Large scale activity in the fruit fly brain

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Objective: Our goal is to measure activity in a whole brain at once, and identify the best techniques to make sense of the data we obtain. Striking a balance between complexity and tractability, the fruit fly is a powerful model system to achieve that goal. It generates complex behaviors -even head fixed-, but it is small enough so that its brain can be observed fully under a microscope. The powerful genetic tools have allowed beautiful work to be done on the function of small parts of the circuit and the anatomy of the whole brain. Whole brain imaging in the fruit fly thus helps bridge the gap between local and global networks, as well as between anatomical and functional networks.

Methods: A calcium sensor –GCamp6– or voltage sensor –Arclight– are expressed, either pan-neuronally, or in subsets of neurons broadly distributed in the brain (including acetylcholine and dopamine neurons). We then observe the whole brain's fluorescence through light field microscopy, and record at a frame rate up to 200Hz with a high-speed CMOS camera. We image in various situations: while the fly is behaving freely (typically walking, grooming, or resting) and in response to a range of stimuli. 3D stacks are then reconstructed from the light field images using wave optics to model point spread functions, before applying 3D-deconvolution. We then use principal component analysis and independent component analysis (with the program MELODIC in Fsl) to find the different sources in the signal.

Results: Although the cell bodies are rarely visible, the components extracted by ICA often coincide with neuritis or neuropils previously identified anatomically - thus isolating them without the need of further genetic intersection techniques-. Figure A and B present z stacks of components (in different colors) for whole brain dopamine neurons (TH-gal4/UAS-GCAMP6F) and central complex cholinergic neurons (Cha-gal4/UAS-GCAMP6F) –the scale bar is 30 μm-. The fluorescence time course (and thus the activity) for each component is also extracted. Figure C presents an example of activity in neurons projecting radially in the ellipsoid body and linearly in the protocerebrum bridge. Slow oscillations (~0.1Hz) observed in calcium data (Cha-gal4 / UAS-GCamp6F) seem to be also present for similar components in voltage data (Cha-gal4 / UAS-Arclight). We also find characteristic patterns of activity in other parts of the brain (large pulses in the noduli, fast continuous activity in the mushroom body, the inner ring of the ellipsoid body and the fan-shaped body for example). As these patterns are observable in the absence of any stimulus, and when the fly is at rest, they could result from spontaneous activity.
We also identify brain regions responding to stimuli such as an odor (in the antennal lobe, lateral horn and mushroom body for example) or an additional flash of light (whole protocerebral bridge for example).

Conclusion: We are thus able to extract functionally relevant signals from whole brain fluorescence measurements. The next step is to characterize the spatial and temporal relationships between components to understand the interactions between them. We also wish to start a public database to facilitate collaboration with experts on different subcircuits in the fly brain.

Significance: This work directly addresses, in a model system, the BRAIN grand challenge 3a: "Produce a dynamic picture of the functioning brain by developing and applying improved methods for large-scale monitoring of neural activity".