Chemical stimulation of single neurons in an in vitro system:
Coupling a force-controlled nanopipette with a MEA recording system

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A major focus in Neuroscience is how action potentials are transmitted between two neurons over chemical synapses. Structural and functional remodeling of the synapse due to certain stimulation patterns are proposed to play a major role in learning and memory. However, the resolution limit of in vivo techniques and the high complexity of brain topology make it difficult to study such changes caused by a defined stimulation pattern. Small neuronal networks cultured on microelectrode arrays (MEAs) offer great advantages in recording activity of in vitro neuronal networks but their applicability to cell stimulation is limited. The presence of large stimulus artifacts and the poorly controlled spread of electrical stimuli in medium are amongst the main disadvantages when studying the role of single cells in small networks. A possible solution to overcome these issues is to interact with individual neural cells by mimicking the chemical signaling itself. Recent developments of systems for precise neurotransmitter release include the use of micropipettes, microfluidic systems, organic electronics, and optical release of caged compounds. However, all these techniques require delicate setups and complex preparatory processes.

In this project we propose to combine MEA technology (Multi Channel Systems GmbH, Switzerland) with novel FluidFM technology. The FluidFM (Cytosurge AG, Switzerland) setup is based on a hollow atomic-force microscopy (AFM) cantilever acting as force-controlled nanopipette to release a molecule of interest. The MEA setup is used to read out the chemically induced local response. This system allows us to directly use any molecule of interest and deliver volumes in the nanoliter range with micrometer precision.We present the ability to locally release neurotransmitters on the cell membrane with precise control over applied force (sub-nN) and spatial position (μm). We used FluidFM cantilevers with 2 μm openings, where the microfluidic channel was filled with physiological solution containing 200 μM glutamate. In order to chemically induce a local electrical response in a culture of dissociated hippocampal neurons from E17 rat, we first brought the FluidFM cantilever in contact with the cell membrane. We then applied pressure-pulses between 50 mbar and 300 mbar with 300 ms duration to locally eject sub nanoliter volumes of the neurotransmitter. Induced activity of the stimulated neurons was shown by extracellular recordings with the MEA and a calcium sensitive dye (Oregon Green BAPTA, Life Technologies Ltd, Switzerland).

Using cantilevers with micrometer-sized openings, the whole neuron surface can be scanned, resulting in a sensitivity map for the applied molecule. These maps would provide comprehensive information about the position of the chemical receptor molecules, and can be an invaluable tool for studying synapse formation and development, plasticity, and testing the effect of different pharmaceutics.

The acquired knowledge in finding the effective way of chemical stimulation allows influencing the network development. Studying small neuron circuits designed to mimic the topology of different brain tissues can reveal the correlation between topology and the properties of network activity, as well as testify the models on the influence of stimulation patterns to the ongoing changes in the synapse.

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