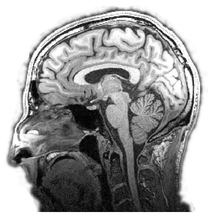

Applying human brain image processing methods to honeybee calcium image data

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Summary

Methods developed for analyzing human brain fMRI data have great potential for application to brain imaging data of different spatial and temporal scales, different imaging methods, and different species. In this work, we demonstrate a simple analysis of honeybee (*Apis mellifera*) brain image data using the Python programming language.

To our knowledge, this is the first application of human brain imaging techniques to an invertebrate. These techniques provide advantages when analyzing intra-individual phenomena, and invertebrates such as the honeybee offer the advantage of harboring a simpler, experimentally more accessible nervous system.

Background

Data in invertebrate studies are commonly pooled across multiple specimens based on a segmentation of the neuropil of interest. For many applications, this approach is powerful because physiological measures are based on a population mean. However, traditional methods [1] are limited when insufficient neuroanatomical information prevents a reasonable segmentation. With the proposed method, no *a priori* segmentation is necessary.

Imaging brain activity in bees

We investigated odor information processing in the brains of honeybees while the bees were awake versus while they were asleep [2]. Using functional imaging with Ca-sensitive, fluorescent dyes (calcium imaging), we measured neuronal activity during these two physiological states. Specifically, we tested the following:

Test 1. effect of odor vs. no odor (**asleep**, max. concentration)

Test 2. effect of odor vs. no odor (**awake**, max. concentration)

Test 3. effect of concentration (**asleep**)

Test 4. effect of concentration (**awake**)

Test 5. effect of asleep vs. awake (maximum concentration)

Image processing

We wrote open source Python software available via Github:
https://github.com/binarybottle/beebrains

Preprocessing steps

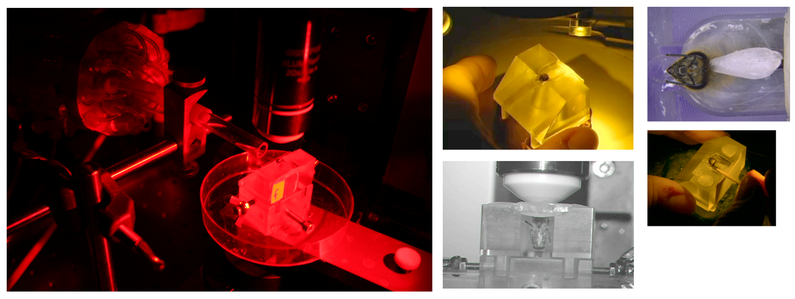
1. Divide images corresponding to one wavelength by images of a second wavelength (aligned with each other).
2. Apply FSL's mcflirt motion correction [3].
3. Smooth each slice image with a Gaussian kernel.

Processing steps

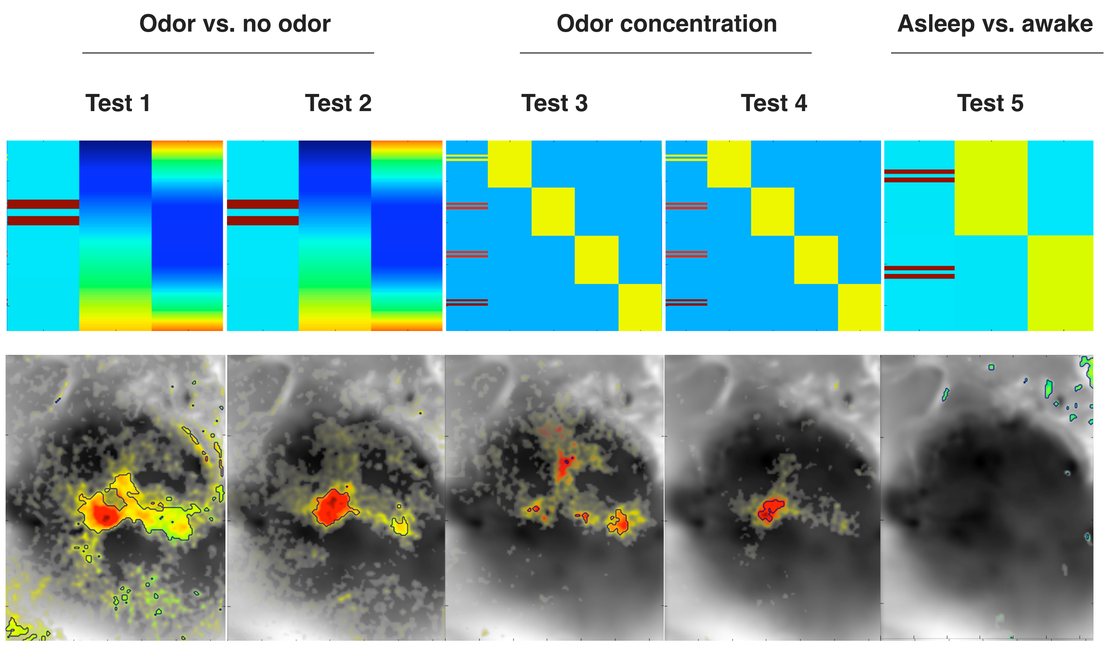
1. Mean-scale, de-mean, and normalize data to span zero to one.
2. Construct a design matrix from conditions, amplitudes, onsets, and durations, with a 2nd degree polynomial drift model to remove linear or quadratic trends in the data [4].
3. Apply a general linear model to all voxels and create a contrast image.

Barrett prepped for an MRI.

Bee prepped for calcium imaging.



Left: Honeybee "f" positioned under microscope lens in front of tube supplying constant air flow punctuated with timed release of odor. Imaging conducted under red light, to which honeybees are less sensitive.
Right: Setting up a study specimen, with grayscale image showing a bee under the microscope lens.



Design matrices (top row):

The five panels represent the design matrices for the five tests. A design matrix represents the different regressors in the context of multiple linear regression, or the general linear model. The vertical axis represents the number of images (Tests 1,2: 232; Tests 3,4: 928; Test 5: 464). 232 sequential images were acquired for a given condition (concentration in Tests 3 and 4, asleep vs. awake in Test 5), and are represented as staggered yellow blocks in these design matrices. The horizontal bands in the leftmost column of each design matrix represent the odor onset times, durations, and amplitudes (increasing from yellow to red in Tests 3 and 4). The gradient columns represent a 2nd degree polynomial drift model to remove trends.

Contrast images (bottom row):

The five panels show contrast images for the five tests conducted on 1 of 6 bees. Color indicates effect size and opacity reflects statistical significance, and the contour is at the statistical threshold. These preliminary results suggest that this bee responds to odors whether asleep or awake (Tests 1 and 2), there was an effect of concentration of the odor whether the bee was asleep or awake (Tests 3 and 4), and that there was no difference in activity between asleep and awake conditions when controlling for concentration of the odor delivered to the bee (Test 5).

The panels show the same portion of antennal lobe in a honeybee, and are images taken by a CCD camera monitoring changes in intracellular calcium levels using fluorescent dyes and excitation wavelengths of 340 and 380 nm. This frame was taken at a single time point within a time sequence of hundreds of frames acquired at a rate of 8 Hz. Taking the ratio of images at the two wavelengths compensates for differences in dye loading of neurons, which improves the detection of changing brightness due to calcium influx and excitation of stained neurons.

References

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