

Project: Brain Endoscopy for the Functional Analysis of Neuronal Networks *in Vivo*

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Introduction

Mouse mutagenesis is one of the most powerful approaches for the analysis of gene-specific functions. However, a critical limitation in the investigation of brain function in, for example, mutant mouse disease models is the lack of efficient, high-resolution *in vivo* analyses of neuronal networks. Up to now such studies have involved, at best, extensive *in vitro* (slice) electrophysiological recordings from individual cells, necessarily isolated from the brains network. The extent to which such *in vitro* studies reflect biologically relevant functions remains in most cases unclear. We propose the development of *in vivo* brain endoscopy for the analysis of neuronal networks with single cell resolution. These approaches involve fluorometric Ca^{2+} recordings through optical fibers and two-photon microscopy.

Fluorometric Ca^{2+} imaging is a powerful and highly sensitive method for monitoring neuronal activity. It makes use of the fact that in living cells most depolarizing electrical signals are associated with Ca^{2+} influx owing to the activation of one or more of the numerous types of voltage-gated Ca^{2+} channels, abundantly expressed in the nervous system. Besides reporting changes in membrane potential, Ca^{2+} imaging directly detects changes in the intracellular calcium concentration. Such Ca^{2+} signals are essential for elementary forms of neuronal communication, including most prominently chemical synaptic transmission. In addition, Ca^{2+} signaling is obligatory for complex processes, like the induction of memory- and learning-related forms of neuronal plasticity. Furthermore, many aspects of development at the beginning of a neuron's life, including gene expression, neuronal migration and neurite outgrowth, require transient intracellular elevations in Ca^{2+} concentration, while, paradoxically, Ca^{2+} transients are also involved in neuronal cell death.

Alterations of the intracellular Ca^{2+} homeostasis have been shown to play a central role in the pathogenesis of several age-related neurodegenerative diseases, like Alzheimer's or Parkinson's disease. The majority of many earlier studies, however, were performed *in vitro* in cultured embryonic cells. It is, therefore, difficult to relate those results to specific patho-mechanisms of the disease, because at early developmental stages mutant animals often lack disease-related phenotypical changes. Thus, the analysis of normal brain function as well as that of the underlying pathophysiological mechanisms of neurodegenerative diseases requires *in vivo* methods for detection of neuronal calcium signaling in adult and aged brains.

To monitor changes in the intracellular calcium concentration, cells are usually stained with Ca^{2+} -sensitive fluorescent indicator dyes. Upon binding Ca^{2+} ions such dyes change the spectral characteristics of the emitted light. The emitted light is collected either by CCD- (charge-coupled device) or video-camera-based systems or by photomultipliers. *In vivo* Ca^{2+} measurements require photomultiplier-based two-photon imaging because it is the most sensitive approach for measuring fluorescence in scattering opaque tissues. So far, however, such measurements have been restricted to anaesthetized or fixed animals and have been outermost performed only in the upper cortical layers.

We propose the implementation of endoscope-like devices for Ca^{2+} measurements to gain access to any brain region of interest. That this is feasible has been indicated by recent work describing the use of a pair of implanted optical fibers, one for the excitation of a Ca^{2+} -sensitive dye, the other one for detection of the emitted fluorescence [1].

In a previous study we found wave-like cortical Ca^{2+} activity in brain slices and whole brain explants of newborn rats and mice, termed cortical early network oscillation (cENOs) [2]. They occur spontaneously and are caused by synchronous activation of a large number of neurons. *In vitro* such Ca^{2+} oscillations were shown to control neuronal maturation, synaptic wiring and regulation of neurite growth as well as regulation of gene expression in the developing brain. However, demonstration of their existence *in vivo*, in the intact brain subjected to a variety of sensory stimuli remained elusive. To conduct such *in vivo* experiments we have developed a technique for bulk loading of cells with membrane-permeable Ca^{2+} indicator dyes [3]. Two-photon Ca^{2+} imaging has shown that stained cells are viable and responding to sensory stimulation such as, for example whisker deflection. However, no spontaneous cENOs are observed in the cortex of neonatal mice suggesting that they might be blocked by the anesthetics.

To monitor Ca^{2+} levels in awake behaving mice we have developed a prototype of an optical fiber-based recording system consisting of a chronically implanted fiber with 200 μm diameter used both for excitation of the dye and for detection of the emitted fluorescence (Fig. 1). This "single-fiber endoscope" allows recording of Ca^{2+} signals from any brain region in anesthetized as well as awake, behaving animals. It offers therefore a unique opportunity for studying brain function and for detecting the action of drugs without the influence of anesthetics. In addition it is also applicable to cultured cells and can therefore be enhanced to an automated high throughput screening (HTS) platform for testing of drug candidates influencing Ca^{2+} channel function.

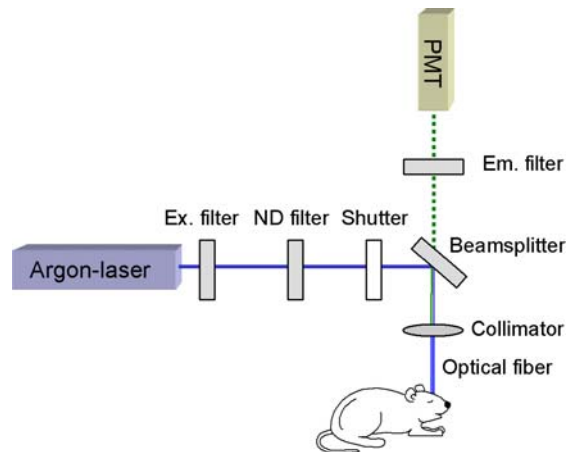


Fig 1: Scheme of the optical fiber based calcium imaging system.

For *in vivo* recordings with the "single-fiber endoscope" we had to modify our staining technique. Under deep anesthesia we opened the skull above the desired brain region with dental drills. We then injected a few microlitres of dye-containing solution [3] through a patch pipette. This procedure yields a roughly cylindrical stained area with the base diameter of approximately 400 μm . About 30 min after the dye-injection an optical fiber with a core diameter of 200 μm was lowered into the same canal where the injection filament has been placed. After fine adjustment of the fiber position it was fixed on the skull with dental cement. For excitation of the Ca^{2+} -sensitive dye the beam of an argon laser was attenuated to 0.25 mW with a neutral density filter

Results/Project Status

and focused into the external end of the optical fiber via a collimator. The emitted fluorescent light was collected with the same fiber and detected with a photomultiplier. With our detection device it was possible to register cENO in the cortex of neonatal mice after recovery from the anesthesia, thus, confirming their existence *in vivo* [4]. During the first two weeks of postnatal development the sensory systems start to function. With the “single-fiber endoscope” we have been able to detect sensory evoked calcium transients. For this purpose the fiber was implanted in the desired primary sensory cortex and stimulation was performed either with light flashes, sound pulses or whisker deflection. Application of short sound pulses (100 ms, 10 kHz, 70 dB) every 10 s resulted in calcium transients evoked with an average delay of 16.3 ms after onset of the stimulus (Fig. 2). The optic fiber was implanted in the primary auditory cortex of an adult mouse (aged P36).

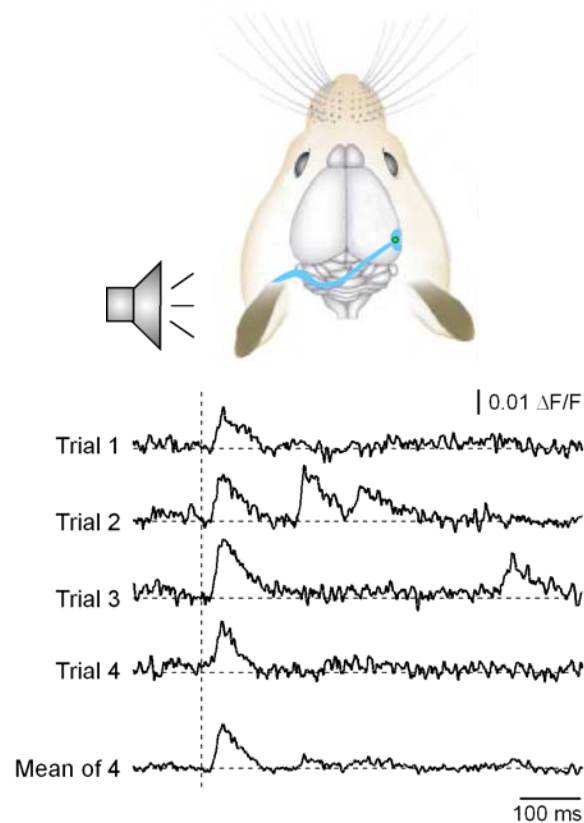


Fig 2: Sound-evoked calcium transients detected with the optical fiber in the primary auditory cortex of an adult mouse.

For detecting light-evoked transients the optic fiber was implanted in the right primary visual cortex. Stimulation was performed with a white LED. To test the specificity of the stimulation the light flashes (100 ms length, 10 s stimulus interval) were applied either through the ipsilateral or through the contralateral eye. Only in the latter configuration stimulus-evoked calcium transients were detected. These appeared with a mean delay of 68.6 ms after onset of the stimulus. The delays of the calcium transients detected after stimulation of the ipsilateral eye varied randomly, suggesting that in this case only spontaneous transients are present. Stimulus-evoked transients could also be detected with the optical fiber in the hippocampus and in secondary cortical regions, like the entorhinal and the frontal cortex. They appeared with delays longer than the ones in the respective primary sensory regions.

Open questions are whether spontaneous and stimulus-evoked transients are generated by the same group of neurons or by distinct populations and the temporal relation between primary and secondary sensory regions and deeper brain regions like the hippocampus and the thalamus. Furthermore the influence of the anaesthesia on the physiological brain signalling has to be investigated by performing recordings in awake animals.

Outlook

Our future work will focus on the development of the following approaches for the functional analysis of physiological and pathological brain function *in vivo*:

1. Establishment of a “double-fiber endoscope” combined with a video tracking system for the simultaneous analysis of calcium signaling in different brain regions and behavior in non-anesthetized mice.
2. Development of GRIN lens based micro-endoscopic devices for two-photon imaging and their implementation for *in vivo* applications. Such devices allow imaging with a high temporal and spatial (=cellular) resolution from any brain region of interest.
3. Alterations in brain calcium signaling will be tested in mutant mouse models of Alzheimer’s disease.

Lit.: 1. Duff Davis M., Schmidt J.J. (2000) *In vivo* spectrometric calcium flux recordings of intrinsic caudate-putamen cells and transplanted IMR-32 neuroblastoma cells using miniature fiber optodes in anesthetized and awake rats and monkeys. *J. Neurosci. Meth.* 99:9-23. 2. Garaschuk O., Linn J., Eilers J. & Konnerth A. (2000) Large-scale oscillatory calcium waves in the immature cortex. *Nat. Neurosci.*, 3, 452-459. 3. Stosiek C., Garaschuk O., Holthoff K & Konnerth A. (2003) *In vivo* two-photon calcium imaging of neuronal networks. *PNAS USA*, 100, 7319-7324. 4. Adelsberger H., Garaschuk O., Konnerth A. (2005) Cortical calcium waves in resting newborn mice. *Nature Neurosci.*, Jul 10; [Epub ahead of print].