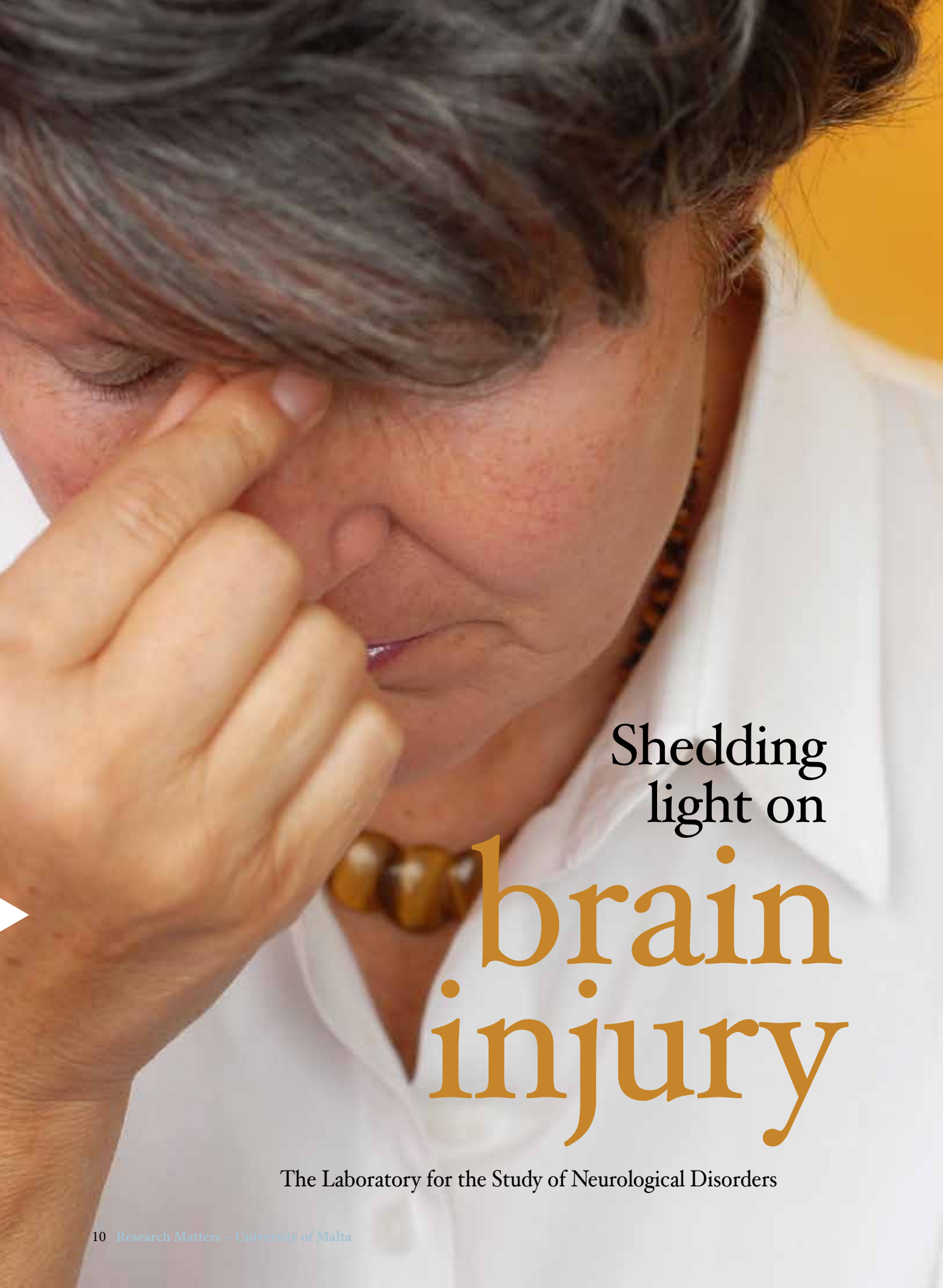


Shedding light on brain injury

The Laboratory for the Study of Neurological Disorders

Each year millions of people worldwide suffer from brain injury. Strokes, heart attacks, physical force and low oxygen levels can all lead to permanent damage. These injuries can lead to emotional, mood and behavioural problems. Speech can become slurred, whilst in the worst cases memory lapses occur, muscle weakness can kick in and it can even lead to death.

The worst thing is that there is little that can be done. Dead neurons cannot be replaced, but this old dogma is falling apart. Neurons can be regenerated, due to a type of cell that can develop into a neuron and replenish our brains. In part, these discoveries are due to multiphoton microscopy that can peer into neurons and discover what makes them tick and what makes that tick stop: the death of neurons.

It is now possible to capture cellular and molecular events deep down in the brain to study how cells communicate, develop, and how injury and reconstruction occurs. Knowledge of the intricate dynamics and cross-talk between neurons, glia and the vasculature is unraveling what causes these cells to die and how they could be made to recover. These and other discoveries will progress into clinical practice, and lead to a revolution in neurology and neurosurgery.

Along with the measurement of the typical ionic and electrical signature of the cells in nervous system, scientists combine state of the art imaging directly into the live rodent brain to observe and quantify changes that occur during injury and recovery and propose the use of new drugs to help in the restoration of brain function and recovery.

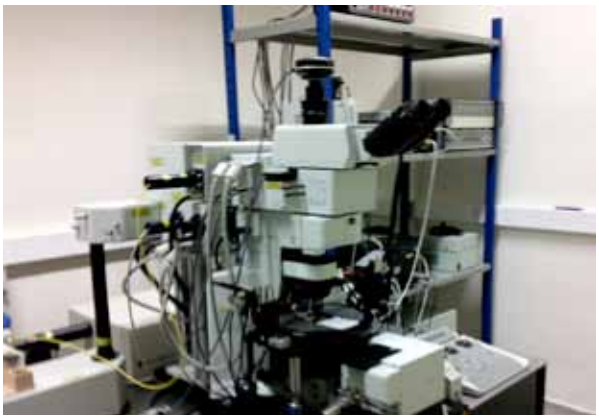
The Laboratory for the Study of Neurological Disorders of the University of Malta was established by Prof. Richard Muscat and Dr Mario Valentino in 2005. Research in this laboratory is directed to understanding cellular mechanisms of damage and the pharmacological recovery in brain injury. Since its establishment, the laboratory has expanded its research and moved its focus to include the broad study of stroke development, using a combination of multiparametric and non-invasive approaches to follow brain injury over time. Experimental methods used in the lab include brain slice, and in vivo stroke models in transgenic mice.

During the past decade, the rapid improvements in the tools available for labelling proteins within cells, has increased the lab's ability to unravel the finer details of cellular events. One significant reason is the development of fluorescent proteins that can be incorporated into proteins by genetic fusion to produce a fluorescent label.

Microscopic techniques include confocal microscopy of thin fixed brain slices, deep tissue imaging of live slices and whole animal preparations under multiphoton microscopy. Other research avenues include spreading depression during stroke and in vivo microdialysis.

Research activity

Normal brain activity critically depends on a continuous supply of oxygen and glucose through cerebral blood flow (CBF). Although cerebral energetic demands are very high, the brain can



The FV1000-MPE Olympus multiphoton system

only store a little energy. Because of this unique characteristic, local brain activity has to be matched by a concomitant increase in local CBF (a phenomenon referred to as functional hyperemia or neurovascular coupling). Functional hyperemia is involved in the pathophysiology of many acute neurological and neurodegenerative diseases, such as stroke, hypertensive encephalopathy, Alzheimer's disease, and vascular dementia. Moreover, functional hyperemia forms the basis of many modern non-invasive functional neuroimaging techniques that use this phenomenon to map brain activity in animals and humans.

Despite the importance of functional hyperemia for clinical neurology and neuroscience, the underlying mechanisms have remained largely undefined. Over the last years, many different transmitters and pathways have been implicated in functional hyperemia, but the relevance of these mechanism *in vivo*, as well as the exact sequence of events and the different cell types involved, remain to be determined.

In such cases, the goal of the research team is to study these mechanisms in the living, intact brain of anesthetized rodents, using molecular *in vivo* imaging techniques. They aim to define the role of different cellular pathways in functional hyperemia, and explore the perturbation of this phenomenon in animal models of neurological disease. The core instrument used in these projects is centred around the use of the two-photon microscope, funded by the European Regional Development Fund. In this way, fluorescently labeled cells are routinely imaged *in vivo* through a cranial window, which has allowed Dr Valentino and his team to precisely record changes in neuronal and glial activity, as well as CBF changes in single blood vessels, in living anesthetized animals with unprecedented resolution.

Role of astrocytes in functional hyperemia

Astrocytes, the star-shaped glial cells found in the brain and in the spinal cord, are in close contact with neuronal synapses as well as with blood vessels, making them ideal candidates to convey changes in neuronal activity to the vasculature. Using *in vivo* two-photon microscopy to probe neuro-glial network activity and CBF, with the help of fluorescent dyes and transgenic animals expressing optical markers of cellular activity, the research team focuses on how the intracellular pathways trigger functional hyperemia.

Astrocytic function and functional hyperemia are severely impaired after a person suffers a stroke. This likely contributes to accumulation of water in the brain, known as celebral edema, and enlargement of the ischemic area, but the underlying mechanisms have remained unclear.

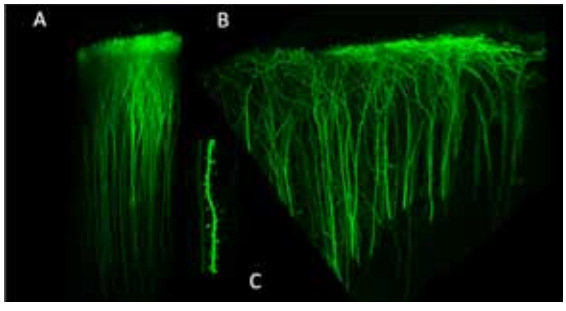
The research group is actively involved in collaborative research projects with Max Planck Institute for Neurological Research in Cologne, with whom they are currently working on a non-invasive method to look at blood flow changes that will allow researchers to image aberrant changes in blood flow that evolve over time in cases of traumatic brain injury, stroke and subarachnoid haemorrhage. Other collaborative research partners include the Department of Cell Physiology and Pharmacology of the University of Leicester, the Laboratory of Eukaryotic Signal Transduction and Gene Expression, University of Ghent in Belgium and the Department of Internal Medicine, University of Perugia in Italy.

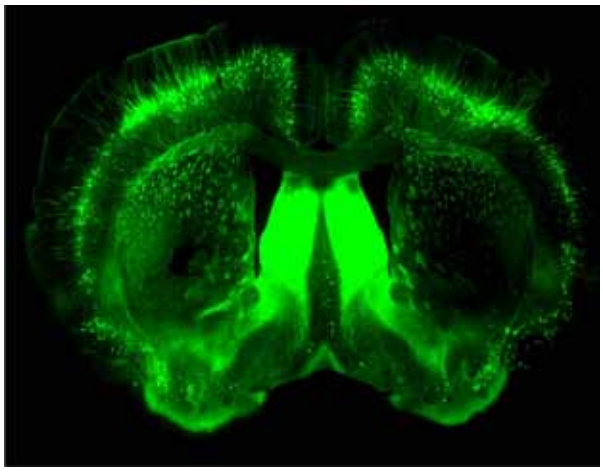
In this project, the research team monitors cellular activity of astrocytes and neurons together with CBF changes in real-time during and after focal cerebral ischemia in mice, using two-photon microscopy. By doing this the team aims to unravel the pathways and molecules involved in these pathological mechanisms, and investigate if pharmacological intervention of these pathways can ameliorate ischemic damage.

Imaging the muse brain in 3D is giving new insight into the development of brain injury that will allow Dr Mario Valentino and his team to visualise the damage step by step. Knowing which brain cells die off first, allows for the development of drugs and techniques to reduce brain injury and guide the surgeons' knife. This research will help millions of sufferers world wide.

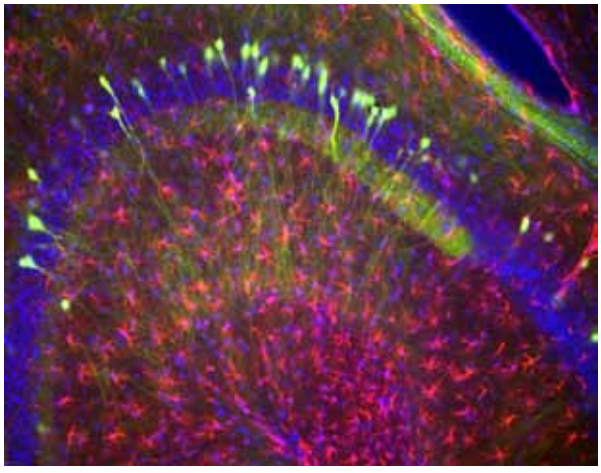
Three-dimensionally constructed images of neurons expressing YFP-H in the cerebral neocortex of a mouse under anesthesia. Images captured using the Olympus Fluoview FV1000-MPE multiphoton system with a 25x objective.

(A) Live imaging of anaesthesized mouse through a cranial window up to 614µm deep below the pial surface showing distinct pyramidal neurons with axons extending down to layer IV and (B) at a of 450µm. In the cortex, most studies of dendritic plasticity have taken advantage of apical tuft dendrites of layer 5 neurons and layer 2 and layer 3 neurons that reach near the surface of the cortex. Near the cortical surface, the tuft dendrites curve and almost run parallel with the pial surface. These parallel running dendrites have spines (C) projecting laterally taking full advantage of two-photon microscopy's good resolution with depth.

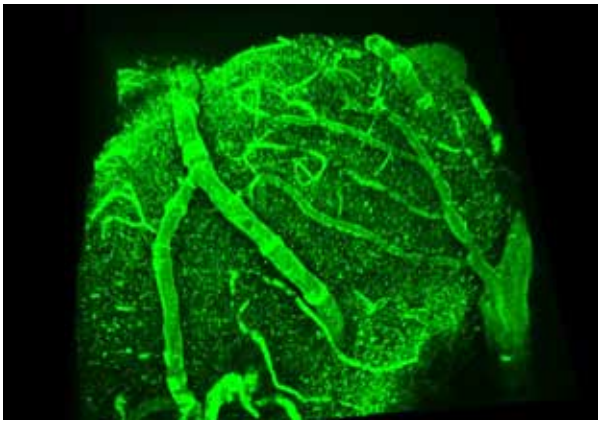




A coronal section from a mouse brain that expresses the fluorescent GFP dye in its nerve cells



Looking closer into the hippocampus (Blue DNA, Red Astrocytes, Green Neurons)



The mouse brain in 3D with blood vessels. The brain from the surface showing an area of around 1mm squared. Highly visible is the vasculature (in green) surrounded by tree-like branched extensions from the nerve cells below the surface (also in green)