Published in final edited form as:

J Neurosci Methods. 2013 October 30; 220(1): . doi:10.1016/j.jneumeth.2013.08.017.

AtlasGuide: Software for stereotaxic guidance using 3D CT/MRI hybrid atlases of developing mouse brains

Xin Li^a, Manisha Aggarwal^a, Johnny Hsu^a, Hangyi Jiang^a, and Susumu Mori^{a,b,*}

^aRussell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

^bF.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, Maryland, USA

Abstract

Stereotaxic operations of the mouse brain are critically important for various types of neuroscience research studies, which include electrical recording of neural activities or site-targeted injection of stem cells, chemical tracers, and vectors, to name a few. To guide such operations, two-dimensional histology-based mouse brain atlases, such as the Paxinos and Franklin atlas, are widely used. Recently, computed tomography (CT) and magnetic resonance imaging (MRI) based hybrid three-dimensional (3D) atlases of developing mouse brains have been introduced. In this study, a new stereotaxic guidance software, called AtlasGuide, is introduced, which was developed to fully utilize the benefits of the 3D atlases for high-precision stereotaxic targeting. The AtlasGuide software provides functions to visualize oblique needle paths in 2D and 3D views, which allow investigators to simultaneously examine brain structures that could be damaged by the needle path and optimize the injection angles for high-precision trajectory selection through critical neural tissue. It allows reorientation and scaling of the atlases dynamically to match the orientation of the animal brain prepared for surgery, thereby eliminating the need to manually align the subject to the atlas, a procedure which is essential while using conventional 2D atlases. In addition, the software enables loading user-defined atlases when researchers need image-based guidance for different age groups, strains, or species. The software with integrated 3D stereotaxic mouse atlases is available for download at the <http://lbam.med.jhu.edu> website.

Keywords

stereotaxic guidance; surgical; atlas; mouse; brain

1. Introduction

The goal of this study was to develop a stereotaxic navigation and user-interface software with integrated electronic mouse brain atlases for image-guided stereotaxic surgical operations. The development of genetic engineering technology in the early 1990s has brought the mouse into a prominent role for investigations of the mammalian central

© 2013 Elsevier B.V. All rights reserved.

*Corresponding author: Susumu Mori, Ph.D., Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, 330B Traylor Building, 720 Rutland Avenue, Baltimore, MD, 21205, USA, Phone: (+1) 410-614-2702, susumu@mri.jhu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

nervous system. In these mouse-based neuroscience studies, stereotaxic surgical operations are essential for targeted delivery of chemicals, vectors, cells, or implantation of electrodes at specific locations in the brain (Athos and Storm, 2001; Cetin et al., 2006; Jones et al., 1977; Slotnick, 1972). Because the targeted delivery is essentially a blind operation, stereotaxic atlases are an essential resource for these surgeries. Namely, the coordinates of specific brain structures of interest are identified from a stereotaxic atlas, and the needle or electrode is inserted to the targeted location.

There are several histology-based atlases that are available for anatomic delineation of the mouse brain (Dong, 2008; Jacobowitz and Abbott, 1997; Paxinos and Franklin, 2003; Sidman et al., 1971; Slotnick and Leonard, 1975; Valverde, 2004). Of these, currently the Paxinos and Franklin atlas (Paxinos and Franklin, 2003) in stereotaxic coordinates is almost exclusively used for the guidance of stereotaxic surgical procedures in the adult mouse brain (Messier et al., 1999). However, histology-based atlases, while they have excellent proven values, also have several limitations. First, they cover only adult mouse brains and at present there is no histology-based stereotaxic atlas available for neonate brains. Second, the two-dimensional histology-based atlases cover only a limited number of slice orientations and locations. From a series of 2D slices, it is difficult to design an oblique needle path in order to avoid damaging important brain structures. Third, the tissue fixation and sectioning process can introduce potential inaccuracy in the precise stereotaxic brain coordinates. In recent years, several 3D mouse brain atlases based on high-resolution magnetic resonance imaging (MRI) have been developed (Dorr et al., 2008; Jacobs et al., 1999; Kovacevic et al., 2005; Lee et al., 2005; Ma et al., 2008; MacKenzie-Graham et al., 2004). Specifically for stereotaxic operations, atlases based on MRI and micro-computed tomography (micro-CT) have been recently introduced (Aggarwal et al., 2009; Chan et al., 2007). Because stereotaxic surgery relies on using external skull-surface landmarks to estimate the locations of underlying brain structures, the CT and MRI co-registered atlases can provide 3D stereotaxic coordinates of brain structures identified with MRI contrasts, relative to cranial landmarks that can be delineated based on micro-CT contrasts.

In this study, we introduce a new navigation software developed for image-guided stereotaxic operations with integrated three-dimensional electronic atlases of mouse brains at different developmental stages. The data are based on CT/MRI hybrid 3D atlases published in our previous report (Aggarwal et al., 2009). Based on the hybrid atlases with high anatomical fidelity, we developed a stereotaxic guidance software, called AtlasGuide, which provides a user-interface for atlas navigation with several advanced features to utilize the potential of the 3D atlases. It provides built-in functions including calculation and visualization of a 'virtual needle path' for pre-operation planning, oblique-angle needle guidance, and dynamic atlas registration to the orientation of the experimental mouse heads, to allow high-precision stereotaxic surgical targeting. In addition, the AtlasGuide software allows users to load animal brain images from their specific studies, extending its capability to other ages, strains or species. In this paper, we describe the details and capabilities of this resource software, which can be freely downloaded from <http://lbam.med.jhmi.edu>.

2. Theory and Methods

2.1. Construction of MRI and micro-CT hybrid stereotaxic atlases

The details of the MRI and micro-CT combined stereotaxic atlases of developing brains have been described in our previous report (Aggarwal et al., 2009). Briefly, the atlases consist of high-resolution ex vivo MR and micro-CT images of C57Bl/6 mouse heads at six different developmental stages: postnatal day 7 (P7), P14, P21, P28, P63, and adult (P140-P160). At each age, T2-weighted and diffusion tensor MR images acquired at a resolution of $0.125 \times 0.125 \times 0.125 \text{ mm}^3$ were co-registered to micro-CT images of the skull, using

landmark-based rigid registration (Zhang et al., 2009). In addition to single-subject atlases for developmental stages, for the adult mouse brain a second-level population-based in vivo MRI atlas was generated, and a postmortem distortion-corrected atlas was developed by nonlinear deformation of the high-resolution ex vivo data to the in vivo atlas. This procedure was implemented to; i) correct for postmortem fixation-related tissue shrinkage, and ii) remove any sample-specific anatomical bias, in order to achieve improved anatomical fidelity and accuracy of stereotaxic targeting in the adult mouse brain. The bregma and lambda cranial landmarks were identified on the micro-CT skull images at each age in order to establish the stereotaxic coordinate reference frame for the atlases, based on the bregma-lambda horizontal alignment convention defined in Paxinos' murine brain atlases (Paxinos and Franklin, 2003; Paxinos and Watson, 1982).

2.2. Structural Segmentation

For the adult mouse brain atlas, 46 anatomical structures were manually segmented using ROEditor (www.mristudio.org). Table 1 shows the list of segmented brain structures with the corresponding abbreviations in Paxinos's mouse brain atlas, which can be three-dimensionally visualized in AtlasGuide.

2.3 Atlas reorientation function

When histology-based 2D atlases are used to navigate stereotaxic operations, the animal head is required to have exactly the same position and orientation as that of the atlas. Specifically for mouse brain studies, the bregma-lambda axis has to be horizontal and aligned to the Y (fore-aft) axis of the stereotaxic device. Operators are required to carefully align the mouse head, and if necessary, reposition the head manually. When the 3D CT/MRI atlases are used, it is theoretically possible to reorient the atlas to match the position of the experimental mouse head. Once the mouse head is secured in the head holder, the coordinates of the bregma and lambda junctions can be read by positioning the probe tip on these anatomical landmarks and reading the respective coordinates from the stereotaxic device display. This two-point reading from the device provides the bregma-lambda vector for the experimental brain, from which the angles of rotation of the atlas about the X and Z axes can be calculated. Rotation of the atlas 3D matrix is then implemented in AtlasGuide as follows:

Suppose $\tilde{v}_1$ denotes the bregma-lambda vector in the atlas coordinate space, and $\tilde{v}_2$ denotes the corresponding vector in the experimental brain coordinate space. Then the angle of rotation with bregma as the origin is given by $\cos\theta = \tilde{v}_1 \cdot \tilde{v}_2 / |\tilde{v}_1| |\tilde{v}_2|$, and the axis of the rotation is given by the unit vector $\hat{u} = (x, y, z) = \tilde{v}_1 \times \tilde{v}_2 / |\tilde{v}_1 \times \tilde{v}_2|$. The rotation matrix with bregma as the origin is then calculated as;

$$[R] = \begin{bmatrix} (1 - \cos\theta)x.x + \cos\theta & (1 - \cos\theta)x.y - z.\sin\theta & (1 - \cos\theta)x.z + y.\sin\theta \\ (1 - \cos\theta)x.y + z.\sin\theta & (1 - \cos\theta)y.y + \cos\theta & (1 - \cos\theta)y.z - x.\sin\theta \\ (1 - \cos\theta)x.z - y.\sin\theta & (1 - \cos\theta)y.z + x.\sin\theta & (1 - \cos\theta)z.z + \cos\theta \end{bmatrix}$$

which is applied to transform the atlas to match the orientation of the experimental animal, thereby eliminating the need to reposition the mouse head for horizontal bregma-lambda alignment. For additional calibration of alignment along all X, Y, and Z axes, landmark readings from more than 2 points including off-midsagittal cranial points are necessary, and can be used to confirm leveling of the medial-lateral axis (rotation about Y axis).

2.4 Oblique needle path calculation

There are two inputs required for the oblique needle or probe path calculation. First is the specification of the needle angle (α). This angle can be hypothetical for pre-operation planning, or the actual reading from the stereotaxic device. Typically, there are two degrees of freedom for setting the angle of rotation movements in commercially available stereotaxic instruments: the tilt angle of the needle and the rotation angle about the main support shaft. Because of the relative spatial position of the animal head and the arm of the probe-holder that supports the needle, the tilt angle is, for most devices, practically limited to movements about the medial-lateral (X) axis, meaning the probe angle can be tilted fore and aft of the device (anterior-posterior of the animal). By using the rotation (arm swing) about the main shaft, it is theoretically possible to create a compound angle, but, in reality, this operation is not practical because the arm swing angle is only limited to a few degrees unless the position of the mouse is moved, which is not practical for most devices with a fixed relationship between the main shaft and the animal head-holder position. Therefore, in the current version of AtlasGuide, support for the fore-aft probe tilt function is implemented. The second input needed for the oblique path calculation are the target tissue coordinates, (s_x, s_y, s_z), in the atlas space. The coordinates can be readily obtained by clicking the brain region to be targeted in the 3D MRI atlas (in any of the sagittal, horizontal, or coronal 2D planar displays). Once these two inputs are entered, the resulting oblique path of the needle is calculated and visualized in real time. The anterior-posterior displacement of the probe from the bregma (along the Y axis) and the distance the probe tip has to be lowered from the bregma-lambda horizontal plane in order to hit the target coordinates are then given by $\Delta y = s_y + s_z \tan \alpha$ and $\Delta z = s_z / \cos \alpha$, respectively, where (s_x, s_y, s_z) are the coordinates of the target structure and α is the tilt angle. By displaying the computed needle path, the coordinates of the skull entry point where the virtual needle intersects the skull can also be directly read. In addition, by visualizing the overlaid 3D locations of various brain structures, a path that avoids important brain structures for the specific study can be designed.

2.5. Stereotaxic device

As a courtesy of Stoelting Co. (Stoelting Co., Wood Dale, Illinois, USA), we borrowed a Digital Lab Standard Stereotaxic device for the testing of our software. All demonstrations in this paper are based on this instrument.

3. Implementation and Results

3.1. CT/MRI atlases of developing mouse brains

Fig. 1 shows the CT/MRI atlases of developing mouse brains from P7 to adult (P140-P160). At each time point, multiple MRI contrasts (T2-weighted, isotropic diffusion-weighted, fractional anisotropy, and direction-encoded colormaps derived from DTI) are provided and each contrast reveals different neural structures. For instance, the diffusion-weighted images clearly show the ventricular structures, while the color-coded orientation maps carry rich gray and white matter anatomical information. The co-registered micro-CT and MRI/DTI data provide a stereotaxic coordinate system based on the skull features identified from CT, which is applied to the brain structures imaged by MRI/DTI. Fig. 2 shows the prominent cranial landmarks and features identified on the adult mouse skull CT image. For stereotaxic guidance in AtlasGuide, the atlases are oriented in the bregma-lambda horizontal position. At P7, the bregma and lambda cannot be clearly identified due to wide sagittal and lamboid cranial sutures at this stage (Zimmermann et al., 1998), hence approximate coordinates for the bregma junction are provided. Although MRI/DTI data of earlier developmental stages are available, the bregma/lambda-based coordinate system is not applicable at these earlier developmental stages because the neonatal mouse skull is not completely ossified. The

atlases shown in Fig. 1 are included in the current version of AtlasGuide and can be selected from a pull-down menu.

3.2. Implementation and user interface

AtlasGuide has been developed in Visual C++ and runs on Windows operating systems (XP, Vista, and Windows7) with support for 32-bit and 64-bit versions. Fig. 3 shows the user interface of AtlasGuide. The viewer consists of three orthogonal 2D planar views and a 3D visualization panel. The “Visualization” section controls the slice locations, 3D atlas rotation and reslicing, grid control, and image magnification. Displayed images can be selected from available multiple contrasts (CT, T2-w, iDW, FA, and color map contrasts) from the drop-down menu. The “Structure Annotation” section allows visualization of the pre-segmented structures (Table 1) in the orthogonal 2D views as well as in the 3D view. Users can choose specific structures to be visualized and their colors and overlay transparency from the list. In the “Coordinate Control” section, users can define the origin of the coordinate system. The bregma and lambda junctions are pre-defined in AtlasGuide, and users can choose the appropriate landmark as the origin of the coordinate system. In addition, users can define any additional coordinates of brain structures in the atlas for selection as the origin. Depending on the user-defined coordinate origin, the (0, 0, 0) point of the atlas is automatically adjusted and updated.

3.3. Pre-operation planning and needle guidance

AtlasGuide can be used to guide stereotaxic operations in a way similar to conventional histology-based stereotaxic atlases; users can simply navigate through the 2D image panels to find the brain structure of interest and read the (x, y, z) coordinates. After the stereotaxic device is calibrated at the bregma or lambda, the probe can be moved and lowered to those coordinates. In this case, AtlasGuide provides several advantages such as three orthogonal views, population-representative coordinates (adult atlas only), and precise skull-brain coordinate matching. However, the real power of the 3D atlases is materialized when users need 3D interactive operations such as pre-surgical operation planning with oblique injection angles. For instance, if one wants to avoid damaging particular structures to target a deep-seated brain region, careful planning of the needle path is necessary, which would be very difficult using paper-based 2D atlases. AtlasGuide offers a ‘virtual needle path’ function for interactive pre-operation planning. In AtlasGuide, an arbitrary tilt angle can be chosen and a target structure can be selected by clicking on the location. The projected virtual needle path is then automatically calculated and visualized (Fig. 4). To investigate the spatial relationship between the projected needle path and adjacent structures of interest, selected structures can be simultaneously visualized three-dimensionally. The angles and target coordinates can be interactively changed while investigating the interference between the needle path and the visualized 3D structures. Fig. 4 illustrates an example of the virtual needle path function using AtlasGuide, in which a thalamic nucleus was targeted while avoiding the hippocampus and the caudo-putamen.

Once the user is satisfied with the pre-operation planning and the calculated needle path, AtlasGuide provides the precise coordinates for positioning the stereotaxic device. Fig. 5 illustrates an example of oblique needle guidance using AtlasGuide. If the tilt angle is zero, the user could use the (x, y, z) coordinates of the target directly from the atlas; i.e., drill a hole on the skull surface at the (x, y) location, position the probe tip at (x, y, 0), and lower the needle by z mm in order to reach the (x, y, z) coordinate. If the tilt angle is non-zero, however, the absolute coordinates of the target location are not usable for oblique needle guidance. In this case, the calculation of the needle coordinates would become more complicated, and is provided by the AtlasGuide interface. As shown in Fig. 5, the user can now directly read the recalculated stereotaxic coordinates and position the probe tip at the

specified (x' , y' , 0) coordinates, drill a hole at the skull entry-point coordinates read from AtlasGuide, and lower the needle at the specified angle (α) by z' mm depth. This ensures that the needle tip will hit the (x , y , z) coordinates of the targeted brain region (Fig. 5).

3.4. Atlas realignment based on the actual brain orientation

Fig. 6 illustrates the automated reorientation of the 3D atlas in AtlasGuide based on reading the coordinates of multiple landmarks from the experimental mouse skull. In this example, the bregma was set as the origin and, therefore, the coordinates of the lambda were read as the second landmark. If the bregma-lambda axis is horizontal and the midline is aligned to the instrument's Y axis accurately, the x and z coordinates of lambda should be zero, while the y coordinate indicates the length of the bregma-lambda distance. The result in Fig. 6A shows that the lambda was 0.3 mm lower along the Z axis, indicating that the bregma-lambda axis was not precisely horizontal after positioning the mouse in the head-holder. This two-point reading from the actual brain was used to compute the transformation matrix to virtually rotate the atlas to match the orientation of the skull.

Fig. 6B shows the results of testing the accuracy of the landmark-based atlas alignment function. After the bregma-lambda orientation was measured by the stereotaxic device, the re-orientated 3D atlas was computed and generated by AtlasGuide. As seen in Fig. 6B, the coordinates of the bregma remain unchanged at (0, 0, 0), and the new coordinates of lambda agree with the physical reading (0, -4.2, 0.3) in this case). From the updated MR brain images in the atlas, the old and new coordinates of other brain structures were also measured before and after the reorientation, to test if they were rotated by the correct angle about the reorientation vector. We tested this function with different positions of mouse heads, and accuracy was confirmed in each case.

3.5. Atlas rescaling based on the actual skull landmarks

In the previous section, the atlas was matched to the actual brain orientation using a rigid rotation operation. However, the landmark coordinate-reading provides information about not only the orientation, but also the relative size of the specimen head. For instance, Fig. 6 indicates that the bregma-lambda distance was measured to be 4.2 mm, while the corresponding distance in the adult CT-MRI atlas (population average) is 4.25 mm, indicating that the atlas well represents the sample brain morphology. However, when mutants or significantly younger mice are used, it is possible that the measured bregma-lambda distance is substantially different, say, 3.8 mm. If the specimen brain is noticeably smaller (or larger), the normal adult atlas may not provide high surgical accuracy. In this case, users have the option to linearly scale the atlas to match the bregma-lambda distance of the specimen. This procedure can be performed by using a scaling factor s such that the rotation operation now becomes $s[R]$. This is a linear scaling based on 2-point measurement. This function was tested by providing various hypothetical bregma-lambda coordinates, and examining the resultant atlas. For example, if the bregma-lambda distance was artificially reduced by 10%, we confirmed that the calculated atlas was indeed 10% smaller.

3.6. Use user-defined MR images for atlas

AtlasGuide architecture is based on the widely-used MriStudio platform (www.mristudio.org) developed at the Johns Hopkins University (Jiang et al., 2006). MriStudio has the capability to read data from virtually all commercial MRI scanners. These include vendor-specific DICOM and other formats such as Siemens Mosaic, Philips REC, as well as non-MRI scanner formats such as Nifti, Analyze, MINC, and NRRD (Fig. 7). AtlasGuide inherits this input/output capability, which is constantly upgraded. This function allows users to load their own images into AtlasGuide for use as the reference atlas.

4. Discussion

In this study, a new stereotaxic guidance software, AtlasGuide, was introduced. This software provides an interface for users to use the 3D CT/MRI atlases of developing mouse brains, which are being developed at Johns Hopkins University (<http://lbam.med.jhmi.edu>), for stereotaxic operations. The software is freely available for download from the website and currently, bundled with developing mouse brain stereotaxic atlases at six ages (Fig. 1). Among these atlases, the inaccuracy, caused by fixation and anatomical bias from the specific mouse used for high-resolution imaging, was reduced for the adult atlas by adopting the coordinates from in vivo population-averaged images as previously described (Aggarwal et al., 2009). Compared to the widely-used 2D Paxinos and Franklin atlas, the 3D atlases and AtlasGuide interface provide various new functions. First, the atlases contain neonatal brain coordinates, which, to the best of our knowledge, are not available from histology-based atlases at present. Second, with the AtlasGuide interface, pre-operation examination of oblique probe insertion paths and identification of structures that could be damaged during the injection can be performed more readily. Third, atlas reorientation and dimension-adjustment are unique features, which are difficult to achieve with 2D atlases. Furthermore, the AtlasGuide interface also allows users to load their own MRI data for stereotaxic guidance.

Anatomical variability is known to exist in the mouse brain across subjects as well as strains (Chen et al., 2006; Sharief et al., 2008; Wahlsten et al., 2006; Wahlsten et al., 1975). This inter-subject variability can introduce inaccuracies in stereotaxic targeting, if absolute atlas coordinates are used for significantly larger or smaller brain specimens. In order to account for global differences in brain and skull size, the AtlasGuide interface enables linear scaling of the atlas based on actual skull measurements. Evaluating the accuracy of stereotaxic targeting with brain size adjustment is, however, a relatively difficult issue. The current rescaling function is based on a two-point calibration using the bregma-lambda distance. If the bregma-lambda distance of the test mouse is substantially different from that of the atlas (4.25 mm for the adult atlas), it makes sense to recalibrate the brain size. However, if the difference is caused by a genetic mutation or strain difference, there is no guarantee that the brain anatomy or proportion remains the same. This is a common issue for all atlas-based stereotaxic guidance operations. If the brain shape is different from that of the C57Bl mouse, but is reproducible within the test group (e.g. a mutant model with an anatomical phenotype), such difference needs to be characterized from actual injection studies. The two-point calibration function could be a practical and effective approach for the initial estimation of the brain coordinates. It should be noted that the current version of AtlasGuide also supports a 9-parameter affine registration function for adjustment of skull dimensions using measurements from more than two cranial landmarks (including off-midsagittal points). This function, however, requires the users to manually define additional reference landmarks on the skull with precision, and for most practical purposes, the two-point measurement based on bregma-lambda distance should be most feasible. We would also like to point out that whether this function could actually improve the accuracy needs to be confirmed for each study with the specific age and strain of mice employed in the study, and thus, initial testing and estimation of potential bias for a given target of interest may be required.

A related issue is the estimation of targeting accuracy and robustness of stereotaxic coordinates based on actual injection studies. It is known that there are multiple sources that can affect the accuracy of stereotaxic surgical operations (Swanson, 2004). Probably the largest contribution to potential inaccuracy stems from anatomical variability. In our atlas, the anatomical variability was estimated from MRI scans of multiple mouse specimens as shown in our previous study (Aggarwal et al., 2009). Nonetheless, the anatomical variability

is influenced by age, nutritional and pathological states, and strains. To ameliorate these issues, the size compensation tool and the interface to load user-defined MRI data are provided. In addition to these measures, measurement of the level of accuracy using actual injection studies can be carried out. However, such quantitative measurements are, in general, difficult to perform and interpret. This is because firstly, each anatomical coordinate, $A[x,y,z]$ is associated with an inherent degree of anatomical variability, $av[x,y,z]$. Measurement of any potential error in the stereotaxic coordinates of anatomic locations derived from the atlas, $ae[x,y,z]$, is likely to be coordinate-specific. In addition, this measurement is often affected by apparent operational error, $oe[x,y,z]$. This error is obvious when, for example, the needle path is not oriented parallel to the midline (i.e., the head was not perfectly aligned in the horizontal plane), but usually cannot be clearly differentiated from av and ae . In such a system, the resulting error is $av+ae+oe$, and reasonable estimation of ae requires a large number of injection trials, and from the results, we need to compute the bias (ae), assuming that av and oe have random distributions. In reality, the apparent oe is often larger than $av+ae$, making high-precision measurement of ae a difficult procedure.

To resolve the issue of sample-specific anatomy for studies using widely different strains or genetic backgrounds of mice, AtlasGuide offers an alternative approach allowing users to load acquired images of the test animal per se. While this can be considered the most accurate solution, there are also difficulties associated with this procedure. For rodent brains, skull features such the bregma and lambda are routinely used as the reference landmarks for stereotaxic guidance, and therefore, our atlases consist of both micro-CT (skull) and MRI (brain) co-registered images. This means that users are required to obtain both CT and MRI images of their mouse brain model. Additionally, because the CT and MRI images are not acquired on the same system, careful three-dimensional co-registration of the two contrasts has to be performed. The empirical calibration described above could be a more practical solution, if users do not have access to these imaging equipments. The ability to load user-defined atlases could also be important for researchers using primate brains. Because of the highly variable cortical anatomy of primates, an atlas based on one representative brain cannot be used for high-precision needle/electrode targeting across subjects. Owing to the relatively high expense of each animal, MRI is increasingly used to scan individual animals in order to accurately characterize the subject-specific brain anatomy. The ability to load and visualize study-specific animal images in AtlasGuide will provide greater accuracy and coordinate precision to drive stereotaxic operations in primate brains.

As shown in our study, the 3D atlases provide improved accuracy and a flexible user-interface for stereotaxic operations compared to histology-based 2D atlases for many studies. However, there are also certain limitations. Firstly, the in-plane resolution of the MRI atlas is much lower compared to histology-based 2D atlases, although it provides higher through-slice resolution (100 coronal sections in the Paxinos and Franklin atlas versus 226 coronal sections in our atlas for the adult mouse brain). With the comparatively lower resolution, very fine neuroanatomical structures in the brain cannot be precisely delineated in our atlases. Likewise, the anatomical definitions based on cyto- or myelo-architecture may not be perfectly identical to the contrast borders (thus the anatomical labels) defined by MRI contrasts; for example, one of the MRI contrasts derived from DTI is based on the underlying orientation of anatomical structures, which could generate anatomical contrasts different from any of the histological staining methods. Therefore, if users want to define the precise coordinates of a specific anatomical region which cannot be unequivocally defined by MRI contrasts, our atlas may provide only an estimation. As a related issue, our current structural segmentation is less extensive than the Paxinos and Franklin atlas. In the near future, with recent advances in MR hardware including higher magnetic-field strength (~ 17 Tesla) preclinical scanners, and image acquisition techniques for rodent brain imaging, it will be feasible to develop stereotaxic CT/DTI atlases with

significantly higher spatial resolutions, which can then also be seamlessly integrated in the AtlasGuide framework developed here.

In summary, we have developed a new stereotaxic guidance software, AtlasGuide, with integrated CT/MRI atlases of developing C57Bl/6 mouse brains at six different ages. The full 3D features of the atlases and guidance interface allow unique operations such as pre-surgical planning for oblique injection paths and high-precision trajectory selection. The atlas reorientation and scaling capabilities can modify the 3D atlases to match the orientation and cranial dimensions of the mouse head prepared for the surgery. The image Input/Output functions allow loading and visualization of user-defined images for subject-specific operation guidance. In the future, as 3D CT/MRI atlases of brains at different ages, strains, or species become available, they can be readily loaded into AtlasGuide using the implemented Input/Output functions. The atlases and software introduced in this study will be a useful resource for neuroscience researchers.

Acknowledgments

This study was supported by National Institutes of Health grant, RO1EB003543. We are also grateful to Stoelting Co, for lending us a Digital Lab Standard device and providing various suggestions and advice for the development of this software.

References

- Aggarwal M, Zhang J, Miller MI, Sidman RL, Mori S. Magnetic resonance imaging and micro-computed tomography combined atlas of developing and adult mouse brains for stereotaxic surgery. *Neuroscience*. 2009; 162:1339–50. [PubMed: 19490934]
- Athos, J.; Storm, DR. Current protocols in neuroscience / editorial board, Jacqueline N. Crawley ... [et al.]. 2001. High precision stereotaxic surgery in mice. Appendix 4: Appendix 4A
- Cetin A, Komai S, Eliava M, Seeburg PH, Osten P. Stereotaxic gene delivery in the rodent brain. *Nature protocols*. 2006; 1:3166–73.
- Chan E, Kovacevic N, Ho SK, Henkelman RM, Henderson JT. Development of a high resolution three-dimensional surgical atlas of the murine head for strains 129S1/SvImJ and C57Bl/6J using magnetic resonance imaging and micro-computed tomography. *Neuroscience*. 2007; 144:604–15. [PubMed: 17101233]
- Chen XJ, Kovacevic N, Lobaugh NJ, Sled JG, Henkelman RM, Henderson JT. Neuroanatomical differences between mouse strains as shown by high-resolution 3D MRI. *Neuroimage*. 2006; 29:99–105. [PubMed: 16084741]
- Dong, HW. The Allen Reference Atlas: A Digital Color Brain Atlas of the C57BL/6J Male Mouse (DVD Edition). Wiley; 2008.
- Dorr AE, Lerch JP, Spring S, Kabani N, Henkelman RM. High resolution three-dimensional brain atlas using an average magnetic resonance image of 40 adult C57Bl/6J mice. *Neuroimage*. 2008; 42:60–9. [PubMed: 18502665]
- Jacobowitz, DM.; Abbott, LC. Chemoarchitectonic atlas of the developing mouse brain. CRC; 1997.
- Jacobs RE, Ahrens ET, Dickinson ME, Laidlaw DH. Towards a microMRI atlas of mouse development. *Computerized Medical Imaging and Graphics*. 1999; 23:15–24. [PubMed: 10091864]
- Jiang H, van Zijl PC, Kim J, Pearlson GD, Mori S. DtiStudio: resource program for diffusion tensor computation and fiber bundle tracking. *Computer methods and programs in biomedicine*. 2006; 81:106–16. [PubMed: 16413083]
- Jones GB, Wahlsten D, Blom G. Precision stereotaxic procedure for the mouse (*Mus musculus*): method and instrumentation. *Physiology & behavior*. 1977; 19:445–8. [PubMed: 343123]
- Kovacevic N, Henderson JT, Chan E, Lifshitz N, Bishop J, Evans AC, Henkelman RM, Chen XJ. A three-dimensional MRI atlas of the mouse brain with estimates of the average and variability. *Cerebral cortex*. 2005; 15:639–45. [PubMed: 15342433]

- Lee EF, Jacobs RE, Dinov I, Leow A, Toga AW. Standard atlas space for C57BL/6J neonatal mouse brain. *Anat Embryol (Berl)*. 2005; 210
- Ma Y, Smith D, Hof PR, Foerster B, Hamilton S, Blackband SJ, Yu M, Benveniste H. In Vivo 3D Digital Atlas Database of the Adult C57BL/6J Mouse Brain by Magnetic Resonance Microscopy. *Front Neuroanat*. 2008; 2:1. [PubMed: 18958199]
- MacKenzie-Graham A, Lee EF, Dinov ID, Bota M, Shattuck DW, Ruffins S, Yuan H, Konstantinidis F, Pitiot A, Ding Y, Hu G, Jacobs RE, Toga AW. A multimodal, multidimensional atlas of the C57BL/6J mouse brain. *J Anat*. 2004; 204:93–102. [PubMed: 15032916]
- Messier C, Emond S, Ethier K. New techniques in stereotaxic surgery and anesthesia in the mouse. *Pharmacology, biochemistry, and behavior*. 1999; 63:313–8.
- Paxinos, G.; Franklin, KBJ. *The Mouse Brain in Stereotaxic Coordinates: Compact Second Edition*. 2. Academic Press; 2003.
- Paxinos, G.; Watson, C. *The rat brain in stereotaxic coordinates*. Academic Press; Sydney: 1982.
- Sharief AA, Badea A, Dale AM, Johnson GA. Automated segmentation of the actively stained mouse brain using multi-spectral MR microscopy. *Neuroimage*. 2008; 39:136–45. [PubMed: 17933556]
- Sidman, RL.; Angevine, JB.; Taber-Pierce, E. *Atlas of the mouse brain and spinal cord* (Commonwealth Fund Publications). Harvard University Press; Cambridge: 1971.
- Slotnick BM. Stereotaxic surgical techniques for the mouse. *Physiology & behavior*. 1972; 8:139–42. [PubMed: 4579152]
- Slotnick, BM.; Leonard, CM. *A stereotaxic atlas of the albino mouse forebrain DHEW Publication*, U S. Department of Health, Education, and Welfare; Rockville, MD: 1975.
- Swanson, LW. *Brain Maps: Structure of the Rat Brain*. 3. Academic Press; 2004.
- Valverde, F. *Golgi atlas of the postnatal mouse brain*. Springer-Verlag; New York: 2004.
- Wahlsten D, Bachmanov A, Finn DA, Crabbe JC. Stability of inbred mouse strain differences in behavior and brain size between laboratories and across decades. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:16364–9. [PubMed: 17053075]
- Wahlsten D, Hudspeth WJ, Bernhardt K. Implications of genetic variation in mouse brain structure for electrode placement by stereotaxic surgery. *The Journal of comparative neurology*. 1975; 162:519–31. [PubMed: 1097481]
- Zhang W, Li X, Zhang J, Luft A, Hanley DF, van Zijl P, Miller MI, Younes L, Mori S. Landmark-referenced voxel-based analysis of diffusion tensor images of the brainstem white matter tracts: application in patients with middle cerebral artery stroke. *Neuroimage*. 2009; 44:906–13. [PubMed: 18852056]
- Zimmermann B, Moegelin A, de Souza P, Bier J. Morphology of the development of the sagittal suture of mice. *Anat Embryol*. 1998; 197:155–65. [PubMed: 9497158]

Highlights

- A new stereotaxic guidance software for high-precision stereotaxic surgery in the mouse brain is developed
- The software allows 3D visualization and navigation through the 3D CT/MRI hybrid surgical atlases of the mouse brain at different developmental ages
- Advanced features include 3D visualization for optimization of injection angles, dynamic atlas reorientation, and user interface for loading study-specific anatomic images
- The AtlasGuide software and integrated atlases will be a valuable resource for neuroscience applications

Figure 1. Micro-CT and MRI hybrid stereotaxic atlases of the C57Bl/6 mouse brain at different postnatal ages. Single-subject atlases for P7, P14, P21, and P28, and the deformation-corrected population-based atlas for the adult brain are shown. Coronal sections through micro-CT images at the level of the bregma ($y=0$) are shown for each age, with crosshairs indicating the location of the bregma landmark. Co-registered MR images show T2-weighted (T2-w), isotropically diffusion-weighted (iDW) and direction-encoded colormap (DEC) contrasts. The colormap derived from DTI is based on the orientation of structural alignment (red: medial-lateral, green: anterior-posterior, blue: dorsal-ventral). This DTI contrast is essential to delineate the detailed neuroanatomy of developing mouse brains.

Figure 2.

3D reconstruction of micro-CT image of the adult mouse skull showing bregma and lambda cranial landmarks used to define the stereotaxic coordinates for the atlases. ns: nasal suture, ss: sagittal suture, ls: lamboid suture, cs: coronal suture, pfs: posterior frontal suture, lbd: lambda-bregma distance.

Figure 3.

The user interface of the AtlasGuide stereotaxic software. The micro-CT contrast is shown as the active image, which can be changed to one of five available contrasts from the pull-down menu. The origin of the stereotaxic coordinates is defined at the bregma junction (B), where the (0, 0, 0) point is located. A grid spacing of 1 mm is selected for display, which can be manually changed by the user. The default atlas orientation with the bregma-lambda (B-L) horizontal line aligned with the Y axis is shown. Several pre-segmented brain structures are selected for visualization using assigned colors in the orthogonal 2D and 3D views.

Figure 4. Pre-operation planning using the Virtual Needle Path visualization function in AtlasGuide. In this case, a needle path (red) to target thalamic nuclei was interactively designed to pass through a narrow region between the hippocampus (green) and the caudo-putamen (yellow). This pre-surgical planning and optimization of the injection angle is possible with the 3D visualization capability and virtual needle path computation implemented in AtlasGuide.

Figure 5.

Oblique needle guidance in the adult mouse brain using AtlasGuide and a digital stereotaxic device (Stoelting Co., Wood Dale, Illinois, USA). There are two steps: synchronization of the actual brain and the atlas coordinates, and needle path guidance. A) The synchronization step sets the origin of the stereotaxic coordinates at (0,0,0) for both the device and AtlasGuide. In this case, the bregma was used as the origin. B) A region in the thalamus with coordinates (1.3, -1.8, 4.1) is targeted at a tilt angle of 30°. Visualization of the virtual needle path with AtlasGuide provides the precise stereotaxic coordinates for placing the burr holes on the skull surface. The needle position and distance to be lowered are also calculated based on the tilt angle and displayed with the original target coordinates. The operator simply dials in the needle coordinates given by AtlasGuide.

Figure 6.

Automated atlas realignment in AtlasGuide to match the experimental skull orientation. A) A function to read multiple landmark coordinates in AtlasGuide. The bregma and lambda landmarks are pre-defined, and additional landmarks can be added. After the bregma is defined as the origin, the lambda coordinates of the mouse skull are read from the device and entered in AtlasGuide, from which the transformation matrix for atlas rotation is calculated. In the example shown, the lambda landmark was located 0.3 mm lower along the Z axis with respect to the bregma. B) Sagittal view showing the atlas (CT skull image) in native coordinate space with bregma (B)-lambda (L) horizontal orientation, and the updated atlas after reorientation to match the experimental mouse skull (lambda 0.3 mm lower along the Z axis with respect to bregma).

Figure 7.

The data-loading interface of AtlasGuide inherited from the MriStudio platform. The capability to read multiple image formats enables users to load their study-specific images as reference atlases in AtlasGuide.

Table 1

List of three-dimensionally segmented structures for the adult mouse brain atlas. Each structure is segmented separately for the left and right hemispheres.

List of segmented structures for the adult brain	
<i>Abbreviation</i>	<i>Structure</i>
A	amygdala
Acb	accumbens nucleus
ac	anterior commissure
CPu	caudate-putamen
Cb	cerebellum
cg	cingulum
Cl	claustrum
cc/ ec	corpus callosum/ external capsule
En	endopiriform nucleus
fi	fimbria
HC	hippocampus
H	hypothalamus
IC	inferior colliculus
ic	internal capsule
LGP	lateral globus pallidus
Cx	neocortex
OB	olfactory bulb
PAG	periaqueductal grey
Pir	piriform cortex
S	septum
SC	superior colliculus
T	thalamus
LV	lateral ventricles