ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

Odour concentration affects odour identity in honeybees

by

Geraldine A. Wright, Mitchell G.A. Thomson, and Brian H. Smith


Electronic appendices are refereed with the text; however, no attempt is made to impose a uniform editorial style on the electronic appendices.
Electronic appendix to Wright et al.

To ensure that odour concentrations were within a concentration range that was detectable by the honeybee, we relied on data from previous studies (Bhagavan and Smith, 1997; Wright and Smith, 2004a). We used an electro-antennogram (EAG) to measure the responses of antennal neurons to odour stimuli; this assay measures the voltage response across a population of olfactory receptor neurons in a manner similar to an electro-olfactogram (EOG) measured from the nasal epithelium of mammals (Knect and Hummel, 2004). The data reported in Wright and Smith (2004) were derived from EAG responses to the following odorants: 1-hexanol, 1-heptanol, 1-octanol, 2-octanone, and geraniol. Each was presented at the 3 different concentrations: 0.0002 M (low), 0.02 M (mid), and 2.0 M (high). We reported that these three concentrations produced significantly different antennal responses as measured by the EAG assay; the low-level (0.0002 M) odorants were detectable above a solvent background (hexane) both as conditioning stimuli and as stimuli in an EAG assay (Wright and Smith, 2004a).

To examine in greater detail the suitability of these stimulus levels for use in the present study, an analysis of the same data was conducted with just the three odorants used in the present experiments (1-hexanol, geraniol, and 2-octanone). The raw data were log-transformed and analyzed using a repeated-measures ANOVA that incorporated both odorant type and concentration as main effects (Figure 3). As reported by Wright and Smith (2004), the EAG electrical potential increased as concentration increased (1-way repeated-measures ANOVA: $F_{1,27} = 60.9$, $p < 0.001$). The overall amplitude of the EAG response averaged across all 3 concentrations was, however, odour-dependent (1-way ANOVA: $F_{2,89} = 5.33$, $N= 30$, $P = 0.007$): the response to geraniol was significantly lower than the response to 1-hexanol (least squares: $P = 0.002$) and 2-octanone (ls: $P = 0.022$), but the response to 1-hexanol was not significantly different from the response to 2-octanone (ls: $P = 0.443$). We also observed that the shapes of the EAG responses as a function of concentration were significantly different across the 3 odorants (1-way repeated-measures ANOVA: $F_{2,27} = 4.08$, $p = 0.028$). For all 3 odorants, the EAG response to the low concentration was significantly different from the high concentration (Table 2), but the magnitude of the difference between the intermediate and high concentration and the intermediate and low concentration was odorant specific (Table 2). In particular, the EAG for geraniol showed the greatest change from the low concentration to the intermediate concentration (Figure 3, Table 2).

The fact that the average EAG amplitude is smaller for geraniol than for the other two odorants may suggest that there are fewer neurons in the antennae that respond to geraniol. The steepness of the geraniol EAG suggests that the neurons that respond to geraniol are fewer in number and more specifically responsive to geraniol than the population of neurons responding to the 1-hexanol and 2-octanone. Our subjects’ failure to discriminate between the intermediate and high concentrations of geraniol may therefore result from the fact that almost no new information is being added from the olfactory periphery as the stimulus concentration increases from intermediate to high.

It is not surprising that this occurs for geraniol, as it is an odor component of the honeybee pheromone, the Nasonov pheromone. Nasonov pheromone is used by honeybees for aggregation (Pickett et al., 1980; Schmidt, 1999). Pheromones often elicit innate behaviors in insects; the tuning of the olfactory system appears to be narrower and
more sensitive for most pheromones in insects than for general, non-pheromonal odors (Smith and Getz, 1994; Shields and Hildebrand, 2001; Angioy et al., 2003). Shields and Hildebrand (2001), however, reported that the ORNs of *Manduca sexta* were narrowly tuned for some plant-derived terpenoids as well. Animals may be more sensitive to odours that are associated closely with highly-predictable events, such as the presence of a host or predator or the presence of a conspecific, and their ORNs may be more narrowly tuned to such odours (Suh et al., 2004).

**Table 2.** Paired-sample t-tests for the natural log-transformed EAG response for each concentration level of each odorant (N = 10 observations per odorant).

<table>
<thead>
<tr>
<th>Comparison</th>
<th>1-hexanol</th>
<th>2-octanone</th>
<th>geraniol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low to intermed.</td>
<td>P = 0.090</td>
<td>P = 0.210</td>
<td><strong>P = 0.005</strong></td>
</tr>
<tr>
<td>Intermed. to high</td>
<td>P = 0.296</td>
<td><strong>P &lt; 0.001</strong></td>
<td>P = 0.207</td>
</tr>
<tr>
<td>Low to high</td>
<td><strong>P = 0.016</strong></td>
<td><strong>P &lt; 0.001</strong></td>
<td><strong>P = 0.005</strong></td>
</tr>
</tbody>
</table>
**Figure 3.** Electroantennogram (EAG) of the honeybee shows that the absolute value of the neuronal response is a monotonically increasing function of concentration. Response of the antenna to: a) 1-hexanol; c) 2-octanone; c) geraniol (N = 10 subjects per odorant).
References:

Bhagavan, S. & Smith, B.H. 1996 Olfactory conditioning in the honeybee, Apis mellifera:
the effects of odor intensity. Physiol. Behav. 61: 107-117.

Behav. 83 (1): 13-19.

Pickett, J.A., Williams, I.H., Martin, A.P., & Smith, M.C. 1980 Nasonov pheromone of
the honey bee, Apis mellifera L (Hymenoptera, Apidae). 1. Chemical characterization. J.

Schmidt, J.O. 1999 Attractant or pheromone: The case of Nasonov secretion and

R., & Anderson, D.J. 2004 A single population of olfactory sensory neurons mediates an

Rev. Ento. 39: 351-75

Wright, G.A. & Smith, B.H. 2004 Different thresholds for detection and discrimination of