphologically indistinguishable forms which differ in the number of chromosomes (diploid set, $2n = 52$, 54, 58 or 60) and occur in Israel in narrow hybrid zones [21,22]. In previous experiments addressing the activity pattern of *Spalax* the karyotype was not specified [27,28], or recordings encompassed only 24–36 h before imposing constant darkness [2,20].

Our purpose was to investigate several aspects of the circadian system on the basis of locomotor activity. We attempted to clarify whether the mole rats show real entrainment to an LD cycle or merely masking by: (1) comparing the phase relationship of activity to a light dark cycle and after release into constant darkness; and (2) applying a single light pulse during the 12-h dark phase according to the paradigm used by Mrosovsky [18] in retinally degenerate mice. Our behavioral recordings were complemented with data of c-fos induction. The availability of *Spalax* of two karyotypes allowed the comparison of their activity patterns.

2. Materials and methods

2.1. Animals and recording conditions

Two chromosomal forms of mole rats, *Spalax ehrenbergi*, whose diploid set of chromosomes was $2n = 58$ or 60 were investigated (15 $2n = 60$ from the region 'Anza', seven $2n = 58$ from 'Muhraqa' Mount Carmel, 'Kabri' and 'Nahal Oren', and four $2n = 58$ or 60 from Muhraqa and Kabri). All 26 animals were juvenile or adult males of unspecified age, captured in the field in Israel and transported to Zürich in three batches in the course of 2 years. Their mean weight at the beginning of the recordings, after at least 40 days adaptation to the environment, was 170.6 ± 8.7 g (S.E.M.). They were kept in an air conditioned and acoustically isolated room with light provided by fluorescent tubes (Philips, TL-D, 58W/33). Ambient temperature was between 21–22°C and relative humidity 36–70%. The first batch of nine animals (Table 1) was initially maintained in an LD cycle of 12:12 h (light 8–20 h; 33–83 lux, minimum and maximum values measured at the bottom of the cages; after day 243 all mole rats were exposed to: L 61–103 lux, superimposed with constant dim red light of approximately 0.01–0.05 lux; 57.0–206.6 $\mu W/m^2$; red plastic; Encap Sulite, Type R20, max. intensity at 610 nm, fixed around a fluorescent tube, Osram L 36W/23, to allow animal care during the 'dark' phase, and then to DD or constant dim red light (DDim). The mole rats were initially housed individualy in polyvinylchloride (PVC) cages and later in aluminum cages ($54 \times 34 \times 31$ cm) placed on four layers of air-bubble plastic to reduce vibrations, with sufficient wood chippings to allow burrowing. The animals were fed with apples, carrots and potatoes ad libitum two to three times per week and cleaning of the cages occurred once or twice a month. Soiled wood chippings were removed and replaced when the animals were fed. Feeding and cleaning was performed at random times of the day.

Motor activity was recorded by a single passive infra red sensor (IR, Profitec SA-215, wide-angle lens) mounted 66 cm over the cage, and in addition, in later experiments by a running wheel (PVC, diameter 28 cm). Each running wheel rotation elicited an impulse activating a contact reed (Hamlin, type MDRR-DT). The number of impulses generated by the IR-sensors and the running wheels was stored on-line on a PC in 1-min intervals (Stanford Software Systems, chronobiology kit).

The period of the free running motor activity rhythm (IR activity values) was estimated for three intervals of at least 7 days after: (1) 15–38 days in DD; (2) 3–14 days in DDim; and (3) 34–63 days in DDim by χ^2-periodogram analysis (Stanford Software Systems, chronobiology kit) or, in a few cases, by visual fitting of a line through the activity onsets.

Table 1
Experiments, corresponding light conditions, total amount of days within a condition or entering the analysis, and number of mole rats with the corresponding figures they contributed to (some individuals contributed to several experiments)

Light conditions		Experiment	Total days	Number of animals (total $N = 26$)	Figure
LD 33–83 lux	12:12 h	Habituation	40	9	
		LD preference 1	35	9	Figs. 5 and 6
		6-h shift of LD		9	Fig. 7
DD	Dark		62	11	
		Circadian period	7–10 (after 15–38 days)		Fig. 8 top
LD 61–103 lux+ constant dim	12:12 h				
		Comparison karyotypes 1		12	Text
		LD preference 2	35	5	Fig. 6
		+Running wheel comparison karyotypes 2	29	6+6	Text
DDim	Dim red	Circadian period	7–10 (after 3–14 days)	20	Fig. 8 middle
DDim	Dim red	Circadian period	7–10 (after 34–63 days)	18	Fig. 8 bottom
	c-fos			10	Fig. 9, Table 2

LD, light–dark; DD and DDim, constant dark or dim red light, respectively.

2.2. Fos immunohistochemistry

After the experiments, the remaining ten animals were maintained on an LD 12:12 h cycle for at least 6 weeks prior to release into DD. Since the phase of the animals could not be clearly assessed in free running conditions, we used the first day in DD to apply a light pulse, when there is little deviation from the exposure conditions of the previous day. Thus on the initial day of testing, individuals received a 60-min light pulse at one of four times including both early and late subjective day and night (ZT4, ZT10, ZT16 and ZT22; where ZT0 was the time of previous light onset). Due to the limited number of animals available, only one to two animals contributed to each time period. Light stimulation was delivered using fluorescence lamps (Philips TLD; 50W/84 HF; 400–500 lux). As dark controls, at each time period, an additional animal was perfused without light stimulation. Animals were deeply anesthetized with methoxyflurane (inhalation) and nembutal (40–50 mg/kg i.p.) and perfused intracardially with heparinized saline followed by 500 ml of 4% paraformaldehyde in phosphate buffer (pH 7.4, 0.1 M) at 4°C. The brains of all animals were post-fixed for 24 h at 4°C.

Histology was performed in Lyon. Coronal sections were made at 40 μm on a freezing microtome. For immunohistochemistry, endogenous peroxidase was suppressed using a solution of 50% ethanol in saline with 0.3% H_2O_2. Free floating sections were briefly rinsed in phosphate buffered saline (0.01M, pH 7.2) containing 0.3% triton and 0.1% sodium azide (PBSTA) and blocked with 1.5% normal goat serum (1 h). Sections were rinsed twice in PBSTA and incubated in the primary antibody (rabbit polyclonal anti-fos, concentration 1/2500 in PBSTA; Oncogene Science, No. PC05) for 3 days at 4°C. Sections were then rinsed in PBSTA, and the presence of fos-like protein demonstrated with the avidin-biotin technique using a secondary biotinylated anti-rabbit antibody (Vectastain, BA-1000), followed by avidin-biotin peroxidase complex (Vectastain, PK6100). Peroxidase was demonstrated by incubation for 10 min in 0.2% DAB with 0.5% ammonium nickel sulfate and 0.003% H_2O_2 in Tris buffer (0.04M, pH 7.6). The sections then were rinsed, mounted on glass slides, dehydrated, and coverslipped in Depex.

Fos-like immunopositive nuclei were counted by direct visual observation and by computer assisted image analysis (Biocom, Les Ulis, France) for detection of nuclear profiles according to density in 11–12 sections per animal. Both methods gave identical results. The identity of the animals were unknown to the observer and all sections of the SCN were counted.

3. Results

3.1. Light–dark preference and activity pattern

Figs. 1–4 illustrate long-term motor activity patterns of individual mole rats. A large variability was observed within and between the animals. The analysis of a 35-day interval after 41 days adaptation to the cages and environment, showed that the majority of the nine animals we had obtained in the first batch was noctur-

nal ($N = 7$; Fig. 5B), while only two were diurnal (see Fig. 5D and F for examples of nocturnal and diurnal individuals, respectively). We determined the stability of the LD preference in the nocturnal animals within the 35 days by subdividing them arbitrarily in 7-day intervals. This resulted in a significant LD difference only in the last 7-day interval (Fig. 5A), because at least one individual in each previous 7-day interval showed a maximum in the light period (see Fig. 5G for an example of an individual with a fluctuating LD preference). Thus the mole rats could be separated into three groups exhibiting: (1) a stable nocturnal activity preference ($N = 4$; e.g. Fig. 5C); (2) a stable diurnal preference ($N = 2$; e.g. Fig. 5E); and (3) fluctuations between a diurnal and a nocturnal pattern ($N = 3$; e.g. Fig. 5G). However, of the five animals which were recorded for over a year (four of the

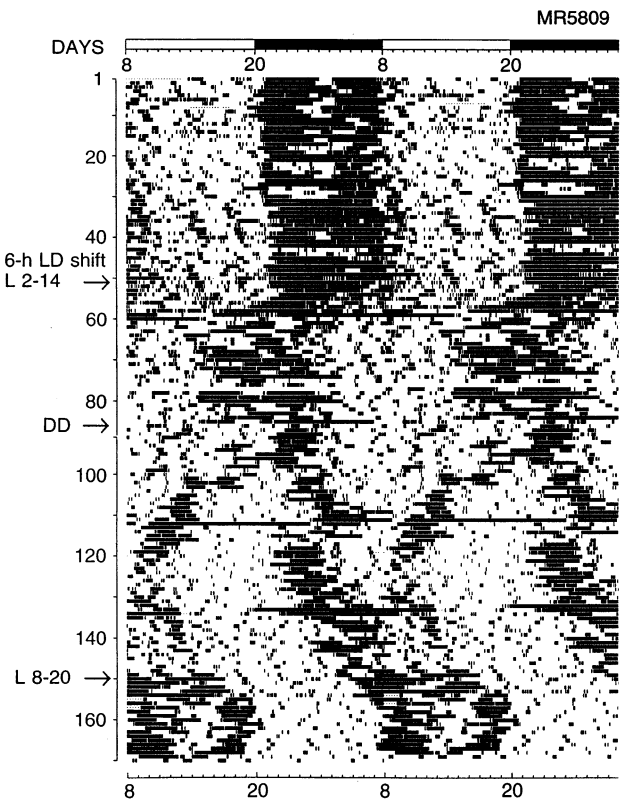

Fig. 1. Individual activity plot of a mole rat (chromosomal form with diploid chromosome set $2n = 58$). Motor activity was recorded by a passive infra red sensor and double-plotted, i.e. the horizontal axis represents 48 h, and every 24-h interval is represented twice. On days 1–50 and 150–170 the animal was maintained in a 12:12 h light–dark (LD) cycle indicated by the white and black bars at the top (Table 1). On day 51 a 6-h LD shift was implemented (light from 02:00–14:00 h), on day 86 the animal was subjected to constant darkness (DD). The horizontal bars represent motor activity based for all 5-min intervals with at least one activity count. The period of the main component of the activity rhythm at the beginning of DD was 24.2 h. The period of a second component was 23.3 h, indicating splitting of the activity rhythm.

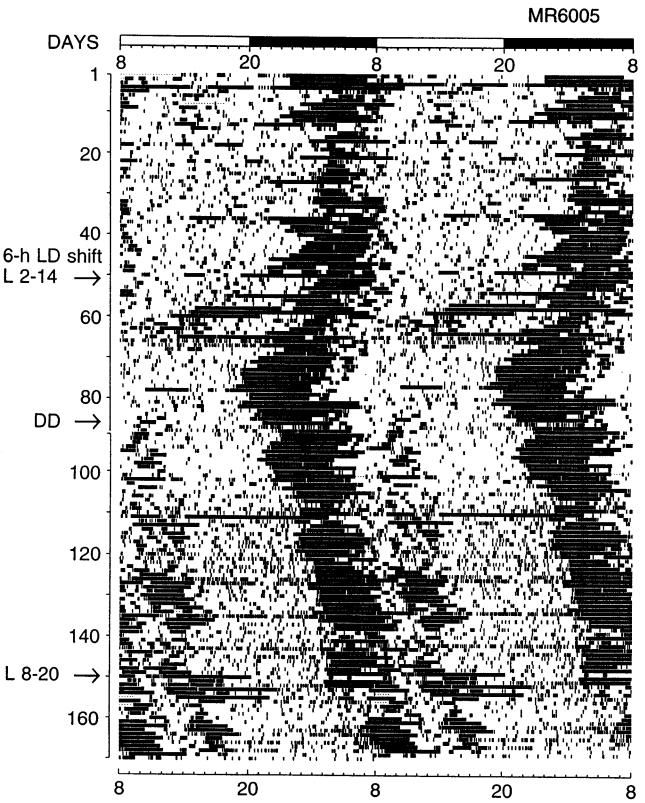

Fig. 2. Individual activity plot of a mole rat (chromosomal form $2n = 60$). Arrows indicate the day of the 6-h shift of the light–dark cycle (LD), the initiation of constant darkness (DD), and the reestablishment of LD. On day 86 the animal was exposed to DD. The period of the activity rhythm at the end of the interval in DD was 24.1 h. For further details see legend for Fig. 1.

stable nocturnal mole rats and one diurnal animal), the analysis of a second 35-day interval 40 weeks after the first interval revealed that the nocturnal animals had become diurnal (Fig. 6) and the diurnal one had not changed its preference (not shown).

The comparison of the activity pattern of six mole rats of each karyotype, after at least 10 weeks in LD 12:12 (Table 1) showed that the majority, independent of karyotype, were diurnal (only one $2n = 58$ and three $2n = 60$ were nocturnal). The running wheel was used vigorously (the highest and lowest maximum value per minute within a 10-day interval attained by single mole rats was 51 and 9, corresponding to 44.9 and 7.5 m/min), but it did not affect the 24-h activity pattern: the IR and running wheel activity profiles were very similar. The chromosomal forms did not differ significantly in the running-wheel activity profile (data not shown), or in total activity within the light period, either in the presence or absence of a running wheel (without running wheel: $2n = 58$: 152.0 ± 6; $2n = 60$: 163.5 ± 8.9; with a running wheel: $2n = 58$: 148.4 ± 5.4; $2n = 60$ 140.6 ± 6.0; values as percent of 24 h).

3.2. Synchronization to the LD-cycle

To determine whether the activity rhythm would synchronize to a shift of the LD cycle, we advanced the LD 12:12 cycle by 6 h (L: 08:20 to 02:14 h). The daily distribution of IR-motor activity in the 35-day interval immediately before the shift was compared with five 7-day intervals after the shift. In addition, the first interval after the shift was analyzed for individual days. The mean L and D values (Fig. 7A–C) include those seven animals whose previous history is represented in Fig. 5A and B. They met two conditions: they were dark active in the mean value of the 35-day interval and in the last 7-day interval preceding the light advance.

The mean activity pattern shifted to the new LD-conditions within approximately 2 weeks in the nocturnal animals (Fig. 7B and C) and immediately in the two diurnal animals (see Fig. 7H and I for an example of a diurnal animal). The shift was verified on the individual actograms (e.g. Figs. 1 and 2). The mean 12-h activity values of the single days immediately after the phase advance of the LD-cycle illustrate the transients. However, the subdivision of the post-shift interval into

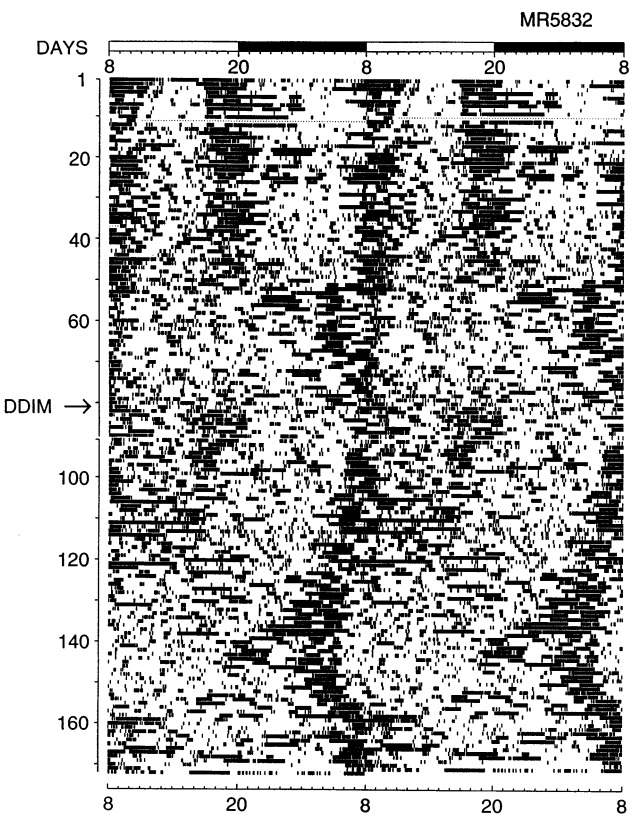

Fig. 3. Individual activity plot of a mole rat (chromosomal form 2n = 58). For details see legend for Fig. 1. On day 81 after beginning the recordings the animal was exposed to constant dim red light (DDIM). The period of the activity rhythm in DDIM was initially 23.9 h and towards the end 24.2 h, with no apparent reason for the change.

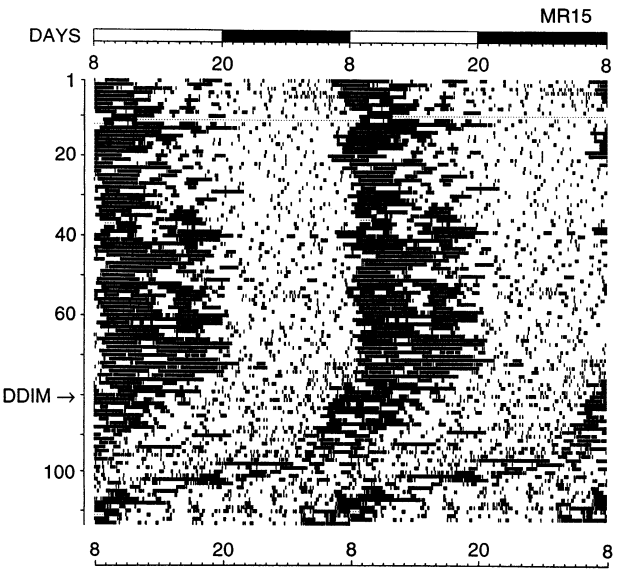

Fig. 4. Individual activity plot of a mole rat (chromosomal form 2n = ?). For details see legend for Fig. 3. The initial period in DDIM was 22.8 h. The animal died before the end of the DDIM experiment, therefore we did not include the second computation of its activity period.

7-day bins revealed that the newly established preference was not stable. Only the second interval showed a significant difference between the activity values of the light versus the dark period because at least one individual exhibited a larger activity value in the light than in the dark in each 7-day interval. Moreover, only four mole rats which previously had been consistently nocturnal shifted their activity and reestablished their preference for the dark phase (e.g. see the individual in Fig. 7D–F). The single consistently light active animal shifted its activity into the light period (Fig. 7G–I). The three animals which previously had no consistent preference for the light or dark (e.g. Fig. 5G and H), did not establish a preference in the 35 days after the shift (not shown).

To investigate whether the synchronization had been affected by the shift, activity onset after lights off (or after lights on in the diurnal animal) was determined for a 7-day interval immediately before the shift and the last 7 days of a 35-day interval after the shift. Activity onset was determined on the basis of the individual 7-day 24-h mean activity profile (based on 30-min bins) and was defined as the time when the activity value of the 30-min bin was at least double the value of the preceding interval.

A large intra- and inter-individual variability of activity onset was evident (e.g. Figs. 2–4). The range before the shift was -8 to $+10$ 30-min bins relative to L or D onset and the mean value $+0.6 \pm 1.9$ S.E.M. 30-min bins ($N = 9$). The mean synchronization after the shift was $+4.3 \pm 2.1$ 30-min bins, and the mean difference was $+3.4 \pm 3.7$ 30-min bins. The range of

the difference between activity onset before and after the LD-shift was 30–600 min. No significant difference was observed in the phase of entrainment to the LD-cycle before and after the shift ($P > 0.4$; paired t-test, $N = 9$).

3.3. Rest-activity rhythm in constant darkness or dim red light

Individual actograms illustrate the activity pattern in constant conditions (Figs. 1–4) and the distribution of the mean periods of activity in three intervals are

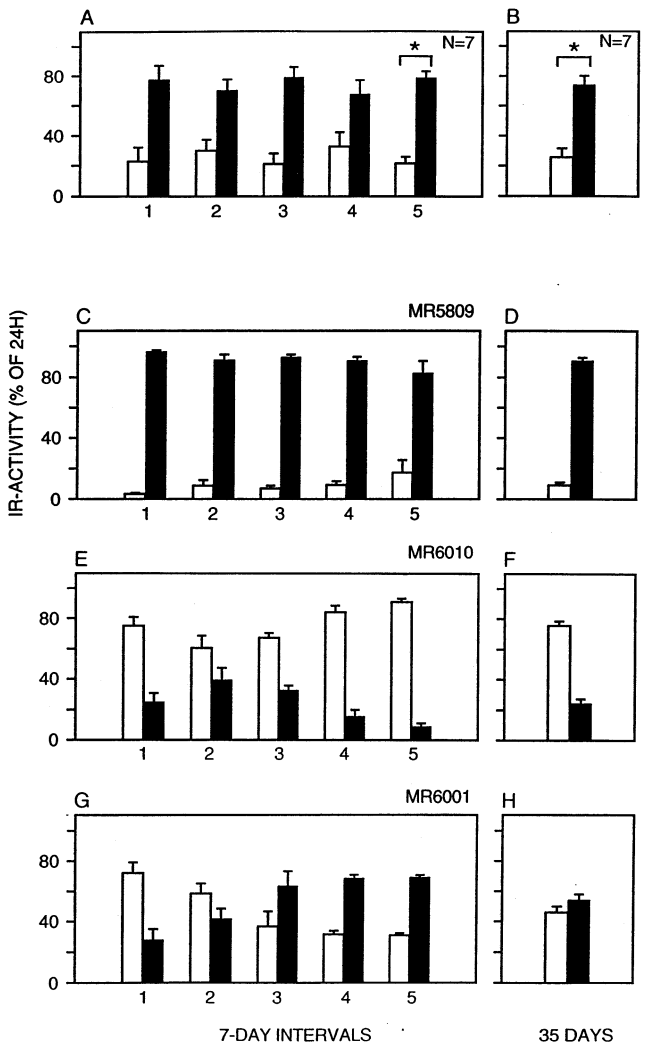

Fig. 5. Motor activity for five consecutive 7-day intervals (1–5) and the entire 35-day recording. Mean values of the nocturnal animals ($N = 7 \pm$ S.E.M.), including the individuals illustrated in C and G, over 7 and 35 days (A, B) and three examples of individuals to illustrate a stable nocturnal, stable diurnal and fluctuating preference (C–H). Bars represent the mean 12-h activity values measured by passive infra red sensors for the light and dark period (white and black bars). The means and S.E.M. in A and B were determined on the basis of the respective 7- or 35-day mean value computed for each individual. * Significant differences between the light and dark period, $P < 0.05$; two-tailed paired t-test.

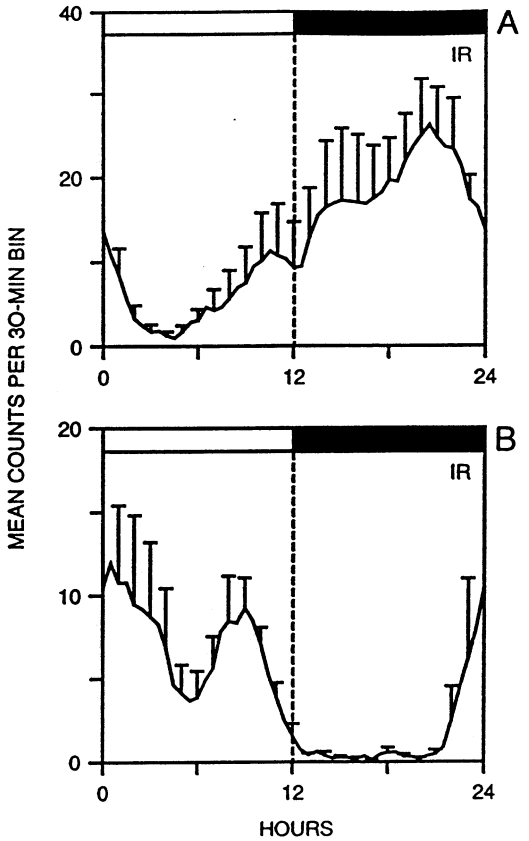

Fig. 6. Daily activity profiles in the course of prolonged recordings. Each panel represents a mean 35-day profile (Table 1). Mean activity counts per minute for 30-min bins $\pm$ S.E.M. ($N = 4$) over 24 h, measured by a passive infra red sensor (IR). The animals had not yet been provided with running wheels.

illustrated in Fig. 8 (Table 1). The activity periods of neither of the two DDim conditions differed significantly between the two chromosomal forms (first DDim interval: 2n = 58, $N = 6$, 23.8 ± 0.2, 2n = 60, $N = 10$, 23.9 ± 0.04; second DDim interval: 2n = 58, $N = 6$, 23.9 ± 0.1, 2n = 60, $N = 9$, 24.1 ± 0.07).

To clarify whether the animals' activity had been merely synchronized to the previous light-dark schedule or whether the endogenous clock was entrained, we attempted to determine the phase of activity onset in DD or in DDim relative to the previous LD-cycle, by eye-fitting a line through the onsets of activity in the first 10 days in the constant conditions. The irregular daily onset of motor activity in LD did not allow to determine reliably the activity onset on day 1 after release of the animals into DDim (e.g. Fig. 3). A similar day-to-day variability was seen also when activity onset was determined by running-wheel activity.

3.4. C-fos immunohistochemistry

The total number of c-fos immunopositive cells in the SCN is listed in Table 2. In the absence of a light pulse

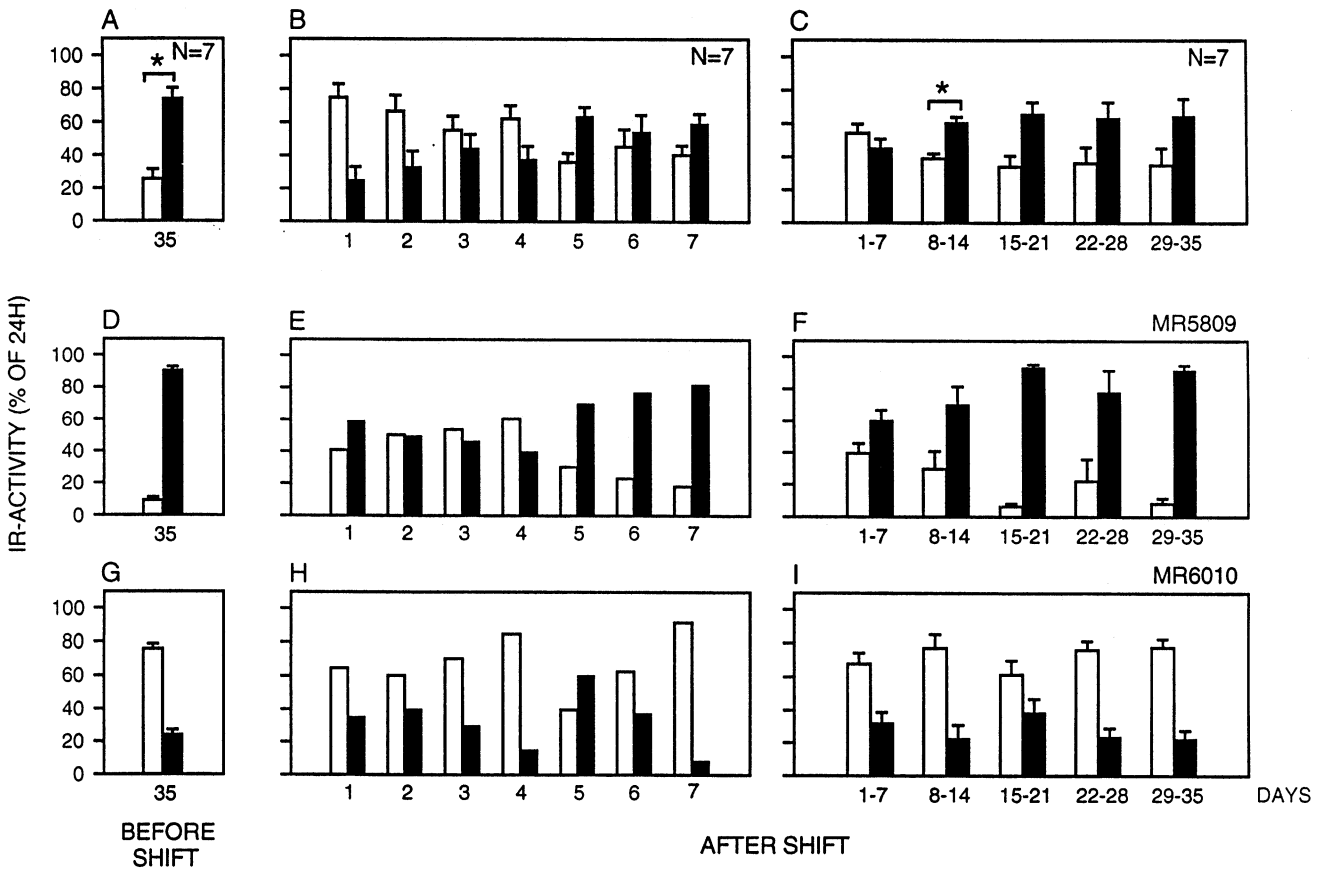

Fig. 7. Motor activity for 12-h periods in the light-dark conditions (LD; white and black bars respectively). A–C: mean activity values ± S.E.M. measured by passive infra red sensors for the 35-day interval before a 6-h LD shift, single days (1–7) immediately after the LD shift and five 7-day intervals after the shift ($N = 7$). The means and S.E.M. in A–C were determined on the basis of the 1-, 7- or 35-day mean value, respectively computed for each individual. D–I: corresponding data of two individuals. * $P < 0.05$, two-sided paired t-test, 12-h light vs. 12-h dark.

(control animals) c-fos immunoreactivity was never observed in the SCN (Fig. 9). In the presence of a light pulse, c-fos immunoreactivity was also absent or very low during the subjective day (ZT4, ZT10). C-fos was markedly expressed following a light pulse during the subjective night (ZT16, ZT22). Only one individual receiving a light pulse during the subjective night (ZT16) failed to show expression of c-fos. However, the computation of the activity period in the last 10 days in LD 12:12 before the light pulse was applied, showed that this animal had become arrhythmic, whereas all others were diurnal.

4. Discussion

4.1. Light–dark cycle

The mole rats were either light or dark active, confirming previous data that they do perceive the change from light to dark or vice versa. However, the large inter- and intra-individual variability in the 24-h activity profiles indicates only a weak coupling to the LD cycle. With the provision of a running wheel we attempted to reduce the variability, but this was not the case. The activity patterns were monophasic (Figs. 1 and 2) or biphasic (Figs. 3 and 4). In field recordings by radio transmitters a monophasic, diurnal activity pattern was prevalent [26], whereas in previous laboratory studies both a diurnal activity pattern [27], and multiphasic activity bouts were observed [2,20].

Since in the field *Spalax* were diurnal [26], it is not surprising that after prolonged adaptation to the laboratory they eventually also became diurnal, as had been the case in two other studies [27,28]. Many individuals may have perceived only the light change at the LD transition. The 6-h light shift elicited a progressive adaptation to the new LD cycle. But in some individuals the LD preference and the activity pattern had changed, and the previous LD or DL activity ratio was not attained even after 35 days in the new conditions. All four animals exposed to a larger LD shift of 12 h and similar light intensity entrained to the new LD cycle within 1–2 weeks [27]. Neither the degree of synchronization nor its stability after the shift was investigated previously. The variability in the adapta-

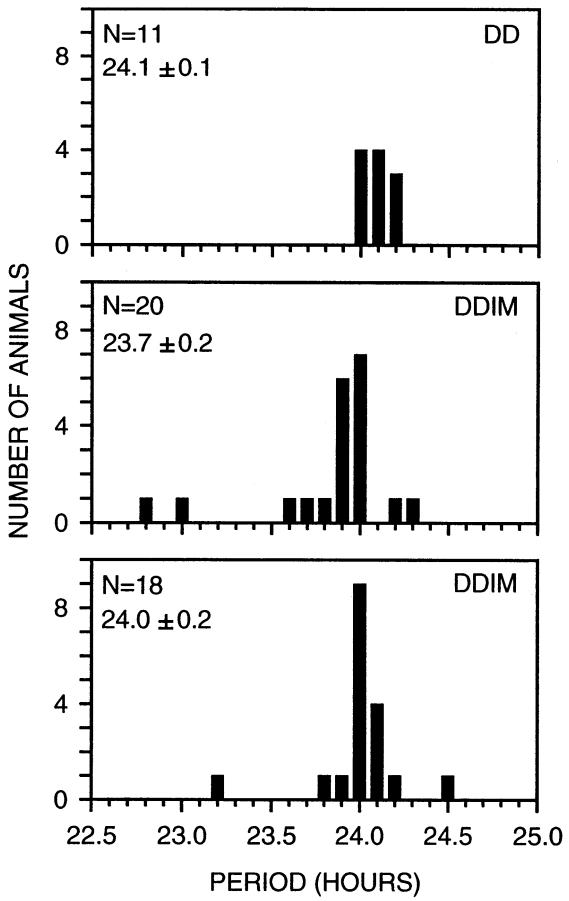

Fig. 8. Distribution of the period of the locomotor-activity cycle in constant darkness (DD) or constant dim red light (DDim). The same individuals contributed to the two DDim conditions (for technical reasons two were no longer included in the bottom panel, and six of them also to the top panel. Within the panels: mean period length in hours ± S.E.M. and *N* is the number of animals.

tion to the advance of the LD cycle, and the failure to detect a clear, stable phase relation between LD-conditions and activity supports the hypothesis that the changes of light intensity at the LD or DL transition were detected, rather than the lighting condition per se. This could be a feature of the subterranean life of these rodents. In other rodents like the rat, mouse and hamster synchronization to an LD advance in the presence of a running wheel is more stable and entails also entrainment of the circadian clock [12,17]. However, it cannot be excluded that the light intensity may have been too low or, alternatively, that light perceiving structures may have incurred some damage by the exposure to light of approximately 100 lux.

It is possible that the circadian clock was not entrained by the LD cycle and that the behavioral response to light was merely due to masking [18]. The variability of activity onset did not allow a reliable assessment of the phase relationship between the LD cycle and the endogenous clock. Our attempt to investigate the contribution of masking by applying a 1-h light pulse during the dark period and alternatively, a 1-h dark pulse during the light period was unsuccessful. Neither experiment allowed to draw conclusions because the day-to-day variability in the activity pattern as well as its variation within a day resulted in many animals sleeping during the pulses, and the comparison of activity during the pulse with the corresponding hour of the day prior to the pulse or with activity in the hour before or after the pulse was meaningless (data not shown).

4.2. Constant lighting conditions

In DD (and in DDim) over 50% of the animals exhibited a free running activity rhythm but a large inter-individual variability of period was evident. This could be an indication for a weak link between the endogenous clock and locomotor-activity behavior. One could speculate that in the subterranean environment the selection for a strong control of the motor activity pattern by the SCN is unnecessary. Previous studies also showed a large variability of activity rhythms in the absence of a LD cycle, but in contrast to our animals, arrhythmicity was often encountered [2,27]. Some of the free running rhythms showed a period of 24 h (Fig. 8). In such cases a hidden 24-h zeitgeber cannot be excluded. However, this is unlikely because individual animals did not show this consistently throughout the experimental periods.

Table 2
C-fos immunohistochemistry

	Controls				Light pulse					
Time (ZT)	4	10	16	22	4	10	16	16	22	22
Karyotype	?	60	?	60	58	58	58	58	60	60
Total no of cells	0	0	0	0	0	47	0	316	295	1140
Mean cell count	0	0	0	0	0	5.88	0	45.14	36.88	103.64

Cell counts of c-fos immunopositive cells in the SCN of ten individuals. The brains were obtained either in constant darkness (controls) or after 60-min exposure to a light pulse at different circadian times (ZT12, previous dark onset) on the 1st day in constant darkness (light pulse). Total number of immunopositive cells and mean cell count over 11–12 sections per animal. Karyotype designates the number of diploid set of chromosomes ($2n = 60$ or $2n = 58$), unless not known (?).

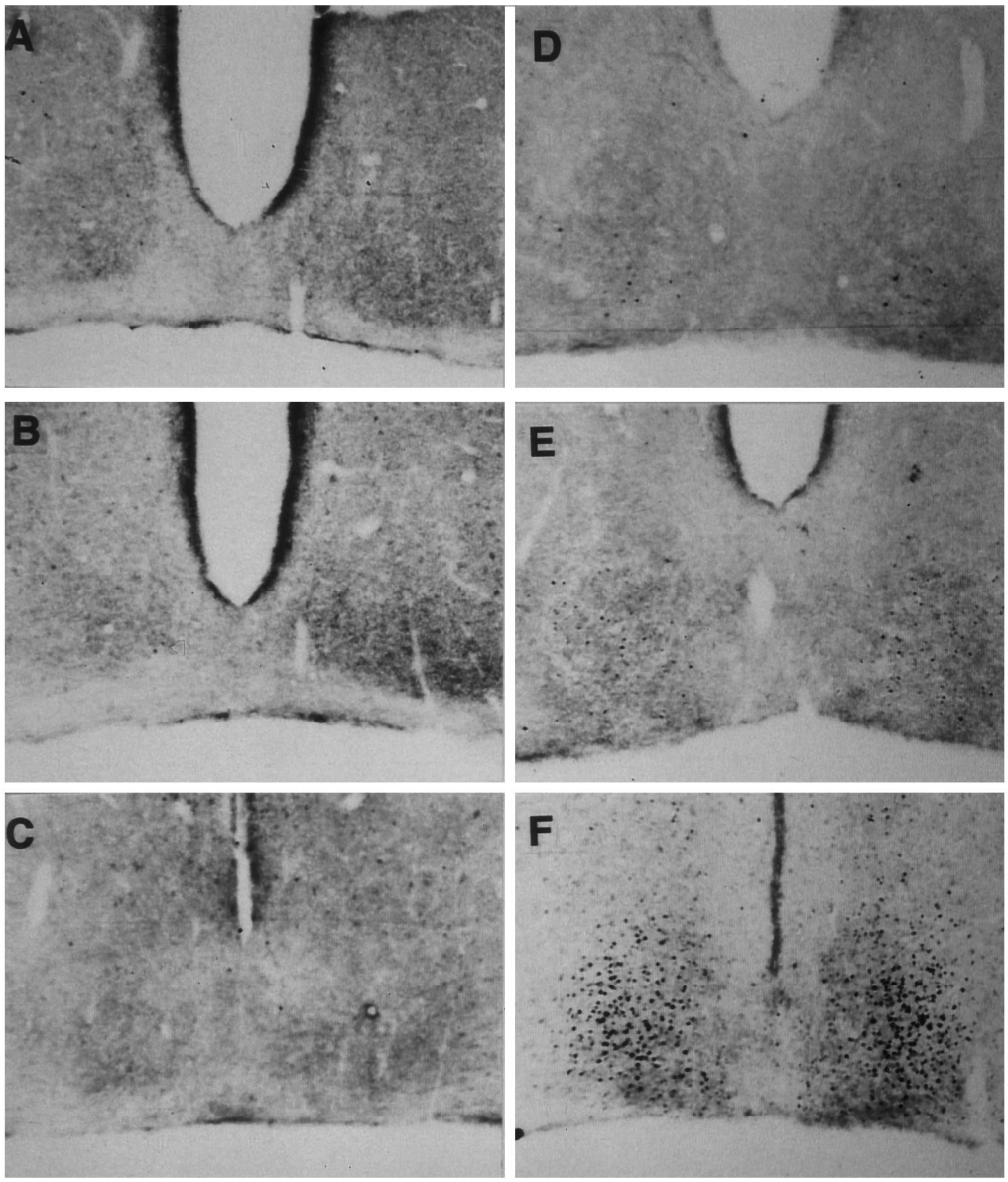

Fig. 9. Light field illustration of the distribution of c-fos like immunoreactivity in the SCN of *Spalax* at different circadian times. c-fos is expressed following a 1-h light pulse during the subjective night only, with the highest expression occurring at ZT22. A, ZT4, dark control; B, ZT16 dark control; C, ZT4 light pulse; D, ZT10 light pulse; E, ZT16 light pulse; F, ZT22 light pulse.

Under certain conditions circadian rhythmicity can exhibit splitting, i.e. a single daily activity band dissociates into two components which temporarily free run at different frequencies [24]. This phenomenon has provided the most direct evidence for a dual-oscillator system controlling circadian activity rhythms [25]. Recently splitting of circadian activity rhythms was observed also in a subterranean rodent, the solitary Cape mole rat [14]. Approximately 50% of our animals exhibited intervals of splitting (see Fig. 1). Splitting in subterranean animals may be more common than in other mammals, indicating a looser coupling of the putative two oscillators.

The question whether the circadian clock was entrained by the LD cycle could not be resolved. The large variability of activity onset after release into DD or DDim could reflect either a weak endogenous pacemaker or a weak link between the endogenous clock and the locomotor-activity output. The c-fos induction experiment did provide some clues. None of the control animals expressed c-fos induction in the SCN, whereas with one exception, all animals exposed to a light pulse in the subjective dark period did exhibit c-fos immunoreactive cells. The number of c-fos reactive cells in all cases at ZT16-ZT22 were relatively low compared to other species [4]. The prior history of the individual which did not respond to the light stimulus applied during the subjective night does not

provide clues for the absence of a response. Our results are consistent with the light-induced c-fos immunoreactivity in the SCN of two female *Spalax* individuals ($2n = 60$) exposed to a 75-min light pulse (300 lux) 1 and 6 h after dark onset in a LD 12:12 [33]. We can tentatively conclude that: (1) light is sufficient for c-fos expression in *Spalax*; and (2) the expression is gated by the endogenous clock, although these results need to be confirmed in a larger number of animals.

The data of the two *Spalax* karyotypes did not differ, and were similar to those obtained in the Cape mole rat, a solitary, subterranean rodent belonging to another family. The cape mole rat also entrained activity to the LD cycle despite retinal degeneration, and exhibited a free running activity rhythm in constant conditions [13,14].

In summary, our main finding was a large intra- and inter-individual variability in: (1) the 24-h activity pattern; (2) the preference for nocturnal or diurnal activity in LD; and (3) in the absence of a LD cycle, in the period of the free running activity rhythm. Nevertheless, a free running rhythm was present in constant conditions in most animals. The results are consistent with the conditions of the natural habitat, where there is presumably little selection for circadian aspects.

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