Behavioural entrainment of circadian rhythms

N. Mrosovsky, S. G. Reebs, G. I. Honrado and P. A. Salmon

Departments of Zoology and Psychologie, University of Toronto, Toronto (Ontario M5S 1A1, Canada)

Summary. This paper reviews the discovery and characterization of a behavioural system for entrainment of circadian rhythms. This behavioural system depends on non-photic inputs but interacts with the light-entrainment system. Non-photic stimuli can be powerful quantitatively: behavioural events can shift rhythms by several hours. Non-photic entrainment offers scope for rephasing biological rhythms in circumstances where light input from the environment is inadequate.

Key words. Behaviour; circadian rhythms; non-photic; social.

Introduction

The traditional view in biological rhythms research is that circadian clocks (pacemakers) control sleep-wake cycles and activity; pacemakers and overt rhythms are in a master-slave relationship. But suppose there were feedback from overt rhythms, then simply by voluntarily controlling one's activity, it should be possible to adjust the pacemaker. Experiments with animals show that it is indeed possible to influence the clock through behaviour. Of course, it has been known for some time that, in addition to environmental stimuli such as light, behavioural experiences are capable of altering the phasing of circadian rhythms. For instance, daily social interaction is capable of entraining (synchronizing) activity rhythms[6,20]. However, such effects have generally been rather inconsistent (not all individuals entrain) and a theoretical framework has been lacking. Recent experiments (with hamsters) supply a theoretical framework and also show that behavioural events can exert quantitatively important effects on circadian rhythms.

The essential element in the theoretical framework is that there is a phase response curve (PRC) for non-photic input[22,27,28], just as there is for light. That is, a behaviourally arousing event occurring at one phase of the circadian cycle affects the pacemaker/oscillator differently from the same event occurring at another phase of the cycle. Phase response curves for light[7,8] have been key elements in understanding its effects on circadian rhythms. We therefore first review the evidence for the existence of a behavioural PRC. We then characterize its properties and show how these properties can explain a variety of phenomena in biological rhythms previously explained in other ways. Synchronization of rhythms by periodic feeding is not discussed in the present review because this phenomenon appears to have different properties from the behavioural effects discussed here, although more within-species comparisons are needed to assess this property. Two features of available studies on periodic feeding are 1) anticipatory activity occurs shortly before the expected time of feeding, and 2) usually only a component of the animal's activity is entrained to the time of feeding; other components continue to free run[1,4,12,33]. These features are not characteristics of entrainment by the behavioural activities described in the present paper.

The behavioural PRC

The first PRC for behavioural events arose out of the initially unwanted observation that the free-running activity rhythms of male golden hamsters kept in constant
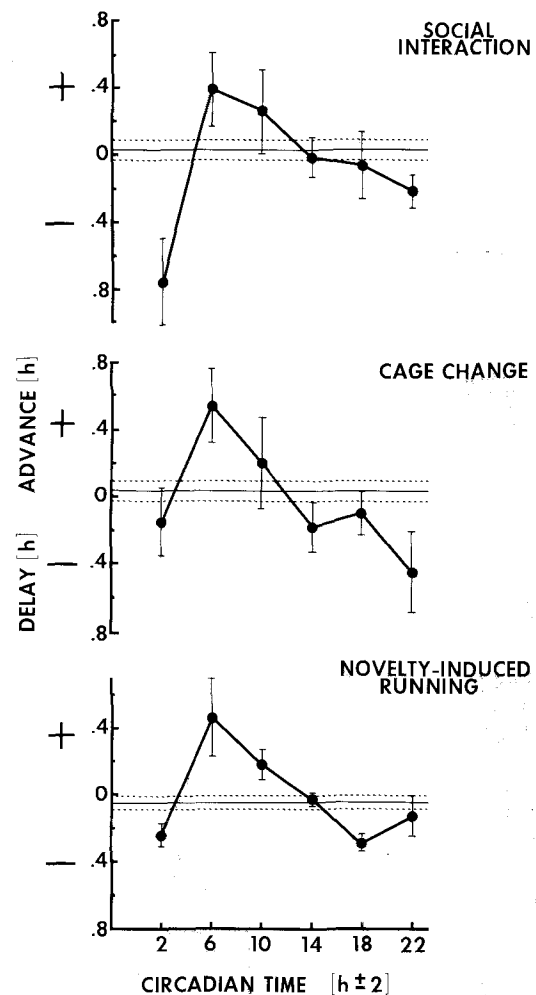

Figure 1. Phase response curves (means ± SEM) for 30-min social interaction (top), cage changing (middle) and 2 h of novelty-induced wheel running (bottom). Circadian times are grouped into 4-h bins. By definition, circadian time 12 is activity onset. Horizontal solid and dotted lines show mean ± SEM for phase shifts after control days on which no manipulations were made. The social interaction and cage changing took place in constant light[22] and the novelty-induced running in constant darkness[28].

conditions were often phase-shifted after cage changing. When the hamster finds itself in a new cage it becomes active, scent marking, scratching, piling up food and running in its wheel. Subsequent planned experiments[22] showed that behaviourally arousing events such as cage changing or social interaction with another male hamster produced phase advances when these events took place during the mid to late subjective day (a time when the hamster is normally inactive). Phase delays occurred when the events took place in the late subjective night or early subjective day (fig. 1). In some cases these shifts were more than 1–2 h, but the averages at the times of maximal advances and maximal delays were about 30 min. In these early experiments the behavioural events were not well controlled in that some hamsters remained active for a few hours after their cage was changed while others settled down quickly. A more controllable behavioural method for producing phase shifts is to confine hamsters to a running wheel for a specified duration. If the wheel is clean and different from the one the animal has in its own cage, the novelty of the situation is usually sufficient to induce running. The PRC for 2 h of novelty-induced running is similar in shape and amplitude to that for cage changing and for social interaction[28] (fig. 1).

When the arousing event is repeated daily, then it is possible to entrain the circadian rhythms of hamsters previously free-running in constant lighting conditions[22, 28]. If the period of the free-running rhythm is long, as is common in constant light, then entrainment is by phase advances and the zeitgeber coincides with the late subjective day. If the period is short, as is common in constant darkness, then entrainment is by phase delays and the zeitgeber coincides with the early subjective day.

Therefore, the phase angles in the entrained condition are consistent with the PRCs (fig. 2).

Non-photic nature of stimuli

When a snoozing hamster is awakened by being placed in a new cage or wheel, or by the intrusion of another hamster, it is likely to keep its eyes open more of the time. This will alter the pattern and intensity of light input. Several points show that the change in photic input resulting from altered behaviour is not what is responsible for the phase shifts. First, even when all photic input is excluded by keeping the animals in constant darkness, novelty-induced wheel running still produces phase shifts[28]; the PRC for hamsters kept in darkness is similar to that obtained for social interactions and for cage changing with hamsters kept in constant light (fig. 1). Second, the shape of the behavioural PRC is quite different from that of the PRC for light pulses. The advance portion of the behavioural PRC peaks about 6 h before the time when the animal normally becomes active. At this phase of the circadian cycle, light pulses are without effect in nocturnal rodents[7, 8, 26]. Light pulses will result in phase advances if given during the second part of the subjective night. At this time arousing events produce phase delays or no change in hamsters. Therefore, the first property of the behavioural PRC is that it is nonphotic.

Non-specificity of the behavioural system

It has already been mentioned that cage changing, social interactions and novelty-induced wheel running have
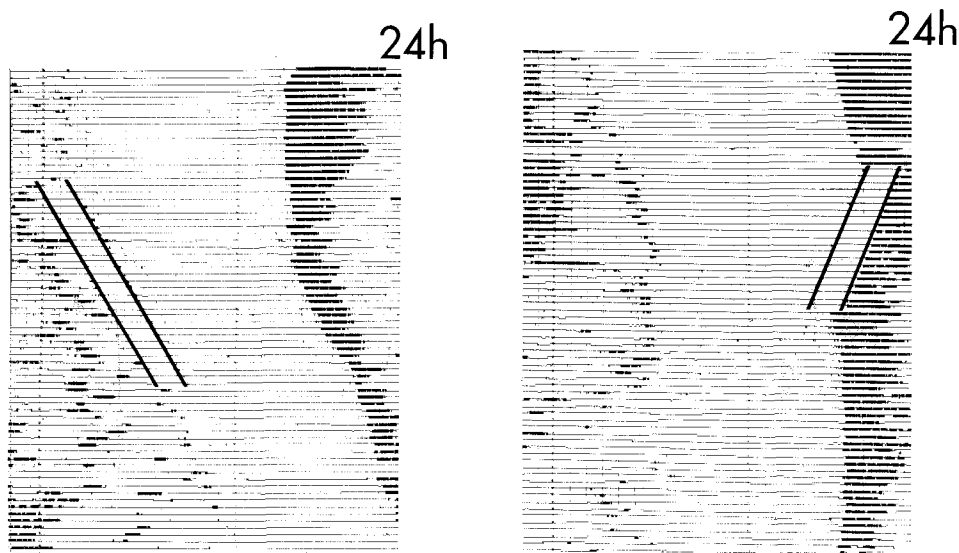

Figure 2. Entrainment of activity (pen marks) rhythms of two hamsters kept in continuous darkness by daily 2-h bouts of novelty-induced running (between dark slanted lines) occurring at intervals of 24.25 h (left) or 23.83 h (right). The hamsters were induced to run by being confined to a wheel other than the one in their home cage. Note that the period of the rhythm during entrainment differs from that before or after the zeitgeber presentation, and that the phase of the post-entrainment rhythm cannot reasonably be predicted by extrapolation of the pre-entrainment rhythm. (Data from Reebs and Mrosovsky[28]; and unpublished).

similar PRCs with maximal advance portions about 6 h before the onset of activity. Some hamsters also entrain to periodic opportunities to hoard seeds[31]. Rather than postulate a separate underlying mechanism for the PRCs in each of these situations, it is more economical to assume that the non-photic system is non-specific. Data from a different testing paradigm reinforce this view. Since an arousing event occurring 6 h before activity onset produces a phase advance (fig. 1), it should be possible to accelerate the adjustment to a phase-advanced light-dark cycle by scheduling such an event at the appropriate time. For example, if the light-dark cycle is advanced by 8 h, starting with an advance of the onset of darkness, then an arousing event introduced 1 h after the advanced dark onset falls about 7 h before the hamster's previous time for activity onset. This should accelerate adjustment to the new light-dark cycle and indeed this is what is found when novelty-induced wheel running is the behavioural event: re-entrainment takes only 1.5 days on average compared with 8.5 days for undisturbed control animals[23, 24]. Sexual arousal produced by putting a female hamster in estrous close to but not accessible to a male leads to a similarly dramatic enhancement of re-entrainment rate[13] (fig. 3). There seems no need to postulate a separate mechanism for these various effects. In fact, common to all these situations is the availability of a wheel in which the hamster runs vigorously. Preventing the wheel from rotating considerably reduces the effects of the estrous female[13].

Although at present the best available predictor of phase shifting is how much the animal runs in its wheel[13] (fig. 4), it is possible that it is not the activity but some
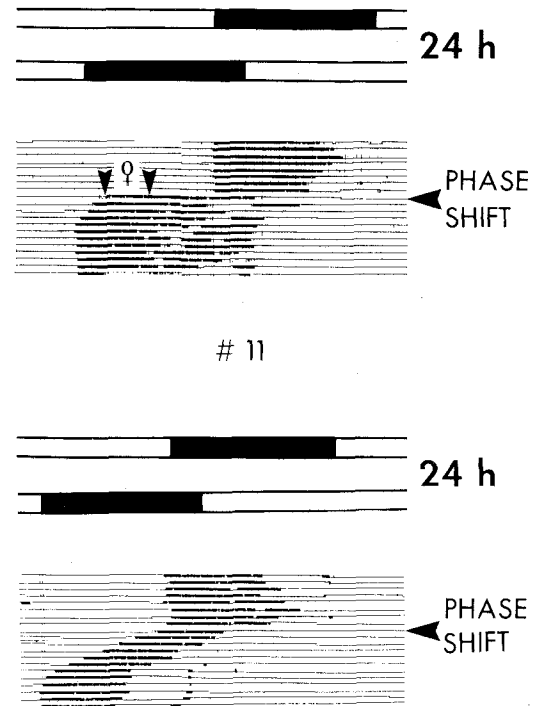

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Figure 3. Phase shifts of a male hamster in response to 8-h advances of a 14:10 h light-dark (LD) cycle on two occasions. On the first phase shift (top) there was a nearby female hamster in estrous condition during the 3 h shown between the arrows. For the other shift (bottom) there was no non-photic manipulation. Actograms show wheel running (pen marks), with successive 24-h periods mounted in order downwards in the conventional way. Open and dark bars above the actograms show the LD cycles before and after the phase shifts (Data from Honrado and Mrosovsky[13]).
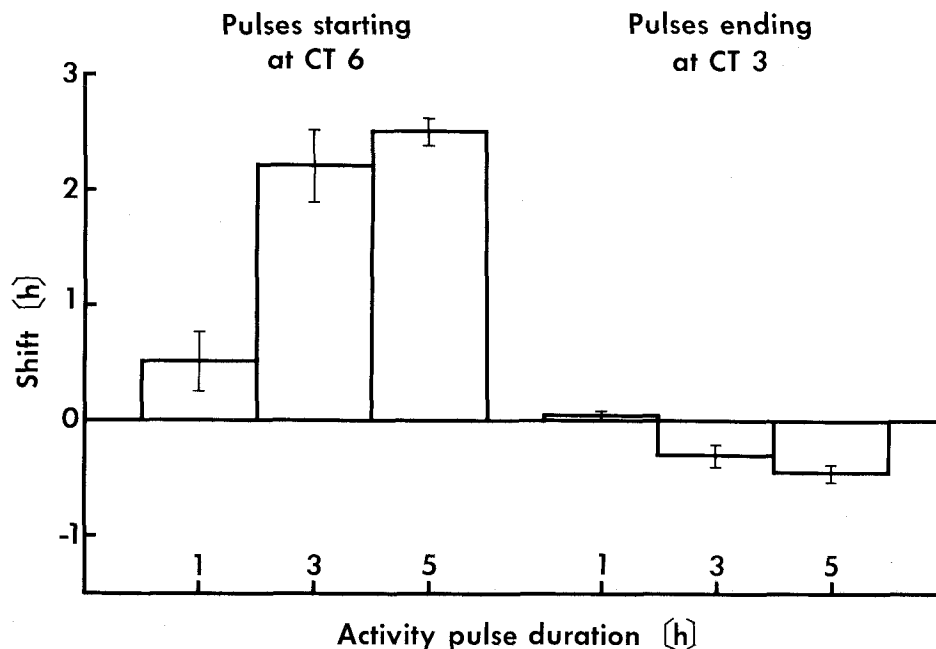

Figure 4. Phase shifts (means ± SEM) obtained by pulses of novelty-induced wheel running of different durations. The pulses were given at different circadian times (CT) to hamsters kept in constant darkness. (Data from Reebs and Mrosovsky[29]).

correlated variable that is important. Factors that might be involved when animals are induced to run at a time they would normally be resting include the following: altered body temperature or hormonal levels, the state of arousal rather than the motor activity itself, and interruption of sleep or rest. The latter may not be critical because slow rotation of a rat's cage, forcing it to move, does not alter the rhythm of spontaneous wheel running [3].

Duration of non-photic stimuli

In our first experiments with cage changing, social interaction, and novelty-induced activity, the amplitudes of the PRC were modest (fig. 1). Maximum advances averaged around 30 min only; some animals did not respond at all. When these stimuli were repeated every day at the same time, entrainment was obtained but only in about half the animals [22, 28]. In these experiments the social interactions had only lasted 0.5–1 h, and the novelty-induced activity only 2 h. Subsequent experiments showed that when novelty-induced activity lasted 3 h, much larger phase advances (mean 2–3 h) in free-running rhythms could be obtained [29] (fig. 4). Increasing the duration to 5 h produced little further effect. The full PRC has not been mapped out yet with these longer pulses of activity, but it is already evident that its amplitude is in the order of a few hours (fig. 4).

Summarizing, there exists a behavioural PRC which can be engaged by non-photic events, is relatively nonspecific but for maximal effects requires the events to last for a few hours. A PRC with these properties can account for a number of phenomena in biological rhythms previously explained in other ways.

Dark pulses

Just as light pulses given to an animal kept in constant darkness phase-shift its rhythm, so can dark pulses given to an animal kept in constant light. It has been said that the dark pulse PRC is a mirror image of the light pulse PRC [5]; this suggests some commonality in mechanism. However, when one looks at the details this mirror image is blurred. In particular with nocturnal species, maximal phase advances to dark pulses are somewhat earlier than in the schematic mirror images for phase delays produced by light pulses [5] and in some experiments delay portions of the PRC to dark pulses are almost absent [9] and by no means mirror images of the sizeable advances produced by light pulses at these circadian times.

There is also a striking difference between experiments with dark and light pulses. A light pulse of 1 h or even only 15 min is commonly used and is effective [7, 8, 26]. In contrast, a dark pulse must last for several hours to phase shift a rhythm [5, 9, 16, 34]. It is evident from looking at the records of experiments with dark pulses that often animals become highly active during these pulses. Most of these experiments have been with nocturnal animals. It is not surprising that a nocturnal animal kept in continuous light becomes excited by several hours of darkness. The necessity for dark pulses to be long, taken together with their activity-inducing effects, suggest that darkness is working through the behavioural non-photic PRC rather than through some mechanisms related to the light pulse PRC. This suggestion is reinforced by the fact that the dark pulse PRC has a peak advance portion around 3–6 h before activity onset and is generally similar in shape to the non-photic PRC. To test whether dark pulses are effective because of the behaviour they induce, we pre-
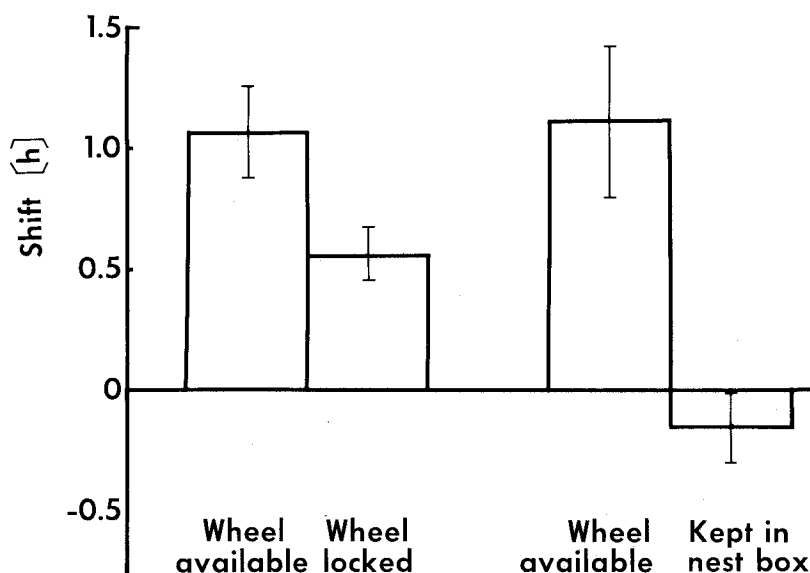

Figure 5. Phase shifts (means ± SEM) produced by 3-h pulses of darkness, starting at circadian time 9, given to hamsters otherwise kept in constant illumination. The animals with their wheels locked were free to move about their cages. This was not the case for those confined to nest boxes. Control experiments showed that confinement in itself at that circadian time produced negligible phase shifts. (Data from Reebs et al. [30]).

vented hamsters from running by blocking their wheels during the pulse. This halved the phase shifts produced. Further restriction of activity by confining the animals to small nest boxes during the dark pulse completely abolished phase shifts[30] (fig. 5). Similar results have been obtained by Van Reeth and Turek[36] who prevented their hamsters from moving during the dark pulses by placing them in a small tube. Evidently there is more to darkness than meets the eye: what the hamster does during the dark pulse determines the phase shift.

Diurnal animals, such as canaries[38] and house sparrows[15], also phase-shift in response to periods of imposed darkness. The photic changes in both these experiments affected behaviour: the animals became less active during dark pulses. It is not known whether such behavioural changes in diurnal animals mediate their phase shifting in response to dark pulses.

Benzodiazepines

Triazolam, a benzodiazepine, can phase-shift the circadian rhythm of hamsters[35]. The PRC is similar in general shape to that for non-photic events. Although triazolam is soporific for people, in hamsters with the doses used to produce phase shifts it often initiates prolonged bouts of wheel running. This suggests[23, 24] that triazolam and non-photic behavioural events have similar PRCs because the drug makes the animals active. The benzodiazepine antagonist, Ro 15-1788, can block the phase-shifting effects of triazolam; this block is evidently accompanied by a block of the activity-inducing effects of triazolam[37]. Other ways of reducing activity also diminish the phase-shifting effects of triazolam, for instance restraining the hamsters in a small tube[36]. This shows that triazolam does not directly induce phase shifts; what occurs depends on the behaviour of the animals and if this is constrained, then the chronotypic effects of the drug can be eliminated.

What applies to triazolam and to dark pulses, namely the importance of the behaviour that occurs in reaction to these stimuli, may well apply to other drugs and to other stimuli affecting circadian rhythms. For example, the increased activity of squirrel monkeys in the warm and decreased activity in the cold might be involved in the entraining effects of cycles of high and low ambient temperatures[2]. If the behavioural entrainment system is non-specific, it would be expected that a variety of behaviour-altering drugs and stimuli from the environment would be capable of producing phase shifts. Direct effects of chronotypic drugs on light-entrainable oscillators should not be assumed without examining the animal's behavioural reactions, or studying the effects of drugs on isolated pacemakers in vitro. Nor should it be assumed at this stage that behavioural mediation of chronotypic effects could only occur with stimulant drugs. If activity during the normal rest period produces phase shifts, then it is conceivable that sleep during the normal active peri-

od might do the same. For example, entrainment by daily melatonin injections in birds may be produced indirectly by its sedative effects[11]. However, it remains to be determined whether in fact there is a PRC for induced sleep.

Species generality

Much of the work discussed so far has been with the golden hamster (*Mesocricetus auratus*). At least for vertebrates, the golden hamster is to biological rhythms what the fruit fly is to genetics. However, the existence of behavioural non-photic PRCs is not confined to hamsters. In sparrows (*Passer domesticus*) there is a PRC for rattling the side of their cages[27]. This noise affects their behaviour: they become alarmed, sleek their feathers and often seek shelter. Advance portions in the sparrow's behavioural PRC occur in the late half of its subjective night as opposed to the late subjective day in the hamster. In contrast to the hamster, however, the sparrow is diurnal. With respect to the rest-wake cycle, the advance portion of PRC is similar to that of the hamster. Another similarity is that the effects are non-specific. In sparrows, entrainment by daily arousal is similar whether the stimulus is cage rattling or playback of conspecific vocaliza-
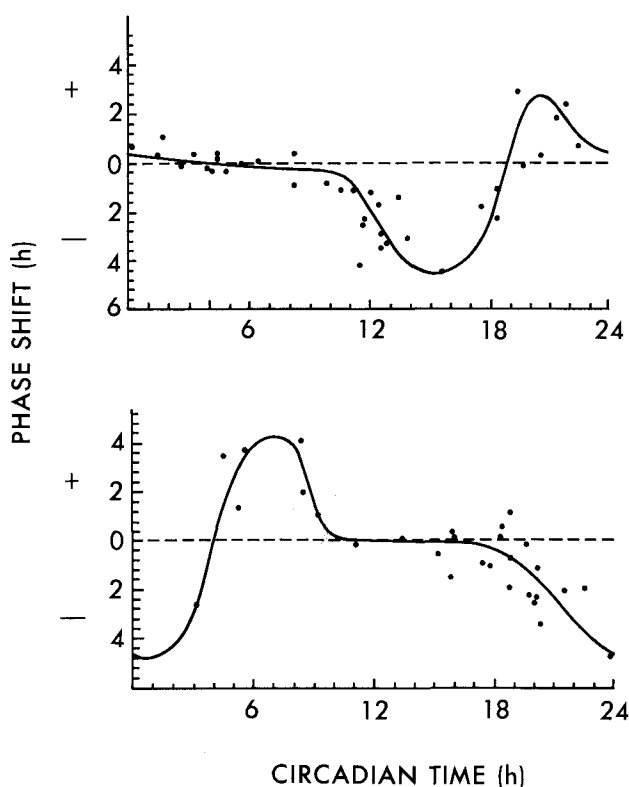

Figure 6. Phase shifts in response to 2-h light pulses of the electroretinogram (ERG) rhythm of scorpions kept in constant darkness, except for 10-ms light flashes every 30 min to measure the ERG. Circadian time 12 is the onset of ERG responsiveness; this coincides approximately with the start of behavioural activity. Top: tests on which scorpions remained calm during the 2-h pulses. Bottom: tests on which scorpions greatly increased their activity during the 2-h pulses. (Redrawn from W. Hohmann and G. Fleissner, pers. comm.).

tion[27]. In finches (Fringillidae) sound from buzzers and shaking of the cage can both act as synchronizing agents[10, 19].

Turning to invertebrates, a spectacular example of the importance of the animal's behaviour comes from work with scorpions (*Androctonus australis*) (W. Hohmann and G. Fleissner, pers. comm.). When kept in continuous darkness, these nocturnal arthropods display a rhythm in the amplitude of their electroretinogram (ERG) response to a brief flash of light. This ERG rhythm can be phase-shifted by light pulses. When all data points are considered together, there is a large scatter and no clear PRC. But when tests are grouped into those in which the scorpions remained inactive during the light pulse, and those in which they became active, then two clear but different PRCs emerge. For occasions on which the scorpions were inactive, the PRC is similar in shape to that found for light pulses with other nocturnal species. For occasions when the scorpions became active, the shape resembles that of the non-photic PRC for hamsters (fig. 6). Evidently scorpions can switch between two modes of responsiveness, with behaviour a determinant, or at least an index, of which PRC is engaged.

Although the formal properties of non-photic entrainment may have many cross-species similarities (as is the case for photic entrainment), the physiological mechanisms must obviously be different, at least between classes. For mammals, it has been suggested that serotonergic input from the raphé to the suprachiasmatic nucleus, either direct or via the lateral geniculate body, mediates non-photic entrainment[14, 22].

Possible applications

Bringing the non-photic system into action may be useful in circumstances where light stimuli are inadequate. An extreme example of inadequacy of light stimuli occurs in blind people whose melatonin and activity rhythms sometimes free-run with periods around 25 h[17, 21]. Investigations of the effects of exercise on rhythms in such cases are being launched.

Even people with normal sight may not always receive sufficiently bright light stimuli to entrain them. Initial attempts to obtain photic entrainment of biological rhythms in people met with limited success[39]. Entrainment was only present when the periodicity of light-dark cycles was close to 24 h. However, if bright light (> 3000 lux) was used, then entrainment over much wider ranges could be obtained[40, 41]. It takes light of about 2500 lux intensity to suppress melatonin levels in people[18] but in modern societies people encounter light of such intensities only sporadically and for short durations[32]. Therefore, it is possible that even for healthy people in the every day population, the scheduling of activity could be useful in entrainment. When physically fit subjects ran 8–12 km before 06.00–08.00 h their body temperature

maxima (acrophases) were about 2 h earlier than when they ran at 17.00–19.00 h before supper[25].

Another circumstance when light-dark cues are inadequate is after crossing time zones; it usually takes at least several days to adjust to new schedules. Non-photic enhancers of the rate of re-entrainment may be of some value here. These enhancers need not be jet lag pills. Research with animals suggests that non-pharmacological events such as activity induced by arousing situations may be just as effective.

Summary: explanatory power

A PRC that is non-photic, non-specific and best engaged by long-lasting stimuli accounts for entrainment by various behavioural events such as social interactions, vigorous activity, and arousal by being in a new environment. It also explains the accelerated adjustments to new light-dark cycles that can be produced by non-photic enhancers. The same process explains the effects of dark pulses (in some nocturnal species at least) and of triazolam on biological rhythms. Not only is there now evidence for the existence of a non-photic PRC with an amplitude in the order of hours, but this PRC has considerable explanatory power. Understanding of this system is likely to be of value in supplementing or substituting for light-dark stimuli when they are inadequate.

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Research Articles

A new marker technique in cineradiography for the recording of movements in small vertebrates – Application to the study of jaw movements in soricids (Insectivora)

R. Dantuma and Ch. Dötsch*

Department of Anatomy and Embryology, University of Groningen, Oostersingel 69, NL-9713 EZ Groningen (The Netherlands)

Summary. Dental parapulpar pins (TMS® Link Series) are reliable markers in cineradiography for the recording of movements in small vertebrates. The application of the pins to an analysis of mandibular movements of soricids allows a reconstruction of the complex movement pattern of both jaws during mastication.

Key words. Marker pins; cineradiography; movement recording; mastication; soricids; shrews.

Cinefluoroscopy often uses the natural landmarks of the skeleton to detect motions, e.g., those of the jaws during mastication[1]. This may, however, be inadequate for the analysis of the complex jaw movements of small verte-