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Social Synchronization of Circadian Rhythms in Deer Mice (*Peromyscus maniculatus*)

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Summary. Deer mice (*Peromyscus maniculatus*), kept in individual cages under constant dim-light conditions, displayed steady free-running rhythms of activity, the period of which varied between individuals.

When two previously isolated mice with different rhythms were placed in a common enclosure, under the same constant light conditions, they soon displayed a mutual synchronization of their activity rhythms. When separated again, the mice lost mutual synchronization (Figs. 1 and 2).

The process by which mutual synchronization was attained in the common enclosure is typical of entrainment by an external synchronizer (*Zeitgeber*). Our results suggest that the activity rhythm of the dominant mouse entrains the activity rhythm of the subordinate, and is thus a 'social Zeitgeber.'

Introduction

Under natural conditions, a circadian biological rhythm is usually 'entrained' by an external synchronizer, or *Zeitgeber*. As a result of this entrainment, the biological rhythm adopts the periodicity of the Zeitgeber, keeping a definite phase relationship with the oscillations of this forcing factor (for references and terminology, see Bünning 1973; Palmer 1976; Saunders 1977).

Although most studies on the entrainment of biological rhythms have been concerned with the role of geophysical factors, such as rhythmic oscillations of light intensity or temperature, several observations and experiments made chiefly on birds and mammals (including man) suggest that social factors can also act as Zeitgebers. Spontaneous or experimentally induced changes of the social conditions in groups of mammals living under standard LD conditions ($T=$ 24 h) have been reported to be followed by mutual adjustments of the activity patterns of the individuals involved (Kavanau 1963; Bovet 1972; Roper and Ryon 1977; Nygren 1978; Farr and Andrews 1978). Beavers (*Castor canadensis*) (Bovet and Oertli 1974; Potvin and Bovet 1975) keep free-running circadian rhythms of activity during certain phases of their life cycles, under natural conditions; these rhythms are well defined for beaver colonies as a whole, which might suggest that the members of a colony synchronize their individual activity rhythms with one another and not with geophysical Zeitgebers. Working under constant light conditions, Gwinner (1966) obtained an entrainment of the activity rhythms of songbirds (*Carduelis spinus* and *Serinus serinus*) on 24-h periodic presentations of conspecifics' songs. Finally, house sparrows (*Passer domesticus*) that had different individual circadian periods of activity when studied in isolation under constant light conditions synchronized with one another when placed in a social situation under the same light conditions (Menaker and Eskin 1966). Testing humans, Pöppel (1968) reported similar findings.

The goal of the present study is to provide further experimental evidence about the possible Zeitgeber properties of social factors. The rationale behind our procedure is that if social factors act as Zeitgebers, two individuals that display activity rhythms with distinct circadian periods when kept separately under constant light conditions should become mutually synchronized when placed in a common enclosure under the same constant light conditions. If social factors do not act as Zeitgebers, each individual is expected to keep its own original rhythm of activity in the common enclosure. Mice of the genus *Peromyscus* appear well suited for this kind of study. Their patterns of activity have been extensively studied (review in Falls 1968). Of particular relevance is that, in appropriate experimental conditions, individual

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mice tend to keep a steady free-running periodicity over several weeks and that in isolated animals, kept under similar constant light conditions, there is a well-marked variability in periods of free-running rhythms between individuals (Rawson 1959; Stinson 1960). These mice are easy to keep in captivity, in isolation as well as in groups. Their maintenance requirements are such that, provided with enough food and water, they can survive without caretaking for many days. This enables the avoidance of biases caused by frequent maintenance visits to the animals. Among the various species of *Peromyscus*, the deer mouse (*P. maniculatus*) was chosen as an object of study because of its local availability.

Materials and Methods

Cages, Enclosures, and Recording Devices

The experiments were performed in two separate rooms. Throughout the experiments, light conditions in both rooms were maintained constant by using four incandescent, 40-W red bulbs, which provided a 0.18-lux illumination at the floor level of the cages or terrarium. Temperature was kept constant at $22° \pm 2$ °C. Room A contained eight $40 \times 22 \times 23$ cm cages, each equipped with a running wheel connected to a multichannel event recorder. Room B contained a 3.2-m-long, 2.3-m-broad terrarium, the floor of which was covered with vegetal soil. The terrarium was divided transversally into two equal compartments by means of a 0.5-m-high partition, the median part of which could be removed. A 35-mm movie-camera loaded with a high-speed infrared film was placed next to the terrarium in order to take pictures of the whole surface of the terrarium, using a reflecting mirror placed above the latter. The camera automatically took a single picture every 6 min (1/30-s exposure time). The event recorder and the camera were working continuously when mice were kept in room A or B, respectively.

Experimental Procedure

We carried out five replicates of the same experiment. Each replicate consisted of three consecutive stages:

Presocial Stage. In a first *selection step* lasting at least 28 days, 6–8 mice were kept each in a separate cage of room A. We then selected the two mice that, according to a gross examination of their running-wheel activity records, showed the most diverging circadian periods τ of free-running activity rhythms. These two mice were then earmarked and transferred to room B. For 4–8 days (*transition step*), each was confined in a separate compartment of the terrarium. The position of the nest (hole, or 'cup' on the surface) that each mouse made within a few hours or, in some cases, minutes from release in the compartment was noted.

Social Stage. This stage began without manipulation of the mice by the removal of the median part of the partition. This created a 1.1-m-wide gap at floor level through which the two mice could freely visit each other's original compartment. Due to the large space available, a mouse that was resting or sleeping in a nest could easily remain undisturbed by the activities of the other mouse. This stage lasted 27–44 days, during which time use of nesting sites and social interactions were recorded by direct observation in several 30- to 60-min sessions throughout this stage. At the end of the stage, the mice were captured in live-traps and transferred back to room A.

Postsocial Stage. Both mice were reintroduced, each in its original individual cage in room A, to spend there a further 21 days.

Animals

The five replicates were made using ten different individuals selected from 32 adult male deer mice tested during selection steps. These 32 mice had been wild caught in several locations in Québec.

Analysis and Presentation of Data

Activity. The data collected by the event recorder or the camera were used to make actograms illustrating the sequences of bouts or 'instants,' respectively, with or without activity (see Figs. 1 and 2). The running-wheel records made during selection steps and postsocial stages were divided into consecutive bouts of 6 min. In any bout, a mouse was considered active if it made at least one running wheel turn, and inactive if it did not. Activity in the terrarium was determined by examining the movie-camera films frame by frame under a binocular. If a mouse was seen outside a nest on a picture, it was considered active, while if in a nest, it was considered inactive. In a transition step, each mouse was identified by the compartment in which it was confined. In a social stage, however, positive identification of the mice was no longer possible, their earmarks not being visible on the pictures. Therefore, the recordings made for a social stage indicated only whether zero, one, or two mice were active at the time any picture was taken. Thus, if only one mouse was active, we could not determine which one. As a consequence, the actograms made for a social stage (see Figs. 1 and 2) should be considered as 'group actograms' that illustrate the sequence of 'instants' with zero, one, or two animals active.

The periods implied in the sequences of data were estimated by means of periodograms (see Fig. 3). Simplified, a periodogram is a series of estimated relative amplitudes of oscillation (measured in ordinate) for several assumed oscillatory periods (in abscissa). The assumed period(s) producing the highest amplitude(s) is considered the best estimator(s) of the period(s) implied in the sequence (see Enright, 1965, for details). All our periodograms covered a range of assumed periods from 20 to 28 h in steps of 0.1 h. The periodograms produced for the transition steps were usually unclear, due to a too small number of days, and the period of the rhythms could only be grossly estimated on the basis of the actograms. The periodograms produced for the social stages were 'group periodograms' made on the basis of sequences of pictures with and without activity, no distinction being made between pictures with one or two mice active. It should be stressed here that if two different rhythms are present within a group's activity pattern (e.g., because the two mice have different rhythms), this should be revealed by the presence of two different peaks in a group periodogram.

Social Ranks. Dominance relationships between the two mice in any replicate were assessed by means of the 'signs of dominance' observed during social stages. 'Signs of dominance' belonged to one of three types, as suggested by previous reports on social behavior in *P. maniculatus* (Eisenberg 1962; Sadleir 1970; Hill 1977; Farr et al. 1978). Type 1 signs were linked with overtly agonistic behavior: chasing the opponent, and having him run away. Type 2 signs were linked with apparently nonagonistic social inter-

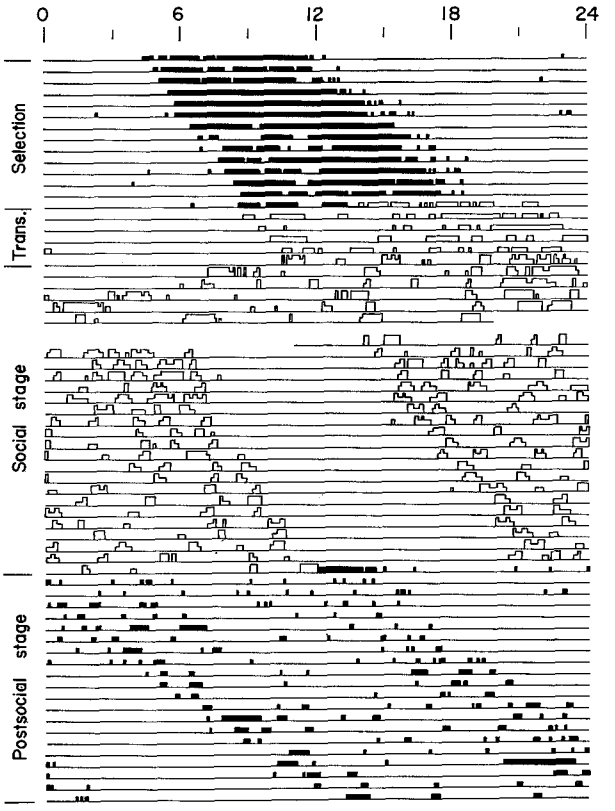

Fig. 1. Actograms for mouse I (dominant, replicate 5). *Horizontally:* Eastern Standard Time; *vertically:* consecutive calendar days. For the selection step, only the last 14 days are shown. *In selection step and postsocial stage*: symbols over the base lines indicate activity, absence of symbols inactivity; the smallest symbols correspond to a bout of 6 min with at least one record of running-wheel activity. *For the transition step and social stage*: symbols indicate activity, absence of symbols inactivity; the smallest symbols correspond to one picture with activity. In the social stage, the records concern the activity of both mice I and J: single-height symbols indicate only one mouse active, double-height symbols both mice active. Absence of base line indicates that recording device was failing. Change from selection step to transition step occurred on day 14 at 1350 hours; from transition step to social stage on day 19 at 1000 hours; from social stage to postsocial stage on day 46 at 1200 hours.

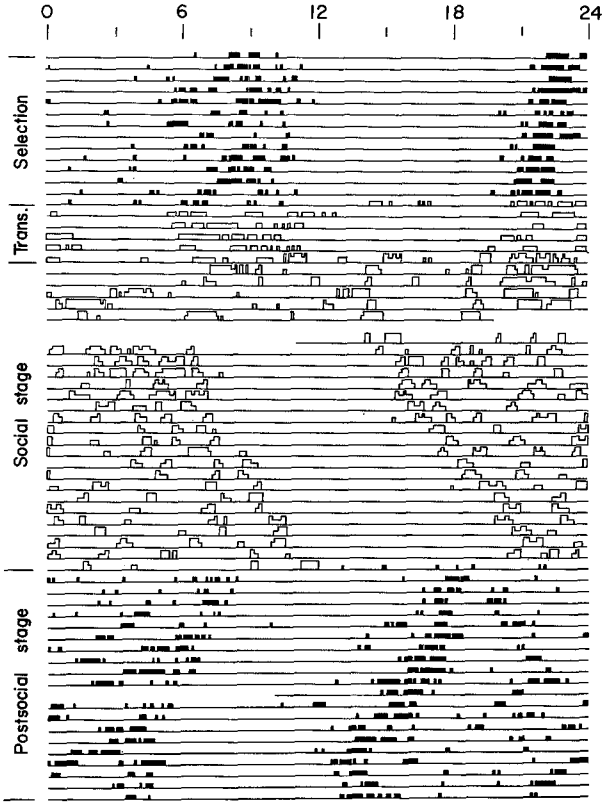

Fig. 2. Actograms for mouse J (subordinate, replicate 5). Explanations: see Fig. 1. Note that the records of the social stage concern the activity of both mice J and I

actions: sniffing, grooming, following, and mounting the other. Type 3 signs were linked with use of a common nest: keeping the nest made before social stage and having the other mouse move into this nest (see Hill 1977). We counted how many signs of dominance of each type were observed for each mouse of a pair. The mouse that showed more signs of dominance of each type was ranked as dominant, the other as subordinate.

Results

The main results of the study are summarized in Table 1. All the social rankings are based on the observation of all three types of signs of dominance, except in replicate 4, where we did not observe agonistic behavior (type 1 signs). All the τ values given in Table 1 correspond to the position of well-defined, single peak in any periodogram (see Fig. 3a, b, and

Table 1. Social ranks, and periods of the activity rhythms (τ) observed in the five replicates

Replicate	Mouse	Social rank[a]	τ (h)			Phase angle (h)
			Pre-social stage	Social stage	Post-social stage	
1	A	D	24.2	24.3	24.2	1
	B	S	23.5		24.2	
2	C	D	23.4	23.8[b]	23.8	9
	D	S	24.2		24.9	
3	E	D	23.8	23.9	—[e]	3
	F	S	24.3		24.2	
4	G	D	24.2	24.2[c]	24.7	8
	H	S	23.6		—[e]	
5	I	D	24.4	24.4[d]	24.7	9
	J	S	23.9		23.8	

[a] *D*, dominant; *S*, subordinate

[b] For last 12 days of the stage; for first 29 days, periodogram shows a major peak at 23.8 and two minor peaks at 23.4 and 24.5, respectively

[c] For last 23 days of the stage; for first 14 days, no clear-cut periodicity

[d] For last 20 days of the stage (see Fig. 3d); for first 7 days, no clear-cut periodicity (see Fig. 3c)

[e] Mouse made little use of running wheel, without apparent periodicity

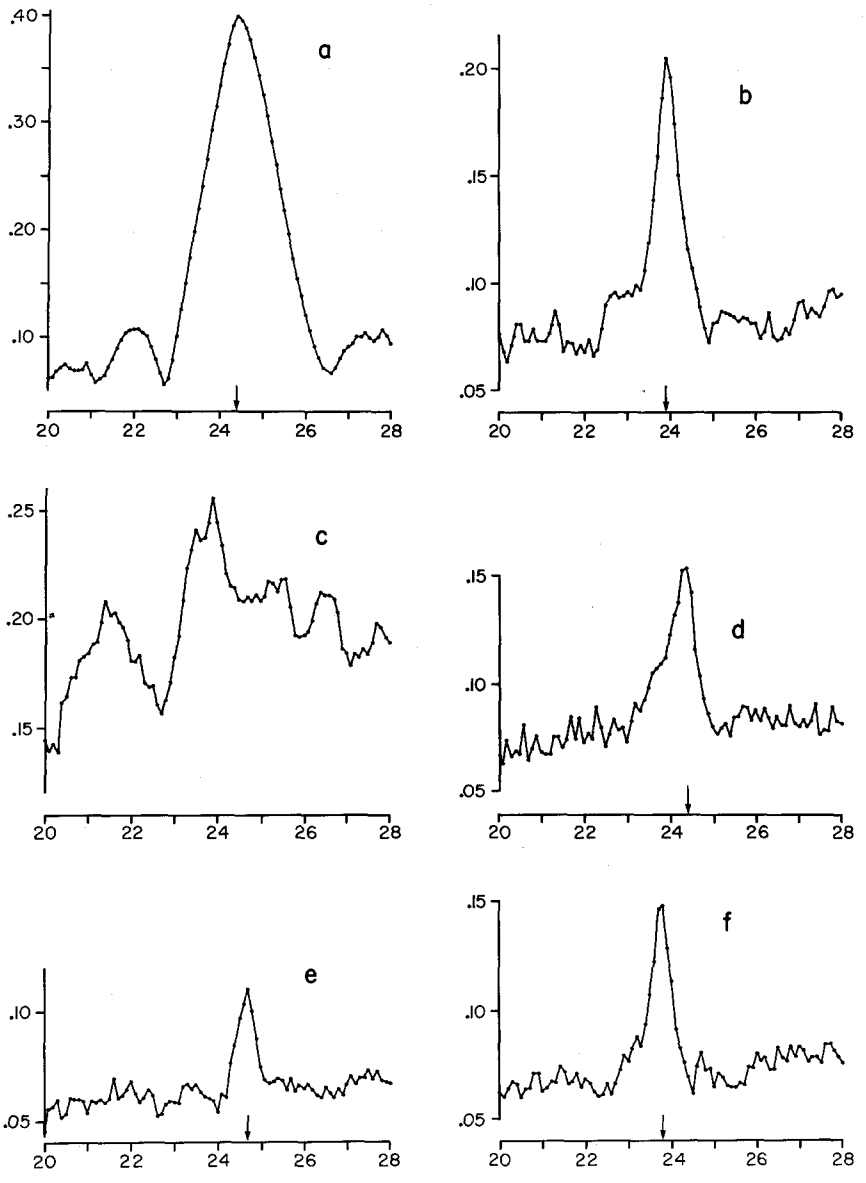

Fig. 3a–f. Periodograms of replicate 5. *Abscissa*: period assumed (hours); *ordinate*: amplitude (see Enright 1965).
a, b Last 14 days of selection step of mice I and J, respectively.
c, d Social stage, first 7 and last 20 days, respectively.
e, f Postsocial stage of mice I and J, respectively.
Arrows in **a, b, d–f** point to the projection of the single major peak on the abscissa

d–f for example). Presocial stage values correspond to the last 14 days of a selection step, postsocial stage values to the whole stage (21 days). Social stage values for replicates 1 and 3 correspond to the whole stage (44 and 28 days, respectively); for replicates 2, 4, and 5, see the appropriate footnotes in Table 1. The phase-angle values are a rough estimate of the time between onsets of activity in the two mice on the last day of presocial stage (transition step). The actograms and periodograms obtained in replicate 5 are shown in Fig. 1–3, as examples of detailed results.

In general terms, the following features appeared in the course of all replicates:

Presocial Stages – Selection Steps. The mice studied in the selection steps maintained steady τ's in their activity rhythms and these τ's varied between individuals by at least 30 min, providing a good basis of selection for two markedly different individuals for the rest of the replicate.

Presocial Stages – Transition Steps. Due to the short duration of these steps, no precise values of τ could be derived from periodograms, but the actograms indicate that the activity patterns of each mouse remained periodic during transition steps. In comparing the actograms of selection and transition steps, it appears that while the change of environment and/or of recording technique affected the apparent total amount of activity, it did not induce any major change in period of the rhythms or in phase position of the gross active and resting phases (see Figs. 1 and 2).

Social Stages. A clear group circadian activity pattern was obtained either from the start of the stage (replicates 1 and 3), or after several 'days of adaptation' during which time no clear-cut rhythmicity in the pair was observed (replicates 2, 4, and 5; see Fig. 3c). The periodograms for days following the onset of a clear activity pattern were all single peaked (see Fig. 3d), indicating that the mice adopted a common period for their individual rhythms. In replicates 1, 3, 4 and 5, this common τ was identical (± 6 min) to that displayed by the dominant mouse during the presocial stage; while in replicate 2, the common τ was intermediate between the two original ones. The presence of 'days of adaptation' appears to be linked with the phase angle between the individual patterns at the end of presocial stage. In two replicates where the phase angle was small (see Table 1), rhythms became synchronized from the start of social stage, whereas in the other three replicates, where the phase angle was large, synchronization appeared only after several days. The adoption of a common period implies that the two mice in a replicate maintained a steady phase relationship with each other. No direct measure of the steady phase angles can be obtained from our records, as we were unable to separate individual rhythms in this stage. However, examination of the social stage actograms suggests that the phase angles were small (i.e., that the two mice were approximately in phase with each other). In replicate 5 for instance (see Figs. 1 and 2, last 20 days of social stage), both mice showed signs of activity in the first hour of 90% of the active phases, after onset of a clear circadian pattern. Corresponding values in replicates 1–4 were 91%, 90%, 100%, and 78%, respectively.

Postsocial Stages. With the exception of replicate 1, the break of the social situation resulted in a loss of mutual synchronization between the two individuals. Other observable effects, such as lengthening or shortening of the periods, varied considerably among individuals and were not apparently related to social rank (see Table 1).

Discussion

Our results show that two deer mice having different individual free-running activity rhythms become mutually synchronized when placed in a social situation, and in general lose mutual synchronization when separated again. When the two individual activity patterns are strongly out of phase at the start of the social stage, several days are required before mutual synchronization becomes established. These findings are analogous to those commonly found in experiments dealing with the entrainment of a free-running rhythm by a geophysical Zeitgeber (e.g., DeCoursey 1961; Aschoff and Wever 1963). Our results suggest that the periodic activity pattern of the dominant animal acts as a social Zeitgeber responsible for the synchronization observed during the social stage. In our experimental situation, however, and in contrast with geophysical Zeitgebers, our social Zeitgebers were free-running themselves, and therefore likely to be flexible and affected by the activity rhythm of the subordinate animal. This explains perhaps why, in replicate 2, it took as long as 31 days of apparent 'competition' between the two individual rhythms before a common rhythm with intermediate period emerged as the only rhythm (see Table 1, footnote b).

The above considerations are essentially based on the τ values obtained in the various stages of the replicates, as determined from periodograms. Due to the nature of our recordings, the occurrence and importance of phase shifts in the course of the experiments are difficult to evaluate and thus to compare with findings of studies on geophysical Zeitgebers. Generally speaking, the social and postsocial free-runs of the dominant mice appeared to be a continuation of their presocial ones, without change in phase (see Fig. 1). No major phase shifts between social and postsocial stages were apparent in the records of the four subordinate mice that maintained rhythmicity in the postsocial situation (mice B, D, F, and J). For mice D and J (see Fig. 2), the phase position observed at the start of postsocial stage resembled not only that of the social stage, but also the phase position expected had they maintained their presocial τ. Thus, the possibility that the circadian rhythms of these two mice were masked rather than entrained by the dominants' rhythms cannot be excluded (see Hoffmann 1969; Loher 1979). However, for mice B and F (see Fig. 4), the differences between the phase positions observed during postsocial stage and those expected if the rhythms had been masked were so great (11 and 12 h, respectively) that entrainment is a more feasible explanation than masking.

While our data indicate the presence of social Zeitgebers, one must ask whether the latter are involved in the regulation of individual activity rhythms under natural conditions when other, geophysical Zeitgebers are present. In a group of animals, there will be a state of mutual synchronization if all individual rhythms are directly entrained by the same geophysical factor. Since each rhythm adopts the period of the geophysical oscillations as well as a steady phase relationship with them, all the rhythms will have the same period and be in a steady phase relation with one another. This kind of mutual synchronization

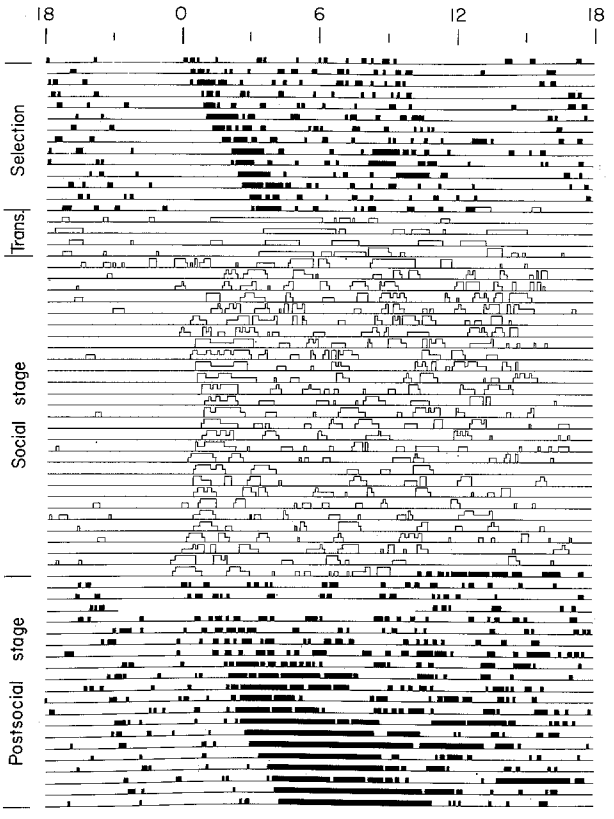

Fig. 4. Actograms for mouse F (subordinate, replicate 3). Symbols: see Fig. 1. The records of the social stage concern the activity of both mice E and F. Change from selection step to transition step occurred on day 14 at 1245 hours; from transition step to social stage on day 18 at 0800 hours; from social stage to postsocial stage on day 46 at 0930 hours

without a social Zeitgeber is a by-product of individual responses to the same geophysical factor. However, the pattern of mutual phase settings thus obtained might not be optimal for social activities and might require adjustment to the social structure of the group, as suggested by Roper and Ryon (1977) in their work on captive packs of wolf × coyote hybrids (*Canis niger* × *C. latrans*). Due to their experimental protocol (under LD conditions), these authors were unable to determine whether the adjustments observed resulted from an entrainment by social Zeitgebers or from a process of normalization ('normalization still implies a process of synchronization mediated by social cues, but suggests that these cues alter the sensitivity of the animals to an external zeitgeber, rather than they constitute a zeitgeber in themselves'; ibid. p 183). Due to the experimental procedure, our results do not provide accurate measures of the mutual phase settings between two mice of a pair. However, they clearly reveal a process of period harmonization that is typical of Zeitgeber entrainment. The findings of Gwinner (1966), Menaker and Eskin (1966), Pöppel (1968), and of the present study, linked with those of Roper and Ryon (1977), strongly suggest that the social cues, responsible for mutual adjustments, act as Zeitgebers. Further research should reveal to what extent geophysical and social Zeitgebers duplicate, complement, and/or suppress the other's role in the establishment of mutual synchronization in groups of animals.

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