Transient spine enlargement is an important event associated with the structural plasticity of dendritic spines. Many of the molecular mechanisms associated with transient spine enlargement have been identified experimentally. Here, we use a systems biology approach to construct a mathematical model of biochemical signaling-mediated transient spine expansion in response to calcium-influx due to NMDA receptor activation. We have identified that a key feature of this signaling network is the paradoxical signaling loop. Paradoxical components act bifunctionally in signaling networks and their role is to control both the activation and inhibition of a desired response function (protein activity or spine volume). Using ordinary differential equation-based modeling, we show that the dynamics of different regulators of transient spine expansion including CaMKII, RhoA, and Cdc42 and the spine volume can be described using paradoxical signaling loops. Our model is able to capture the experimentally observed dynamics of transient spine volume. Further, we use the model to make experimentally-testable predictions on the role of different components of the network on spine dynamics

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Nano-Mechanical Probing of Synaptic Activity at Dendritic Spines

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Dendritic spines are small structures found covering the dendrites of neurons, forming excitatory synapses throughout the brain. These structures are a fundamental element in neural circuits, allowing many synaptic inputs to be processed by a single neuron. Due to their sub-micron size, however, it is very difficult to study the individual synaptic properties of spines using conventional methods such as electrophysiology and optical measurements. In particular, direct measurement of the membrane depolarization that occurs in the spines is needed to fully understand their function. While probing spines with Atomic Force Microscopy (AFM) we have observed spontaneous, mechanical events at the spine head which last for ~10ms. During these events, the apparent height of spines increases abruptly with a temporal pattern that exhibits features similar to action potentials. Using different AFM recording modes we have measured changing forces between the AFM tip and spine head that accompany these height changes. We have used these measurements to test a mechanical model of the spine that can explain the observed behavior. Our observations suggest a strong electromechanical coupling phenomenon occurring specifically at spines that may facilitate non-invasive probing of individual synaptic activity by AFM.

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Development of Fast-Response GCaMP6 Calcium Sensors for Monitoring Neuronal Action Potential

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Green Fluorescent-Calmodulin Proteins (GCaMPs) have been the reporters of choice for visualizing neuronal network activity *in vivo*. With the GCaMP6 generation sufficient brightness was achieved for single action potential (AP) detection[1]. However, the Ca^{2+}-response kinetics of GCaMP6 probes remained limiting to separating individual APs. The aim was to accelerate the Ca^{2+} response kinetics of GCaMP6 probes by point mutations in the Ca^{2+}.CaM.RS20 complex[2]. A series of probes retaining high fluorescence dynamic ranges was generated with *in vitro* dissociation constants (K_d) for Ca^{2+} in the μM range (0.1-3.3 μM) and Hill coefficients of 1.7 to 4.2. Two highlighted probes termed GCaMP6$_{fast}$ and GCaMP6$_{bright}$ had *in vitro* half times ($t_{1/2}$) at 37°C for Ca^{2+} rise of 1.3 ms and 3.3 ± 0.2 ms and decay $t_{1/2}$-s of 2.8 ± 0.1 and 3.5 ± 0.1 ms, respectively, compared to the 10 ± 1 ms rise and 63 ± 6 ms decay times for GCaMP6f. Fluorescence changes on Ca^{2+} association were highly cooperative and characterized by a rate limiting conformational change. *In vivo* Ca^{2+} responses associated with AP firing patterns were tested in cultured hippocampal slices by two-photon imaging at 28°C. The kinetic performance of GCaMP6$_{fast}$ and GCaMP6$_{bright}$ was compared with that of GCaMP6f. Ca^{2+} decay kinetics were determined by monitoring fluorescence changes evoked by 5 APs fired at 100 Hz. $t_{1/2}$ values for GCaMP6$_{fast}$ and GCaMP6$_{bright}$ were 58 ms and 126 ms, respectively. With an up to 7-fold faster *in vivo* decay kinetics than GCaMP6f, GCaMP6$_{fast}$ and GCaMP6$_{bright}$ are promising tools for monitoring brain activity.

[1]Podor et al., *Neurophotonics,* Epub 2015 Apr 30.
[2]Helassa et al., *Scientific Reports*, accepted.

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OptoMEA: A Platform for Analyzing Signaling Efficiency of Neuronal Circuits using Multi-Location Extracellular Electrophysiology, Optical Imaging and Optogenetics

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Investigation of network level dynamics from neuronal circuits requires technologies that can probe many neurons simultaneously. Traditionally, multi-electrode array (MEA) recording and calcium imaging have been used for this purpose. We combined these two technologies to overcome some of their intrinsic shortcomings, and have recently established an investigative tool - OptoMEA - that allows for optical stimulation of channelrhodopsin-2 expressing neuronal networks with blue light pulses, and simultaneous electrophysiological and optical readout of neuronal activity from multiple locations of the network. For measuring optical response from these networks, we are using RCaMPh, a genetically encoded calcium sensor that fluoresces in red and is excitable by green light. We have successfully used this combinatorial platform to simultaneously examine dynamics of many single cells and the entire neuronal network in which these cells are embedded with good spatio-temporal resolution. We are using this approach to induce, detect and track short- and long-term changes in spontaneous and light-evoked electrical activity and calcium response in neuronal circuits. Additionally, we have developed several multi-parametric assays for determination of signaling efficiency from neuronal circuits exposed to various perturbations, image analysis codes, and algorithms for characterization of frequency specific neuronal dynamics and network synchronization. The OptoMEA platform has enabled us to connect the signaling efficiency of a neuronal network to its ''health''. As proof-of-concept demonstration of this technology, we have generated comparative large-scale network circuit data from normal and diseased neuronal networks. We have used an in vitro network model of neuronal inflammation and neurodegeneration for this study. Our results indicate that diseased networks show compromised evoked electrical response to optogenetic stimulation and markedly different calcium dynamics.

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Light Sheet Microscopy for Functional Imaging of Brain Activity Evoked by Natural Sensory Stimuli

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Light sheet microscopy and the zebrafish emerged recently as exciting tools for studying brain activity with cellular resolution in a vertebrate model system. High temporal and spatial resolution can be achieved in 3D with light sheet microscopy using either single-photon (1p) or 2-photon (2p) excitation. However, effects of the imaging itself on the observed neural activity are rarely addressed, which is of particular concern when the excitation laser power is high, as is needed for fast imaging. Here, we use the brain response evoked by natural sensory stimuli to assess how imaging conditions, such as excitation modality and laser power, affect the observed brain activity in unanesthetized zebrafish larvae. We show that red (630 nm) light flash-induced response is diminished when imaging with 1p-excitation of blue (488 nm) light, a wavelength that is visible to the fish larvae. This effect cannot be explained by saturation of blue-photoreceptors by excitation light, and points to an interference mechanism occurring further downstream of the visual processing. We also assess the effect of the high power of 2p-excitation, required for high speed imaging, to the observed brain activity, and establish a threshold power level above which the neural response starts to be affected, possibly due to the interference between the visual and heat/pain responses. Importantly, the interference effects described above occur at power levels that are not very high (~ 10 uW and ~ 100 mW, for 1p and 2p-excitation, respectively), where gross photo-damage effects (e.g. tissue ablation) are not visible. Staying below the perturbation thresholds of laser powers, we proceed to map the brain-wide activity due to evoked responses, providing the necessary