Monitoring brain function by *in vivo* 2-photon microscopy

Gian Michele Ratto

gimmi@in.cnr.it

Collaborators
R. Brambilla
L. Cancedda
G. Carmignoto
M. de Curtis
C. Lodovichi
G. Mandolesi
I. Skaliora

The mission of the in vivo microscopy lab is to provide NEST with the ability of studying in anaesthetized animal models the function and structural plasticity of the brain and the physiological response to exogenously delivered agents. These techniques offer the capacity of studying at subcellular resolution and at high time resolution (1 Hz or better) the interactions between brain cells and nanofabricated devices.

The instrument at the core of this activity is the 2-photon microscope. This is a scanning microscope where the light source is provided by an ultrafast laser capable of delivering short light pulses (about 150 fs). Thanks to the high peak power, the near IR radiation provided by the pulsed laser is capable of exciting fluorofores that are normally excited at visible wavelength. The 2-photon microscope has several crucial advantages over the one-photon microscope: these features are instrumental in allowing imaging in thick samples:
1) 2-photon excitation is a non-linear phenomena that occurs only at the focal plane, therefore, there is no need for a spatial filter to remove out of focus excitation with a consequent increase of detection efficiency.
2) Infrared excitation and its localisation at the focal plane decrease phototoxicity.
3) Infrared excitation is far less scattered by the tissue increasing the power that can be delivered at the focal plane, and the image contrast.
The implementation of this technique require the simultaneous solution of a wide variety of problems, ranging from the proper alignment and tuning of the microscope, to the design of custom systems for the maintenance of the animal under the microscope, to the application

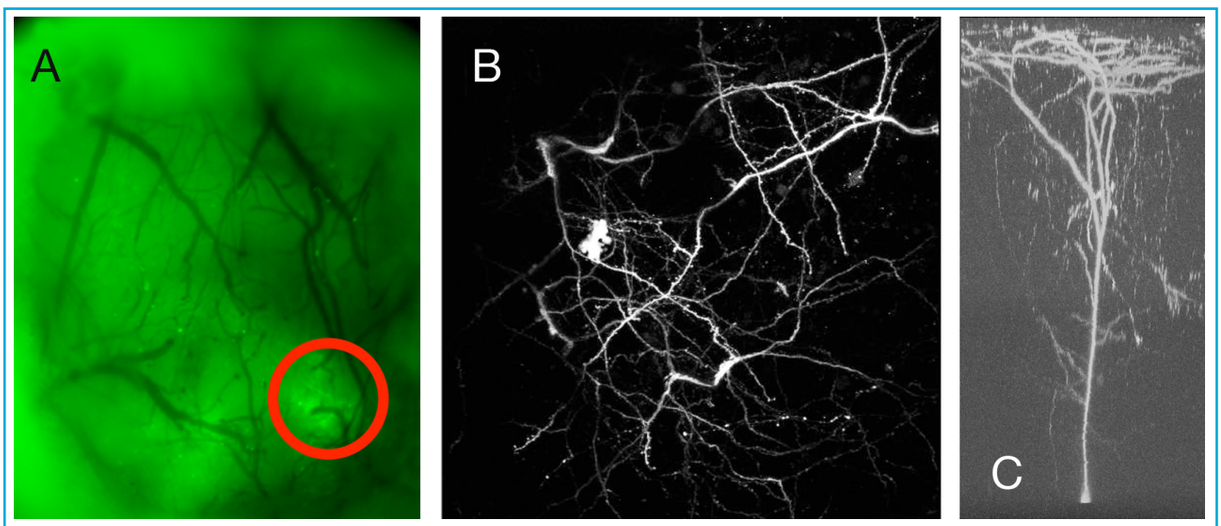

Fig. 1
A) Low magnification of the cortical surface. The pattern formed by the blood vessel is used to record the position of the imaged fields. This allow to record day after day changes occurring at specific cortical structures. The area marked by the red circle is shown at 2-photon excitation in B. C) transversal reconstruction of a brain neuron. The cell body is placed in cortical layer V at a depth of over 600 micron from the surface.

of proper procedures for the surgery and anaesthesia. Currently, we have devised protocols and the appropriate hardware components for in vivo imaging on visual and somatosensory cortices and on the olfactory bulb.

In figure 1 are shown examples of *in vivo* imaging of brain neurons in transgenic mice expressing the Green Fluorescent Protein GFP in a subset of cortical pyramidal cells. The optical access is obtained by implanting a recording chamber over the mouse skull. At the center of the opening a small craniotomy is cut in sterile conditions, the brain is exposed and then it is covered with a layer of transparent gel topped by a glass coverslip. In this way the recording chamber is sealed and can be kept on the mouse for days or even weeks. This procedure allows the longitudinal study of single identified features. We produced alternative designs of the imaging chambers to allow the microinjection of pharmacological agents during the chronic imaging sessions.

Functional imaging is performed after the delivery to the cells, either by genetically encoded products or by microinjection, of fluorescent probes. In the example shown in figure 2 two fluorescent dyes are microinjected. The red fluorophore (Sulphorodhammine 101) selectively stain and identify a population of brain cells, the astrocytes. The green dye is the calcium indicator Oregon Green BAPTA 1, and provides a florescent signal proportional to the calcium load of each cell. The calcium indicator becomes fluorescent only after loading into healthy cells, and therefore the injecting microelectrode appears only red.

Imaging of the brain with Ca indicators is an especially powerful technique because electrical activity of neurons brings about an increase in Ca load. In

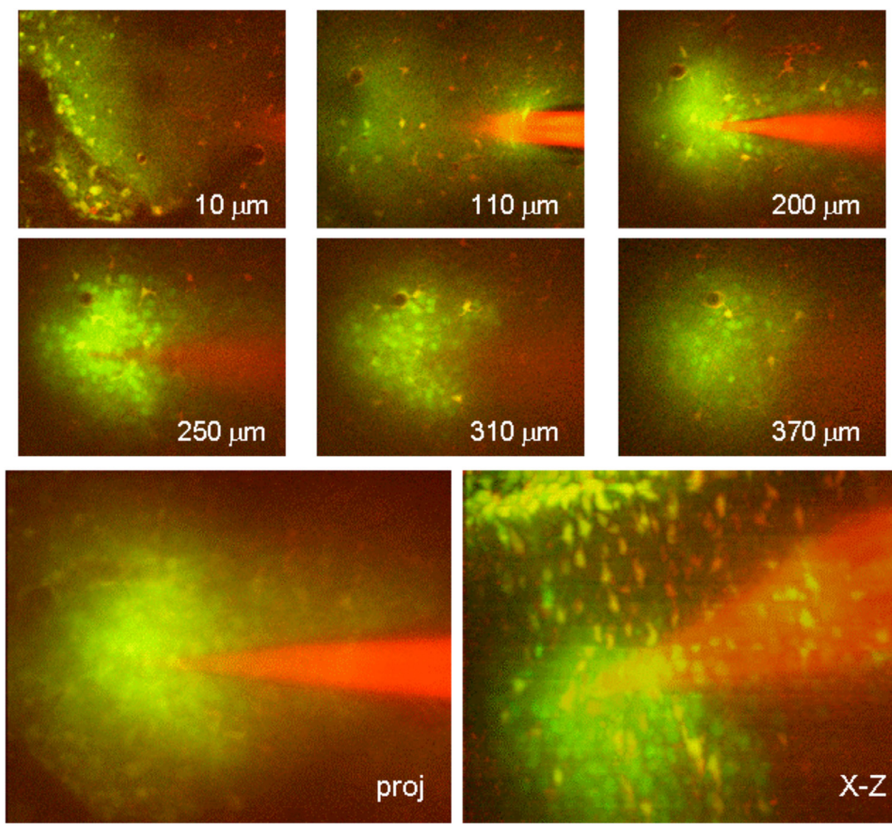

Fig. 2
Microinjection of fluorescent reporters in the somatosensory cortex *in vivo*. The upper images show the fluorescence at various depth. The maximum projection and the X-Z reconstruction show that the injected bolus fill a volume about 250 µm in diameter. Several stained astrocytes can be seen outside of this volume because the dyes diffuses through the gap junctions existing between these cells.

astrocyte, increase of Ca is associated to their activation with consequent release of neuro-active substances. Therefore, these experiments can return information shedding lights on processes as varied as cytotoxicity, neuronal computation, astrocyte physiology. Furthermore, we can combine the imaging with electrophysiological recordings obtained by a sharp electrode placed in the imagine volume. An example of such recording is provided in figure 3.

These techniques are crucial to study basic cellular mechanisms operating in pathological conditions in mouse model of neurological diseases. Presently we are employing the combination of Ca imaging and electrophysiology to analyze the events at the basis of neuronal synchronisation during epileptic seizures. In this study, presently under review, we have demonstrated the existance of an excitatory loop between neurons and astrocytes that is instrumental for the onset of epileptic seizure. Furthermore, we have demonstrated that the pharmacological inhibition of this loop reduces the probability of enduring an epileptic seizure.

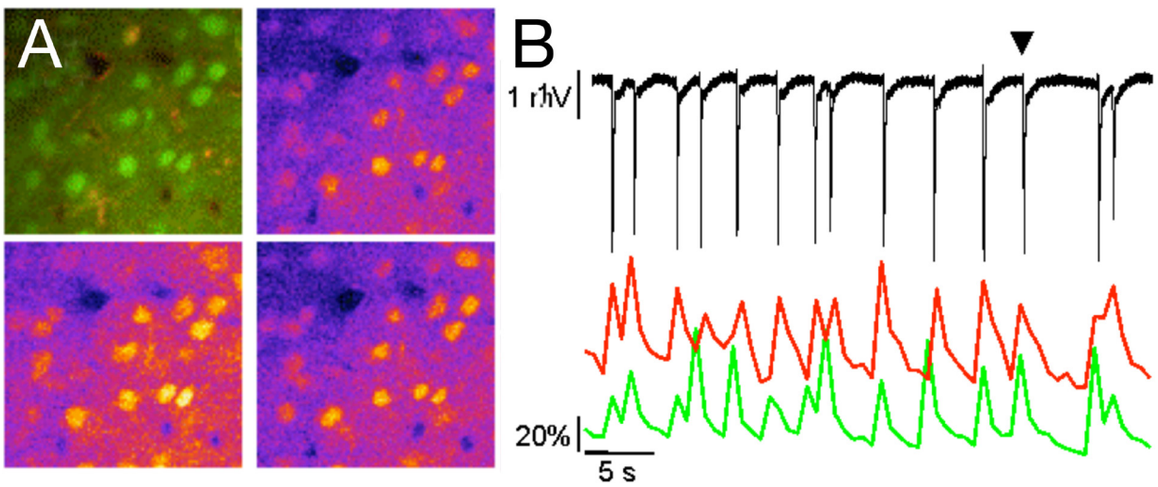

Fig. 3
A) Astrocytes appear red or orange, while neurons are green. The three frames show the changes of the Ca signal before during and after the event indicated by the arrowhead in B. B) The upper trace shows the electrical field recording dysplaying repetitive electrical activity characteristics of the phases preceding epileptic seizure. The two lower traces show the Ca imaging signal from two cells of the field. Although there is good correlation between Ca imaging and the electrophysiological recording, it can bee seen that the cells not always respond during the electrical event.