3D GENERALIZATION OF BRAIN MODEL TO VISUALIZE AND ANALYZE NEUROANATOMICAL DATA

by

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Neuroscientists present data in a 3D form in order to convey a better real world visualization and understanding of the localization of data in relation to brain anatomy and structure. The problem with the visualization of cortical surface of the brain is that the brain has multiple, deep folds and the resulting structural overlap can hide data interweaved within the folds. On one hand, a 2D representation can result in a distorted view that may lead to incorrect localization and analysis of the data. On the other hand, a realistic 3D representation may interfere with our judgment or analysis by showing too many details. Alternatively, a 3D generalization can be used to simplify the model of the brain in order to visualize the hidden data and smooth some of the details. This dissertation addresses the following research question: Is 3D generalization of a brain model a viable approach for visualizing neuroanatomical data?

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PREFACE

First, I would like to express deepest appreciation to my committee chair, Professor Michael Lewis. His insight into life and breadth of knowledge has continually and convincingly conveyed a spirit of adventure in regard to both research and life. Without his relentless support and guidance this dissertation would have not been possible.

Thanks to my colleagues in School of Information Sciences and Systems Neuroscience Institute at the University of Pittsburgh and in the Robotics Institute at Carnegie Mellon University for their hard work, creativity, support, and encouragement. Special thanks to Dr. Peter Strick for giving me the privilege to work at SNI and use the resources in his lab. This work would have not been possible without his kind support. Thanks also to the members of my committee, for their effort and time in guiding me. Thanks to Dr. Karimi for his valuable advice in incorporating my ideas and work into this dissertation; thanks to Dr. Hirtle for his astute judgment and advice; and thanks to Dr. Dum, for his professional insights and advice in neuroscience. I am immensely grateful and deeply indebted to them.

Finally, I would like to thank my family for their emotional support and understanding throughout my graduate studies. I am forever grateful to my parents for their confidence in me and for giving me the support to explore a new world. Most importantly, a special thanks to my wife, Umme Zarin, for always having faith in me and pushing me further than I thought I could go.

LIST OF ABBREVIATIONS

AIP   Anterior Intraparietal Area

AS   Arcuate Sulcus

CA   Calcarine Sulcus

CS   Central Sulcus

CB   Cerebellum

CMA   Cingulate Motor Area

CgS   Cingulate Sulcus

IPS   Inferior Intraparietal Sulcus

IOS   Intraoccipital Sulcus

LS   Lateral Sulcus

Lu   Lunate Sulcus

M1   Primary Motor Cortex

S1   Primary Somatosensory Cortex

PS   Principal Sulcus

PMd   Secondary Motor Area Dorsal

PMv   Secondary Motor Areas Ventral

STS   Superior Temporal Sulcus

STS   Superior Temporal Sulcus

SMA   Supplementary Motor Area

V1   Visual Area 1 (Primary Visual Cortex)

1.0   INTRODUCTION

1.1   BACKGROUND

Visualization is indispensable in promoting an understanding of different representations of experimental, simulated, and observational data. Visualization can be applied at all stages of the problem-solving process, from development of a hypothesis, through data analysis, representation of data and evaluation. With the advancement of computer hardware and software, we now have a vast amount of digital data that needs to be analyzed in order to answer complex questions that arise from these data. The human visual system can be a powerful processing system when combined with technologies such as image processing, computer graphics, animation, simulation and virtual reality. Over the years, especially in Geographic Information Science (GIS), cartographers have focused on transforming data into a more understandable and simplified form. These simplifications are often two-dimensional (2D) flattened representations of more complex multidimensional data. At the same time, a more realistic three-dimensional (3D) representation has been sought after to provide the viewer with the ability to sense depth and maximize the level of details. Both of these representations have their own set of shortcomings. For example, a 2D representation of the earth's surface requires transformation of the latitudes and longitudes of locations onto a plane. This creates a significant amount of spatial distortion. A classic example of area exaggeration is the comparison of landmasses on the map.

Greenland appears bigger than South America and comparable in size to Africa, while in reality it is about one-eight the size of South America and one-fourteenth the size of Africa. Similarly, a 3D reconstruction of the human brain (as shown in Figure 1-1) can be extremely difficult to visualize due to its structural complexity. The cortical surface of the brain has multiple folds and structural overlap that can make it difficult to identify different layers of the surface and the cells interweaved within the folds. A primate brain has relatively simple structural properties (Figure 1-2, insets) and has been intensively studied in neuroscience.

Figure 1-1: The many folds and valleys of a single human brain surface (Credit M. F. Glasser and D.C. Van Essen for the WU-Minn HCP Consortium)

2D representation of the primate brain has been used for decades in neuroscience research involving neuronal tracer substances including neurotropic viruses to label neurons and demonstrate connectivity. With the advancement of computer hardware and software, researchers are now inclined towards a more realistic 3D representation. The shift from a 2D representation to a 3D representation is probably due to the fact that successful visual inspection or analysis is highly dependent on identifying key anatomical landmarks. The more realistic and concrete the visual representation is, the easier it is to identify the landmarks. But the problem associated with each of these representations still exists. On one hand, a 2D representation can give a distorted view (Figure 1-2 shows the flattened representation of the primate cortex) that may lead to incorrect analysis of the data. On the other hand, a 3D representation may clutter our judgment or analysis by showing too many details. One way to solve the latter problem is to generalize away from reality. "Generalization" in GIS means decreasing the Level Of Details (LOD) to simplify the representation for the viewer (Ruas, 2000). 3D generalization has been used in GIS to simplify virtual city 3D models by grouping building models and replace them with cellblocks, while preserving local landmarks. Although the structural complexity of the brain is not nearly comparable to the city 3D model, one can apply the concept of generaliozation to simplify the model of the brain. In addition, a formal evaluation of the technique used to simplify the complex model is essential to understand how effective these methods are.

Figure 1-2: Color-coded representation of different cortical areas on the cortical map of the complete hemisphere of the macaque monkey. Insets show lateral and medial views of the hemisphere (Drury et al., 1996)

Spatial visualization is deeply rooted in the visualization of Neuroanatomical data. Surface modeling and cluster analysis as part of the spatial data visualization is employed to represent and draw inference from the data. Visualization of neuroanatomical data often involves visual inspection of different layers of data (i.e. labeled cells) in relation to the anatomical data layer (Bakola, Passarelli, Gamberini, Fattori, & Galletti, 2013; Borra, Gerbella, Rozzi, & Luppino,

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2011; Dum & Strick, 1991, 2005; Yuqing Liu, Yttri, & Snyder, 2010; Prevosto, Graf, & Ugolini, 2009; Van Essen et al., 2001; Zaborszky et al., 2015). Since judging the spread of the labeled cells and how dense the cells are in identifiable regions elucidates brain connections, it is important that the representation provides the viewer with accurate information about brain anatomy and the location of the labeled neurons . Both 2D and 3D data modeling have been used for representing and conveying the knowledge to the user. However, the effectiveness of these modeling techniques has rarely been studied. With the added Z dimension, representation can be tricky since occlusion, shading, surface texture and perception of depth from the 3D model plays a crucial role in drawing inference from the data. 3D representation of data may seem intuitive but the cognitive, perceptual, and technological cost of the added dimension can outweigh the benefits (Fabrikant, Montello, & Mark, 2010; Ware, 2010). In addition, studies (Montello, Fabrikant, Ruocco, & Middleton, 2003) have shown that perception of width and height in 3D is different from the 2D. A review of the literature shows that the most common representations are: 3D, inflated 3D (generalized), and 2D (flattened). For the reasons mentioned above, it is advantageous to find how effectively these different representation techniques convey the right message. In this research, I focus on developing ways to generalize and visualize neuroanatomical data. In addition, I designed a quantitative human factors study to evaluate the effectiveness of the different representation techniques used.

1.2 RESEARCH OBJECTIVE

Establishing a fundamental framework is the key to understanding the human neuroanatomy. To establish this framework, one needs to successfully interpret the results of the imaging

technology used for humans (Dice, 1945; Fiez, Damasio, & Grabowski, 2000; Inoue, Madhyastha, Rudrauf, Mehta, & Grabowski, 2014; Zhang, Kimberg, Coslett, Schwartz, & Wang, 2014)). Noninvasive techniques like fMRI (functional magnetic resonance imaging) and DTI (diffusion tensor imaging) has been used widely for human subjects but it is difficult to trace connection or identify lesion locations at the microscopic level without intervention. Observations from neuroanatomical studies in non-human primates provide the required intervention and aid in interpreting fMRI and DTI in human subjects. Since techniques (i.e. viral tracing) used for elucidating brain connection or separation of lesioned from non-lesioned tissue (lesion segmentation) in non-human primates are different from techniques used for human, it is essential to correctly interpret homologies between the non-human primate brain and the human brain from different representation used for different methods (Mah, Jager, Kennard, Husain, & Nachev, 2014). In this dissertation, I have developed ways to represent volumetric data acquired from histology, which conventionally uses 2D illustration, and represent them in a form that is analogous to the 3D representation generally used for fMRI or DTI. The generalization of the homologies between the non-human primate brain and the human brain provides ancillary benefits to interpret and translate the primate data in relation to similar displays of human imaging data. Since anatomical data can be very complex, the generalization is done in multiple steps. An application using Java and a combination of self-developed algorithms and Visualization Toolkit (VTK) library functions is developed to generalize and visualize the neuroanatomical data in 3D. To answer the research question in this dissertation, an approach, with a generalization component and a visualization component, is proposed. The primary research question in this thesis is:

"Is a 3D generalization of the brain a viable approach for visualizing neuroanatomical data?"

The generalization of the 3D models helps us to visualize cells deep inside the structure in the context of the brain without completely distorting the anatomical structure. Landmark identification is the key to observe variations in the data from one animal to another. Statistical analysis is often not possible because data are compared between two animals with significant variation in anatomical structure. A visual inspection of the density relative to landmarks is the key to analyzing such data. Since there is a tradeoff between exposing the hidden data and identification of key landmarks using the generalization technique, the proposed research is approached by:

1. Conducting a thorough literature review of neuroanatomical data visualization and visualization in general

2. Developing algorithms for the generalization of neuroanatomical data

3. Developing a tool that implements the proposed visualization methods

4. Conducting a pilot study to refine the dependent variables list gathered from Section 3.B when the three (2D, Generalized 3D, and Full 3D) conditions are applied

5. Conducting an experiment to find the effectiveness of the three (2D, Generalized 3D, and Full 3D) conditions on visualizing neuroanatomical data.

Overall, in this work I will provide insight into how techniques that are used to draw insights from a 2D representation compare with those in a 3D representation of neuroanatomical data and whether the viewer's knowledge gain can be enhanced by the proposed generalized display technique of the 3D brain.

2.0   BACKGROUND WORK

Considerable effort has been directed at elucidating the connections between different areas of
the brain. Understanding these connections is important to unravel the functional mechanisms of
each area of the brain. This understanding, in turn, may help us tackle diseases that are affected
by these areas. Several methods have been used to demonstrate the connections in the brain.
Neurons can be labeled by injecting tracers that travel from one neuron to the other. These
labeled neurons then can be identified by taking sections of the brain and marking them under
the microscope. However, it is important to understand how data are acquired using this
technique and why surface modeling and cluster analysis are employed to analyze the data in
details. I surveyed several seminal papers that used rabies virus as tracers and investigated
different visualization techniques used to represent the data. The first section in this chapter
focuses on how data are currently analyzed and displayed and what challenges we encounter in
visualizing such data. The purpose of this survey is to gather domain specific knowledge about
the visualization techniques used. The following section gives an overview of techniques used to
generalize 3D city and brain models. The last section identifies specific visualization challenges
that we encounter when visualizing complex structures (i.e. cortical surface).

2.1 VISUALIZATION IN NEUROSCIENCE

Visualization of neuroscientific data is met with similar challenges when dealing with data from other domains, like storage and retrieval of big data, reconstruction, visualization of multimodal data - to name a few. Although the obvious problem of such data is the sheer size of it (in petabytes), the crux of the problem with dealing such data lies in its interpretation. Since all the analysis and techniques evolve around the characteristics of biological data, it is imperative to understand in detail what it is and why certain analysis is important for such data.

2.1.1 Biological Data Characteristics

Most biological volume datasets are acquired by scanning biological object of interest using Magnetic Resonance Imaging (MRI), Computer- aided Tomography (CT), Positron Emission Tomography (PET), and/or Sonogram machines. Another source of volume dataset is through laser scanning confocal and other high power microscopes. However, a downside of histological data acquisition using a microscope is that the biological specimen usually must be sacrificed to capture the underlying phenomena or to simply fit it under the microscope. It is important to make the distinction between these two types of data because histological data often requires additional spatial aligning due to distortion inherent in tissue processing. Analysis of multiple specimens of the same species is also difficult because of different anatomical shape and sizes. Data from multiple specimens are prone to registration errors and data at microscopic level is spatially dislocated during the alignment correction phase. The first step involving such spatial data analysis involves volume rendering. Volume data usually represents the anatomical structure of the biological sample.

2.1.2 Challenges of Visualizing Biological Data

The visualization of biological data can be quiet challenging due to the anatomical and functional complexity. Within the nervous system alone, type of cells and their functions varies a lot and often these varied population of cells coexist which makes it difficult to track both functional and structural connectivity within those cells. Jeff Lichtman (Lichtman & Denk, 2011) identified five general problems in understanding the relation between the structure of the nervous system and its function. The first problem in analyzing biological data, especially neurobiological data, is the immense diversity of nervous cell types. Although it is possible to identify different cell types using immunohistochemistry, the plotting and mapping pose a big challenge. The second problem is the imaging of electrical and chemical activity. This problem poses a challenge of mapping multimodal (electrical & chemical) responses of the nervous system. The third problem is that neurons extend over vast volumes. This can be a difficult focus + context problem to solve because simple interaction of localized volume may not be sufficient in understanding the extent of the functional response. Another similar and fourth problem is the level of details. The critical details of neuronal connectivity occur at the level of the synapse. It is easy to forget the structural context when mapping very minute levels of details. The fifth and the final problem identified by Lichtman is the need for even more dense and saturated construction to avoid functional overlaps.

Imaging and functional data are often multidimensional, noisy and dense. Reconstructing such high volumes can be difficult and require considerable time to execute queries on them. The biggest challenge is to understand the functional activity in the context of the entire brain. In addition, answering domain specific knowledge-based queries can be challenging due to different processing techniques. The final output of the analyzed data may not be identifiable by

experts from one domain to the other. For example, reconstruction of fMRI data and histological data are quite different due to lack of high-resolution data in fMRI. Histological volume reconstruction contains cellular level details that are absent in fMRI. Finally, producing the tools for interacting with a 3D model is not easy. The popularly used ray casting method can identify objects right on the 3D display screen but it is not entirely accurate, especially when occlusion and noisy data are present.

2.1.3   Towards Effective Visualization

One must consider the characteristics of human visual processing in order to create an effective visualization. Colin Ware (Ware, 2013) describes the human visual thinking process and its components. Based on his description of these components and their limitations, we can map out an effective solution for the visualization problem. A few key areas where effective visualization methods have been applied are discussed below.
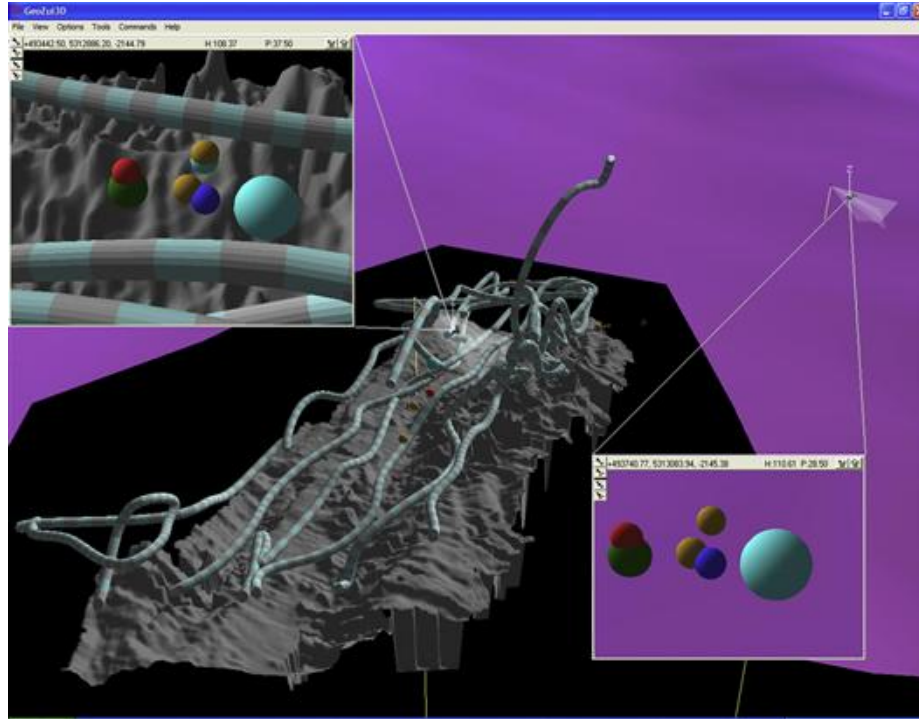
Figure 2-1: Sub-windows allow details to be compared while the overall context can also be seen (GeoZui3D interface). (Ware, 2013)

Maintaining "*Focus + Context*" when visualizing the level of details is a big challenge in anatomical data visualization. The brain is organized over six orders of magnitude. The functional activity can be seen at the micron level whereas even the mouse brain is 100,000 times larger. . One way to overcome this problem of viewing fine levels of detail while maintaining context is to apply distortion-oriented presentation techniques. This kind of technique involves distortion of the information space by showing certain information larger and shrinking others. The polyfocal display (Kadmon & Shlomi, 1978) , fisheye view (Furnas, 1981), bifocal display (Spence & Apperley, 1982) and perspective wall (Mackinlay, Robertson, & Card, 1991) are examples of such display techniques. Although these techniques can hide important information, this problem can be mitigated by implementing a user determined degree-of-

relevance factor. The generalization technique used to abstract information for both the 3D city model and the brain model (as proposed in this work) tackles some of the challenges that are inherent to the "Focus + Context" problem. Another interesting way of exploring details while maintaining context is the multiple window approach (Plumlee & Ware, 2002) which takes into account the limitations of the visual working memory. The authors show that having multiple windows working as a placeholder for the working memory increases the user's ability to compare more patterns in larger space. This can theoretically alleviate the problem of visualizing multiple data sets (varied anatomical models) in a large space.

Interacting in 3D with the nodes that represent data points or objects can be tricky. Most of the interaction in neuroscientific data visualization focuses on dealing with neuronal connection represented in a form of graph. Novel ways to select nodes of the network data have already been applied in NAViGaTOR (McGuffin & Jurisica, 2009), a software package for visualizing and analyzing biological networks. The authors show that rearrangement of selected nodes and compact context menu help maintain context. However, these kinds of selection techniques are useful when observing short chain networks. The interactions involved in inspecting 3D models are a little different since the user has to constantly view the model from different perspectives while maintaining context. The interaction technique must also engage the user in eliminating the obscuring of data by solid body (Livingston et al., 2003).

Dynamic queries are also part of an interaction technique to visualize and navigate through discrete multidimensional data. An interface developed by Ahlberg (Ahlberg & Shneiderman, 1994) shows how dynamic queries are used to narrow down points using range sliders. Another nice way to construct sliders is to use the distribution of the variables to combine distribution information of the objects along with the ability to even select

discontinuous regions (Eick, 1994). Usage of magic lens filter for dynamic query is also useful. Fishkin in his paper (Fishkin & Stone, 1995) showed that stacking magic lenses to reveal the chain transformation can be an enhancement to dynamic queries. Although these interaction techniques are useful in some data exploration task, they are highly subjective and applying them to neuroanatomical data visualization remains to be investigated.
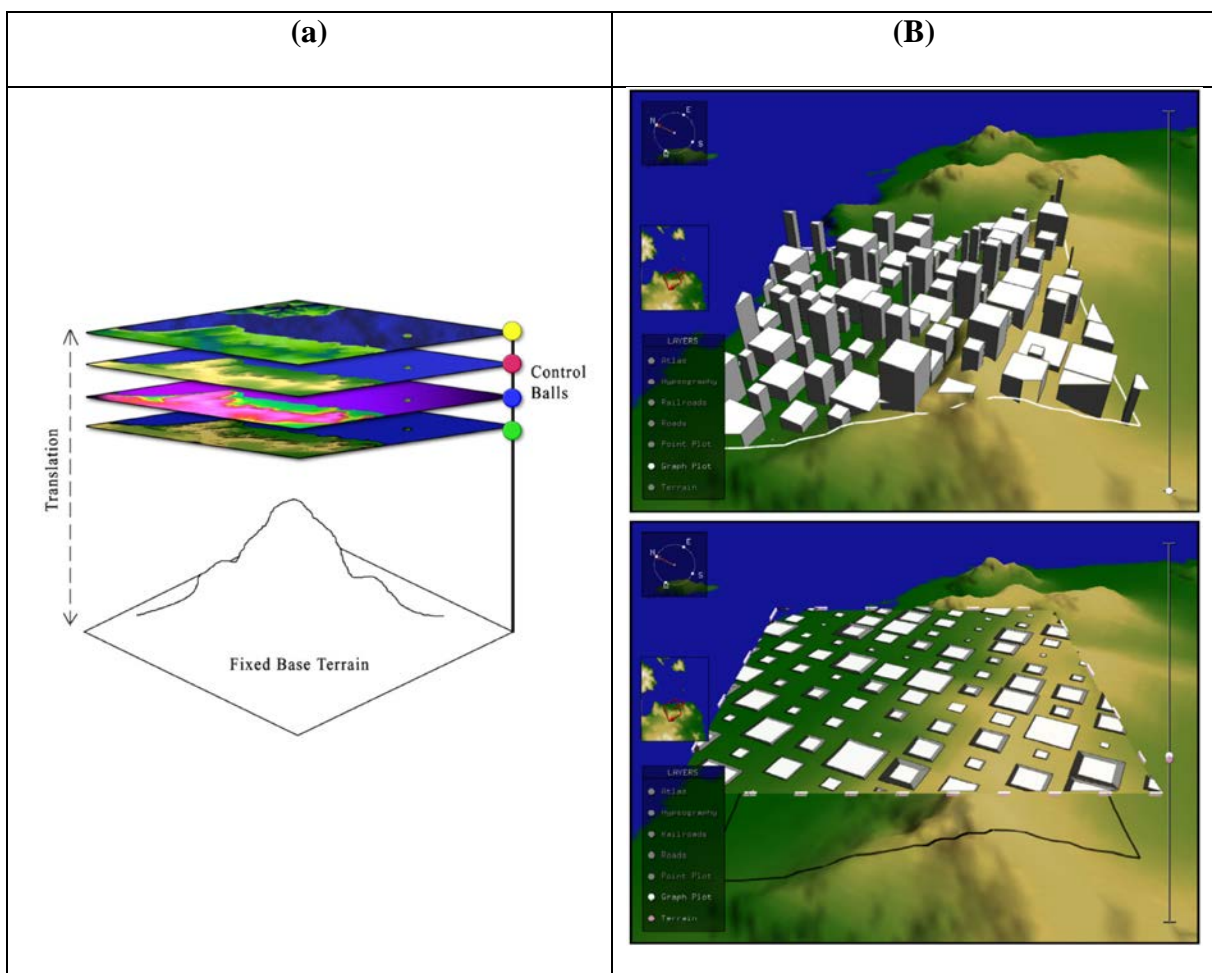
Figure 2-2 : 2D/3D hybrid visualization(a) Multiple 2D/3D layers in hybrid display over a 3D base terrain. The vertical translation of each layer is set with its associated control ball. (b) A landmark layer a ground-level (top) and at the flattening level (bottom) (Brooks & Whalley, 2008)

Gaining insight from 3D spatial datasets can be particularly challenging. Self-occlusion prevents the user from viewing data all at once. It can be even more challenging when multivariate data need to be presented in a meaningful way. Another challenging part of 3D visualization is to find the best way to simultaneously visualize multiple types of data (i.e different functional data). In geographical information systems (GIS), a lot of research effort has been made to find a solution to this problem. Studies show that 2D views are often used to establish precise relationships, while 3D views help in the acquisition of qualitative understanding (Springmeyer, Blattner, & Max, 1992). Since both viewing modes have distinct advantages, a hybridized viewing mechanism may be useful in interpreting the spatial relationship. Following this concept, Brooks & Whalley (2008) came up with a unique visualization system for spatial data. In their hybrid system, the 3D terrain can be projected into a 2D plane (and vice versa). This process allows the user to view the 2D data in direct relation to the 3D view on the same screen. In their system, the user also has the ability to construct multiple 2D/3D layers that can be separated and viewed for analysis. Figure 2-2(a) shows the two different layers in the same view. Figure 2-2(b) demonstrates the projection of the building layer to a 2D layer. In volumetric data visualization, this type of overlay technique can useful after the surface has been constructed and different data layers have been registered to the corresponding surface and landmarks. Unlike the earth's surface, the volumetric object can have complex structure (i.e. brain cortical surface), which makes overlay of different data layers very complex and tricky. The specific challenges are discussed in later chapters.

2.2   ELUCIDATING BRAIN CONNECTIVITY: HOW, WHAT, AND WHY

Revealing the organization of multi-synaptic circuits in the central nervous system has been a ubiquitous quest in neuroscience research. Some of this research has included development of new techniques to elucidate the circuitry in the brain. The use of neurotropic viruses to trace neural connections is one such technique. For example, rabies virus is capable of infecting nerve cells and moves from neuron to neuron exclusively at the synaptic connections.   After transferring across a synapse, rabies virus moves from the axon terminals to the cell body in the retrograde direction. In the cell body, the rabies virus replicates and thereby, amplifies the tracer signal. This process can be repeated at subsequent synaptic connections to reveal chains of synaptically linked neurons in a time dependent manner . This process has been termed "retrograde transneuronal transport" of virus. The fact that rabies virus rarely causes the infected cell to rupture and contaminate the extracellular space makes this virus a good candidate for transneuronal tracing. The rabies virus allows us trace the connections from a given brain region back to the source of the signal. Since this connectivity cannot be traced in real time, the animal is sacrificed at different stages of virus infection and connections are deduced from multiple experiments. Figure 2-3 shows labeled Purkinje cells in the cerebellar cortex (source) when the rabies virus was injected into the primary motor cortex (destination) of the animal.
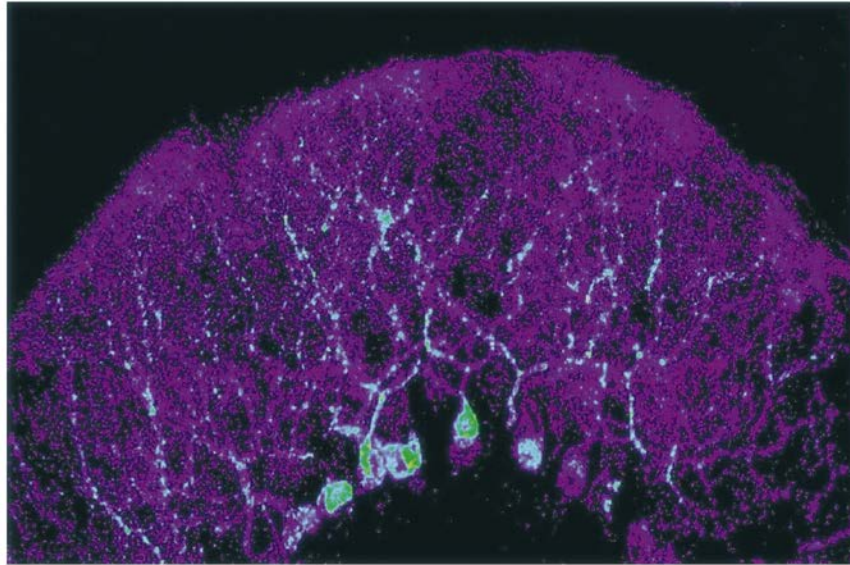
Figure 2-3. Purkinje cells in the cerebellar cortex labeled through retrograde transneuronal transport of rabies from the arm area of the primary motor cortex of a Cebus monkey.Transport of three orders of interconnected neurons can be visualized. From first-order neurons in the thalamus to 'second-order' neurons in the dentate nucleus to 'third-order' Purkinje cells in the cerebellar cortex (Kelly & Strick, 2000)

In addition to locating the primary origin of the signals that control our movements, scientists are increasingly interested in learning the spatial organization within the regions that control one particular type of movement. These topographic divisions help neuroscientists understand and locate areas that can in turn be used to treat diseases involving those areas in the brain. For example Dum, Li, and Strick (2002) reported a topographic map of the functional output to the monkey dentate using the herpes simplex virus (HSV1), another virus that is transported transneuronally. In their study, when the monkeys were injected with the HSV1 into specific parts of the primary motor cortex (M1), the viruses traveled through the neuronal connections to reveal the output source of those targeted areas of M1. A flattened map of the dentate was created in order to visualize the density. Figure 2-4 shows the flattened map of the

dentate nucleus, where compact clusters of neurons were identified that project via the thalamus

to representations of the leg, arm and face in M1.
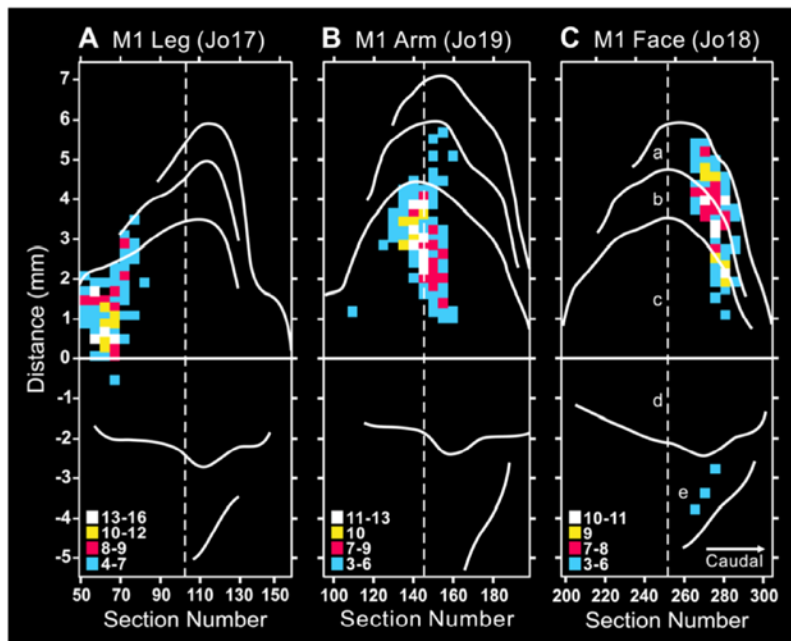
Figure 2-4. Somatotopic organization of dentate output channels to M1.Unfolded maps of the dentate illustrate the neurons labeled after HSV1 injection into the leg (A), arm (B), and face (C) representations of M1.

From these density maps (Figure 2-4 A,B and C) and further experiments with injections into non-motor regions of cerebral cortex, the authors were able to create a topographic map of the dentate nucleus as shown in Figure 2-5. On the map (Figure 2-5A), the authors placed a label at the location of the greatest density from the density maps (Figure 2-4). The map reveals, as the authors report, that the dentate provides afferents to both motor and non-motor areas of the cerebral cortex. This is in contrast to the classical view that the dentate provides afferents only to motor areas of the cerebral cortex. Not only that, the map shows that there are spatially

segregated domains that target the motor and non-motor areas of the cerebral cortex. The representation of the data (a 2D map in this case) provides a way for the viewer to understand this segregation. However, the map by itself does not provide any context to the viewer with respect to the brain.
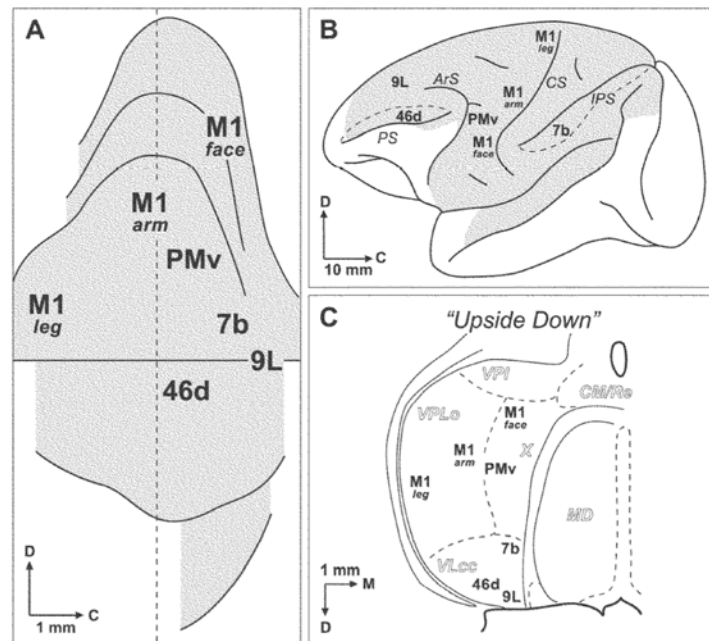
Figure 2-5. Topography within the cerebello-thalamocortical circuit.(A) Dentate output channels. Labels mark the location of the highest concentration of the dentate projections to each cortical area studied. (B) Selected cortical targets of cerebello-thalamocortical circuit. Color shding designates regions of the lateral hemisphere that project to the cerebellum via pons. (C) Upside down view of the ventrolateral thalamus to indicate the correspondence between the topography and that of dentate.

The use of transneuronal tracing with viruses has led to changing views of brain function. One of the classical views is that the cerebellum and basal ganglia are separate subcortical systems. . Recent studies have shown that the basal ganglia and cerebellum are reciprocally

connected . Bostan et al. (2013) reported the existence of synaptic pathways connecting the cerebellum with the basal ganglia. These authors found that the projections from motor and non-motor regions of the subthalamic nucleus terminated in both motor and non-motor regions of the cerebellar cortex. This observation indicates that basal ganglia influence both motor and non-motor functions within the cerebellum. This continuing expansion of connectivity maps of the brain may play an important role in identifying and treating neurological diseases. For example, neuroimaging studies have shown the involvement of the basal ganglia in reward prediction and reward-based learning with the cerebellum. Since the cerebellum is thought to be involved in adaptive modification of behaviors, functional MRI studies on addicted individuals provide compelling evidence (Figure 2-6C) that cerebellum is active when addicted individuals interact with conditioned drug cues that increase craving. This evidence also may explain why lesions in both regions impair reward-based learning and explain functional interaction between the cerebellum and basal ganglia. Activation in the cerebellum and basal ganglia in Tourette syndrome tic generation has also been shown in other neuroimaging studies (Figure 2-6D), confirming the existence of a connection between the cerebellum and basal ganglia. The finding of a connection between different functional regions of the brain has led us to better explain why abnormal activity in one can have a pervasive effect on the other.

Observations from neuroanatomical studies in non-human primates provide a fundamental biological framework for understanding human neuroanatomy and are particularly informative for interpreting fMRI and tract tracing methodologies (DTI) in human subjects. Neuroscientists who are engaged in fMRI and tract tracing in human subjects are often unfamiliar with the primate neuroanatomy and brain morphology. Results from neuroanatomical studies in non-human primates are qualitative and displayed in relation to key morphological

features of the primate brain. Determining and interpreting the homologies between the non-human primate brain and the human brain is impeded by their complex cortical structures. Our goal in producing a generalized brain model for displaying non-human primate neuroanatomical results is enable the viewer to interpret and translation the primate data in relation to similar displays of human imaging data.
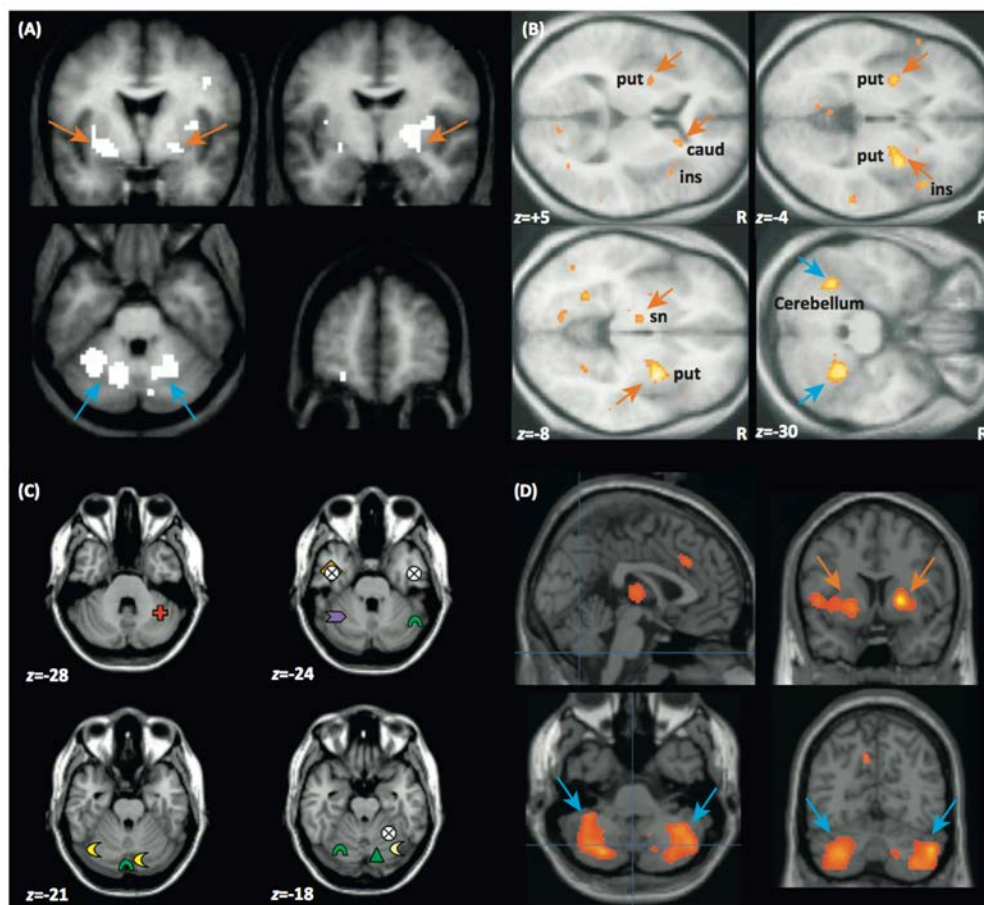
TRENDS in Cognitive Sciences

Figure 2-6. **The image shows cerebellar activation associated with learning paradigms and neuropsychiatric conditions.(A) Functional MRI study of appetitive conditioning with a pleasant taste reward. Activation (white) in the basal ganglia and the cerebellum correlates with temporal difference prediction error. (B) Functional MRI study of higher-order aversive conditioning. Activation (yellow/orange colors) in the basal**

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ganglia and cerebellum correlates with temporal difference prediction error. (C) Cerebellar involvement in addiction. Summary results of cerebellar activation associated with cue-induced craving. Different shapes indicate results from different studies. (D) Tics in Tourette syndrome (tics minus sleep contrast) activate both the cerebellum and basal ganglia. In all panels, blue arrows point to sites of cerebellar activation and orange arrows point to sites of basal ganglia activation.

2.3   3D GENERALIZATION

Generalization is essential for effective visualization. 3D generalization can be used to remove unnecessary details that may interfere with the viewer's successful insight into the data. Generalization can also reduce the level of details (LODs) for more efficient storage and computation. Generalization is a well studied process in cartography and is defined as a method that represents information on the map in way that adapts to the scale and the purpose of the map (Ruas, 2000). Although the development of the generalization process in GIS has increased dramatically, the generalization concept is rarely used when visualizing a 3D brain model.
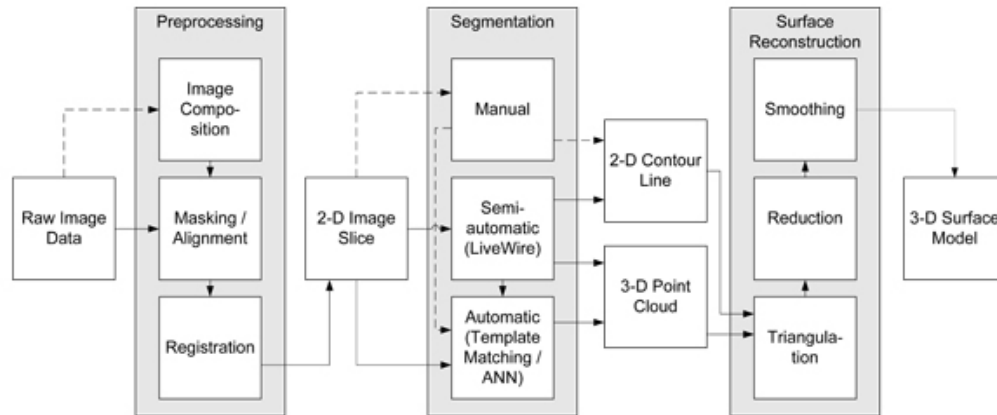
Figure 2-7 : The graph shows the interplay of different modules in reconstruction and generalization towards high-resolution 3-D models. Dashed lines indicate optional processing steps (Schoor et al., 2009)

The processing pipeline for creating 3D models of the brain and of cities are similar with the exception of the data acquisition process and preprocessing. Figure 2-7 shows the general pipeline for creating a 3D model for biological data. The raw data are converted into 2D contours lines or 3D point clouds from 2D image slices. The source for these image slices is typically MRI image stacks or brain histology slices. Similarly, as shown in Figure 2-8, the 3D city model is converted into a 3D point cloud from Image data, GPS or range data. To construct a surface from a set of structured or unstructured points, triangulation algorithms are used. In both cases, the generalization process must take place after the digital model has been created where features can be extracted for generalization.
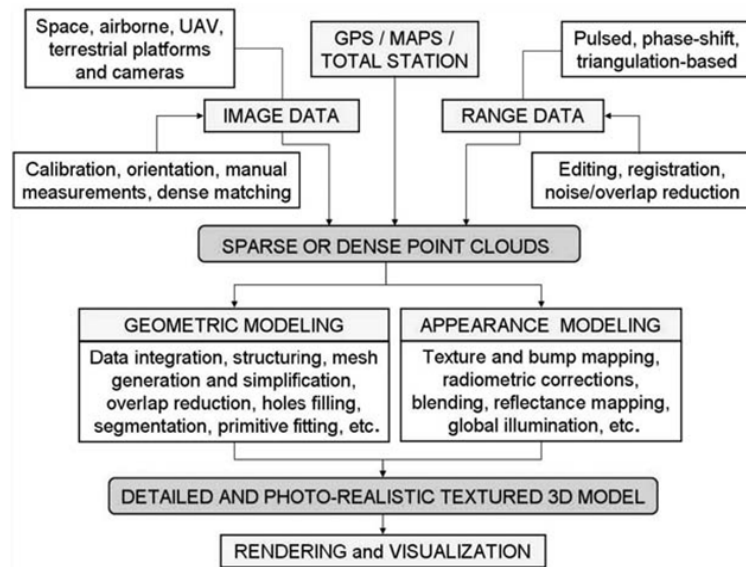
Figure 2-8 : The multi-sensor and multi-resolution 3D modeling pipeline based on passive and optical active sensors for the generation of point clouds and textured 3D models (Fabio, 2003)

For 3D generalization of city models, existing approaches focus on simplifying complex structures, like buildings. Kada (2002) demonstrated a generalization method that uses a least squares adjustment to preserve building regularities. The process involves building a constrained model as the first step. Once the coplanarity, parallelism and rectangularity of two faces in the model are detected, the planes are grouped to form a hierarchy of constraints. Complex features are generated from the hierarchy of constraints and removed. After removing the features, a least square adjustment is applied to the original points to achieve the final shape. Thiemann and Sester (2004) proposed a different approach where they segment the volumetric model of the building with one or more planes. All segmented parts are evaluated with respect to model scale and resolution. Once segmented, the generalization is done in two steps. The generic step excludes any feature that is "sticking out" from the 3D-body depending on the size. The second step takes the specific characteristics of the segments into account and ranks them. For example,

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characteristics such as inclination of the plane, horizontal or vertical orientation, and height of a feature over ground level are ordered. Once the segments are arranged, they are stored as a cell model, which represents the topological adjacency of the features, and as a CSG (Constructive Solid Geometry) tree, which represents the history of the segmentation. The cell model and the CSG tree are then used to merge the features and create a generalized model of the building.

Mathematical morphology has also been used to generalize 3D city models. Kimia, Tannenbaum, and Zucker (1995) introduced a methodology called the *reaction-diffusion-space* that sequentially combines a reaction step and a diffusion step . In the reaction part, mathematical morphology is used to incrementally shift the segments inwards or outwards. The second step, used for more complex structures, utilizes the curvature information of the shape to determine the direction of the shift. Forberg (2007) used a similar scale-space approach to generalize the shape of 3D building structures. The method involves computing the distance between the parallel facets and moving them towards or away from each other, given a distance threshold (Figure 2-9).
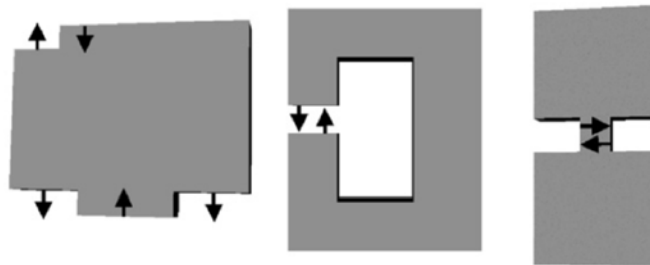
Figure 2-9 : Parallel facets under a certain distance are shifted towards each other (Forberg, 2007)

Generalization methods used for more complex polyhedral building models have been discussed by Rau, Chen, Tsai, Hsiao, and Hsu (2006). In their method, the generalization takes place in two steps: 1) Polyhedron Merging, and 2) Wall collapsing with Regularization. The polyhedral merging is achieved by flattening the sloped building rooftops, merging two connected polyhedrons with a small height difference and removing smaller enclosed polyhedrons. In the wall-collapsing step, the principle structure of the building is determined to maintain the structural integrity and the rest of the segments are collapsed to simplify the structure. These methods applied in GIS are useful to know when reconstructing the cortical surface of the brain from a set of contours because individual contours (i.e. overlaps) can be corrected prior to reconstruction.

In GIS, generalization falls under focus + context visualization, where the viewer is engaged in understanding a location in the context of a map while focusing on certain areas that are identified as key landmarks to be preserved in the process of generalization. In computational anatomy (Grenander & Miller, 1998; Miller et al., 1997), the focus is placed on developing mathematical and software tools to visualize and understand the variability of brain anatomy in humans and primate monkeys. This goal is accomplished by generalizing the structure into an amenable form, often into a flat map. The problem with deforming a complex structure, like the brain, is that the key landmarks can be deformed to a point where the viewer can no longer identify them. However, this is not the only problem with deformation. Data associated with the structure can also be misinterpreted as the structure is deformed to a simpler form. Since reconfiguration and representation of the brain surface is essential to visualize and identify variability of brain anatomy, a variety of methods are available for generalizing the cortex.

The cortical surface is complex and non-convex. To study the functional architecture and neural maps embedded in them, the curved and convoluted surface is often flattened to a planar representation. This is difficult to achieve because fine tessellation is needed to preserve the topological integrity of the original surface. Schwartz, Shaw, and Wolfson (1989) were able to flatten the cortical surface by first constructing a matrix of the geodesic distances of each vertex to other vertices. The surface was then projected randomly to a plane that minimizes the mean-squared error between the original distance matrix and that of the flattened surface. Dale and Sereno (1993) used a different approach where local forces were estimated based on the curvature information for each vertex. The surface was then relaxed towards minimal surface tension, and thereby, minimizing the local geometric distortion (shown in Figure 2-10).
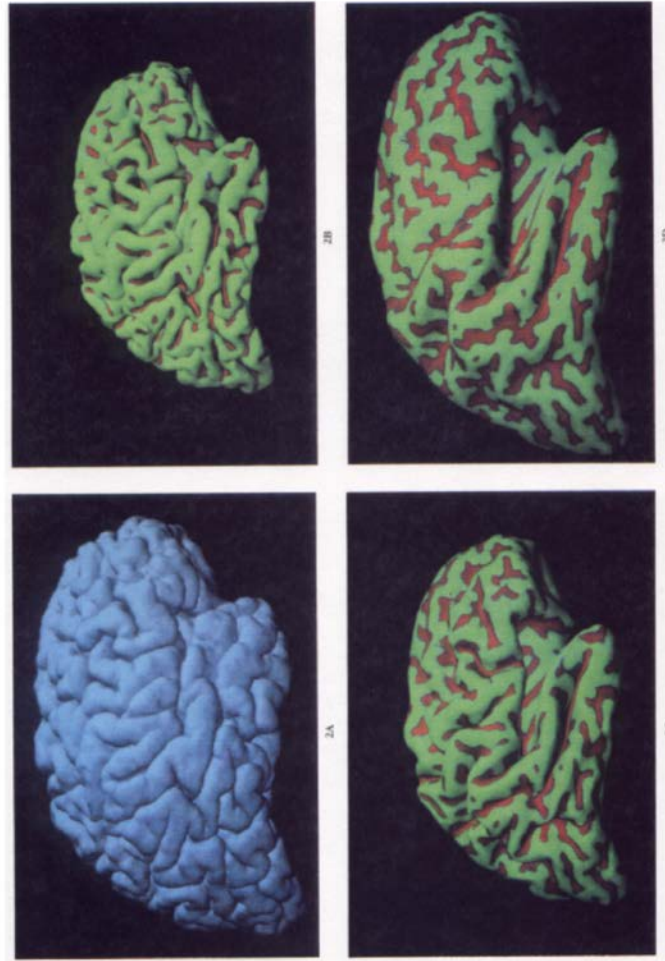
Figure 2-10 : Partially flattened representation of the cortex. Locations on the initial folded surface with large positive curvature (i.e. sulci) are colored red, while location with large negative curvature are colored green.

Similar methods to generalize the surface were also used by Carman, Drury, and Van Essen (1995), and Drury et al. (1996). One advantage to using such methods to flatten the surface is that it can be applied to the entire cortical hemisphere. In addition, the partial flattening has the advantages that it exposes hidden sulcal cortex and retains the global shape. Seminal work has been done by Fischl et al. (1999) where the cortical surface was inflated to expose the activity buried inside the sulci using spring forces to smooth the surface and metric-preservation to retain

some of the original topology. The authors also described methods to completely flatten or transform the original surface into a sphere for the purpose of better visualization and for mapping coordinates to facilitate quantitative analysis.
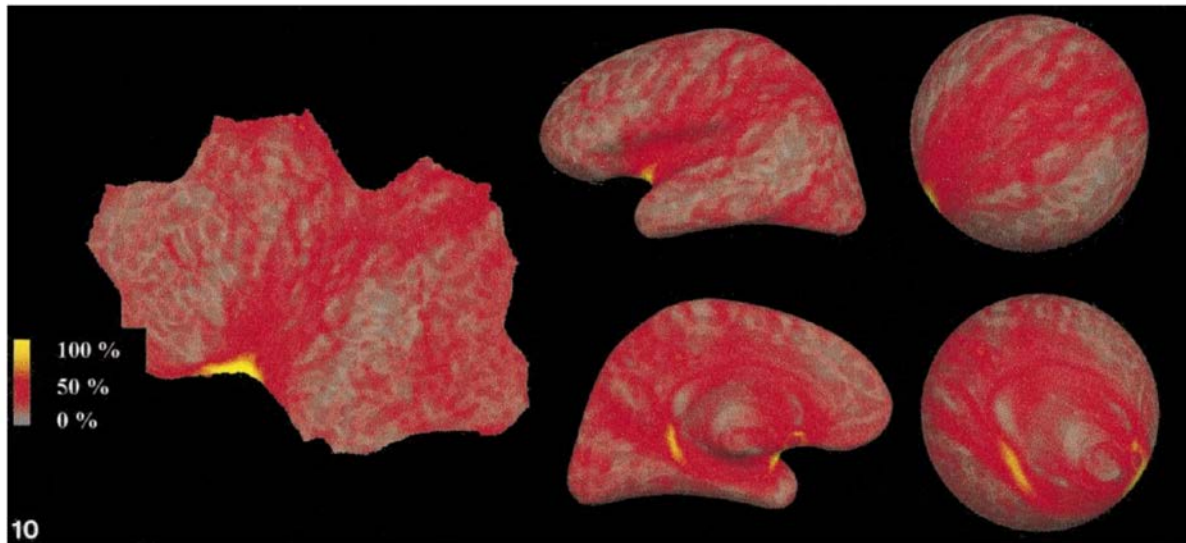
Figure 2-11: Flattened (left), inflated (center), and spherical (right) representations of the same surface

(Fischl, Sereno, & Dale, 1999)

The generalization techniques described above for both generalizing 3D city models and cortical surfaces have one thing in common – abstraction. In both cases, by minimizing the level of details, we are able to remove the unnecessary clutter and visualize data without having to interact too much with the data. However, as evident from Figure 2-11, the different representation requires significant effort from the viewer to transform the distorted view into a more natural shape that is easily recognizable.

2.4   VISUALIZATION OF CORTICAL STRUCTURE

Visualization of the cortical structure is difficult due to its structural complexity. As part of the survey of papers that best represent visualization of neuroanatomical data using both conventional and transneuronal tracer, I attempted to outline some of the challenges and current methods that are being used to overcome these challenges.

Context

Mapping of labeled neurons provides an accurate representation of the topographic distribution of neurons in the brain. A simple plot of the neuron density tends to distort the actual representation of the density. The raw data comprises typically of slices that are arranged at an interval. Figure 2-12A shows the plot of labeled cell distributions, which makes it difficult to visualize the density and the spatial location of the distribution without any frame of reference. A frame of reference or context can instantly make the data more recognizable, as shown in Figure 2-12B.
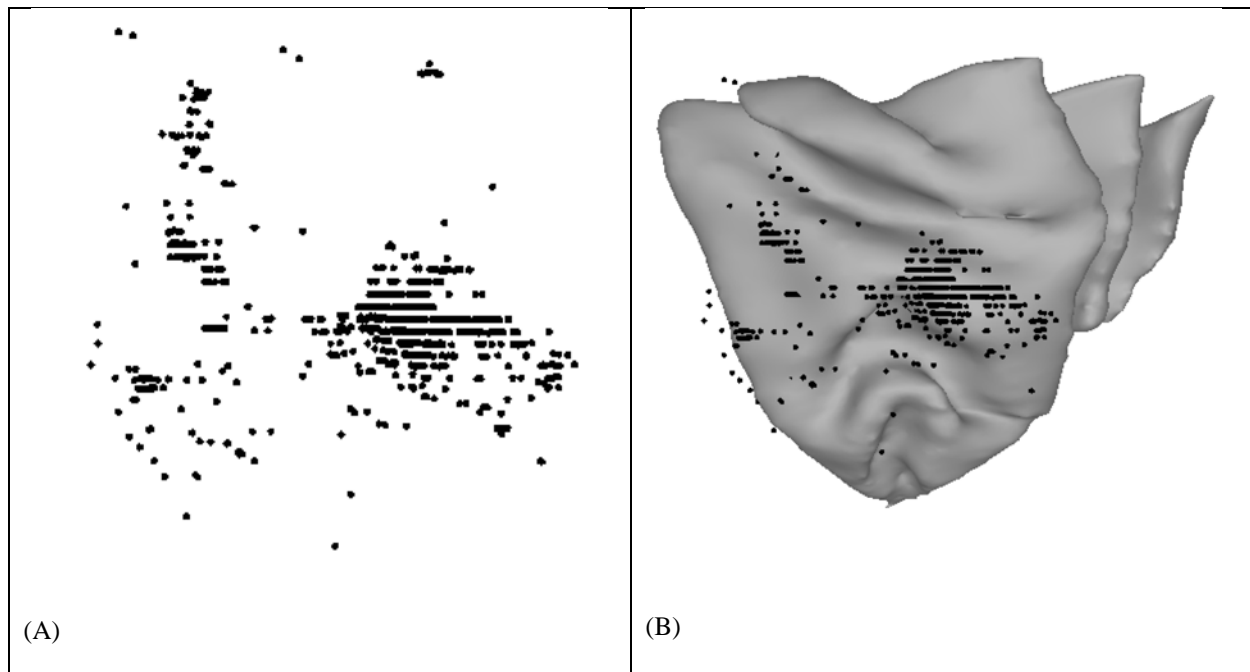
Figure 2-12 (A) A simple plot of labeled neuronal cells (B) The same cell distribution with reconstructed surface for frame of reference.

Visualizing the hidden data

Occlusion of neurons on serially arranged histological sections poses a big problem in visualizing the density. To overcome this problem, many techniques are used to accurately represent the neuronal density. For example, Dum and Strick (1991); (Zaborszky et al., 2015) used flattened reconstruction and histograms to represent neuronal density. In the paper, the authors extended the functional mapping of motor control in the frontal lobe. Using a retrograde conventional tracer, the authors showed that the primary motor cortex and six premotor areas in the frontal lobe project to cervical segments of the spinal cord. After inoculating the non-human primate with the conventional tracer in the spinal cord, the animal was terminated and brain sections were processed to reveal the labeled neurons. For visualization purposes, the sections

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were further processed to create flattened map by taking each individual sections and "unfolding" them to reconstruct a flat map as shown in Figure 2-13.
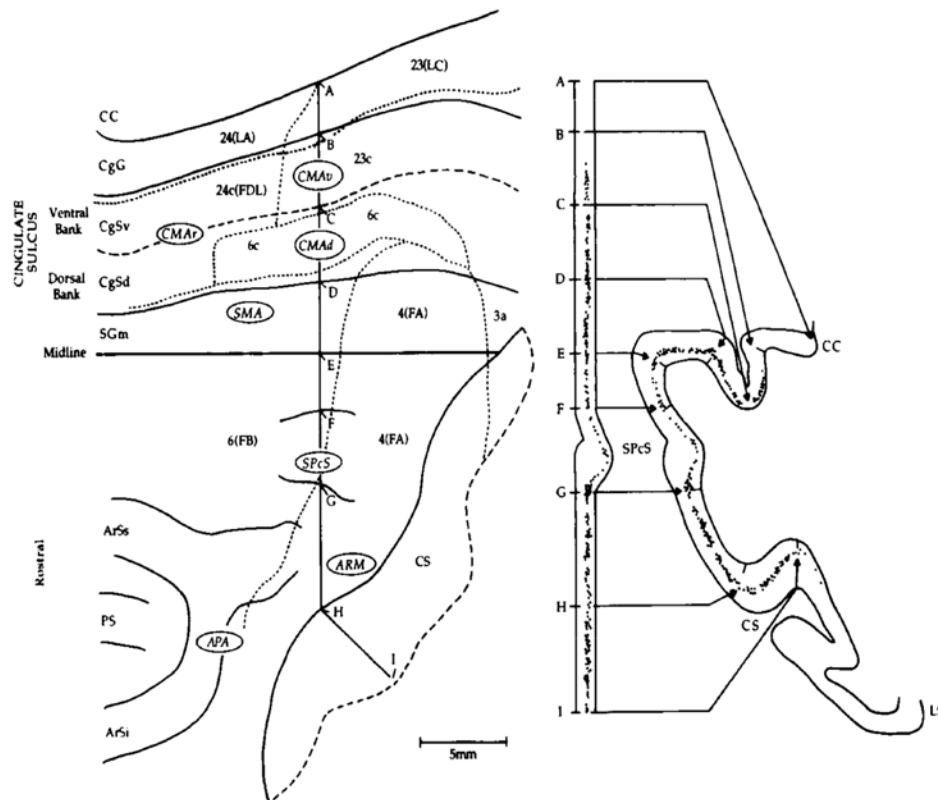
Figure 2-13. Schematic diagram of motor areas in frontal lobe.Individual coronal sections of cortex (far right) were straightened (middle) to form a flattened reconstruction (left) of the frontal lobe. The corresponding locations are shown with capital letters on the schematic diagram.

The reconstructed flat map, Figure 2-14, showed that the corticospinal projections to cervical segments of the spinal cord originate from the same areas of the premotor cortex that project to the arm area of the primary motor cortex.
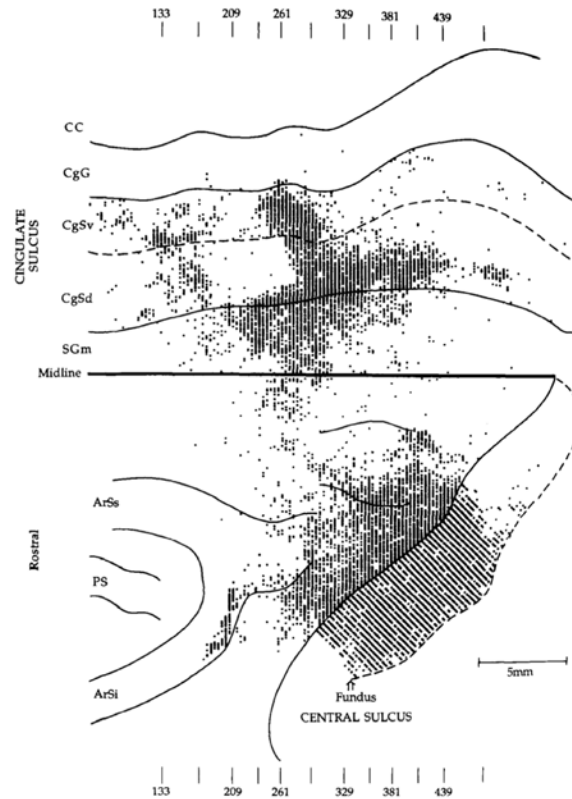
Figure 2-14. **Reconstructed flat map of corticospinal neurons in frontal lobe. Every fourth section was used to construct this map.**

The spatial locations of the labeled neurons provide location and extent of the arm representation of the premotor cortex. These areas include SPcS (superior precentral sulcus), SMA (supplementary motor area), CMAd (caudal cingulate motor area, dorsal bank), APA (arcuate pre-motor area), CMAr (rostral cingulate motor area) and CMAv (caudal cingulate motor area, ventral bank). They created a density mapby dividing the map into small bins and then counting and color-coding each bin based on the number of cells in each bin (Figure 2-15). These quantitative measures indicated that the size of the corticospinal system from the premotor areas equals or exceeds that from the arm representation of the primary motor cortex. The analysis showed that as much as 40% of the corticospinal projection originate from the premotor area

identified in the paper. In other words, these premotor areas have direct access to the spinal cord that influences the generation of movement independently of the primary motor cortex, which had been was considered to be the main source of motor signals in the corticospinal tract. This precise identification of premotor areas may help further understand the complex mapping of motor control in the brain.
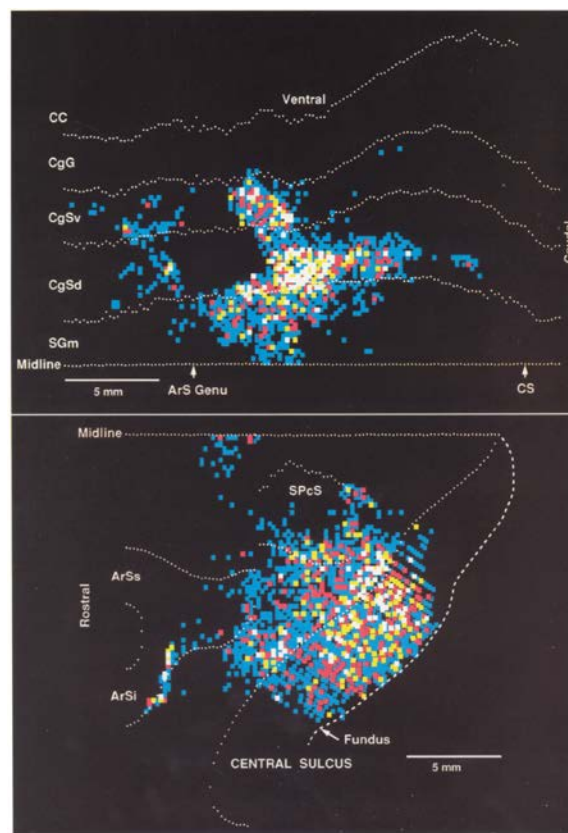
Figure 2-15. Density of corticospinal neurons in frontal lobe: medial wall of hemisphere(top) and lateral surface (bottom). The color blocks represent density bins . (i.e. bins in which the number of labeled neurons was in the upper 10% of the total sample of 200-um bins)

Visualizing without depth

Representing the data using flat maps has been an effective way to visualize the distribution and density of labeled cells. Dum and Strick (2005) used these techniques to further understand the organizational structure of the connections within the brain. With the help of conventional fluorescent tracers, the paper expands the functional mapping of the brain between the primary motor cortex (M1), the dorsal premotor area (PMd), and the ventral premotor area (PMv). After identifying the digit representation areas using electrophysiological stimulation, the authors injected non-human primates with distinct retrograde tracers (fluorescent dyes) and analyzed the density of labeled cells using the two dimensional reconstruction technique described in their previous paper (Dum & Strick, 1991). Figure 2-16, Figure 2-17 and Figure 2-18 show the injection site and the spread of tracers in pertinent areas of the frontal lobe.
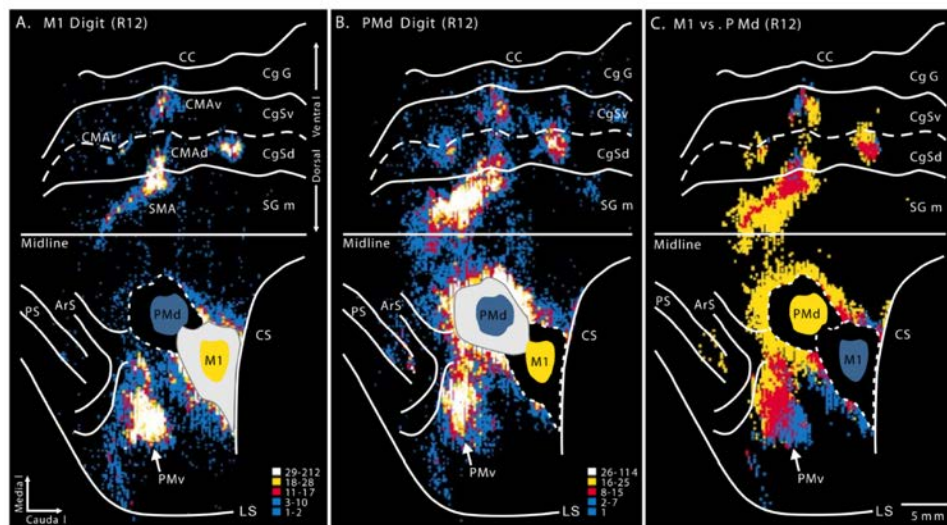
Figure 2-16. Frontal lobe input to the digit representations of M1 and PMd.A) Location and density of neurons labeled after DY (diamidino yellow fluorescent dye) injections into the digit representation of M1. Yellow shading around the injection site in M1 indicates zones 1 and 2 of the spread of the DY. The region surrounding the injection sites where neurons were too dense to plot is indicated by gray shading. B) Location

The result shows that primary motor area (M1) receives strong input from premotor areas (PMv and PMd) of the brain as shown in Figure 2-16A and Figure 2-17A. The overlap map shows bins (red shading in Figure 2-17C and Figure 2-18C) where there is a high concentration of neurons projecting to the injection site in the PMv and to the injection site in the PMd. . Analogously, M1 projects to the digit representation of PMv and PMd as shown in Figure 2-18.
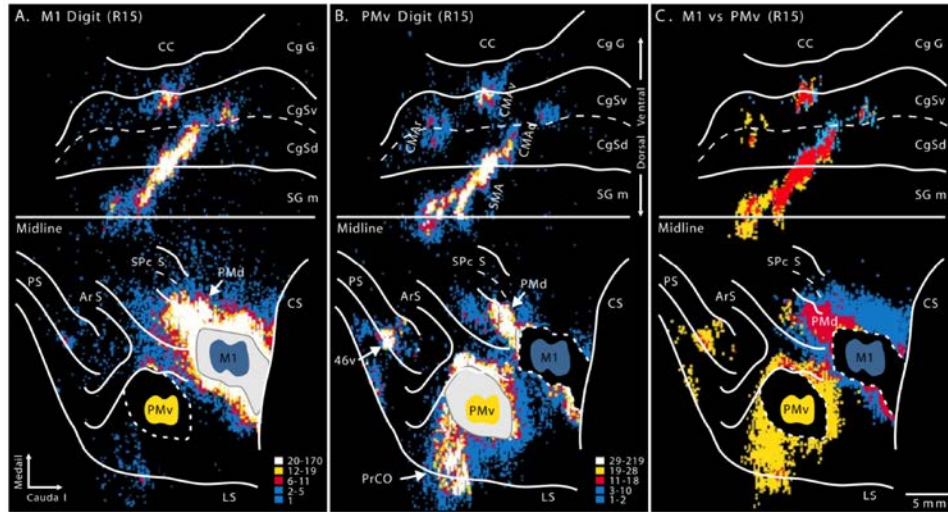
Figure 2-17. Frontal lobe input to the digit representations of M1 and the PMv.A) Location and density of neurons labeled after FB injections into the digit representation of M1. B) Location and density of neurons labeled after DY injections into the digit representation of the PMv. C) Overlap of the input to the digit representations of the PMv and M1. Red shading (overlap bins) indicates bins that have a high density of cells

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This result substantiates that both PMv and PMd are interconnected with the hand area of M1. In addition to the interaction between these three areas, the authors observed that the supplementary motor area (SMA) projects more strongly to PMv and PMd than M1 (Figure 2-16, Figure 2-17, Figure 2-18).
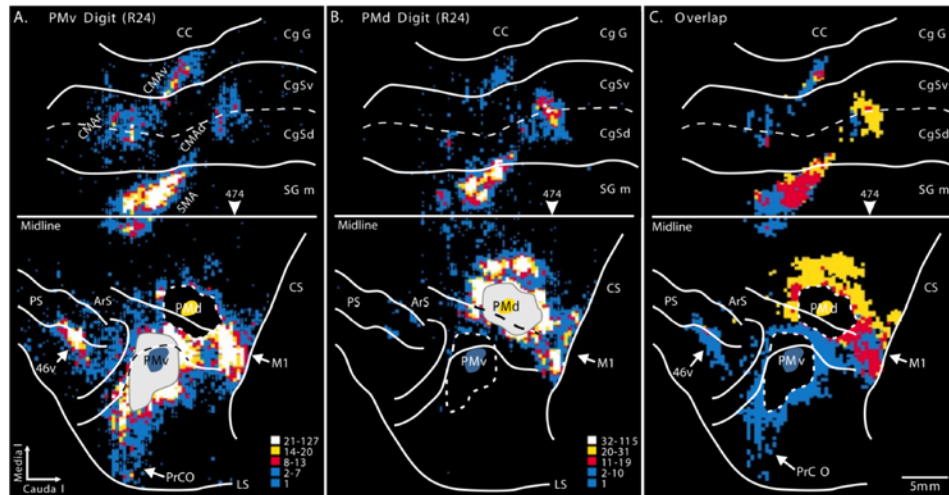
Figure 2-18. Frontal lobe input to the digit representations of the PMd and PMv.A) Location and density of neurons labeled after FB injections into the digit representation of the PMv. B) Location and density of neurons labeled after DY injections into the digit representation of the PMd. C) Overlap of the input to the PMd and PMv. Red shading (overlap bins) indicates bins that have a high density of cells projecting to each injection site. Blue shading represents bins that have a high density of cells projecting to PMv. Yellow shading represents bins that have a high density of cells projecting to the PMd.

A quantitative analysis of relative inputs to M1, PMv and PMd is shown in Figure 2-19. The analysis of the density map along with the quantitative analysis is then used to create a schematic diagram showing the interconnected network for the generation and control of hand movements (Figure 2-20). These result supports the view that the premotor areas have the potential to influence spinal cord mechanisms and motor output through pathways that are independent of M1 since PMv and PMd seems to operate at the same level as M1.
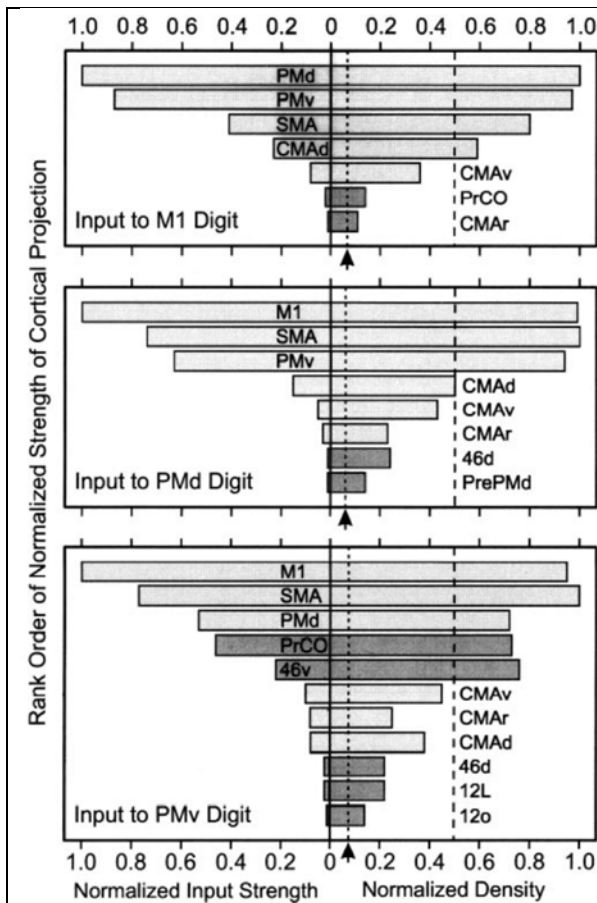
Figure 2-19. **Normalized strength and density of input from cortical areas in the frontal lobe to the digit representations of M1, the PMd, and the PMv.The strength (numbers of labeled neurons in an area) and density (average number of labeled neurons per bin in an area) were normalized to the cortical area with the most labeled cells or the highest density. The lowest possible value for the normalized density (equivalent of 1 cell per bin) is indicated (dashed line marked by arrow). Light shading indicates cortical motor areas. Dark shading indicates prefrontal areas.**
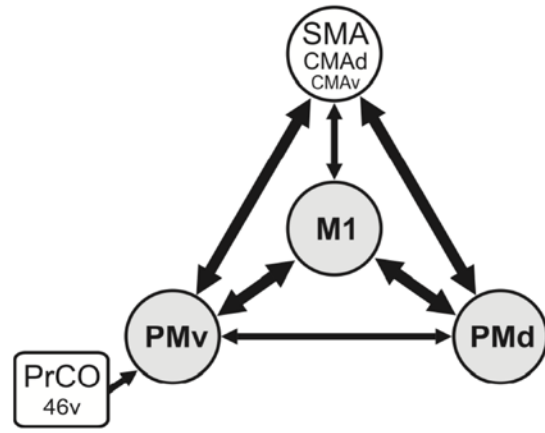
Figure 2-20. **Frontal lobe network for hand movements.The size of the arrows indicates the relative strength of an input. Shaded circles indicate motor areas on the lateral surface. Unshaded circles are motor areas on the medial wall. The square indicates prefrontal areas of the cortex. Abbreviations are as in Figures 1 and 4.**

3-D visualization and problem with depth

Although effective, data representation in 2-D is gradually being taken over by 3-D representation or more appropriately - 2-D projection of 3-D data representation. However, lack of interaction can hinder understanding of 3-D data. For example, a wire frame diagram of 3-D data offers some spatial context but offers no sense of depth. Prevosto et al. (2009) illustrated the distribution of labeled cells with such diagram (Figure 2-21).
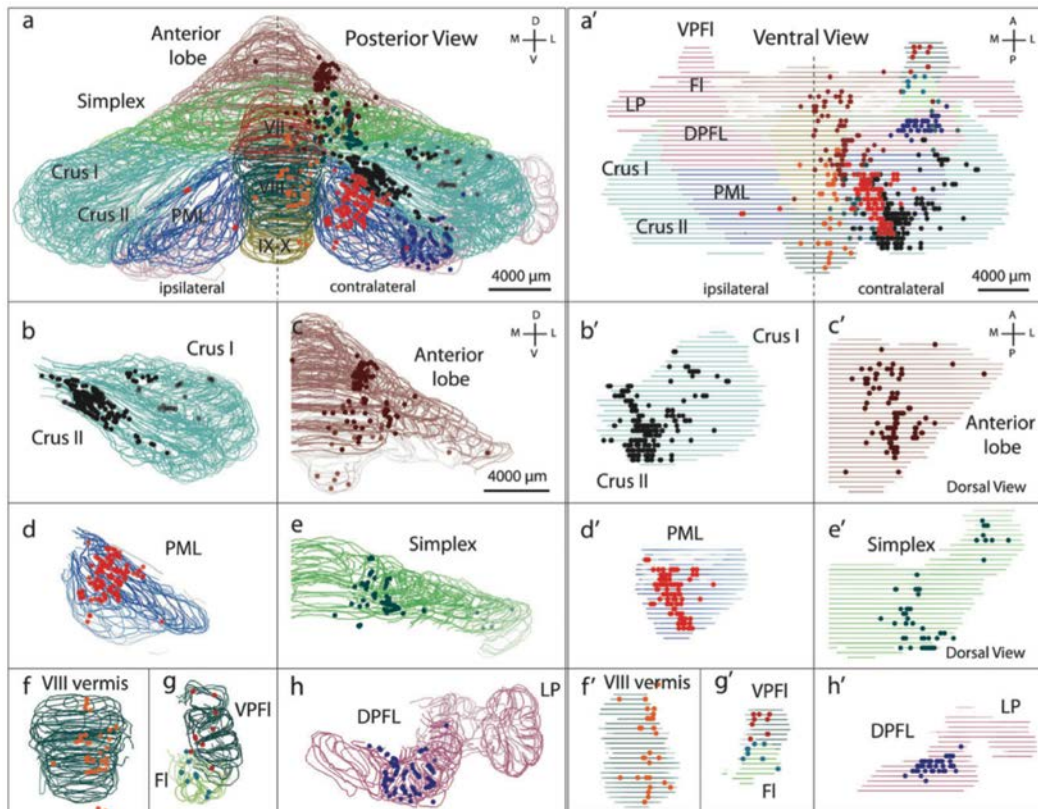
Figure 2-21. 3-D reconstruction of the cerebellar cortex, showing the organization of labeled Purkinje cells providing trisynaptic inputs to MIP (3 days). 3-D reconstructions were made using Neurolucida.

This type of wireframe illustration is difficult to follow, due to the lack of hidden surface removal that prevents determination of the viewpoint orientation. Shaded models, on the other hand, may be a better choice for representing anatomical structures since shading and rendering can greatly enhance the realism of the model.

3-D surface visualization

In a recent paper (Borra et al. (2011), the authors used both 2-D and 3-D reconstruction images to show cell distribution and density. They reported that the non-human ventrolateral prefrontal (VLPF) Area 12r is involved in higher-order nonspatial information processing. After injecting the animal with retrograde tracers, the distribution of the cells were presented using 3-D reconstruction and 2-D drawings of brain slices as shown in Figure 2-22.
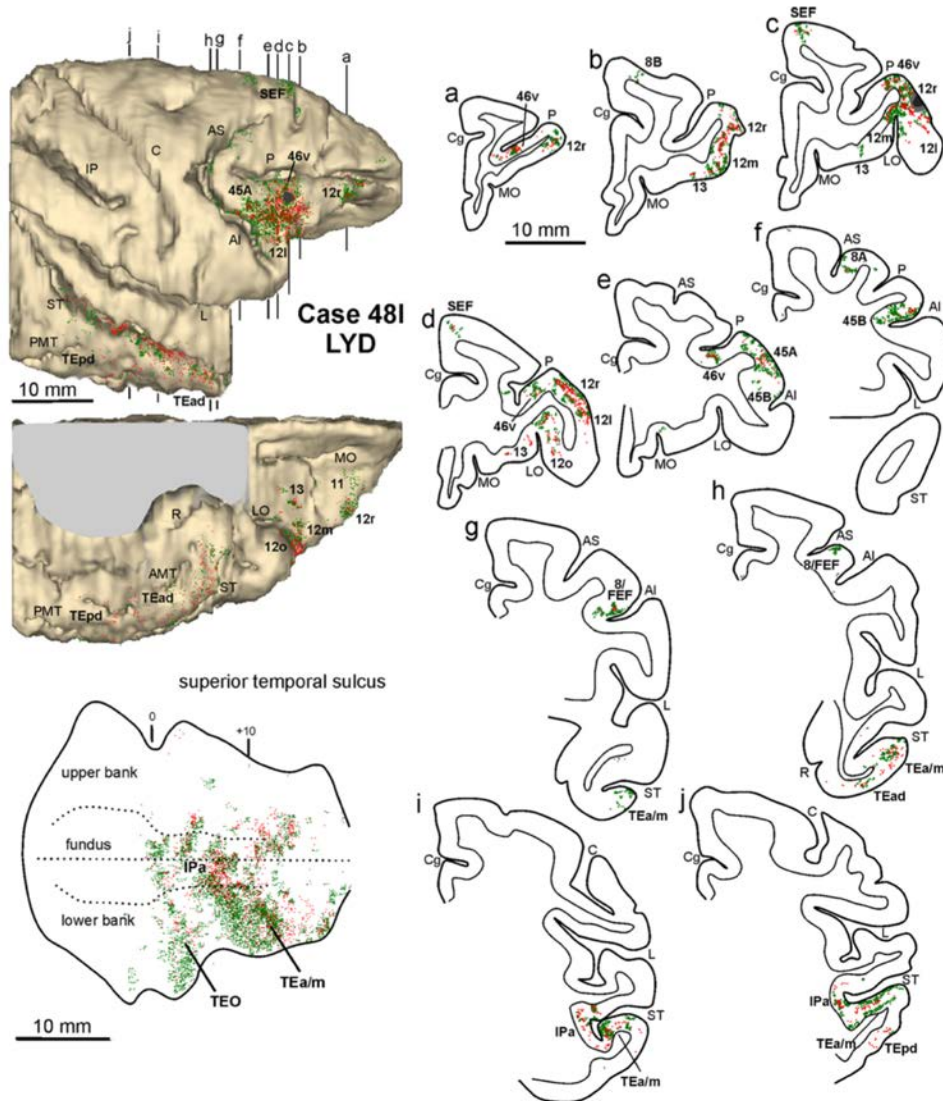
Figure 2-22. Distribution of the retrograde (red) and anterograde (green) labeling observed after injections in caudal area 12r, shown in dorsolateral and bottom views of the 3-D reconstructions of the injected hemispheres (top left), in 2-D reconstructions of the STS (bottom left), and in drawings of coronal sections arranged in a rostral to caudal order (a–j; right). PMT, Posterior middle temporal sulcus.

The 3-D reconstruction shows the overall distribution of the cells whereas the 2-D drawings show retrogradely labeled neurons located in the superficial (II–III) versus deep (V–VI) layers of the cerebral cortex.   The 2-D images not only show the laminar distribution of labeled cells

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that is not visible in the 3D reconstruction but also allow interpretation of the hierarchical architecture of the cortico-cortical connections. These representations along with the quantitative analysis, Figure 2-23, show connections between different parts of the brain and three distinct zones of Area 12r (rostral, intermediate, and caudal). The topographic map shows that the intermediate zone of area 12r is densely connected with area 46v and several orbitofrontal areas.
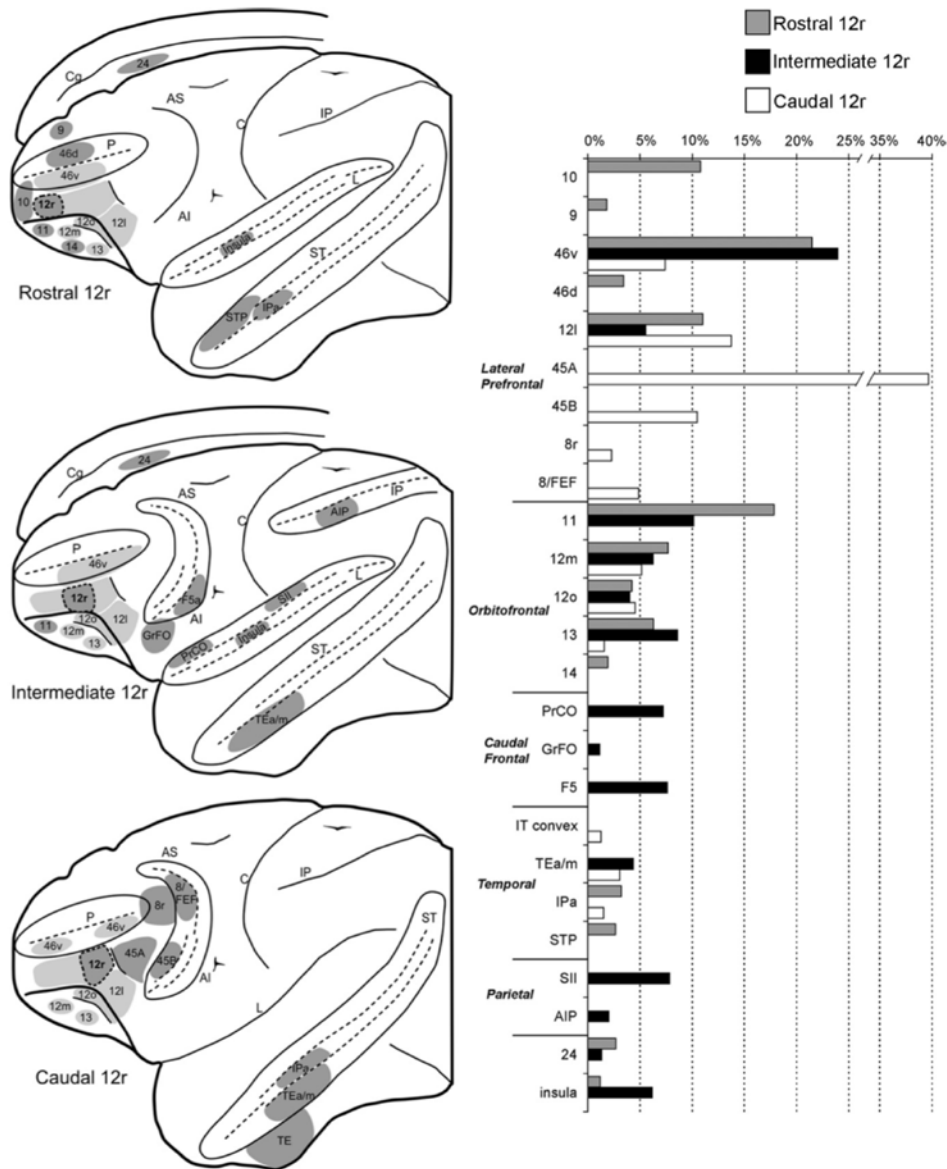
Figure 2-23. Summary view of the major ipsilateral cortical connections of the rostral, the intermediate, and the caudal area 12r sector and mean percentage distribution of the retrograde labeling.In the brain drawings, areas connected with one or two area 12r sectors are shown in darker gray, and those connected to the entire area 12r are shown in lighter gray. In the bar graph, only connections with mean value 1% are shown.

In contrast, the rostral zone has strong connectivity with rostral prefrontal areas. This heterogeneity suggests that the different zones of 12r contribute to different functions. One

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important thing to notice is that in the topographic map (Figure 2-22), the fundus of each sulcus (inward folds) has been exposed but the cells on the walls of each sulcus are obscured. This kind of visibility problem is common when projecting a 3-D scene onto a 2-D plane. To overcome this problem, Bakola et al. (2013) (Figure 2-24) presented a flattened map of the reconstructed 3-D surface to display the density of the cells on both surface and within sulci. The authors looked at the corticocortical afferent projections of area PE of the non-human primate brain. Since area PE is believed to be involved in goal-directed movement, the authors injected at various areas within PE with retrograde tracer and observed the projection to PE. Figure 2-24 shows the sagittal sections after the injection and the two-dimensional reconstructions of cortex.
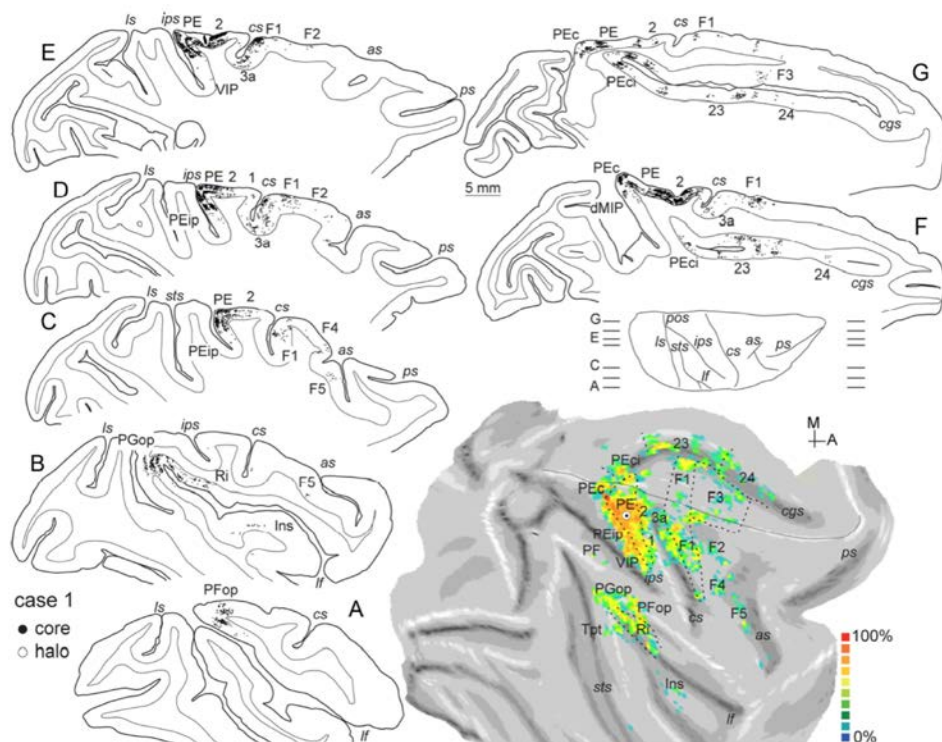
Figure 2-24. Location of injection site in lateral PE and cortical distribution of retrograde-labeled cells.Sagittal sections (A–F) were taken at the levels indicated on the brain silhouette. Bottom right, Two-dimensional reconstruction illustrating the distribution and density of labeled cells in case 1. Color scale

indicates the relative density of labeled cells as a percentage of the maximum unit value. Darker shades of

gray on the flat maps represent sulcal depth; intermediate shades of gray, cortical convexity; white color,

sulcal lip.

The reconstruction and the density were obtained with the CARET software (Van Essen et al., 2001). The density maps were created by projecting the location of each neuron to the nearest mid-thickness contour, along with marks noting the architectonic borders of cortical areas. After comparing several density maps, where the different areas of PE were injected with tracers, the authors were able to compose a topographic projection map to area PE as shown in Figure 2-25.
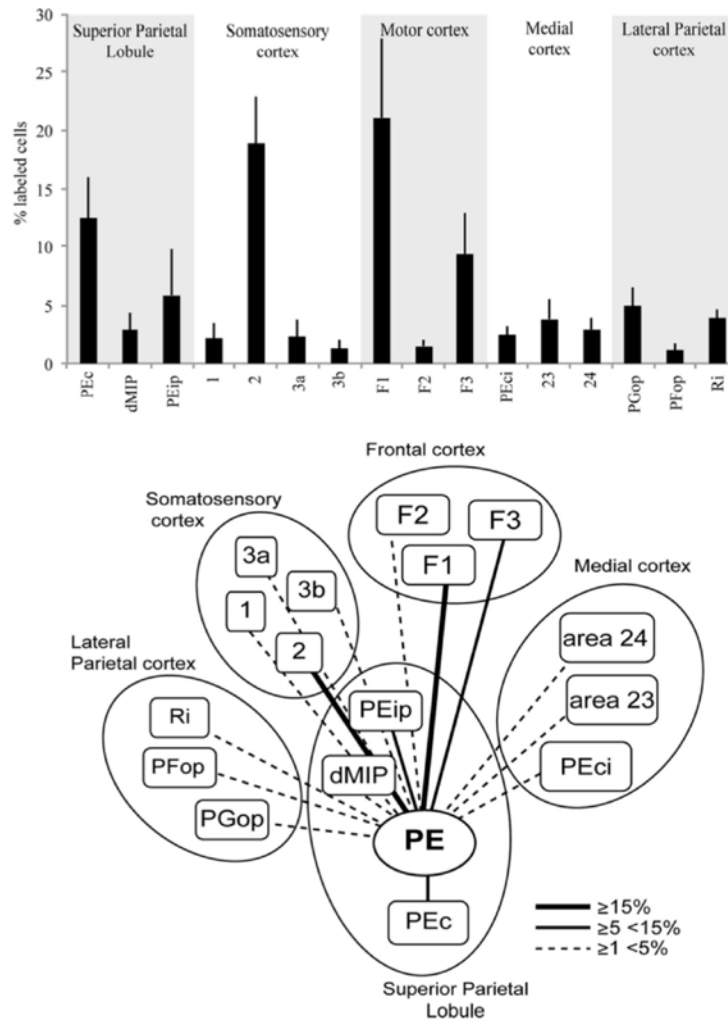
Figure 2-25. Summary of significant projections to area PE.Top, Average percentages of labeled cells in different cortical areas after all tracer injections within PE. Bottom, Boxes represent cortical areas with significant numbers of labeled cells and are organized according to their approximate location in the brain (medial to the right, caudal to the bottom). The thickness of the bars shows the proportion of cells in each of the areas connecting with PE

The authors concluded that, area PE receives nearly 60% of its projections from the parietal areas that are involved with the somatosensory system. Another 30% arrives mainly from primary motor and medial premotor cortex. In contrast, the authors did not find any direct connection

between PE and visuomotor parietal areas involved in reaching or grasping movements. This type of representation exemplifies the problem of distortion where the anatomy can get distorted beyond recognition. Moreover, since every brain exhibits some morphological variation, finding spatial similarities and identifying landmarks in the flattened representation is difficult.

Hidden data problem with 3-D and transparency

3-D reconstruction of histological slices is becoming a mainstay for communicating spatial context to a broader audience with superficial understanding of the brain anatomy. One way to represent labeled cell density in anatomical context without having to distort the anatomical structure, is to "cut out" certain parts of the brain to expose data in hidden structures . Zaborszky et al. (2015) represented the neuronal projections in the basal forebrain (BF) by reconstructing the outermost layer of the BF and then cutting out chunks of it to expose the labeled cell density. Since the basal forebrain area is anatomically complex and its involvement in cortical activation is not well understood, the authors injected distinct retrograde tracers into various frontal and posterior cortical areas, and mapped retrogradely labeled neurons in the BF. Visualization of the reconstructed surface along with the cell distributions, color coded according to different cortical targets, reveals band like structures (Figure 2-26).
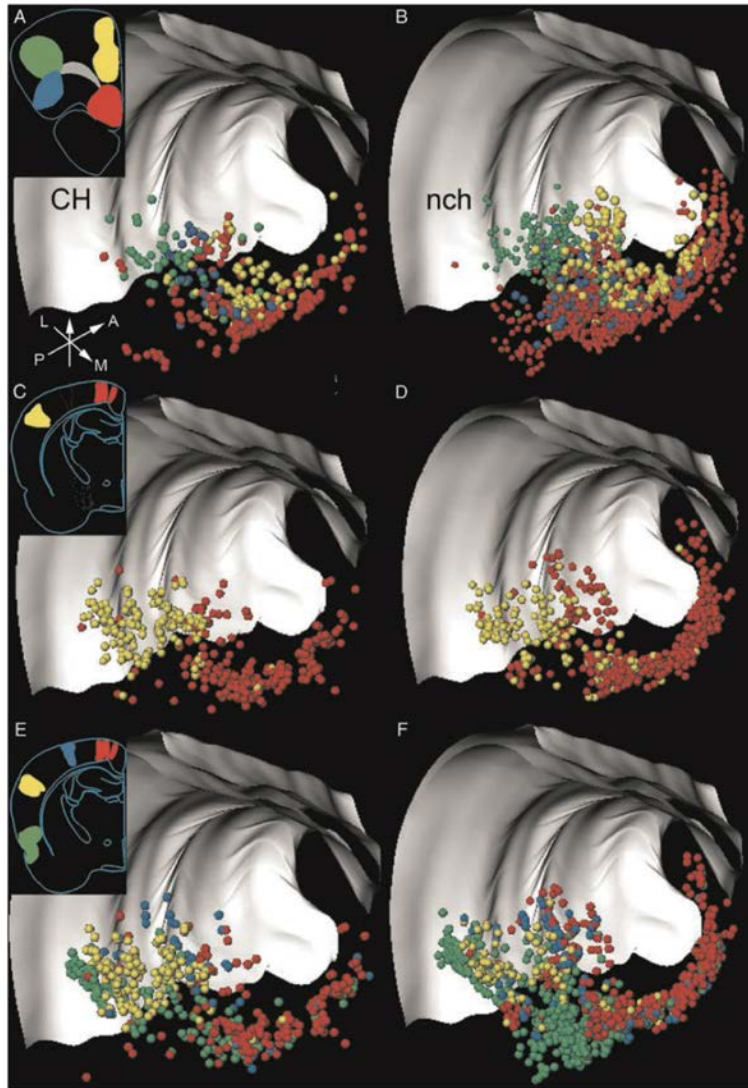
Figure 2-26. Comparative 3-D distribution of cholinergic and noncholinergic projection neurons in the BF demonstrating the gross topography of projection.A,B: neurons projecting to medial targets in the frontal cortex are located medially and rostrally in the BF, while cells projecting to more lateral targets in the frontal cortex occupy more lateral and caudal locations in the BF. C,D: similar mediolateral topographical organization can be observed among cells in the BF that project to different mediolateral sectors in more posterior cortical areas. E,F: show the distribution of cells projecting to mediolaterally located caudal cortical areas.

This distinct topography shows that laterally located targets in the frontal cortex receive their input from more caudal levels of the BF. From this observation, the authors postulated that the populations of projecting neurons in the BF are not diffuse but segregated. Since the different populations of labeled neurons can be obscured by visual occlusion one way to distinguish the different population visually is by using variable transparency when rendering the surface of 3-D data objects. An example of the use of transparency for hierarchical data visualization is shown in the Figure 2-27 (Zaborszky et al. (2015).
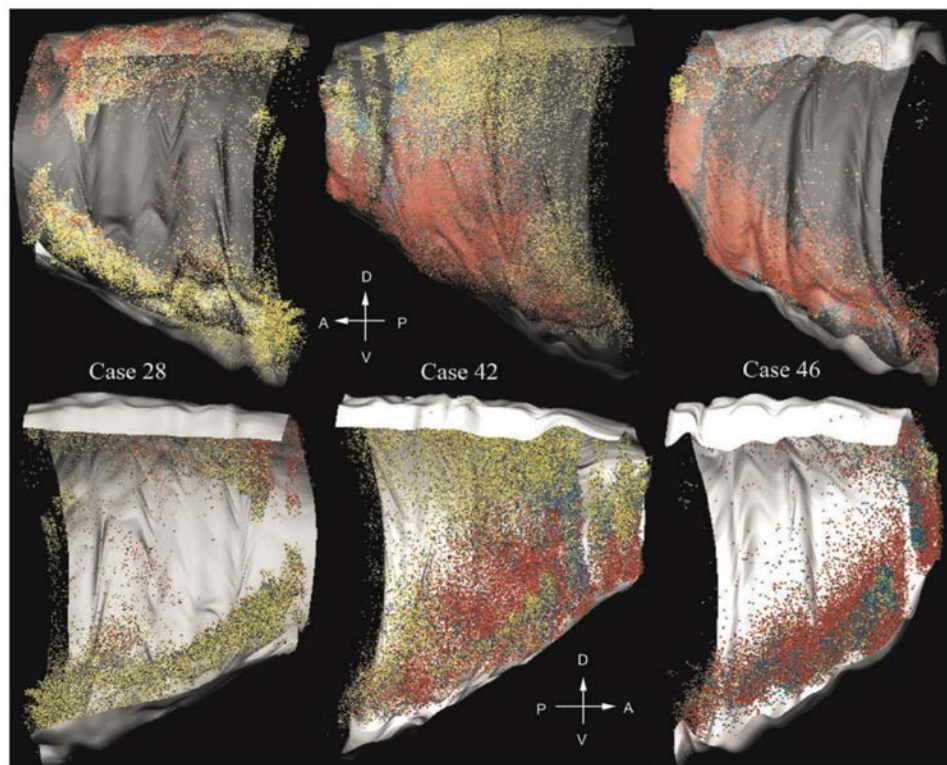
Figure 2-27. Distribution of retrogradely labeled cells in the cortex using 3-D surface rendering tools of the Micro3-D software.Upper row: outside-in view: the labeled cells are viewed through a semitransparent layer that is interpolated from the section contour outlines, lower row: inside-out view: the labeled cells are in front of a surface that represent the outside contours of the sections. The gray-white surfaces represent a rendering generated from the outlines of the mapped sections. Case numbers are indicated in the middle.

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Location, Location, Location

This review of the literature suggests that a major advantage of visualizing surface in 3-D is that the spatial location of the labeled cells can be easily ascertained. Identifying the exact location of the distribution can have a significant impact on understanding the connectivity within the same region of the brain. In this context, 3-D visualization of anatomical data must take into account the following important attributes:

- *Context* is essential to provide a frame of reference that can promote instant recognition of the object being displayed (i.e. the brain).

- *Revealing hidden data* is important since complex anatomical structures (especially 3D) can obscure data located inside the folds of the brain.

- *Visualizing without depth* can hinder our ability to identify key landmarks

- *3D representation* can provide a sense of realism that promotes the perception of depth necessary for identifying landmarks.

- *Accurate localization* of the data on the rendered object, especially in relation to identifying spatial landmarks, is crucial for comparison between different subjects. In such comparisons, regions of activation (fMRI) or neuroanatomical labeling (non-human primate research) are often judged according to their distance from known landmarks.

In conclusion, any visualization method used in interpreting complex structures should address the challenges mentioned above. In this dissertation, all of these challenges have been addressed and evaluated for a 3D generalization model of the non-human primate brain.

3.0   METHODOLOGY

Visualization of cortical surface of the brain difficult because it has multiple folds and structural overlap that can hide data interweaved within the folds. A flattened 2D representation can give a very distorted view that may lead to incorrect analysis of the data. On the other hand, a 3D representation may clutter our judgment or analysis by showing too many details. We want to minimize the level of details so that we can visualize the hidden data and reduce distortion at the same time. In this dissertation I have developed ways to represent volumetric data in simpler form that can do exactly that. First we "fix" the contour to make a complete surface and then simplify it. Since anatomical data can be very complex, the simplification is done in multiple steps. An application using Java and a combination of self-developed algorithms and Visualization Toolkit (VTK) library functions has been developed to generalize and visualize the neuroanatomical data in 3D. To answer the research question in this dissertation, an approach, with a generalization component and a visualization component, was implemented.

3.1   3D GENERALIZATION OF BRAIN MODEL

The generalization component deals with reconstruction of the brain surface from a stack of polygons that have been traced from brain histology.

The steps of the generalization component are:

- The vector data is gathered from the histological sections of the brain, and is then used to create individual polygons for each section

- The polygons are then inspected and corrected for overlaps and minimum distance required between folds to create a surface without disconnected components or "islands"

- The polygons are then stacked together to create a set of contours

- The "vtkVoxelContoursToSurfaceFilter" from the Visualization Toolkit (VTK) library is used to create a structured points dataset of signed floating point number and applies contouring filter to generate 3D surfaces from a stack of 2D contour distance slices

- "vtkSmoothPolyDataFilter" from VTK library is used to smooth or "relax" the mesh. The method uses Laplacian smoothing. Laplacian smoothing was selected because it reduces high frequency information in the geometry of the mesh thus reducing features and "opening" up the overlapped structures

- "vtkLinearSubdivisionFilter" from VTK library is used to further subdivide the surface triangles to give it a smoother appearance

3.1.1 Contour Generalization

Diffeomorphic methods have been studied for the purpose of registering one brain to the other. In this research the inspiration of "inflating" the surface comes from GIS or more specifically mathematical morphology used in GIS to generalize the model. The cortex is like a sheet of tissue adjoined on its inner side by subcortical white matter (see Figure 3-1).
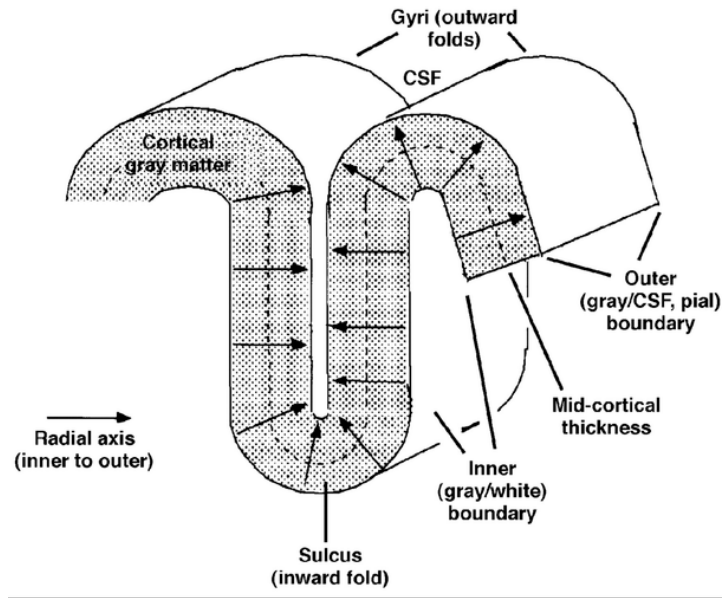
Figure 3-1: Schematic representation of key structural features of cerebral cortex (Van Essen et al., 2001)

Each cross-section of the cortical surface gives a polygon from which the iso-surface is generated. Since the cortical walls are so close to each other in certain locations, two major problems arise when reconstructing the surface. First, the contour lines can overlap due to deformation of the brain tissue slices when they are physically mounted onto the microscope slide. This overlap results in the "filling" of the gap at the top of a sulcus (inward fold) (Figure 3-2.a).
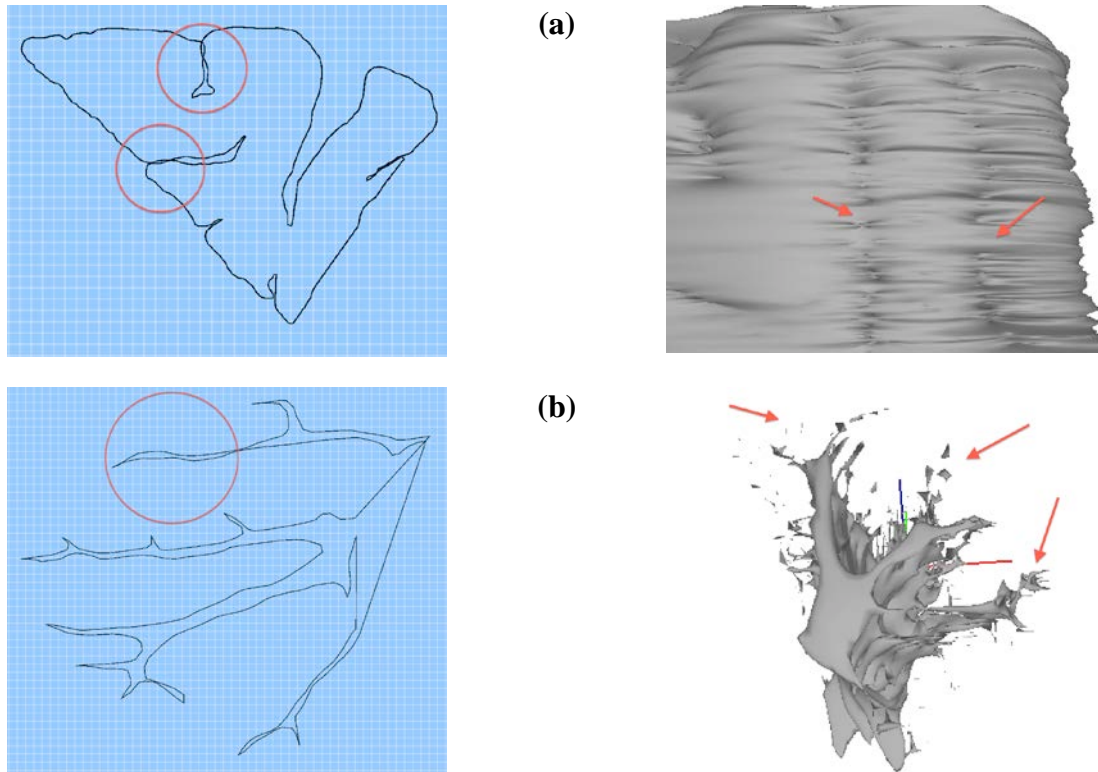
Figure 3-2 : (a) Shows Contour overlap due to mounting that results in filling effect on the right (b) Shows disconnected areas (islands) because the contour walls are too close.

Second, the triangulation algorithm ignores areas that are too close to each other resulting in "islands" (Figure 3-2.b). This problem can be solved by employing the dilation operator used in mathematical morphology. Mathematical morphology is well suited for biological image analysis because it offers ways of extracting image components. These components in turn allow us to represent regions of interest and manipulate them for further pre- or post-processing.

Morphological operations have widely been used in computer vision and image processing. Examples include problems in nonlinear filtering, noise suppression, and contrast enhancement (Heijmans, 1994; Maragos, 1998; Maragos & Schafer, 1990; Serra, 1983). In this research, we

are only interested in the dilation operator, which is one of the two basic operators in mathematical morphology. The basic effect of the dilation operator is to enlarge gradually the boundaries of the original structure. The calculation of the new point for dilation is described as follows (for sets in R^n):

Definition (Van Den Boomgaard & Smeulders, 1994): Let X be the compact set of the original structure where the boundary of a set X is denoted by ∂X and S is the structuring element. Let $x \in \partial(X \oplus S)$ then $\tilde{S}_x$ hits the boundary of X. According to the definition of dilation,

$$X \oplus S = \{x \mid X \cap \tilde{S}_x \neq \emptyset\}$$

So, for a point x on the boundary of $X \oplus S$ such that $X \cap \tilde{S}_x \neq \emptyset$. $\tilde{S}_x$ with $x \in \partial(X \oplus S)$ may hit ∂X at infinite number of points. In this research, we consider the dilation of set $X \in R^n$ with disk of radius ρ (denoted as ρB). Point $P \in \partial(X \oplus \rho B)$ is chosen such that $(\rho B)_y$ hits ∂X in one point, so that $X \oplus \rho B$ has a unique normal at P denoted as $N_{X \oplus \rho B}(y)$. We assume that our structuring element is convex (i.e. circle), and there exists a well-defined normal at each point x. We further limit our dilation only to points that are close to edges that are not immediate to the point. Alternatively, we only dilate points that test positive in searching for edges close to it after excluding edges that are connected to the point x. Under these assumptions, the dilated point can be calculated as:

$$P = x + \rho N_{X \oplus \rho B}(x)$$

As illustrated in Figure 3-5.a, the contour line of a square (blue area) can be dilated (indigo area) by moving a disk of radius r along the contour line. In this research, the dilation technique is used on the contour line to dilate only the areas that are within the radius of the disk. Since the distance between any three points are fairly consistent and vertices are ordered, the normal N at

point x is calculated the following way. A detailed explanation of the projection methods described below can be found in Beyer (1978); Eisenberg and Sullivan (1996); Foley and Van Dam (1982); Gellert (2012); Honsberger (1985); Kern and Bland (1938); Singer (1995).

Definition: Let A, B & C be the three points of an arc, where, B is is assumed to be the exact middle of the arc. The normal N between points A and C is given by,

$$N = \langle (y_A - y_C), (x_C - x_A) \rangle$$

N is the normal vector perpendicular to the line connecting points point A & C. The magnitude of the vector is given by,

$$\|N\| = \sqrt[2]{N_x{}^2 + N_y{}^2}$$

The normal vector can further be normalized by,

$$\boldsymbol{n} = \left\langle \left(N_x / \|N\| \right), \left(N_y / \|N\| \right) \right\rangle$$

So, for a structuring element with radius ρ the point P can be calculated at point B as:

$$P = B + \rho \, \boldsymbol{n}$$

$$P(x, y) = (B_x + \rho \, \boldsymbol{n_x}, B_y + \rho \, \boldsymbol{n_y})$$

The normal is calculated for every point that's exceed the minimum distance from an edge. In this work, each point is checked against every edge making algorithmic complexity of finding the edge $O(n^2)$. Since the number of vertices are not exceedingly big, an implementation of a Quad Tree (Finkel & Bentley, 1974), which can reduce the complexity to $O(\log n)$ time, has not been implemented. So, for a set of vertices V and their corresponding set of edges E, the algorithm can be written as shown in Figure 3-4.
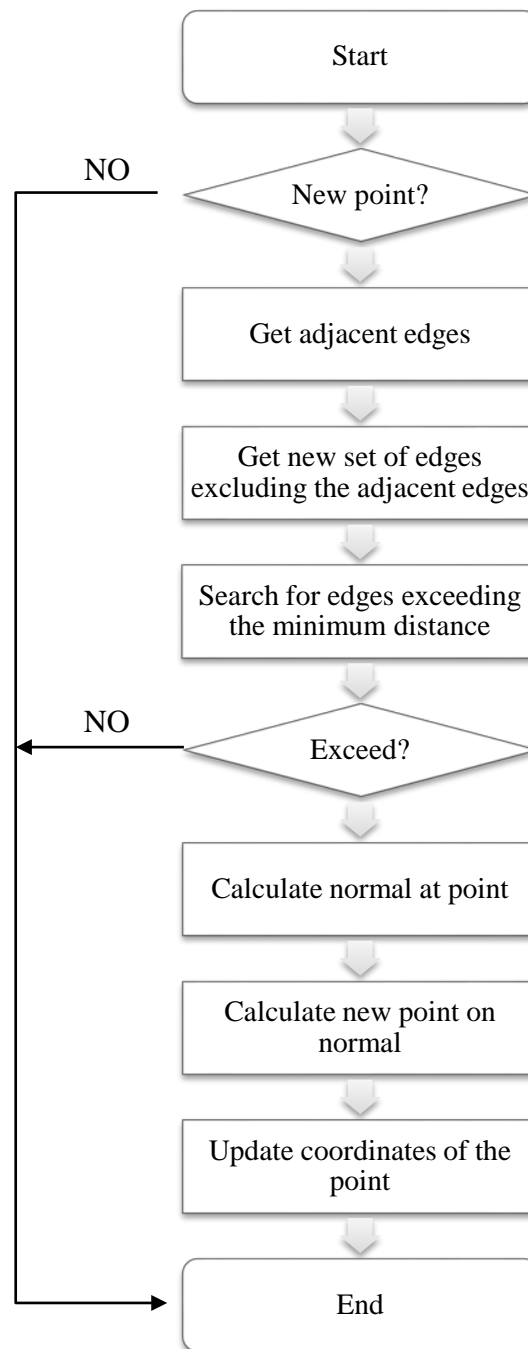
Figure 3-3 : Flowchart for inflating contour points using dilation

```
Algorithm for dilation:

for each v ∈ V, in linearized order {

    get new set E' ⊆ E with adjacent edges to v excluded

    for each e ∈ E' {

        dist(v) = distance from point v to edge e

        if(dist(v)< minimum distance) then {

            normal(v) = calculate normal at point v

            d_adjusted = minimum distance - dist(v)

            point(v) = new point on normal(v) at d_adjusted

            v = point(v)

        }

    }

}
```

Figure 3-4 : Dilation algorithm used to inflate the contours

Figure 3-5.b shows a small segment of the cortical contour line where the distance between the two walls creates a gap when reconstructed (Figure 3-5.d). When dilation is applied by moving a circle along the contour line that creates enough gap for the reconstruction algorithm to create a whole surface (Figure 3-5.e) and gives an inflated look. This step fixes the overlap and proximity of the walls problem mentioned earlier. Contour generalization is the first step towards generalization of the reconstructed surface. Once the contours are generalized, the next step is to reconstruct the surface using contouring filter available in the VTK library.
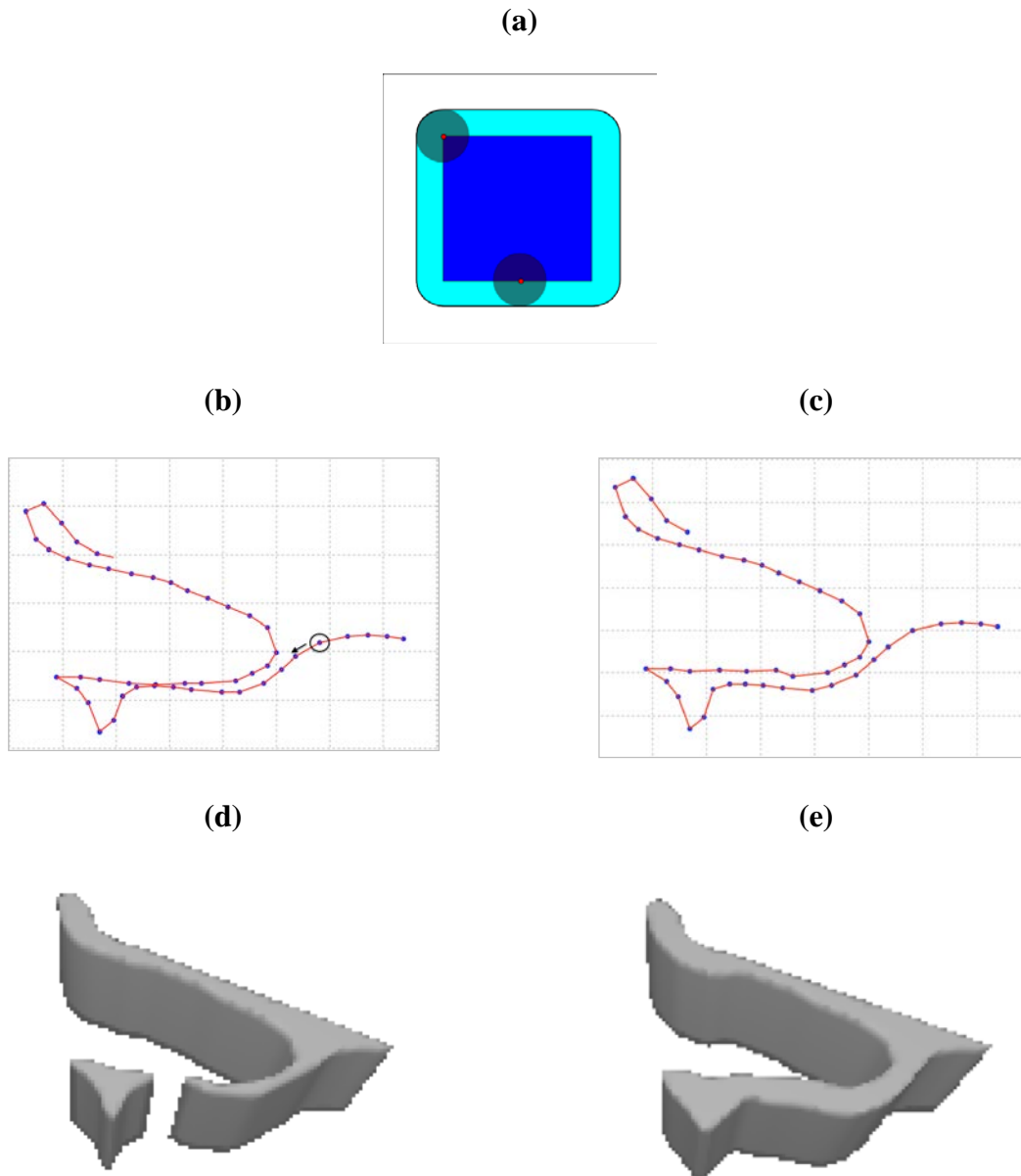
Figure 3-5: Dilation in visualization(a) The dilation of dark-blue square by a disk, resulting in the light-blue square with rounded corners (b) A small segment of the cortical contour showing how close the contour walls can be (c) Dilation is applied to the contour shown in "b" which opens up the gap (d) The reconstruction of the contour without dilation creates an island (e) Reconstruction of the dilated contour fixes the island problem

Visualization Toolkit (VTK) library (Martin, Schroeder, & Lorensen) is used to create the final surface. The "vtkVoxelContoursToSurfaceFilter" takes a set of polygons and applies a contouring filter to generate a 3D surface. This surface is then smoothed using "vtkSmoothPolyDataFilter". As mentioned earlier, the filter uses Laplacian smoothing. Laplacian smoothing is widely used for mesh smoothing because of its ability to stay true to the original geometry. In Laplacin smoothing, local information (immediate neighbors) is used to select the new position of the vertices.
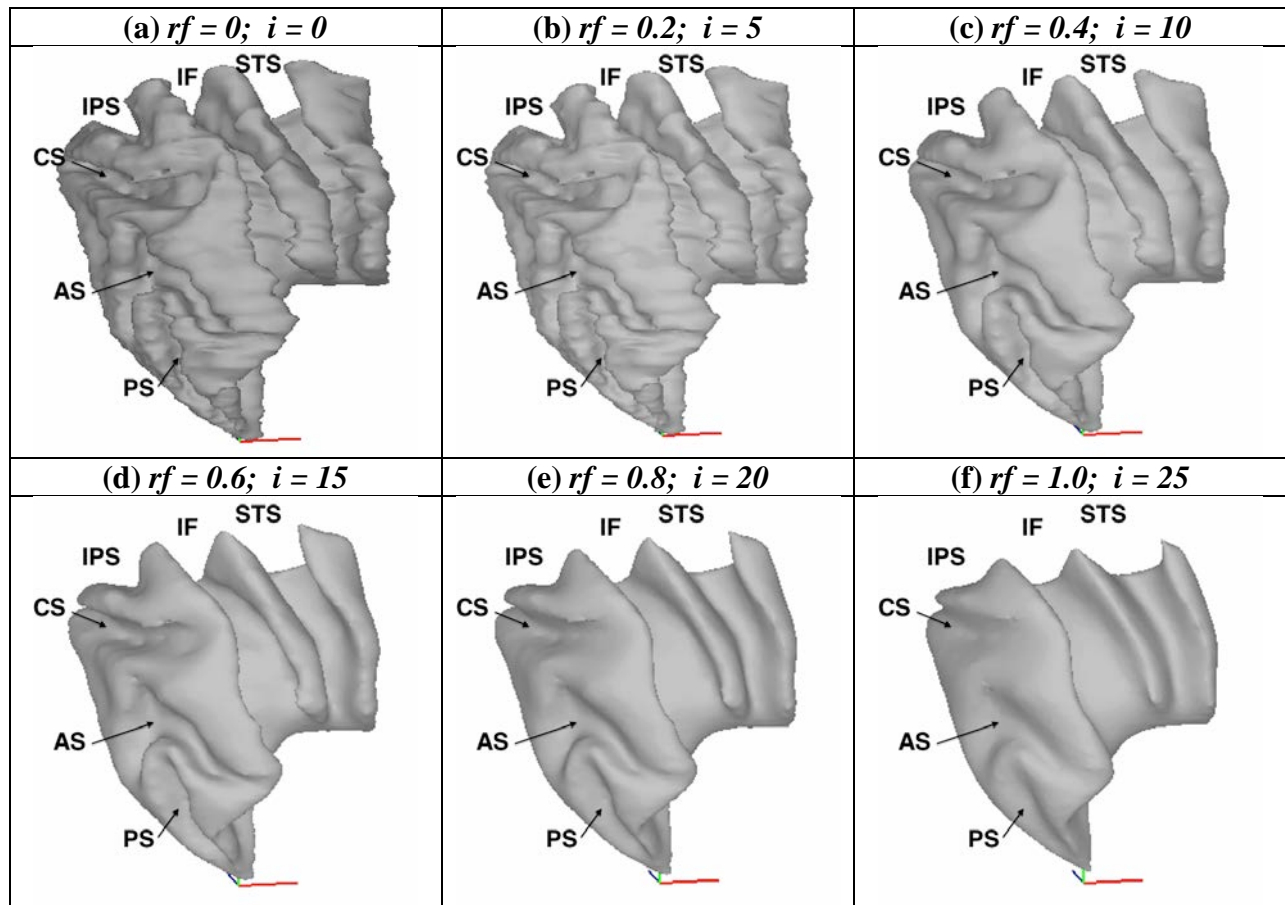
Figure 3-6: A reconstructed model of a primate frontal lobe with different *RelaxationFactor (rf)* and

NumberOfIterations (i)

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The biggest advantage is that the Laplacian operator depends on the mesh topology and not the location of the vertices thus making it an ideal candidate for the smoothing needed to generalize the surface. In VTK, the relaxation of the surface is controlled by "*RelaxationFactor*" and "*NumberOfIterations*". The *RelaxationFactor* controls the amount of displacement of the vertex over number of iteration specified. It is important to note that there is a shrinkage factor associated with the number of iteration. Figure 3-6 shows the smoothing effect on reconstructed primate cortical surface for different *RelaxationFactor* and *NumberOfIterations*. The progressively smoothed surface shows that the primary landmarks are still visible with a very high value for *RelaxationFactor* and *NumberOfIterations* while opening up the sulcal bank. This effect is especially noticeable in the inferior frontal sulcus (IF) and the superior temporal sulcus (STS) area.

3.2   VISUALIZATION OF BRAIN MODEL

The visualization component deals with visualization of the neuroanatomical data or the labeled cells after the reconstruction process has gone thru the generalization steps.

The steps of the visualization component are:

1. Once the generalized surface is generated, the labeled cells (hidden data) are projected onto the smoothed surface by calculating first the surface normal and then finding the projection point onto the surface,

2. Once the cells are projected to the surface, a hierarchical clustering algorithm is used to spatially cluster the cells in order to create a density representation,

3. Step two is reiterated with different values to progressively shrink the cluster size,

4. The density map is transferred to the surface of the brain by determining which surface vertices belong to the area enclosed by each cluster and colorizing them.

3.2.1 Cell Projection On To Cortical Surface

A customized method similar to the surface relaxation method described earlier is used to project labeled cells to the generalized surface. For each cell, the closet surface normal is calculated. The cell is then projected to the normal projection point given a threshold to avoid cells being projected beyond the desired depth. The calculation of normal is similar to the contour inflation method. One of biggest advantage of using an iso-surface for projection of the cells is that the normal of the triangles is pre-computed during the tessellation process. This normal is calculated for the triangle plane that is perpendicular to the triangle facing the outward direction of the surface. The normal of a triangle is computer the following way:

Definition: Let A, B & C be the three points of a triangle. Two possible directional vectors representing the plane of that surface are:

$$v_1 = \langle B_x - A_x, B_y - A_y, B_z - A_z \rangle$$

$$v_2 = \langle C_x - B_x, C_y - B_y, C_z - B_z \rangle$$

The cross product of these two vectors will yield the normal perpendicular to the plane,

$$N_x = v_1 y \times v_2 z - v_1 z \times v_2 y$$

$$N_y = v_1z \times v_2x - v_1x \times v_2z$$

$$N_z = v_1x \times v_2y - v_1y \times v_2x$$

The magnitude of the vector is given by,

$$\|N\| = \sqrt[2]{N_x^2 + N_y^2 + N_z^2}$$

The normal vector can further be normalized by,

$$\boldsymbol{n} = \left\langle \left({N_x}/{\|N\|} \right), \left({N_y}/{\|N\|} \right), \left({N_z}/{\|N\|} \right) \right\rangle$$

This normal is already provided for each triangle as mentioned earlier. The projection is done by finding the point on the plane parallel to the normal and not the centroid of the triangle. The calculation is done the following way:

Definition: For a plane given by three points A, B & C,

$$ax + by + cz = d$$

and its normal $\boldsymbol{n} = \langle a, b, c \rangle$, we want to calculate the projection, $x = (x, y, z)$, of a point $x_0 = (x_0, y_0, z_0)$ on the plane. Since the normal and the vertices are known, one way to calculate d is,

$$d = A_x\boldsymbol{n}_x + A_y\boldsymbol{n}_y + A_z\boldsymbol{n}_z$$

So, for a point that lies on the plane it need to satisfy the equation of the plane $ax + by + cz = d$. Alternatively, we need to find a parameter t such that,

$$x = x_0 - t\,\boldsymbol{n}$$

t can be obtained by

$$t = x_0\boldsymbol{n}_x + y_0\boldsymbol{n}_y + z_0\boldsymbol{n}_z$$

Once we have t, the projected point can be calculated as,

$$x = x_0 - t\,\boldsymbol{n}_x$$

$$y = y_0 - t\,\boldsymbol{n}_y$$

$$z = z_0 - t\,\boldsymbol{n_z}$$

The calculation of projection for n cell on m triangle is at the order of $O(nm)$. The complexity can be reduced in several ways. In this research, however, we used a conditional operator to reduce the number of calculations (of normal projection) needed for each triangle by defining a rectangular cube around the cell and only calculating for the triangles bounded by the cube. The process is shown in Figure 3.7.
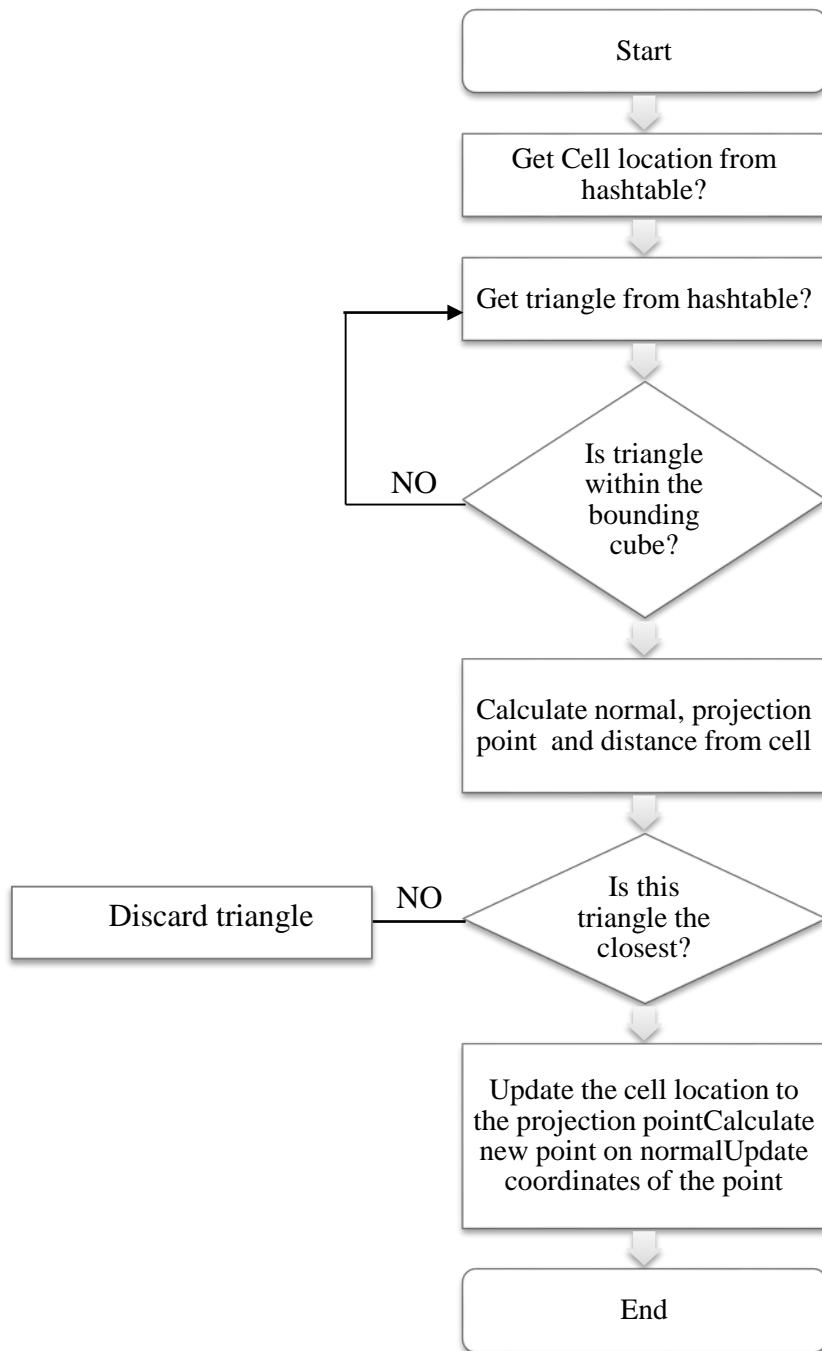
Figure 3-7: Flowchart for projecting cells onto the surface

The algorithm shown below describes the process mentioned above for a set of cells C to be projected onto the surface described by a set of triangle T. The algorithm checks if the triangle is within the projection range first. This is done because anatomically the cells can only be within certain distance of the contour. For example, a cell closer to the frontal lobe should be projected on to the frontal lobe and not the occipital lobe even if a projection exists in latter lobe. Once the projections has been found for the candidate triangles, the closest triangle is picked.

```
Algorithm for cell projection:

for each cell c ∈ C {

    minimum projection distance from cell to triangle, d;

    d = set a value that is greater than minimum projection

    for each triangle t ∈ T {

        if(t is within the bounding cube of c) then {

            normalprojection(c) = calculate normal projection of

            c on triangle t

            d_{c,t} = calculate distance for each t from c

            if(d_{c,t} < minimum projection distance, d){

                d = d_{c,t};

                c = normalprojection(c);

            }

        }

    }

}
```

Figure 3-8 : Dilation algorithm used to inflate the contours

For the cells that cannot be projected onto the surface (i.e. edges), are projected to the closest

vertex. This is reasonable since anatomically the cortical surface does not have sharp unnatural

edges.

This method is distinctly different from the 2D flattening method because the cell follows

the 3D surface as opposed to being perpendicularly projected onto the contour polygon. This

projection method eliminates the "stack effect" and improves the distribution of the cells (Figure 3-9: (A) Shows The "Stack Effect" Of Cells In 2d (B) Shows The Stack Effect In 3d (C) The Stack Effect Is Minimized By Projecting To The Surface).

(A)   (B)   (C)
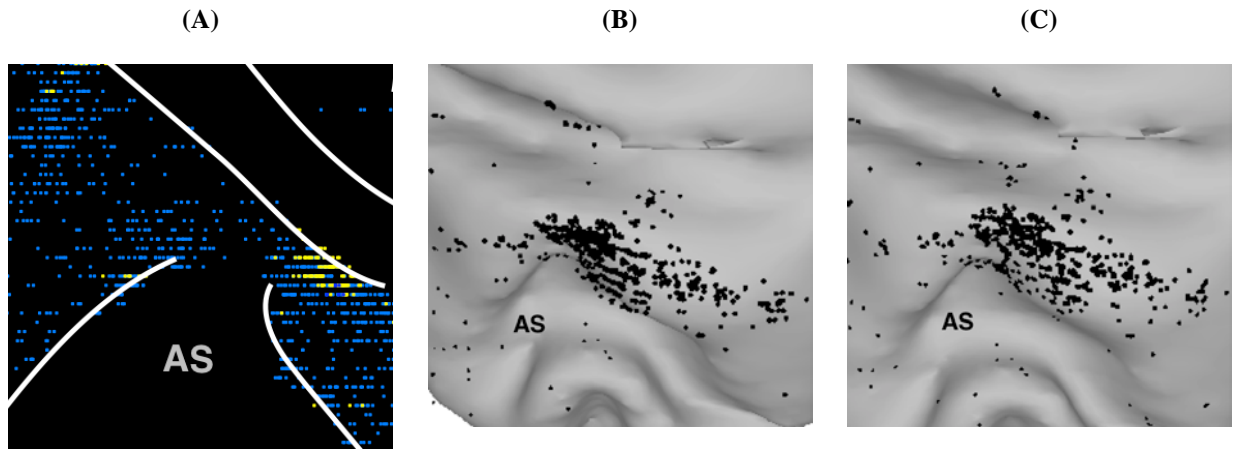
Figure 3-9: (A) Shows The "Stack Effect" Of Cells In 2d (B) Shows The Stack Effect In 3d (C) The Stack Effect Is Minimized By Projecting To The Surface

The DBSCAN (Peter & Antonysamy, 2010) clustering algorithm (density based spatial clustering algorithm) is used to cluster the projected labeled cells because it finds clusters starting from the estimated density distribution. It groups points that are closely packed together. The advantages of using DBSCAN are:

1. As opposed to k-means algorithm, it is not required to know the number of clusters in the data,

2. It can find arbitrarily shaped cluster. This feature is useful because the cluster shape can stay true to the data distribution and is reflected on the surface density,

3. Domain experts can set the parameters used for clustering. This helps to fine tune the map if the data is well understood.

The clustering process creates multiple clusters with different population of cells. These clusters are then mapped onto the surface by identifying the area of the cluster and then checking if the surface vertices belong to that area. Once identified, the vertices are colorized according to the user defined color map. Figure 3-10 (a) shows the labeled cell distribution on the reconstructed surface. After performing DBCSAN, each cluster area was transferred onto the surface. In the figure, we see different levels of color (ranging from red to yellow to white) to show the different levels of density. The initial density (red area) values are set by the domain expert and are then progressively constricted to show the peak of density (white area).

(a)   **(b)**
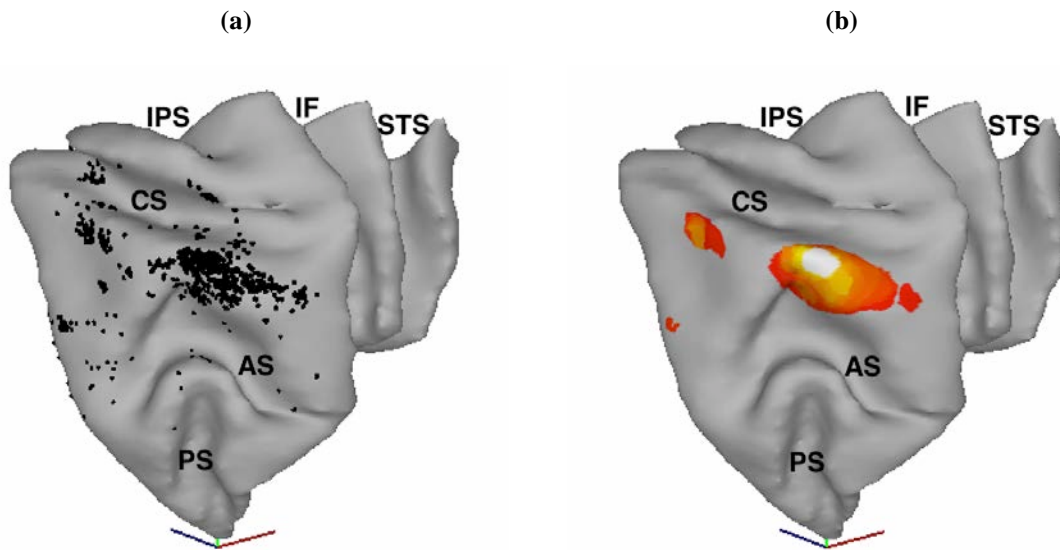
Figure 3-10 : (a) Distribution of labeled cells that has been projected onto the reconstructed surface (b) Density mapping of the labeled cells onto the surface

3.3 CASE STUDIES

Two data sets from Systems Neuroscience Institute at the University Of Pittsburgh were generalized and visualized to show the ways in which the visualization is improved with the proposed method.

Case 1: Labeled neurons in the frontal lobe after retrograde transneuronal transport of rabies virus from a neck muscle:

Figure 3-11 shows a comparison of different representations used to distinguish the labeled neurons in the frontal lobe of the primate brain following retrograde transneuronal transport of rabies virus from a neck muscle. Figure 3-11 (a) is the 2D flattened representation, where the labeling of the cells hidden deep inside the arcuate sulcus (AS) and central sulcus (CS) are shown separately. They must be shown separately, because it is not possible to display the data without deforming the 2D representation. The 2D map is divided into small bins, and the density representation in the 2D map is achieved by counting the number of cells in each bin (white represents 4–10 cells per bin). Figure 3-11 (b) shows a generalized 3D view of the same data. The density map overlaid on the reconstructed surface reflects the density shown in the 2D map. The inside of the central sulcus can be clearly seen in the generalized 3D view. The accuracy of the spatial density in 3D is comparable to the 2D density mapping using a binning system. The "hot spots" visible inside the arcuate sulcus, the central sulcus, and on the lateral surface are visible on the 3D view. This shows that the two maps are not only comparable, but also that in 3D, the density shows peaks where the population is the highest. In addition, the 3D view provides a context with respect to the whole brain that enables the viewer to easily recognize key landmarks on its surface. For example, the central sulcus in the 2D flattened

representation needs to be imagined from the two bounding lines, and determining its context within the brain requires piecing together the flattened CS map (Figure 3-8a, right) with the 2D map of the whole brain (Figure 3-8a, center). In the 3D representation, that mental transformation is not necessary, as the activation of the central sulcus in the bank can be instantly visualized in the appropriate context.

(a)

2D map of the Arcuate Sulcus **2D map of the Lateral surface of the hemisphere** **Unfolded map of the Central Sulcus**
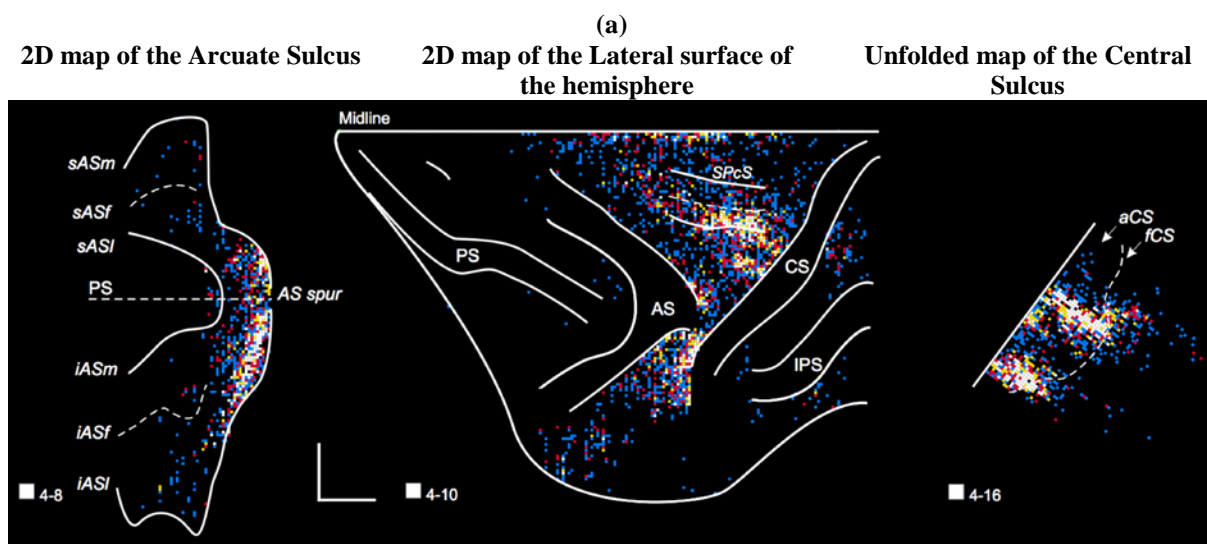
(b) Generalization of the hemisphere and density mapping of labeled cells
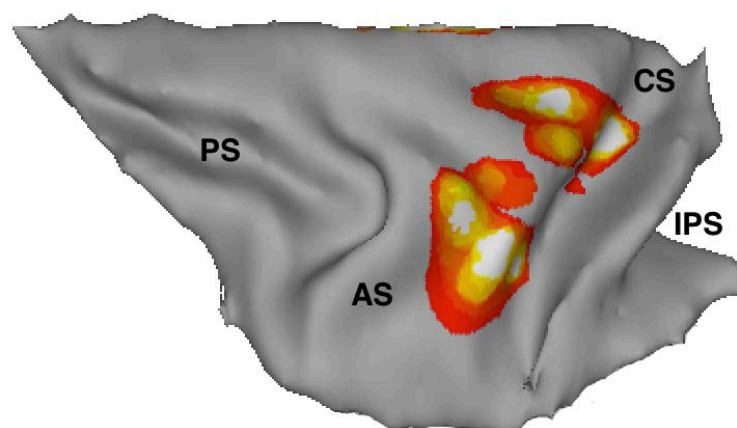
Figure 3-11: Barebrain visualization of Case 1(a) A flattened representation of the frontal lobe; density showing labeled neuron inside the arcuate sulcus, central sulcus, and on the lateral surface; (b) A generalized representation of the same data with density mapped on the reconstructed surface

Case 2: Labeled neurons in the frontal lobe after retrograde transneuronal transport of rabies virus from the adrenal medulla:

Figure 3-12 shows the data representation of labeled neurons after the rabies virus was injected into the adrenal medulla. The images on the left show a 2D flattened representation and a generalized 3D representation of the lateral surface. The images on the right show the 2D flattened representation (Figure 3-12(a), left) and a generalized 3D representation (Figure 3-12(b), left) of the medial wall. A corresponding cluster of high-density labeling in each representation is indicated with an asterisk (*) symbol. In this 2D dataset, the central sulcus has not been unfolded, since the labeling inside the CS is not the focus of this study. However, labeling can be seen inside the CS without changing the perspective of the 3D generalized view. In both representations, three distinct hot spots can be seen on the lateral surface. On the medial wall, multiple hotspots can be seen in both representations. In the generalized representation of the medial wall (Figure 3-12), the cingulate sulcus (CC) can be instantly recognized, and the density can be seen in the bank of cingulate sulcus. This observation is nearly impossible to view in the 2D flattened map (Figure 3-12(a), right) without the boundary drawings. Similar to Case Study 1, the key landmarks are instantly recognizable in the generalized 3D representation, while landmarks in the 2D flattened representation can only be recognized by their outlines and text labeling.

(a)

2D flattened representation of the lateral surface of the hemisphere

2D flattened representation of the medial wall of the hemisphere

(b)

Generalized representation of the lateral surface of the hemisphere

Generalized representation of the medial wall of the hemisphere
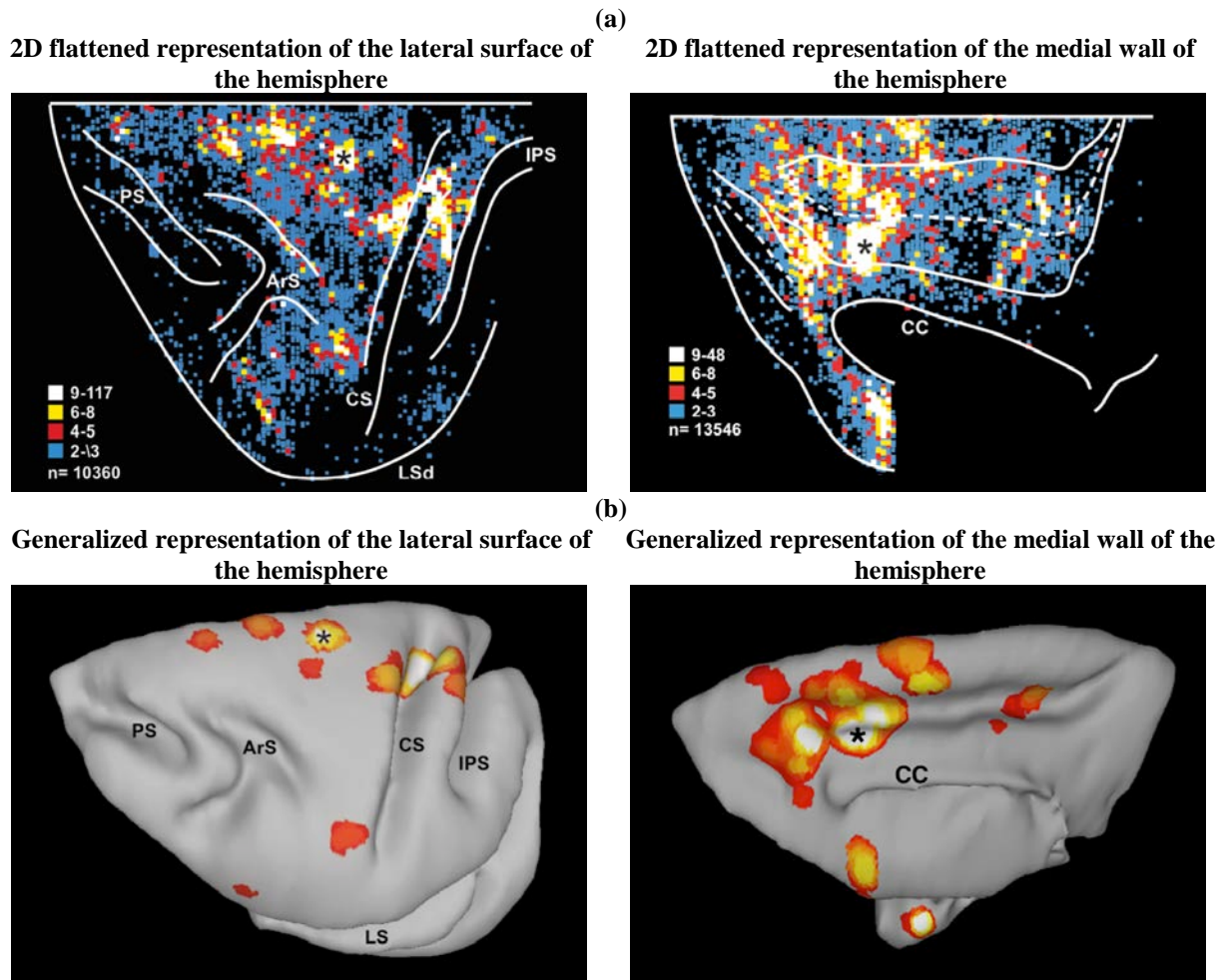
Figure 3-12: Barebrain visualization of Case 2(a) A 2D flattened representation of the frontal lobe – lateral surface (left) & medial wall (right); density showing labeled neurons on the lateral surface; (b) A generalized representation of the same data with density mapped on the reconstructed surface - lateral surface (left) & medial wall (right). The correspondence of density is shown with "*" on the maps.

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3.4   ANALYSIS OF EXPERT INTERVIEWS

Guided expert interviews were conducted (Appendix B) to gather domain expert requirements, in order to accurately represent experimental data. This is an important step, since the analysis varies widely, depending on the type of data and the acquisition process. A typical exploratory analysis is rendered ineffective in neuroanatomical data analysis, since regions of interest are already known; and statistical analysis cannot be done, due to expense and scarcity of the data. Therefore, the most effective way to find out how visualization techniques can be improved is to gather an experimenter's query into the data and what message he or she is trying to convey with its representation. Figure 3-13 shows the analysis of interviews using a "wordcloud" (Fellows, 2012) shows the commonality of words used during the interview. A "wordcloud" is used to highlight the most commonly used word in a text; in our case, this text is the transcribed interviews. The frequency of each word is counted, and the more frequently a word is used, the larger and bolder it is displayed. The guided interview focused on experimenter data and current representation methods, as well as what an experimenter expects to see or expects others to see with their representation. It also focused on how the representation can be improved to gain better understanding of the data. The "wordcloud" obtained shows that the experimenters want to have a representation that enabled them to see different regions of the brain by mapping. It also shows that interpretation of the data is closely associated with correct identification of the anatomical structure, due to anatomical variation within the same species and mapping data hidden deep inside complex anatomical structures. Further analysis of the interviews can be summarized in queries that a domain expert wishes to perform when analyzing or showing the data.

Figure 3-13: A "wordcloud" representation of the expert interviews showing the commonality of words being used.

These queries include:

1. Can we identify the areas of the brain that are affected by the data?

2. Can we localize the areas where the density is the highest?

3. Can we identify which part of the brain we are viewing when we view a small portion of the brain?

4. What hypothesis can we construct by viewing the data?

5. How much do we understand about the data?

6. Can we describe patterns in the data? Does the data follow any particular anatomical structure?

7. When looking at labeled cells, can we assess the amount of labeled cells in the data?

These queries are representative of the data sense making process that the domain expert utilizes when viewing experimental data. While conducting the interviews, the users expressed an increasing interest to represent data in three-dimensional form. The primary reason for that is a better grasp of context and understanding of anatomy. Due to structural complexity, the interviewees found that two-dimensional flattened representation of the brain creates distortion and cannot show true data distribution. This leads the question whether a three-dimensional representation can really aid in data interpretation. One immediate benefit of the three-dimensional reconstruction is that the viewer does not have to perform mental transformation of the two-dimensional flattened model to fit the generic three-dimensional model of the brain regardless of the variation among species. The brain model can be readily recognized as a 'brain' and the viewer can move on to perform more complex analysis by comparing various structural landmarks. However, exposing data that are hidden by the surface can be a challenge. Unfolding the cortical surface is made difficult by varied shape and size of the brain and its complex overlapping structure. With help of generalization, we should be able to show data in context of a quasi brain model without the structural complexity. On the other hand, the generalized brain model, which is also in 3D, is not without flaws. "Conversely, a cost associated with using 3D displays is that any projection of a 3D world inevitably produces an inherent perceptual

ambiguity" (Y Liu, 1997). Infinite 3D perspective for a single point in 2D hinders the user's judgment on relations or values along any axis, especially when attempting to judge the distance between two points along the line of sight (Liu, 1997). In addition, 3D displays are susceptible to clutter (Garner, 1970). 3D choice may seem like a natural choice to display biological data, but a 2D display may aid in judging distance. With the help of the generalization, we expect to lessen some of the underlying issues with 2D and 3D representations. A human factors experiment is proposed to find exactly this performance gain across all three representations.

3.5   PILOT STUDY

Experimental Conditions

We designed two conditions for the purpose of this dissertation. One is the 2D flattened condition, where the user visualizes the flattened brain map, and the other is the generalized 3D condition, where the user is presented with an generalized view of the brain. The two were compared in a counterbalanced repeated measure design. The pilot study questionnaire was designed, based on the expert interview that was previously discussed. Both conditions were created using identical data sets. The 2D flattened reconstruction was produced by "straightening" the layer IV of the cortical structure, redrawn from the histological slices. The layer IV on the medial wall of the hemisphere was unfolded and reflected upward, and the lateral surface of the hemisphere was unfolded and reflected downward (Dum & Strick, 1991). The generalized 3D reconstruction was generated by first correcting for contour overlaps, and then dilated using the smoothing technique described in the "3D GENERALIZATION of brain model" section. In the flattened condition, the operator can rotate the model to get the right

perspective. In the generalized condition, the operator has the six degrees-of-freedom (DoF) required to view the model from any perspective.

Participants & Procedure

10 participants were recruited from the University of Pittsburgh who had a prior knowledge of primate brain anatomy. Participants read the description of both conditions and were instructed on how to control the camera view for the second (generalized) condition, followed by a 15-minute training session. It was verified that the participants understood the different cytoarchitectonic areas of the brain that were used to score the answers during the training. The participant then spent time answering the questionnaire for each condition. In the first condition, the participants spent their time observing the 2D flattened model and the density map that was plotted on the map. For the second condition, the participant spent their time moving the generalized 3D model and inspecting the density map projected on the generalized surface.

In both conditions, participants were instructed to answer specific questions about the model to access their ability to draw inference from the model (Table 1). These questions were designed after an analysis of the expert interview. Participants were provided with a schematic diagram of the brain map with cytoarchitectonic areas of the brain labeled for answering questions that required localization of areas of activation or density. The amount of time spent on answering questions about each representation was also recorded. A "Spatial Orientation Test" (Hegarty & Waller, 2004) was given prior to the training session to test the user's spatial ability

to imagine different perspectives or orientations in space. Answers were scored based on correct answer provided by the expert analysis.

Table 1 : Questions asked during the evaluation of the 2D Flattened and Generalized 3D conditions

Number	Questions
1	Identify which part of the brain this is
2	Identify key landmarks
3	Localize areas with highest density
4	Identify closest landmark(s) to the highest density
5	Identify location of the highest density in a given area that follows the following structure? I) Surface II) Sulcus & III) Sulcal Bank
6	Identify the area with highest density by judging the shape and size of cluster

Results and Discussion

Data were analyzed using a repeated-measures ANOVA analysis that compared the 2D Flattened condition to the Generalized 3D condition. Since the sample size was too small to obtain statistical significance using ANOVA, Pearson correlation coefficients were also measured against the user's spatial test score, and are shown in Table 2. The general idea of the pilot study was to understand the effect of each condition on visualizing neuroanatomical data. When the user was asked to identify the part of the brain that was represented, there were no statistical significance between the 2D Flattened and the Generalized 3D conditions. When users were asked to identify key landmarks (Question No. 2) in the represented brain, there was a

marginally negative correlation (-0.143) between the 2D Flattened condition and the user's spatial abilities: in short, users with higher spatial abilities did poorly in identifying landmarks in the flattened representation. A total score of correctly identified landmarks in this condition also shows an advantage over the flattened representation (Figure 3-14.a). Similarly, when the user was asked if the density mapped in the 2D Flattened condition followed any anatomical structure (namely, the sulcal bank of the central sulcus, as in Question No. 5), users with high spatial ability performed the worst in the 2D Flattened condition. (-0.406).

Table 2: Pearson correlation coefficients against the user's spatial test score. Values are between +1 and −1 inclusive, where 1 is total positive correlation, 0 is no correlation, and −1 is total negative correlation.

Number	Questions	2D Flattened	3D Generalized
1	Identify which part of the brain this is	0.132	0.132
2	Identify key landmarks	-0.143	0.092
3	Localize areas with highest density	0.418	0.0
4	Identify closest landmark(s) to the highest density	0.083	0.250
5	Identify location of the highest density in a given area that follows the following structure? I) Surface II) Sulcus & III) Sulcal Bank	-0.406	0.056
6	Identify the area with highest density by judging the shape and size of cluster	-0.263	0.263
Total time spent		0.208	-0.376

A similar effect can be seen in Question No. 6, where the user was asked to identify the location of highest density (-0.263). However, the average score between the two conditions for identifying the location of the highest density showed no significant difference (Figure 3-15.a). A positive correlation (0.418) for Question No. 3 indicates that users with a higher spatial score did better in localizing areas with the highest density. The total scores calculated for correctly identifying areas with the highest density also shows that users scored better in the generalized representation (Figure 3-14.b). One notable observation in this pilot data analysis is the total time it took for the user to complete the questionnaire (Figure 3-15.b). It shows that users with higher spatial ability took less time and that users with low spatial ability took more time. The negative correlation coefficient (-0.376) found in the generalized condition also supports this observation.
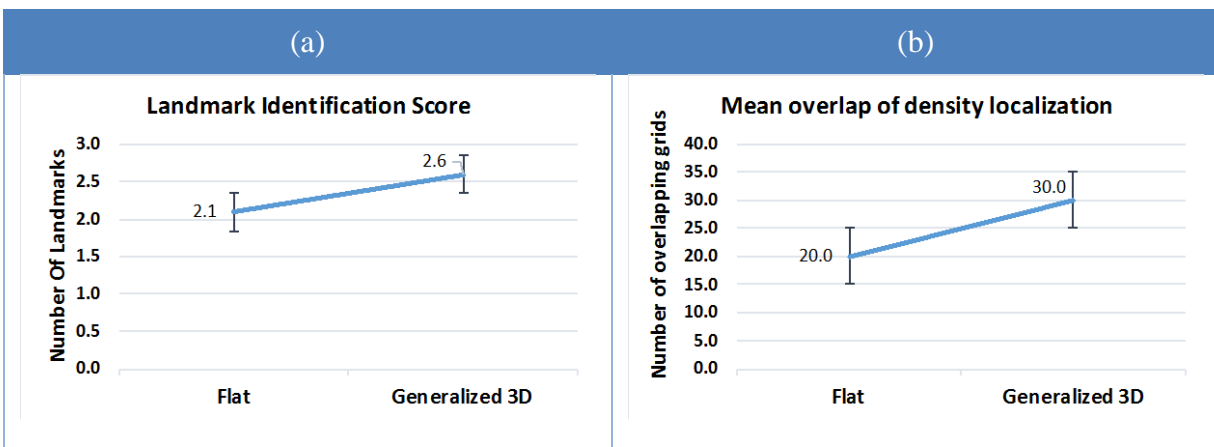
Figure 3-14: (a) Score of total landmarks identified for each representation; (b) Score of correctly identified areas with the highest density.

The effect (or the absence of it) of the generalized condition is what we would expect form the pilot study indicating that regardless of spatial ability, all users scored comparably. The fact that users with low spatial ability performed better could be due to the similarity of the schematic diagram provided during the answer and the 2D Flattened representation. This can be avoided by rotating the initial representation in a counterbalanced manner. Also, stricter scoring rules should be applied when checking the answers for marking areas of high density, and a better schematic diagram showing all the folds should be provided for the answers.
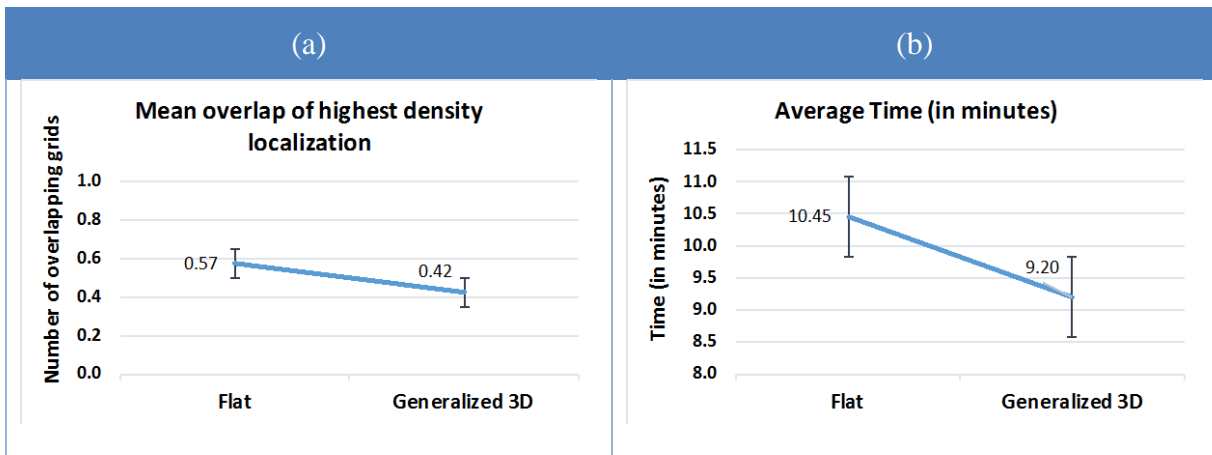
Figure 3-15: (a) Scores for correctly identifying the area with highest activation for each representation; (b) Total time spent for each condition.

From the results of the pilot study, a quick calculation of the sample size shows that 35–40 subjects should confirm the effect of the generalized condition (reject the null hypothesis H_0; μ = mean of flattened condition score) for a two-tailed test, with a 95% confidence interval and a 70% probability of correctly rejecting the null hypothesis when it is false.

4.0 EVALUATION

To evaluate the visualization technique used for neuroanatomical data in this research, we decided to conduct a series of laboratory studies. User requirements gathered from the expert interview, along with the results from the pilot study, were used to evaluate the usability of the visualization method.

The research question we hoped to answer from the studies were appropriate for an exploratory approach that allowed us to learn users' search strategies to effectively visualize neuroanatomical data, and explore possible directions and design guidelines for future visualization technologies. From a thorough (although not exhaustive) literature review of neuroanatomical visualization, we found that there is a limited amount of previous information available on similar problems regarding factors or design guidelines that lead to the effective interpretation of neuroanatomical data. In addition to examining the design guidelines, the effect of being a novice versus an expert on neuroanatomical data visualization was explored.

4.1 APPARATUS

The following reported study and evaluation experiment were conducted using the BareBrain application developed for this dissertation with a combination of Java, a number of self-

developed algorithms and the Visualization Toolkit (VTK) library. Data used and conditions compared for the experiment are discussed in details below.

Data

Three different sets of data were used in a counterbalanced experimental design. The first data set was comprised of neurons in the frontal lobe of the primate brain that were labeled by retrograde transneuronal transport of rabies virus injected into a neck muscle. The other two data sets were comprised of labeled neurons that were labeled when rabies virus was injected into adrenal medulla. The brain was then extracted and further processed to identify the labeled cells. The contour of the cortical surface and locations of the cells were drawn using a microscope and saved in a file that contained the vertices of the contour and cells.

Conditions

Three different representations were created for each digitized histological data.

The *Flat* map was created using ReconWin (Dum & Strick, 1991) application by taking each individual sections and "unfolding" them and mapping density based on the relative location of the cells on the unfolded surface. The 2D flattened reconstruction was produced by "straightening" the layer IV of the cortical structure redrawn from the histological slices. The layer IV on the medial wall of the hemisphere was unfolded and reflected upward, and the lateral surface of the hemisphere was unfolded and reflected downward (Dum & Strick, 1991). In the flattened condition, the operator could only view the representation and did not interact with the map.

The *Generalized* map was created using the BareBrain application where an abstract cortical surface was created in 3D. The generalized 3D reconstruction was created by correcting for contour overlaps first and then dilated using smoothing technique described in "3D

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GENERALIZATION of brain model" section. After generating the generalized surface, labeled cells were projected onto the surface. Spatial density clustering to reveal densest areas and mapped onto the surface. In the generalized condition, the operator had the 6 degrees-of-freedom (DoF) to view the model from any perspective.

The ***Full 3D*** map was also created using the BareBrain application where no generalization methods were applied to the cortical surface. For the full 3D representation, the cortical surface was reconstructed without any inflation of smoothing filters. The cell density was mapped similarly to the generalized representation. The map in full 3D contained all the landmarks and were unaffected by generalization. The operator had the 6 degrees-of-freedom (DoF) to view the model from any perspective.

All three representations were presented to the user on a 2560 x 1600 LCD Retina display. The computer used for the displaying the representations was a MacBook Pro with an Intel Core i7 processor with 8 GB of RAM using Intel HD Graphics 4000 with 1536 MB VRAM. Participants interacted with the software using a standard mouse and keyboard.

2D map of the Arcuate Sulcus **2D map of the Lateral surface of the hemisphere** **Unfolded map of the Central Sulcus**
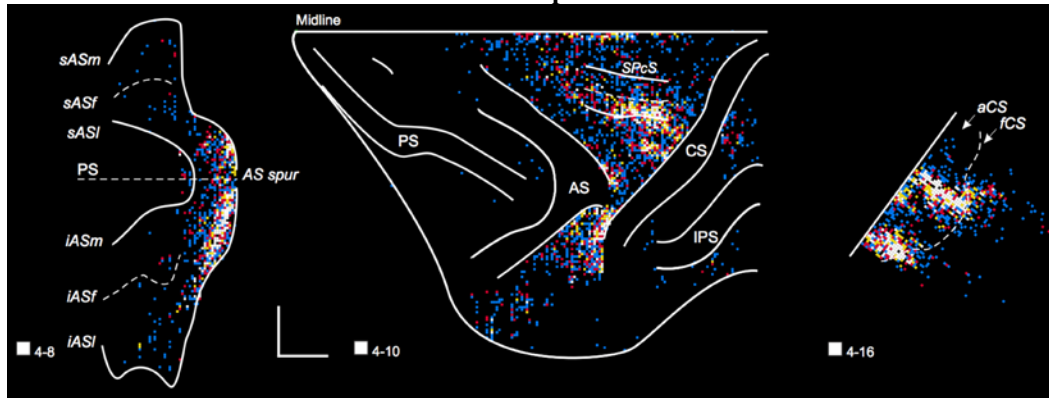
(b) Generalized 3D model of the hemisphere and density mapping of labeled cells
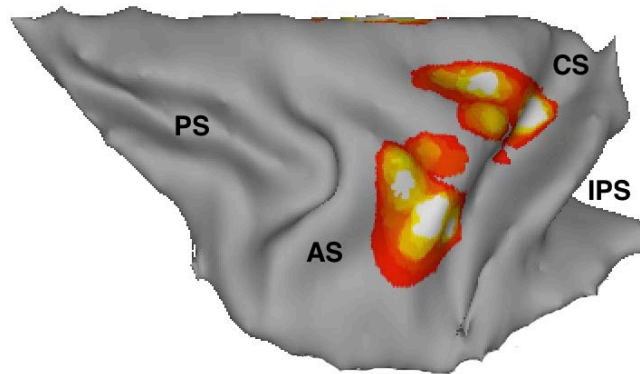
(c) Full 3D model of the hemisphere and density mapping of labeled cells
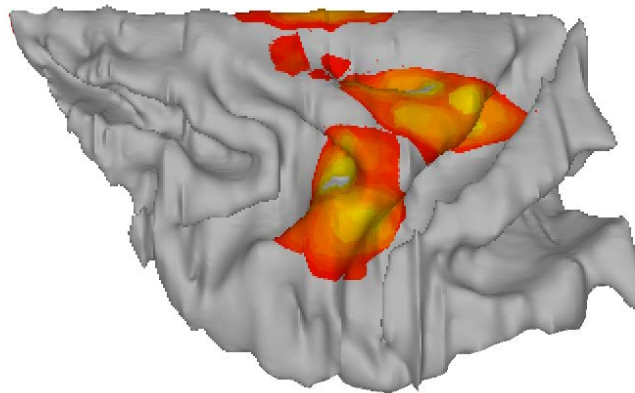
Figure 4-1: Experimental conditions(a) The Flat map condition where the user visualizes the map and makes inference. The map is a representation of the frontal lobe; density showing labeled neuron inside the arcuate sulcus, central sulcus, and on the lateral surface; (b) A generalized representation of the same data with density mapped on the reconstructed surface; (c) A Full 3D map of the same data with density mapped on the reconstructed surface. ** The density scale is not the same across representations.

4.2   VISUALIZATION USER STUDY

4.2.1   Survey Question Design and Measures

In all three conditions, participants were instructed to answer specific questions about the brain and the represented areas of activation in order to access their ability to draw inference from the model. These questions were designed following an analysis of several interviews with experts in the field of neuroanatomical neuroscience. These guided interviews focused on the scientist's data and current representation methods, and what the scientist expects to see or wants others to see with his or her representation. From the analysis of the transcribed interview, we focused on two essential factors for interpreting the data: correct landmark identification and accuracy of locating density. It is important for the user to correctly identify the exact location because activation in the sulcal bank, for example, is not the same as activation that occurs deep inside the sulcus. Since our results from the pilot study showed a plausible effect, we retained our questions from the pilot study and added measures to keep track of the time and number of keystrokes used for the Generalized 3D and the Full 3D representations. We also measured performance for the between-animal variation, where the users were shown maps (either Flat or Generalized 3D) from two different animals with corresponding areas of activation. Users were asked to identify common areas of activation when they compared maps of two different brain. These types of between-animal comparisons are important when comparing and/or validating the results from a control study.

4.2.2 Participants

To understand the accuracy of different representations of the reconstructed brain, we recruited two different groups of participants from the University of Pittsburgh community. Each group was balanced between genders and each participant was offered $15 per hour compensation. Twenty participants were recruited for the "Anatomy" group. These participants had prior experience in visualizing anatomical data where 2D flat maps are commonly used. Twenty participants were recruited for the "fMRI" group. These participants had prior experience in visually evaluating fMRI data, where 3D brain displays are more common than in the visualization of neuroanatomical data. In addition, the fMRI group had more experience in visualizing human brains, as opposed to visualizing the primate brain.

4.2.3 Procedure

Participants were given general information about different models of the brain and how neuronal cells are activated in different areas of the brain. A paper-based Spatial Orientation Test (Hegarty & Waller, 2004) was used to assess participants' spatial ability (SpA), especially regarding perspective. The test was followed by two training sessions where the user was given a thorough description of primate anatomy and of where to find key landmarks in the brain. In the trial sessions, the user familiarized themselves with a sample of each of the three representations. The participant then practiced drawing areas of activation on the standardized map. For each question, the standardized map was provided to mark the area/s of activation when present. The standardized map contained marking showing the cytoarchitectonic areas of the brain. In the training session, participants used specific instructions to learn to manipulate the 3D interactive

models (Generalized and full 3D). Three test sessions followed where each subject answered visualization questions for all three different representations. The experimental conditions and data were counterbalanced between participants in each group.

4.2.4 Analysis / Performance Metrics

Area of activation:

The area of activation drawn by the participants on a paper-based standardized map was evaluated by counting the total number of $1mm^2$ squares against the correct answer (area of activation) identified by the expert. We measured the following:

Overlapping Area:

- True positive (TP): Overlapping area/s of activation

- False positive (FP): Non-overlapping area/s of activation

- Total mean response: Total area/s identified as activation

(a) Expert Response (mm^2)   **(b) User Response (mm^2)**

(c) Measurement of overlapping and non-overlapping (mm^2) areas
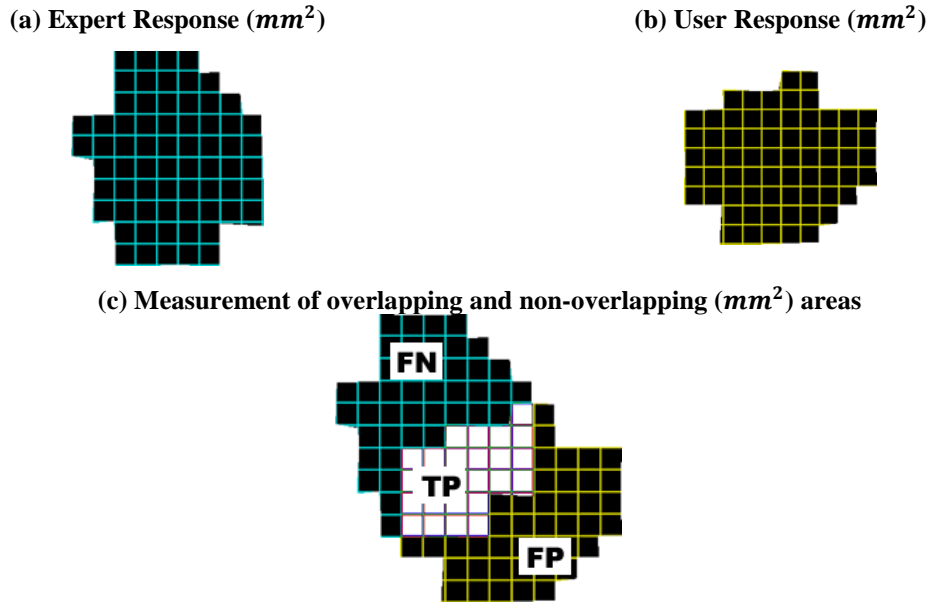
Figure 4-2: Measure of agreement with expert response(a) The expert response was used for the correct area of activation; (b) Activation area marked by the user; (c) The two responses provided on the standardized maps were compared. The overlapping area was identified as a true positive (TP). Areas that that did not overlap, were identified as a false positive (FP).

We measured the answers at three different levels. First, we checked the answers against the area of activation that was drawn by the expert. Second, we checked the participant's answers against the correct area of activation that had its area increased by 1mm in all directions (relaxation of criteria). Second, we checked the participant's answer against a correct answer that was increased by $2mm^2$. Figure 4-3 illustrates this increase.
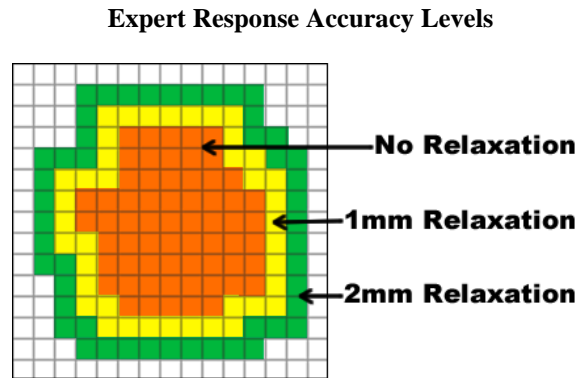
Figure 4-3: Expert response with different levels of relaxation. Each block represents an area of $1mm^2$.

Highest area of activation:

The participants were asked to identify the most intensely active area. Again, the overall accuracy was measured.

Number of landmarks:

Each represented dataset had a set of landmarks that the user had to identify correctly. Type I & II measures were taken for correctly identified landmarks.

Time:

The time taken by the participants to analyze the data for each representation was recorded in minutes.

Number of Keystrokes:

The total number of keystrokes that the participant used during the interaction with the Generalized 3D model and the full 3D models were recorded. The 2D representation did not involve any interaction, so the number of keystrokes was not recorded in this condition.

Spatial Ability (SpA) Score:

A paper-based Spatial Orientation Test (Hegarty & Waller, 2004) was scored and used to measure the participant's spatial abilities.

Variability In Overlap Across Two Brains:

We measured overlap of the participant's localization of density with the expert answer when representations (Flat condition and Generalized condition) of two different brain from same species were compared. True positive (overlapping areas) and false positive (non-overlapping areas) results were reported.

4.2.5 User Response Bias:

Human information processing capabilities are limited and systematically biased. In this research, the fMRI group is familiar with 3D visualization whereas the Anatomy group is familiar with 2D visualization of brain maps. To deal with this bias, we chose the repeated measures design for our evaluation. All three conditions were counterbalanced with three different datasets. The datasets were particularly chosen to have significant anatomical variation and activation from different sources (namely, neck muscle and adrenal medulla). There is a possibility of transfer bias when the user identified the areas from 2D map to a standardized map. 2D maps used for evaluation in this dissertation varied significantly from the standardized map since they were created from different animals and had all the inherent anatomical variation. Also, in one 2D representation, the sulci were unfolded and shown separately where the user had to mentally piece together these maps creating contrast with the standardized map.

4.2.6   Results

The data were analyzed using a repeated-measures ANOVA that compared the 2D Flattened representation with the Generalized 3D and Full 3D representations between the fMRI and Anatomy groups.

Areas of activation:

As discussed above, we measured the area of overlap of the response provided by the users from both groups and the expert answer. A true positive result indicates the overlap. Figure 4-4 shows the mean overlap or the area (in mm^2) that was identified by the user. Figure 4-4 (a-left) shows that when the accuracy of the expert answer is not relaxed, there was no significant difference between the fMRI or Anatomy group ($F(1,38)=3.30$, $p=0.08$) including no significance between representations ($F(2,38)=1.56$, $p=0.21$) or interaction between group and representation ($F(2, 38) = 0.45$, $p = 0.64$). The percentile of the true positive result shows that the users were able to identify an average of 10.7% of the total true positive answers (expert answers) in the Flat condition and 15.3% in the Generalized condition (Figure 4-4 (a-right)). When we relaxed the accuracy of the expert answer by 1mm, we see a marginal difference between representations ($F(1, 38) = 2.96$, $p=0.06$), with users from both groups scoring higher in the Generalized condition, but no difference between groups ($F(1, 38) = 2.96$, $p = 0.09$) or interactions between group and representation ($F(2, 38) = 0.48$, $p = 0.62$). Users were able to identify an average of 8.5% of the total true positive answers in the Flat condition and 13.6% in the Generalized condition (Figure 4-4 (b-right)). When we relaxed the accuracy of the expert response by 2mm, users scored significantly higher in the generalized condition ($F(2, 38) = 3.65$, $p = 0.03$) but there was still no difference between groups ($F(1, 38) = 2.48$, $p = 0.12$) or

interactions between group and representation ($F(2, 38) = 0.661$, $p = 0.519$) . When accuracy was relaxed by 2 mm, users were able to identify an average of 7.4% of the total true positive answers in the Flat condition and 13% for the Generalized condition (Figure 4-4 (c-right)). The percentage shrinks as the accuracy is relaxed, because the total number of true positives (expert answers) increases against the user response.
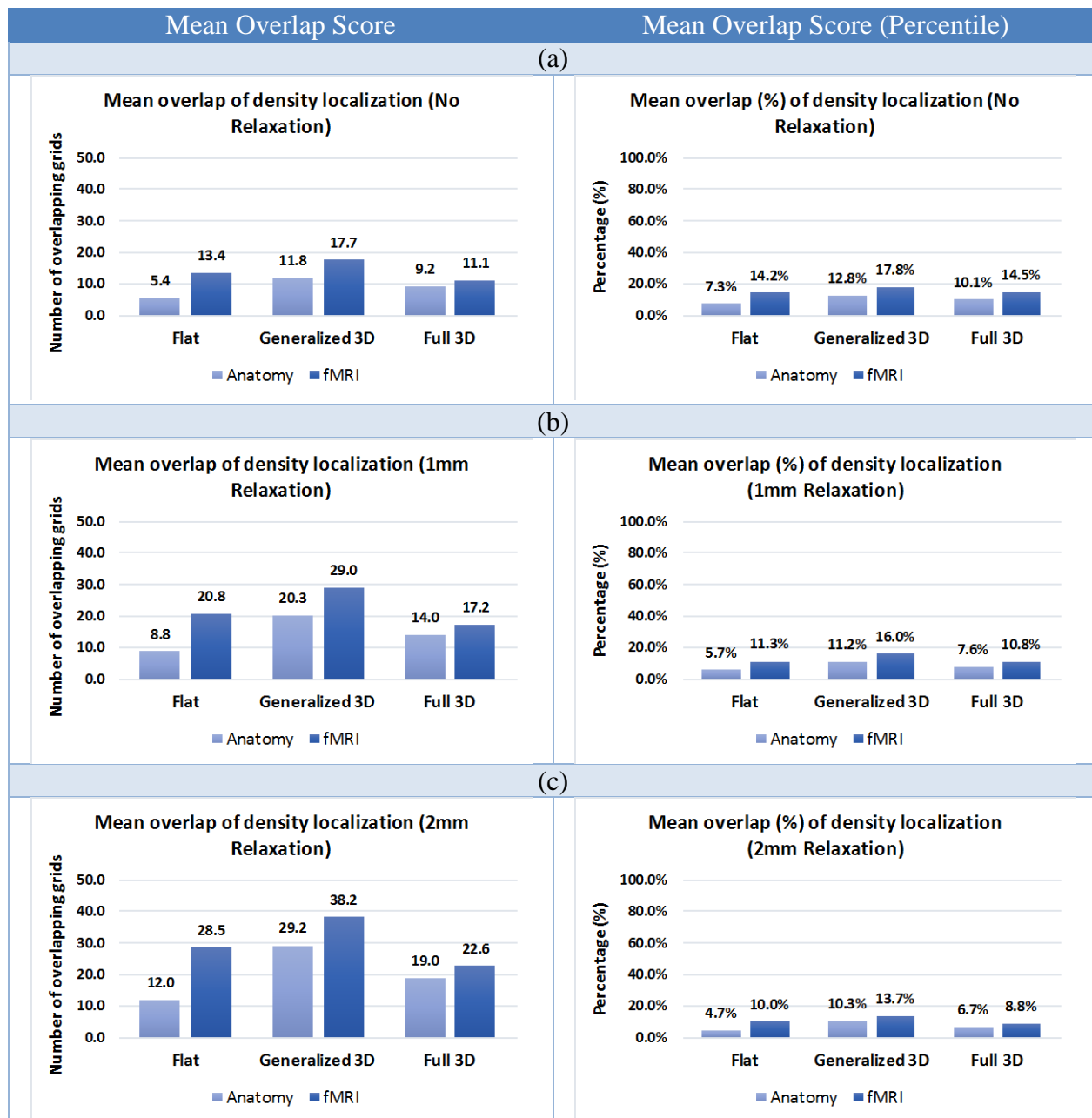
Figure 4-4: Shows the mean accuracy (true positive) or area correctly identified by the users from both groups;the left column shows the mean true positive score and the right column shows the percentile of true positives. (a) Shows the mean response when accuracy was not relaxed; (b) Shows the mean response when accuracy was relaxed by 1mm; (c) Shows the mean response when accuracy was relaxed by 2mm.

The false positive results show the area/s that did not overlap with the expert answered.
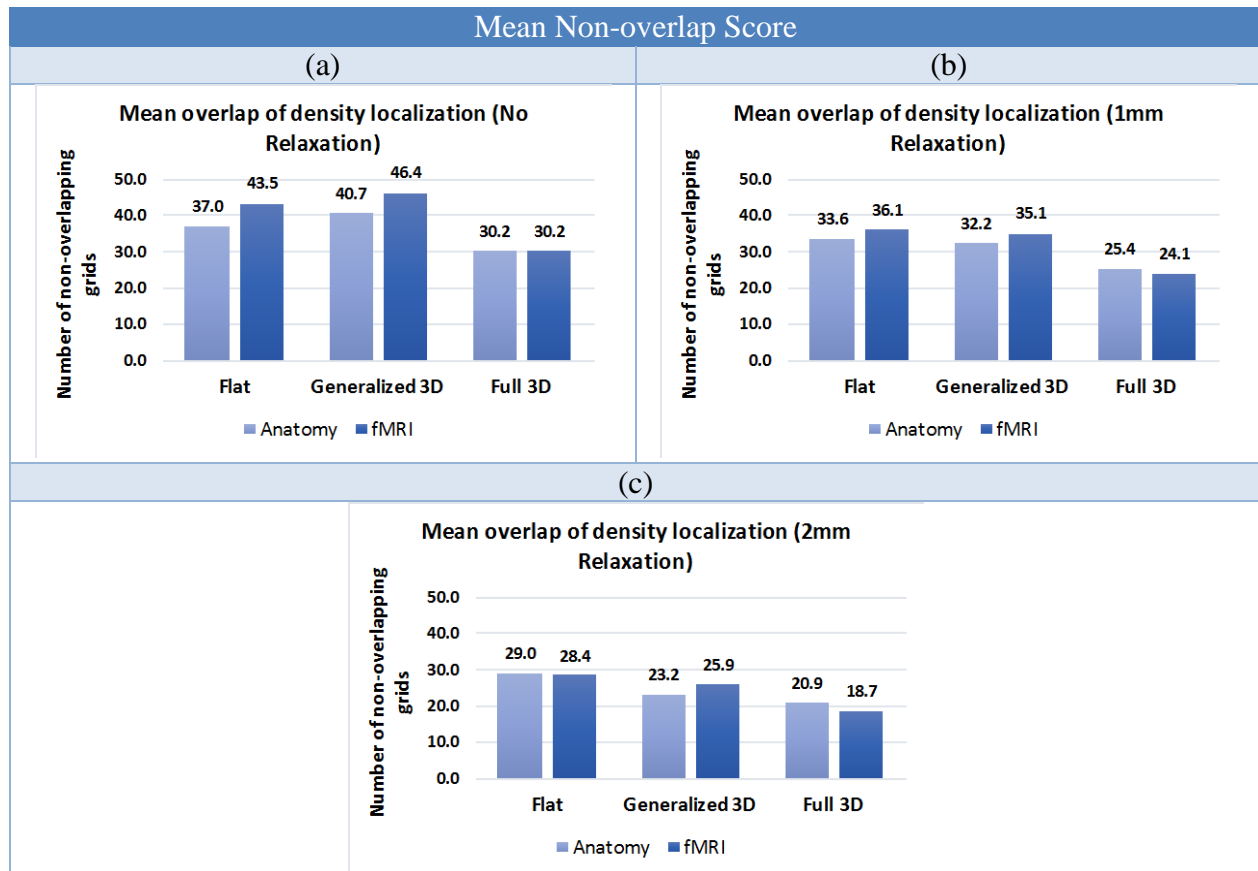
Figure 4-5: Shows the mean non-overlap (false positive) identified by the users from both groups.(a) Shows the mean response when accuracy was not relaxed; (b) Shows the mean response when accuracy was relaxed by 1mm; (c) Shows the mean response when accuracy was relaxed by 2mm.

The result shows that when the accuracy of the expert answer is not relaxed (Figure 4-5 (a)), there is no significant difference in non-overlapping areas between the fMRI or Anatomy groups ($F(1, 38) = 0.34$, $p = 0.57$), including no significance between representations ($F(2, 38) = 2.481$, $p = 0.09$) or interaction between group and representations ($F(2, 38) = 0.16$, $p = 0.85$). When we relaxed the accuracy of the expert answer by 1mm, we still see no difference between groups

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(F(1, 38) = 0.05, p = 0.82), including no significance between representations (F(2, 38) = 1.84, p = 0.17) or interaction between group and representations (F(2, 38) = 0.16, p = 0.85) (Figure 4-5 (b)). Similarly, no significance was observed between the fMRI or Anatomy groups (F(1, 38) = 0.00, p = 0.99), including no significance between representations (F(2, 38) = 1.30, p = 0.29) or interaction between group and representations (F(2, 38) = 0.10, p = 0.90) (Figure 4-5 (c)).

The results shown above also prompt us to question if the total response or size of the response given by the users were different in all the conditions. As a result, we measured the total mean response of the users for the Flat, Generalized 3D, and Full 3D conditions. The results shown in Figure 4-6 indicate that although the responses across representations are not statistically significant (F(2, 38) = 0.56, p = 0.57), the users in the Generalized condition responded about 15% more.
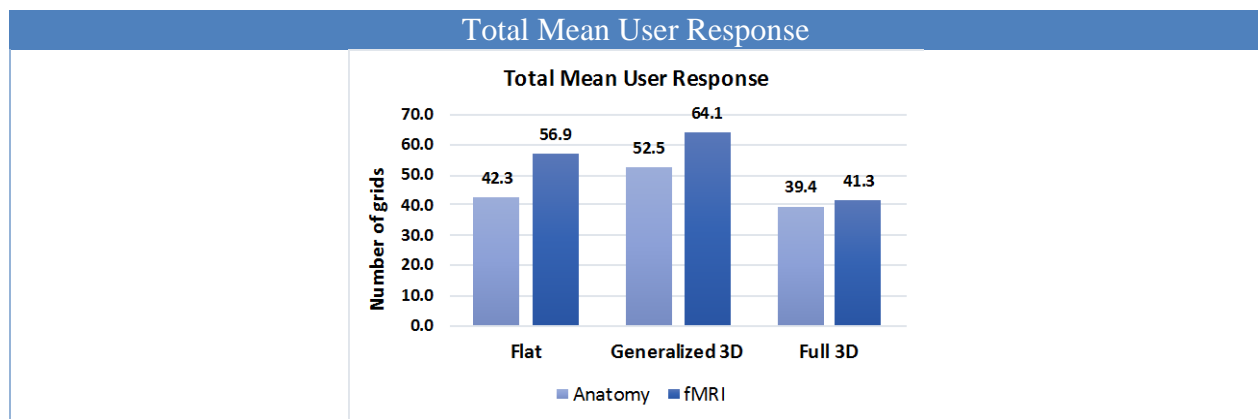
Figure 4-6: The mean response (mm^2) given by the users from both groups

The area of highest activation:

Since it was essential for the user to be able to identify the area with highest activation, we asked the users to identify one area that they considered to be the highest. We again measured the true

positive response with three different levels of accuracy – no relaxation, 1mm relaxation and 2mm relaxation. The results show that when the accuracy of the expert answer is not relaxed, the fMRI and Anatomy groups $(F(1,38)= 5.53, p = 0.02)$ scored differently(Figure 4-7 (a-left)). The fMRI group scored high in both the Flat and Generalized conditions with an average of 23.1% of the total overlapping area, while Anatomy scored 11.9% (Figure 4-7 (a-right)).
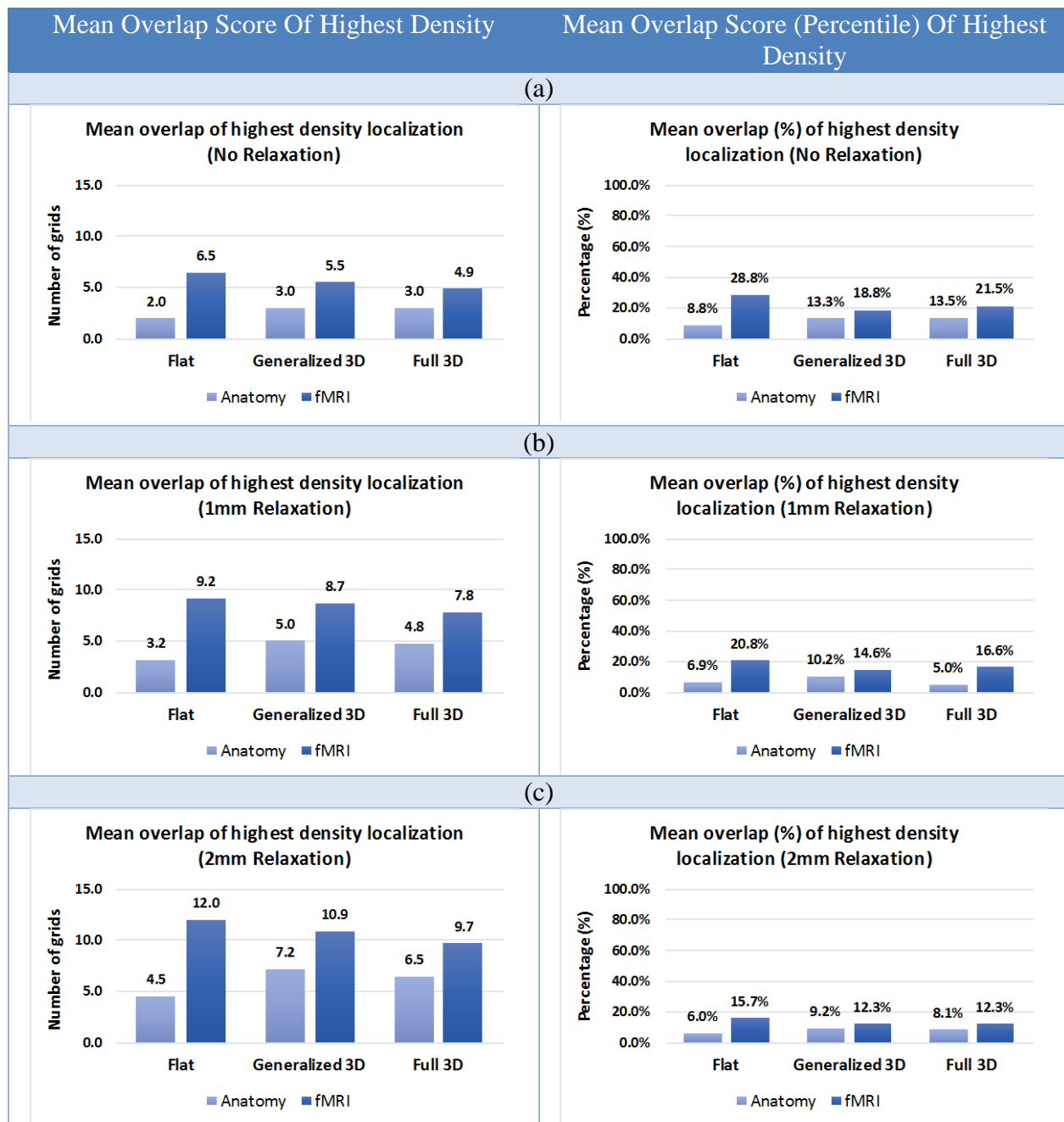
Figure 4-7: The mean overlap (true positive) identified by the users from both the groups; the left column shows the mean true positive score and the right column shows the percentile of true positives. (a) Shows the mean response when accuracy was not relaxed; (b) Shows the mean response when accuracy was relaxed by 1mm; (c) Shows the mean response when accuracy was relaxed by 2mm.

No significance between representations ($F(2, 38) = 0.02$, $p = 0.98$) or interaction between group and representation ($F(2, 38) = 0.34$, $p = 0.71$) was found. When we relaxed the accuracy of the expert answers by 1mm, we again see the fMRI and Anatomy group ($F(1, 38) = 4.99$, $p = 0.03$) scoring differently (Figure 4-7 (b-left)). The fMRI group scored high in both the Flat and Generalized conditions with an average of 17.3% of the total correct answers, while the Anatomy group scored only 7.3% (Figure 4-7 (b-right)). No significance between representations ($F(2, 38) = 0.05$, $p = 0.95$) or interactions between group and representations ($F(2, 38) = 0.203$, $p = 0.817$) was found. When the accuracy was relaxed by 2mm, we see a similar effect with both the fMRI and Anatomy groups ($F(1, 38) = 3.92$, $p = 0.05$) scoring differently (Figure 4-7 (c-left)). The fMRI group scored high in both the Flat and Generalized conditions with an average of 13.4% of the total correct answers, and the Anatomy group scored only 7.7% (Figure 4-7 (c-right)). No significance between representations ($F(2, 38) = 0.06$, $p = 0.95$) or interactions between group and representations ($F(2, 38) = 0.31$, $p = 0.73$) was found.

Number of landmarks:

We asked users to correctly identify a total of seven landmarks in all three representations. Correct identification of these landmarks required some knowledge of brain anatomy. It is important to mention that some features or landmarks do partially exist in each representation, since it is impossible to draw an exact boundary in biological data, especially in 3D. A true positive result shows the number of landmarks that the users were able to correctly identify; a false positive result shows the number of landmarks that the users incorrectly identified; and a false negative result shows the number of landmarks that were present in the model but that the user did not identify. For a true positive score, the results shows no significant

difference between the fMRI or Anatomy groups ($F(1, 38) = 0.48$, $p = 0.49$), including no significance between representations ($F(2, 38) = 1.07$, $p = 0.35$) or interaction between group and representation ($F(2, 38) = 0.29$, $p = 0.75$) (Figure 4-8 (a)). In contrast, the false positive result shows a significant difference between representations ($F(2, 38) = 5.84$, $p = 0.004$), but no significant difference between the fMRI and Anatomy groups ($F(1, 38) = 1.31$, $p = 0.26$) or interactions between group and representations ($F(2, 38) = 0.089$, $p = 0.92$) (Figure 4-8 (b)). However, it is important to note that the average number of incorrectly identified landmarks does not exceed 1 in either case. The number of landmarks that were not identified by users even though they existed (false negative) shows that there was no significant difference between the fMRI or Anatomy groups ($F(1,38) = 0.48$, $p = 0.49$), including no significance between representations ($F(2,38) = 1.07$, $p = .35$) or interaction between group and representations ($F(2, 38) = 0.29$, $p = 0.75$) (Figure 4-8 (c)).
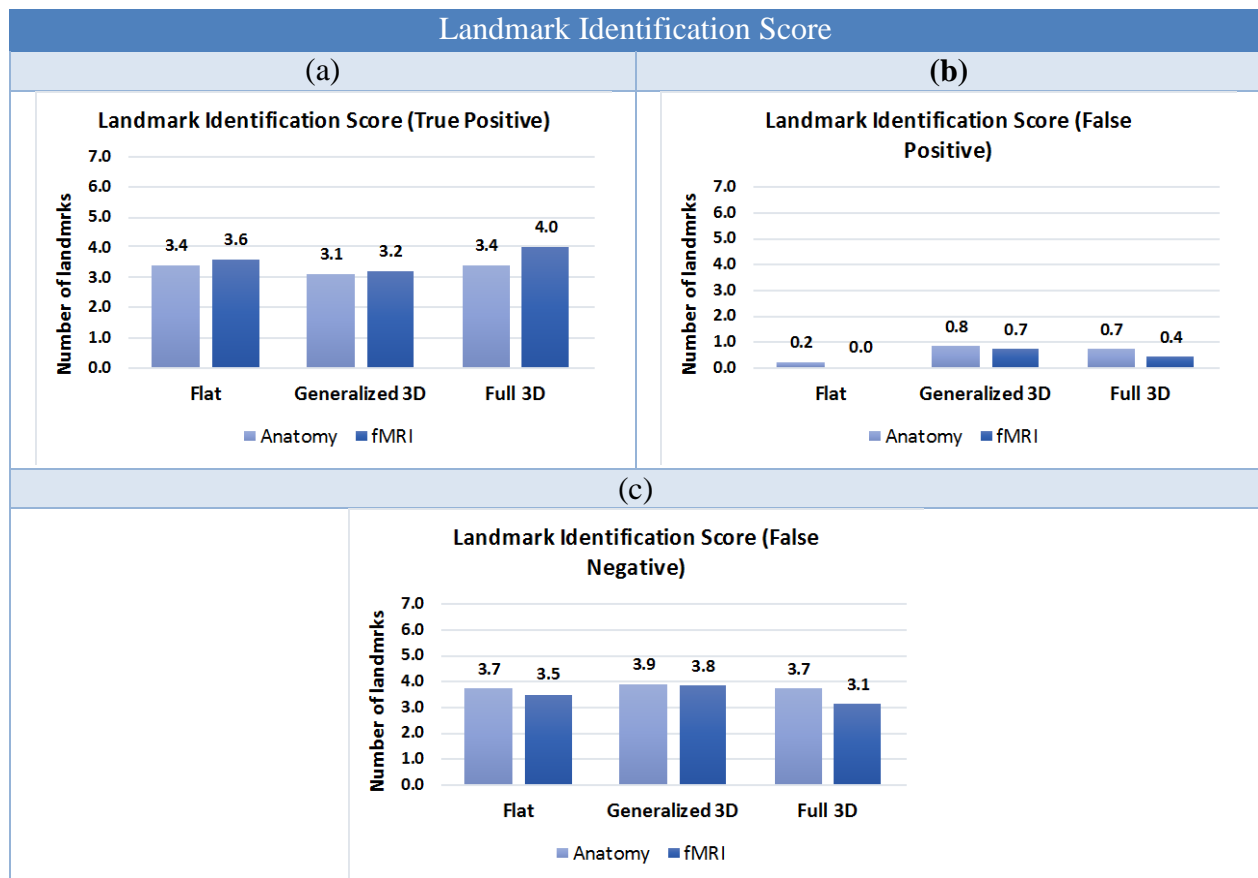
Figure 4-8: The mean landmark identification scores(a) Shows landmarks correctly identified, or a true positive result; (b) Shows landmarks incorrectly identified, or a false positive result; (c) Shows landmarks not identified even though they existed, or a false negative result.

Time:

The total time spent for each representation was recorded. Results show a significant difference between representations ($F_{(2, 38)}$ = 5.14, p = 0.008) with users in the Generalized condition spending more time(Figure 4-9). However, no significant difference was found between the fMRI and Anatomy groups ($F_{(1, 38)}$ = 1.88, p = 0.18) or interaction between group and representations ($F_{(2, 38)}$ = 0.99, p = 0.38).
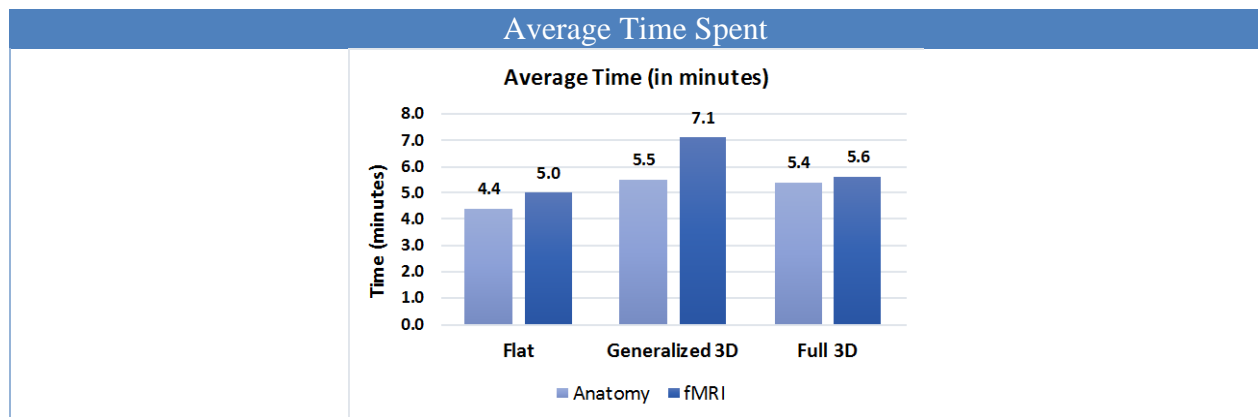
Figure 4-9: Shows the average time spent for each representation

Since users in the Generalized condition spent more time, but scored higher in accurately identifying area of activation, we looked at the correlation between time spent and overlap score. No correlation was found: $r(38) = -0.12$, $p > 0.05$. However, there was a significant correlation (Figure 4-10) between the landmark identification score and time spent in the Generalized condition, with $r(38) = 0.31$, $p < 0.05$. No other correlation with time was found.
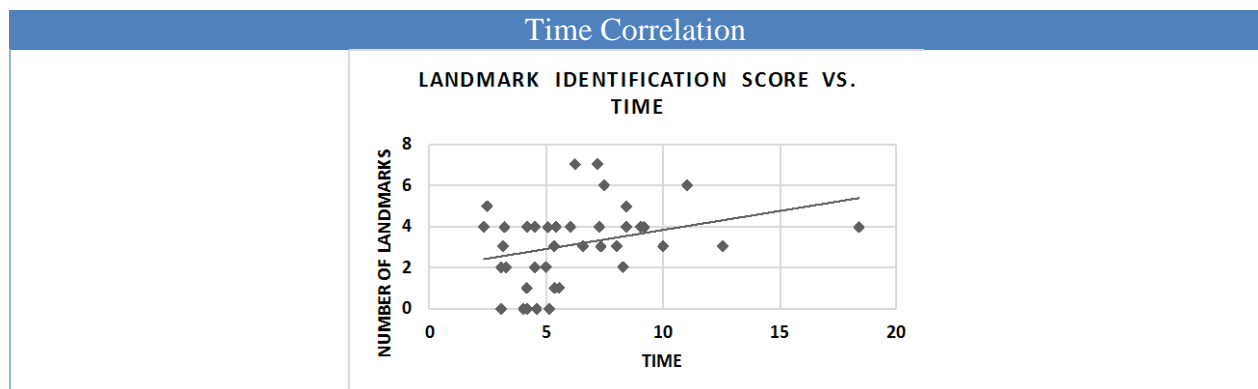
Figure 4-10: Correlation between landmark identification score and time spent

Number Of Keystrokes:

The total number of keystrokes was recorded for the Generalized condition and the full 3D condition. The interaction with the model demonstrates how much the user is engaged with visualizing the model. A higher number of keystrokes may explain the greater difficulty in visualizing a complex structure. Results show no significant difference between the fMRI or Anatomy group ($F_{(1, 38)} = 0.006$, $p = 0.94$) including no significance between representations ($F_{(2, 38)} = 0.21$, $p = 0.66$), or interactions between group and representations ($F_{(2, 38)} = 0.16$, $p = 0.69$) (Figure 4-11).
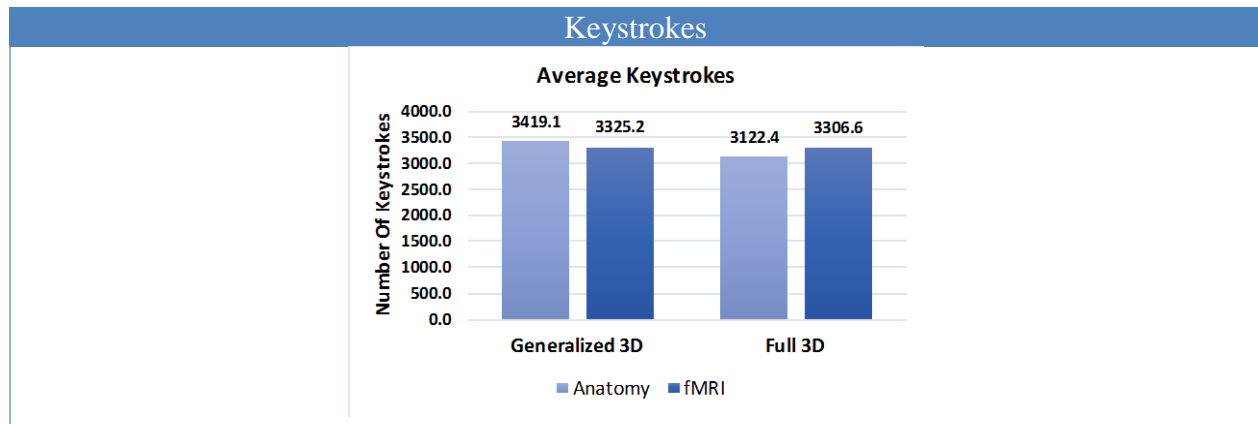
Figure 4-11: Average number of keystrokes for the Generalized 3D and Full 3D conditions.

Spatial Ability (SpA) Score:

The spatial ability score was obtained by administering a paper-based spatial orientation test (Hegarty & Waller, 2004), but the score shows no significant correlation between mean activation area accuracy and SpA for the Generalized condition. No correlation exists when accuracy is not relaxed: $r(38) = 0.2$, $p > 0.05$. Additionally, a correlation does not exist when the accuracy is relaxed by 1mm ($r(38) = 0.08$, $p > 0.05$), as well as when accuracy is relaxed by 2mm ($r(38) = 0.17$, $p > 0.05$), as shown in Figure 4-12.

SpA Correlation	
(a)	(b)

DENSITY LOCALIZATION ACCURACY VS. SPA (NO RELAXATION)

DENSITY LOCALIZATION ACCURACY VS. SPA (1MM RELAXATION)

(c)

DENSITY LOCALIZATION ACCURACY VS. SPA (2MM RELAXATION)
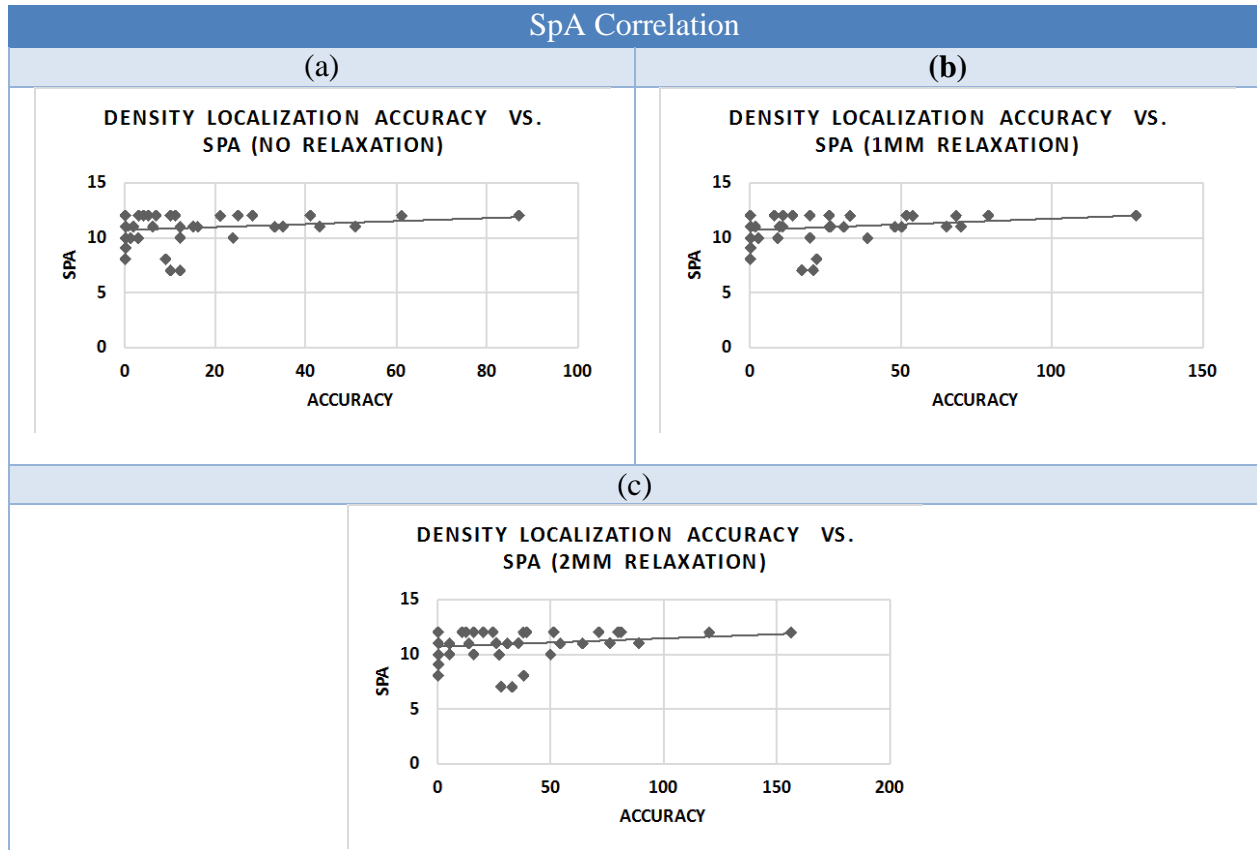
Figure 4-12: Correlation between density localization accuracy vs. SpA score in the Generalized condition.

Variability In Overlap Across Two Brains:

As discussed above, we measured the overlap of the response, provided by the users against the expert response, from both groups in only the Flat and Generalized conditions. The true positive result indicates that the user identified area overlapped with expert response. Figure 4-13 (a) shows that when the accuracy of the expert answer is not relaxed, there is no significant difference between the fMRI or Anatomy groups (F(1,38)=0.76, p=0.39) including no significance between representations (F(1, 38) = 1.73, p = 0.2) or interactions between group and representations (F(1, 38) = 0.000, p = 0.99). When we relaxed the accuracy of the expert answer by 1mm, we see no significant difference between the fMRI or Anatomy groups (F(1,38)=1.96, p=0.24), including no significance between representations (F(1, 38)=1.73, p = 0.17) or

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interactions between group and representations (F(1, 38) = 0.08, p = 0.78) (Figure 4-13 (b)). When we relax the accuracy of the expert answer by 2mm, we still see no significant difference between the fMRI or Anatomy groups (F(1, 38) = 1.47, p = 0.23), including no significance between representations (F(1, 38) = 2.89, p = 0.1) or interactions between group and representations (F(1, 38) = 0.005, p = 0.94) (Figure 4-13 (c)).
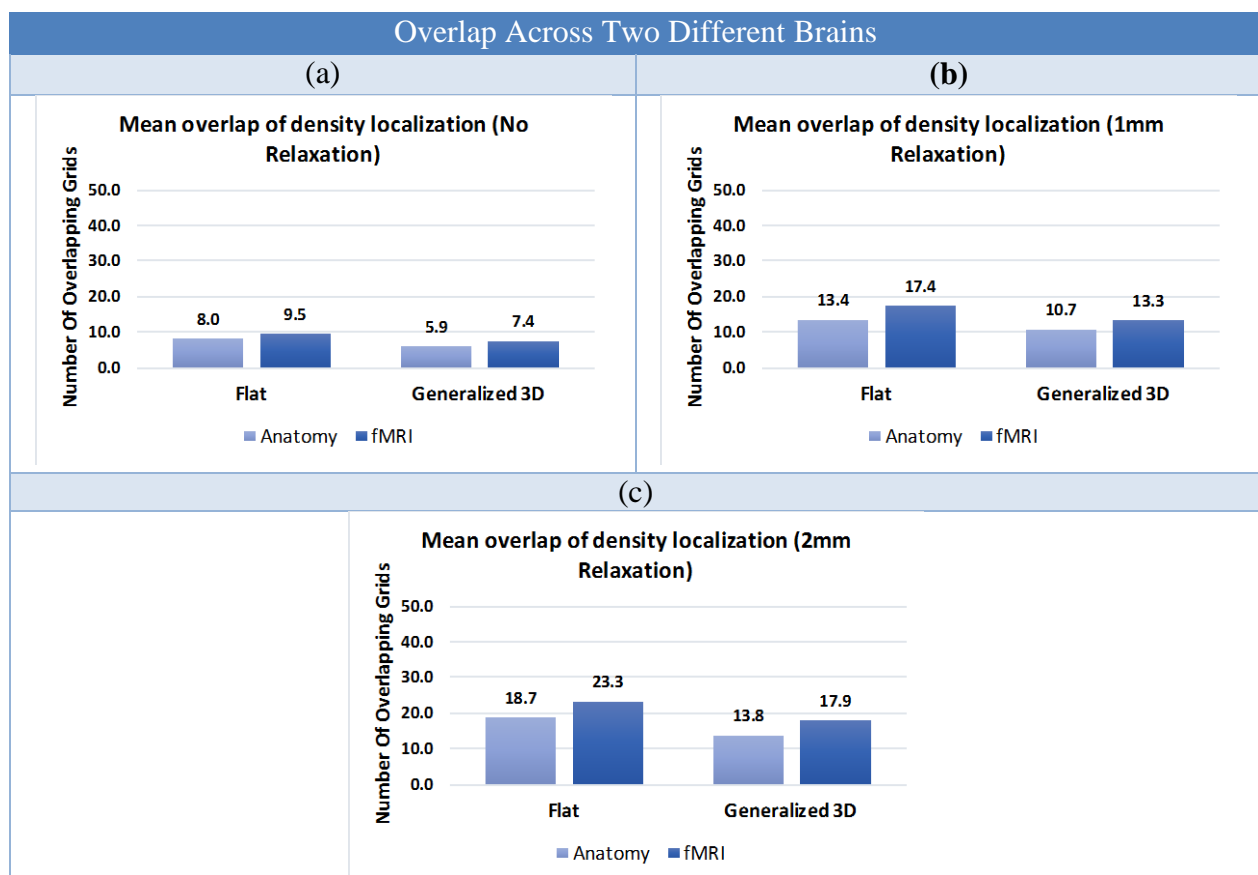
Figure 4-13: Shows the mean overlap (true positive) or area (mm^2) correctly identified by the users from both the groups.(a) Shows the mean overlap when accuracy was not relaxed; (b) Shows the mean overlap when accuracy was relaxed by 1mm; (c) Shows the mean overlap when accuracy was relaxed by 2mm.

Non-overlapping areas were also measured for density localization between two brains. The statistical result shows no significant difference when the accuracy of expert answers is not relaxed. However, when the accuracy is relaxed by 1mm, we see a marginal significance in interaction between group and representation ($F_{(1, 38)} = 3.83$, $p = 0.058$), but no significance between the fMRI or Anatomy groups ($F_{(1, 38)} = 0.11$, $p = 0.74$), including no significance between representations ($F_{(1, 38)} = 2.89$, $p = 0.1$). When the accuracy was relaxed by 2mm, we see a significance in interactions between group and representations ($F_{(1, 38)} = 4.59$, $p = 0.039$) but no significance between the fMRI or Anatomy groups ($F_{(1, 38)} = 0.26$, $p = 0.62$), including no significance between representations ($F_{(1, 38)} = 2.0$, $p = 0.17$). However, this result is trivial since the interaction is significant and disordinal. We also performed a Oneway ANOVA analysis to examine each of these variables and did not find any difference between groups for either the Flat condition ($F_{(1, 39)} = 1.08$, $p = 0.31$) or the Generalized condition ($F_{(1, 39)} = 1.02$, $p = 0.32$). A through explanation of these results can be found in the discussion section.
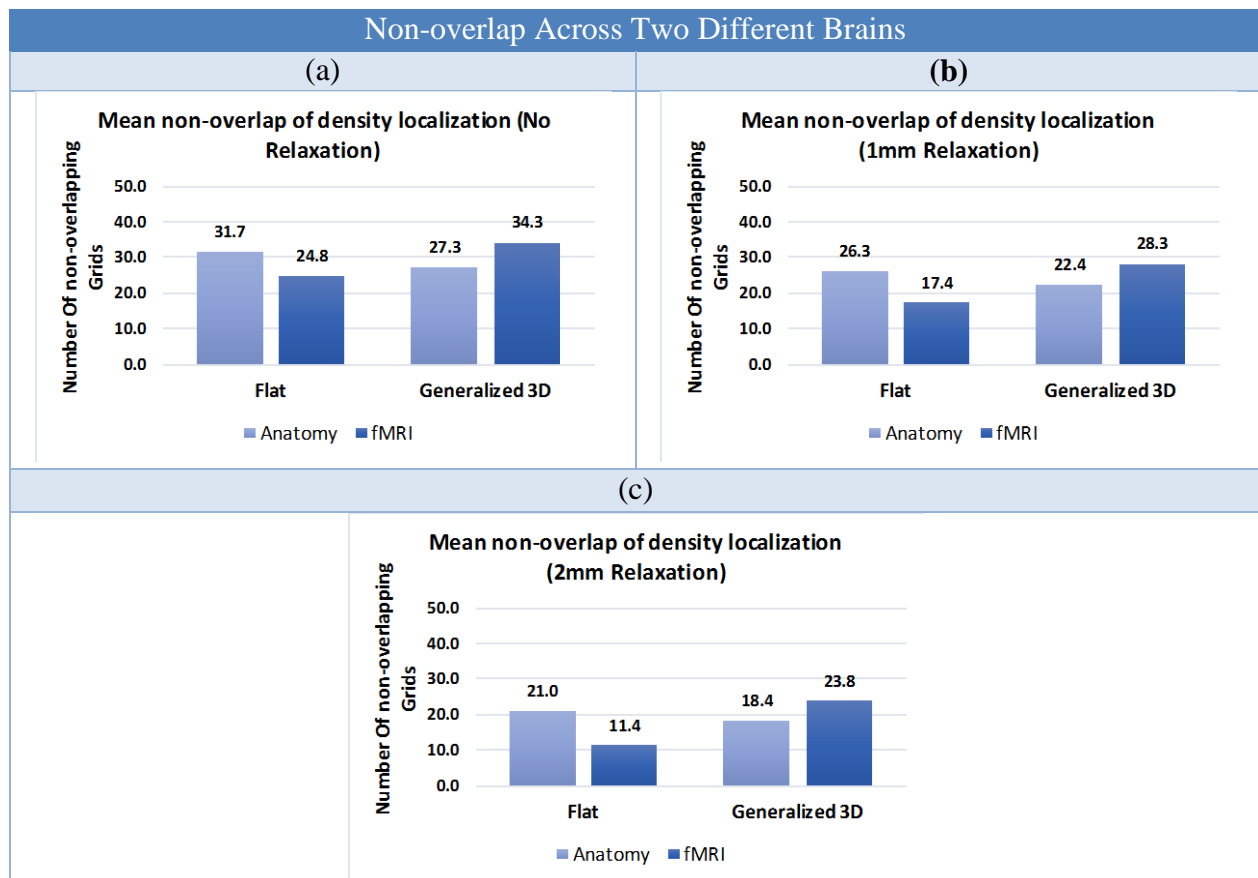
Figure 4-14: Shows the mean non-overlap (false positive)(a) Shows the mean non-overlap when accuracy was not relaxed; (b) Shows the mean non-overlap when accuracy was relaxed by 1mm; (c) Shows the mean non-overlap when accuracy was relaxed by 2mm.

4.2.7   Discussion

Areas of activation:

Assessing how accurately the user can identify areas of activation is important, as we found out from our expert interviews. Correctly identifying activated areas can help us understand which part of the brain contributes to certain body movements, or to understand connectivity within the brain and ultimately understand this connectivity at a much granular level. Our results show that

the agreement between the expert and the user (area of overlap) improves in the generalized condition when we relax the accuracy by both 1 and 2 mm. Users from both groups were able to consistently capture about 13% of the overlap in the Generalized condition, as compared to about half of the overlap in the Flat condition. A noticeable difference is that in the Full 3D condition, users did not perform as well as in the Generalized condition, which validates the hypothesis that 3D is not always better when visualizing neuroanatomical data, due to overall structural complexity. Also, users in the fMRI group performed consistently better in all three conditions, as compared to the Anatomy group, which shows that a good knowledge of neuroanatomy and experience with volumetric data has a positive effect on visualization. Despite good performance in the Generalized representation, users in this group did not do so great in capturing overall areas of activation. There might be other factors that affect this overall result. One explanation is that our representations of the brain in all three conditions are a fabrication of the real data. In each representation, the data is manipulated in some ways that creates significant distortion. This is not the case for MRI data and representation created from MRI images. MRI datasets have very little distortion, but do not have the resolution required to identify cellular level activation. Since histology is the only way to track cellular activity, such distortion can be minimized, but not entirely avoided. By reconstructing the histology in 3D and generalizing it, we can stay as close to the ground truth as possible while minimizing overall distortion.

The non-overlapping results supports the positive effect of the Generalized condition when visualizing anatomical data. The false positive results in all three conditions were of comparable magnitude. However, the inaccuracy in Flat condition is not as adverse as we expected it to be. It can be argued that users in the Generalized condition did better simply because they identified a bigger region. This is unlikely, because when we analyzed the total user

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response for each representation, we did not find any statistical significance between representations.

The area of highest activation:

Results for identifying the highest area of activation show that the fMRI group was superior in localizing, scoring almost twice as higher than the Anatomy group. Users in the fMRI group have a good knowledge of neuroanatomy in addition to visualizing volumetric data that involves perceiving activation or lesion from 2D slices and volumetric reconstruction in 3D. In other words, users in the fMRI group are more experienced observers than those in the Anatomy group. Fiez et al. (2000) found similar results that slightly favored the experienced users. In their task of identifying lesions to a standardized brain map, the experienced users did slightly better, which indicates that naïve users can learn and become proficient, given sufficient anatomical knowledge.

Volumetric data involves 2D slices and orthographic projections of 3D volumes. The general problem of visualizing such data (namely, through CT, MRI, and fMRI) involves an accurate estimation of size, shape, and location of the activation or lesion. Studies have shown that slice visualization and projection are not an optimal way to understand shapes or relative positions in 3D space (Rehm et al., 1998; Tory, 2003). John, Cowen, Smallman, and Oonk (2001) have found that 3D displays hamper judging relative positions, but facilitate shape understanding. Our task of identifying areas of activation is essentially a relative position identification task where the user has to identify position of the activation on the cortical surface. Users in our Generalized condition did better than the Flat (2D) and 3D condition. This result supports our hypothesis that generalization is a viable approach for improving the presentation of

brain neuroanatomy. Additionally, effective training can increase a user's ability to comprehend both shape and size in 3D (Osborn & Agogino, 1992). In our case, the task of identifying the area of highest activation involves understanding the shape and size of the activation. Our result shows that the fMRI group who had more training visualizing data in both 2D and 3D indeed performed better in all three conditions. This result indicates that providing effective training to the user can enhance user performance.

Number of landmarks:

Identifying anatomical landmarks from different representations primarily depends on the user's anatomical knowledge and overall ability to understand the spatial landscape. 3D displays are useful when trying to understand the general shape of the object (Frisby, 1979; John et al., 2001). In our result of landmark identification, users in the Generalized condition did not perform better than those in the Flat condition. As a matter of fact, users in the Generalized condition incorrectly identified landmarks that were not present on the model. When we take a closer look at the user response, we see that users often mistakenly recognize the reconstructed frontal and partial parietal lobe as a full brain, though it does not include the occipital lobe and landmarks contained within. We can find two plausible explanations for this. First, our Generalized model contains a partially reconstructed parietal area and its landmarks. It is impossible to draw the exact boundary for anatomical data, which makes the reconstruction of only the frontal lobe virtually unattainable. The partially reconstructed landmarks in the Generalized model often lead to false positive results—though this is not the case in a flat map. A flat map can easily be manipulated by simply not drawing the landmarks that are of no interest. Second, the smoothing of the reconstructed model in the Generalized condition blurs the exact boundaries of the

landmarks. The more we smooth, the more we lose edges. This can create a misperception of the reconstructed model, as users perceive the blob-like structure as a whole brain. This is especially the case when a user does not know the exact anatomy of the primate brain. One way to overcome this problem is to put markers that define the boundary of landmarks on the Generalized model. This will help the user identify the exact boundary of the landmarks, while maintaining an overall context of the brain. Drawing these markers is much easier and accurate in a 3D model, since we can identify these landmarks in the histological sections and reconstruct without distorting the location. Figure 4-15 shows an illustration of edges being drawn on the reconstructed surface. We can clearly identify the sulcus (dotted line) and see that one of the areas of activation is right in the middle.
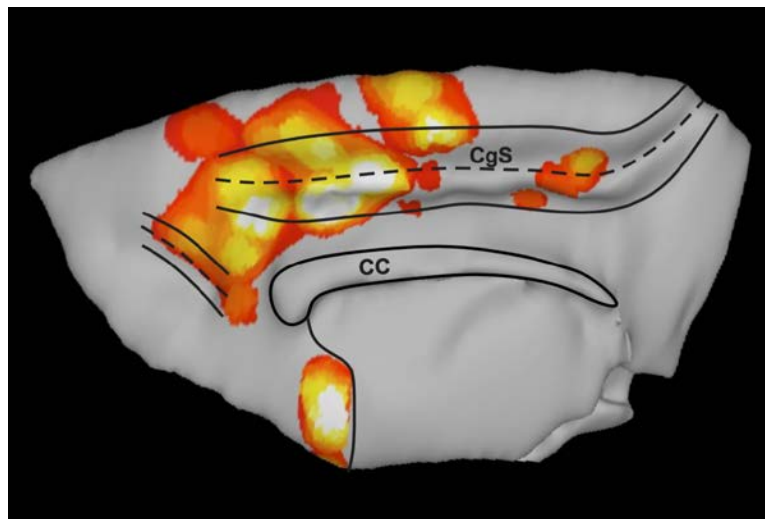
Figure 4-15: Illustration of reconstructed medial wall with landmark edges drawn on the surface. Cingulate sulcus (CgS); corpus callosum (CC).

Time:

There was a significant difference in the total time spent in the Flat condition and the Generalized condition. Users spent more time in the Generalized condition and identified more landmarks as they spent more time. Although this does not mean that users who spent more time in the Generalized condition did better than those in the Flat condition when identifying landmarks, examining the interaction time may lead to a better understanding of the Generalized model and also lead to better accuracy.

Number of Keystrokes:

User engagement with the model shows that there was no difference between the Generalized condition and the 3D condition. We also did not find any correlation with engagement and any of the other dependent variables.

Spatial Ability (SpA) Score:

No significance was found between the user's spatial ability and accuracy scores. One reason for this effect (or lack of effect) is that there was little diversity of spatial ability in our population (M = 10.9, SD = 1.39). Velez, Silver, and Tremaine (2005) ran into similar issues in trying to explain how spatial ability relates to visualization comprehension. The authors concluded that for visualization experiments, one needs to have a large enough sample of subjects to observe any effect. In our case, the participants were from a population group where the ability to effectively perceive data in different modalities is a must. Hence, it is not surprising that there was very little diversity in the results.

Variability In Overlap Across Two Brains:

Judging the corresponding areas of activation between two brains of the same species can be challenging, because the overall shape and size of the brain may vary significantly. When analyzing and comparing peak activation areas within species, this variation can have a huge effect in understanding the corresponding areas. Identifying similar areas can corroborate a functional connectivity. However, this comparison is often done between two brains and is infrequently standardized, due to structural variability. In our task of finding corresponding areas of activation between two brains, we did not find any significance in overlap (with the expert response) between the Flat condition and the Generalized condition. This shows that the Flat representation is as good as the Generalized representation when finding corresponding peaks and drawing them on the standardized map. A remarkable result is that both the Anatomy group and the fMRI group performed similarly when mapping activation from multiple brains to a standardized map. Our result is consistent with the results of the study conducted by Fiez et al. (2000). Fiez found small differences between the intraobserver variability for the experienced observer vs. intraobserver variability for the inexperienced observer for lesion segmentation. In our experiment, the fMRI group was more experienced in visualizing volumetric data and performed slightly better in accurately marking areas of activation.

The main effect of the false positive result is unremarkable, because of the disordinal interaction effect. A disordinal result involves crossing lines, and in our study, the fMRI group has fewer false positives in the Flat condition than in the Generalized condition. On the other hand, the Anatomy group showed an opposite effect. Because of this result, we performed a Oneway ANOVA test and did not find any significance between groups.

5.0   CONCLUSION

This dissertation focuses in the generalization of the 3D models to help us visualize cells deep inside the structure in the context of the brain without completely distorting the anatomical structure. Viewer's agreement with the domain expert hinges on accurate landmark identification and representation of the data. Since a qualitative analysis of the neuroanatomical study is often used to understand and communicate results, presenting high-resolution visualizations of the brain in a way that can facilitate a Neuroscientist's efforts is worth exploring.

5.1   THESIS CONTRIBUTION

As stated in Section 1.2, the objective of this research is to provide insight into how techniques that are used to draw insights from a 2D representation compare to those used in a 3D representation of neuroanatomical data and whether the viewer's knowledge gain can be enhanced by the proposed generalized display technique of the 3D brain.

This work contains several contributions to neuroanatomical data visualization. First, a comprehensive review of state-of-the neuroanatomical data visualization, methods and integration in addition to a formal interview of domain experts been provided. Second, generalization algorithms were developed to tackle inconsistencies in the data. Third, a visualization tool that implements the proposed visualization methods has been developed, with

a fully functional interface, and is currently being used at the Systems Neuroscience Institute at the University Of Pittsburgh. Fourth, a user study was conducted to find the effectiveness of all three conditions (2D, Generalized 3D, and Full 3D) on visualizing neuroanatomical data.

From our analysis of the data gathered from the user study, we discovered a few guidelines that may help when designing future visualization applications. They are:

1. Generalization of complex neuroanatomical structures aids in end user visualization in 3D. Generalization combines the strategies employed by the user in both 2D and 3D. Thus, generalization promotes better localization accuracy while maintaining correct landmark identification.

2. Adding a third dimension provides the user with the realism of the biological structure, but can confuse a user who is analyzing partially reconstructed structures. Users with limited knowledge of the structure under analysis may confuse a partial structure for a whole. This is especially noticeable in the Generalized model, since the edges of the structure are not as salient, which can make the user's perception of boundaries non-uniform.

3. Effective training can increase a user's ability to understand the anatomical data. In our research, experienced users performed better across modalities.

4. The Generalized model can be enhanced by explicitly identifying the edges of the landmarks by either coloring different areas or by simply drawing lines.

5. Determination of the degree to which a user's spatial ability has an effect on visualization comprehension requires a large and diverse enough sample to include users with a range of spatial abilities.

5.2   FUTURE WORK

There are a few limitations to this research, which require further investigation. First, the source of neuroanatomical data and its analysis varies among experimental approaches. Even within one approach, the data varies depending on the question the researcher is asking. For example, histological data may not require a 3D reconstruction if the research only focuses on a number of cells in a focused area. In that case, there is no contour information available to create the surface. A wide array of algorithms is required to implement a complete visualization tool and accommodate different data types. However, it is useful to find visualization guidelines that enhance end user performance and to refine them, and our research focuses on exactly that issue.

Second, anatomical structures can be much more complex (for example, the cerebellum) than those presented in this research. Our algorithm is ineffective for data that includes severe deformations or overlaps in the slice. Standardization to an atlas is one way to solve this problem. However, in recent work, we have found that a multistep generalization technique can create a much more coherent surface. This multistep process involves using a morphological operation for contour data (as described in this research as a first step), converting the cleaned contour to a binary image, performing a morphological operation on the image, and then converting it back to contour for reconstruction. Third, it would be exciting to see how our guidelines affect end user performance. By increasing the sample size, we would hope to introduce a degree of diversity in users' overall spatial abilities and observe its effect.

Finally, a robust landmark-based slice registration technique would be useful for reconstruction. We cannot use an off-the-shelf registration algorithm, since our data is often messy, due to human error, and widely available rigid-body registration cannot be applied to our dataset. Currently, we manually align slices based on different recurring landmarks. Another big

challenge is that we almost always reconstruct a partially cut brain in the lab. A block of the brain is extracted from the full brain and sliced. These slices are then individually processed, thus losing any alignment information, except for the block plane. When drawing from these individual slices, the experimenter often ignores the plane and focuses on the cortical structure, which makes accurate registration even more difficult.

APPENDIX A

CYTOARCHITECTONIC AREAS OF THE PRIMATE BRAIN

The map of the brain is shown below with labeled areas discussed in this article. The numbers refer to cytoarchitectonic areas of the brain.
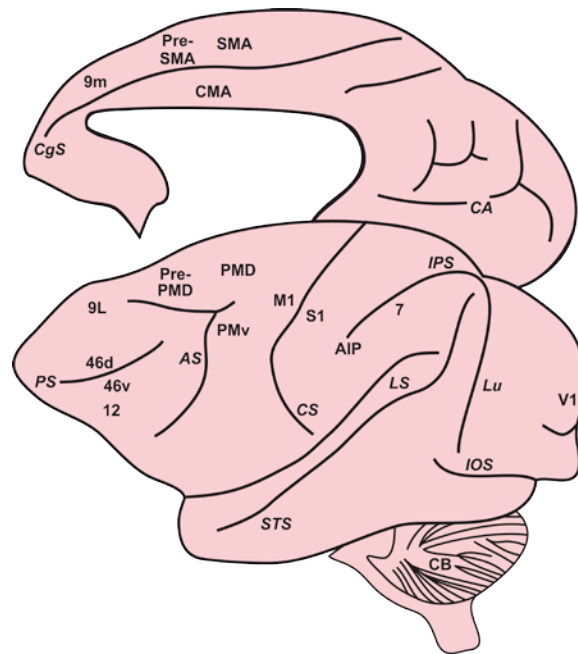
Figure AppendixA-1: Cytoarchitectonic map of the Macaque Monkey brain. Modified from (Bostan, Dum, & Strick, 2013)

AIP	Anterior Intraparietal Area
AS	Arcuate Sulcus
CA	Calcarine Sulcus
CS	Central Sulcus
CB	Cerebellum
CMA	Cingulate Motor Area
CgS	Cingulate Sulcus
IPS	Inferior Intraparietal Sulcus
IOS	Intraoccipital Sulcus
LS	Lateral Sulcus
Lu	Lunate Sulcus
M1	Primary Motor Cortex
S1	Primary Somatosensory Cortex
PS	Principal Sulcus
PMd	Secondary Motor Area Dorsal
PMv	Secondary Motor Areas Ventral
STS	Superior Temporal Sulcus
STS	Superior Temporal Sulcus
SMA	Supplementary Motor Area
V1	Visual Area 1 (Primary Visual Cortex)

GUIDED INTERVIEW DATA

This appendix contains transcripts of domain expert interviews recorded with the interviewees permission. An important value of the recorded interviews was having access to the exact words spoken by the interviewees. The names of the interviewees were initialized for confidentiality purposes.

B.1   INTERVIEW 1

S: You can describe what data you use now and what kind of representation you use now. So what do you think? What is your data? Do you use rabies virus?

AB: Yeah, so we have the rabies virus, and I mostly use 2-D representations right now, in the cortex, and other parts of the brain.

S: So when you inject the rabies virus and you see the infection at any point of time, at any order, what do you expect to see, for your purposes?

AB: Just the distribution of neuron, of points in the space.

S: In which part of the brain?

AB: I look throughout the whole brain, basically.

S: Ok, Not just basal ganglia or anything?

AB: No. I mean, sometimes. Depending on the experiment and the question.

S: Why do you look at the whole brain, is it because you're trying to find connections between areas in the whole brain, or – what is the purpose?

AB: Yeah, basically. To look at connections.

S: Any particular example that you want to give me?

AB: Well, so for example, you can look at, say, inputs to the cerebellum and you're looking in cortex to see what kind of network projects to the cerebellum. So it's usually, it can be an interconnected cortical network, and then, so you see the distribution of the whole   ??? (1:41).

S: I mean, why – and this is something I was reading in the paper, and I guess it makes sense, but I want to hear it from you. I guess we have pre-defined regions where we inject the retrograde or anterograde virus, and we want to see where it ends up, right?

AB: Right.

S: Why does it necessarily mean that there's a connection between those two areas?

AB: Well, because the virus transports at synapses.

S: But does it necessarily give you functionality, or a sense of functionality, or are we just saying that area A is connected to area B? What is the objective here?

AB: The objective can be different depending on the experiment. So for example, when I inject in, say, the cerebellum to look at the cortical inputs or outputs, it's like, there's parts of the cerebellum that we don't really understand their function. And basically the function, whatever it does, it does through its connection to these other areas that we have more

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information about. So this will give us an insight into its function, seeing the areas with which it's connected, can provide us some insight into what its function may be.

S: The review paper that you have, I read through it, and you were also talking about, the cerebellum is connected to the basal ganglia, and the cerebellum is connected to the motor area, so I guess there are three different regions that are interconnected, and I that makes sense because… I guess the reason that you want to look at the connection is because if there is a disease that affects one area, we should be able to figure out if it affects the other areas that are connected?

AB: Yeah. And how, and the idea is, if you have a disease in the basal ganglia, then the abnormal activity in that area will cause abnormal activity in other areas that it's connected with, or other areas that it's connect with may be able to compensate for deficits, so, there's different things that you can look at.

S: The rest of the question is to discuss, why do we use flat maps? Let's say if you're showing your data to me, and I have very little understanding of the brain anatomy, what do you want me to see? Is it just, hey, look, I have labeling in basal ganglia, or do you want to look at sub-regions in the basal ganglia?

AB: A lot of the reasons for showing these maps is to show that, yes, it's connected to this area. But we also use it a lot to compare between different experiments. So, say I inject two cerebellar regions and one is connected to the motor regions of the basal ganglia cortex and the other is connected to more ??? (5:04) regions. You want to be able to see that difference in the maps that you're showing. And a lot of times that's kind of the main reason for doing these maps.

S: When you're looking at those maps, are you looking for particular sublocations or, I should

say, cytoarchitectonic areas? In the brain, from one animal to the other, it's always this

gray line between the areas, but you can always find out what the motor areas are, right,

they're bigger regions, but how important are the subregions? Like, say, SMA, or PMV,

PMD ?

AB: Well, you can have connections with all of the motor areas or just some motor areas, and

that may provide insights into function, too. So it really depends on the…

S: I guess what I'm trying to get at is, let's say, in one animal, the motor area is not so prominent

anatomically, and in another monkey…

AB: In terms of labeling?

S: Right, in terms of labeling. Well, not in terms of labeling, but anatomically, they're not the

same.

AB: I don't think that happens.

S: You don't think it happens that much?

AB: No.

S: Some landmarks can be very prominent, as opposed to, in other animals, they might not be

that prominent?

AB: Some, yes. So say that –

S: So how do you locate those areas? Let's say the labeling could be the same. Do you always

have a control, and then you have an experimental brain, or do you always have two

animals?

AB: The anatomy, at least in the animals that we use, is more or less consistent, in the cortex,

say, or in the basal ganglia, thalamus. There's a lot of consistency in what we're looking

at. But there are individual differences in sort of size and organization. But I think they're not as …

S: As diverse?

AB: No.

S: But let's say there are individual differences. How do you look at your data? How do you compare?

AB: You can use cytoarchitecture, or just landmarks, so like, sulci, and things that are more consistent than others. So that's what we usually use, the central sulcus and then you know you're in the motor area, and then, the arcuate sulcus, and in front of that there's cognitive areas and behind that there's motor areas.

S: So you're always eyeballing where those areas are?

AB: Yes.

S: There are no defined regions?

AB: No, and one way to look at it is based on seeing connections, too. So, say you're looking at a motor area. So then, when you inject the muscle, you will get labeling in the motor area, so that's how you define the motor area. And then regions that are connected with that, are, say, pre-motor areas. And so that's a way to define the regions, based on connections. Based on previous experiments and so on. But you can't identify all the areas functionally in all your monkeys. I mean, you could, but I don't know how you would do that.

S: So any disadvantage that you have right now – I always hear Richard saying that whenever I'm presenting this data to an M.D., the guy doesn't have any idea about –

AB: The flattened maps. The flattened maps are really hard to explain.

S: Why is it hard?

AB: Because it's not obvious.

S: Is it so you can identify those landmarks.

AB: Well, so there's two reasons. One, I guess, is familiarity with the model. So maybe they're not as familiar with the monkey brain because they don't look at it every day. And the other is that once you stretch things out in a flattened map, it doesn't really look like a brain anymore.

S: So it's distorted?

AB: Yeah. And a lot of M.D.'s and people are used to looking at human imaging data, and that's usually presented on the brain so that they know where it is and how to translate it.

S: I guess we talked about how you draw inference, you look at the anatomical regions and then – ok. Do you think – I guess it's a redundant question – 3-D might help the limitations of 2-D? Do you want to add anything to it? Why do you think if we present the data in 3-D, it will help us?

AB: Well, it'll be more straightforward. It'll be easier for people to recognize –

S: To recognize the brain as a brain.

AB: Yes. So, the only disadvantage that I can see is, you have to interact with it, because otherwise you – if you have a flat map you can see the midline and the outside and the underneath, kind of, even though you have to put them together in your head, but you have to be able to interact with a 3-D structure. Otherwise it obscures some of the data.

S: Right. So the occlusion is a problem. In 2-D all the data is represented all at once, as opposed to, in   3-D you have to have multiple perspective.

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AB: And also, stuff that's buried in the sulci sometimes, depending on how your 3-D presentation is. If most of your data is within sulci and what you're showing is just the surface…

S: I guess in 2-D, what I have seen in Richard's data, again, or any data that we produce, is that, in the 2-D, sometimes you have to open it and present it that way.

AB: And that makes it harder to know what you're looking at, even more.

S: The other problem with 3-D is that, if you have data in different layers. Do you have data in different layers?

AB: I try not to.

S: Because you cannot see it?

AB: Because you can't see it, and also, once you get to, say, if you're looking at outpost from cortex, if you get to the third layer, it can be connections within different cortical – you get into another level of connectivity that is harder to …

S: So for example, Richard was saying that from layer 2 to layer 5, obviously layer 5 is going to have more … more or less? I forget.

AB: Layer 5 is the outpost, so layer 5 always has more. And then layer 3, is basically, based on connections –

S: A projection of layer 2 to layer 3, or the other way?

AB: 3 projects to 5, yeah. So then you'll get, if you have an extra layer you can have layer 3. But then once you have lots of things in layer 3, it can be connections between, not your injection site, but different cortical areas talk to each other too, so it won't be as informative. But it could be, so…

I have a lot of, within brains, basal ganglia data, which are like nuclei. So for them, it would be, we don't have 3-D maps, because we don't do that. But those would be useful, too, because it's hard to…. So if I have a ball structure and then I'm just showing sections through it. It's harder to compare between two animals, because you get rotation, you know what I mean? So if you have a picture of the 3-D structure, it might be more useful to look at and compare.

S: In context of… it'll give you more context – is that what you're saying? That you have the anatomical structure and you have the presentation of the data, and comparing them both it might give you more information and understanding?

AB: Mmm-hmm. Because if you flatten it, it gets really hard to look at. So if you have a C-shaped structure that starts like this, and then it goes up, and then it goes down, so there's a bit more variability. But if you flatten it, then it's hard to tell if you have a rostral-caudal distribution. It's hard to show rostral-caudal and dorsal-ventral distributions if you just flatten it all together. And there can be differences that are hard to show, if you don't have the 3-D… Because with the flattened map you're getting like a maximum intensity plot, kind of thing. And it can look the same if you have lots of cells rostrally, or lots of cells caudally, when you flatten it, it's going to look very similar.

S: All projected to a plane.

AB: Yeah.

S: I guess that concludes with number 10, if you had an ideal world and you wanted to present your data, how would you represent it? I guess your answer would be, in 3-D.

AB: Yeah. Well, I'd like one of those, you know, like a hologram, where you can move it around.

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S: And you can manipulate the data, sort of like in an immersive display technique where you can manipulate the data?

AB: Yeah, and you can take some layers off and you can look at it. That's what would be ideal, but it's not going to happen.

S: What if you were going to take off some data and keep some data? What would be those data that you would take away and visualize? Would it be a different population of labeling?

AB: It would depend on the experiment, really. So say if you had the composite data from many experiments, so different questions, it would be nice to be able to take out part of it.

S: Sort of like a dynamic query.

AB: Yeah. Something like that, I think.

B.2 INTERVIEW 2

S: Let's start.

C: So I guess we'll just start with what I expect to observe.

S: You can say that there's a data set that you are working with, and if you want to add to that, that's fine. What inference do you draw from looking at the data?

C: Okay. Since what I'm doing is a muscle injection, what I'm looking for is the neural substrate that controls that particular muscle. And now I know that, because of the way that rabies infects, it's going to bloom a little bit but we know at some point any of the labeled cells are projecting down to that muscle, that there may be other projections that we're also picking up. So I guess that's kind of what I'm expecting to see.

S: So when you cut open the brain and you slice them, what do you expect to see? Obviously you have a defined region or a target region that you already have in mind. In addition to that, there are other places where the virus might spread?

C: To some extent you have a target region, but you also don't want to go in thinking, the place I'm going to find cells will be here, because you could be wrong. That's why we're doing the work. And, I mean, yeah, if you inject it into the eye, you should really see labeled cells in very specific places along the pathway, but it turns out, when they did the work, they found these other places that had never been picked up before. So that's why you have to do it.

So I try to go in with low expectations, because I don't want to have – at least with the first case. After you've done a few cases, it's really hard to do that because you kind of know what pattern you should see. But at least with the early cases you try to go in with low expectations, if you can.

I: It's almost like a double blind – you don't want to bias your work because otherwise you'll catch your reporting – sorry.

S: So I guess you have, I guess that's what I meant by target region that you already have. But then, if you're showing this data to somebody, what do you expect them to see?

C: For my work, what I want them to see is some of the stuff that hasn't been reported before, because I have cells that are down in the sulcus that, from the previously published physiology, they would say, that shouldn't be there, but it's clearly connected to the muscle, and the physiology was done on the surface, so of course they didn't get anything in the sulcus. They couldn't have found it with the previous techniques. So now we're adding this new technique to find something new.

S: So I guess then the second question is, what kind of representation do you use now?

C: Right. So, for this data, what I'm doing right now is reconstructing the plots into the 2-D flat map. Kind of the standard map that we've been using.

S: Why do you use the flat map?

C: The advantage of using the flattened cortex is that you can compare within an area and across areas and see the whole aerial pattern of projections at once. Whereas, if you're just looking at a single slice, you can see the cells that are on that slice, but you don't know what's going on before or after. So you can see the global picture of where all your labeled cells are. And if you have multiple different things injected, you can see a sense of topography. It's something I used to do a lot of. You'd inject different colors across a whole-body representation, and I could see that all the projections from these four areas are organized, but from this one it's all mixed together. And is there physiology data, is there perception data that explains why you would see those differences between the projection patterns of those areas?

S: So you're sort of generalizing in terms of the global overview of where the labeling is.

C: Yeah. And within that, if you're writing a paper, you're going to have your global overview and then you're going to go and point out your specific things that you think are important. But you just want to show everything, because somebody else may be doing something and be like, wait, there are cells there? That's really cool, and I'm going to drill down on that.

S: So this is another thing that we want to measure, is how detailed – so the cytoarchitecture, there's a gray line from animal to animal, and I guess if we see spread of infection, then how do we identify each sub cytoarchitectonic area? How do you identify those areas?

C: in terms of that for now, I'm using some of the information from my cytos, which are just Nissl stains, and so you can, it's a good way to find the middle, most classical part of your area, but yeah, on the borders it's going to be fuzzy. You can add other types of stains. That's one way to do it. But right now I'm using a lot of cues from my Nissl stained sections, and then also measurements from previously published - Because even though there's a range, you can kind of take that and fit that to your brain and get a good idea. And then if we have something where we're like, well, this is really interesting, we might again go back to the cyto and try to get something more precise.

S: Number five is – we talked about the advantages and disadvantages. What kind of inference do you draw – what I mean by that is, for your data, when you're injecting into the muscle, you expect to see…

C: Cells throughout the cortex, yeah.

S: Okay. What does it tell you?

C: Well, it tells me, you know, what is the network that's projecting to that muscle, and then it gives me interesting nodes within that network. Like, what are the earliest areas that project to that? So then I can go in and inject there, and ask more questions and see if there are loops through subcortical regions. And then, I have cases who went too far, so I'm labeling quite a lot of the brain at that point. But you can see the hotspots – those would be the earliest areas. How many steps do we think we've crossed, so, how many steps, for instance, since I'm doing vocalization, how many steps does it take from the auditory cortex that processes vocalization to get that information to the motor cortex so that you can make a response?

S: Then I move on to the 3-D map, and what do you think – we don't do 3-D maps now in the lab, but I don't know if you've used any previously?

C: I haven't, because I used to just randomly unfold the cortex, so some of the problems were avoided with that. But I can see an advantage to 3-D. When you're actually cutting the brain, and we are, you're – you have to cut the brain in stereotyped ways, and so, you can kind of get different looks to your 2-D map from doing that. So a 3-D, if you can put it all there and you can – okay, I can just rotate this around and I could cut it here or I could cut it here, or I can cut it this way, so I can change how I'm viewing things. Which is really nice.

S: So a different perspective matters.

C: And then also when you have these – something that's on the surface or continues into a sulcus, or, there's whole regions where there's a lot of debate about which three areas are in the sulcus, in 3-D that's going to be a lot easier to understand, because you have parts where it kind of curls back on itself or whatever, so that can be kind of hard to figure out from the sections alone. But when you put them all together –

S: It makes sense, right. So when you have a sense of depth in an actual model of the brain, you can see that the data follows a certain contour.

C: Right. And you can tell the difference between, if a sulcus is shaped like a U, or more like a hockey stick, that could really throw off where you think your cells are in a flat map. But then, when you can actually see that, oh, yes, this is in M1 and it hasn't crossed into 3a yet because 3a is over here, not right here, then that's really helpful.

S: All right. That's important. The next thing that I was wondering is, obviously 2-D versus 3-D, in both types of representation, it's always projected data.

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C: Right.

S: So the obvious question is, in 3-D you have the ability to see the data in layers. But for our purposes, we don't have data in different layers. Or do we?

C: Well, it depends on how far - so I have one case that's my good case that I did the grant on – and I do have cells in layer 3. And now that I have this other case that seems to be earlier, so there are cells in layer 3, but it's like tenfold fewer, if not more than that. So at some point, Peter's going to want me to compare the two layer-3 maps, and see if that gives us a clue where the virus got first. So, having a way to do that layer information in turn - view all your cells, and right now I just have them in two different colors, so, view all your cells all together and then view just your layer 5 and just your layer 3 and then I can do a comparison that helps you tell how far the virus went, and where it got first.

S: But doing it in 3-D, do you think, the only way to do it is some sort of change in transparency – do you think that would add anything to the visualization of it?

C: Do you mean, when you do the projection, they become, angled?

S: Right, everything projects to the surface. One way to do it is to do two different kinds of density and see if there's an overlap. A cluster overlap. That's one way to do it. The other is to keep them separate, have two or three different surface layers, and visualize the density that way. But that doesn't seem as intuitive as –

C: No. Even in a projection – what they're doing now, they get intermingled together. The layer 3 goes on top of the layer 5, and if you hide that…   So that's less of an issue as long as you can independently control what you're seeing and see either both or none, it's fine, it can be useful.

S: So if you had an ideal world where you could represent your data however you want, what would it be?

C: I would be able to have a 3-D map that I can then flatten the way I want to so that it doesn't distort the brain. Because that's the issue with flat maps that are computer-aided, they distort the brain. Whereas, like I said, I used to physically unfold the cortex, and so, the distortions you get there are cuts and tears that you put in, so that you know, at some point the tissue just doesn't have the strength to hold together, it just won't. So you put a cut in and you know exactly what's what, and there's stereotyped ways to do that so that you get the best overall picture of the brain, so that continuous areas are the most continuous.

S: But flattening it would always create distortion, right?

C: It does. Like I said, it's dependent of what distortion. Because the distortion that caret makes when you flatten it are really extreme, and so it can make two things that are supposed to be next to each other look like they're not.

S: Okay. So spatial location is very important.

C: Right, because if you have, say you're going through sequential areas that are next to each other, and all of a sudden this third area is over, seemingly in a different part of the brain, it makes a little bit less sense because then people are going, well, why is that so far? Because there's a bias against long connections. It's a metabolic issue. The longer the connection, the more metabolically expensive, so it seems like evolution has tried to shorten the connection length. So if you all of a sudden see this thing that looks like a really long connection, it's suddenly interesting, when in fact, they're just next to each other. And it's just going stepwise.

S: That's it.

B.3 INTERVIEW 3

RD: "When presented with the data where the brain has been infected with rabies, what do you expect to observe?"

So we want to observe the location of cells and their density, and we'd like to plot them with respect to known cytoarchitectonic areas. And there's even been times when we've plotted relative to, say, a physiological experiment to identify an area ahead of time. Or, it could be post-experiment. With rabies it would probably be ahead of time.

S: This is one question that I had. Those specific areas that we just talked about, architectonic areas, how specific are they? The line between VM1 and V – the paper that you have -

RD: Sure. There's easily a millimeter of uncertainty. If you're in the center of one cytoarchitectonic region and move to the center of another one, you can get a fairly good idea that they're different. But where the exact border is, there's a region of clear uncertainty in transition.

S: Because when I thought of doing the experiment – let's say we would present the data to a potential user and a person who's looking at the data, trying to figure out… What do you want them to see?

You want them to see where the density is and be able to identify that location, right

RD: Right, to be able to identify the location in the quickest manner possible, and, to identify it, but also be able to compare it to work of previous authors. So the comparison – that's part of science, is comparison.

S: That brings me to a different question. We're talking about building knowledge here. Something that you already know, prior to seeing this data. And that affects a lot, that means a lot in terms of discovering what we see. So what do you think is the prior

knowledge that we need for our data? Because often Peter has said, "I want to convey the message of anatomy," and that's not usually enough, because a person, let's say a biology student who has some idea of what the brain looks like and what the areas are. But they're not going to know why we're doing this without the background and the story behind it. So what do you think is the knowledge that we need?

RD: Well, I do think that's a difficult question, because I've gotten to the point that I have such a knowledge base that there are so many things I assume already. And that's what makes it difficult.   People need to know something about the basic structure of the brain. The different regions, the concept that different regions tend to do different things, and that there are parts that are motor, somatosensory, visual areas, auditory areas, and some areas that kind of – they call them association cortex, but they meld various disparate forms of information that come in through separate sensory systems and they're designed to get it out through the motor system. As well as, there's an autonomic nervous system that controls all the internal functions of the body. So that's some of the basic knowledge.

S: Because ultimately, we are trying to understand connection, right?

RD: Yes. Another thing that people do is simply just do a connectome. It's really a matrix of who's connected to whom.

S: How is that different from what we do?

RD: Well, that can actually just - it looks like a checkerboard, and then, it could be a plus or minus for yes or no, or it could some gradation of the strength of the connection. I think they call it "graphical." I could show you a paper of where they've done that.

S: I know from Harvard, Jeff Mitchum, is the guy who did the brain bowl.   But that's anatomically tracing –

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RD: Anatomically tracing, and I think it's mostly in a mouse. And we haven't – and that's because they're able to genetically manipulate the mouse. We can't genetically manipulate the least higher primates. Whether that would be possible in the marmoset, I don't know. But even the marmoset brain is a lot bigger than the mouse brain. But there are possibilities along those lines.

S: When I went to the talk they were literally slicing these brains and reconstructing it through 3-D.

RD: Right.

S: The traced or marked neurons helped them figure out which neurons – how should they connect, one layer to each other layer.

RD: You can look at both the layers. We're not doing a lot of layer-to-layer connections for ours; our is a more macro organization.

S: Right. We're most interested in certain areas of the brain and how they're connected.

RD: Well, we've concentrated on motor areas, but we've done some sensory areas and we keep branching out.

"What type of representation do you use now?" Well, typically we're using flat maps and sometimes we show individual sections. So historically people had – there were various ways of doing it, but some people have plotted it out, so that they would look under the microscope and actually do x-y plotting, which is what we do to get the basic knowledge for each individual section. And then, the way people have reconstruct is, I think they would look at those sections and they would have a schematic of the brain, some average brain that maybe they took a photograph when they took one out, and then they would put dots on that brain, what they thought was the density.

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So that's how location was, it wasn't very precise, density was difficult to represent because it was a density of dots and it was what you saw on a plot of individual sections, to putting the right place on this map, and then getting the density right. And some people then also did other forms of density, where they, well, you can put dots or you can put lines about how much, so you can have this varying-width line. Or you could just plot cells. And I think I gave you one paper where they just plot cells.

But what I find, and I don't really know what the psycho-physical transformation is, but really dense…

What happens, I think is that areas of a few cells tend to be more prominent than they should be, relative to the areas that are really dense. And you can't really represent the level of density that you see.

An example is that people would say they would use a plus system, like, 1 plus – low level, 2 – medium, 3- pretty high, and 4 - the most dense. And my experience of having done quantitative analysis, I think it's more like a squared projection. The two plus's is really 4, and 3 is really 9, and 4 is really 16, times as many cells, as a rough estimate of what was going on. But each person also would be doing their own estimation.

So quantitation has become more important so that you can get some idea of the relative balance between different areas that project, one area to another.

So we've used a flat map, and there are advantages and disadvantages.

One is, when you flatten the map, then it's like flying sort of over the surface and looking down at it directly. So you see what's directly underneath you. Just in comparison to a 3-D map, your perspective is very important to what it looks like, and it looks more like the brain that you see and that's certainly useful if you're trying to go back in and record, or compare to

other – but you don't always get the view that you wanted every time. You might have to turn it in ways that foreshorten some parts and exaggerate other parts, which is one of the things that I'm noticing. And then the flattened map also allows you to do a density – you've collapsed all the cells to a theoretical layer 4 or layer 3 to 5 junction, and then you can count the number of cells along whatever amount of distance you want. 200 microns is what we've often used, but it may be bigger, anywhere from 200 to 500 microns.

It's all how fine-grained you really want your map to be. And then you can say, how many cells were there, and form some kind of representation of the density of what you're visualizing.

S: We also have the same problem with 3-D, right? Because of the surface, we can only see one layer, and it's always the projected one.

Yes, it's always the projected layer. Now, you could leave it in 3-D so that you've looked at it with a semi-transparent surface, but then when you look at it, your perspective becomes even more important. Because when you look through, you'll see a surface one place, and you'll be looking directly at – you'll lose a sense of depth, and I've never found that very useful, in my experience.

S: The last paper that you gave me, or the latest, they had a similar representation where they increased the transparency, and you just see a cloud, and you have no idea which is in the front and which is in the back.

RD: Right, you just see a cloud. And particularly for cortex, I think that's a big problem.

Another problem with 2-D maps is that when you're cutting through a sulcus – so the cingulate sulcus is 90 degrees to the plane of section. So when you fold it, it folds very nicely. But there are some that start to become parallel to your plane of section. And that means that you're getting more cells there, and when you flatten it, it gets in a distorted position. So

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you have to do some kind of transformation, like when we do the central sulcus, sometimes we rotate that so it's perpendicular to the lip of the sulcus, is one way to do it. But then you can't display the whole thing at once.

Every one has ways in which it's blocking some of the information or making it more accessible. So you have to trade off these advantages. And also when you then do something like that – the cingulate sulcus because it's perpendicular or 90 degrees to your plane of section – well, everything stretches out and distorts a bit, but it's all distorted similarly. If you do that on the lateral sulcus, or the lateral surface, there are places where, there's a sulcus and if you go down to the sulcus and flatten that out, that's going to greatly distort that piece of tissue, and then the next one may not have as much sulcus, and then the tissue will have something really big, and then it'll get small, and they'll lose their relationship to each other . There's various ways around it – you can cut out that sulcus and display it somewhere else, but then people are forced to piece things together in their minds. I don't think any way is completely ideal, or solves all these problems.

The 3-D one is also useful in that it is somewhat easier to relate it to the human functional imaging data that is often shown. So that people often do maps where they show things on the surface of the human brain. So that is one way to make it slightly easier for people to go between the two. If they're not familiar – functional imaging data sometimes inflates brains and things like that, and it takes away some of the, all the convolutions of the human brain and makes it simpler, and then it may be easier to correlate between the two different animals.

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So that's one reason why we'd use a 3-D map. But I've already seen – because we're seeing a perspective as you rotate it. If you want to see something in the sulcus, then you have to rotate it and you can't see things on the surface and other places.

So typically we haven't done real fine-layer data on our brains. It's hard to do just even when you're looking at the cytoarchitecture alone, just to decide where the layers are. And when you're actually plotting it, it's possible to do what we would call super-granular, the dorsal half vs the ventral half. I think that's do-able, and I have done that sometimes in the plotting. You could compare what the two are in separate maps, side by side. But I haven't really figured out a way to place that in 3-D. I probably could think of some ways. It probably requires some kind of change in programming a little bit, but you say oh, I want to make one on top of the other, like you do a density of the deep stuff, and then maybe a density of the superficial stuff, so that you could say, well, look, some places have superficial, and other places only have deep.

S: Sort of like an overlapping –

RD: Yes, sort of like an overlapping. But then it would give you the much the same information that a density map would do also. They would probably correlate very well, because if you have cells - when you have cells of the superficial layers, they often are because they're projecting to the deeper layers. It's the typical columnal organization of the cortex. So the superficial layers project to the deeper layers, and then, because you have this stack of cells you're going to have a higher density. Simply because they're all through all layers. If they're mostly in the output layer, which is layer 5, then they're going to be thinner, because it's not as thick a layer, there are just fewer of those cells.

So as the infection progressed, you're going to get these vertical columns appearing, and of course they're denser. It makes lots of sense.

So really, changing to the different layers probably may give a little additional information but I'm not sure what the usefulness is at this point.

The other thing is, to do it truly properly, you have to have the cytoarchitecture at the same time.

You have to figure how to designate those cells at that time. Or we'd have to come back in and do some cytoarchitecture put that in the next section and then group those cells and rename them. And it'd be really laborious, and you'd have to say, what is the purpose of this laborious work. Is it going to give me some information that I really need to know, or is it going to be somewhat… Is the effort really going to be worth the gain? I'm not sure that it is.

So, we talked about the different layers. We talked somewhat about the advantages and disadvantages of the 2-D. And we talked a little about the complexity of the data. There's really no ideal way to show the data. I suppose the ideal way is that someday it will be, for instance, if it's a 3-D, you would actually just have encapsulated the 3-D image there that the person could rotate on their own.

S: So interaction or perspective or the way of looking at the data is important in 3-D?

RD: Yes.

S: Whereas, in 2-D, the lack interaction is, it's an advantage -

RD: It's an advantage for flat print.

S: And in terms of understanding the knowledge, that's something that maybe we can measure. Does it really work.

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RD: So it works in flat print but it does create a somewhat distorted map, and there's also the

   carat method of creating a flat map, and that causes a different kind of distortion. I don't

   know if it's greater or less. Some things don't stay in true relationship to each other and

   it's very complicated to look at. I'm sure that people who do Carat all the time say, oh,

   yeah, I know this. But when I look at it, it takes me a long time to do it. I look at my flat

   map and of course I've been living with it so it's very easy for me to do it. But, when you

   show it to someone else, sometimes….

S: There's a learning curve.

RD: There's a learning curve.

S: And we need to minimize that

RD: So I think the advantage of 3-D is theoretically it has the lowest learning curve, because it

   looks the most like the whole brain that's somebody's going to show in a picture. So that

   would be the theoretical thing. Except for the disadvantage of that it's not always – you

   can't have it in interactive form on the flat page.

S: Yeah, that kind of summarizes…

RD: … What you're thinking. And so part of is hopefully that making the 3-D brain makes it

   more accessible to more people, so they don't have to go into the details of understanding

   how a flat map has been constructed.

S: If we wanted to show details of a flat map versus details on a 3-D map, what would be that

   detail? I'm just thinking out loud here. The detail information is that in 3-D, I don't know

   what's behind the surface, so that's one detail that we're missing unless we move it, rotate

   it, figure it out, but in flat map, it's instantly available, but it's distorted. So…

RD: So one advantage that I have noticed with the 3-D map is, say, the arcuate sulcus is a problem, because it sits like this, so you're cutting it very tangentially. And so if there are cells all along this face, when you come here, you're going to get a very high density of cells. And the way we cut things, well, that might actually project up onto the surface for a 2-D map.

And some of them might show up in the sulcus, but it won't be an accurate representation of their density. Because you're taking this flat surface and now you put it like this and then you get all these extra cells in it. So you're gathering the concept there. And therefore, you're projecting a flat surface, the end of a flat surface, up to a single location. And the nice thing about your 3-D program is that then it has a surface out there, it projects to the closest surface, not to an artificial surface. It's created one, it's supposed to be. And therefore, it's a more accurate representation of density in location.

Really, I do not think there's any adequate way to convey it in a flat map. And it's just a matter because, you cannot cut, you want to cut different parts of the brain at different angles. And then on the flat map you just can't piece them all together. But there's no way to do that, but you can take all these slices and now you can reconstruct the brain. So it is one of the nice things that I enjoyed about using the 3-D program. It placed them in the proper perspective.

B.4   INTERVIEW 4

I: "With rabies virus as a neural marker, what do you expect to observe?"

S: Right, that's question number one. Yes.

I: I'm expecting to see infection. And what is infection, is infected neuron. I'm expecting also, linked to that, an organizational level. So organization, distribution of labeling, and eventually density of labeling, difference of density from one area of the cortex to the other. So there is topography, and there is a density. Make sense?

S: Yeah.

I: "What type of representation do you use now?" So there is different kind of representation. There is what we call, the section by section representation, so those are raw data, r.a.w. .

And the other representation is, what I've been spending a month in doing, is flat map, ok? So you want to represent all the regions of the cortex, and you have the flat map, the 2-D, and eventually the 3-D.

"Do you use flattened map?" yes. It gives you a sense of region and density in one part of the cortex. Whatever you choose.

"What are the advantage and disadvantage of 2-D flat map?"

Advantage is to show the general organization and density of your labeling.

Disadvantage is, if you're not a neuroanatomist, it's very difficult to figure out where you are at the level of the cortex or wherever. I'm working at the level of the cortex. But it's really difficult to nail down exactly the region you are situated. And what I was telling you before, we really want 3-D, because we want our friend, physiologist or whatever, to put their electrode there and get to the next step, into the interpretation and analysis of data.

"What inference do you go from your" - you make interpretation.

S: So when you see density in a certain location, what does it tell you?

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I: It tells me this region in particular is connected to the region I injected. After multi-synaptically connected neuron is what is sensed first. This region of the brain is connected to where I inject.

S: What if there are multiple activation?

I: So, multiple activation means they are connected to my injection site. Now, the next step to understand is, is there an order into those sites, that counting, labeling. Meaning, between all those guys, those regions, is there a region connected before or earlier than the other regions?

So it's for this that we reach the level of maybe 5 first, so when you see labelling of maybe two and three, supra, I mean, as what I was saying -

S: So these are time dependent?

I: That's right. It's like an order, a neuronal order. Time dependent is how the virus is working. But the first thing that comes into your mind is, all those regions are connected to your injection site. Then you work to a different level of interpretation. Which one comes before the other.

And to know that, is what I tell you, is the difference of a layer in which the labeling is. But also, you're using all the prior literature that we're using just mono-synaptic tracer and told you, this region is connected to that region, this part is connected to that part, so is an ensemble, not only of the virus that I'm injecting into the layer, but also what we have in the literature, what we know from prior work.

So you conjugate both of those and you have your answer.

"Would using 3-D map help overcome the limitation?"? Definitely. There is a huge limitation. When I see the 3-D of the brain, I know exactly where I am. So it's, attach your data to something most of the people in the world know.

S: It's more recognizable.

I: Recognizable. I know how look a brain. A flat map is really tough. Now you can use your 3-D and from the 3-D get to the 2-D to have that micro-organization. So you would not use the same organ.

You want first your audience to understand where is the labeling and what is connected to the injection site and so on. The versatility of the 2-D map can answer other questions.

Microorganization

"Why would you use 3-D maps" so we have discussed about this, the recognition is the same.

"Do you have data at different layers?" Of course.

"How do you represent this data?" So what we saw is, there is different way of doing this. Either you say, ok, I'm going to separate my layers in my different representations, and layer 5 being that first stage, and then 2, 3, and 6 is like one step further.

Or, you have the way of using the raw data that you present in different color, your different layers.

So let's imagine black will be layer 5, and then 2, 3, 6 in red. Because you're expecting to have much less labeling in 2, 3, and 6, since they are additional order of labeling than your layer 5.

So you can do that, and you can represent them in density. If you do it in density, you need to separate your data.

There is another way you can represent it. When you want to demonstrate there is columnar organization in the cortex, you use overlap. So you set a threshold or a cutoff. Meaning, I want to represent just 75% of my population of cells. So you cut off 25%.

And I'm going to represent, for example, here, in my case, the 75% of the eye in blue, and the 75% of the neck in yellow.

And what is overlapping - or even eye, 2, 3, and eye 5 in different color, and what is overlapping can demonstrate either overlap between two different systems or whether there is a columnar organization between the different layers of labeling.

So it depends on the question you want to answer, but layers, either it's raw data, different color of cells, or you want density, you separate your brain.

S: How do you think 3-D would help in understanding this columnar distribution?

I: I don't think it would bring more to the 2-D, it's just always having a model that is more recognizable. Effectively, when we discussed last time we were thinking of opacity and changing maybe the opacity of the cortex, so that you can see transparency. Things are, it is very difficult to put this on paper or in a publication. Unless you have really an interactive brain and you are turning around…

The 2-D map according to me is better in this respect, because you're really flattening things and seeing if they can respond. So you don't have to play with the 3-D, or with the volume.

I: Question nine - "What type of complexity in the data prevent you from conveying your message across?"

S: Are there complexity that you run into that you cannot represent your data the way you present it now?

I: I don't see any for now.

S: Ok. So, anything that you produce are instantly represented -

I: Yes, there is a complexity, sorry about this. I see one. Did you see when I tried to represent the gyrus and the sulcus at the same time, ok? So I need to clean up some part of the map I don't want so I can plug in or I can approximate my flat map that is in the sulci. So there is no sense really of depth, or whatever, and it can be very tough to really imagine that curve and how is the labeling, you know?

S: If the density or the data follows a certain curve, why is it important?

I: It's not systematically that it is important, what I'm telling you is that the neuron of this region that follow each other from gyrus to – are part of the same system. A part of labeling, they arrive at the same time there. They may be a functional unit within themselves. So they may give you a sense of boundaries within your cortex. Or it can be, M1, and M1 what we know about it, there is a part that is the pre-central gyrus, but there is a part also in the gyrus, in the anterior, back, and here we said, huh, it's a unit, it's M1. So it can also help you in drawing those boundaries, and then, instead of saying, well, there is this part at the surface, and this part, you see what I mean? So it's much more in terms of functionality.

"What would be the most ideal way to represent your data?" I think there is good and bad in 2-D and 3-D. A combination of both, plus, I didn't insist enough about this, I told you about section, showing row data on a section, because it's what neuroanatomy is, first off, everything, we re-construct from individual sections. So it's good to go back to the roots of the problem.

Just to show the rawest form of the data?

That's right. And then I give it a black dot of a certain diameter, and it would represent a square 200x200 micrometer. So, it's not just a surface, it's not just a density. It's also a

representation of, where is my cells? Basically, what would be most ideal would be everything converged. Everything is good; it depends on the message you want to convey.

B.5   INTERVIEW 5

S: I'll let you start with the first question, I guess. For all our data, everybody I've asked, we all pretty much use some kind of tracer. It doesn't necessarily have to be rabies, it can be any tracer. So for your data, what do you expect to see when you inject an animal with the tracer and you're looking at the brain? What do you see or what do you expect to see? The other question would be, what do you want the potential observer to see?

M: So we want to see what regions in the brain contain labeled neurons. And that tells me what regions are connected to my injection site.

S: So what kind of representation do you use now?

M: So I use multiple 2-D flattened maps, and the reason I use multiple flattened maps is because my data spans the whole brain, and you can't see all sides of the brain at once. I have data on the medial surface, the lateral surface, the dorsal and ventral surfaces of the brain. And so, in order to view those in 2-D, I have to, the best way to do it is to break it down into understandable chunks and then flatten those chunks.

S: I guess one thing I ran into when I was talking to Mike yesterday is, 2-D shows you everything at once. So why do you want to do 3-D? Or do you want to do 3-D? If 2-D is showing the data you want to show, then why do you even bother to show 3-D?

M: When you flatten the brain, you lose information. When you flatten the brain, you stretch certain parts, you make it more difficult to recognize that, to recognize the brain, but a

picture in 3-D is easier to recognize as a brain, and you can better identify the locations within the brain that are labeled.

S: So I guess that answers the question of what are the advantages and disadvantages of 2-D flat maps. The advantage would be to see everything all at once, the disadvantage would be that the brain is kind of deformed, it doesn't have the canonical form of the brain. And we don't know which part is which.

M: And what happens is that each lab kind of develops their own version of flattening, and that makes it hard to compare your data to others' data. That's another disadvantage of the 2-D map.

S: So now, about the inference that you make from the 2-D map – what do you observe when you look at density against some anatomical region in the 2-D map, what inference do you draw?

M: So, I can tell which regions of the brain contain the most labeled neurons. In other words, which regions of the brain have the densest projection to my injection site. And I know where those densely-labeled regions are, because when I look at my 2-D map, I can see landmarks that are common across brains, like particular sulci or gyri will show up in the flattened map, and those are my landmarks that will help me say, oh look, here is the primary motor cortex. I know it's the primary motor cortex because it's this far away from this sulcus and this far away from this sulcus. So it's density and location.

S: Together.

M: Mmm-hmm.

S: Okay. So number six is, would using 3-D map help overcome these limitations, do you think?

M: Yeah, absolutely.

S: How?

M: The 3-D map can look like a brain. And that's a universal image that's understood by brain researchers, at least for a particular animal. Even if it's across species, all brains, or many brains, look a little bit similar. So I can understand more looking at a 3-D representation of a mouse brain than I would be able to take away from looking at a 2-D representation of a mouse brain, even though I don't study the mouse.

S: What about landmarks, like certain areas of the brain that are – maybe certain features of the brain, across animals, the same species, may be different?

M: So you're talking about individual differences?

S: Individual differences, yes.

M: So the way to get around that would be to have some kind of an average brain, such as the brain that they use for MRI data analysis.

S: So, now let's talk about the important stuff, which is, I was talking to Mike again yesterday and he was saying that - I thought of the idea of using the 3-D brain because there are multiple layers of data and we're visualizing. The 2-D doesn't allow you to see multiple unless there's as Venn Diagram kind of deal, where you see only overlaps. But the moment you, even if you do it in 2-D and if you overlay information, and you have some sort of transparency set up, then that becomes a 3-D, and it has the same 3-D visualization problem that an actual 3-D representation has. So how do you – well, first of all, do you have information in different layers?

M: I have labeling on the surface of the cortex, I have labeling inside sulci, which are generally below the surface, and I also have labeling in deep brain regions that don't show up on a -

that aren't usually mapped at all, they have their own special maps. So I wouldn't show
those with the cortex, which is most of my other data.

S: So how do you visualize them now?

M: I haven't analyzed them, but if I was to analyze them using the techniques that we have now,
I would create a whole new type of map that showed the sections in a different way. And
I'd have to learn a whole new way of looking at that particular part of the brain.

S: So essentially you would create a 2-D map of each individual area of interest?

M: Yes. That's the best we can do right now.

S: Because one thing that Jean-Alban was saying that right now, the problem with 2-D is, a. for
cortex, which is relatively simple in terms of anatomical structure, if you flatten it the
distortion is minimal. However, there are complex structures that cannot be – what we do
is, we do region by region to minimize the distortion. So I guess the same thing applies in
your case.

M: Yes, the same thing would.

S: So that was the answer to number nine. So if you had an ideal world, how would you present
your data?

M: So the best way to show my data would be in like a video format, where all of the cortical
neurons were labeled and you could spin the brain around so that you could see the
different regions that are labeled. And to show the deep structures, then I would want to be
able to poof away the cortex and leave the core, deep-brain structures, make them
transparent and be able to spin them around and show where the labeling is. And, at the
same time, I'd want to have a second brain doing the same thing so that you can see across

animals or across two different types of experiments, how the labeling differs between animals.

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S: So going to the first question…

JA: So basically when we do this type of experiment, we inject the virus somewhere, and the virus is neurotropic – meaning that it grows and is transported in neurons, or nervous system cells, and because of that property, and because it grows in them, replicates so the signals amplify, we can look at regions that contain the virus and that are connected in some ways with the site of injection.

So what we expect to observe when we do these types of experiments are the regions in the brain that are connected through the known or specified number of synapses with the place you injected.

So, for example, you inject a limb muscle, you can look at the region of the brain that are connected with that muscle, and therefore that can control the action of that muscle.

"What type of representation do we use now?" Mostly 2 representation. The 2 derives from processing the tissue. So, we extract brain tissue and we cut it in sections, we slice it, and we keep the slices in order. That's how we know where they're placed in the brain or the block that we cut. And one representation is just through the section that you cut, so you look at a microscope and you look through the section, and you look for the neurons that contain the rabies virus, so, there's a way to show that, and we can see sections and location of neurons.

But then, from this, we might want to have a better idea of the 3-D spread of populations of

neurons, and therefore we need to look at multiple sections that have neurons, and

understand where these neurons are located in the 3-D space of the brain.

So one way to do that, and that addresses question 3, is to use a flattened map. That's one way.

But really, I think the main constraint of how we're going to represent this 3-D volume

depends on which structure you look at. So, the cortex, a good way to represent it is a flat

surface, because the cortex is a sheet of tissue in rodents, in some animals, it's a sheet of

tissue that's relatively flat, that covers the brain. So, it's not flat, but it's not folded, at

least. It's kind of smoother along the brain. Which makes a representation, a flattened

reconstruction, relatively easy. But in higher monkeys, the cortex is folded, and it makes

the flattened unfolding a little bit trickier. But because it's a sheet of tissue, it's a valid way

of representing. If you were to look inside a nucleus, for instance, which could be like a

sphere or an ovoid or something, more like a ball, distorted or not, but more like a ball,

then maybe a flattened reconstruction would not be appropriate and you would need to

devise a different way to represent.

So usually that's when we use a cross-section and just pure drawing. That might be a way we can

show a different level. So we show three or four sections to convey that. That's one way of

showing it. But maybe it might be interesting to create a 3-D model of that region.

"What are the advantages and disadvantages of the 2-D flat map?"

So the main advantage is, it's the same space as a sheet of paper, so it enables us to print or

publish in regular papers, the maps, and, again, I insist, if you were to do a flat map of a rat

brain, there are limited distortions because it's a relatively flat surface, so, although it's not

quite equal, it's very similar to the actual brain to do a flat map of the rat brain.

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The disadvantage is that the more unfolding you do to flatten, the more distortion you create, and then you enter into the whole problematic of people making a geographical map – how do you represent a 3-D volume on a sheet of paper?

"What inference do you draw from 2-D flat maps?" I'd say multiple, but ….

S: So, for instance, are people looking at – obviously they're looking at densities, right? So there's a region, it's a topographic map. There are certain regions in the brain, and we want to see where the density is. So, I guess, location, or the subsection of the brain, are important, and where the density, compared to that section is important.

JA: So a few things that I've looked at – on the map, we can represent the location of the borders between different regions, and so, you can look at the first of all the spread of labelling in a particular region, but also, as you said, the actual density or the relative number, as compared to another region in the same animal. So that's one thing, yes.

So the spread of the whole population is something that you might want to look at. If the population makes one blob, or one peak, or several peaks, is another thing you might want to look at. You can color-code your labeling on a map or a 2-D map and get a very quick visual of this relative density in the different regions as well. And then, another idea that just pops into my mind, is that you can make your individual maps fit a model of a map and try to overlap the data from different animals and see if labeling from two different injection sites are in the same region or in different regions.

S: But why would you want to fit it to a model? Is it because the anatomy is different across animals?

JA: So, as you move across the phylogenetical line, yes, the differences between animals will increase. So, in rats, for instance, from a rat to another rat it's very consistent, the shape

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and size and location of the different regions in these animals are very similar. In a monkey, it's another story. The folding of the brain follows general guidelines, but the exact shape of each fold can vary (10:46) ????

Some little dimples in the brain are very pronounced, very well-marked in some animals, and others don't have them. But the type of inference that we can draw from a 2-D flat map is, comparison across animals as well, yes. So intra-animal comparison, but also, across-animal comparison.

"Would using a 3-D map help overcome [limitations], and why would you use a 3-D map?" So, I said a few things about it. But I think the problem we're working on together can be touched here – one problem when cutting a brain, in particular a monkey brain, is you have to select a plane of section, and that plane of section will be optimal to make a 2-D reconstruction of a particular region, but might make a different region of the brain not optimal at all to make a 2-D reconstruction, just because the plane of section, the way it's cut through that region, makes it very difficult to reconstruct and know where you are in the brain. You can lose your references. To explain to a child that, I would like to, I don't know if I can make an image, but -

I don't know, if you cut a banana and want to make slices, you can cut the banana to make round slices or you can make oval slices or you can make a band of bananas. And if you have bands of bananas, figuring out, without knowing the banana at all, that, if you were to cut a different direction, you would get circles. It becomes something really difficult to represent in your brain. So, making the circles appear from bands of bananas is tricky. So that's kind of similar, problematic. So by making a 3-D reconstruction, the hope is that you can erase some of the distortion due to cutting and due to flattening, when you do 2-D

maps, and therefore regain something that's not entirely lost, but regain a vision of where labeling in an outside region of your first area of interest is coded in the brain.

S: This is a question that I run into. The other purpose of doing 3-D, other than anatomy, is that if you have labeling in different layers – so, for example, if this is a surface, you have labeling here and you have labeling up top. Obviously in 3-D, you don't see the labeling at the bottom.

JA: You're perfectly right. Making a 3-D map, you lose one dimension.

S: And it's the same with 2-D. 2-D would be, either you do supra or you do infra, and then you compare side by side.

JA: And that's what I'm saying, with 2-D you lose a dimension. You lose the z-dimension. And so yes, the cortex is a sheet of tissue but it's layered, and so, as you said, if you want to know, if your labeling in that sheet of tissue in a certain layer, in a 2-D map you have to trick the system. So, for instance you can color-code cells that are in a certain layer, a different color for a different layer, and then you can kind of represent your three layers on that same map. But still you lose the dimension – ok, it's in that layer, but is there a dip in that layer, or is it on the surface in that layer?

S: Is that important?

JA: It could be. It depends what you're looking at. But to some extent you shrink that z-dimension. And so, if the essence of the data you want to present or talk about is in that z-dimension, than the flat map is no longer ideal. You could use a cross-section. The cross-section will lose another dimension, but will show you the dimension. So it's not necessarily impossible, just with cross-section and 2-D, to show something about that z-

dimension. But if you want to do similar things that you are doing with the map, then you can't.

S: So then question number 9 was, how complex is your data?

JA: So, I think that touches on something I've wanted to talk about, or is implicit, but when you do 2-D reconstruction on a monkey brain, because of the unfolding that you have to do and the distortion that it creates, you basically have to do small region by small region of cortex, and kind of a mosaic of – you can't unflatten the whole monkey brain, because at the edges of your map, you don't know what you're looking at, it's too distorted, it's too weird. And a 2-D map, you can unfold the sheet of cortex, but then you lose whatever is inside the brain, the deeper structures, so if you want to show that, that's a different figure, that's a different type of labeling, that's a different reconstruction, so one of the main advantage of a 3-D representation is to show everything at once, if possible, if you can see that 3-D model of the brain, that you can zoom in if you want to see a particular region, you can rotate to appreciate the distribution, you can see everything in one same space.

S: You can make a mental model by looking at all of these locations simultaneously.

JA: Right. So you have everything there at once.

S: Which you still do in 2-D, but it's kind of distorted, or sometimes not possible because you said you have to do smaller regions.

JA: Right. In 2-D you can't have that everything together. So that's one complexity that 3-D will help appreciate, is what's the big picture, what's the general thing. And the other part is, because of the 2-D unfolding and the fact that you have to plot things together, regions that have no to very little cells, you tend to ignore, you don't show them, there is nothing in there. And so, one thing that you would appreciate in a 3-D reconstruction is maybe how

localized the labeling is, in the brain, because everything is there and there is nothing in another region for that injection. Or, in that half of the brain, it's scattered all over, but in the other half there's nothing, or something like that. So you would also appreciate the places that don't have labeling.

So what would be the most ideal way to represent your data? My answer is, there is not one ideal way. The question to me is, the key word is "represent." So when you want to represent data, it already is implicit that you're making a choice, that you no longer show the real data, which is the section or which is the brain tissue that you have. You are already transforming that real data in a representation. And a representation is biased towards whatever it is that you want to say. And so depending on what it is you want to say, depending on what you feel is appropriate, you want to say, and depending on the media, the origins, any type of parameters, it will modify your representation. And so the idea is to find the best one, the one that you think is the best, to convey what it is you want to say, but there is not one solution for that.

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