OPINION

Localization in the brain: new solutions emerging

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Descriptions of neuroanatomical locations are often ambiguous. With the greatly increasing volumes of imaging data that are being produced and the increasing need to generate databases for the efficient analysis of these data, neuroscientists need to avoid such confusion in nomenclature. Here, I discuss the theory and practice on assigning locations to anatomical data, with a focus on data that have been collected from microscopic sections.

Localization of function in the brain has been a topic in experimental brain sciences since the classical electrical stimulation studies on the dog cortex that were published in 1870 by Fritsch and Hitzig[1]. Parcellation of the brain into structural domains took a major leap forward with the microarchitectonic investigations of Brodmann[2] in 1909. The issue of localization in the brain is now, more than ever, a topic of importance, as modern neuroscience collects huge amounts of data that often have no meaning without a precise description of location.

Making sense of data that are collected from the brain is a demanding task. One of the main concerns in interpretation of neuroscience data is localization. From which cortical area, brainstem nucleus or cerebellar zone were the data derived? From where within an area, nucleus or zone were the data obtained? And what was the quantitative distribution of the elements or features recorded? These questions apply not only to classical neuroanatomy, but also to a wide variety of disciplines. When gene expression data from the brain are analysed, functional interpretation relies on exact information about location in the brain. Precise identification of electrode positions during electrophysiological recordings profoundly increases the value of the collected data. Data about distributions of neurons or other elements that are labelled with the use of immunocytochemistry or *in situ* hybridization make sense only when they are mapped in relation to well-defined landmarks and boundaries. The same applies to activation patterns that are observed in positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies. Here, I discuss the theory and practice related to the task of assigning locations to brain data, with a focus on data collected from microscopic sections.

Why is localization complicated?

The issue of localization is far from trivial. Several factors make it difficult to assign an accurate position to a given set of data. The brain is divided into numerous structurally and functionally different regions. The neuroanatomical nomenclature consists of several thousand terms that are used to describe localization (see listings in brain atlases; for example, REFS 3–5). Different terminologies are used to describe a given brain region, and boundaries of many areas and nuclei are not unambiguously defined. Individual variability further complicates interpretations of localization. In many investigations, only a coarse description of localization can realistically be achieved.

Brain atlases

Atlases are important reference tools for researchers who seek answers to questions about localization in the brain, and brain atlases on numerous species are available. They typically provide a nomenclature that is defined in relation to manually segmented two-dimensional cross-sections, which are taken at intervals of a few hundred micrometres or millimetres. The plane of sectioning used in the atlas (coronal, sagittal or other) is usually considered as a 'standard plane'. Researchers often attempt to reproduce the atlas planes, to facilitate comparison of their sections to those of the atlas.

Defining the location of a given section in relation to a brain atlas might, however, pose problems. The angle of sectioning in the experimental material might — deliberately or not — be different from the 'standard angle' of the atlas. The angle of the atlas section might not even be optimal for visualization and analyses of a specific brain region. Furthermore, conventional atlases do not contain information about structural variability. Individual variability, particularly in the detailed anatomy of the primate cerebral cortex, poses a substantial challenge for comparison of experimental sections with those in atlases, and for comparisons of data across animals.

Three-dimensional reconstruction

Three-dimensional (3D) reconstruction and subsequent computerized visualization and analysis are powerful strategies for dealing with the issue of localization at several scales, including data from microscopic sections[6–8] (FIG. 1). The 3D approach brings together the information that was separated by serial sectioning and facilitates the understanding of spatial relationships within and among structures. However, 3D reconstruction from microscopic sections is an elaborate process and is usually only realistic if efficient, data-acquisition procedures are available. Software for segmentation of section images is used to automatically detect surfaces, principal boundaries and landmarks and, to some extent, experimental data. Registration of the

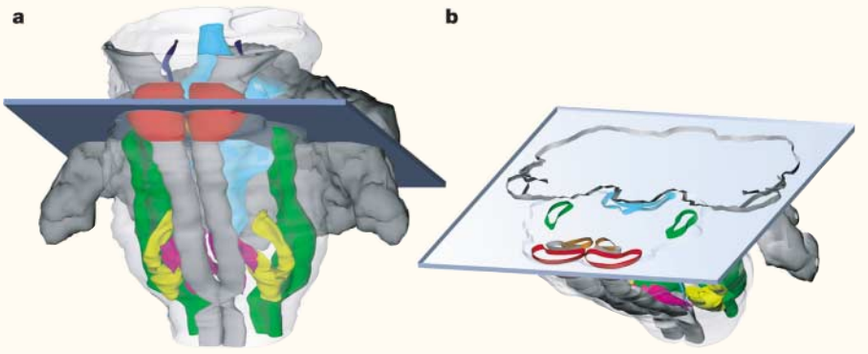

Figure 1 | **Example of a partial 'next-generation atlas'. a** | Three-dimensional model of the rat brainstem with selected, cerebellum-related regions shown in different colours. The image shows a plane of sectioning that is different from a standard atlas plane. **b** | The model is rotated to show the structures of interest in the selected section plane.

segmented images into a 3D stack can be at least partly automated. Some techniques, including confocal microscopy, even allow direct 3D reconstruction of data from optical sections through small tissue blocks[9]. But for many types of analysis, semi-automatic or manual data-acquisition methods are required. Hundreds of neuroscience laboratories now use image-combining computerized microscopy — for example, the Neurolucida system (MicroBrightField, Vermont, USA) — to acquire accurate information about the morphology of individual neurons and localization of neurons or other elements in tissue sections. Commercial and custom software tools are used to reconstruct the spatial distributions in 3D.

Image analysis and 3D reconstruction alone, however, are not sufficient to analyse fully spatial organization in brain sections. To compare data from different brains efficiently and reliably, common coordinate systems and dynamic atlasing approaches are needed. The neuroimaging community has developed several methods and strategies to deal with these issues.

Coordinates and dynamic atlases

Coordinate systems are used to describe locations in the brain. Structures in the brain, or landmarks in the skull, are used to define global coordinate systems. Conventional atlas line drawings are usually shown in a skull-based coordinate system[3–5]. Local coordinate systems cover only smaller parts of the brain and are defined by boundaries and landmarks in the tissue. A local coordinate system does not necessarily have to fit the exact size and shape of the analysed region, as long as it is based on distinct and easily reproducible boundaries or landmarks. For anatomy at the light microscopic level, local coordinate systems are particularly useful for assigning exact locations to distribution data. Local coordinate systems also facilitate comparison of data collected in different experimental animals.

One example of a local coordinate system is the recently introduced 3D coordinate system for the rat pontine nuclei[7,8,10] (FIG. 2). The pontine nuclei form the main cell group that is intercalated in the pathways from the cerebral cortex to the cerebellum. The coordinate system for these nuclei uses a cuboid bounding box with a specific orientation that is fitted to cytoarchitectonic boundaries and other nearby landmarks. The location of the bounding box is defined in relation to standard atlas coordinates, allowing transfer of data to other coordinate systems[10]. The pontine-nuclei 3D coordinate system has been

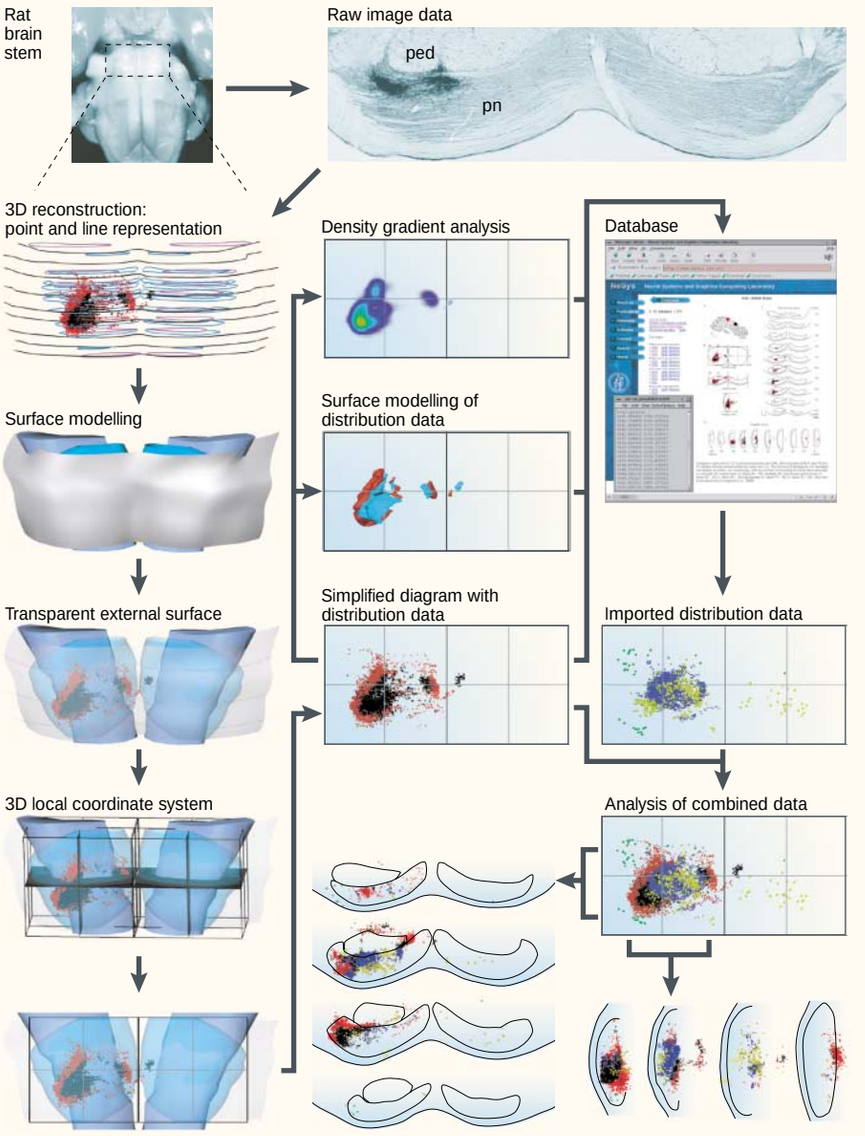

Figure 2 | **Example of computerized analysis of neuronal distribution data.** A part of the brain in an experimental animal is isolated and sectioned. Images from serial sections are used as a basis for collecting information about the distribution of specific neuronal elements. In this example, the elements are axonal plexuses in the pontine nuclei that were labelled after tracer injections into individual whisker barrels of rat primary somatosensory cortex (data from REF. 8, available in the NeSys Database). In the initial analysis, lines represent the external brain surface and boundaries of brainstem regions and fibre tracts, whereas points represent experimental distribution data (two categories of tracer-labelled terminal fibres, shown as red and black dots). Computerized methods for visualization and analysis are used. A three-dimensional (3D) coordinate system is introduced to define the region of interest. This regional coordinate system is made up of a bounding box with a specific orientation and location that are determined by local landmarks and cellular and fibre architecture. A simplified diagram is used to show the distribution data in the local coordinate system. Examples of further computational analysis of the distribution data (surface modelling of the main clusters of points and density gradient analysis for one of the point categories) are shown. The new experimental data are deposited in a database. Data from the same brain region and different experimental animals are superimposed in the same coordinate system. From the database, different data sets can be downloaded and combinations of data viewed and further analysed. In this example, the data downloaded from the database (blue, yellow and green dots) represent distributions of neuronal cell bodies that are labelled after tracer injections into another part of the brain (the cerebellum, which receives input from the pontine nuclei). Dynamic subdividing of the combined data sets into sections of chosen thickness and orientation (lower right) add a further dimension to the analysis of the 3D topographic map. Ped, peduncle; pn, pontine nuclei.

used successfully for presentation of data that was obtained from different brains. Data from 70 animals have so far been superimposed in this coordinate system, analysed with software tools for advanced 3D analysis and made available in web-based archives. These archives will, in turn, serve as a basis for developing a digital and dynamic atlas of structure–function relationships in this specific part of the brain. Computerized tools that are suitable for analysing combinations of distribution data (available in the same 3D coordinate system) include density-gradient analysis, surface modelling to illustrate shape and size of clusters of cells or other elements, and re-slicing at chosen angles of orientation[11] (FIG. 2).

Three-dimensional coordinate systems offer distinct advantages for studying deeper structures of the brain and are also used for analysing cerebral cortical distribution data. Two-dimensional (2D) coordinate systems, however, provide an alternative for the cerebral cortex. The cortex is a laminar structure that is topologically equivalent to a 2D sheet. Advanced software for flattening the complicated folded primate cortex has been developed and used in several anatomical and physiological investigations that are based on light microscopic section data[12,13]. Several new tools that are aimed at assisting in the analysis of fMRI data have been developed to further facilitate cortical distribution analysis. They include optimized flattening software, tools for the transfer of data from the cortex of different brains onto an average representation and new surface-based coordinate systems[14]. The neuroimaging community, which relies on powerful tools for analysis of a rapidly increasing pool of data, is a driving force in this endeavour.

Individual variability is an important concern for all brain distribution analysis. In the example of the rat brainstem (FIG. 2), this variability is limited. An accurate analysis can be carried out by directly superimposing data from different animals in the same internal nuclear coordinate system, adjusting only for size differences (linear warping). For example, repeated, identical experimental manipulation in a series of rats might produce almost identical distribution patterns of data that are superimposed in the same local coordinate system[8]. However, the nervous systems of higher mammals are known to have larger individual variability, which requires various measures to allow comparison of data from different animals.

In the cerebral cortex of primates, variability is particularly pronounced for gyral folding, and for size, shape and distribution of structurally and functionally defined areas.

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Cortical variability is a research topic in itself, as it presumably accounts for many of the differences in behaviour that define human individuality. Variability is therefore one of the factors that have motivated the establishment of probabilistic human brain atlases that are based on large populations of subjects[15–17]. A crucial aim of these atlases is to provide, for any given location in the brain, probability distributions and confidence limits for the assignments of structural and functional characteristics, including descriptions of imaging activation data in relation to cortical locations. Toga[18] has reviewed, in detail, the complex methods that are involved in the creation of probabilistic atlases, including warping of images from different experimental data sets.

Databases

The neuroimaging field provides examples of different strategies for creating digital atlases and databases. Recently, functional images of the human brain have been made available through web-accessible databases. Several consortia of researchers have set up mechanisms that allow the incorporation of new imaging data for analysis and comparison with information that is already available in the database[19,20]. One example is the NEUROGENERATOR initiative, which is linked to the European Computerized Human Brain Database[21]. This is a 3D database for relating imaging data, primarily PET and fMRI data, to cortical microstructure and, hence, area localization. The database contains detailed information about cortical cellular architecture and neurotransmitter receptor distributions, as revealed by image analysis of post-mortem brain sections. The database uses a new concept. It creates daughter databases with homogeneously processed data, which are then distributed back to the submitters, allowing advanced meta-analysis and modelling of the human brain at the systems level on the basis of homogeneous functional data. This approach allows the creation of flexible and distributed databases, which are closely linked to the specific topics that are studied in the individual neuroscience laboratories. This and other imaging databases and tools might serve as examples for several disciplines that rely on analysis of section-based data sets.

Concluding remarks

Here, I have taken a 'localization' perspective on microscopic data from the brain. Neuroanatomists are used to dealing with brain architectonics and classical 2D atlases for assigning locations to their data. But descriptions of locations are often ambiguous. With the greatly increasing amount of neuroscience data that are being produced, neuroscientists need to avoid valuable data being lost in a state of spatial and nomenclature confusion. Examples of measures that should be considered are the use of more efficient and reliable data-acquisition methods, the use of 3D reconstruction when possible, presentation of distribution data in global or local coordinate systems and sharing of data through web archives or databases. Several tools and approaches that have been developed by the neuroimaging community could be adopted by other neuroscience disciplines, and could help to improve analysis of brain distribution data. From the perspective of the investigator of the rodent brain, the small size of the mouse and rat brains is particularly encouraging, as it makes 3D reconstruction much easier. In addition, the relatively small individual variability of the rodent brain invites investigators to use coordinate systems that will facilitate precise description of localization and comparison of data across animals and laboratories.

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Online links

FURTHER INFORMATION
BrainMap: http://ric.uthscsa.edu/brainmap/
Cerebellum Database: http://www.cerebellum.org/
Encyclopedia of Life Sciences: http://www.els.net/
bioinformatics | biological data centres | mining biological databases
European Computerized Human Brain Database: http://fornix.neuro.ki.se/ECHBD/Database/index.html
fMRI Data Center: http://www.fmridc.org/
International Consortium for Brain Mapping: http://www.loni.ucla.edu/ICBM/index.html
MIT Encyclopedia of Cognitive Sciences: http://cognet.mit.edu/MITECS/
magnetic resonance imaging | positron emission tomography
NeSys Database: http://www.nesys.uio.no/
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NEUROGENERATOR: http://www.neurogenerator.org/
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