

fied and have been shown to function in zebrafish gastrulation movements (25, 26). As Wnt activity can stimulate calcium release through activation of Rfz2 (15, 23), we tested whether loss of PDE activity would cause defects in cell movement. Embryos treated with the PDE inhibitors dipyridamole and zaprinast during gastrulation stages lacked extension along the A-P axis, a hallmark for cell movement defects during gastrulation, which results in transient reduction of body length (Fig. 6, table S3) (20). Decreased dorsal convergence was demonstrated by the medial-lateral broadening of *MyoD* expression, a somite marker (27). Relative to wild type (Fig. 6A), we observed severe lateral expansion of *MyoD* expression or epiboly defects in 76% of embryos incubated at doses of 10 μ M dipyridamole (Fig. 6B) ($n = 38$). We observed moderate defects in embryos (fig. S4A) incubated in lower doses (fig. S4B) (20) ($n = 90$) and less severe defects in zaprinast-treated embryos (fig. S4C) (20) ($n = 84$). Reduced extension of the A-P axis is apparent by the increased density of somites. Dashed lines highlight the length from somite 1 to 6. Somites are packed closer together, and thus the distance is reduced in PDE inhibitor-treated embryos (Fig. 6, A and B; fig. S4, B and C). The full

details of the whole mount in situ analysis of *MyoD* expression and alterations in morphology are provided in (20).

This work identifies a novel role for a signaling pathway that largely was thought to be confined to the visual pathway. Our data reveal a key role of PDE and of cGMP in Wnt-Frizzled signaling (fig. S5) (20). Activation of Rfz2 by Wnt5A leads to activation of G protein-mediated downstream signaling, culminating in the activation of phospholipase C and PDE, integrating calcium and cGMP signaling.

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Materials and Methods

Figs. S1 to S5

Tables S1 to S3

9 May 2002; accepted 7 October 2002

Resetting the Circadian Clock by Social Experience in *Drosophila melanogaster*

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Circadian clocks are influenced by social interactions in a variety of species, but little is known about the sensory mechanisms underlying these effects. We investigated whether social cues could reset circadian rhythms in *Drosophila melanogaster* by addressing two questions: Is there a social influence on circadian timing? If so, then how is that influence communicated? The experiments show that in a social context *Drosophila* transmit and receive cues that influence circadian time and that these cues are likely olfactory.

Circadian clocks in animals regulate the timing of molecular, physiological, and behavioral rhythms. Environmental features such as photoperiod and temperature cycles reset these biological oscillators, enabling

anticipation of dawn, dusk, and season (1–6). Other kinds of cues (“nonphotic”) also influence clock time (7). For example, studies on humans (8), rodents (9), fish (10), and bees (11) have demonstrated social influences on rhythmicity, but underlying sensory mechanisms remain unexplained. It is nonetheless clear that multiple sensory pathways transmit ambient temporal information from the periphery to clock cells in the brain (7).

We investigated social influence on circadian timing in the fruit fly *Drosophila melano-*

gaster. We initially hypothesized that the circadian phases [marked by the peak of locomotor activity in DD (constant darkness)] would be more coherent for *Drosophila* living together (group-housed) than those of isolates, because groups of flies might agree about the time of day even without photic cues. Locomotor activity rhythms from group-housed wild-type individuals were compared to those of sibling isolates. After an initial 5 days in 12 hours of light and 12 hours of dark (LD 12:12), isolates and group-housed subjects were maintained for 2 weeks in DD. Isolates were then placed in activity monitors, whereas the group-housed flies were separated and monitored in DD to assess the effects on individual rhythmicity (12).

The effect of this treatment on phase coherence was analyzed with the use of circular statistics (Fig. 1A) (13, 14). The resulting vector angle indicates the mean peak time for each group, and its magnitude indicates phase coherence, with longer tails denoting a tighter distribution of phase estimates around the day (0, no correlation; 1, perfect correlation) (13, 14). The difference in phase coherence was significant ($P = 0.02$), and there was no effect on phase angle (timing) ($P = 0.64$), suggesting that the clocks of group-housed individuals in DD are more synchronized than those of isolates (12).

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This finding implies that housing arrhythmic mutant individuals (visitors) together with wild-type hosts could destabilize host phase. After 5 days in LD 12:12, we placed a group of wild-type controls (40 per vial) or a group of wild-type hosts plus arrhythmic *per⁰* visitors (32 plus 8, per vial) in DD for 5 days (12) (*per⁰* is a loss-of-function *period* mutation causing locomotor arrhythmicity). Locomotor activity was then assessed individually in DD for these two groups.

There was a large effect of social experience on the phase of the hosts (Fig. 1B). Wild-type hosts joined by *per⁰* visitors showed dispersed phase coherence ($P < 0.02$) and mean peak time ($P < 0.01$) as compared to controls. This further suggests an interaction between circadian clock function and social experience, because coherence and phase (and also strength; fig. S1) of locomotor activity rhythms are influenced by the genotypic characteristics of the biological clocks (or lack thereof) within the social mix (12).

We next expanded our question to ask whether phase among hosts could be influenced by visitors from another "time zone" (12). Two LD 12:12 cycles with the start of the light phase (lights-on) occurring 6 hours apart were established, with "early" and "late" control individuals housed in vials in the respective incubators for 5 days. On the fifth day, visitors (8 per vial) from one of the incubators were mixed with hosts (32 per vial) from the other incubator 9 hours after lights-on in the early incubator (3 hours after lights-on in the late incubator). The controls and mixed groups were placed immediately in DD for another 5 days, and activity was individually monitored in DD for 5 days thereafter.

Here (Figs. 2 and 3), the analysis was extended to include stability and timing of individual peaks for each animal. Points correspond to estimates of mean phase and its variability for an individual subject. The arrows shown in Figs. 2 and 3 summarize each group of points; length describes the mean estimate of the dispersion of the peak phase, and direction indicates mean timing of occurrence of the daily peak in locomotor activity. A significant overall difference between the vectors can result from differences in phase angle, phase coherence, or a combination of the two; thus, this analysis is more general and more conservative than the method used in Fig. 1, because it is not linked to a hypothesis about effects on phase coherence or mean phase time (12).

Figure 2 depicts comparisons between early and late control individuals, early controls and early hosts, and late controls and late hosts. After 5 days in DD followed by behavioral monitoring, early and late control

groups maintained a mean difference of 4.2 hours (a reduced difference from 6 to 4.2 hours presumably stems from variability among the individual free-running circadian clocks during DD). There were significant differences between the early and late controls (Fig. 2A; $P < 0.01$), a weaker, nonsignificant effect of the late visitors on the early hosts (Fig. 2B; $P = 0.12$), and a significant effect of the early visitors on the late hosts (Fig. 2C; $P < 0.01$). The effect is directional: Early visitors affected phase among late hosts, whereas late visitors did not affect early hosts as strongly, implying that social influence on locomotor rhythms depends on

when (subjective time) the stimulus is provided. Time-varying responses underlie clock resetting in general (5, 6), suggesting that phase response curves could be developed for these social interactions (7, 9).

Would a similar effect on phase occur between mutant strains? A fast-clock (*per^S*) and a slow-clock (*per^L*) *period* mutant display, respectively, advanced and delayed evening activity peaks in LD 12:12 (15). Experimental design was the same as in the previous experiment, except only one incubator and one LD cycle were used. For the studies with *per⁰* (Fig. 1B) and early and late visitors (Fig. 2), we maintained a ratio of 32

Fig. 1. Phase coherence of *Drosophila* locomotor activity rhythms is affected by housing and genotype. (A) Group housing increases phase coherence. Phase analysis of locomotor activity rhythms is on the basis of individual records in constant darkness (13, 14). Mean phase estimates for the isolates (asterisks; $n = 58$) as well as for the group-housed subjects (open circles; $n = 87$) are plotted in a 24-hour dial. (B) Arrhythmic *per⁰* visitors disperse the phase of wild-type hosts. Open circles represent phase estimates for hosts ($n = 34$); asterisks indicate wild-type controls ($n = 9$).

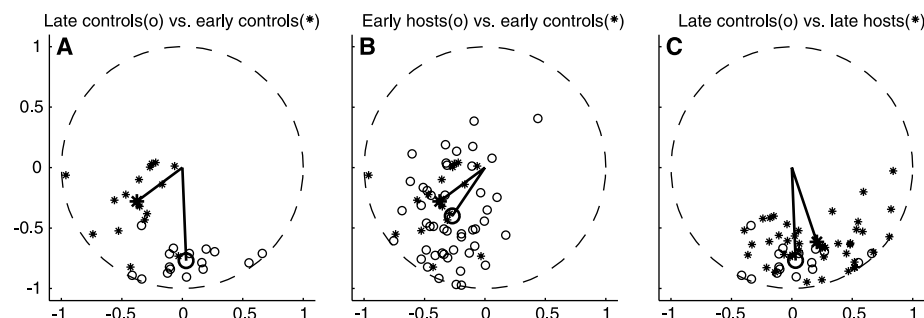
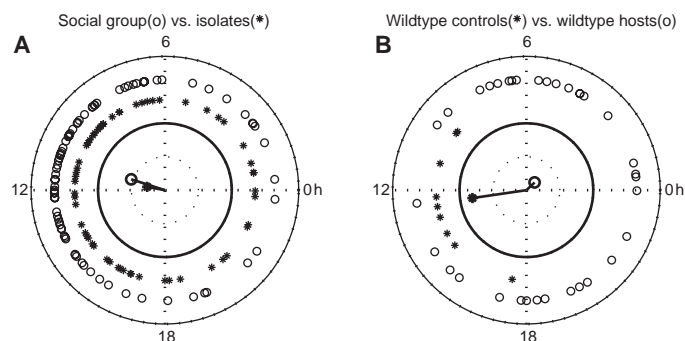


Fig. 2. The interaction between flies from different "time zones" alters circadian phase. (A) Open circles show late controls ($n = 16$); asterisks mark early controls ($n = 16$). (B) Early control individuals (asterisks; $n = 16$) were compared with early hosts (open circles; $n = 47$). (C) Late control individuals (open circles; $n = 16$) were compared with late hosts (asterisks; $n = 42$).

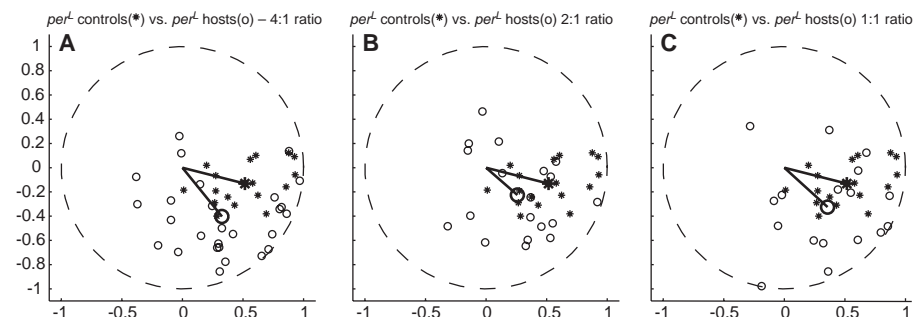


Fig. 3. Effects of *per^S* visitors on *per^L* hosts depend on the host:visitor ratio. Controls (asterisks; $n = 17$ in all panels) and *per^L* hosts (open circles) (A) at a 4:1 ratio ($n = 27$), (B) at a 2:1 ratio ($n = 19$), and (C) at a 1:1 ratio ($n = 17$) were compared.

hosts to 8 visitors or 4:1. Here, we examined the effects of 2:1 (27 hosts:13 visitors) and of 1:1 ratios, maintaining a constant number of 40 flies per vial in DD, to assess whether the size of a social subgroup matters. Analysis for hosts versus control (*per^L* alone) was the same as in Fig. 2.

Consistent with our results from early visitors and late hosts (of similar genotype), *per^S* visitors had a significant effect on *per^L* hosts at ratios of 4:1 (Fig. 3A; $P = 0.01$) and 2:1 (Fig. 3B; $P = 0.04$) but had a weaker (non-significant) effect at 1:1 (Fig. 3C; $P = 0.1$). In addition, and consistent with the previous early-versus-late experiment, there was no effect of *per^L* visitors on their *per^S* hosts at any ratio. These experiments indicate that the composition of the social group plays a role in the communication of timing signals.

What sensory mechanisms underlie social effects on circadian function? Visual or thermal cues are unlikely; social interactions and locomotor activity measurements took place in darkness and at constant temperature. Studies on courtship in *Drosophila* demonstrated that communication between flies may occur over a short distance by means of endogenous volatile chemical signals (16, 17). We asked whether chemosensory signaling could synchronize the phase of circadian activity in wild-type flies. Humidified air was pumped through either a vial containing food plus 10 to 15 wild-type flies ("fly air") or a similar vial without flies ("neutral air"). Both vials were simultaneously maintained in a 24-hour LD cycle at 25°C. Outflow from each vial reached individuals in an otherwise completely isolated activity monitor such that half received fly air and half received neutral air. Individuals receiving fly air were synchronized, whereas those receiving neutral air were more dispersed ($P = 0.05$; Fig. 4A). This indicates that chemical signals generated by wild-type *Drosophila* can synchronize in-

dividuals maintained in constant darkness and further suggests a cue that is rhythmically produced and short-lived.

We used the allelic olfactory mutants *para^{sbl-1}* (18) and *para^{sbl-2}* (19) to ask whether the sense of smell might be involved. The *para* locus encodes a voltage-gated sodium channel, and the *sbl* alleles produce generalized deficits in olfactory responses (19, 20), including to odors emanating from other flies (21, 22). Individuals from these strains were capable of detecting sucrose and light on the basis of assays of gustation and phototaxis (12, 23). Hypothetically, if smell detects the timing signal, then *per⁰* visitors would not disrupt phase in the mutants as they disrupt phase in the wild type (Fig. 1B). There was no effect of arrhythmic visitors on the phase of locomotor rhythms in *para^{sbl-2}* hosts ($P = 0.4$; Fig. 4B), *para^{sbl-1}* ($P = 0.3$), contrary to wild-type visitors in a control experiment ($P = 0.02$).

We cannot rule out parallel involvement of auditory or tactile cues, but because olfactory mutant responses are not disrupted by *per⁰*, olfaction is likely required for the social effect observed in the wild type. However, there is another possibility: Although clock cells in the central brain regulate locomotor activity rhythms, autonomous circadian clocks reside in a variety of *Drosophila* tissues, including several associated with sensory structures (24). We considered whether temporal regulation of sensory input (as opposed to the input per se) might be required for effective social communication. Accordingly, we employed a transgenic strain, *per^{7.2}* (12), in which *per⁺* expression is restricted to certain clock neurons within the central brain (25). Behavioral rhythmicity is normal in this strain (25), but temporal regulation of responses to odors by the antennal nerve is eliminated (26). Figure 4C shows no disruptive influence of *per⁰* visitors on the phase of

per^{7.2} hosts ($P = 0.5$; the wild-type positive control as above, Fig. 4B). This suggests that recognition of a social cue depends on the temporal control of sensory input by peripheral clocks.

These findings show that circadian clocks may be reset by social communication in *Drosophila*; that this communication may reflect genotype, experience, and composition of the group; and that the mechanism underlying these effects is likely chemosensory. In addition, this mode of social communication appears to rely on the distributed property of the circadian system, whereby temporal gating of peripheral sensory input informs the central clock-controlled regulation of behavior. Finally, neural mutations and gene manipulations in *Drosophila* can now be used to dissect social rhythm-regulating interactions.

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Supporting Online Material

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Materials and Methods

Fig. S1

References and Notes

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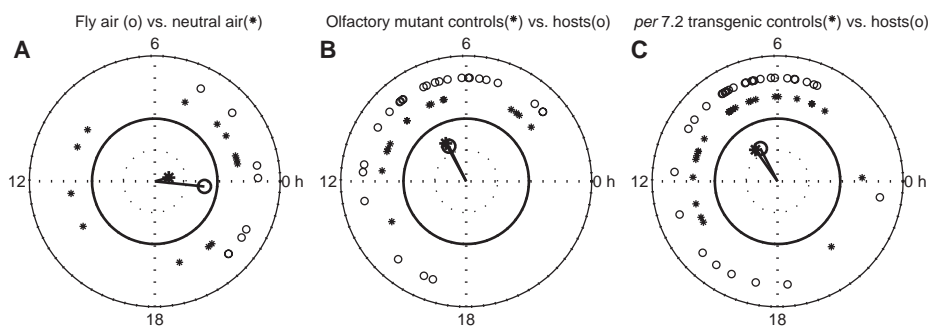


Fig. 4. Chemosensory signaling mediates social influence on circadian timing. (A) The peak phases of locomotor activity rhythms generated by isolated wild-type individuals receiving neutral air (asterisks; $n = 13$) are significantly more dispersed than those generated by individuals receiving fly air (open circles; $n = 8$). (B) Disruptive effects of *per⁰* visitors are not evident on the peak phase of olfactory mutants. The *para^{sbl-2}* controls (open circles; $n = 26$) are indistinguishable from sibling hosts (asterisks; $n = 15$). (C) Effects of *per⁰* visitors are not evident on the peak phase of *per^{7.2}* transgenic hosts (open circles; $n = 32$); transgenic controls indicated by asterisks ($n = 25$).