



Analysis of odor processing in the mushroom bodies of the honeybee[☆]

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Abstract

To study the neuronal dynamics in the mushroom bodies (MB) of the honeybee *Apis mellifera* we simultaneously recorded local field potentials (LFP) from different sites within the MB. Based on a wavelet analysis of the LFPs we found in the absence of odor stimuli ongoing 8–27 Hz oscillations which are coupled between both hemispheres of the brain. Odor stimuli give rise to faster waves in the range of 16–45 Hz, which seem to be decoupled between the hemispheres and appear either instead of or superimposed on the spontaneous waves. A modified correlation analysis shows, however, that even during odor processing there is a significant coupling between the two hemispheres of the brain. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Understanding the neural basis of olfactory signal processing is a major open problem in the neurosciences. Honeybees are ideally suited to study this problem because they are highly olfactory animals whose neural responses to natural odors can be measured in vivo. Various brain regions process the olfactory stimuli but the

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precise dynamical interplay, for example, between the left and right hemispheres is largely unknown.

Odor processing in insects induces neural activity patterns which are structured in space and in time. In the antennal lobe (AL) odors are represented as specific spatial patterns of glomerular activity [1,2,6]. Moreover, odor specific oscillatory synchronizations of AL neurons have been described [4,8]. In contrast to the relatively stable glomerular code these temporal patterns of AL-neuron ensemble activity are highly variable. The oscillatory activity of AL neurons is supposed to underlie field potential oscillations which can be recorded in the mushroom bodies (MB), a neuropil which integrates sensory information of different modalities.

We studied the neuronal dynamics in the MB network of the honeybee *Apis mellifera* by simultaneously recording local field potentials in the MB and unit activity of MB output neurons. We then performed a wavelet analysis of local field potentials and applied a new method inspired from non-linear systems theory to study the dynamics of the recorded brain areas and their interaction.

2. Experiment

Honeybees (*Apis mellifera*) were used in an in vivo preparation. To avoid muscular artifacts we used Philanthotoxin-343 (a component of the sting toxin of the digger wasp *Philantus triangulum*) to paralyze the muscles in the head. The head capsule and thorax were fixated with dental wax in the recording tube. The basis of the antennae were immobilized with a silicon polymer. Preparation consisted of removing large parts of the cuticle of the head capsule. Glands in the head had to be removed. Unipolar recordings of field potentials (FP) and extracellular recordings from single neurons in the α -lobes of the mushroom bodies were performed with borosilicate-glass microelectrodes (resistance: 3 M Ω). Before further processing FPs have been band-pass filtered from 5 to 100 Hz.

3. Time-frequency analysis

A wavelet analysis [7] was carried out to reveal the temporal variations of the frequency content of the LFP signals from the MB (Fig. 1). As mother wavelet we have chosen a Morlet wavelet of order $\omega_0 = 5$

$$\psi(\eta) \propto \sin(\omega_0 \eta) \exp(-\eta^2/2).$$

We then computed the wavelet transform W of the discrete time series x_n (the field potential sampled at intervals δt)

$$W_n(s) = \sum_{n'=0}^{N-1} x_{n'} \psi^* \left[\frac{(n' - n)\delta t}{s} \right],$$

for all desired scales s and translating along the localized time index n .

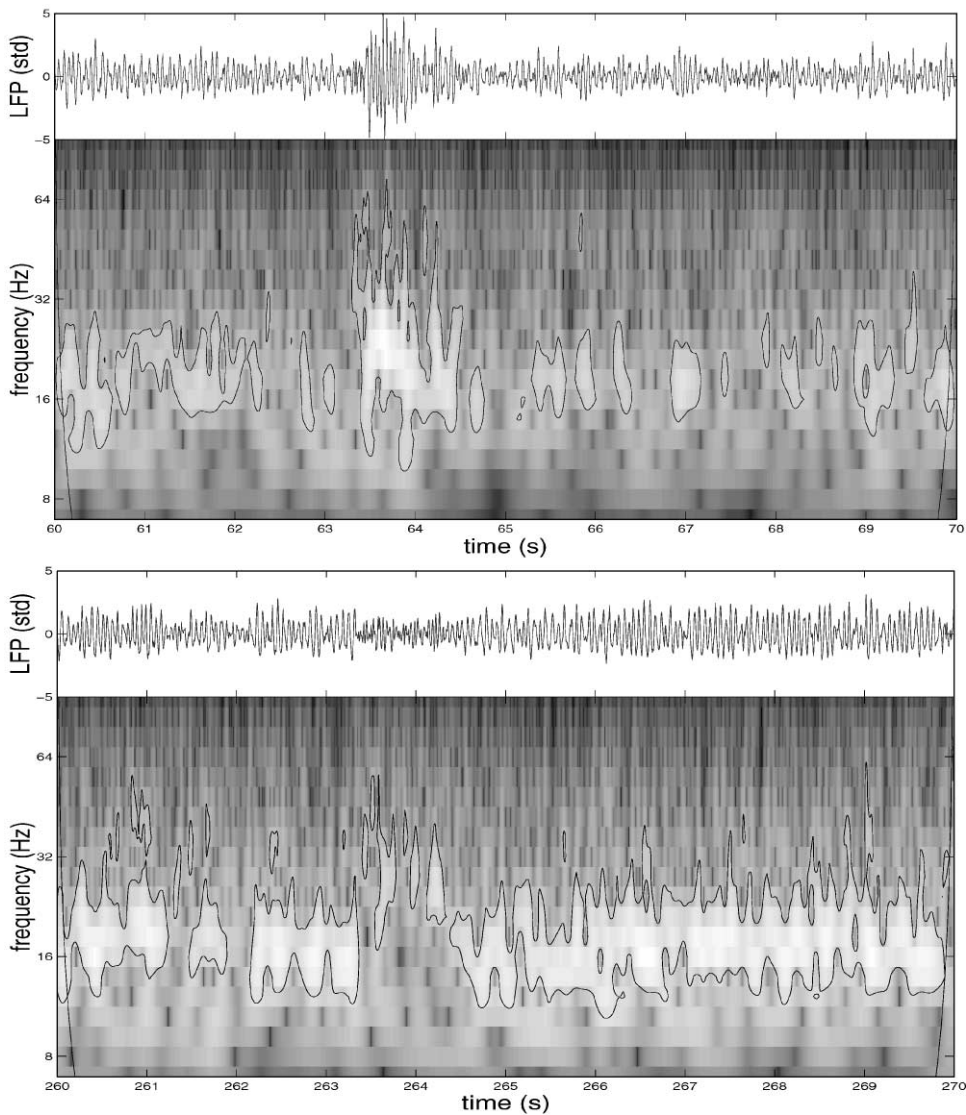


Fig. 1. Local field potential (top traces): An odor was presented from $t = 63$ s until $t = 64$ s (upper part) and from $t = 263$ s until $t = 264$ s (lower part). Units are in standard deviations of the signal ($50 \mu\text{V}$). Wavelet spectrum (lower panels): Contour plot of the wavelet power (logarithmic scale) showing the time-resolved frequency content of the signal above. The thick contour encloses regions of significant deviation from the best matching AR(1) process (a red noise process with the same lag-1 correlation coefficient; sampling rate 208 Hz; 95% significance level). Note the logarithmic frequency scale.

In the absence of experimentally applied odor stimuli we found ongoing 8–27 Hz oscillations which are coupled in both hemispheres of the brain (see below). Odor stimuli either abolish this spontaneous activity and give rise to faster waves in the

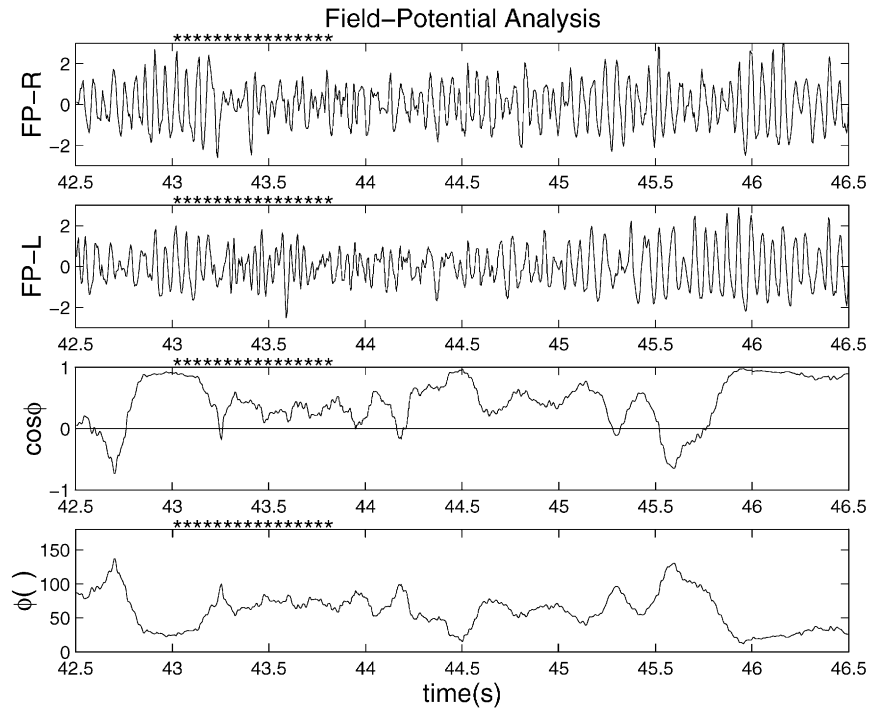


Fig. 2. Voltage traces from two different recording sites (upper two panels; from left and right hemisphere respectively). An odor was presented from $t = 43$ s until $t = 44$ s. Units are in standard deviations of the signal. Lower two traces: The instantaneous correlation ($\cos \phi$) and the angle in phase space (ϕ) between the two signals.

range of 16–45 Hz, (Fig. 1; lower part), or these faster waves appear superimposed on the spontaneous waves (Fig. 1; upper part). Extracellular recorded MB output neurons often fire with the frequency of these oscillations and are phase-locked to the MB field potential (data not shown).

4. Measuring statistical dependence

Next, we performed a phase-space reconstruction through delay embedding [3]. A suitable embedding dimension turned out to be $m = 30$ reflecting a typical time scale of 150 ms. Unfortunately, the system turned out to be non-stationary, so it was impossible to find any attractors of the system or to estimate the mutual information between two signals [5].

To quantify the instantaneous correlation between two simultaneous recordings we instead referred to the angle enclosed by the vectors representing the two signals in phase space at each moment in time. The calculation of this angle is straightforward.

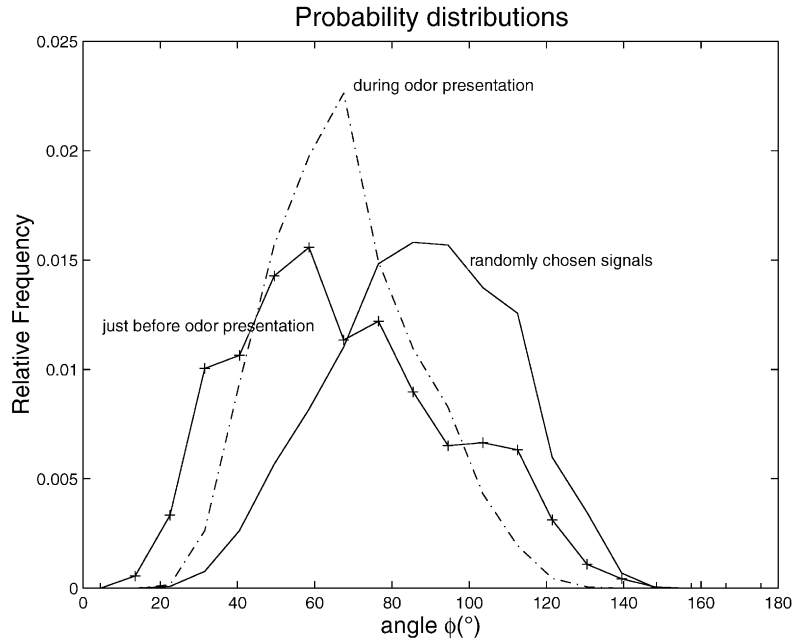


Fig. 3. The distribution of phase angles for three different cases: on-going activity just before odor presentation as well as during odor presentation are significantly correlated in contrast to traces from different experiments.

According to linear algebra

$$\cos \phi = \frac{\mathbf{x}_t \cdot \mathbf{y}_t}{|\mathbf{x}_t| |\mathbf{y}_t|},$$

where \mathbf{x}_t and \mathbf{y}_t are the delay coordinates of the signals, i.e. $\mathbf{x}_t = (x_t, x_{t-\delta t}, \dots, x_{t-(m-1)\delta t})$. So $\cos \phi$ is basically a running correlation coefficient. This correlation and the underlying angle in phase space are shown in the lower two traces of Fig. 2.

Since this correlation measure highly fluctuates we next considered the distribution of the angle for three different cases: parts of the signals (i) where no odor has been presented, (ii) during odor processing and as null hypothesis to test against (iii) signals from different trials. These distributions are shown in Fig. 3. According to the symmetrized Kullback–Leibler distance both distributions from simultaneously recorded signals are significantly different from the shuffled ones indicating a weak coupling between the two hemispheres of the brain.

5. Conclusion

Together, these results support the hypothesis that information processing in the MB network makes use of a temporal coding scheme but that the two different

hemispheres are processing stimuli largely independently. The exchange of information between both hemispheres is rather frequent in general, but not directly provoked by an olfactory stimulus.

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