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Letter to the Editors

Letter to the Editors: Does cockroach sensitivity to odors vary ten fold with circadian rhythm?

The recent Journal of Insect Physiology article by T.L. Page and E. Koelling (2003) caught our interest for two reasons. The finding that amplitudes of EAG depolarizations of the cockroach, *Leucophaea maderae*, exhibit a robust, light-entrained, circadian rhythm is novel and potentially important to insect neurobiology. We commend the authors' discovery and documentation of this phenomenon. However, we find highly suspect the major conclusion that the sensitivity of cockroach antennae changed 5-10 fold during these circadian oscillations. The concentrations of odorants (ethyl acetate, octanol, or fenchone) actually delivered to the antennae were never quantified in this study. Rather, the authors inferred that the stimulus concentrations were rising in 10-fold steps because the dilution of odorants in mineral oil increased by 10-fold increments.

Without seeing direct proof, we are reticent to accept the conclusion there was a 10-fold change in antennal sensitivity of this cockroach during a circadian interval. This interpretation may be based on a flawed assumption about consistency of the relationship between liquid loading concentration and concentration of odorant delivered in the vapor above the liquid. We became aware of this problem in a recent study of the EAG responses of tortricid moth antennae to low molecular weight phytochemicals (e.g., hexanal, limonene, benzaldehyde) (Stelinski et al. 2003; Fig. 6); gas-chromatographic quantification of changes in delivered concentration of odorants with 10-fold loading increases in mineral oil revealed only ca. 10% (rather than 10-fold) increases with each 10-fold increase in liquid concentration (range 1 ng to 10 mg in 100 microliters of mineral oil). Regrettably, the units on the abscissa of our Fig. 6 should have read ng/planchet rather than mg/planchet. Park et al. (2001) also directly quantified the emission rates of plant-volatiles (cis-3-hexenol, cyclohexanone, indole, caryophyllene, and others) delivered from filter paper housed in Pasteur pipettes, one common odorant delivery device used for EAG measurements. Loading dosages that increased by 10,000 fold on filter paper yielded only ca. 300-1200 fold increases in the emission rates of the delivered odorants. These authors also cautioned that establishing antennal sensitivity requires direct measurement of emitted vapor concentrations. Finally, a very recent paper by Cometto-Muniz et al. (2003) establishes definitively that proportionality between the liquid- and vapor-phase concentrations of low-molecular-weight volatile organic compounds (including ethyl acetate) dissolved in mineral oil does not hold at concentrations greater than ca. 1% v/v. Rather, these curves plateau such that there is very little increase in vapor-phase concentrations of odorants despite massive increases in concentration of solute in mineral oil.

Cometto-Muniz et al's. Fig. 2 establishes that proportionality between vapor and dissolved concentrations for ethyl acetate in mineral oil is lost and the curve plateaus beginning at and extending above 10^{-2}. Thus, at least two of six data points in Page and Koelling's Fig. 5 are invalid for making indirect interpretations of sensitivity to odor because they fall above the zone of proportionality for ethyl acetate. Unfortunately, these points featured powerfully in the argument for the substantial shift in sensitivity to odors with circadian rhythm. Moreover, why the EAG data in these graphs did not abruptly plateau above an ethyl acetate concentration above 10^{-2} is itself puzzling, given the Cometto-Muniz et al. data. Lack of congruence across these studies reduces confidence that the relationship between odor concentration and EAG amplitude is sufficiently regular throughout to permit indirect generalizations about the magnitude of sensitivity shift over time. Additionally, for the single cockroach (representing two others) generating the data of Page and Koelling's Fig. 5 (top panel), there was no difference in EAG amplitudes at all three dosages of ethyl acetate below 10^{-2}. Thus, the data from the top panel of this Fig. 5 representing three out of four total animals so tested are not supportive of the proposed large shift in peripheral sensitivity to odor. This argument then hinges on only two elevated data points (10^{-3} and 10^{-4}) (from just one cockroach; Fig. 5 Bottom) falling clearly within the zone of proportionality for ethyl acetate. However, the force of these two data is blunted by the finding of no elevation at the adjacent loading of 10^{-5}. Collectively, we find the current case for a 10-fold shift in peripheral sensitivity of L. madarae far from convincing.

Until direct quantifications of stimulus concentrations for their study are completed and the case is bolstered by a substantial increase in the numbers of replicate measures, readers are cautioned that the estimate of Page and Koelling for change in peripheral sensitivity of the cockroach with circadian rhythm might be vastly inflated. We feel obligated to raise this concern because

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whether or not peripheral sensitivity changes as much as 10 fold over time is of central interest and importance to understanding sensory physiology of insects and animals generally. Changes this large are likely to have meaningful behavioral consequences while changes of only a few percent might not. The data used to support such an important argument need to be robust, compelling, and immune to challenge before the conclusions based upon them gain broad acceptance.

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