Combining two-photon microscopy and electrophysiology leads to exciting scientific breakthrough

Electrophysiological techniques have been used for studying cells and tissues for over 70 years; with Hodgkin and Huxley reporting their achievement of recording an action potential within a nerve fibre for the first time in 1939. In contrast, the first two-photon microscope was not created until 1990 by Winfried Denk, and there was no commercially available system until 1996.

Since they were first described, both research methods have been developed to overcome many technical problems and there are now many scientists using the techniques around the world.

Electrophysiologists can now create several recording sites on one dendrite to measure different levels of excitability in multiple locations. Two-photon (or multiphoton) microscopy is now used to get clear images of cells, at over 1 mm depth, *in vivo* without harming the tissues.

When used together electrophysiology and two-photon imaging can be extremely powerful, particularly when investigating phenomena in the nervous system. Described below are two examples of researchers making breakthroughs in neuroscience by integrating both of these techniques in their work.

Dr Long-Jun Wu – Microglia-neuron communication in the brain

The main aim of Dr Wu's lab at Rutgers University is to understand microglia-neuron communication in the healthy and diseased brain. To achieve this they use data acquisition equipment (amplifiers, digitiser and software) and a multiphoton imaging system. With this set-up they image microglia and neurons in brain slices and *in vivo* whilst simultaneously recording or stimulating electrical activity.

In a study recently published in *The Journal of Neuroscience*, Dr Wu and colleagues looked at changes in microglia morphology after neuronal hyperactivity (e.g. after an epileptic seizure) and the underlying mechanisms of the response.

Using two-photon imaging they found that induced seizures led to a significant increase in microglial process extension towards neurons. They then elucidated the molecular process that leads to the increase in microglial process extension. They found that glutamate released during increased neural activity opens calcium channels on post-synaptic membranes through the NMDA receptor. This intracellular calcium influx causes a release of ATP from the post-synaptic neuron which is detected by P2Y12 receptors on the microglial processes, promoting growth towards increasing concentrations of ATP.

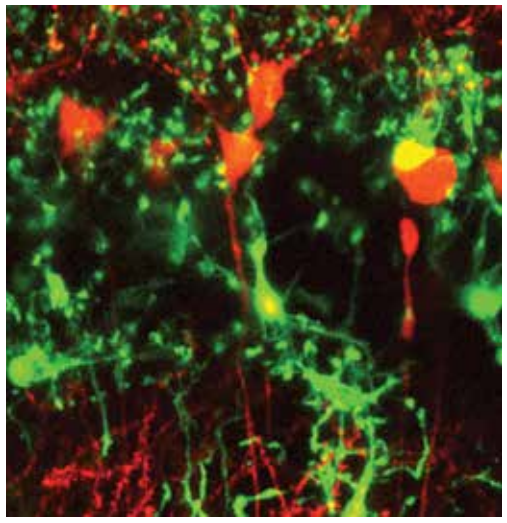


***Figure 1**: Two-photon image of a hippocampal slice from a double transgenic mouse expressing GFP in brain microglia (green) and YFP in pyramidal neurons (red). The image is a projection of several z-stack images through a 45µm thickness of the brain slice taken after five minutes of glutamate treatment. This glutamate treatment activates neurons that indirectly attract microglial processes through ATP release to make bulbous contacts with neurons. During conditions of extensive neuronal activity such as seizures, this contact is presumed to serve neuroprotective functions.*

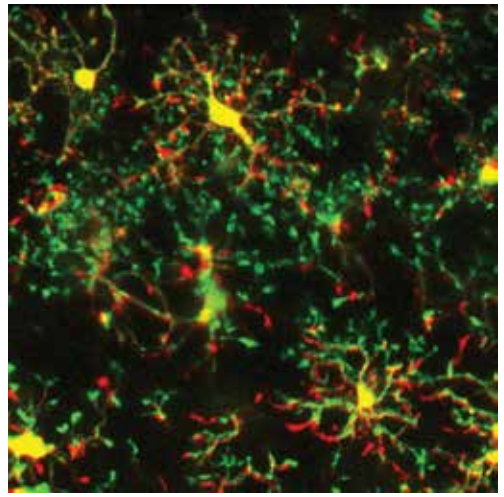


***Figure 2:** A color-coded merge of two-photon images of hippocampal microglia in a mouse brain slice before (red) and 15 minutes after glutamate treatment (green). The image is a projection of several z-stack images through a 45µm thickness of the brain slice and shows that in response to neuronal activation by glutamate, microglia extend their processes (green) elaborately. Such extending processes contact neuronal elements and presumably serve neuroprotective functions that are especially relevant during epileptic contexts.*

By looking at P2Y12 knockout mice they found that the P2Y12 receptor is necessary for process extension during neuronal hyperactivity, as shown by a lack of morphological change in these mice. Furthermore, P2Y12 knockout mice showed worse levels of seizures when induced through delivery of Kainic Acid (KA). This suggests that microglia have a neuro-protective function during periods of hyperactivity in epilepsy.

Dr Wu is now interested in studying the effects of direct stimulation of neurons followed by imaging microglial motility and recording the electrical properties of the cells. This study will allow the researchers to directly test the idea of the potential functional of microglial interaction with neurons.

Dr Wu said: "Our research will open a new avenue for studying microglia-neuron communication in the brain and allow study of the potential therapeutic targets in microglia for seizure pathologies.

"I have always found using Molecular Devices data acquisition hardware and software very user friendly and it combines easily with Scientifica's equipment to achieve the cutting-edge techniques required for my research."

Reference:

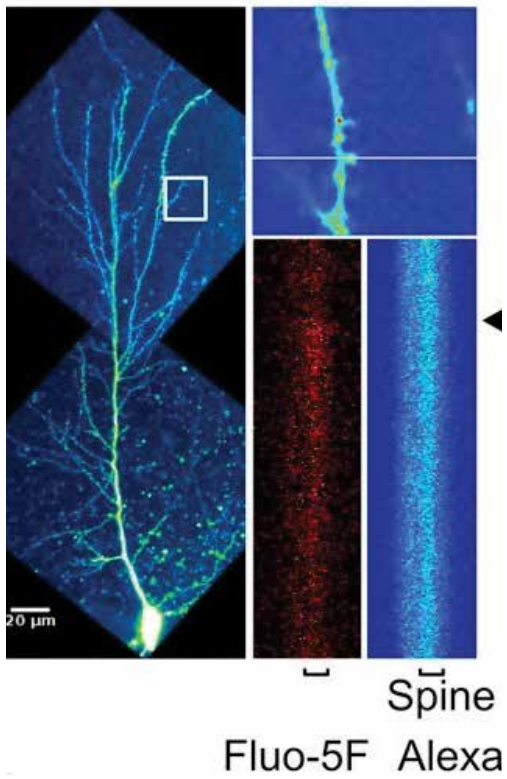
Eyo UB, Peng J, Swiatkowski P, Mukherjee A, Bispo A, Wu LJ (2014). Neuronal Hyperactivity Recruits Microglial Processes via Neuronal NMDA Receptors and Microglial P2Y12 Receptors after Status Epilepticus. *Journal of Neuroscience* 34(32), 10528-40.

Dr Jack Mellor & Dr Cezar Tigaret – Synaptic physiology and cognitive functions

Jack Mellor and Cezar Tigaret, at the University of Bristol, use a two-photon microscope and electrophysiology equipment to measure calcium transients in dendritic spines during the induction of synaptic plasticity.

They are interested in the mechanisms of synaptic plasticity in the hippocampus, a cellular process that underlies associative learning. Excitatory synapses in the hippocampus persistently change their response to stimuli when action potential discharges from the post-synaptic cell are closely time-correlated with synaptic input. Synaptic stimuli and back-propagated action potentials trigger minute transient changes in cytosolic Ca^{2+} concentration at dendritic spines, specialized protrusions on neuronal dendrites where these synapses occur.

Integrating electrophysiology with two-photon fluorescence imaging of spine Ca^{2+} transients in hippocampal slices provides the unique possibility to test whether these Ca^{2+} signals encode the



***Jack Mellor - Figure 3**: Spine EPSCaT recordings from a CA1 pyramidal neuron in a hippocampal slice. (Left) Montage of maximum intensity projections of two TPLSM z-stacks of a CA1 pyramidal neuron in a hippocampal slice loaded with the fluorescent Ca2þ dye Fluo-5F and Alexa Fluor 594 (Alexa). (open rectangle, zoomed in top right) Location of the scanning line across a spine head. (Bottom right) Primary data consisting of line-scan XT images with Fluo-5F and Alexa channels (0.08 mm/pixel 1200 lines/s, 1 s, temporal axis vertical) synchronized with a single stimulus (arrowhead) delivered via the extracellular electrode. The spine region of interest is labelled beneath the Alexa line-scan image.*

magnitude and direction of synaptic plasticity. It also refines the spatial dimension of the research such that synapses can be studied in the context of their location. By correlating the synaptic plasticity with the size of the Ca^{2+} signals elicited by plasticity-inducing stimulations they have uncovered mechanisms which explain the requirement for post-synaptic action potentials in the induction of plasticity in the mature hippocampus.

Dr Mellor said: "The Scientifica two-photon system has a stable optical build and reliable components in a small footprint which requires very little maintenance. Of particular advantage to us is the use of a Matlab framework, which allowed us to adapt the acquisition software to our needs and incorporate custom written scripts."

Reference:

Tigaret, CM, Tsaneva-Atanasova, K, Collingridge, GL & Mellor, JR (2013). Wavelet Transform-Based De-Noising for Two-Photon Imaging of Synaptic Ca^{2+} Transients. *Biophysical Journal* 104, 1006-17.

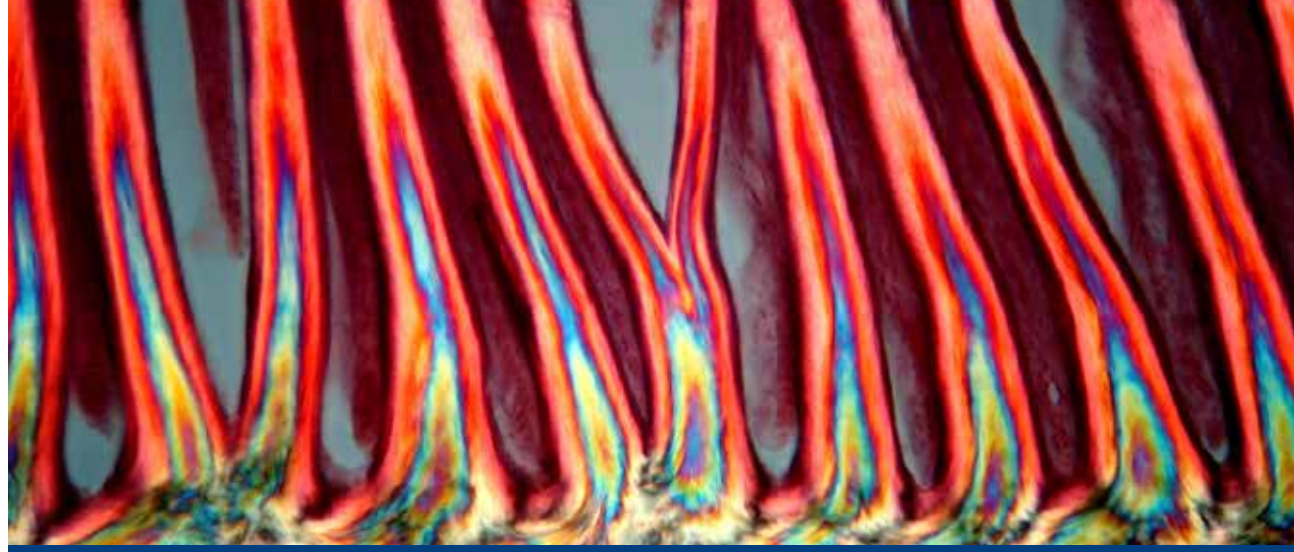
Conclusion

By using two-photon imaging and electrophysiology together, researchers around the world are learning new insights about many different properties of neuronal networks. They are now able to visualise neurons at high-resolutions deep inside living tissues and simultaneously gain knowledge of their electrical nature.

The equipment and technology used in both of these labs were sourced from Scientifica http://www.scientifica.uk.com/products/two-photon-imaging and Molecular Devices http://www.moleculardevices.com/systems

About the Author

George Ward is a biology graduate from the University of Manchester and a fully trained journalist. He currently works for Scientifica Ltd as an in-house copywriter and marketing executive where he focuses on articles related to electrophysiology, two-photon microscopy and optogenetics. More articles from this author can be seen at www.scientifica.uk.com



Hoof of Sheep

Photographed using polarized light. An 1867 Prize Medal slide by A.C. Cole, from the collection of the Northamptonshire Natural History Society. Wild M20 microscope with a Wild x10 Fluotar objective and x10 Olympus wide field eyepiece.

Mike Gibson *Northamptonshire Natural History Society*

2016 RMS Calendar: Call for Images

The Royal Microscopical Society produce a calendar every year which is distributed to our members as well as at RMS events and courses. We are proud to include a range of images across life and physical sciences to brighten up desks, offices and homes throughout the year! We are now looking to find our set of images for the 2016 calendar, so, if you would like one of your images to feature in next year's calendar, please email a low resolution version along with a title, description and your contact details to **Mel Reedman (mel@rms.org.uk)**

Landscape images work best for the calendar, and if your image is selected then we will require a high resolution version.

The closing date for entries is Friday 16th October. Successful submitters will be informed by the end of October. If you have any queries or would like more information then please contact Mel on the email address above.

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