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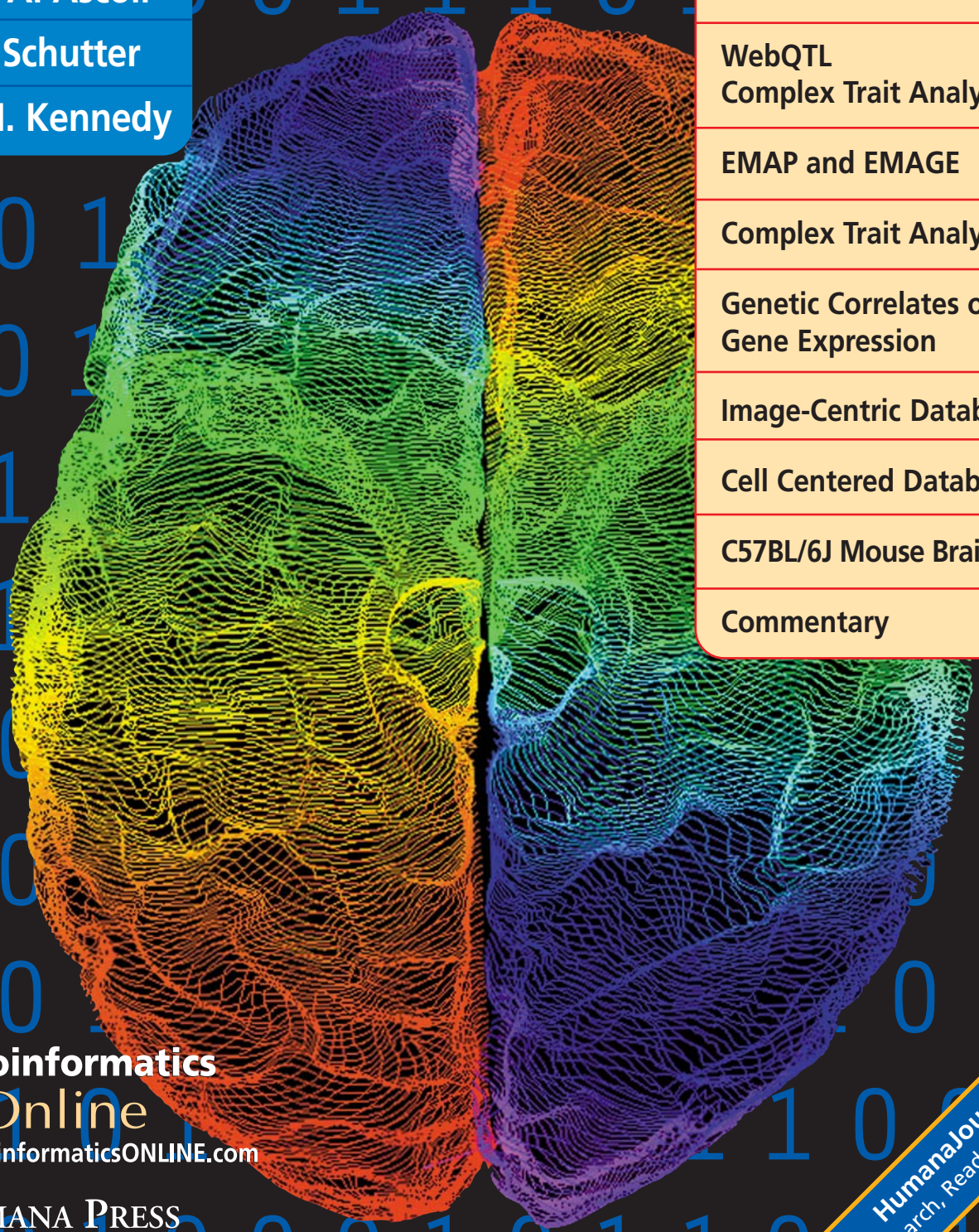

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Original Article

The Informatics of a C57BL/6J Mouse Brain Atlas

Allan MacKenzie-Graham,[1] Eagle S. Jones,[1] David W. Shattuck,[1] Ivo D. Dinov,[1] Mihail Bota,[2] and Arthur W. Toga[*,1]

[1]Laboratory of Neuro Imaging, Department of Neurology, University of California,
[2]NIBS—Neuroscience Program, University of Southern California, Los Angeles, CA

Abstract

The Mouse Atlas Project (MAP) aims to produce a framework for organizing and analyzing the large volumes of neuroscientific data produced by the proliferation of genetically modified animals. Atlases provide an invaluable aid in understanding the impact of genetic manipulations by providing a standard for comparison. We use a digital atlas as the hub of an informatics network, correlating imaging data, such as structural imaging and histology, with text-based data, such as nomenclature, connections, and references. We generated brain volumes using magnetic resonance microscopy (MRM), classical histology, and immunohistochemistry, and registered them into a common and defined coordinate system. Specially designed viewers were developed in order to visualize multiple datasets simultaneously and to coordinate between textual and image data. Researchers can navigate through the brain interchangeably, in either a text-based or image-based representation that automatically updates information as they move. The atlas also allows the independent entry of other types of data, the facile retrieval of information, and the straightforward display of images. In conjunction with centralized servers, image and text data can be kept current and can decrease the burden on individual researchers' computers. A comprehensive framework that encompasses many forms of information in the context of anatomic imaging holds tremendous promise for producing new insights. The atlas and associated tools can be found at http://www.loni.ucla.edu/MAP.

Index Entries: Anatomy; atlas; brain; C57BL/6; gene expression; mouse; MRM.

*Address to which all correspondence and reprint requests should be sent. E-mail: toga@loni.ucla.edu

Introduction

The mouse is a living encyclopedia, a repository for gene functions known and unknown. In an effort to expand our understanding of gene expression and function, the Mouse Genome Project (MGP) has undergone the task of sequencing and cataloging the entire genome of a single strain of mouse. This strain of mouse is the C57BL/6J. In addition to being the canonical mouse for the MGP, the C57BL/6J is one of the most commonly used stains of mice for scientific research. Most mutant phenotypes (naturally occurring, transgenics, and gene targeted knockout animals) are crossed back onto this stain, making it a commonly used control.

However, each year more mutant mice are generated, and with that production comes a concomitant increase in the amount of information used to describe them. Genetic maps have localized genes to specific sites on chromosomes, but their dynamic patterns of expression have only begun to be catalogued. In fact, there is no common framework to house and correlate gene and protein expression, let alone anatomic and molecular information drawn from traditional and novel imaging technologies. Digital atlases can provide the framework for the organization, analysis, and publication of large collections of data.

Atlases are composed of graphical reconstructions that highlight important anatomical detail, descriptions of anatomical structures and nomenclature, and a standardized coordinate system enabling structures to be referenced. Traditional atlas construction typically involves sectioning, staining, and recording of photomicrographs, but recent advances have expanded the atlas concept (Toga and Thompson, 1998). However, in book form, the intrinsically 3D brain must be viewed as a series of 2D sections, making it difficult to follow 3D structures or compare one's own invariably oblique sections with the orthogonal planes of the atlas. 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 better understand their relationships with one another. Additionally, a multimodal digital atlas can encompass many different kinds of data, allowing the investigator to visualize covarying patterns simultaneously, such as the expression of two genes, or functional activation with gene expression. 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 the superimposition of image volumes, but easily extend to text-based information such as nomenclature, descriptions of gene expression, or even literature citations. One could even link to images of microarrays associated with an anatomical region or one defined by functional criteria.

At least two commercially available CD-ROM mouse atlases (Hof and Young, 2000; Paxinos and Franklin, 2001), two rat atlases (Paxinos and Watson, 1998; Swanson, 1998), and other non-commercial CD-ROM undertakings (Ghosh et al., 1994; Smith et al., 1994) have been created with some of these benefits in mind. A couple of world-wide-web sites present a variety of two-dimensional data (Mouse Brain Library [Rosen et al., 2000], www.nervenet.org/mbl/mbl.html; High Resolution Mouse Brain Atlas, www.hms.harvard.edu/research/brain) and some even aim towards being 3D atlases (Toga et al., 1995). The Edinburgh group has made a significant effort to create a gene expression database (Ringwald et al., 1994). However, the wide array of data available to today's researcher requires more than most of these electronic atlases can offer. What is required is a neuroinformatics framework that can coordinate these disparate forms of data and present them to the researcher in a clear and concise way.

The Mouse Atlas Project aims to produce such a framework, in the form of a digital atlas of the C57BL/6J mouse brain, and a set of tools for interacting with it. Our objectives are: to implement a framework for mapping gene expression in the brain and to allow researchers to compare gene expression patterns with digital brain maps. Researchers will be able to import their own gene and/or protein expression data into the atlas in the form of micrographs, or even images of gels and microarrays. Slices and 3D volumes can be brought into the atlas space using the GEM Importer pipeline within the LONI Pipeline environment, whereas other forms of data can be imported within BrainGraph. Once the data is in the same space as the atlas, researchers will be able to make comparisons between it and the atlas, across their own data, or with data acquired in other laboratories.

We intend the atlas to be used as a repository for gene and protein expression maps, all in the context of structural and functional brain maps generated by MRM and novel imaging technologies. We believe that the synthesis of genetic imaging and time-varying anatomy in an accessible and interactive digital form will greatly accelerate our understanding of how gene and protein expression patterns are related to brain structure and function.

Methods

Mice

One hundred day-old male C57BL/6J mice (The Jackson Laboratory) were used for the atlas. All animals were housed and treated in accordance with the UCLA Animal Research Committee guidelines.

MRM

Mice were initially anesthetized and magnetic resonance imaging was done on an 11.7 T Bruker Avance imaging spectrometer (Bruker Instruments) at The California Institute of Technology. Typical spatial resolution was approx 60 μm^3 per voxel.

Blockface

The mice were then sacrificed by an overdose of halothane (Sigma) according to procedures approved by the UCLA Animal Research Committee. Sections were cut serially in 50 μm thick transverse (coronal) sections 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 (approx 6.7 μm/pixel) in 24-bit color.

Histology

Sections either were Nissl-stained (thionin) (Simmons and Swanson, 1993), or myelin-stained using a modified myelin impregnation stain (Gallyas, 1979). Gene expression maps were generated to neuropeptide Y (Carson et al., 2002). Protein expression maps were generated (Kahn et al., 1999) using antibodies to GFAP (Dako). Stained preparations were digitized using a 1.25X objective on an AX70 microscope (Olympus) with a DMX-1200 digital camera (Nikon) at a resolution of 3840 × 3072 (approx 3 μm/pixel) in 24-bit color.

Image Processing

The two dimensional digital images of the stained sections were segmented with MouseMask (Laboratory of Neuro Imaging). MouseMask operates on histological images using a combination of downsampling, thresholding, and mathematical morphology to identify a mask representing the brain tissue within the image. The 3840 × 3072 images were downsampled by factor of 16 in each direction, where the downsampled pixel was taken is the minimum value of the 16 × 16 block of pixels in which it was located. This has the effect of merging regions corresponding to stained structures, which appear as a number of

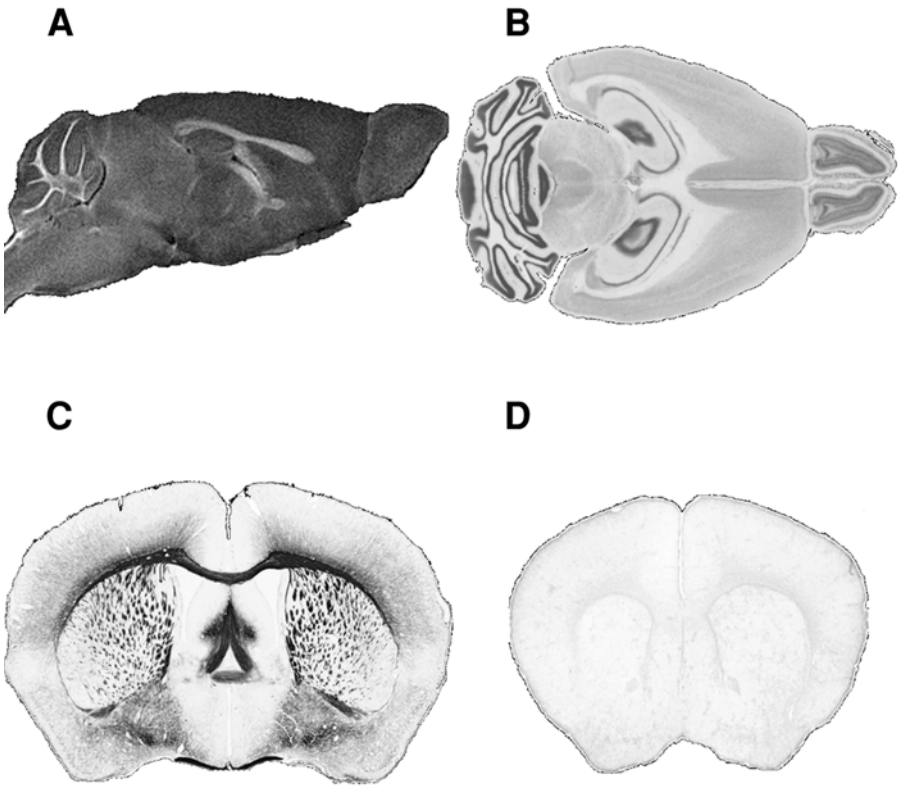

Fig. 1. Multiple modalities and planes of section. Data are shown in several planes of section to demonstrate the inherently 3D nature of the atlas. **(A)** An MRM scan of a 100 day-old mouse brain using a z-direction diffusion-weighted imaging protocol. **(B)** A horizontal section from a Nissl-stained volume of a 123 day-old mouse brain. **(C)** A transverse (coronal) section from a myelin-stained volume of a 100 day-old mouse brain. **(D)** A transverse (coronal) section from a glial fibrillary acidic protein stained volume of a 100 day-old mouse.

disconnected dots within the section. This allows MouseMask to compute a threshold, apply it to the image, and select the connected component corresponding to the imaged tissue. MouseMask then applies a mathematical closing operation, which fills breaks in the boundary of the tissue mask. MouseMask then expands the mask back to the size of the original image. The boundary of this mask will have block artifacts, hence we apply a threshold operation to the 16×16 blocks on the edge of the image. This allows MouseMask to more closely follow the boundary of the tissue. The 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. The registered images were reconstructed into 3D volumes using Reunite (Automated Image Registration 4.0) (Woods et al., 1998a; Woods et al., 1998b). Three-dimensional digital volumes were subsequently brought into register with a diffusion-weighted MRM in a common coordinate system (defined by the mid-sagittal plane and the interaural line [Paxinos and Franklin, 2001]), once again using Baladin. All image processing was done on either a 32-processor

Onyx 200 or 64-processor Origin 3000 supercomputer (SGI).

Nomenclature and Delineations

In the development of a comprehensive, standardized, and mutually exclusive nomenclature (Bowden and Martin, 1995; Bard et al., 1998) and anatomic delineation, our primary references were the mouse brain atlases of Hof (Hof and Young, 2000) and Franklin and Paxinos (Franklin and Paxinos, 1997) and inconsistencies were resolved by Swanson, 1998 (Swanson, 1998). Neural structures (including cell groups, fiber tracts, and gross anatomical features such as the ventricles) were determined under the microscope from the histologically stained sections. Anatomic delineations were prepared by tracing digital images from these serially stained sections using BrainSuite (University of Southern California) (Shattuck and Leahy, 2002). Three-dimensional surfaces were reconstructed in BrainSuite from the delineations.

Results

Diffusion-weighted MRM mouse brain images were acquired over several hours in a high-field magnet. Diffusion-weighted volumes show a great deal of gross anatomical detail and good contrast between gray and white matter (Fig. 1A).

Nissl-stained sections provide critical information about cortical lamination and subcortical nuclei (Fig. 1B). Myelin-stained sections complement the cytoarchitectural data, delineating fiber tracts and helping to define nuclei (Fig. 1C). Protein maps are a crucial aspect of the atlas. Thus far, immunohistochemistry for various neuronal and glial markers (neurotrophin-3 receptor TrkC, Glial Fibrillary Acidic Protein, Myelin Basic Protein) has been carried out on serial sections (Fig. 1D). Complete volumes range from a spatial resolution of approx $100 \times 100 \times 100\ \mu m^3$ ($128 \times 256 \times 128$ voxels, 4.2 Mb uncompressed) for a low-resolution grayscale MRI volume to $3 \times 50 \times 3\ \mu m^3$ ($3840 \times 330 \times 3072$ voxels, 11.6 Gb uncompressed) for a high-resolution full-color Nissl-stained volume.

GEM Importer

The GEM Importer is a pipeline for use in the LONI Pipeline Environment (Rex et al., 2003) specially designed to allow users to readily import their image data into the atlas. Researchers can use it to register and reconstruct their 2D slice images of gene and protein expression data into 3D volumes in the same space as the atlas. Since they occupy the same space, volumes generated by the GEM Importer can be loaded into the LONI_Viz atlas visualization program locally, granting the user full access to its features. The process is automatic; the user inputs a set of Tagged Image File Format (TIFF) images, voxel dimensions, and the GEM Importer masks, registers and reconstructs the images into a 3D volume. Thus a researcher could bring their *in situ* hybridization images or immunohistochemistry images, acquired on different mice, into a common space for comparison.

Visualization

The primary form of interaction with the atlas is through one of two atlas viewers: LONI_Viz and the Synchronized Histological Image Viewing Architecture (SHIVA). In addition to one of the viewers, the downloadable atlas contains three data volumes (a Nissl-stained volume, a blockface imaging volume, and a diffusion-weighted imaging MRM volume) and a label volume containing anatomical delineations. Researchers can load and view their own data volumes, volumes generated by the GEM Importer, or volumes downloaded from the MAP volume database, using either visualization package. We expect that the atlas will be most useful to users for the compari-

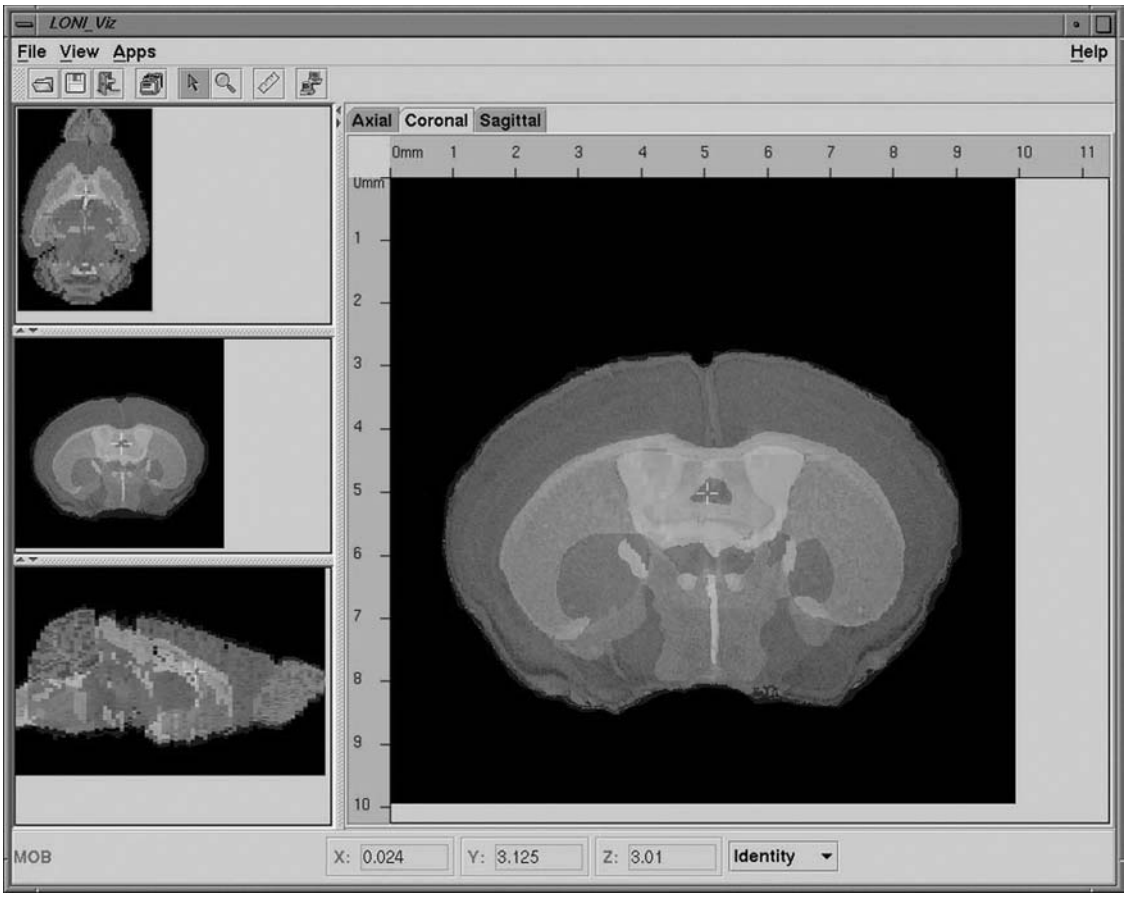

Fig. 2. LONI_Viz: orthogonal sections and high-resolution display. Sagittal, transverse (coronal), and horizontal sections through two volumes, a Nissl-stained volume from a 100 day-old mouse and a color-coded delineation volume, shown overlaid. The actual name of the structure that the cursor identifies shown in the lower, left-hand corner. Small, low-resolution thumbnails are for navigation, whereas, a high-resolution view of the same data allows one to visualize both nuclei and white matter tracts.

son of gene and protein expression data in a defined space, or to an anatomical standard.

LONI_Viz is a self-contained, platform-independent software tool written in Java capable of visualizing multiple 3D datasets simultaneously at several levels of magnification (Fig. 2).

Anatomic delineations in the form of either contours or surfaces are overlaid on the data, allowing the investigator to select an arbitrary plane of section and view the delineations as 2D line segments. Users can also draw their own delineations and use them to make quantitative measurements of the volumes. LONI_Viz is fully integrated with BrainGraph and the Brain Architecture Knowledge Management System allowing the user to query an online database for nomenclature, connections, cell types, and further information about any structure based on the position of the cursor.

SHIVA is also a Java-based imaging framework. It has a powerful and flexible message-passing architecture, which allows simultaneous display and manipulation of

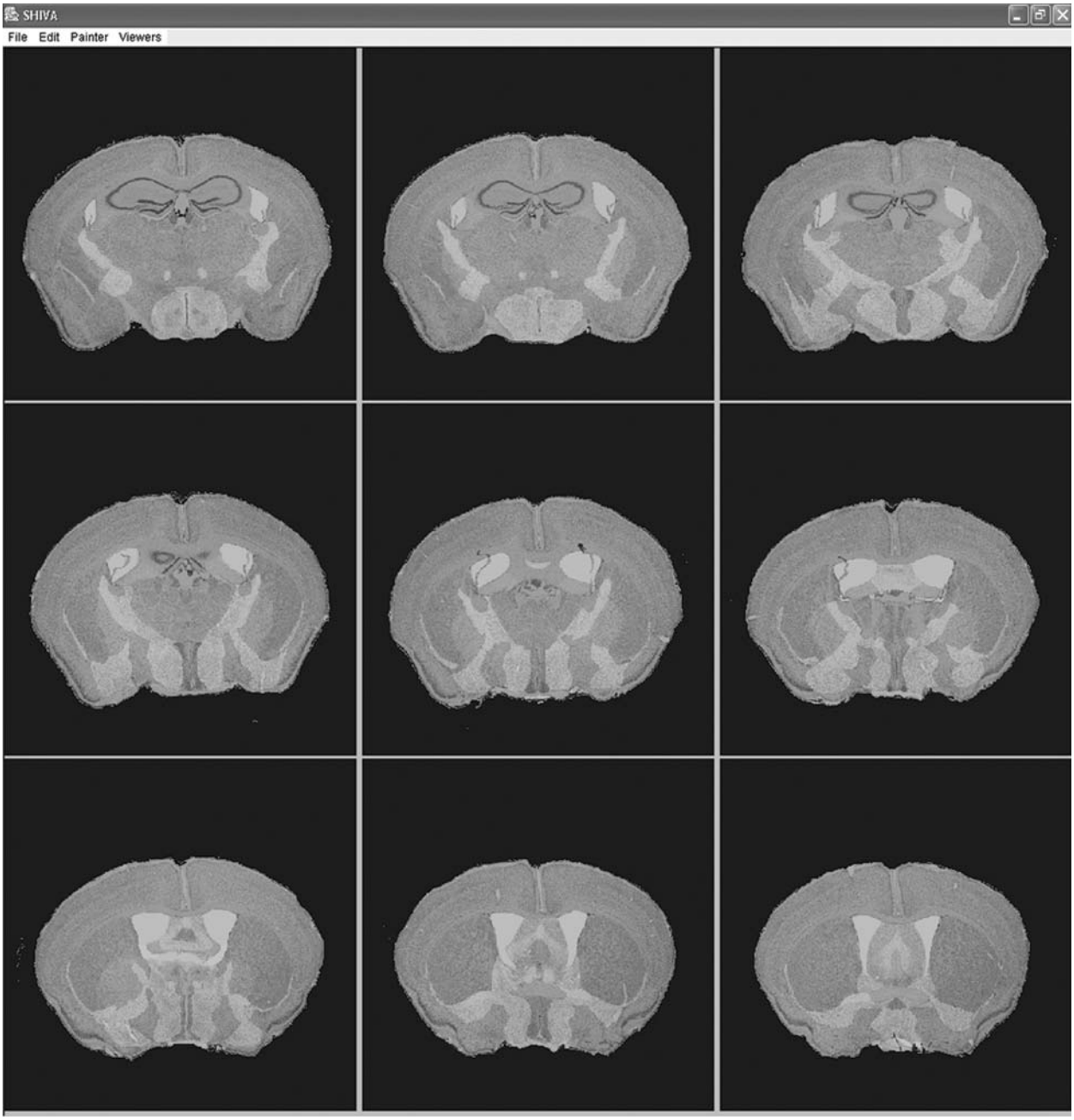

Fig. 3. SHIVA. A set of transverse (coronal) sections through a Nissl-stained volume form a 100 day-old mouse with a set of color-coded anatomic delineations overlaid upon them. The data are displayed in "light-box" format, one of several visualization plug-ins available. Several volumes may be visualized simultaneously, either in the same or different plug-ins, all synchronized.

multiple high-dimensional datasets. We have developed visualization plug-ins to load and manipulate various 3D volume and surface-based file formats. Messages are passed between plug-ins so that the viewers are synchronized, and the data can be compared and manipulated. The architecture seamlessly extends to make every plug-in network-aware, loading data and coordinating viewers over the Internet.

We designed SHIVA to be general and flexible, reducing the difficulty of developing image-processing software. New techniques and algorithms can be prototyped and tested within the framework much more rapidly than it is possible to develop entirely new applications. SHIVA's interface can be modified by plug-ins or scripts, allowing the powerful core feature set to be used in different ways by different audiences. Experienced researchers can be presented with a multi-window interface with complete control over plug-in communication and data manipulation. Individuals working on a single task (such as labeling volumes or counting features) are presented with a single-window, task-specific interface, expressing only needed features (Fig. 3).

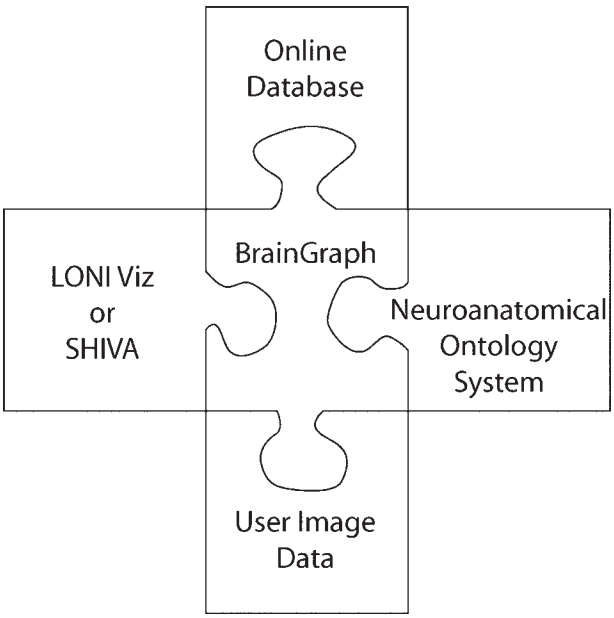

Fig. 4. Synchronization. An illustration of the how the different components of the Mouse Atlas Project communicate with one another, synchronizing the display of information. BrainGraph forms the hub of a set of visualization and analysis tools, allowing the user to access both image and text-based data.

LONI Atlas Information Server

Higher resolution views are available to both LONI_Viz and SHIVA from a central server. Magnification of up to 8X is attainable on the volumes downloaded with the atlas by connecting to the LONI Atlas Information Server (LATIS), a central atlas server application with access to the higher resolution volumes. Users can also load their own data volumes for viewing in the MAP Atlas Viewers. If the volume occupies the same space as the atlas, it can benefit from all of the atlases capabilities.

Anatomic Delineations

Anatomic delineations are fundamental to the atlas and help orient the user with graphical representations of important anatomical detail and provide a standard description and nomenclature for a region of interest. In addition, our anatomical delineations provide the basis for the interaction between image-based data volumes and text-based information networks. The delineation volumes allow us to reference the name of a given structure and synchronize the location of the cursor with the appropriate structure in either BrainGraph and/or the Brain Architecture Knowledge Management System (Fig. 4).

Thus, the researcher is always viewing information about the same structure simultaneously, be it image-based data (such as a histologically-stained volume) or text-based data (such as what kind of neurons are found in this structure).

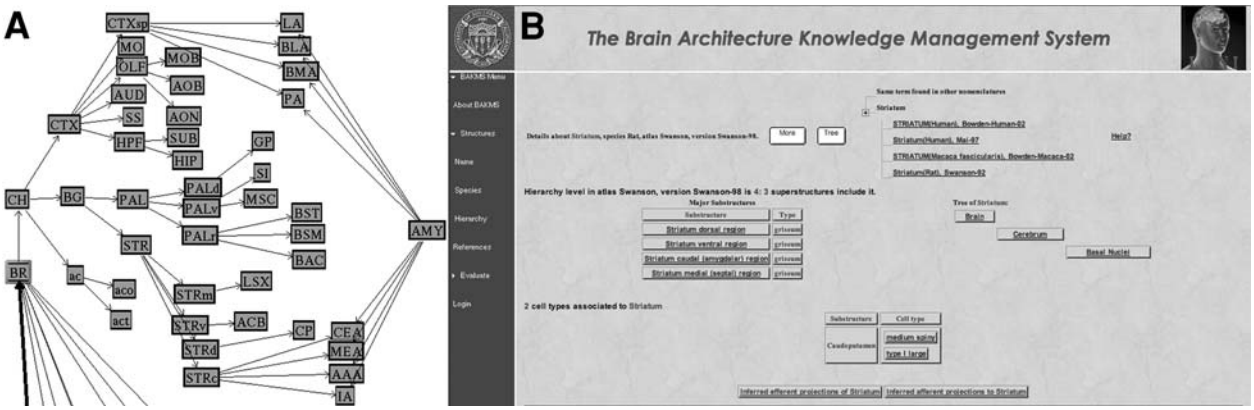

Fig. 5. BrainGraph and the Brain Architecture Knowledge Management System. **(A)** BrainGraph representation of the cerebral hemispheres showing the superstructure amydgala as parent to both the cortical and basal amygdalar nuclei. **(B)** The BAMS contains information about the superstructures that contain the region of interest, substructures contained in it, and its inferred cytological profile.

BrainGraph

BrainGraph is the hub of the Mouse Atlas Project neuroinformatics framework. It controls and coordinates the interaction between both image-based and text-based data. It is a general, flexible, graph-based data model that integrates, organizes, and provides direct access to external structural, functional, histological, genetic, and contextual brain information. Purely hierarchical organizations based on anatomical containment are insufficient to represent complex interrelations between different regions of the brain. BrainGraph allows simultaneous storage of multiple labeling schemes and graph traversal schemes. Each structure has a number of predefined (or user-specifiable) description categories (e.g., functional connectivity, anatomical relations to neighbors, developmental information, genetic information, literature references, and other external contextual information) permitting the user to navigate through their data in a variety of ways (Fig. 5A), accessing information over the Internet directly through BrainGraph.

Users can even add links to BrainGraph in the form of URLs, enabling any form of data to be associated with any structure in the brain, regardless of how it is defined. Thus a researcher could link an image of a microarray of the cortex or the image of a gel run on cortical tissue to the cortex in the atlas.

Brain Architecture Knowledge Management System

The Brain Architecture Knowledge Management System (BAMS) (Bata et al., 2003) can be used as a source of information pertaining to brain structures collated from the literature, as a system for evaluation of the neuroscientific data characteristic of a given structure, and for relating brain cell groups defined in different parcellation schemes within or across different species. We have designed the knowledge base of BAMS in such way that it allows the online insertion, processing, and retrieval of neuroscientific information characteristic of different levels of organization of the mammalian central nervous system: from functional networks of brain structures, to

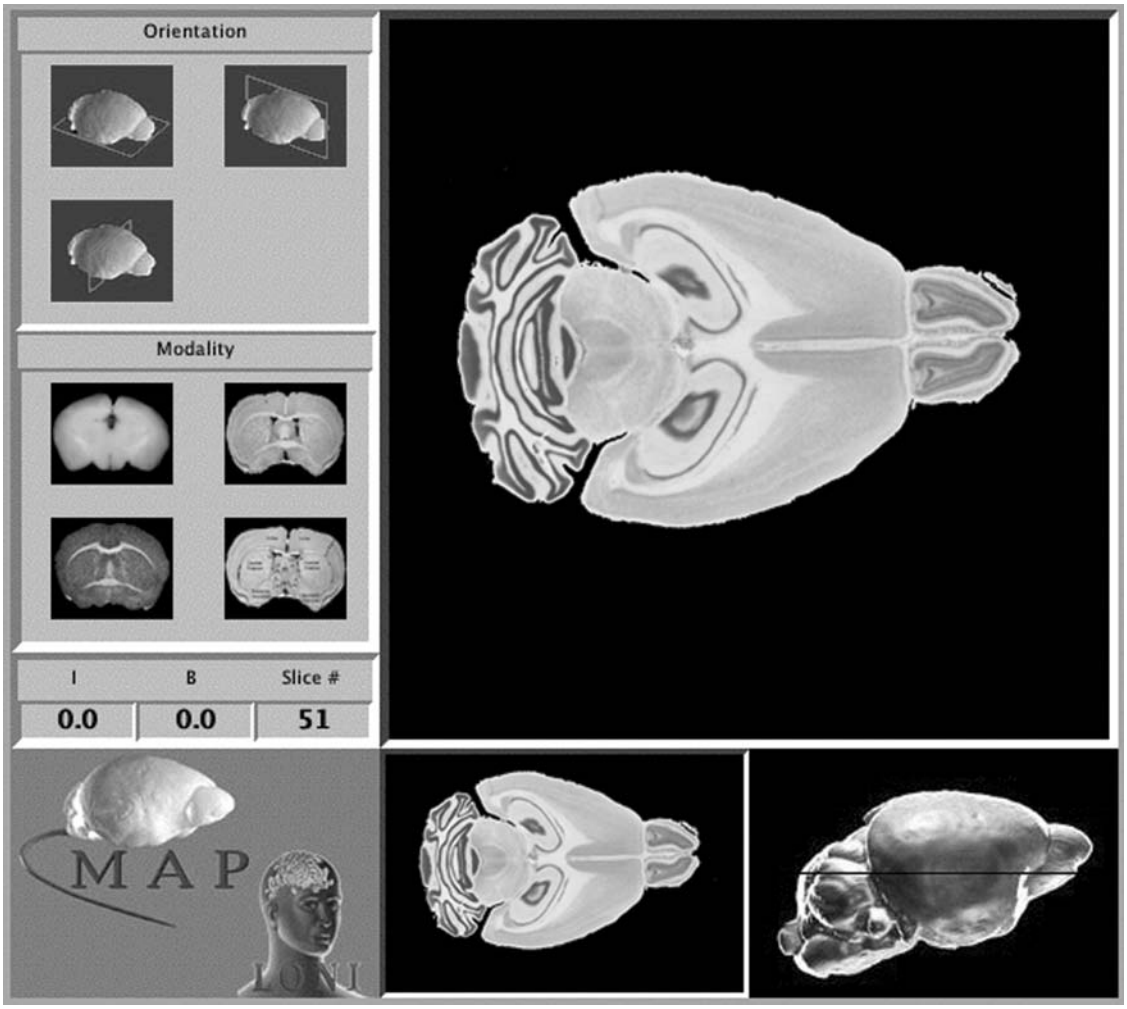

Fig. 6. The MAP-2D Atlas Viewer Applet. The MAP atlas can be visualized online in the form of a Java applet. MRM, Nissl-stained, and blockface imaging volumes may be paged through as 2D slices, in addition to a set of anatomical delineations.

cytoarchitecture and connectivity patterns of different brain nuclei, to distributions of neurotransmitters and receptors.

BAMS contains a set of inference engines for relating neuroanatomical atlases and brain hierarchies proposed by different researchers (Stephan et al., 2000), for evaluation of neuroanatomical connections found in a specific parcellation scheme, for translation of morphological cell types and of connections in different brain atlases in the same species, and for evaluation of the neural homologies of brain structures from different species (Nicolelis et al., 1990; Stephan et al., 2001) by taking into account a set of similarity criteria (Bota and Arbib, 2002; Fig. 5B).

The system currently contains more than 1000 reports related to brain structures in mouse and human. It is accessible from both BrainGraph in the form of a hypertext link and may be accessed independently on the web at http://www.brancusi.usc.edu/bkms.

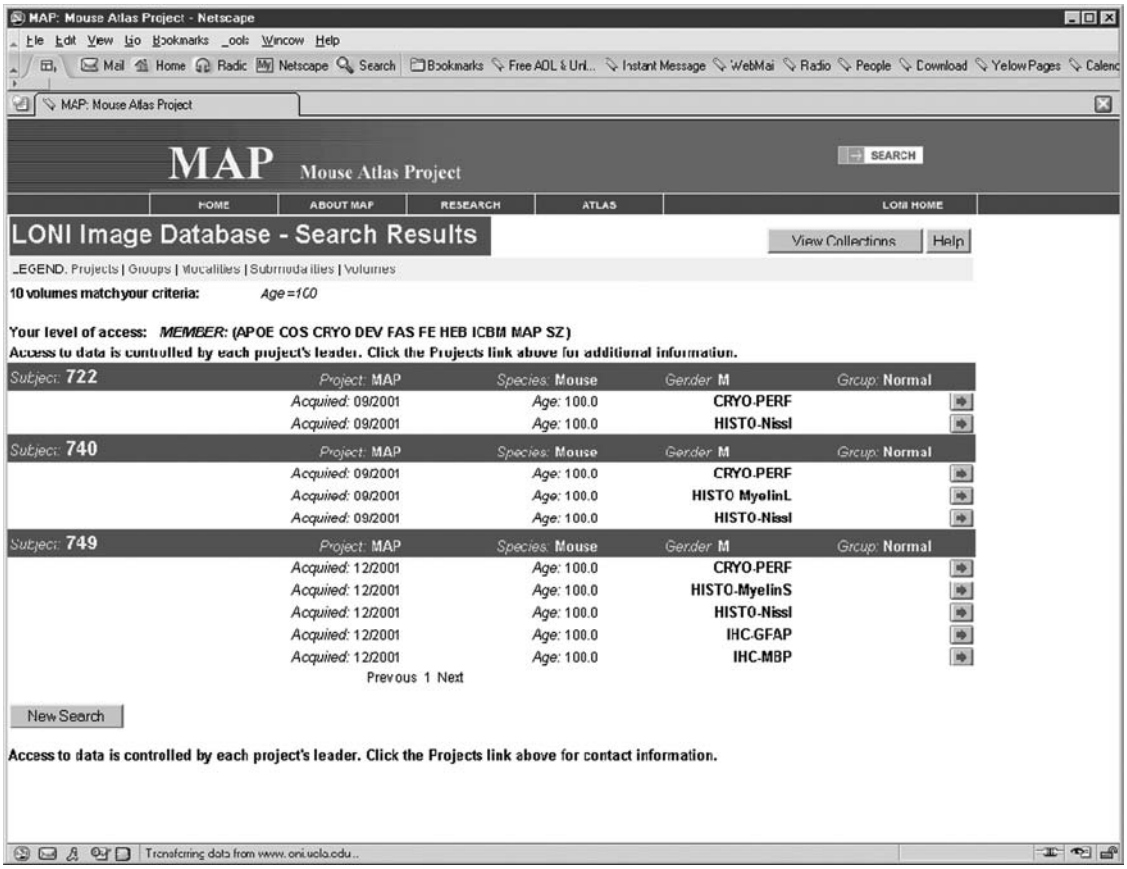

Fig. 7. Database. The Mouse Atlas Project is comprised of over 130 experimental animals and over 350 volumes of data collected using multiple modalities. In order to facilitate the process of selecting and analyzing volumes of interest we have developed a web-accessible, relational database to manage and catalog images and related data. The results of a search of the MAP Volume Database for histological image volumes from 100 day-old mice. The arrows are links to more information on the individual data set, a link to a simple java applet for inspecting the data, and a link to download the data volume.

MAP Website

The Mouse Atlas Project (MAP) website is available through the world-wide web at http://www.loni.ucla.edu/MAP. In addition to the downloadable version of the MAP visualization tools, the atlas can be viewed from the website by using a Java applet. The MAP-2D viewer is a web-based 2D slice viewer that visualizes single slices at a time (Fig. 6).

The user can page through different volumes of slices as thumbnails, clicking on an image to view it at full-size. Since this is strictly a slice viewer, orthogonal views are only available when data has also been acquired in orthogonal planes.

The site also contains a number of resources for teaching and research. There are numerous models and animations ranging from demonstrations of the techniques for sectioning and staining the mouse brain to models of a mouse embryo derived from MRIs. Descriptions of the methods and software used to develop the atlas are also readily available.

MAP Volume Database

A database of volumes is available from the Mouse Atlas Project website (http://www.loni.ucla.edu/MAP), as a supplement to the core MAP volumes. They represent different developmental timepoints and methods of preparation. To facilitate the process of selecting and analyzing image data from this collection, we have developed a relational database application to manage and catalog the images and related data. Attributes of the subject such as age, strain, weight, and anatomical structure are recorded, as are image volume properties such as modality, resolution, and anatomical structure. As the relevant values are stored in the database, the image files are systematically catalogued and stored in a central repository. Once deposited, image data can be queried, viewed, downloaded, or selected for processing, either individually or as a collection. The MAP-2D slice viewer can be used to browse the contents of a 3D volume prior to downloading for use with the MAP Atlas Viewers (Fig. 7).

Discussion

There are many difficulties involved in the implementation of such an ambitious project. Systemic issues had to be resolved, such as how to incorporate disparate forms of data and how to handle data with many different resolutions. Digital atlases and their associated tools were well-suited to the task of collating and cataloging large volumes of data and linking them to other sources of information. BrainGraph ties different kinds of data together and LATIS makes high-resolution data available over a network. Technical issues also had to be resolved, such as how to prepare the tissue for several different kinds of imaging and the creation of mechanisms for automatically and robustly masking and registering multimodal image data.

These issues, once solved, allowed us to produce a tool for the visualization, analysis, and manipulation of varied sets of data: a digital atlas of the C57BL/6J mouse brain.

The atlas will facilitate collaboration by providing researchers a common framework for comparing results. Data acquired from disparate sources will be directly comparable within the atlas. An example of such a study would be finding the histological correlates of an MRM signal, or the comparison of gene or protein expression data collected in different laboratories. One of the main uses of atlases is the localization of gene or protein expression data to an anatomical structure, a task made difficult in paper atlases by 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.

Atlases can be generated that combine data across a large number of animals and modalities. Nissl- or silver-stained atlases can be generated from the average of many specimens, as can maps generated from MRM or the expression of genes. Developmental atlases can be made, mapping changes that occur over time, helping to elucidate the processes that produce a normal animal. Additionally, one could generate atlases that capture changes in anatomy, gene, or protein expression as the result of environment or experience. Furthermore, these atlases do not have to be limited to normal animals. Atlases of genetically modified or mutant animals can produce great insights to the effect of genetic manipulation and mutation on the phenotype of the mouse. Additionally, atlases representing disease states can be made to better understand the processes and effects of the disease.

All of these atlases in turn can be linked to other sources, such as the Neuroanatomical Ontology System (NOS) and the Gene

Expression Database (GXD). These databases can greatly enrich the user experience by bringing together information that was previously disparate, and allowing the data to benefit from the synergy.

Keeping all of the atlas information current would require the existence of 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 extending this work would be to include the rest of the nervous system (spinal cord, peripheral nerves, and even retina) into a complete atlas of the nervous system. Though this would be technically challenging, the benefits would be far-reaching. Additionally, the creation of a curated collection of atlas data would be beneficial to the community at large. Such a collection would be an extension of the volume database, composed of submissions from the scientific community at large. Standardized protocols for *in situ* hybridization and immunohistochemistry will facilitate the importation of data into the atlas. This collection could encompass not only the C57BL/6 mouse, but other strains of mice, including genetically-modified animals, mutants, and disease models. With the establishment of individual and group permissions, it could become a model for the dissemination of data and the establishment of collaborative efforts.

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