CONSTRUCTION OF A POPULATION-BASED DIGITAL ATLAS OF THE FETAL SHEEP BRAIN

by

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Abstract

Introduction

The brain may be the most complex, and therefore the least understood, organ in the body. Recent advances in imaging and computing technology are bringing innovative new tools to study the human brain. One such tool is a brain atlas, which contains numerous features allowing for greater insight into the brain's structure and function. Various forms of atlases exist. Some answer questions such as where is a given structure and what is its shape, while others seek to quantify the difference between a normal and diseased brain or measure variability within a given population. Newer atlases have enabled powerful new pathways to integrate disparate data sets creating correlative studies. The imperative step in integrating these distinct data sets is registering the image sets into one common space. Registration templates were built to provide a means for spatial normalization. Several templates exist in the human realm. Nonhuman species are more appropriate for many studies, yet the development of such targets for animal models have lagged behind. The fetal sheep brain is a widely used model in many research efforts, especially in the study of Cerebral Palsy. The existence of a population-based fetal sheep atlas would greatly benefit those studying this animal model.

Procedure

Five age-matched fetal sheep brains were selected to construct the atlas. The images were acquired at Washington University in Saint Louis, Missouri. These images were processed to extract the tissue form non-tissue. The widely used Automatic Image Registration tool was then used to build an affine atlas. The images were further aligned

by the Flow warping algorithm found in AMIRA, another separate image visualization and manipulation package. Landmarks were the driving force for the warping algorithm. The use of several imaging tools necessitated the utilization of image file conversion programs.

Discussion

Essentially, two population-based atlases were built, an intensity-based affine atlas and a deformation-based atlas. The majority of non-human atlases are affine atlases. However, the more aggressive approach utilizing warping algorithms creates well-defined images. The sheep brain is ideal as a study model partly due to the onset of sulcation. A superior registration target is the result of the additional effort taken to align the sulcal/gyral patterns found on the individual brains. While there is no gold standard for the number of images necessary to build a template atlas, this project may benefit by the registration of additional age matched images. This step is necessary if the project were to be expanded to quantify the variability found in the fetal sheep brain.

Conclusion

This project has built the foundation on which to integrate numerous image data sets of the fetal sheep brain. Its existence opens exciting new pathways in which to study the population. Researchers now have a tool to replace disparate and focused studies with a more correlative approach.

Introduction

Brain Atlases

The brain may be the most complex, and therefore the least understood, organ in the body. The purpose of this project is to contribute to the efforts studying the brain by constructing a population-based digital brain atlas of the 90-day fetal sheep brain. The atlas is an instrumental tool in numerous studies of the human and non-human brain. The types of atlases created vary with the number of studies that produce them. The Merriam Webster definition of an atlas is "a bound collection of maps often including illustrations, informative tables, or textual matter" (1). Brain atlases are used to define spatial and functional characteristics. Road atlases are used to navigate the numerous streets in a city or state. In the same manner, brain atlases are used to navigate the numerous elements of the brain. Like road atlases, brain atlases provide information about particular elements of the brain relative to other brain features (2). These features may be structural or functional. A structural atlas provides anatomical boundaries, where functional atlases provide information on regional brain activity.

Questions such as where is a given structure and what is its shape can be answered. Other answered questions include what is the difference between a normal and diseased brain or what is the variability within a given population (3). Traditional atlases answer only a few of these questions well. These traditional atlases are comprehensive maps, comprised of graphical reconstructions, highlighting important anatomical details. Examples of anatomical atlases included Talairach and Tournoux 1988 (4) and Ono et al. 1990 (5). The majority of these early atlases were derived from one or, at best a few,

specimens. These specimens were post mortem extracted brains (3). The classic brain atlas is a structural atlas consisting of serial cross-sections. Anatomical regions are designated with pointers or annotated with numbers. Newer atlases utilize Magnetic Resonance Imaging (MRI) to capture native space image sets. These are then annotated to define structure. While the prior mentioned atlases define anatomy, others map function. These atlases use Positron Emission Tomography PET or functional MRI (6).

While these atlases have proven instrumental for the purposes they were built, they remained separate endeavors. They were built to answer a specific question. "Unfortunately, each of the brain maps contained in these atlases (post mortem and in vivo) has a different spatial scale and resolution, emphasizes different structural or functional characteristics, and is inherently incompatible with the other." (3) The integration of these independent efforts has opened completely new opportunities in the study of the brain's structure and function. Relatively recent advances in imaging and computing technology have enabled powerful new pathways to integrate these data sets influencing researchers to have a more correlative approach. These advances in technology allow for the construction of multi-subject, multi-modal brain atlases. The imperative step in integrating these disparate data sets is that of building a target in which to register the various data into one common space. This common space is a registration template to which researchers can spatially normalize several images. Though spatial registration allows for the analysis of disparate data sets, significant challenges exist in using such a space normalization technique. When spatially normalized, the individual

integrity of a single brain must be maintained while capturing variability found among populations and across ages, gender, and disease states (7).

In response to this need, population based digital brain atlases were developed. A population- based atlas is essentially a probabilistic atlas derived from a well-defined and carefully selected group. Probabilistic atlases encode inter-subject variation and are constructed using three primary approaches: density-based, label-based and deformation-based (8). The Density-based approach combines multiple MRI scans to create an average intensity data set. The selected MRI scans are "linearly transformed into stereotaxic space, intensity_normalized, and averaged on a voxel-by-voxel basis"(9). The average 305 brain developed by the Montreal Neurological Institute is a density-based atlas of 305 normal brains. This approach leads to blurred-out areas; especially where spatial variability is at its greatest. The highly convoluted cortex from the variable sulcal/gyral pattern of the human brain is one such area. The label-based approach creates probability lobes of manually segmented regions. Native space images are segmented, and then linearly aligned into a stereotaxic space. Probability clouds are then calculated from the regions of interest (8). Finally, deformation-based atlases align brain structures in one scan to corresponding structures in others. This technique utilizes non-linear registration, also referred to as image warping.

The Fetal Sheep Model

The use of brain atlases and registration algorithms has been the focus of intense efforts in the study of the human brain. Numerous templates exist in the human realm. However, nonhuman species are more appropriate for many studies, such as lesion models and pharmaceutical trials. The development of such registration targets and the use of other neuro-imaging techniques have lagged behind (10). The advantages of multi-subject and multi-modal studies found in the human field are not being realized in research studies using animal models. These advantages include "the ability to detect signals in regions not known a priori, reduced influence of individual anatomic variation, ease of analysis, and increased sensitivity to low-magnitude responses" (10). Only a few examples of nonhuman population-based atlases exist, they include the Macaque (11), Baboon (10, 12) and the mouse (13, 14).

The fetal sheep brain is a widely used model in many research efforts. Its use is especially prevalent in experiments that induce hypoxic ischemic events for the study of Cerebral Palsy. The experimental techniques applied vary greatly between studies. One group used functional Magnetic Resonance imaging to study blood oxygenation during a hypoxic event (15). Others used fluorescent microspheres to quantify blood flow during the hypoxia and histology to quantify cell death (16, 17). In these three studies alone, three disparate image sets are created. The integration of the three techniques may lead to powerful new analysis in the study of Cerebral Palsy. Only traditional anatomical sheep atlases exist. Published formats include a complete adult atlas (18) and a partial atlas for the fetal sheep forebrain (19). More recent anatomical atlas are available online

(20, 21). The benefits of a registration target for images of the fetal sheep brain and data on aspects of brain function in the form of a population-based atlas are clear.

Procedure

The methodology used to build the population-based digital atlas followed well-known techniques within the digital atlas-building field. Prominent algorithms used, originated from neuro-imaging research laboratories such as the Laboratory of Neuro-Imaging (LONI) at the University of California, Los Angeles and the McConnell Brain Imaging Center (BIC) at the Montreal Neurological Institute. The image visualization and manipulation tool, AMIRA, was also extensively used.

Sheep

Five mixed Western time-bred sheep were used, each at the gestational period of 88-91 days. The full term gestational period is 145 days. The brains are the twin control of the experimental fetus in the Riddle et al. hypoxic/ischemic study (16).

Tissue Handling

Following brain extraction the tissue was immersion fixed for 48 hours at 4°C in 4% parafomaldehyde in 0.1 *M* phosphate buffer, pH 7.4. The tissue was then anchored in agarose gel, packed with gauze and submersed in PBS.

Magnetic Resonance Imaging

Scanner

All scans were acquired at Washington University in Saint Louis, Missouri and were derived from an Oxford Instruments 200/400 (4.7 T, 40 cm clear bore) magnet equipped with a 10-cm inner diameter, actively shielded Magnex gradient coil (60 G/cm, 100 _s

rise time). The magnet, gradient coil, and Techron gradient power supply were interfaced with a Varian UNITY-INOVA console controlled by a Sun Microsystems Blade 1500 workstation.

Sequence

The MRI sequence protocol was a 2D spin echo. The relaxation time was 10 seconds and the echo time was 40 milliseconds. The voxel dimension was 0.23mm x 0.23 mm x 0.5 mm.

Image Pre-Processing

Masking - MNI Display

Once the images were acquired, they were processed to separate tissue from non-tissue. This was achieved by using Display, an image visualization and manipulation software developed and distributed by the McConnell Brain Imaging Centre of McGill University. The tissue was manually masked in Display. Basic utilities also available at the BIC were then used to extract the brain tissue from the remaining signal. They are mincresample and mincmath (23).

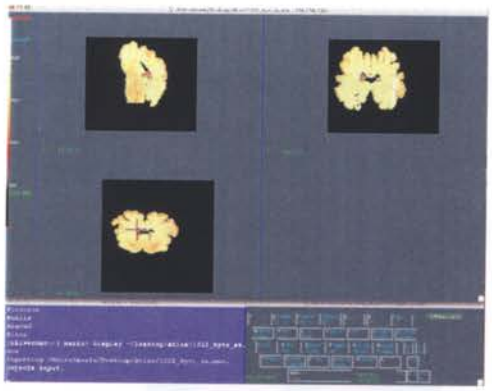


Figure 1. Screenshot of the MNI tool Display.

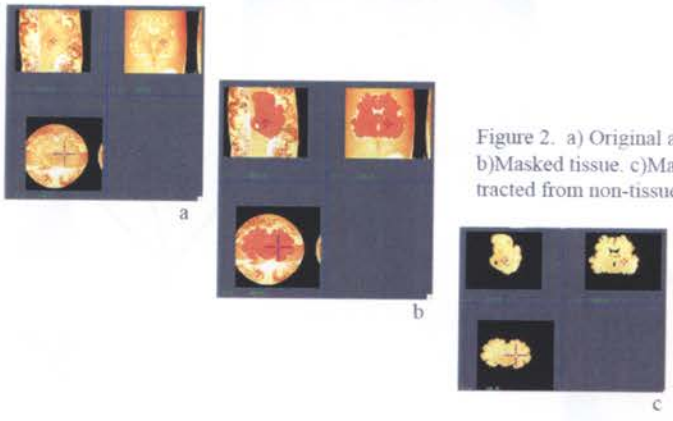


Figure 2. a) Original acquired image. b)Masked tissue. c)Masked tissue extracted from non-tissue.

Intensity Correction

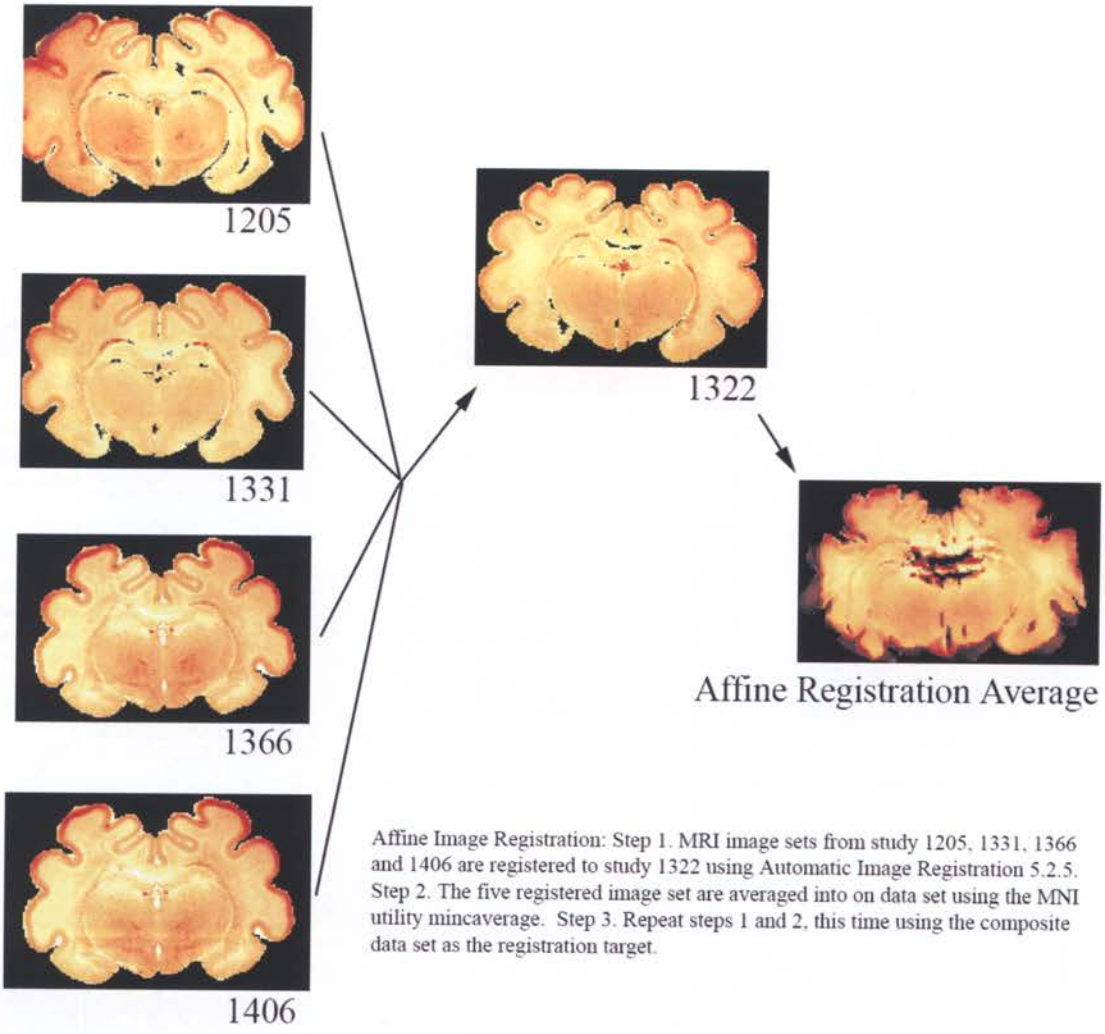
The program nu_correct was run on the five image sets to correct for any intensity non-uniformity found within the MRI volume.

Image Registration

Automatic Image Registration - 12 parameter registration

The widely used Automatic Image Registration AIR package 5.2.5 was used to create an initial affine atlas. One image series, 1322, was initially chosen to be the registration target of the other four. It was chosen due to its image quality and nearly orthogonal orientation. Once all five were aligned and averaged into one data set, the process was

repeated. This time the newly created average was the registration target. This created a density-based atlas, similar in creation to the earlier mentioned MNI 305 normal atlas.



Affine Image Registration: Step 1. MRI image sets from study 1205, 1331, 1366 and 1406 are registered to study 1322 using Automatic Image Registration 5.2.5. Step 2. The five registered image set are averaged into on data set using the MNI utility mincaverage. Step 3. Repeat steps 1 and 2, this time using the composite data set as the registration target.

MNI – register

MNI register was used to verify the resulting image registration. Register is another tool developed and distributed by the McConnell Brain Imaging Center (23). Register is a three-panel tool. The first two panels consist of the individual images, while the third displays a merged image of the two. Failed alignments were clearly discovered and the registration step using AIR 5.2.5 was repeated.

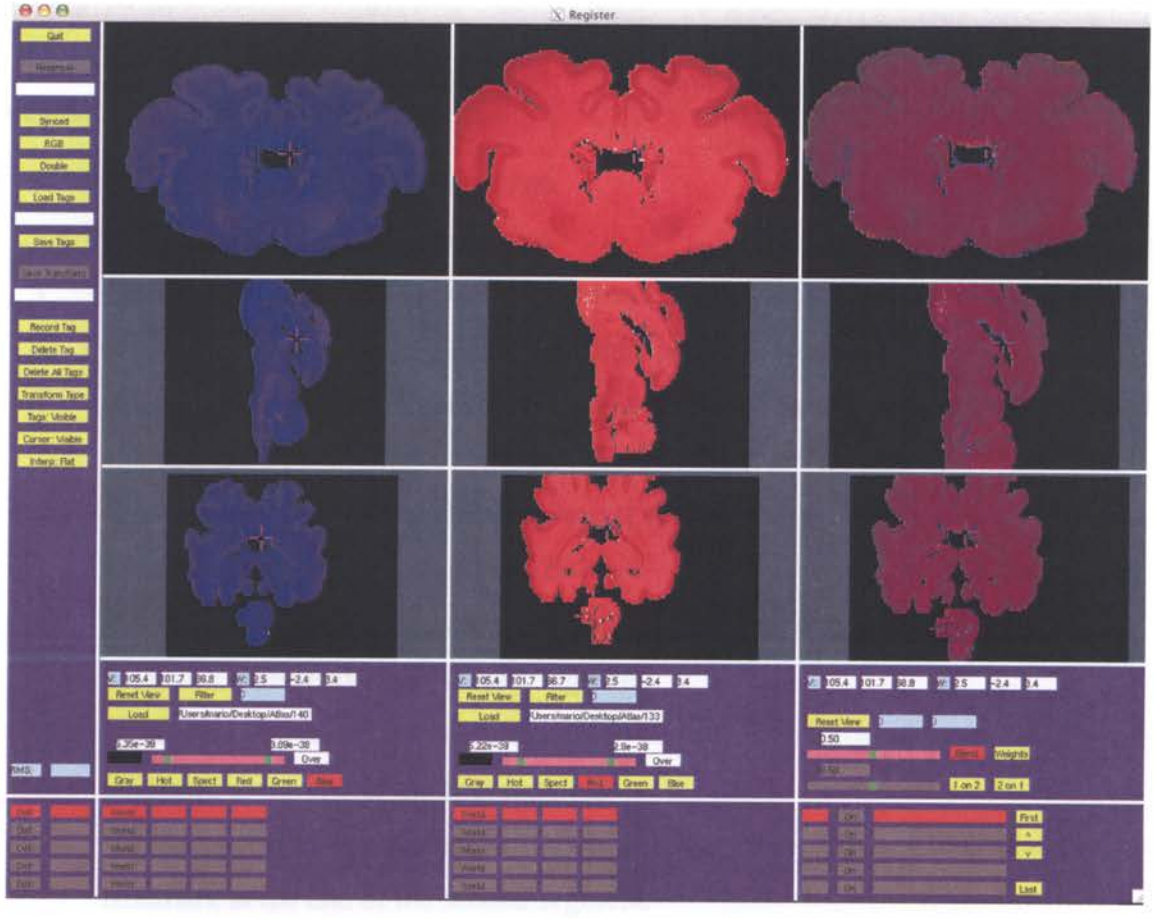


Figure 4. MNI Register: Column one (blue) and two (red) are individual image sets. Column three depicts the blended image of the two. Purple depicts the overlap

Image Warping

AMIRA landmark warping

The registered data sets are then imported to another visualization and manipulation tool, AMIRA available from TGS in San Diego, CA. Once in AMIRA, surface renderings were produced, detailing the cortical patterns on each brain. Predetermined areas were chosen on which to place the landmarks. These landmarks anchor the non-linear registration (warping) step. The area on which to place the landmarks were chosen using traditional adult sheep anatomical atlases (18, 20, 21). Points were chosen along well-distinguished sulci. (Appendix A)

1. Dorsal Extent of the Sylvian sulcus.
2. Caudal extent of the Sylvian sulcus.
3. Branching point of the Sylvian sulcus where the sulcus moves dorsal and caudal.
4. Point of intersection of the Diagonal sulcus and the Ansate sulcus.
5. Branching point of the Caudal Ectomarginal sulcus where the sulcus moves dorsal and caudal.
6. Caudal extent of the Caudal Ectomarginal suclus.
7. Most mesial point of the Marginal Sulcus.
8. Divide the Marginal Sulcus into three parts. Moving rostral to caudal, place a landmark at the end of the second segment.
9. Divide the Ectomarginal sulcus into three parts. Moving rostral to caudal, place a landmark at the end of the first segment.
10. Divide the Ectomarginal Sulcus into three parts. Moving rostral to caudal, place a landmark at the end of the second segment.
11. Most rostral point of the Coronal sulcus before it moves laterally.
12. Most dorsal point of the Calcarine sulcus.

13 & 14. Most rostal and caudal points of the olfactory suclus.

15 & 16. Most rostal and caudal points of the periamydgaloid cortex.

AMIRA Warping Algorithm

The LandmarkWarp Module was used to warp individual data sets to the affine atlas. The Flow option, with the beta parameter set at 4 and the norm at L2, was chosen among the three available (Rigid, Bookstein and Flow). The Flow warping method uses scattered data interpolation (23).

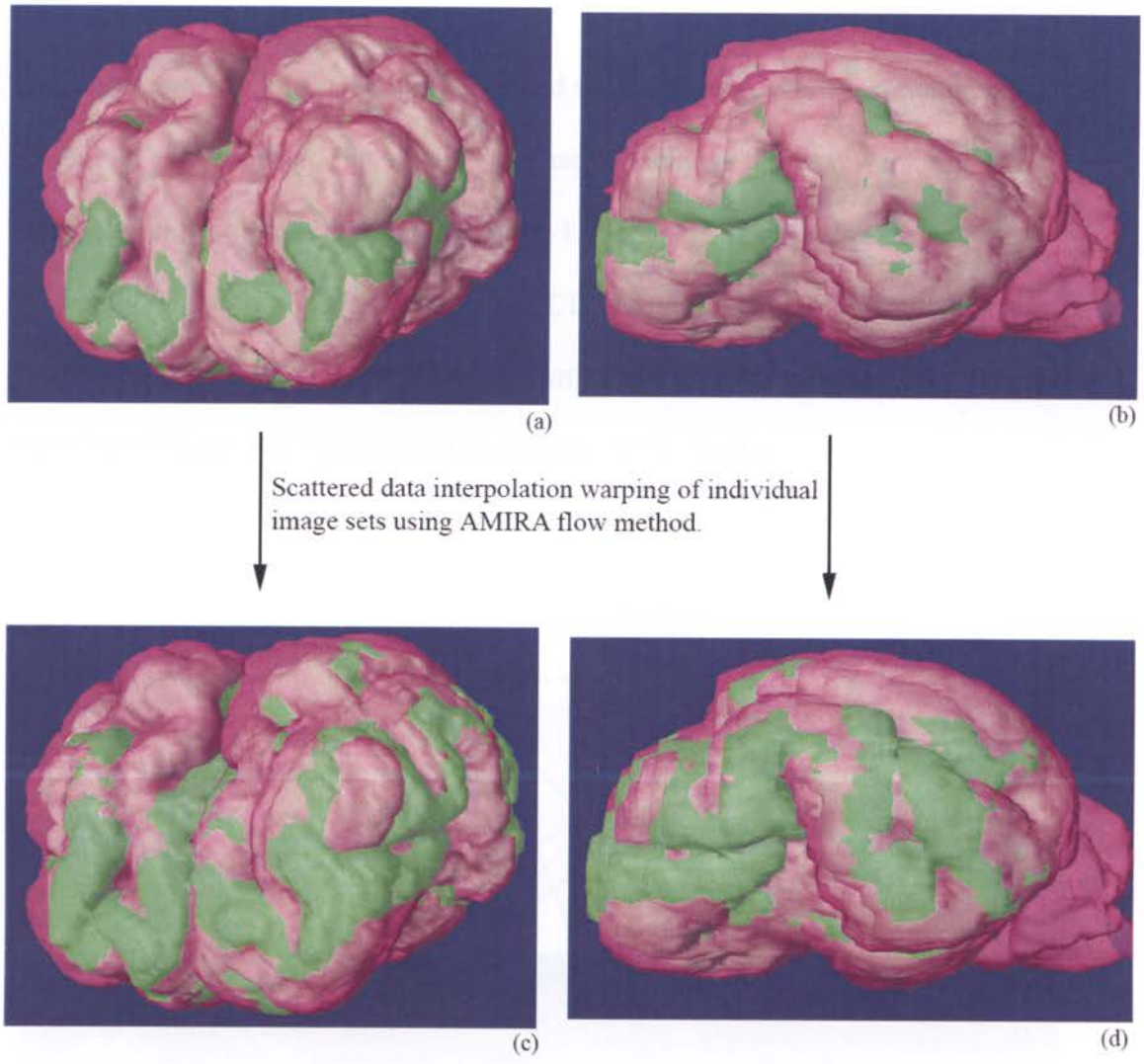


Figure 5. The purple (transparent) brain is warped to the green brain. Pictures a and b are affine registrations only. Pictures c and d are the results of the Flow warping algorithm within AMIRA.

mincaverage

The warped images were exported from AMIRA in analyze format. The BIC utility mincaverage was used to average the warped images into one comprehensive data set.

Format Conversion Tools

The use of numerous software packages and work environments necessitated numerous file format conversions. Three tools were used to achieve these conversions: LONI Debabeler, rawtominc and minctoraw. The Laboratory of Neuro Imaging at the University of California at Los Angeles (UCLA) developed LONI Debabeler. It is readily available on the UCLA Laboratory of Neuro Imaging web site (24). Rawtominc and minctoraw are BIC utilities included in the MINC package.

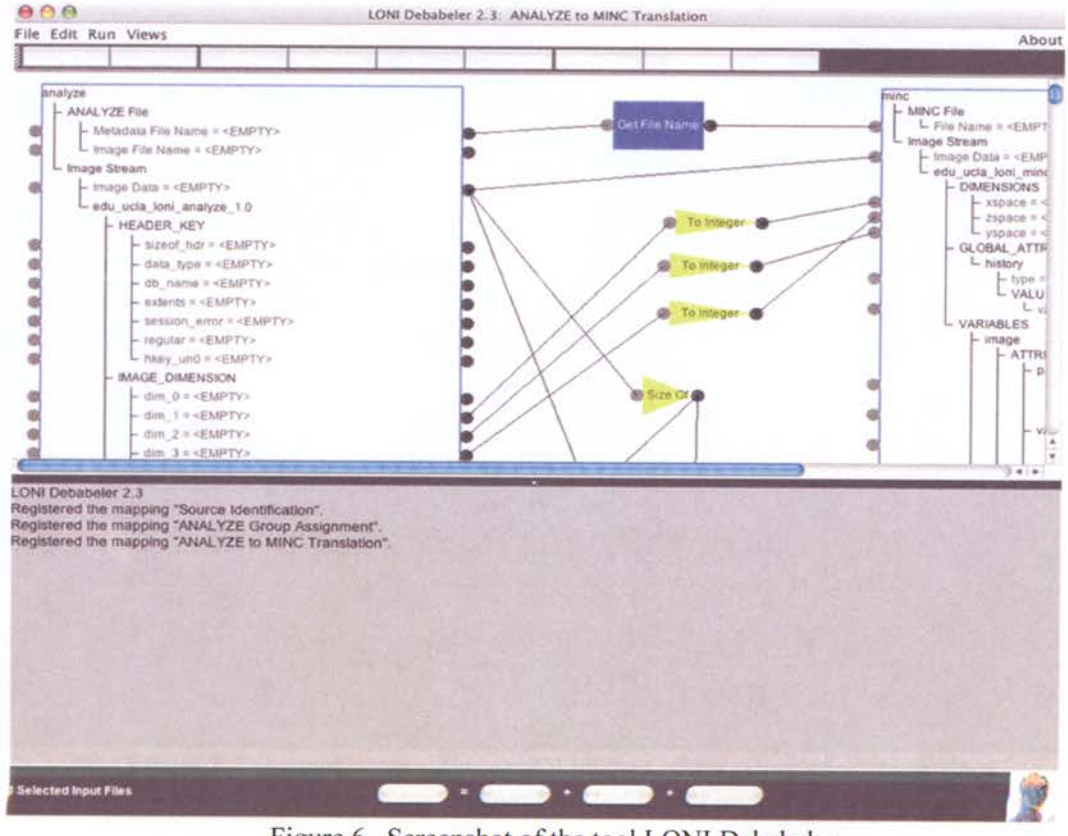


Figure 6. Screenshot of the tool LONI Debabeler.

Results

Affine Atlas

Essentially two registration templates, affine and deformation-based, were created. The image below is the result of a 12-parameter intensity-based registration algorithm using AIR 5.2.5. The aligned image sets were then averaged into one composite set using the BIC utility mincaverage.

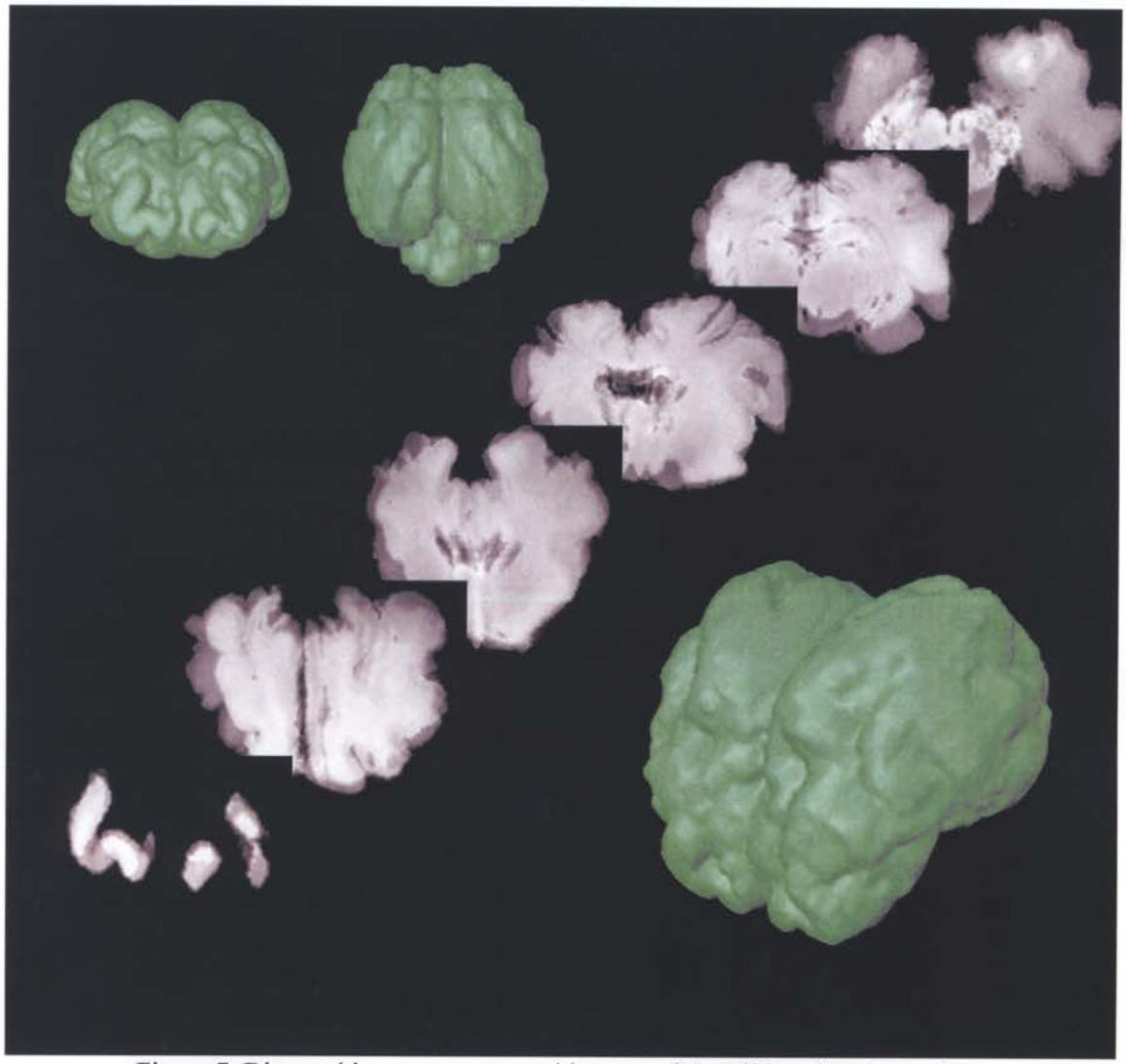


Figure 7. Diagonal images are coronal images of the affine atlas. Isosurface renderings surround the individual images.

Deformation Based Atlas

The image below is the result of the Flow warping algorithm found in AMIRA. The warped image sets were again averaged into one composite set using the BIC utility mincaverage.

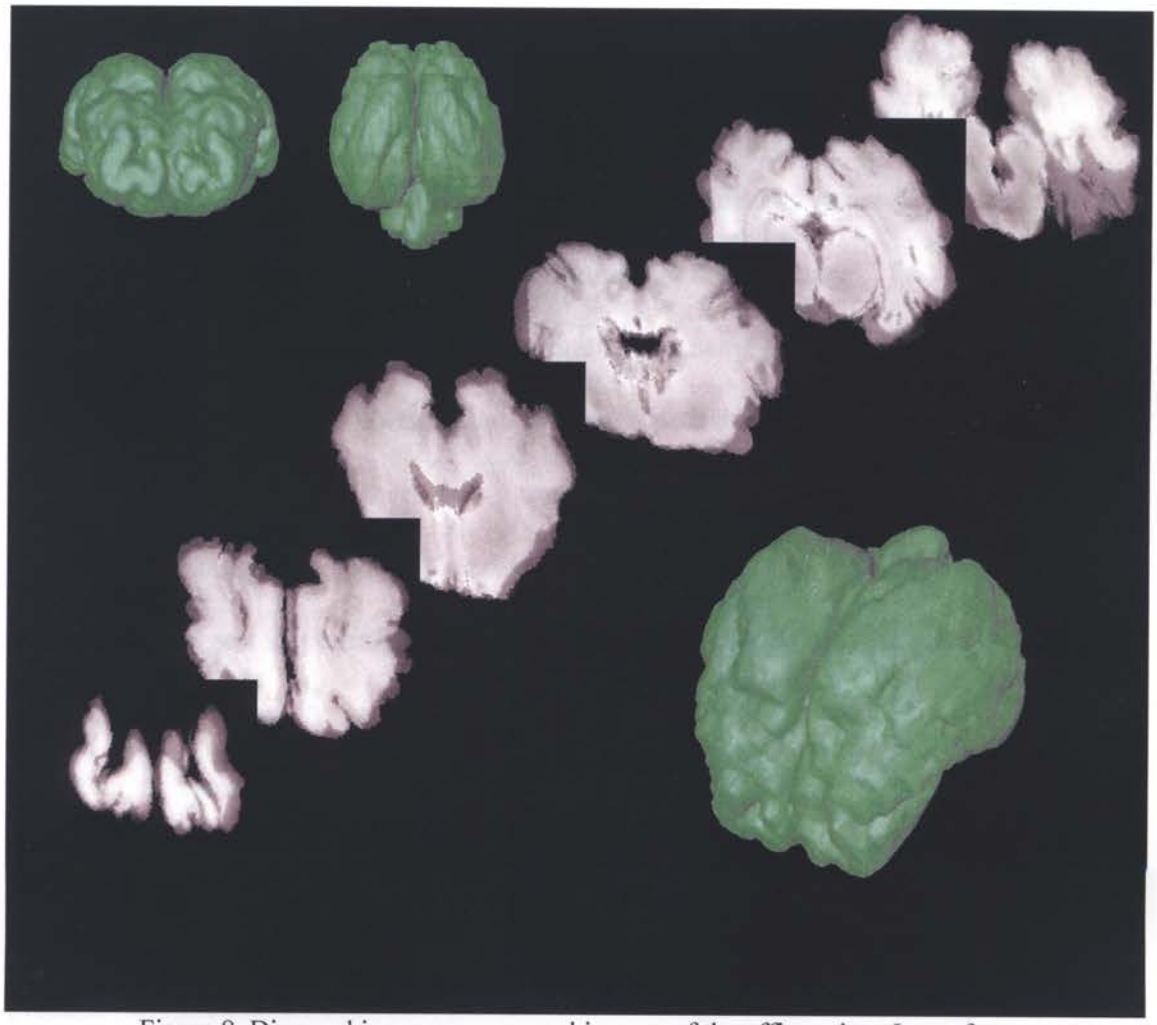


Figure 8. Diagonal images are coronal images of the affine atlas. Isosurface renderings surround the individual images.

Discussion

Impact on the Research Community

As a registration template this atlas should serve as a powerful tool to the research community that studies the fetal sheep brain. It can now be the target to integrate the disparate results of the numerous studies using the fetal sheep model. It is an ideal target because it "extracts commonalities among individual brain anatomies and filters out idiosyncrasies" (13). Researchers are able to conduct correlative studies such as, functional MR images quantifying blood oxygenation during a hypoxic event registered to images derived from a technique using fluorescent microspheres. In addition to registering two disparate image sets investigating the same question, one can register diseased state brains to the normal atlas creating a path to quantify the differences between the two states. Furthermore, it may serve as a framework to correlate gene expression, as suggested by MacKenzie-Graham et al. (14) and their building of a mouse atlas. They suggest importing gene expression maps (GEMS) into the atlas to localize gene expression to an anatomical structure.

In terms of providing anatomical detail, the MR atlas cannot match the resolution of a histology section. However, the shape of the stained slice is altered in the histology process. Distortions are introduced in the tissue cutting and in the necessary dehydration and rehydration steps. The high-resolution histology sections can be registered to the atlas, therefore utilizing the detail of stain in a correct spatial alignment. In this function the MR atlas serves as the framework to correct any spatial distortions of histology sections introduced when staining (14).

Comparison to other Non-Human Atlases

Past animal model atlas development efforts, whose purpose was to create a target template for registration, stop with the affine atlas using only the intensity-based registration algorithm (10, 12). However, the fetal sheep model is ideal for many studies due to the onset of cerebral sulcation. The variability in the pattern of this sulcal/gyral pattern is unknown. However a high amount of variability would cause a blurring effect around the atlas perimeter. I therefore, decided to further align the image sets using a landmark-based warping algorithm. Thereby, the atlas was taken a step beyond the intensity-based atlas to a deformation-based atlas and eliminating the possibility of blurry edges. Definitive sulcal pattern were used, similar to the technique used at the Laboratory of Neuro Imaging (25).

Despite the additional effort, it appears the deformation-based atlas is only slightly better than the affine atlas. This is most likely the result of averaging only five image sets. It is possible that with an 'n' of five, the cortical overlap due to the sulcal/gyral variability is not enough to completely blur the edges, as is the case with the MNI 305 atlas. An alternative explanation is that at this stage of development the variability is not great enough to cause the blurring. Further investigation is needed to determine a definitive reason.

Limitations

While there is no gold standard for the number of images necessary to build a template atlas for a defined population, a larger 'n' should equate to a greater capturing of the population variability. However, published non-human atlases have been created with as little as two subjects, (11), while the major fall with in the range of six to nine (10, 12,13) An n of five should suffice for the purposes of this project.

The method in which the affine atlas was created was widely used in the creation of past intensity-based atlases. The accompanying literature in the AIR 5.2.5 package instructs the user to choose one 'representative' brain and register the other to it. Among others Greer et al. chose this method as well (12). However, Kovacevic et al. argue that, while producing an image of high resolution, it may be biased due to the dependency of one choice image to which the others are registered (13).

Public Availability

The atlas will either live online or be available for download on a local server. The immediate plan to publicly announce this atlas is to incorporate it in a biological research study, informing the larger community in the publishing of the study. In this study, registered methyl-green stained histology sections will be used to vet the anatomical layout of the atlas. The atlas will then be the target registration template for numerous images created from varying modalities, such as fluorescent microsphere images, used to calculate cerebral blood flow and TUNEL stained sections, which quantify cell death.

Future Research

Possible future endeavors include the creation of a sub-volume probabilistic brain atlas of the fetal sheep brain, much like the one Mega et al. built for the elderly and demented human population (26). Such a tool would automate the laborious step of manually segmenting a brain into regions of interest (ROI). The use of probability clouds accommodates for anatomic variability and possible registration errors introduced. Depending on the number of ROI's this would take a significant amount of time in segmenting the spatial normalized individual brains and constructing the probabilistic clouds. Additionally, the number of image sets used may need increasing to provide a greater representation of the population. However, once completed, any future study will significantly benefit from its availability in decreased labor and increased anatomical confidence.

Conclusion

The rapid growth in imaging and computing technology has lead to powerful new pathways to study the brain. Intense efforts to capitalize on this technology have focused on applications to the human brain, resulting in a wealth of knowledge. This project applied the established knowledge to the efforts studying the fetal sheep brain. By creating a population-based atlas of the fetal sheep brain this project has built the foundation on which to integrate several image data sets and register brain research data. Its existence opens powerful new pathways to further summarize, evaluate and compare fetal sheep brain images and research results in disease and health.

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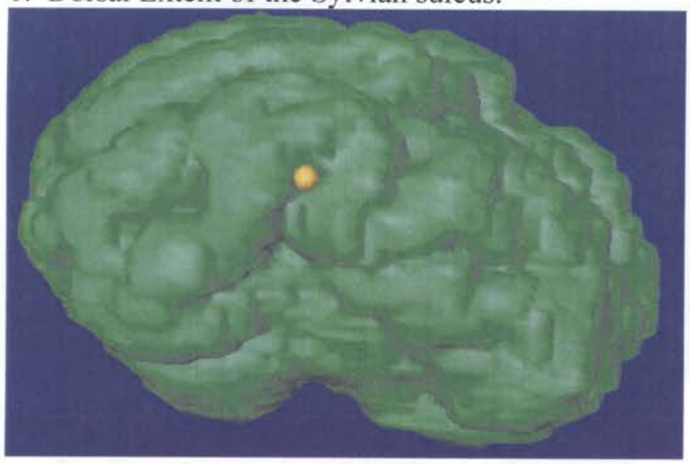
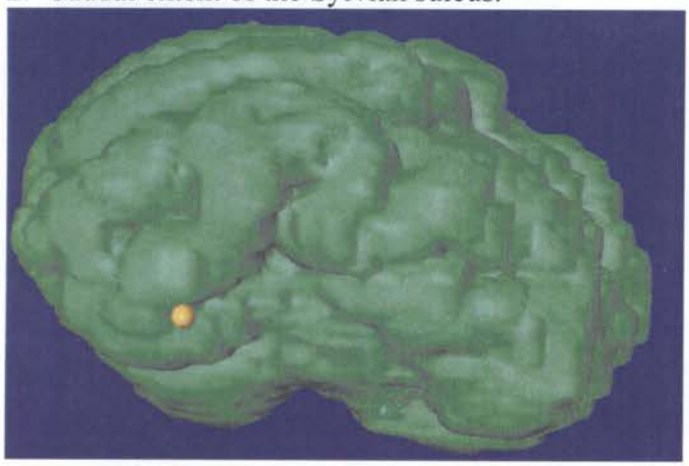
Appendix

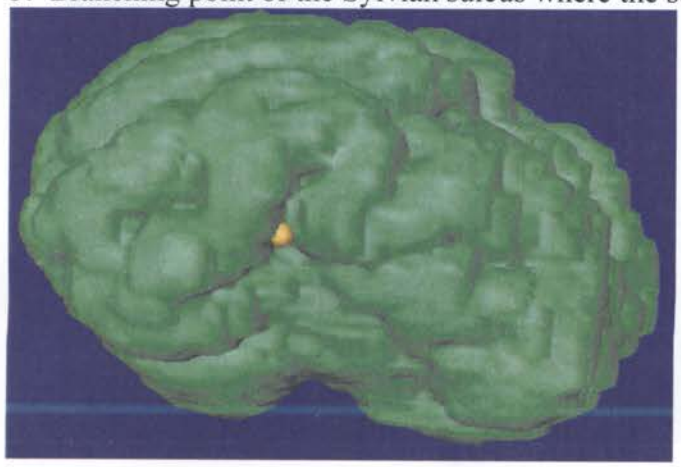
1. Dorsal Extent of the Sylvian sulcus.



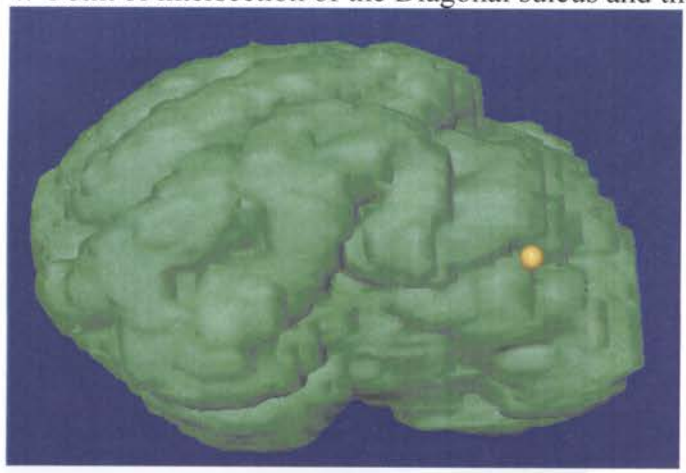
2. Caudal extent of the Sylvian sulcus.



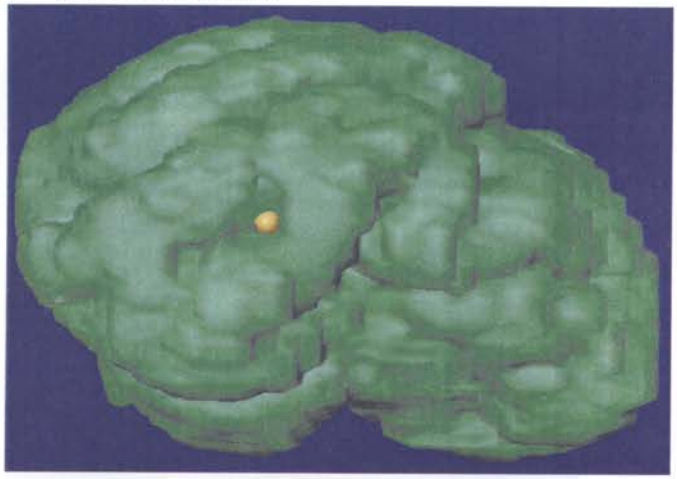
3. Branching point of the Sylvian sulcus where the sulcus moves dorsal and caudal.



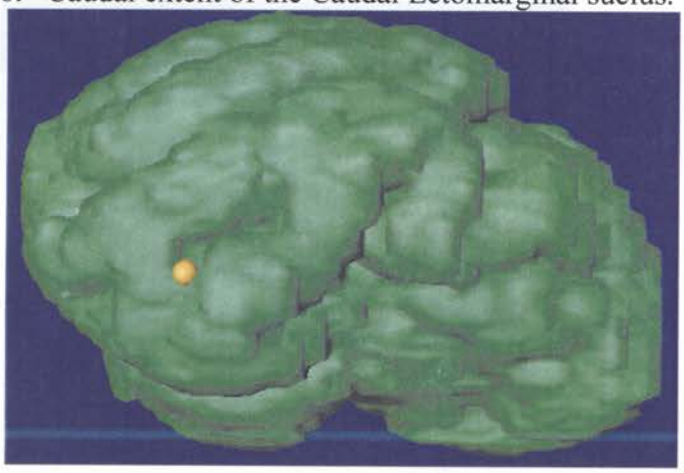
4. Point of intersection of the Diagonal sulcus and the Ansate sulcus.



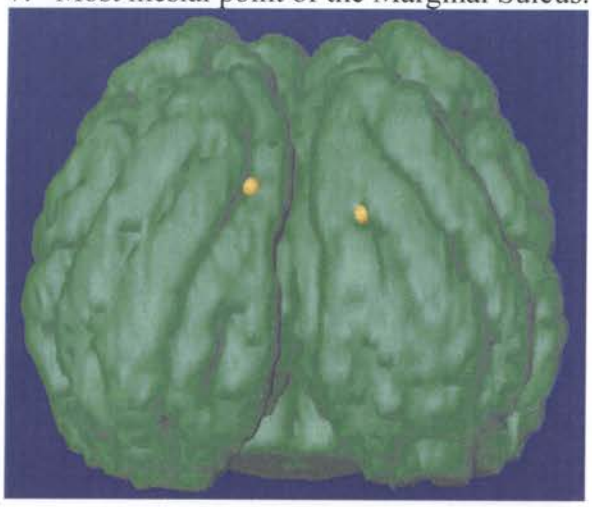
5. Branching point of the Caudal Ectomarginal sulcus where the sulcus moves dorsal and caudal



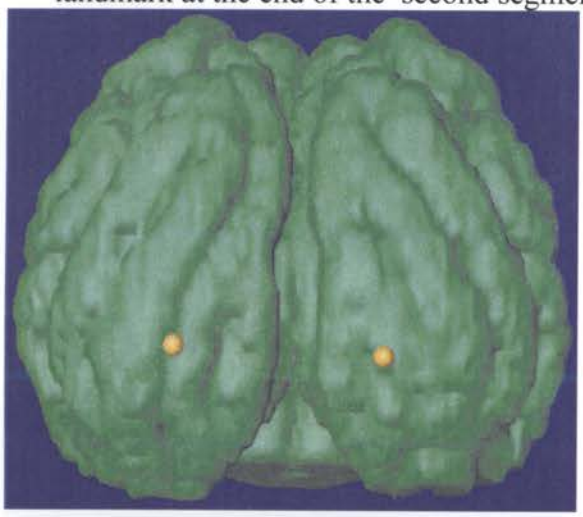
6. Caudal extent of the Caudal Ectomarginal suclus.



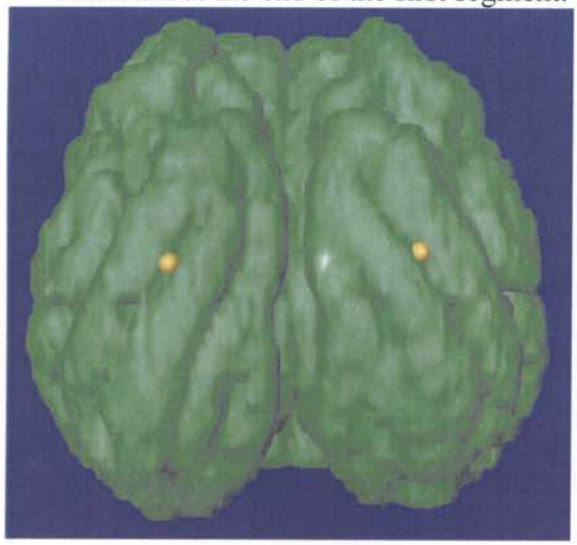
7. Most mesial point of the Marginal Sulcus.



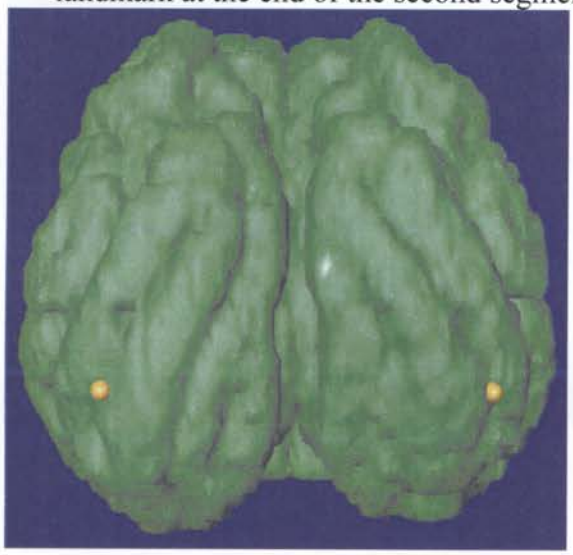
8. Divide the Marginal Sulcus into three parts. Moving rostral to caudal, place a landmark at the end of the second segment.



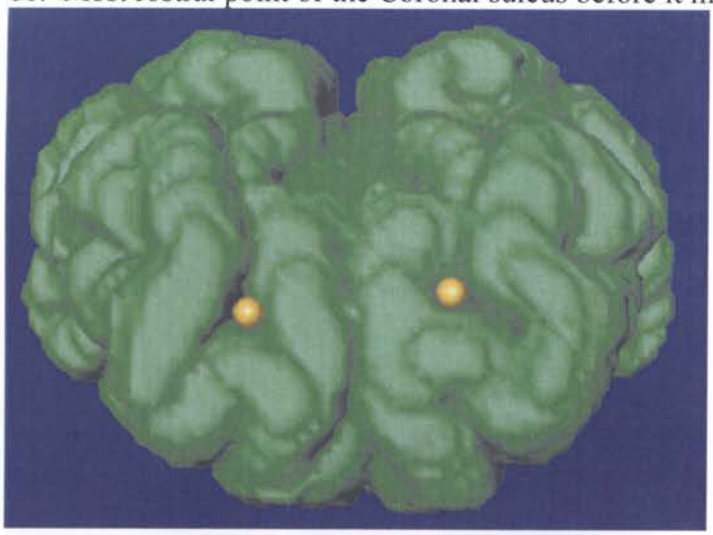
9. Divide the Ectomarginal sulcus into three parts. Moving rostral to caudal, place a landmark at the end of the first segment.



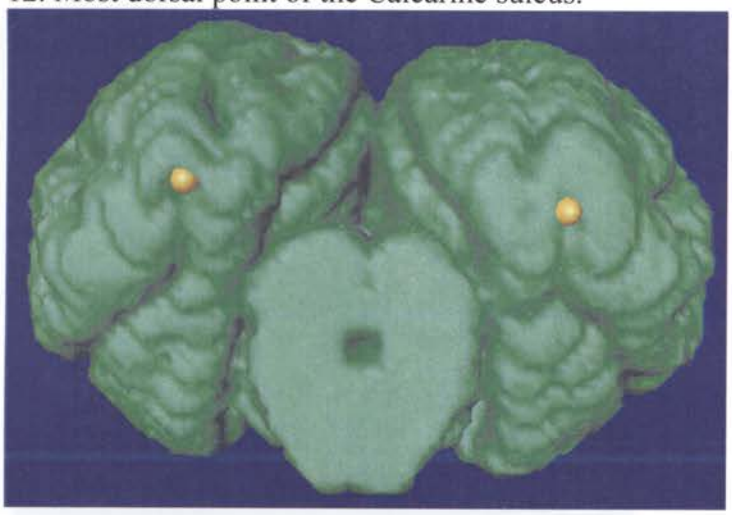
10. Divide the Ectomarginal sulcus into three parts. Moving rostral to caudal, place a landmark at the end of the second segment.



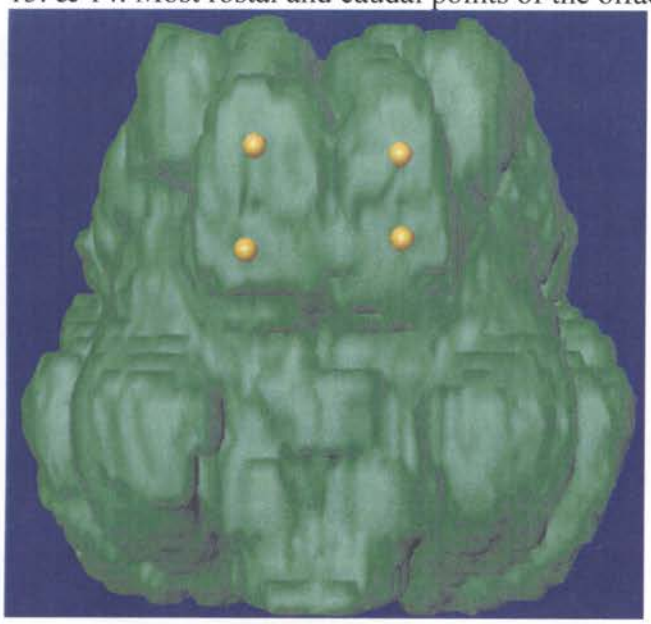
11. Most rostral point of the Coronal sulcus before it moves laterally.



12. Most dorsal point of the Calcarine sulcus.



13. & 14. Most rostal and caudal points of the olfactory suclus.



15. & 16. Most rostal and caudal points of the periamydgaloid cortex.