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EXPLORING COMPUTATIONAL NETWORKS ACROSS AN ENTIRE BRAIN AT SINGLE CELL RESOLUTION

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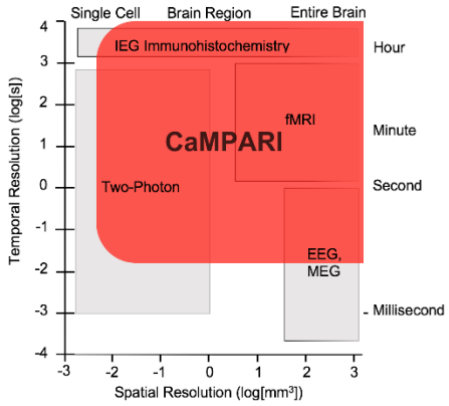
Large scale monitoring of neuronal activity in mouse models currently depends on either live imaging techniques or activity snapshots based on the expression of endogenous immediate early genes (IEG) correlated with neuronal activity. These two strategies differ in their spatial versus temporal resolution. Live imaging techniques are fast but their spatial resolution is limited by either photon-scattering or quickly decaying activity signals. In contrast, snapshots can capture activity across an entire brain, but have high-background and poor temporal resolution due to hour-long delays between cell activity and maximal IEG expression. In order to overcome the limitations of IEG-based activity snapshots, we have generated a mouse line for cre-recombinase dependent expression of the newly developed CaMPARI reporter. CaMPARI is derived from the stony corral protein EOS, which irreversibly shifts its fluorescence—that is to say, it photoconverts—from green to red in the presence of violet light (Fosque et al., 2015). By fusing calcium binding calmodulin and a calmodulin/Ca^{2+} binding peptide to either end of EOS, photoconversion (PC) becomes calcium dependent. High intracellular calcium is found naturally in active neurons, while PC light can be delivered by the experimenter when and where an activity snapshot is required. In proof-of-principle experiments, we have tested our new mouse line by restricting CaMPARI expression to excitatory neurons of the neocortex. Pulsed, high-power PC light was supplied transcranially to the right hemisphere. Brains were extracted, fixed, sliced and imaged for green versus red fluorescent neurons using a confocal microscope. In the hemisphere without PC light, green but no red fluorescence was detected. In contrast, both green and red florescence was present in neurons receiving PC light. Despite the fact that all cells express CaMPARI from the same engineered gene, we observed variable CaMPARI-expression levels in our reporter mouse line. We compensated for the bias of expression on the readout by using the ratio of red versus green fluorescence as the activity measure. Moreover, we have shown that saturating PC light intensities can be delivered deep into the brain (≥ 1 mm) without damaging neuronal tissue. To summarize, we have demonstrated that our newly developed CaMPARI-reporter mouse can indeed be used to monitor neuronal activity across large brain volumes in genetically defined cell types.



Spatio-temporal resolution of CaMPARI-based activity snapshots; IEG: immediate early gene; fMRI: functional magnetic resonance imaging; EEG: electroencephalography; MEG: magnetoencephalography.