Animal behaviour

Social synchronization of circadian rhythmicity in female mice depends on the number of cohabiting animals

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Communal animals often engage in group activities that require temporal synchrony among its members, including synchrony on the circadian timescale. The principles and conditions that foster such collective synchronization are not understood, but existing literature hints that the number of interacting individuals may be a critical factor. We tested this by recording individual circadian body temperature rhythms of female house mice housed singly, in twos (pairs), or in groups of five (quintets) in constant darkness; determining the daily phases of the circadian peak for each animal; and then calculating the cycle-to-cycle phase relationship between cohabiting animals over time. Significant temporal coherence was observed in quintets: the proportion of quintets (4/7), but not pairs (2/8), that became synchronized was greater than could be achieved by the complete simulated reassortment of all individuals. We speculate that the social coupling of individual circadian clocks of group members may be adaptive under certain conditions, and we propose that optimal group sizes in nature may depend not only on species-specific energetics, spatial behaviour and natural history but also on the mathematics of synchronizing assemblies of weakly coupled animal oscillators.

1. Introduction

Many animals live naturally in groups [1]. Some of the proposed benefits of group living (e.g. more efficient thermoregulation by huddling and protection from predation by a ‘safety in numbers’ effect) require that individuals’ rest–activity cycles be synchronized, but little is known about the underlying processes responsible for such coordination. Social regulation of the individual circadian clocks of cohabiting animals would be one appealing mechanism, but past investigations of this issue have been plagued by problems in experimental design and interpretation as well as inconsistent results (for review, see [2]). Of note, however, are reports of robust temporal synchrony in shoals of fish [3], colonies of bats [4] and honeybees [5], and groups of fruit flies [6], suggesting that a critical factor favouring collective synchronization might relate to the number of interacting individuals, a hypothesis consistent with theoretical models that suggest that network strength increases with the number of coupled phase oscillators (for review, see [7]). As an initial test of this idea, we continuously recorded the body temperature (Tb) rhythms of inbred female mice—housed singly (singlets), in twos (pairs) or in groups of five (quintets)—in constant darkness (DD), and analysed the data using a rigorous method that does not demand invariance of period and amplitude over time. The results suggest that the number of cohabiting animals is key for achieving social synchronization of circadian rhythms in laboratory mice.

2. Material and methods

Sixty female BALB/cByJ mice arrived from Jackson Laboratories at four weeks of age and were initially housed 3–5 mice cage−1 in a 12 h light : 12 h dark (LD) cycle. To ensure a spread of rhythm phases when later grouped, mice were housed in one of
two opposite LD cycles, sp., lights went off at noon local time for half of the mice and at midnight for the other half. At eight weeks of age, mice were implanted with intraperitoneal temperature dataloggers (ibuttons; Dallas Semiconductor DS1922, Maxim Integrated Products, Inc., Sunnyvale, CA, USA; as in [8]) and then single-housed in their pre-surgical LD cycle. One week later, all mice were single-housed in constant darkness (DD). iButtons were programmed to record Tb every 15 min beginning five weeks after implantation (four weeks after transfer to DD). Circadian Tb rhythms were recorded for a total of 85 days in DD: 10 days before grouping, 68 days of cohabitation (n = 8 pairs and 7 quintets), and 7 days following separation (figure 1a). Pairs and quintets comprised individuals that were not previously housed together in our facility. For pairs, individuals were previously

Figure 1. Social synchronization in a female mouse quintet. Panel (a) illustrates the timeline of experimental procedures. Panel (b) depicts individual double-plotted body temperature (Tb) actograms of five cohabiting mice before, during and after cohabitation along with a 'composite' actogram of their combined Tb rhythms (lower right). The overlaid black box indicates the interval of cohabitation. Note the out-of-phase Tb rhythms at the start of the composite record gradually cohere into a single approximately 24 h rhythm. Acrophases of the circadian component of Tb rhythms were determined using wavelet analysis as in (c), which depicts a representative 7-day Tb rhythm of an individual mouse (top) along with its wavelet transformation (bottom). In (d), circular distributions of acrophases at the start of cohabitation (left) and before separation (right) are shown for the quintet in (b). The resultant vectors show the magnitude as the length of the vector and the phase by its orientation; in this example, coherence is 0.42 (initial) and 0.93 (final). L : D and D : L, the two 12 h : 12 h light : dark cycles; DD, constant darkness. (Online version illustrates each cohabitant’s activity record and initial/final coherence plots with a specific colour.) (Online version in colour.)
housed in opposite LD cycles; for quintets, three individuals were from one LD cycle and the remaining two were from the other.

Raw data (figure 1b) were transformed using the complex-valued Morlet continuous wavelet function (figure 1c), daily phases of the circadian peak were determined for each animal (see supplementary online material in [9]) and the cycle-to-cycle phase relationship between cohabiting animals was expressed as coherence. Thus, a pair with a coherence = 1 represents rhythms with identical phase, and a coherence = 0 represents rhythms in anti-phase; for a quintet, with the five individual phases plotted as points on a unit circle, coherence was defined as the resultant vector, representing the magnitude and angular dispersion of the circular distribution (figure 1d). Group coherence could thus be calculated each day, and the change of daily coherence values over time represented the trajectory of coherence. The predicted trajectory of coherence over the duration of the experiment, as extrapolated from the linear regression of phases from 9 days before cohabitation, was calculated along with the 95% prediction interval (sp., the area in which 95% of all data points would be expected to fall based on the regression line). Synchronization was defined as an average coherence for days 44–75 (the second month of cohabitation) of greater than or equal to 0.8 and outside the 95% prediction interval.

3. Results

All mice exhibited free-running circadian Tb rhythms in DD. There was no evidence of fighting (e.g. wounds) in the pairs or quintets, and body mass did not differ between singlets, pairs and quintets at the time of grouping, separation or sacrifice (data not shown).

Figure 2 illustrates the coherence plots of representative non-synchronized (figure 2a,b) and synchronized (figure 2c,e) pairs and quintets, respectively. Synchronization was achieved in 2/8 pairs and 4/7 quintets. To analyse these proportions, and the likelihood that spontaneous changes of circadian period might lead to apparent synchronization in the absence of actual social interactions between co-housed individuals, we performed a systematic grouping with all permutations, viz., we used the transformed temperature data of our 16 mice in pairs and 35 mice in quintets to construct a series of ‘simulated’ pairs and quintets in silico. For pairs, there were 112 possible simulated combinations (that did not include the actual eight pairs of the experiment) and 103 possible simulated sets composed of eight unique pairs/set. The proportion of simulated pairs in simulated sets that met our definition of synchronization is shown in figure 2c; our actual 2/8 result could be reproduced in silico in over 20% of the simulated sets. For quintets, there were 2520 possible simulated combinations (that did not include the actual seven quintets of the experiment) and 404 possible simulated sets composed of seven unique quintets/set. The proportion of simulated quintets in simulated sets that met our definition of synchronization is shown in figure 2f; there was no combination of simulated quintets that could reproduce our actual 4/7 result. Hence, the actual proportion of synchronized quintets, but not pairs, was greater than or equal to the complete simulated reassortment of all individuals.

Synchronization did not appear to depend upon the circadian attributes of the individual quintet cohabitants. The average free-running circadian period (τ) of non-synchronized (3/7) and synchronized (4/7) quintets was no different at the start of cohabitation, as calculated from the pre-cohabitation τ’s of mice before their quintet assignment (mean ± s.d. = 23.81 ± 0.09 h versus 23.82 ± 0.06 h, respectively; linear
regression of acrophases), and the mean intra-quintet variation in $\tau$ of individual mice also did not differ between the two groups ($0.19 \pm 0.05$ h versus $0.18 \pm 0.12$ h, respectively). There were no pre-cohabitation differences in rhythm amplitude or in the cycle-to-cycle precision of amplitude or $\tau$. The average coherence at the start of cohabitation was no different for quintets destined to be non-synchronized or synchronized ($0.37 \pm 0.26$ versus $0.37 \pm 0.18$, respectively). By days 44–75 of cohabitation, both non-synchronized and synchronized quintets displayed a lengthened average $\tau$ ($23.96 \pm 0.15$ h versus $23.96 \pm 0.06$ h, respectively), similar to that exhibited by mice in pairs or singly housed over this duration. As expected, the mean intra-quintet variation in $\tau$ of individual mice had become smaller for synchronized (0.07 ± 0.03 h) than for non-synchronized (0.30 ± 0.16 h) groups, although this did not reach statistical significance ($p = 0.087$, Mann–Whitney $U = 0.0$ ($U = 12$), $n_{sync} = 4$, $n_{non-sync} = 3$).

4. Discussion

Behavioural synchrony in group-living animals could be achieved through several mechanisms. At short timescales, animals might match their behaviour to that of others in the group, a process called allelomimetic (e.g. the synchronized grazing/resting bouts of some herd animals [10]). At the circadian timescale, synchrony could arise in the absence of socially regulated circadian clocks, either by the entrainment of individual clocks to a common environmental factor (e.g. the light–dark cycle) or by the modification of individual rhythm phases and waveforms by circumventing or overriding the clock (a mechanism referred to as masking).

Conceivably, social cues could promote behavioural synchrony, or fine-tune photic entrainment [11], by altering the $\tau$’s of cohabitant clocks, but studies of this putative mechanism have yielded inconsistent results. We have previously reported that social influences on $\tau$ likely depend on individuals being housed unrestrained in direct physical contact for a relatively long period of time [8]. A survey of the literature and the results from this experiment suggest that another crucial element is the number of individuals in the group—with 25 fish [3], 100 bees [5], 40 flies [6], many bats [4], and, in this experiment, five mice. We do not yet know the nature of the coupling mechanism(s), but the importance of unrestrained physical contact and our results in constant conditions suggest that it involves direct physical contact between individuals (which presumably is increased in larger groups). Further comparative studies in inbred mouse strains may also elucidate the influence of individual circadian attributes; BALB/c mice, used here, appear to show a relatively loose circadian organization [12], which might favour entrainment by weak social cues.

Species natural history strongly influences the kinds of behavioural interactions between cohabitants [13], and female mice are renowned for their communal nesting and nursing behaviour [14]. Notably, pair-housing naturally solitary male Syrian hamsters can alter the $\tau$’s of the cohabitants but does not lead to significant synchronization [8]. The effects of housing hamsters in greater numbers, and whether the result would be temporal segregation [15] rather than synchronization, have yet to be tested.

We can only speculate on the significance of group-mediated circadian synchrony under natural conditions, especially when group members are each able to individually entrain to the light–dark cycle. Coupling of individual clocks within the group may provide greater precision or altered phases of entrainment for group-directed behaviours (e.g. huddling, foraging). It also may be adaptive under certain challenging conditions, e.g. during hibernation [16], under attack [17] or when geophysical cycles are weak [18,19]. The optimal group size may thus depend not only on species-specific energetics and natural history but also on the mathematics of synchronizing assemblies of weakly coupled oscillators.

References


