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A multimodal, multidimensional atlas of the C57BL/6J mouse brain

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Abstract

Strains of mice, through breeding or the disruption of normal genetic pathways, are widely used to model human diseases. Atlases are an invaluable aid in understanding the impact of such manipulations by providing a standard for comparison. We have developed a digital atlas of the adult C57BL/6J mouse brain as a comprehensive framework for storing and accessing the myriad types of information about the mouse brain. Our implementation was constructed using several different imaging techniques: magnetic resonance microscopy, blockface imaging, classical histology and immunohistochemistry. Along with raw and annotated images, it contains database management systems and a set of tools for comparing information from different techniques. The framework allows facile correlation of results from different animals, investigators or laboratories by establishing a canonical representation of the mouse brain and providing the tools for the insertion of independent data into the same space as the atlas. This tool will aid in managing the increasingly complex and voluminous amounts of information about the mammalian brain. It provides a framework that encompasses genetic information in the context of anatomical imaging and holds tremendous promise for producing new insights into the relationship between genotype and phenotype. We describe a suite of tools that enables the independent entry of other types of data, facile retrieval of information and straightforward display of images. Thus, the atlas becomes a framework for managing complex genetic and epigenetic information about the mouse brain. The atlas and associated tools may be accessed at http://www.loni.ucla.edu/MAP.

Key words brain atlas; HRI; infomatics; mouse database; C57BL/6J map.

Introduction

Atlases not only have immense pedagogical value, but also provide a framework for researchers studying normal, mutant and transgenic animals. Traditional atlas construction typically involves sectioning, staining and recording of photomicrographs, but recent advances have expanded the atlas concept (Toga & Thompson, 1998). Atlases incorporate three critical elements: (1) graphical reconstructions highlighting important anatomical detail; (2) nomenclature and description of anatomical structures; and (3) a three-dimensional (3D) coordinate system. Franklin & Paxinos (1997) and Hof et al. (2000) have published this type of mouse brain atlas.

In book form, the intrinsically 3D brain must be viewed as a series of 2D sections, making it difficult to follow 3D structures and to compare one's own invariably oblique sections with the orthogonal planes of the atlas. Data acquired from different individuals at different ages, with subtle variations in technique, or in different planes of section, make it nearly impossible to compare results accurately. Digital atlases obviate both

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of these vexing problems (Toga et al. 1995; Gibaud et al. 1998; Swanson, 2001).

In a digital atlas, complex structures can be navigated and computationally sectioned at arbitrary angles. They can be viewed independently or in conjunction with other structures to understand better their relationships with one another. Additionally, a multimodal digital atlas can encompass many different kinds of data, allowing the investigator to visualize co-varying patterns simultaneously. Maps can be generated that amalgamate data from various experimental techniques, and quantitative measures of anatomy can be determined (e.g. structure volume, cross-sectional area, orientation or complexity). These data are not limited to image data, but easily extend to text-based information such as nomenclature, descriptions of gene expression or even literature citations. All are easily accessible from electronic databases and links to sources on the web.

Magnetic resonance imaging has revolutionized our ability to investigate brain structure and function. Techniques are now available to capture features of anatomy and function at both the molecular and the whole-brain scales. Thus, neuronal dynamics and gene expression patterns can be mapped at microscopic resolutions in normal adult and developing animals, as well as in pathological or genetically modified states (Ahrens et al. 1998; Benveniste et al. 1998, 2000; Jacobs et al. 1999; Dhenain et al. 2001). A comprehensive framework that encompasses genetic information in the context of anatomical imaging holds tremendous promise for producing new insights into the relationship between genotype and phenotype.

Temporal and spatial gene and protein expression patterns, axonal trajectories, patterns of vasculature and specific functional responses all can be combined to obtain a standard or canonical representation. The resulting atlas can easily be extended to include the entire animal. Such a data set could potentially embody all quantitative information known about the organism in a digital framework.

Motivated by such benefits, several digital atlases have been created. There are at least two commercially available CD-ROM mouse atlases (Hof et al. 2000; Paxinos & Franklin, 2001), several rat atlases (Swanson, 1992, 1998; Franklin & Paxinos, 1997) and other non-commercial CD-ROM undertakings (Ghosh et al. 1994; Smith et al. 1994). A number of Internet sites present a variety of 2D data: the Mouse Brain Library (Rosen et al. 2000; http://www.mbl.org/) the High Resolution Mouse Brain Atlas (http://www.hms.harvard.edu/research/brain) and the Edinburgh Mouse Atlas Project (http://genex.hgu.mrc.ac.uk). Several software packages exist for visualization of different data modalities and brain alignment (the Neuroterrain project, http://www.neuroterrain.org) as well as some with an aim towards being 3D atlases (Toga et al. 1995). The Mouse Atlas and Gene Expression Database Project has made a significant effort to create a gene expression database (Ringwald et al. 1994) based upon the Atlas of Mouse Development (Kaufman, 1992). The work of the Informatics Center of the Mouse Neurogenetics (http://www.nervenet.org) includes a comprehensive library of Nissl-stained images of brains of over 100 strains of mice and a set of software tools for 2D and 3D visualization and reconstruction of different brain regions of interest. In the same direction, the High Resolution Mouse Brain Atlas project developed at the Harvard Medical School aims to construct 2D and 3D atlases of the brain of the C57BL/6J mouse strain fully accessible over the Internet.

We have produced an electronic atlas of the rat (Toga et al. 1995) and a multimodality atlas of the Nemestrina monkey (Cannestra et al. 1997) incorporating magentic resonance imaging (MRI), positron emission tomography (PET), computer tomography (CT) and blockface imaging data. In our laboratories, the effort of the International Consortium for Brain Mapping (ICBM) is based on digital 3D representations of a population's anatomy (Mazziotta et al. 1995). We have also created a variety of human atlases that describe anatomical detail from multiple modalities and disease states (Thompson & Toga, 1997; Toga et al. 1997). This paper reports the development of a multimodal, multidimensional atlas of the C57BL/6J mouse brain.

Methods

Mice

C57BL/6J male mice 100 days old (Jackson Laboratories) were used for the atlas, although a volume database contains data from mice of various ages. All animals were housed and treated in accordance the UCLA Animal Research Committee guidelines.

Magnetic resonance microscopy (MRM)

Mice were anesthetized initially with ketamine/xylzaine and then maintained on isofluorane for the duration

of the imaging experiment. Magnetic resonance imaging was done at 37 °C using an 89-mm vertical bore 11.7-T Bruker Avance imaging spectrometer with a microimaging gradient insert and 30-mm birdcage RF coil (Bruker Instruments). Typical imaging parameters were as follows: T2-weighted RARE 3D imaging protocol (eight echoes), matrix dimensions = 256 × 256 × 256; field of view (FOV) = 3 cm × 1.5 cm × 1.5; repetition time (T_R) = 1500 ms; effective time (T_E) = 10 ms; number of averages = 4. The images were padded with zeros to double the number of time domain points in each dimension, and the Fourier transformed to yield a matrix of 512 × 256 × 256. This procedure is commonly called 'zero-filling' and is a well-known interpolation method (Farrar & Becker, 1971; Fukushima & Roeder, 1981). Typical spatial resolution was approximately 60 μm^3 per voxel.

Blockface and histology

The mice were then euthanised by an overdose of halothane (Sigma) according to procedures approved by the UCLA Animal Research Committee. The animals were perfused intracardially using a Minipuls II peristaltic pump (Gilson) at very low pressure with chilled phosphate-buffered saline (PBS) for approximately 2 min and FormaldeFresh (Fisher) for 15 min. The brains were removed and post-fixed in FormaldeFresh for 16 h. After post-fixation the brains were dipped in a mixture of india ink (Pelikan) and 5% gelatin (Sigma) to simplify segmentation of tissue from background later. The brains were cryoprotected in a solution of 20% sucrose for 16 h to prevent freezing artefacts. The brains were then embedded in OCT compound (Sakura) at 4 °C and snap-frozen at –70 °C in a 2-methylbutane/dry ice bath. The brains were attached to a chuck with OCT compound (Sakura), and serial 50-μm-thick transverse (coronal) sections were cut on a modified CM3050S cryostat (Leica). A DMCIe digital camera (Polaroid) captured images of the blockface prior to each section at a resolution of 1600 × 1200 (approximately 6.7 μm pixel^{-1}) in 24-bit colour. Sections were Nissl-stained (thionin) as described (Simmons & Swanson, 1993), myelin-stained using a modified myelin impregnation stain (Gallyas, 1979) or acetylcholine esterase stained as described (Vacca, 1985). Immunohistochemistry was performed on free floating sections with either anti-human MBP1-118 (1 : 10 000) (Accurate) or anti-bovine GFAP (1 : 500) (Dako). Stained preparations were digitized using a 1.25× objective on an AX70 microscope (Olympus) with a DMX-1200 digital camera (Nikon) at a resolution of 3840 × 3072 (approximately 3 μm pixel^{-1}) in 24-bit colour. The images were acquired using a macro imaging system that provided undistorted high-resolution images with even illumination across the entire field of view.

Image processing

Two-dimensional digital images were brought into linear register with Baladin (Institut National de Recherche en Informatique et en Automatique) (Ourselin et al. 2001) using a rigid-body transformation. Baladin registers image volumes by comparing many corresponding small windows across both volumes, calculating a transformation to bring each pair of windows into register and then generating a transformation for the entire volume based on the sum of all the window transformations. The algorithm begins by registering a subsampled representation of the volumes and repeats the process at progressively higher resolutions until the volumes are registered at full resolution. The registered images were reconstructed into 3D volumes using Reunite (Automated Image Registration 4.0) (Woods et al. 1998a,b). Three-dimensional digital volumes were subsequently brought into register with a diffusion-weighted MRM in a common coordinate system (defined by the midsagittal plane and the interaural line; Paxinos & Franklin, 2001), again using Baladin. All image processing was done in the LONI Pipeline Processing Environment (Rex et al. 2003) on either a 32-processor Onyx 200 or 64-processor Origin 3000 supercomputer (SGI).

Nomenclature and delineations

Neural structures (including cell groups, fibre tracts and gross anatomical features such as the ventricles) were determined under the microscope from the histologically stained sections. Three-dimensional label volumes were 'painted' onto co-registered MRM, Nissl-, myelin- and acetylcholine esterase-stained volumes using BrainSuite (Shattuck & Leahy, 2002). Anatomical delineations were prepared by tracing digital images from these serially stained sections using Illustrator 9.0 (Adobe). Three-dimensional surfaces were reconstructed by exporting the delineations from Illustrator to LightWave (NewTek) and manually building models from the delineations.

Results

The Mouse Atlas Project

We have developed, and continue to refine, a multimodal, multidimensional mouse brain atlas to address the limitations of traditional book-form atlases and their static digital counterparts. The goal of the Mouse Atlas Project (MAP) is to empower researchers with the ability to compare gene expression patterns with a conveniently accessible inventory of digital brain maps. It aims to produce a powerful imaging framework to house and correlate gene expression with anatomical and molecular information drawn from traditional and novel imaging technologies. Genetic data acquired *in situ* or from tissue samples will be directly comparable with structural, metabolic, physiological and genetic imaging data from well-characterized populations.

We have collected data from the adult C57BL/6J mouse using multiple imaging modalities. The MAP atlas incorporates MRM, blockface imaging, classical histology and immunohistochemistry. Complete volumes range from a spatial resolution of approximately $100 \times 100 \times 100\ \mu m^3$ ($128 \times 128 \times 256$ voxels, 4.2 Mb uncompressed) for a low-resolution greyscale MRM volume to $1 \times 1 \times 50\ \mu m^3$ ($14400 \times 13200 \times 330$ voxels, 178 Gb uncompressed) for a high-resolution full-colour Nissl-stained volume. These volumes are available for download at http://www.loni.ucla.edu/MAP/Atlas/Databases.html. The data were reconstructed into 3D volumes, transformed quantitatively into a defined and common coordinate system, and described anatomically. A set of visualization, database and mapping tools complete the atlas.

Modelling, comparative neuroanatomy, measurements of tissue and cell dimensions, and analyses of gene influences on strain differences all require original, undistorted representations of tissue. However, all histological processing methods introduce distortions. In order to minimize histological distortions, we froze brains embedded in OCT embedding compound, rather than dehydrating and embedding them in celloidin or paraffin. In addition, our data were registered to an MRM volume of the same animal obtained prior to sectioning. This allowed us to represent the brain in a form closer to its *in vivo* morphology.

MRM images were recorded using Rapid Acquisition Relaxation Enhancement (RARE) and diffusion-weighted MR imaging sequences. RARE MRM images were acquired from both *in vivo* and post-mortem subjects. Diffusion-weighted volumes show a great deal of gross anatomical detail and good contrast between grey and white matter (Fig. 1a).

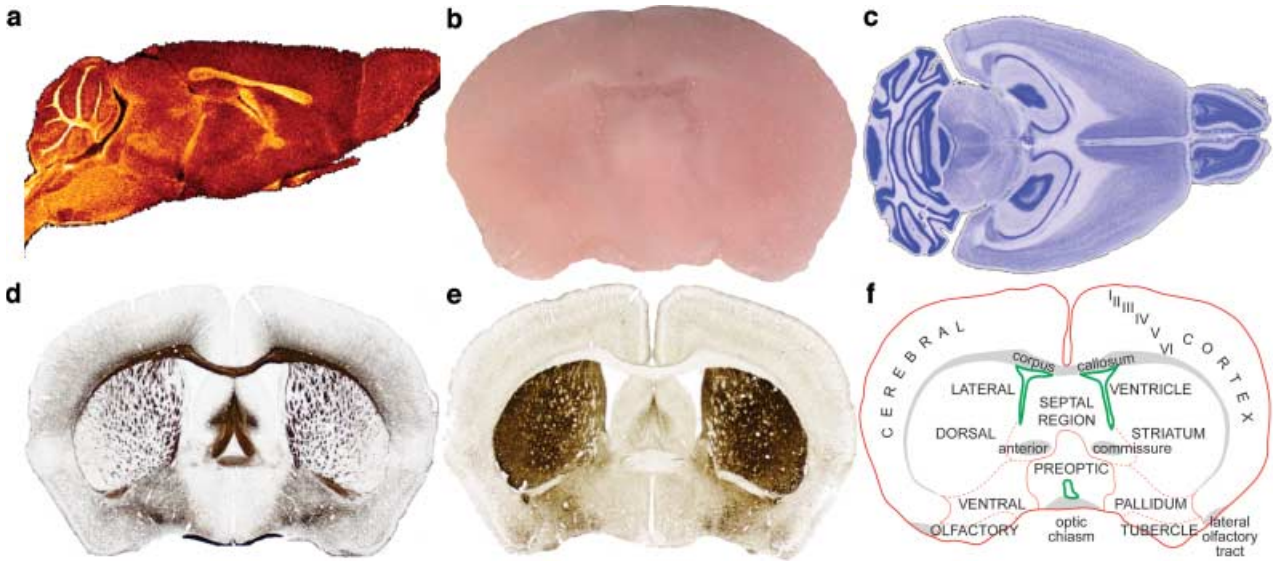

Fig. 1 Multiple modalities and planes of section. Data are shown in several planes of section to demonstrate the inherently 3D nature of the atlas. (a) An MRM scan of a 100-day-old mouse brain using a *z*-direction diffusion-weighted imaging protocol. (b) A transverse (coronal) section from a blockface imaging volume of a 100-day-old mouse brain. (c) A horizontal section from a Nissl-stained volume of a 123-day-old mouse brain. (d) A transverse (coronal) section from a myelin-stained volume of a 100-day-old mouse brain. (e) A transverse (coronal) section from an acetylcholine-esterase-stained volume of a 100-day-old mouse brain. (f) A transverse (coronal) section from a volume of delineations of a 100-day-old mouse brain. The image has been greatly simplified for clarity. Text labels have been added for illustrative purposes only.

Blockface imaging is a colorimetric imaging modality free of many of the spatial artefacts that affect serially stained sections mounted on glass slides: shatter, tears, bubbles and other mechanical distortions. High-resolution colour images of the blockface are acquired as it is sectioned, relying on the inherent contrast of white and grey matter to discriminate anatomical boundaries. A number of gross structural subdivisions of the brain are visible, even without the benefit of histological staining. For example, the internal capsule and the hippocampus are clearly discernible (Fig. 1b).

Atlases traditionally have been composed of collections of histologically stained sections produced to visualize anatomy. Nissl-stained sections provide a wealth of information about cortical lamination and the topography of subcortical nuclei (Fig. 1c). Myelin-stained sections complement the cytoarchitectural data with myeloarchitecture, delineating fibre tracts and helping to define nuclei (Fig. 1d). Acetylcholine-esterase-stained sections provide additional information about the chemoarchitechture of the brain (Fig. 1e).

Anatomical delineations serve to help orientate the user, with graphical representations highlighting important anatomical detail and providing a standard description and nomenclature of the region of interest (Fig. 1f). The delineations depict asymmetries present in the sections, making them more immediately useful than if they were stylized. Delineation of brain nuclei requires an expert neuroanatomist to draw on high-level knowledge, accumulated over a lifetime of careful study of disparate materials (Swanson, 1998). Consequently, manual input was necessary for even approximate compartmentation of brain in its fine details. In the development of a comprehensive, standardized and mutually exclusive nomenclature (Bowden & Martin, 1995; Bard et al. 1998) and anatomical delineation, our primary references were the mouse brain atlases of Hof et al. (2000) and Franklin & Paxinos (1997) and inconsistencies were resolved by Swanson (1998).

Gene expression maps (GEMs) are a crucial aspect of the atlas. Visualizing and measuring gene expression patterns may aid in discerning the relationship between genotype and phenotype. Thus far, immunohistochemistry for various neuronal and glial markers – Neurotrophin 3 Tyrosine kinase receptor C (TrkC), Glial Fibrillary Acidic protein (GFAP), and myelin basic protein (MBP) – has been carried out on serial sections (Fig. 2). The atlas was developed with the intent of allowing researchers to import their own GEMs in to the atlas. The GEM Importer (see below) will permit users to register their GEMs to the atlas, allowing them to access all of the atlas tools for use with their data.

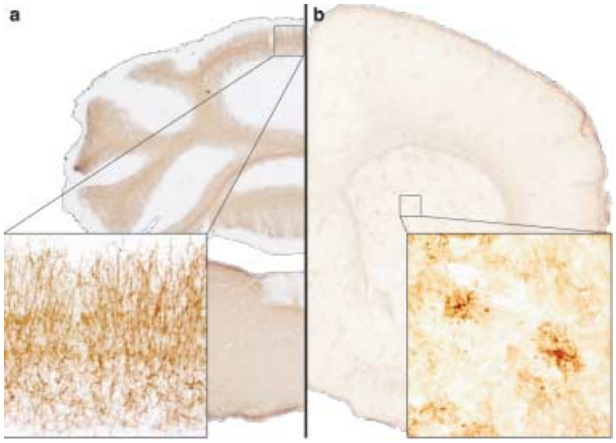

Fig. 2 Gene expression maps: immunohistochemistry. (a) A low-magnification (0.5×) image of a transverse (coronal) section from a myelin basic protein-stained volume of a 100-day-old mouse. The inset is a magnified (10×) image demonstrating individual myelinated fibres visible in the cerebellar cortex. (b) A low-magnification (0.5×) image of a transverse (coronal) section from a glial fibrillary acidic protein-stained volume of a 100-day-old mouse. The inset is a magnified (10×) image demonstrating individual staining astrocytes visible in the dorsal striatum.

LONI visualization environment: MAP

The data that constitute an atlas may take many forms, from the traditional collection of histological images to anatomical ontologies to lists of genes expressed in a given structure. Displaying image data is relatively straightforward, but displaying multiresolution data, associating it with an essentially text-based representation of the brain and keeping everything synchronized is not. In order to fulfil the goal of producing a framework that would incorporate not only image-based data, but also text-based representations and non-volumetric data (such as the image of a Northern blot or the results of a microarray), we developed the LONI visualization environment: MAP (MAP Atlas Viewer). The MAP Atlas Viewer is a self-contained, platform-independent visualization package developed for the display of both text- and image-based atlas data. It is capable of visualizing multiple volumetric and 3D surface datasets simultaneously at multiple resolutions, updating information automatically. Three imaging volumes are distributed with the MAP Atlas Viewer, as well as a set of anatomical delineations in both volumetric and 3D surface representation. Through BrainGraph

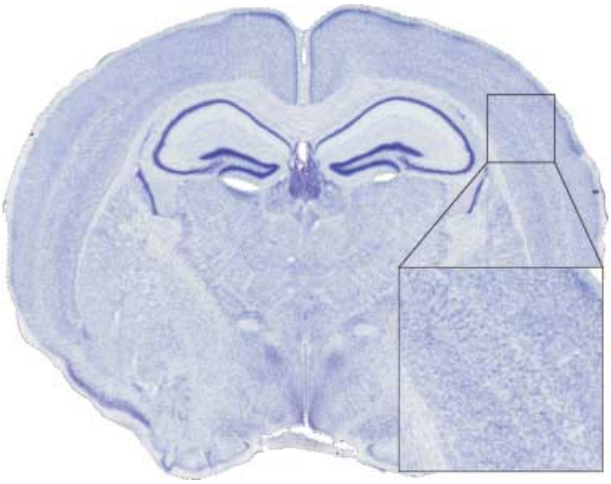

Fig. 3 Very high-resolution display. A transverse (coronal) section through a Nissl-stained volume from a 100-day-old mouse. Volumes are acquired at very high resolution, and although displayed locally at limited resolution, can be viewed at full resolution (inset) upon demand.

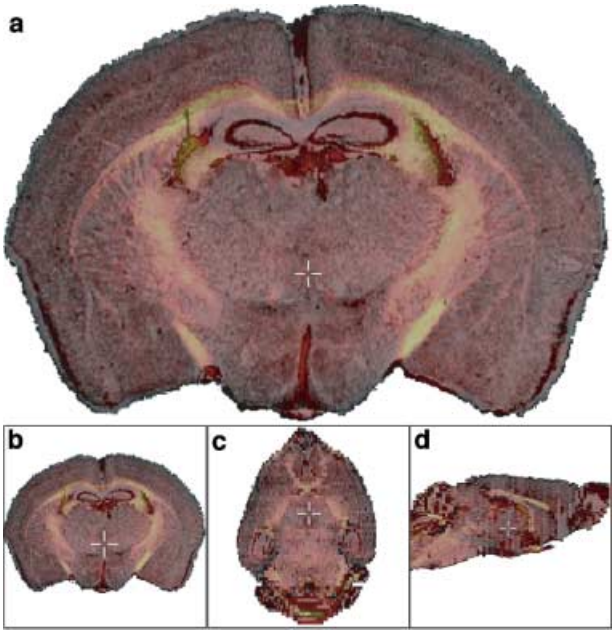

Fig. 4 A digital atlas: high-resolution display and orthogonal sections. A high-resolution view of the image data, an MRM volume and a Nissl-stained volume from a d100 mouse (shown overlaid), is presented the user, much like a traditional atlas. Small, low-resolution thumbnails are for navigation; **b**, sagittal, **c**, transverse (coronal), and **d**, horizontal sections through two volumes.

(see below), the user can query the Brain Architecture Management System (BAMS), a knowledge management system, for nomenclature, connections, cell types and further information about any structure (Bota et al. 2003).

The MAP Atlas Viewer is distributed with three sample volumes: a blockface imaging volume, a Nissl-stained volume and a diffusion-weighted MRM volume, each at a resolution of $60 \times 60 \times 60\ \mu m^3$ ($256 \times 256 \times 256$ voxels, 16.8 Mb uncompressed). More volumes, including a collection of GEMs, are available through the MAP volume database at the MAP website (http://www.loni.ucla.edu/MAP/Atlas/Database.html). Magnification of up to 8× ($3\ \mu m \times 3\ \mu m$ resolution) is attainable on the sample volumes by connecting to a central atlas server application with access to the higher resolution volumes (Fig. 3).

Anatomical delineations are fundamental to the atlas. Two sets of delineations are bundled with the MAP Atlas Viewer, a set of volumetric labels and a set of 3D surfaces. The label volume provides the basis for the interaction between image-based data volumes and text-based information networks. They allow us to reference the name of a given structure and synchronize the location of the cursor with the appropriate structure in both BrainGraph and BAMS. Three-dimensional surfaces may be viewed in relation to each other or sectioned at arbitrary angles, permitting the user a more intuitive grasp of the neuroanatomy of the C57BL/6J mouse.

The basic user interface is composed of four panels (Fig. 4). As shown in Fig. 4, three panels represent orthogonal sections through the volume, the fourth a high resolution view of the data, all of which contain superimposed images of the currently loaded volumes. The user can navigate through the volume by moving the crosshairs. A status bar at the bottom of the window displays the stereotaxic coordinates and the name of the anatomical structure that contains the crosshairs. Three tabs over the fourth panel are higher-resolution views of the orthogonal planes; the views may be changed by selecting the appropriate tab. Three more tabs display the 3D Viewer, BrainGraph and BAMS, respectively.

The MAP Atlas Viewer is capable of volume and surface rendering, greatly facilitating the visualization of data acquired at arbitrary angles. The user can select an arbitrary plane of section to view a volume-rendered representation of the data. The anatomical delineations, stored as surfaces overlaid upon the data volumes, can then be viewed as 2D curves upon that plane. Thus users can view delineations regardless of the angle at which the data were collected (Fig. 5).

GEM Importer

GEMs can easily be imported into the atlas. We have developed a mechanism for automatically masking,

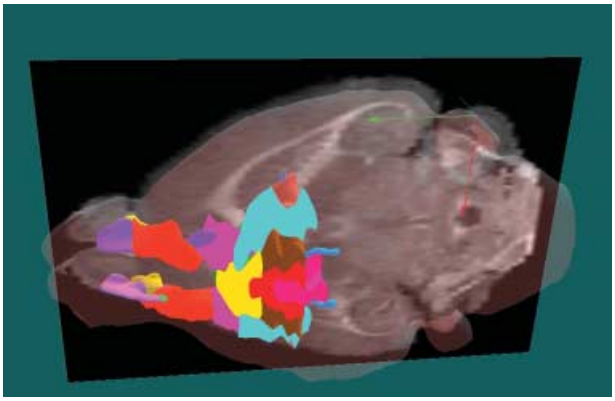

Fig. 5 Three-dimensional delineations intersected by an arbitrary plane. Three-dimensional surface reconstruction of anatomical delineations showing only structures in the anterior end of the brain for clarity. The outer surface of the brain and cortical structures have been rendered transparent and a yellow cube has been added to aid in orientation. The reconstruction has been cut with an arbitrary plane (not visible). The inset is an image of the contours that are the result of the intersection of the surfaces with that arbitrary plane.

registering and reconstructing a collection of 2D slices into a 3D volume occupying the same image space as the atlas. GEMs can be imported into the atlas using the GEM Importer within the LONI Pipeline Processing Environment (Rex et al. 2003). Specially designed pipelets take as input a directory containing a series of tagged image file format (.tif) images, segment them, align them, reconstruct them and bring them into register with the atlas. Thus, a researcher can import their data into the atlas and take advantage of its tools. Other forms of data, such as textual descriptions, microarray images and electron micrographs, may also be imported into the atlas by means of the BrainGraph (see below).

BrainGraph

Nomenclatures are text-based representations of anatomy arranged in a format that emphasizes a hierarchical relationship among the structures of the brain or even an entire organism (Bowden & Martin, 1995; Bard et al. 1998; Swanson, 1998). This kind of hierarchy can provide a standard framework for the visualization of data of differing levels of resolution and against which to reference many kinds of neuroanatomical information.

However, neuroanatomical labelling schemes differ significantly in their organization (Swanson, 1998, 2000). In addition to anatomically (Swanson, 1998) and developmentally (Bard et al. 1998) defined parcellations, there also exist approaches that systematically organize the hierarchy of structures based on cytoarchitectural (Riedel et al. 2002), chemoarchitectural (Colby et al. 1988) or functional connectivity (Van Essen et al. 1998). In addition, purely hierarchical ontologies based on anatomical containment are often insufficient to represent the complex relations between different regions of the brain.

BrainGraph is a flexible, extensible, graph-based data model that integrates, organizes and directly accesses external structural, functional, histological and genetic data. It allows simultaneous storage of multiple labelling schemes and study-specific graph traversal schemes. Each node (structure) and each edge (relationship) has a number of predefined, or user-specifiable, description categories (e.g. functional connectivity, neurotransmitters, anatomical relation, developmental relation, gene expression or literature references) permitting the user to navigate through their data in a variety of ways (Fig. 6a). The researcher can also add their own links to other forms of information in the form of URLs. They can add connections to other web-based databases or can add links to image data, such as pictures of microarray results. Each node in the BrainGraph is in turn linked to other sources of text-based information, such as the BAMS.

BAMS

The BAMS is an extensible text-based framework that can be used to navigate the brain and access various forms of textual information (Adelman & Riedel, 1997; Bota & Arbib, 2000, 2002; Bota et al. 2003). It can be used as a source of information pertaining to brain structures collated from the literature, and as a system for organizing the neuroscientific data characteristic of a given structure. The knowledge base of the BAMS provides for the online insertion, processing and retrieval of neuroscientific information characteristic at different levels of organization of the mammalian central nervous system: from functional networks of brain structures, to cytoarchitecture and connectivity patterns of different brain nuclei. BAMS allows users (Fig. 6b) to view the hierarchical organization of different brain nomenclatures as well as the cytological profiles of structures of interest. The system currently contains more than 1000 reports related to brain structures in mouse (adapted from Swanson, 1998) and human (adapted from *Nomina Anatomica*, International Anatomical Nomenclature Committee and International Congress

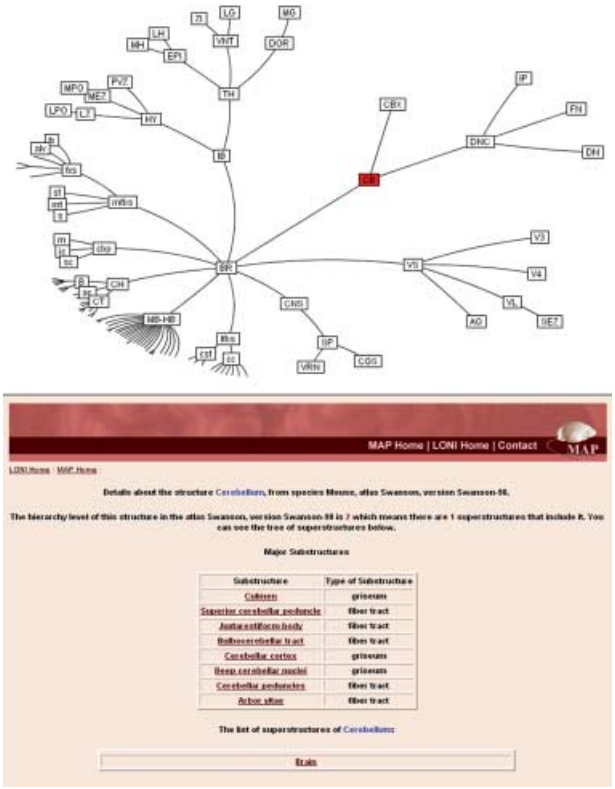

Fig. 6 BrainGraph and the Brain Architecture Management System. (a) BrainGraph representation of the cerebellum showing the superstructure brain and substructures cerebellar cortex and deep cerebellar nuclei. (b) The BAMS contains information about the superstructures that contain the region of interest, substructures contained in it and an inferred cytological profile of it.

of Anatomists, 1983)). It is accessible from BrainGraph and may also be accessed independently at http://brancusi.usc.edu/bkms.

Discussion

One of the primary uses of atlases is the localization of gene or protein expression data to an anatomical structure, a difficult task with a paper atlas owing to the variability of cutting planes and tissue processing. Using the GEM Importer tool, gene or protein expression data can be aligned to anatomical delineations within the atlas, allowing for unambiguous localization. The atlas will facilitate collaboration by providing researchers with a common framework for comparing results. Examples of such studies would be the comparison of gene or protein expression data collected in different laboratories, or finding the histological correlates of an MRM signal.

However, maintaining all of the currently existing atlas information would require a centralized atlas server. We are developing such a server, capable of maintaining multiple atlases simultaneously. Users would request which version of the atlas they required (delineations and nomenclature based on Paxinos and Watson, or Bloom and Hof), and the server would provide a set of delineations appropriate to the request. The atlas server would also maintain high-resolution images of the atlas data that could be sent to users upon request.

Possible future directions include the continued refinement of the atlas, extending it to cellular resolution and increasing its connectivity to other electronic databases such as the Gene Expression Database (GXD). This would permit the investigator to generate lists of genes expressed within a given anatomical structure that were automatically updated.

Another natural extension would be the inclusion of the rest of the nervous system (spinal cord, peripheral nerves and even retina) into a complete atlas of the mouse nervous system. Although this would be technically challenging, the benefits would far outweigh the difficulties. The creation of a curated collection of atlas data would be very beneficial to the community at large. Such a collection would consist of submissions from the scientific community at large. Over time, it could become a model for the dissemination of data and the establishment of collaborative efforts. This collection could encompass not only the C57BL/6J mouse, but also include other strains of mice, especially genetically modified animals, mutants and disease models.

Atlases can be generated from disease states as a way of better understanding the process and effects of disease. Additionally, atlases can be made from genetically modified or mutant animals. These atlases could produce great insight into the effect of genetic manipulation or mutation on the phenotype of the mouse. This perhaps will lead us to a greater understanding of the connection between genotype and phenotype.

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