In vivo two-photon calcium imaging with single neuron resolution in the honeybee

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The primary olfactory area of insects, the antennal lobe, is divided into functional subunits, called the olfactory glomeruli. The input to glomeruli is provided by olfactory sensory neurons that all express the same receptor type. The output of each glomerulus consists of multiple projection neurons (PN) (5 to 8 in honeybees). Most of them innervate only one glomerulus (uniglomerular PN) and transmit odor information to higher brain areas. Thus, a single input channel is split into multiple output channels. The function of this channel splitting is not known. Channel splitting could either duplicate information (e.g. in order to provide different targets with the same information), or serve for parallel processing of different stimulus parameters (such as concentration and temporal structure). In the former case, the responses of different PNs of the same glomerulus would be highly correlated. In the latter case, PN response properties would differ qualitatively and/or quantitatively.

Conventional calcium imaging techniques were limited to superficial neuronal tissue and a low spatial and temporal resolution. Electrophysiology is limited to a small number of cells and poor spatial dimensionality. Here we show that high resolution two-photon calcium imaging is a suitable approach to investigate the function of channel splitting by parallel recording of multiple PNs in several glomeruli. It combines the advantages of calcium imaging (recording of spatial response patterns) with advantages of electrophysiology (recording at a single cell resolution). We selectively stained honeybee PNs with the calcium indicator Fura-2 dextran. We were able to record calcium responses from single PN neurites in the antennal lobe. We achieved a penetration depth of 100 µm, which is sufficient to image from clearly separated neurites, which are intermingled on the surface of the antennal lobe but separate increasingly to the center. Furthermore, there was practically no photobleaching during two-photon excitation. Recordings of the size of a whole glomerulus (60 µm) could be performed with a spatial resolution of 1 µm/pixel and a temporal resolution of 20 Hz. Preliminary results suggest that PNs from the same glomerulus rather respond in the same way than differently.