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In Vivo Imaging of Migrating Neurons in the Mammalian Forebrain

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Introduction

The forebrain periventricular region of the adult mammalian brain contains neural stem cells (Alvarez-Buylla and Lim, 2004) and several *in vitro* studies have indicated that these cells exhibit multipotency (Kempermann *et al.*, 2004). However, approaches performed *in vivo* have demonstrated that neural stem cells were endowed with a rather limited fate. For example, *in vivo* subventricular zone (SVZ) precursors were found to generate primarily neuronal precursors that can either die or give rise to neurons (see Kuhn *et al.* in this issue). Those precursors are unusual in that they exhibit neuronal characteristics as shown by their expression of neuronal markers. In addition, unlike other immature neurons, precursor cells of the SVZ migrate tangentially without the aid of radial glial cells along a tangential pathway, called the rostral migratory stream (RMS), to populate the olfactory bulb where they differentiate into local interneurons.

Although the temporal sequence of physiological maturation of newborn neurons has been recently described (Belluzzi *et al.*, 2003; Carleton *et al.*, 2003), the way of migration *in vivo* remains elusive. For instance, it is still unclear whether migrating cells move homogeneously towards the target tissue. Filling this gap has been impeded largely by the inability to collect brain images, neither from the adult neurogenic zone, nor from the migratory pathway or their integration site. All these areas in the adult brain are too deep for conventional fluorescence microscopy. The aim of the present study was to track, *in vivo*, newborn neurons migrating in the adult mouse forebrain. We developed a method based on an integrated fibered confocal fluorescence microscope, the Cell-vizio (see Figure 1A). For the first time, after labeling a large number of SVZ progenitor cells, we were able to track neuroblasts migrating *in vivo* in the adult forebrain.

Materials and methods

Labeling

Cells were labeled by stereotaxic injection of GFP-expressing lentivirus (stock concentrtion was 10^{10} infectious particles/ml) or of 200 nl of Cell Tracker Green CMFDA (Molecular Probes), in the SVZ. Injection coordinates and protocol were adapted from Carleton *et al.* (2003).

Imaging

Two to five days after labeling, mice were positioned on a stereotaxic apparatus. A small skull window was made to allow penetration of a ProFlex fiber optic endoscope probe (300 µm in diameter with a beveled tip) connected to the Cell-vizio (Mauna Kea Technologies; Le Goualher *et al.*, 2004; Perchant *et al.*, 2004). The Proflex endoscope probe was hooked to a vertical micropipette guide of the stereotaxic apparatus that allows positioning of the probe to the appropriate coordinates within the brain. The penetration of the probe in the brain was achieved slowly, by 10 µm steps, with the bevel perpendicular to the RMS, to avoid perturbing the migration of cells en route to the bulb.

Results

We used Cell-vizio to follow adult-born neuroblasts migrating from the SVZ to the olfactory bulb in living mice. Two to five days after labeling cells in the SVZ by stereotaxic injection of a GFP-expressing lentivirus or of Cell Tracker Green, the ProFlex fiber optic endoscope probe was inserted into the brain of anesthetized animals. Three different sites of recordings were chosen: (i) at the beginning of the RMS (close to the ventricle); (ii) at the horizontal limb of the RMS; and (iii) in the core of the bulb. Labeled cell bodies were detected at all these positions (see Figure 1D in the RMS) and their migration speed range from 40 to 80 µm/h. Only rarely could part of the leading process of migrating cells be detected (not shown). After image acquisition, the brain was fixed and sagittal sections were performed to analyze the position of the probe (Figure 1B,C). The slices confirmed that the probe was positioned right above labeled neurons migrating along the RMS. The acquisitions made with the Cell-vizio could therefore provide information about migrating adult-born neurons *in vivo*.

Discussion

We describe here the use of fibered confocal fluorescence microendoscopy for imaging neurons in deep brain structures of living mice. Combined with a replication incompetent recombinant lentivirus expressing GFP, or with Cell Tracker Green, to label adult-born neurons, the Cell-vizio system has proven its ability to visualize cells *in vivo* in real time in the adult forebrain of anesthetized animals, with a micron-scale resolution.

Although multi-photon microscopy has come to be one of the most powerful tools in life sciences, its application to the intact animal remains limited to imaging several hundred micrometers below the surface. Therefore, a continuous stream of technological developments is yet required to resolve some of its limitations. Any new technical development should extend the limits of imaging depth, speed, resolution, and time applied to intact animals. Here, we show that the use of fibered confocal fluorescence microendoscopy enables minimally invasive, long-lasting recording several millimeters deep in an anesthetized animal. Applying this technology to the forebrain allowed us to visualize, for the first time, migrating adult-born neurons in living animals.

Deep brain imaging with minimal invasiveness

So far, non-invasive methods for imaging deep brain structures in intact animals include techniques such as ultrasound, MRI and PET,

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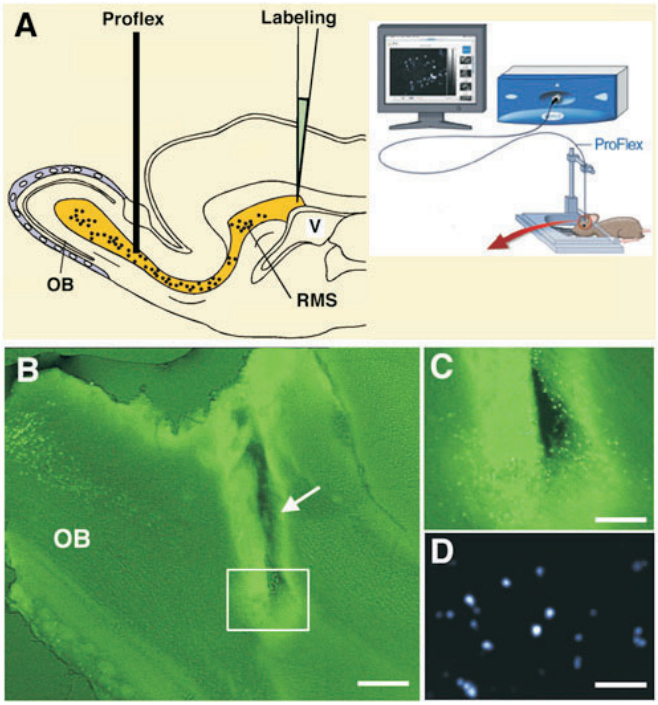

Figure 1 *In vivo* imaging with the Cell-vizio of adult-born neurons labeled with CMFDA. **(A)** Schematic representation of a sagittal view of the brain showing the site of labeling, in the SVZ, adult-born neurons (black dots) migrating from the SVZ to the olfactory bulb (OB) along the RMS, and the site of imaging with the Proflex endoscope probe, used to obtain the results shown in (B–D). **(B)** Sagittal slice of the brain after imaging. The path of the probe can be seen (arrow). **(C)** Magnification of the box shown in B, showing the presence of labeled cells migrating along the RMS, right under the tip of the probe. **(D)** Image acquired with the Cell-vizio, showing labeled neuronal cell bodies. Penetration depth: 3.4 mm. Scale bars in B: 400 μm; C: 200 μm and D: 100 μm.

whose resolution and acquisition times are far less efficient than those of microscopy. Optical imaging techniques can combine micrometer-scale resolutions with functional imaging. These include conventional bench-top confocal and multi-photon fluorescence microscopy. These techniques can be applied to the whole brain but are limited in penetration depth. Fibered confocal fluorescence microscopy offers the advantages of optical imaging adapted to a fiber optic that can be applied *in vivo* with minimal invasive access to deep brain structures. Tissue damage could be evaluated from the path left by the Proflex fiber optic probe on brain slices of explored animals (Figure 1B). The penetration of the Proflex endoscope probe in the brain obviously caused an irremediable separation of tissues. Nevertheless we could observe that, when lowering the probe under the acquisition mode of the Cell-vizio, no cell was fractionated or distorted as seen by the absence of fluorophore leakage from cells. In fact, labeled cells seemed to slide along the bevel of the fiber. We conclude from these observations that fibered confocal fluorescence imaging allows minimally invasive access to deep brain structures of living animals.

Long-term and real-time imaging

Dynamic processes in the brain frequently occur rapidly over long periods of time. As a result, most dynamic cellular processes can only be observed by time-lapse imaging over extended periods of time. The fibered confocal fluorescence microscopy system offers as an optical imaging system the advantage over, for example, PET, MRI or ultrasound, of fast acquisition times compatible with most cellular events. In addition, the probe, once inserted in the brain, can be used for long recording sessions, with minimal bleaching and phototoxicity. We have been able to record labeled cells up to 6 h continuously. Thus, the Cell-vizio allows imaging sessions compatible with cellular processes that can be investigated over prolonged periods of time.

Overall, the results of the present study show that our fibered confocal microendoscopy system provides a direct, rapid and accurate visualization of fluorescent signals in living brains. Using this technique, we were able to track multiple cells simultaneously *in vivo* and moving from the SVZ to the olfactory bulb.

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