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Mapping of neuron soma size as an effective approach to delineate differences between neural populations

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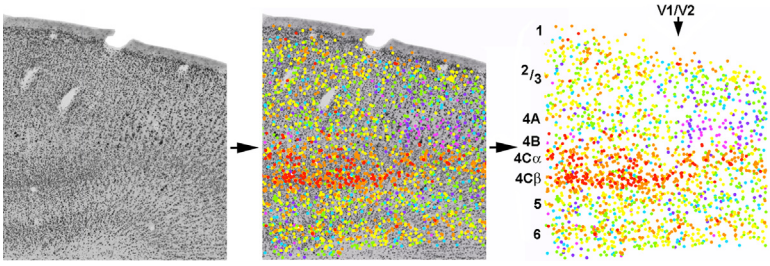
HIGHLIGHTS

- A method for display and analysis of cytoarchitectonic data is presented.
- Neurons from fixed tissue sections were assessed for soma area and in situ position.
- Multi-dimensional mapping revealed distinct neural populations.
- Superior sensitivity revealed small differences between groups of neurons.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: A single histological marker applied to a slice of tissue often reveals myriad cytoarchitectonic characteristics that can obscure differences between neuron populations targeted for study. Isolation and measurement of a single feature from the tissue is possible through a variety of approaches, however, visualizing the data numerically or through graphs alone can preclude being able to identify important features and effects that are not obvious from direct observation of the tissue.
New method: We demonstrate an efficient, effective, and robust approach to quantify and visualize cytoarchitectural features in histologically prepared brain sections. We demonstrate that this approach is able to reveal small differences between populations of neurons that might otherwise have gone undiscovered.
Results & comparison with existing method(s): We used stereological methods to record the cross-sectional soma area and *in situ* position of neurons within sections of the cat, monkey, and human visual system. The two-dimensional coordinate of every measured cell was used to produce a scatter plot that recapitulated the natural spatial distribution of cells, and each point in the plot was color-coded according to its respective soma area. The final graphic display was a multi-dimensional map of neuron soma size that revealed subtle differences across neuron aggregations, permitted delineation of regional boundaries, and identified small differences between populations of neurons modified by a period of sensory deprivation.
Conclusions: This approach to collecting and displaying cytoarchitectonic data is simple, efficient, and provides a means of investigating small differences between neuron populations.

1. Introduction

The stripe of Gennari refers to a dense band of myelinated fibres that runs parallel to the cortical surface distinguishing layer 4 of the primary visual cortex (V1) as well as lower layer 3 and

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upper layer 5 (Braitenberg, 1962). Its discovery in humans in 1776 by Francesco Gennari represented a major shift in thinking about how the cerebral cortex was organized anatomically as well as functionally (see Glickstein and Rizzolatti, 1984). Over a century later, the parcellation of cortex into many distinct areas that serve specific functions was advanced by the work of Korbinian Brodmann (1909), whose delineation of brain regions in 1909 continues to guide both functional and anatomical investigations in present day. Brodmann's division of the human cortex into 43 distinct areas was conducted on the basis of regional differences in cytoarchitecture that included the local positioning, size, and distribution of cells (Brodmann, 1909). As has been pointed out (Amunts and Zilles, 2015), Brodmann was ahead of his time in speculating that regionally specific cytoarchitecture was underlying of functional differences between cortical areas. While more contemporary methods have proven that cytoarchitecture alone cannot account for the complexity of regional subdivisions within cerebral cortex (reviewed in Nieuwenhuys, 2013), and while cortical functioning is far more complex than originally predicted by Brodmann's hypotheses (Zilles and Amunts, 2010; Amunts and Zilles, 2015), his anatomical work remains a testament to the inextricable relationship between brain anatomy and function.

Despite now being well beyond Brodmann's seminal discoveries, the natural aggregation of brain cells with common characteristics continues to inform the delineation of boundaries between groups of cells, as is the case for distinguishing between cortical layers and cortical areas (Balaram et al., 2014). The ability to identify distinct cell populations and regional boundaries using cytoarchitectonic criteria to a large extent depends upon the marker's ability to reveal the constellation of cellular characteristics that are unique to a particular cell group, as well as upon additional characteristics the marker makes visible within the tissue. Issues related to the identification of specific groups of cells likewise applies to experimental manipulations and disease states that can have an isolated effect on brain areas containing a heterogeneous and uniformly distributed population of neurons. In such instances, alterations in cytoarchitecture can emerge from activity-driven processes that have a selective influence on brain cells. For example, the division of primate visual cortex into ocular dominance domains derives from the distribution of eye-specific thalamic inputs onto cortical neurons rather than from an inherent difference in the cytoarchitecture of cortical neurons populating left and right eye columns (LeVay et al., 1975); although it should be noted that transient markers for eye-specific columns do exist very early in development (Tomita et al., 2013). Following monocular deprivation (MD) of patterned vision, cortical and thalamic neurons serving the deprived eye exhibit structural modifications that can be subtle and below detection through microscopic observation (Duffy et al., 2007). In the current study, we describe a method for the visualization and quantification of cytoarchitectonic data that enabled a superior means of distinguishing between cortical areas and subregions, as well as detection of subtle experimentally-induced neural modifications within the thalamus and cortex. We demonstrate the value and sensitivity of this approach by mapping the cross-sectional area of neuron somata within the visual system of cat, monkey, and human. Following a period of MD we show that the mapping of cross-sectional soma size was sensitive enough to identify a small difference between deprived and non-deprived neuron populations, and was sufficient to reveal the two-dimensional pattern of ocular dominance bands in human cortex that simply could not be appreciated from direct observation of the tissue alone. Benefits of this approach include a wide-ranging applicability that extends beyond use in the visual system, and can lead to more reliable and reproducible estimates of anatomical boundaries particularly when other data are not available.

2. Materials and methods

2.1. Animals and rearing histories

Two-dimensional maps of neuron soma areas were produced from sections of cat, monkey, and human visual system. All tissues used in this study were obtained from specimens that were subjects in previous publications. We investigated the distribution of soma areas from (1) the dorsal lateral geniculate nucleus (dLGN) of a normal kitten (postnatal day 30) and 2 kittens monocularly deprived at the peak of the critical period for either 3 or 7 days (Duffy and Slusar, 2009); from (2) the primary visual cortex of a normal adult rhesus macaque monkey (Duffy and Livingstone, 2005); and from (3) V1 of a monocularly deprived adult human (Duffy et al., 2007). All procedures applied to experimental animals were approved by either the University Committee on Laboratory Animals at Dalhousie University (Duffy and Slusar, 2009), or by the Harvard Medical Area Standing Committee on animals (Duffy and Livingstone, 2005). For our human subject, tissue collection was permitted in accordance with institutional guidelines for protection of human subjects from the Massachusetts General Hospital (Duffy et al., 2007).

2.2. Cat surgical procedures

For kittens, MD was performed beginning at the peak of the critical period for ocular dominance plasticity (4 weeks postnatal; Olson and Freeman, 1980) and lasted for 3 or 7 days. Under general gaseous anesthesia (3–4% isoflurane in oxygen) the upper and lower palpebral conjunctivae of the left eye were closed using vicryl suture material, and the eyelids were pursed together and closed with silk suture (Murphy and Mitchell, 1987). Anesthetized animals were provided a subcutaneous injection of Anafen for post-procedure analgesia, local anesthesia with Alcaine sterile ophthalmic solution (proparacaine hydrochloride), and were also administered a broad-spectrum topical antibiotic (1% Chloromycetin) to mitigate infection.

2.3. Human clinical details

Area V1 was examined from the brain of an 86-year-old woman with long-standing monocular blindness. The cause of her death was not disclosed. Immediately following removal, the brain was placed in phosphate buffered saline (PBS) solution containing 4% formalin. Clinical information that was available at the time of autopsy did not provide an indication of the duration of the MD beyond only indicating that it was long-term. The severity of the monocular impairment was evident upon examination of the optic nerves, which revealed that the affected side was reduced in volume with a gray appearance, in addition to severe atrophy of neurons within the LGN connected to the impaired eye (Duffy et al., 2007). Microscopic examination confirmed the severity and chronicity of the impairment, as there was a pronounced loss of axons and myelin sheaths without any evidence of recent injury on the affected side, compared with a normal appearing contralateral optic nerve.

2.4. Histology

Experimental animals (cat and monkey) were given a lethal injection of sodium pentobarbital (150 mg/kg), followed by transcardial perfusion with approximately 150 mls of PBS and then approximately 150 mls of PBS containing 4% dissolved paraformaldehyde. The fixed brain was then immediately extracted and both the visual cortex and thalamus were dissected from the remainder of the brain so as to prepare each for sectioning and histological processing. Blocks of tissue containing the dLGN (cat),