A virtual atlas unveils the cellular distribution of two fluorescent receptors in the mouse brain

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The Institut de la Clinique de la Souris (ICS) **(Mouse Clinical Institute – MCI)** founded in 2002 by Pierre Chambon and directed by Dr Yann Hérault provides a comprehensive set of specific services to academic and industrial users and is a major player in the European post-genomics era programs. ICS is specialized in the generation of customized and ready-to-use genetically modified mice as well as standardized, comprehensive or advanced functional characterization of mouse models. Phenotypic profiling includes a comprehensive histopathological analysis of mutant or treated mice to detect and systematically analyze organ defects and tissue alterations at precise stages during mice development and life. The histopathology and embryology facility therefore acquired the Nanozoomer 2.0 HT, with its fluorescence module, to fulfill challenging requirements in terms of image quality, high throughput screening and interactive data management with customers.

Cellular Distribution of two fluorescent receptors in the mouse brain

Dr Dominique Massotte, Department of Neurogenetics and Translational Medicine, IGBMC, pioneered the creation of a virtual atlas presenting the distribution of two fluorescent receptors in the mouse brain, the mu and delta opioid receptors.

Opioid receptors and endogenous opioid peptides are broadly expressed throughout the nervous system. They form a complex neuromodulatory system that regulates numerous physiological functions with important involvement in pain control, mood disorders and drug abuse. Mu and delta receptors belong to the G protein-coupled receptor family that constitutes 35% of the current drug targets on the market. Changes in mu and delta receptor functional properties have been postulated to result from their co-expression in specific neurons. However, the existence of such neurons in the brain remained controversial.

We generated genetically modified mice that express functional fluorescent versions of the mu and delta opioid receptors respectively in fusion with a red or a green fluorescent protein. *In vivo* fine cartography of the resulting bicolor double mutant line was essential to depict the distribution of the two receptors but collecting data throughout the entire brain proved instrumental to generate global maps. A fluorescent microscope, while achieving high magnification, only enable to view a small field within the sample. Therefore, reconstructing the overall distribution of the signal in an entire brain section turns out as extremely cumbersome. The Nanozoomer 2.0 HT is unique since it provides a single image of the whole tissue section with the possibility to zoom in anywhere to visualize the signal of interest with subcellular resolution. Consequently, an organ as complex as the rodent brain can be screened at high magnification for up to six different fluorescent probes simultaneously. Here, collecting images at three different wavelengths without interference and recombining them make also acquisitions with the Nanozoomer extremely valuable for fine immunohistochemical mapping with different specific markers (Figure 1).

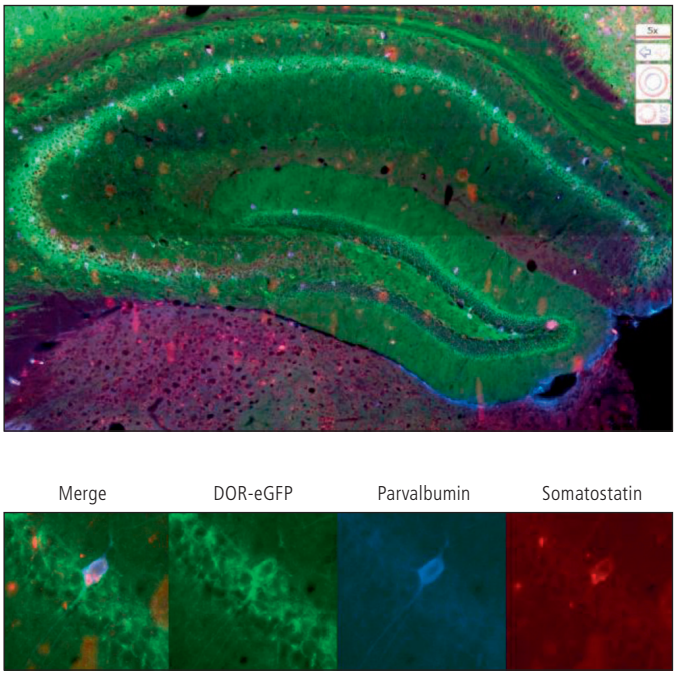

Figure 1: Identification of the neuronal subtypes expressing DOR-eGFP in the hippocampus by co-localization with specific neuronal markers. General view of the hippocampus (above) and individual neuron co-expressing DOR-eGFP, somatostatin and parvalbumin (below).the right are shown from the side.

The possibility to optimize image acquisition for each fluorescent probe to take into account its actual intensity represents another major advantage. Combining the bicolor double mutant mouse line and high resolution image acquisition with the Nanozoomer (magnification 40x resolution 0.23 µm/pixel) made then possible to map the two fluorescent receptors throughout the brain and to single out neurons in which the two fluorescent signals were simultaneously present (Figure 2).

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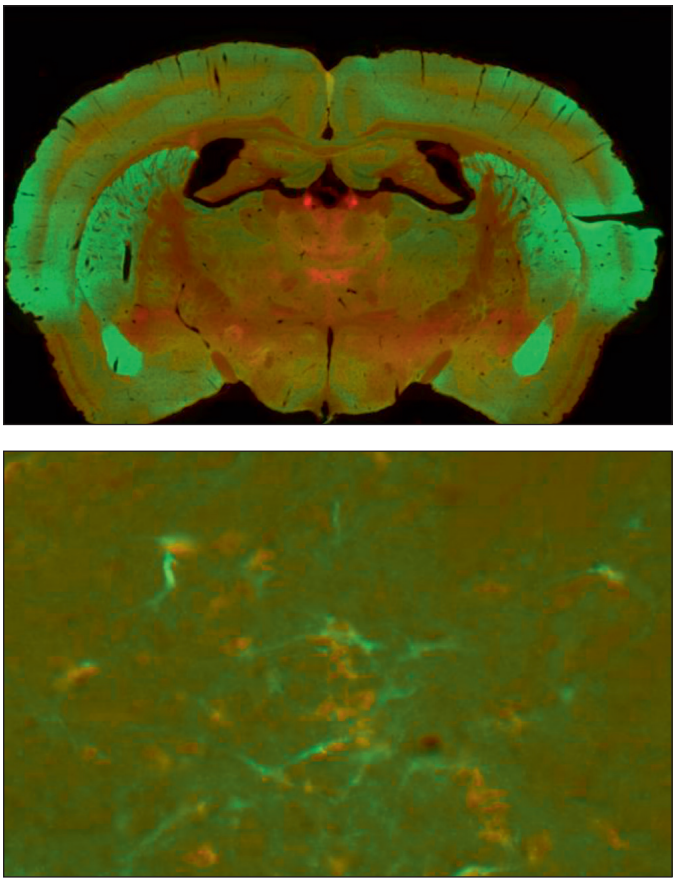

Figure 2: Brain coronal section showing the distribution of the fluorescent signals associated to DOR-eGFP (green) and MOR-mcherry (red). General overview (above) and individual neurons (below) using 40x objective.

Accessing freely to the virtual atlas

Using the NDP.Toolkit program, each image, corresponding to a single brain section could be isolated and used for integration in an interactive resource. All the images can then be accessed freely, using NDP.Serve interface, coupled with NDP.View at https://mordor.ics-mci.fr/.

Following Receptor Internalization

Another interesting application of the Nanozoomer 2.0 HT is the possibility to map brain areas where receptors get activated in response to a physiological challenge. Indeed, in vivo activation of some neuronal networks promotes endogenous opioid peptide release, that activates the receptors. Receptor stimulation in turn induces receptor relocation from the cell surface to intracellular compartments or so-called internalization. Visualization of the fluorescent receptors at subcellular resolution when acquiring images at a 40x magnification (resolution 0.23 µm/pixel) (Figure 3) led to identify brain regions in which internalization had taken place upon a given physiological challenge which represents a powerful approach to study endogenous GPCR physiology.

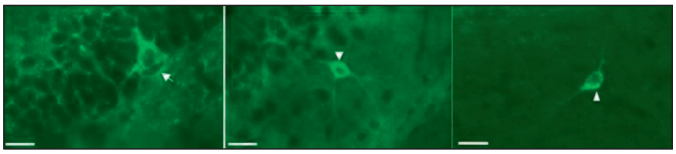

Figure 3: DOR-eGFP is present at the surface of the neuron under basal conditions (arrow, left image) and gets redistributed inside the cell upon activation. Importantly, the internalization profile looks different after physiological activation resulting from endogenous peptide release (arrow head, central image) or pharmacological activation by the agonist SNC 80 (arrowhead, right image).es Scale bar 10µm.

For further information see:

Faget L., Erbs E., Le Merrer J., Scherrer G., Matifas A. Noble F., Benturquia N., Kessler P., Koch M., Vonesch J.-L., Kieffer B.L. and Massotte D. (2012) *"In vivo visualization of delta opioid receptors upon physiological activation uncovers a distinct profile."* J. Neurosci. 32, 7301.

Erbs E., Faget L., Scherrer G., Kessler P., Hentsch D., Vonesch J.-L., Matifas A., Kieffer B.L. and Massotte D. (2012) *"Distribution of delta opioid receptor expressing neurons in the mouse hippocampus."* Neurosciences 221, 203.

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