Raw Data Description.

Experimental details and data analysis are explained in **Prada et al. (2018)** *An open source tool for automatic spatiotemporal assessment of calcium transients and local 'signal-close-to-noise' activity in calcium imaging data.* **PLOS Comp. Biol.**

Primary neurons (raw data-Fig 1 embryonic motoneurons; raw data Fig 3 – 10, hippocampal neurons) were prepared as described in earlier studies [1-3].

Calcium indicator dye loading and Ca^{2+} imaging was performed in artificial cerebral spinal fluid (ACSF) under continuous perfusion. The calcium indicator Oregon Green 488 BAPTA-1, AM was used in all experiments. Changes in OGB/calcium-fluorescence were monitored with the help of an upright microscope and a 40x 0.8NA objective. Images (8-bit) were captured at 2.5 Hz (motoneuron) or 10 – 20 Hz (hippocampal neurons) with a streaming camera under continuous illumination with a 470nm LED light source. The raw data (tif-format) was converted to an avi-format without compression.

- Fig 1. Motoneuron. Motoneurons shift spontaneously in their activity states. One movie shows the low activity state, the second movie shows the switch to the high activity state.
- Fig 3. Hippocampal neurons. Hippocampal neurons were cultured for 24 days in vitro. At this age, neurons develop glutamatergic synapses with mature hallmarks [2] and become spontaneously active. Cells were loaded with OGB1-AM and one neuron was investigated with patch clamp recording. Calcium imaging at 20 Hz.
- Fig 4. Hippocampal neurons. Imaging was performed under low-light conditions. Under control conditions, spontaneous spiking is observed. Spiking behavior was acutely blocked.
- Fig 5. Hippocampal neurons. Control; neurons show a certain number of local activity events in the periphery. cLTP; neurons are stimulated with a chemical LTP solution.
- Fig 6. Noise videos. A homogenous fluorescence signal was imaged. This allows to evaluate the camera noise.
- Fig 7. Hippocampal neurons. Control; spontaneously fast spiking neuron. Spike blockade; spiking behavior was acutely blocked.
- Fig 8. Hippocampal neurons. Control; spontaneously spiking neuron. Spike blockade; spiking was acutely blocked, however, growth cone like structures and activity hot spots show calcium activity under spike block conditions.
- Fig. 10. Signals close to the noise level. Hippocampal neuron. Acute removal of extracellular calcium and acute re-addition of calcium reveals homeostatic resting calcium fluxes (see also [1].

References

1. Samtleben S, Wachter B, Blum R (2015) Store-operated calcium entry compensates fast ER calcium loss in resting hippocampal neurons. Cell Calcium 58: 147-159.
2. Andreska T, Aufmkolk S, Sauer M, Blum R (2014) High abundance of BDNF within glutamatergic presynapses of cultured hippocampal neurons. Front Cell Neurosci 8: 107.
3. Subramanian N, Wetzel A, Dombert B, Yadav P, Havlicek S, et al. (2012) Role of Na(v)1.9 in activity-dependent axon growth in motoneurons. Hum Mol Genet 21: 3655-3667.