Laser Photolysis of Caged Calcium (NP-EGTA)

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Description:

Hippocampal slices of P16 mice were stained with the cell-permeant calcium-sensitive dye Fluo-4 AM and additionally loaded with cell permeant NP-caged EGTA AM. This photolabile chelator releases Ca^{2+} upon UV illumination due to its K_d increase from 80 nM to >1 mM. Uncaging of calcium was achieved by the application of brief (500 ms) and focally restricted flashes of UV light (diameter ~1 µm) to astrocytic somata during two-photon-imaging of these cells. The uncaging unit consisted of a DL-355/10 UV-laser, operated by a UGA-40 galvanometric controlling system (Rapp OptoElectronic). This unit was coupled to a custom-built multiphoton laser scanning microscope (based on a Fluoview 300, Olympus; 60x/1.1, NIR Apo, Nikon, water immersion objective), connected to a tuneable Ti:Sa laser (MaiTai, SpectraPhysics).

Setup:

- Microscope: Olympus Fluoview 300
- Objective: Nikon 60x NIR Apo NA 1.1 water immersion

Rapp OptoElectronic:

- System: UGA-40 – point scanning device
- Light source: DL-355/10 diode laser

⇨ Approx. sample spot size: 1 µm

Combined techniques:

- Slice preparation of hippocampus (P16 mice)
- Multiphoton laser scanning microscopy
- Intracellular calcium uncaging
- Calcium imaging

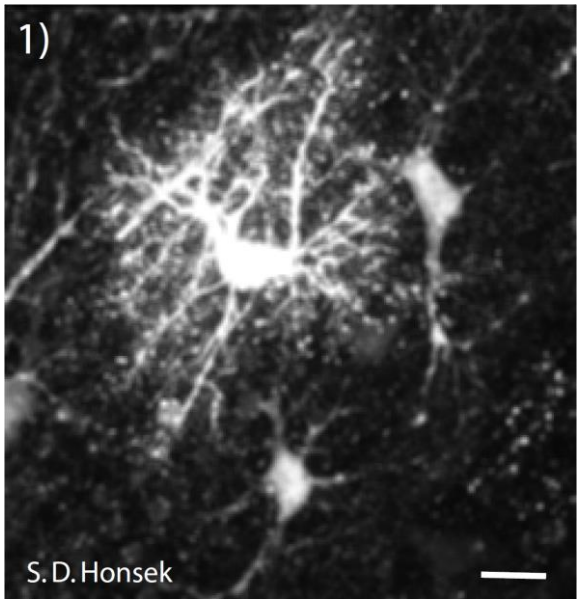

Figure 1: Multi-photon-image of astrocytes in the stratum radiatum of a mouse hippocampus. To specifically identify astrocytes in the hippocampal slice preparation, slices were loaded with the astocyte specific vital dye sulforhodamine 101 (SR 101). Because Fluo-4 fluorescence is very dim at low intracellular baseline calcium levels, a single astrocyte was additionally filled with AlexaFluor 594 to visualize its fine processes. (Scale bar = 60 µm)

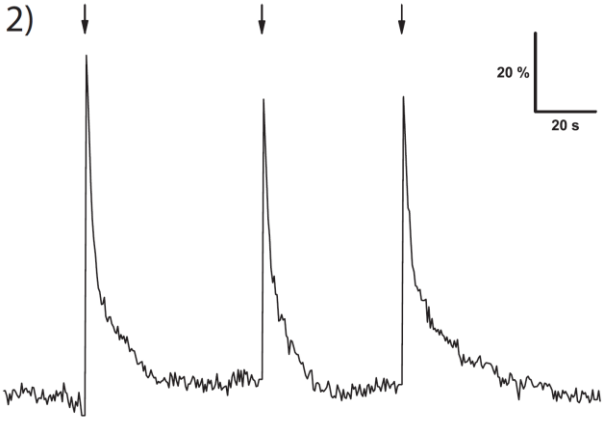

Figure 2: Calcium transients as indicated by changes in fluorescence (ΔF/F0). Uncaging UV flashes (500 ms) were aimed at the soma of the astrocyte.

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