LATTICE LIGHT SHEET IMAGING AND PHOTO-STIMULATION IN BRAIN SLICES

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1. INTRODUCTION

The number of neurotransmitter receptors concentrated in synapses sets the efficacy of synaptic transmission and is dynamically regulated. Understanding the resulting synaptic plasticity is key as it is thought to underlie the processes of learning and memory [1]. This dynamic has been almost exclusively studied in the reduced experimental system of 2D primary neuronal cultures that does not recapitulate the complexity of the brain. This limitation originates largely from the poor optical access in brain tissue.
Lattice light sheet (LLS) fluorescence microscopy is a powerful recent technique for in vivo imaging of single and multicellular samples at very high spatio-temporal resolutions [2]. We built a LLS microscope in which we added a photo-stimulation path to perform all-optical neurophysiological studies in rodent hippocampal brain slices.

2. RESULTS

To image receptor trafficking at endogenous expression levels, we have generated a constitutive knock-in mouse expressing a biotin acceptor peptide tag (bAP) on the extracellular N-terminus of the GluA2 subunit of AMPA type glutamate receptors. Labeling specificity is achieved by directed expression of the enzyme biotin ligase (BirA), which selectively biotinylates the bAP tag on GluA2-containing AMPARs (bAMPARs). Thanks to the photo-stimulation path, we could achieve fluorescence recovery after photobleaching (FRAP) at spatially and temporally controlled regions of interest **on endogenous receptors**. Several fluorescence labelling protocols were employed depending on the imaged structure. Sub-micrometric neuronal elements such as spines or dendritic vesicles could be imaged down to ~20 µm below the surface. We demonstrate the performances of LLS in measurements of AMPA receptor surface diffusion at single spines and vesicular transport in dendrites.

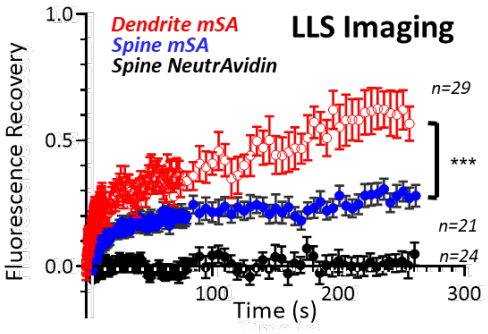

Figure 1: Measurement of AMPAR receptor dynamic in brain slices with FRAP. Endogenous AMPAR are labeled with fluorescent monomeric Streptavidin (mSA). Tetravalent Neutravidin blocks AMPAR mobility.

3. REFERENCES

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