This application note describes the use of a novel Fibered Confocal Fluorescence Microscopy system, the Cell-vizio, for the imaging of fluorescently labeled cells in deep brain structures of living mice. Combined with a stereotaxic apparatus, the Cell-vizio has proven its unique capabilities to image deep brain cells in vivo on anesthetized animals, with micron-scale resolution and minimal invasiveness.

Summary

This application note describes the use of a novel Fibered Confocal Fluorescence Microscopy system, the Cell-vizio, for the imaging of fluorescently labeled cells in deep brain structures of living mice. Combined with a stereotaxic apparatus, the Cell-vizio has proven its unique capabilities to image deep brain cells in vivo on anesthetized animals, with micron-scale resolution and minimal invasiveness.

Introduction

Recent developments – new models of GFP and YFP animals, availability of new fluorophores that can label subsets of neurons durably – combined with acute or chronic brain slice preparation for microscopic fluorescence imaging (Brendza et al., 2003; De Marchis et al., 2001), have led to new insights on the dynamic processes occurring in rodent brains.

Moreover, techniques for in vivo microscopic imaging have emerged, mostly in the form of multi-photon microscopy, paving the way to dynamic in vivo studies. To date multi-photon imaging has been able to acquire images (at a rate of 1 to 2 images per second only) at depths up to 500 microns in the adult mouse brain (Helmchen et al. 2001). Nevertheless, cortical structures deeper than layer 2-3 and, a fortiori, sub-cortical areas remain out of reach.

In vivo deep brain imaging is therefore limited to non invasive yet less performing techniques like MRI and PET, the spatial resolutions of which are more than one order of magnitude lower than that of microscopy, while their acquisition times are more than two orders of magnitude greater (Rudin et al., 2003).

The object of the present application note is to demonstrate the possibility to image fluorescent objects in vivo and in real time, inside deep brain areas and at the sub-cellular scale.
Materials & Methods

Animal Preparation
C57Bl/6 adult mice were anesthetized with intraperitoneal injections of ketamin-xylazin (160 mg/kg + 24 mg/kg resp.) and positioned in a stereotaxic apparatus. A small skull window (diameter of 1 mm) was created with a high-speed drill, where needed.

Labeling
Transgenic TgCaMK2tTA/GFPtetO7lacZ mice that express GFP under the control of the CaM-Kinase promoter in some parts of the rostral brain were used to image the striatum and hippocampus.
For neuron precursors imaging, migrating cells were labeled by stereotaxic injection of 200 nl of Cell Tracker Green CMFDA (10 mM in dimethylsulfoxide; Molecular Probes) in the subventricular zone. Injection coordinates and protocol were adapted from Carleton et al., 2003.

Imaging
The animal was placed on a stereotaxic apparatus and imaged with the Cell-vizio. For both mouse models, the ProFlex was positioned onto a micropipette guide of the stereotaxic apparatus and lowered stereotaxically to the desired coordinates. (See Fig. 1)

The chosen ProFlex for this application were either of the S-0650 or the S-0300 type, which have diameters of 650 and 300 microns respectively. In order to minimize tissue damage, the S-0300 ProFlex was beveled at the tip. Cell Tracker Green-labeled mice were observed at days two to five after injection.
Results

In Vivo Imaging of GFP Neurons in Sub-Cortical Structures of Transgenic Animals

Transgenic animals expressing fluorescent proteins are very valuable tools to explore neurodegenerative diseases. Murine models for both Alzheimer’s and Parkinson’s disease are now available (Backsai et al., 2001; Brendza et al., 2003; Ende at al. 2002) as well as transgenic mice with dopaminergic and GABAergic fluorescent neurons (Kessler et al., 2002; Sawamoto et al., 2001; Oliva et al., 2000; Meyer et al., 2002).

Here we show that the Cell-vizio system equipped with a small-diameter ProFlex (650 microns) makes it possible to image GFP labeled cells in living animals, including deep structures of the brain otherwise not accessible with micron-scale imaging technologies. GFP expressing neurons from the mouse model TgCaMK2tTA/GFPtetO7lacZ were acquired at various locations including the cortex, hippocampus and striatum. (See Fig. 2)

Fig. 2: Cell-vizio images of GFP expressing neurons in the TgCaMK2tTA/GFPtetO7lacZ transgenic mouse strain. The tip of a S-0650 ProFlex, with a diameter of 650 microns, was placed stereotaxically into (a) the hippocampus and (b) the striatum.

In Vivo Imaging of Neuron Precursors in the Adult Brain

Cell Tracker Green was shown to label neuron precursors when injected in the sub-ventricular zone (De Marchis et al., 2001). The dye is incorporated into the cell’s cytoplasm at the site of injection and remains detectable throughout the precursors’ migration towards the olfactory bulb.

We recently developed a new ProFlex intended to minimize tissue damage. Its diameter is 300 microns and its tip is beveled at 45°. Combining this ProFlex (S-0300) and a stereotaxic apparatus, we could detect neuron precursors in the olfactory bulb of a Cell Tracker Green-labeled mouse, at days two to five post dye injection. (See Fig. 3)

Fig. 3: Imaging of neuron precursors labeled with Cell Tracker Green (Molecular Probes). The fluorophore was injected in the sub-ventricular zone. Cell-vizio images were acquired after stereotaxically lowering the beveled tip of a 300 micron-diameter optical probe, into the olfactory bulb.

Control images of the labeled brains were taken, after fixation, with a standard epifluorescence microscope, showing positively labeled cells in the rostral migratory stream (RMS) and in the olfactory bulb. The ProFlex’s invasiveness was evaluated from microscope images of the explored brain. (See Fig. 4)

The rostral migratory stream is only about 80 microns in diameter, therefore, imaging neuron precursors at this location is within the system’s capabilities, but requires the prior knowledge of the exact coordinates. This is still ongoing work.

Fig. 4: Epifluorescence microscope images of fixed brain slices from animals used for Cell-vizio imaging. Cell Tracker Green-labeled neuron precursors can be seen in (a) the olfactory bulb and in (b) the horizontal limb of the rostral migratory stream. Photo (c) shows a high magnification image of the ProFlex probe path.
Discussion

Imaging modalities like MRI, PET and ultrasound feature resolutions of the order of a few tens of microns to a few millimeters. The depth of acquisition of such modalities is usually not limited, and they are non invasive. While MRI and Ultrasound provide only anatomical information, PET allows functional imaging. However, the list of functional contrast agents for PET is much more limited than the existing library of fluorescent markers.

Optical imaging techniques have the power to combine micron-scale resolution with functional imaging. Such modalities include conventional benchtop confocal and multi-photon fluorescence microscopy. In the field of neurobiology, microscopy is either applied to fresh brain slice preparations, and is therefore quite invasive, or to the whole brain, but then limited in penetration depth to 500 microns.

Optical modalities have another advantage over MRI, PET and ultrasound, in that their typical acquisition times are more than an order of magnitude shorter, thus approaching time scales comparable to that of cellular biological events.

Fibered confocal fluorescence imaging brings the benefits of in vivo observations on top of a sub-cellular resolution and the capability to perform dynamic molecular imaging with minimally invasive access to deep brain structures. The feasibility of the image acquisition of fluorescently labeled cells in deep brain structures such as the hippocampus, striatum and olfactory bulb has been described in this note.

Tissue damages can be evaluated from the path left by the ProFlex in the brain of explored animals, as seen on microscope images of brain slices. Cells are obviously irremediably separated. Nevertheless, we could observe that when lowering the ProFlex no cell was fractionated or distorted and there was no fluorophore leakage from cells. As a matter of fact, cells seemed to slide along the bevel.

Acknowledgements and Credits

We are most grateful to the following people, with whom the work described here was performed:
- M. Davenne and P.M. Lledo, Pasteur Institute, Laboratory of Perception and Olfactive Memory, Paris, France
- U. Maskos, J.P. Bourgeois, Pasteur Institute, Laboratory of Receptors and Cognition, Paris, France

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