Imaging brain activity of honey bees sensing odors during sleep

Barrett A. Klein[1], Lisa Rath[2], Arno Klein[3], Satrajit S. Ghosh[4], C. Giovanni Galizia[2], Christoph J. Kleineidam[2]

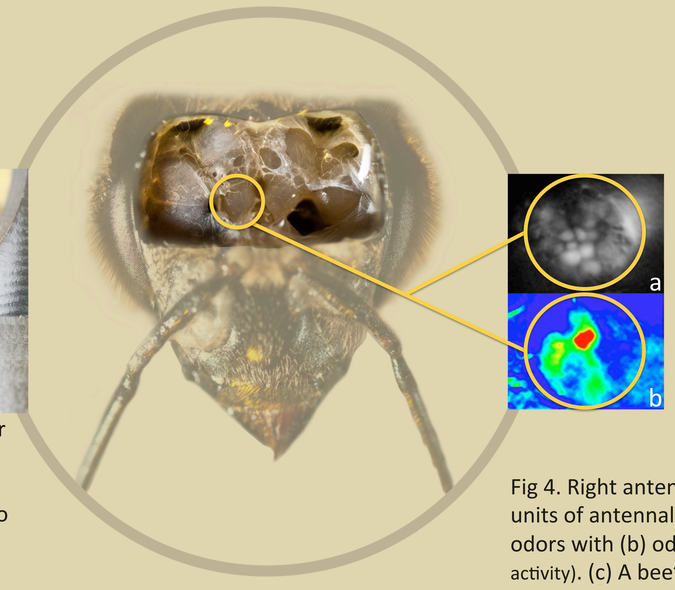
[1]Dept of Biology, University of Wisconsin – La Crosse, WI, USA barrett@pupating.org
[2]Dept of Biology, University of Konstanz, Germany
[3]Dept of Psychiatry & Behavioral Science, Stony Brook University, NY, USA
[4]McGovern Institute for Brain Research, Massachusetts Institute of Technology, MA, USA

Introduction

Honey bees (*Apis mellifera*) smell odors with their antennae, and this information is processed in the antennal lobe and in higher brain regions, eventually leading to behavioral responses. Odors elicit an odor-specific, combinatorial activation pattern in the antennal lobes of awake bees (Fig 4). We hypothesize that honey bees process odors during sleep, and we address the question of how odors are represented at the level of the antennal lobe when bees are asleep.

Fig 1. Honey bee workers in hive.

Fig 2. Forager in holder, ready for surgery. Head capsule opened, a fluorescing dye is injected into neurons that transport the dye to the bee's antennal lobes.



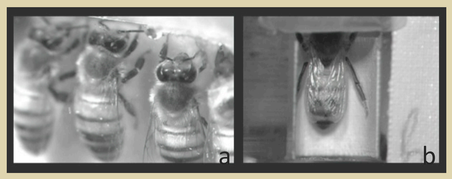


Fig 3. Sleeping honey bees (a) in hive & (b) prepared for brain imaging under microscope. Sleeping behavior (discontinuous ventilation) was indistinguishable between 2 conditions.

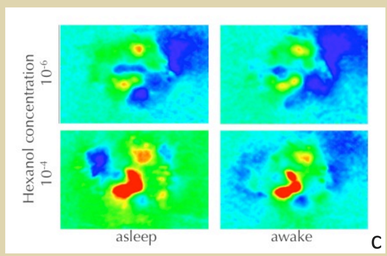


Fig 4. Right antennal lobe of honey bee brain. (a) Functional units of antennal lobe (glomeruli; round structures) respond to odors with (b) odor-specific activation patterns (red = greatest activity). (c) A bee's response to hexanol when asleep & awake.

Methods

We measured neuronal activity in antennal lobes of 7 forager honey bees when presented with odor stimulation, using a calcium-sensitive fluorescent dye. We loaded neurons that carry odor information from the antennal lobe to higher brain regions with the dye, and monitored each bee's brain while she was awake and asleep. Wake-sleep states were videotaped and determined by abdominal respiration patterns (discontinuous ventilation, Fig 3). Data were analyzed from bees that were lively and whose brains responded to hexanol throughout the study, and for which we had both sleep and wake recordings. Images were processed using R, and our own open-source Python software (http://www.binarybottle.com), originally developed for analyzing human brain fMRI data (Fig 8).

Fig 5. Forager bees were collected from indoor, winter-active colonies, and outdoors (Konstanz).

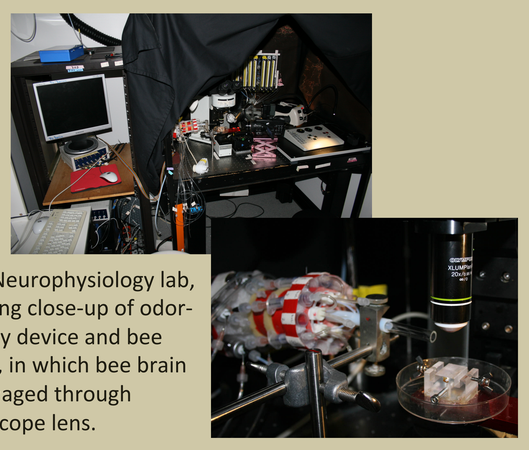


Fig 6. Neurophysiology lab, including close-up of odor-delivery device and bee holder, in which bee brain was imaged through microscope lens.

Conclusion

To our knowledge, this is the first calcium imaging of a sleeping invertebrate, and the first application of human brain imaging techniques to an invertebrate (Fig 8). We believe that imaging a sleeping brain in an insect that is an exceptionally capable learner will pave the path for future investigations of the value of sleep in the context of memory consolidation and learning.

Results

467 honey bee foragers were prepared for the experiments.
7 bees met our criteria for further analysis. All 7 bees showed distinct activation patterns in the antennal lobe with odor stimulation, whether awake or asleep. We found:

1. It is possible to perform calcium imaging of a honey bee's brain while she is asleep without waking her.
2. Response to higher concentrations of odor appeared to be stronger in brains of sleeping bees than when bees were awake (Fig 9a).
3. Odor representation was more distributed (more spatially widespread) in sleeping brains than when bees were awake (Fig 3c, 8, 9b-c). The strongest response to odor stimuli did not differ between sleep and wake, but secondary regions of response were stronger in sleeping bees (Fig 9c), helping to explain result #2.
4. Awake bees showed more spontaneous activity than when asleep (suggesting differences below were not due to the signal wearing down).

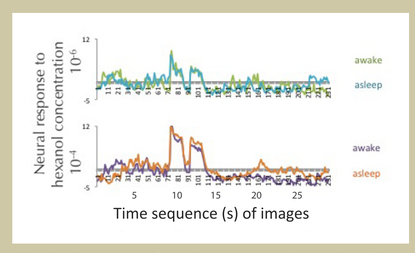


Fig 7. Traces of activity to odor stimuli (odor at image 73 & 93; bee from Fig 4c) over time at 2 different concentrations, with sleep traces superimposed over traces taken when the bee was awake.

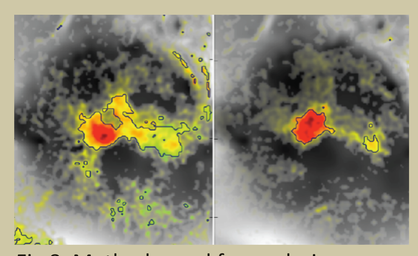


Fig 8. Methods used for analyzing human brain fMRI data applied to a bee that is asleep (left) and awake (right). Contrast images show bee responded to odors whether asleep or awake. Color indicates effect size and opacity reflects statistical significance.

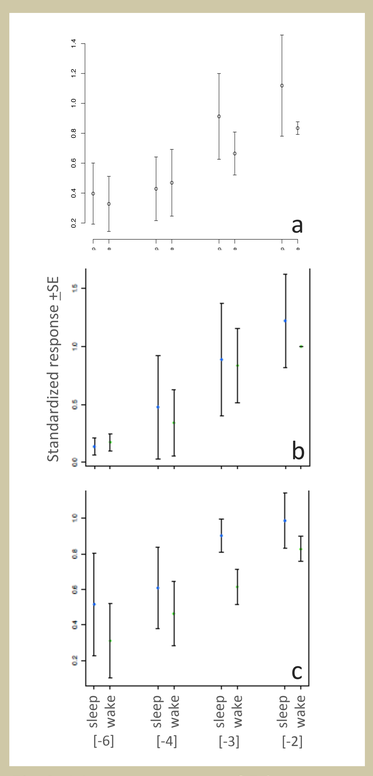
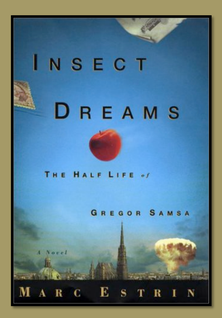


Fig 9. Mean response of 5 bees at increasing concentrations of hexanol when asleep vs. when awake
(a) across 4 regions of interest (ROI)
(b) strongest ROI, (c) 3rd strongest ROI.



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