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# Scheduled wheel access during daytime: A method for studying conflicting zeitgebers

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**Abstract**

It is often stated that light is the primary environmental cue (zeitgeber) for entrainment of circadian clocks. Here, we use a new conflict test design in Syrian hamsters comparing the strength of a photic zeitgeber to that of a non-photic cue, i.e. wheel availability. Re-entrainment to an inverted LD cycle was significantly slowed down in the nocturnal hamster by restricting wheel access to the light phase of the inverted LD cycle. This effect is more pronounced if the illuminance level of the entraining lights is 0.1 lx compared to 6 lx. In this conflict design, the hamsters did not re-entrain to an inverted LD cycle for up to four weeks (when the experiment ended), but voluntarily ran during the light phase. This approximates the situation in people subjected to shift work or jet lag.


*Keywords:* Circadian rhythms; Conflict; Entrainment; Hamster; Jet lag; Non-photic; Photic; Shift work; Wheel access; Zeitgeber

## 1. Introduction

Endogenous circadian clocks, which can be found in most living organisms, are of no use unless they can synchronize or entrain the organism to the cyclic environment. Most prominent of these daily changes is the light/dark cycle caused by the earth's rotation around its axis. Many papers on biological rhythms state that light is the primary or principal entraining agent, or zeitgeber [1–8]. But what exactly is meant by primary? A variety of non-photic cues have been found that can also act as zeitgebers, e.g. temperature [9–11], food availability [12–14], social cues [15,16], sound [17] and wheel running [18]. Saying that light is primary could mean that light is the entraining agent most used by animals in natural circumstances in the wild, or it could mean that light is a stronger entraining agent than non-photic cues. The latter possibility deserves further study in mammals given that, in Syrian hamsters, a species in which non-photic clock resetting has been studied intensively, the amplitude of non-photic phase response curves (PRCs) can be as great as that of photic PRCs, suggesting comparable abilities to phase shift [18]. However, this may not be a valid point as double-pulse experiments show that phase shifts cannot be treated in an additive way [19,20]. A better approach to the question of relative zeitgeber strength is to set up competitions between zeitgebers. Setting up competitive situations, however, will not enable general statements to be made about whether photic or non-photic zeitgebers, as a class, are more powerful. This is because the units for specifying a photic stimulus, such as lux, are of a different quality as those specifying non-photic clock resetting events. Indeed, with non-photic stimuli involving locomotion and/or arousal, it is not even known what are the critical variables leading to resetting of the clock. For example, Duffy et al. [21] found that light pulses facilitate adjustment to an inverted rest-activity schedule, but that social stimuli and meal schedules do not. The light pulses were 5 h of illumination of 7000–13,000 lx and given at times known from previous work to produce shifts. The social contact consisted of a technician entering the room to administer tests and provide meals or a urinal. Moreover, the social events occurred during a 16-h wake period that included 5 h of darkness in the middle. It is not known whether this procedure was optimal for eliciting non-photic

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shifts in people, and whether by having the dark period in the middle of the social stimulus advance and delay shifts might have cancelled each other out. Therefore, it might be premature to conclude that "inversion of the sleep–wake rest–activity and social contact cycles provide relatively minimal drive for resetting the human circadian pacemaker" [21].

Nevertheless, it is still valuable to study the effects of particular co-occurring photic and non-photic stimuli. Although it has been widely recognized that non-photic events play an important role in clock resetting, the interactions between the two types have been much less explored. Some of these interactions were not predicted, and were of significant magnitude [18,22]. As in real life situations photic and non-photic stimuli are likely to interact, it is desirable to continue empirical investigations in which the relationship of non-photic events to LD cycles is altered (e.g. [23]).

In this general context, the present paper explores an interaction between photic and non-photic cues in a conflict situation. The overall strategy was first to entrain hamsters to a light/dark cycle with 14 h of light per day (LD 14:10), with a running wheel being available at all times; then, after stable entrainment, to invert the LD cycle but now restrict the wheel availability to 10 h in the middle of the light portion of the LD cycle. Dominance of light over wheel availability was assessed by whether and how fast locomotion activity measured with passive infrared detectors entrained to the new LD cycle. The power of non-photic events was assessed by whether and for how long wheel access only during the light phase prevented or slowed down re-entrainment to a LD cycle inversion. Specifically, we asked two questions: (1) If the wheel is only available in the light phase after an LD cycle inversion, is the time to re-entrain invariant of the light intensity? (2) Can wheel running in the middle of the light phase (i.e. the "wrong" time of day for nocturnal mammals) slow down re-entrainment after an LD cycle inversion?

## 2. Materials and methods

### 2.1. Animals

We used two batches of animals each consisting of 12 male Syrian hamsters (*Mesocricetus auratus*) obtained from Harlan (Indianapolis, USA). Upon arrival from the breeder, the animals were 6 weeks old and were maintained in a sound attenuated and temperature controlled room at 22 ± 1.5 °C. Animals were held individually in polypropylene cages (44 × 23 × 20 cm) equipped with a plastic mesh [24] surrounding the running wheel (17.5 cm in diameter) on standard bedding (Beta Chip, USA) with food (LabDiet #5001, PMI, USA) and tap water available ad libitum.

### 2.2. Apparatus design and data acquisition

In addition to the wheel, a passive infrared motion detector (PID, HVW Technologies, Calgary, Alberta, Canada) was installed for recording general activity as a second phase marker for the animals' clock. The PID was placed over the side of the cage at the opposite end to the running wheel. Both, the wheel revolutions and the signals from the PIDs were continuously recorded by a computerized data acquisition system (DataQuest III, Mini Mitter, Bend, Oregon, USA).

A hook shaped brass rod hanging into the cage could block the wheel automatically when activated by a motor outside the cage (Fig. 1). The motors were controlled by an interface board, which could individually control up to 16 motors; this board was connected to the parallel port of a personal computer. The timing of the motor movements was set by custom-made software.

The incandescent light sources were dimmed to appropriate light levels by addition of neutral density filters (Cinegel #3404, Rosco, UK). Illumination at the level of the cage floor was measured with a EX2 lux meter (B Hagner AB, Sweden).

### 2.3. Experiment 1

Prior to the onset of Experiment 1, the first batch of hamsters ($n = 12$) were held in a dim LD 14:10 for 21 days. The illumination at the cage floor was 5–6 lx.

Then, the running wheels were blocked for 14 h starting at the onset of the light phase. Simultaneously, the LD cycle was inverted by having 1 day in constant light, i.e. 26 h of light starting at the onset of the light phase, with the new dark phase beginning 2 h after the former lights on (Fig. 2). Thus, the dark phase was delayed by 12 h. Starting on the first inverted day, the wheels were unblocked only throughout 10 h centered in the middle of the new light phase, which was identical with the former dark phase (Fig. 2). After 17 days of re-entrainment to the new conditions, animals were transferred to constant darkness (DD) at the onset of the last night in LD in order to assess the phase of

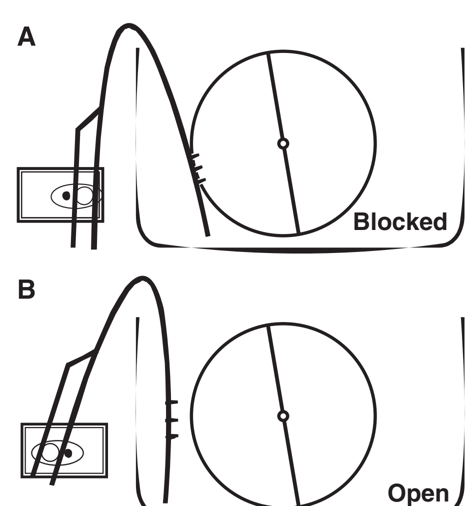


Fig. 1. Schematic drawing of the blocking apparatus and running wheel in (A) a blocked and (B) an open position.