Supplemental Data

Spike Amplitude of Single-Unit Responses in Antennal Sensillae Is Controlled by the *Drosophila* Circadian Clock

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Supplemental Experimental Procedures

Fly Strains

Entrainment of flies for single-unit recording in both LD and DD conditions was done as described [S1]. The *Or83b* mutant is a targeted deletion that fails to express *Or83b* RNA and protein [S2]. The *Δhalo* mutant is a synthetic deletion that removes both the *Or22a* and *Or22b* genes [S3]. A *Gprk2* mutant containing a *P* element insertion in exon 1, $Gprk2^{6936}$ [S4], reduces GPRK2 protein levels, inhibits OR localization to OSN dendrites, and reduces the amplitude of EAG responses [S5]. A second *Gprk2* mutant, $Gprk2^{pj1}$, further reduces GPRK2 levels and the amplitude of EAG responses [S5].

Recording of Single-Unit Responses

Flies (3–7 days old) were mounted in a specially designed apparatus [S6], which was modified such that a fine glass capillary tube was used to both maneuver the antenna on the surface of the coverslip and hold the antenna in place. The antennal surface was observed under 1500× magnification that allowed individual sensillae to be resolved clearly using a BX-51W scope (Olympus). Recording in the dark was made possible with a filter with a cutoff of less than 600 nm (Leeds). Action potentials were recorded with glass electrodes filled with 0.17 M NaCl with tip drawn to less than 1 μm diameter. The indifferent electrode was inserted into the eye of the fly and the recording electrode was inserted into the base of the sensillum so that the electrode is in contact with the sensillar lymph that bathes the dendrite. These electrodes were positioned with Huxley-style manual micromanipulators with fine controls (1 μm steps). Signals from the electrodes were fed into a differential amplifier (DP 301, Warner Instruments) and alternating current (AC) signals were recorded (300HZ–10KHz) and amplified 1000×. Recordings were made from at least three different OSNs per fly. For all experiments described below, a minimum of four flies were measured. Single-unit recordings were stopped when signs of neuron damage characterized by a high frequency burst of firing were seen. Odorant stimulation was achieved by delivery of a quantifiable odor pulse, which interrupts a constant stream of air flowing over the preparation. The number of spikes initiated by the odor pulse was counted manually over 500 ms duration. Spike traces were analyzed with Axoscope (Axon) in offline mode, and peak-to-trough amplitudes of individual spikes were computed with software controls. Rate of spike firing was expressed as number of spikes/s. We could not measure rhythms in cVA-evoked activity in T1 sensillae because cVA-induced spikes could not be reliably distinguished from spontaneous non-T1 spikes having a similar frequency.

Immunostaining

Fly antennae were immunostained and imaged as described [S5]. The primary antibody was Guinea pig anti-TIM diluted 1:1000, and the secondary antibody was Cy3-conjugated anti-Guinea pig diluted 1:200. Images are representative of 10 to 15 antennae analyzed.

Statistical Analysis

Statistical analysis was done with MS-EXCEL (Microsoft) and Statistica (Statsoft). ANOVA analysis was done with Statistica and MS-EXCEL. Post hoc comparisons were done with Scheffe's test ($\alpha = 0.05$). Student's t test was used to compare values at peak and trough time points.

Supplemental References

S1. Krishnan, B., Dryer, S.E., and Hardin, P.E. (1999). Circadian rhythms in olfactory responses of Drosophila melanogaster. Nature *400*, 375–378.

S2. Larsson, M.C., Domingos, A.I., Jones, W.D., Chiappe, M.E., Amrein, H., and Vosshall, L.B. (2004). Or83b encodes a broadly expressed odorant receptor essential for Drosophila olfaction. Neuron *43*, 703–714.

S3. Dobritsa, A.A., van der Goes van Naters, W., Warr, C.G., Steinbrecht, R.A., and Carlson, J.R. (2003). Integrating the molecular and cellular basis of odor coding in the Drosophila antenna. Neuron *37*, 827–841.

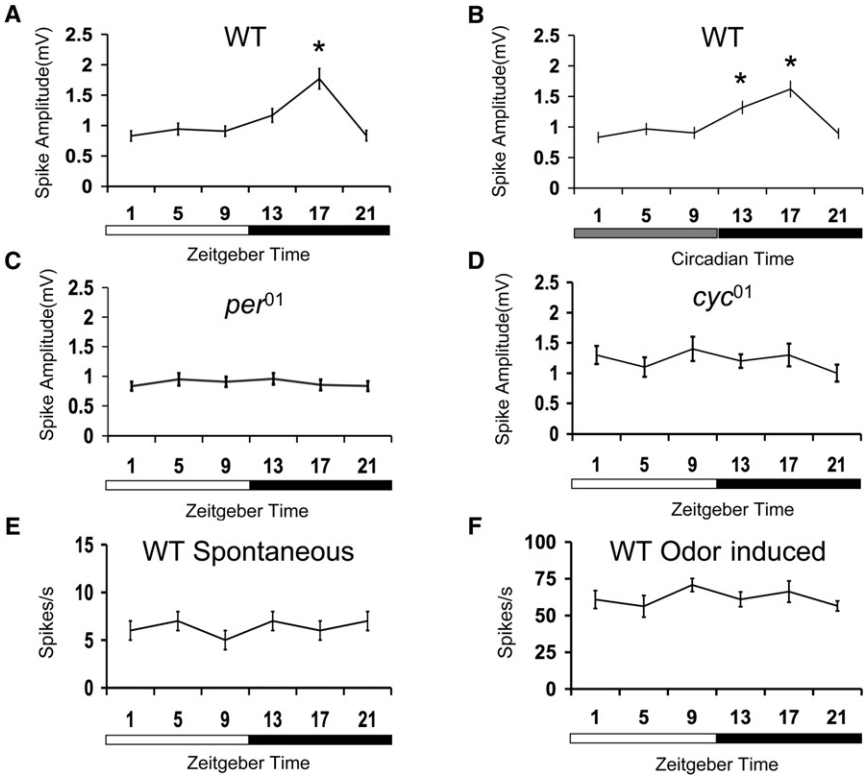

Figure S1. Spontaneous Spike Amplitudes Are under Circadian-Clock Control in the ab1 Sensillae

Single-unit recordings of ab1 sensillae were made from flies entrained for at least 3 days in LD cycles. Spike amplitudes were quantified from the A neuron. WT denotes Canton S flies. Error bars indicate ± SEM.

(A and B) Spontaneous spike amplitudes in wild-type flies during LD cycles (A) or constant darkness (B). The overall effects of time of day is significant by one-way ANOVA ($p < 0.001$) in (A) and (B). Asterisks indicate a significant ($p < 0.01$) increase in EAG responses at ZT17 (A) or CT13 and CT17 (B) compared with responses at all other times of day.

(C and D) Spontaneous spike amplitudes in per^{01} (C) and cyc^{01} (D) flies during LD cycles. Post hoc analysis indicated no significant ($p > 0.6$) difference in per^{01} and cyc^{01} mutants. Each time point represents amplitudes calculated from a minimum of 70 individual spikes in (A)–(D).

(E) Spontaneous firing frequency is not rhythmic in WT flies ($p > 0.4$).

(F) Odor-induced firing frequency in response to a 10^{-4} dilution of ethyl acetate is not rhythmic in WT flies ($p > 0.4$). Responses from a minimum of six OSNs were used to compute firing rate.

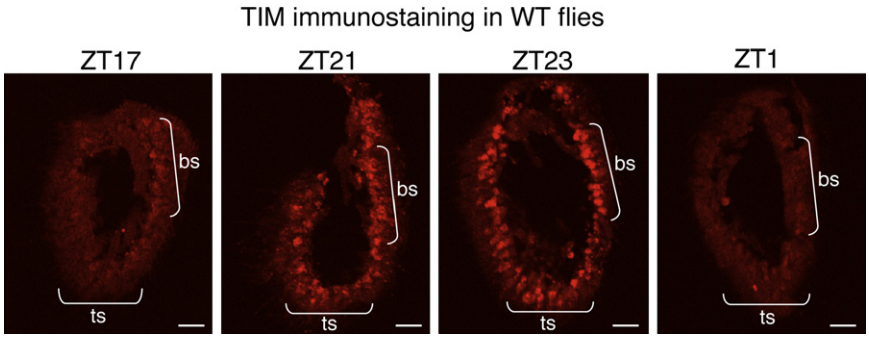

Figure S2. TIM Cycles in the Same Phase in Basiconic and Trichoid Sensillae

Wild-type (WT) flies entrained for at least three LD cycles were collected at the indicated times and immunostained with TIM antiserum. TIM immunoreactivity is seen in red. Regions with concentrations of basiconic sensillae (bs) and trichoid sensillae (ts) are bracketed. The scale bar represents 20 μm.

S4. Crosby, M.A., Goodman, J.L., Strelets, V.B., Zhang, P., and Gelbart, W.M. (2007). FlyBase: genomes by the dozen. Nucleic Acids Res. *35*, D486–D491.

S5. Tanoue, S., Krishnan, P., Chatterjee, A., and Hardin, P.E. (2008). G protein-coupled Receptor Kinase 2 is required for rhythmic olfactory responses in Drosophila. Curr. Biol., this issue, ■■■–■■■.

S6. Clyne, P., Grant, A., O'Connell, R., and Carlson, J.R. (1997). Odorant response of individual sensilla on the Drosophila antenna. Invert. Neurosci. *3*, 127–135.

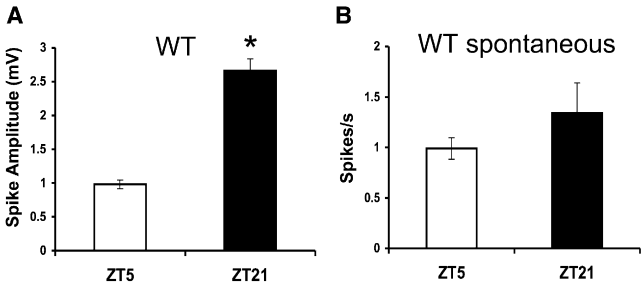

Figure S3. Spontaneous Spike Amplitudes Are under Circadian-Clock Control in T1 Sensillae

Single-unit recordings of T1 sensillae were made from flies entrained for at least 3 days in LD cycles. WT denotes Canton S flies. Error bars indicate ± SEM.

(A) Spontaneous spike amplitudes in wild-type flies during LD cycles. The asterisk indicates a significant ($p < 0.0001$) increase in EAG responses at ZT21 compared to ZT5. Mean amplitude was calculated from a minimum of 13 spikes at each time point.

(B) Spontaneous firing frequency is not rhythmic in wild-type flies at ZT 5 and ZT21 ($p > 0.3$). Responses from a minimum of five OSNs were used to compute firing rate.