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An automated workflow for the anatomo-functional mapping of the barrel cortex

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HIGHLIGHTS

- Here is a new tool to map functional data onto the barrel cortex structure.
- It realigns histological slices and reconstructs the barrel map in 2-D.
- Slice realignment by rigid transformations is computed using detected blood vessels.
- Barrel map reconstruction is obtained by gradient fusion.
- Its application is exemplified for voltage sensitive dye imaging experiments.

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ABSTRACT

Background: The rodent barrel cortex is a widely used model to study the cortical processing of tactile sensory information. It is notable by the cytoarchitecture of its layer IV, which contains distinguishable structural units called barrels that can be considered as anatomical landmarks of the functional columnar organization of the cerebral cortex. To study sensory integration in the barrel cortex it is therefore essential to map recorded functional data onto the underlying barrel topography, which can be reconstructed from the post hoc alignment of tangential brain slices stained for cytochrome oxidase.
New method: This article presents an automated workflow to perform the registration of histological slices of the barrel cortex followed by the 2-D reconstruction of the barrel map from the registered slices. The registration of two successive slices is obtained by computing a rigid transformation to align sets of detected blood vessel cross-sections. This is achieved by using a robust variant of the classical iterative closest point method. A single fused image of the barrel field is then generated by computing a nonlinear merging of the gradients from the registered images.
Comparison with existing methods: This novel anatomo-functional mapping tool leads to a substantial gain in time and precision compared to conventional manual methods. It provides a flexible interface for the user with only a few parameters to tune.
Conclusions: We demonstrate here the usefulness of the method for voltage sensitive dye imaging of the mouse barrel cortex. The method could also benefit other experimental approaches and model species.

1. Introduction

The rodent primary somatosensory cortex is a very convenient model for studying the cortical processing of sensory information because of its well defined structural and functional layout that is invariant from animal to animal (Welker and Van der Loos, 1986; Meyer et al., 2013; Egger et al., 2012). In its layer IV, neurons are gathered into clusters called barrels that respect the same topology as the whiskers on the snout of the animal (Woosley and Loos, 1970). Each barrel is dedicated primarily to the processing of the input coming from its corresponding whisker (Fig. 1A,B). When

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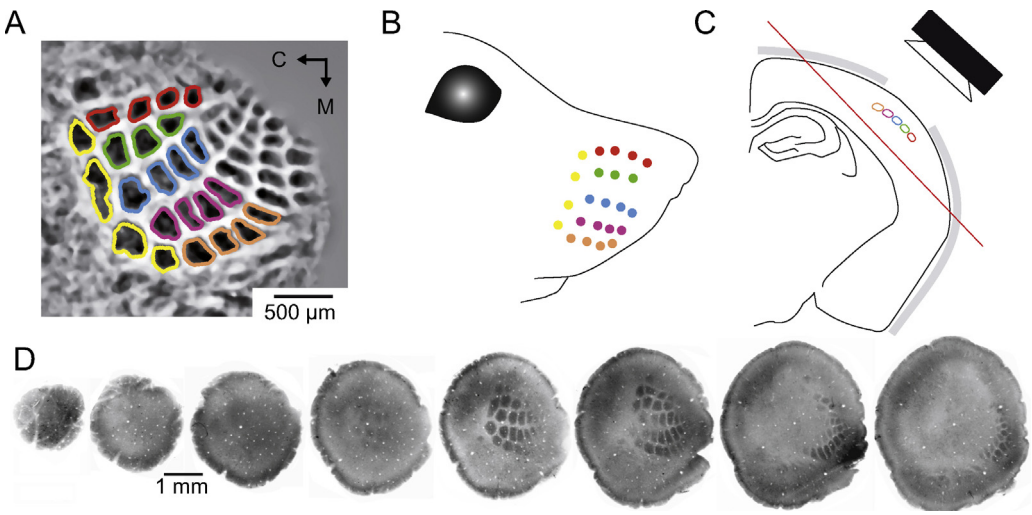

Fig. 1. Cytochrome oxidase staining of tangential sections from the mouse primary somatosensory cortex reveals the structural organization of layer 4 barrels that mirrors the arrangement of the vibrissae on the snout. (A) Following the registration of tangential histological slices and the reconstruction of the barrel map, one can see that the spatial organization of the layer 4 barrels matches the layout of the vibrissae on the snout of the animal (B). (C) Drawing of a coronal section of the left hemisphere of the mouse brain illustrating the position of layer 4 barrels within the primary somatosensory area of the cortex. After in vivo imaging of barrel cortex activity, sections are cut tangentially to reconstruct the layer 4 barrel map (cutting plane indicated by the red line). (D) A series of tangential histological slices stained for cytochrome oxidase. On the first slice one can see superficial blood vessels. On the other slices, one can see white circular to elliptic spots that correspond to sections of plunging blood vessels. Depending on the exact axis of the cut, barrels can be spread over several slices.

studying sensory processing in the barrel cortex either with electrophysiological or imaging methods, it is therefore of great interest to superimpose the recorded activity onto the underlying barrel topography, which can be reconstructed from the post hoc alignment of tangential brain slices stained for cytochrome oxidase. In order to optimize this anatomo-functional mapping, which is usually accomplished manually, we developed an automated workflow for the registration of the histological slices of the barrel cortex and the 2-D barrel map reconstruction.

Here we focus our attention on voltage sensitive dye imaging (VSDI) of the mouse barrel cortex to illustrate the usefulness of the approach. However, the method can be extended to the study of the rat barrel cortex and applied to other techniques such as 2-photon calcium imaging.

The traditional first step to recover the map of the barrel cortex after imaging experiments is: brain fixation by perfusing the animal with a solution of paraformaldehyde, followed by the cutting of tangential slices (~100 μm thick, with or without previous flattening of the cortex), which are subsequently stained for cytochrome oxidase using classical histological procedures that reveal the barrel arrangement in layer IV (Fig. 1C and D (Land and Simons, 1985)).

Next, using digital microphotographs of the slices, it is necessary to:

1. register the slices;
2. fuse the registered slices to define a reconstructed barrel image.

In this article we provide an automated solution for these two steps which are the most time-consuming tasks of the workflow when using conventional manual methods. After completing these steps, it is then relatively simple to define the barrel map by segmenting the reconstructed barrel cortex image. The superimposition of the map with the functional data can be finally achieved by using the superficial blood vessels as anatomical landmarks (Fig. 2). The proposed anatomo-functional mapping tool significantly speeds up the overall process and provides more accurate anatomo-functional mapping.

1.1. Registration of histological slices

A typical example of a series of images obtained after the histological process is shown in Fig. 1D. Depending on their depth, the histological sections present different properties: in the first

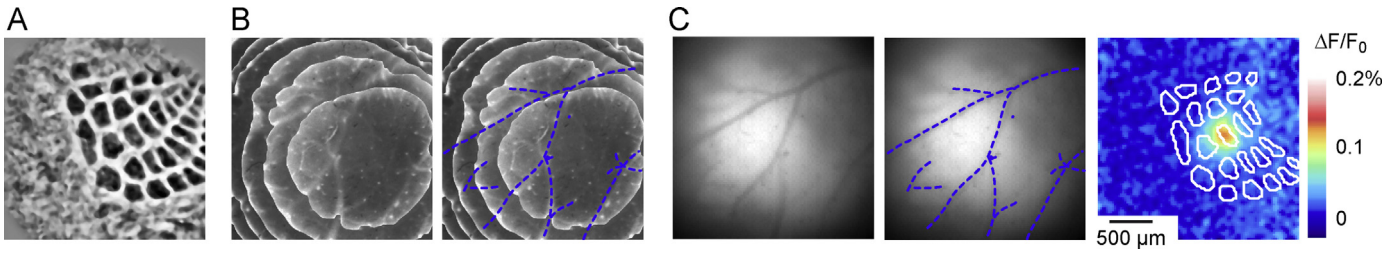

Fig. 2. Alignment of the barrel map with VSDI data using the superficial blood vessels as blueprints. (A) Barrel map reconstructed from the histological slices shown in Fig. 1D, using our anatomo-functional mapping method. (B) Superimposition of the registered histological slices, allowing the delineation of the superficial blood vessels (in blue). (C) The superficial blood vessels also appear in vivo on the fluorescent images taken during the VSDI session. The VSDI data can therefore be aligned with the underlying structural map of the barrel cortex using the blood vessels as anatomical landmarks. On the right, the cortical activity is shown imaged at 10 ms following a single C2 whisker deflection with the voltage sensitive dye RH1691 under urethane anesthesia. The barrels outlined from the reconstruction in A are superimposed on the image as white lines.