10. MULTI-PHOTON MICROSCOPY

BALÁZS RÓZSA, Professor; BALÁZS CHIOVINI, Head of the laboratory; DÉNES PÁLFI PhD, GÁBOR JUHÁSZ PhD, MIKLÓS MADARÁSZ labmembers; GERGELY KATONA PhD, Supervisor

IN VITRO 2D TWO-PHOTON MEASUREMENT OF NETWORK ACTIVITY (PPKE)

Sharp wave-ripples (SPW-R) activity is involved in the process of memory consolidation. We investigate spontaneous single cell neuronal activities during SPW-R in the hippocampus CA3 region under *in vitro* conditions. Fast spiking (FS), PV+ basket cells (BCs) as the clockworks for neuronal oscillations are important elements of hippocampal neuronal networks. Thus, we focus on PV+ interneurons to reveal the dendritic calcium dynamics during SPW-R. Recently we have extended our interest of these measurements with the principal, pyramidal neurons to the better understand of the spontaneous neuronal network activity.

To achieve this, we combine two-photon microscopy, local field electrophysiology, single cell electrophysiology, and dendritic patch clamp recordings. To measure the pharmacological background of calcium dynamics in single cell dendrites, we use focal synaptic stimulation and two-photon uncaging of novel, own developed glutamate and GABA caged compounds.

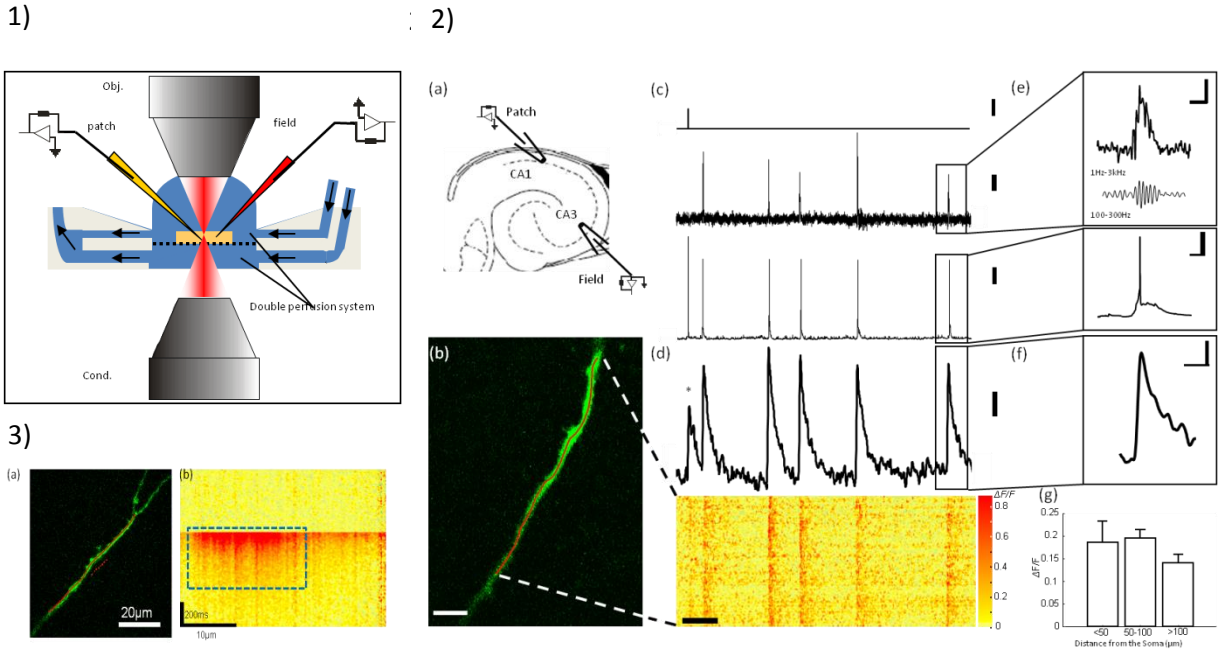

Fig. 1 ***1)*** *Experimental arrangement to generate spontaneous network activity in in vitro conditions.* ***2)*** *Simultaneous two-photon calcium imaging, whole cell patch clamp recording and local field potential recording in in vitro measurement.* ***3)*** *Two-photon uncaging and calcium imaging. (Chiovini B et al. 2010 Neurochem Res.)*

IN VIVO 3D TWO-PHOTON MEASUREMENT OF NETWORK ACTIVITY

To understand the fast computational mechanisms of the brain, one needs to be able to perform rapid measurements at several sites along a single neuron as well as to image large populations of neurons. Traditional two-dimensional measurements are severely limited for such kinds of endeavors since neurons are located in three dimensions. To overcome this problem, we have developed new solutions to perform three-dimensional functional imaging with large scanning ranges along the z direction. With our three-dimensional microscopes we are able to maintain random access point scanning with a short pixel dwell time. The speed and scanning volume of our technique in combination with the ~ 800 µm penetration dept of two-photon technology makes our methodology very convenient for *in vivo* measurements of neuronal populations, too.

Populational activity has long been studied in the visual cortex. We conduct *in vivo* two-photon imaging of the V1 area after using multicell bolus loading of a calcium indicator dye. Neuronal network responses are followed after visual stimulation using a moving bar or moving grating protocol. In addition, active cells are selected based on the previously recorded somatic activity and their dendritic responses are followed along with the network activity in three dimensions by using whole-cell patch clamp techniques.

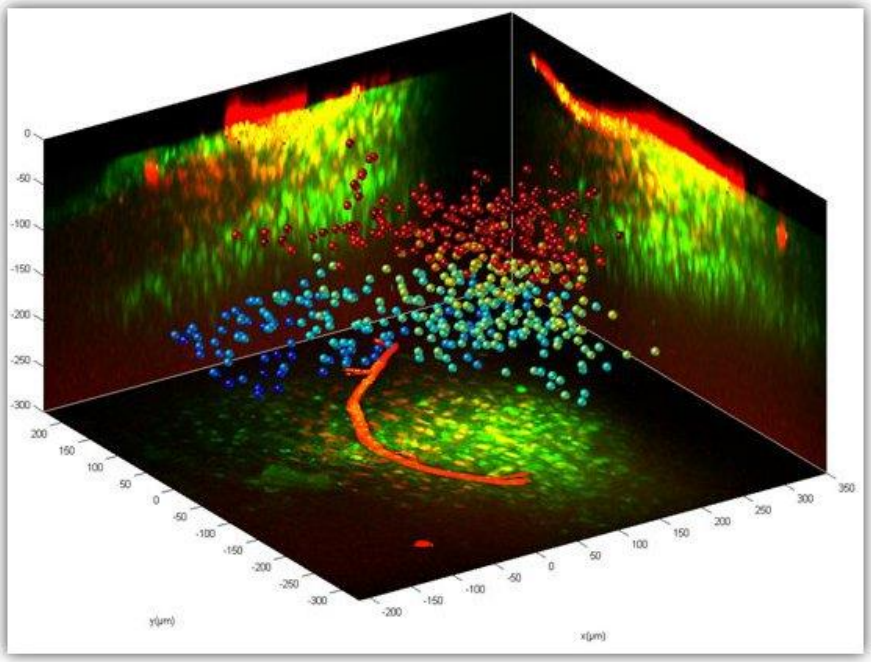

Fig. 2 *Projection of the 3D volume with multicell bolus loading of a calcium indicator dye. Points represents 251 simultaneously measured cells.*

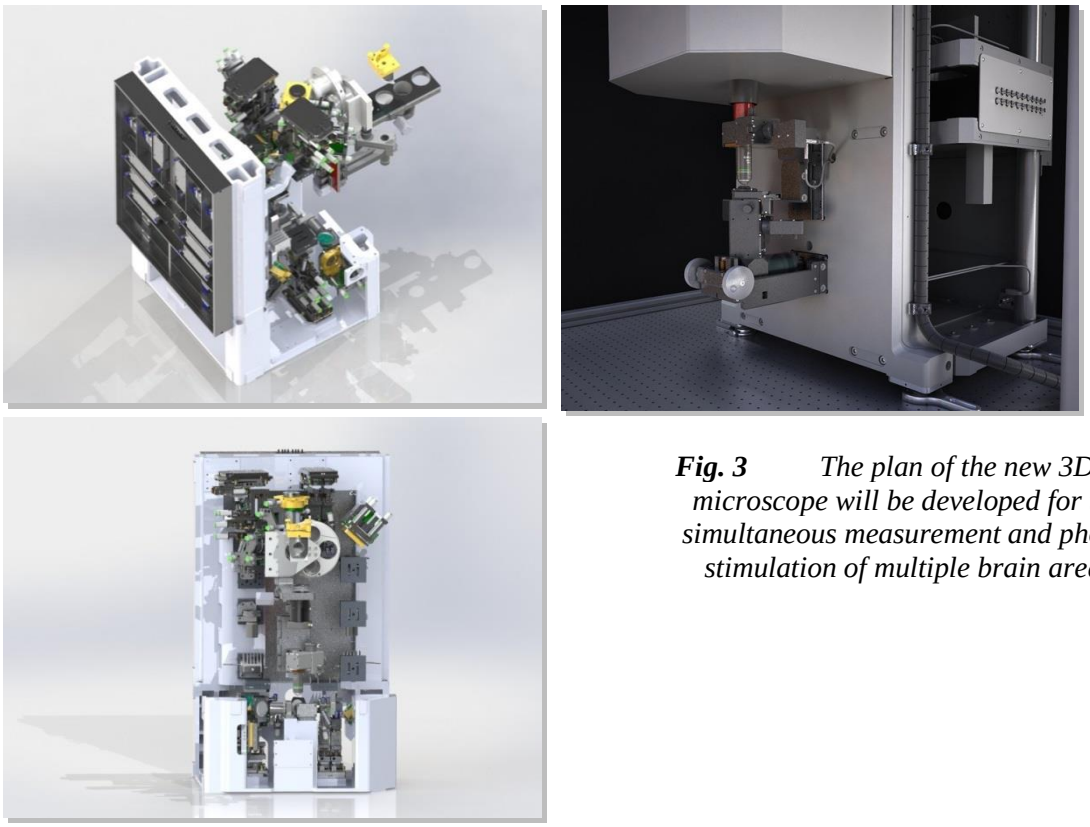

Fig. 3 *The plan of the new 3D microscope will be developed for the simultaneous measurement and photo-stimulation of multiple brain areas*

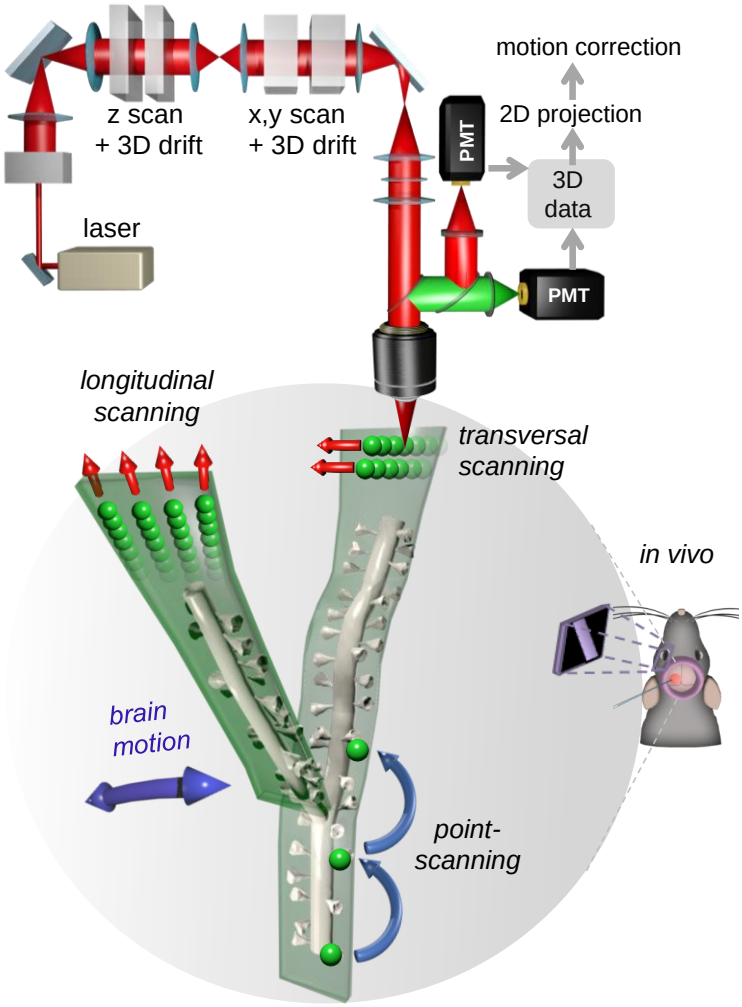

Fig. 4 *New microscope technologies were developed for the 3D measurements of the moving brain of behaving animals.*

PUBLICATIONS

[1] Katona G, Szalay G, Maak P, Kaszas A, Veress M, Hillier D, Chiovini B, Vizi ES, Roska B, **Rozsa B** (**2012**) Fast two-photon in vivo imaging with three-dimensional random-access scanning in large tissue volumes, .***Nature Methods*** *IF= 25.95*

[2] Balázs Chiovini, Gergely F. Turi, Gergely Katona, Attila Kaszás, Dénes Pálfi, Pál Maák, Gergely Szalay, Mátyás Forián Szabó, Gábor Szabó, Zoltán Szadai, Szabolcs Káli, **Balázs Rózsa** (**2014**) Dendritic spikes induce ripples in parvalbumin interneurons during hippocampal sharp waves, ***Neuron*** *IF=16.48*

[3] Adrian Wertz, Stuart Trenholm, Keisuke Yonehara, Daniel Hillier, Zoltan Raics, Marcus Leinweber, Gergely Szalay, Alexander Ghanem, Georg Keller, **Balázs Rózsa**, Karl-Klaus Conzelmann, and Botond Roska (**2015**) Single-cell–initiated monosynaptic tracing reveals layer-specific cortical network module, ***Science*** *IF =33.61*

[4] Jan Tønnesen, Gergely Katona, **Balázs Rózsa**, & Valentin Nägerl (**2014**) Nanoscale spine neck plasticity regulates compartmentalization of synapses , ***Nature Neuroscience*** *IF=15.25*

[5] Noemi Holderith, Andrea Lorincz, Gergely Katona, **Balázs Rózsa,** Akos Kulik, Masahiko Watanabe, Zoltan Nusser (**2012**) Release probability of hippocampal glutamatergic terminals scales with the size of the active zone, ***Nature Neuroscience*** *IF=15.25*

[6] Wolfgang G. Bywalez, **Balázs Rózsa**, et al. & Veronica Egger (**2015**) Electrical compartmentalization in olfactory bulb granule cell spines/Local postsynaptic sodium channel activation in olfactory bulb granule cell spines , ***Neuron*** *IF=16.48*

[7] Marko A. Popovic, Nicholas Carnevale, **Balazs Rozsa** & Dejan Zecevic (**2015**), ***Nature Com.*** *IF=11.47*

[8] G. Katona, A. Kaszás, GF. Turi, N. Hájos, G. Tamás, E. S. Vizi, **B. Rózsa** (**2011**) Roller Coaster Scanning Reveals Spontaneous Triggering of Dendritic Spikes in CA1 Interneurons , ***PNAS*** *IF=9.7*

[9] Williamson A, Ferro M, Leleux P, Ismailova E, Kaszas A, Doublet T, Quilichini P, Rivnay J, **Rózsa B,** Katona G, Bernard C, Malliaras GG. (***2015***) Localized Neuron Stimulation with Organic Electrochemical Transistors on Delaminating Depth Probes, ***Advanced Materials*** *IF=15.4*

[10] Bouhadfane M, Kaszás A, **Rózsa B**, Harris-Warrick RM, Vinay L, Brocard F. (**2015**) Sensitization of neonatal rat lumbar motoneuron by the inflammatory pain mediator bradykinin., ***Elife*** *IF=9.322*

[11] Lőrincz* A, **Rózsa B***, Katona G, Vizi ES, Tamás G (**2007**) Differential distribution of NCX1 contributes to spine-dendrite compartmentalization in CA1 pyramidal cells **= contributed equally* , ***PNAS*** *IF=9.7*

[12] Chiovini B, Turi GF, Katona G, Kaszás A, Erdélyi F, Szabó G, Monyer H, Csákányi A, Vizi ES, **Rózsa B.** (**2010**) Enhanced Dendritic Action Potential Backpropagation in Parvalbumin-positive Basket Cells During Sharp Wave Activity. , ***Neurochem Res.*** 35(12):2086-95

[13] **Rózsa B**, Katona G, Kaszás A, Szipöcs R, Vizi ES. (**2008**) Dendritic nicotinic receptors modulate backpropagating action potentials and long-term plasticity of interneurons, ***Eur J Neurosci.*** *2008 Jan;27(2):364-77*