3D Printing a Data Driven Model of the Drosophila Brain

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Abstract

The structural anatomy of the *Drosophila* brain is complex with many non-overlapping and clearly defined regions. Traditional visualisation tools usually involve computer modelling and fail to actively engage the sense of touch. This project created a physical model of the fly brain with 54 individual parts which can be taken apart and reassembled. Results indicate the tactile engagement is very useful and particularly as a learning tool. Constructing the model was limited by the computational capacity of design software and conversion of raw volumetric data to surface meshes. Furthermore, the 3D printers used limited the model to a resolution of 0.2 mm but provided much greater financial freedom than would be possible with a higher resolution 3D printer. This project lays groundwork for combining 3D printing with *Drosophila* bioinformatics and suggests further applications in areas such as interspecies comparison of insect brain regions and integrating neural connectivity data.

Acknowledgements

Many thanks to my supervisor Professor Douglas Armstrong for all his help, Dr Rob Court for his assistance, and Dr Adam Stokes for his collaboration, and access to his Fabrication Lab for 3D printing. A big thank you to Dr Tom Barraclough for all his help 3D printing the parts for this fly brain model. Thanks also to Andy Jarman, Barry Denholm, Manuela Marescotti, Petra zur Lage and Valentina Ferlito for their participation in evaluating the model.

Finally, thanks go to my friends and family for their support.

Declaration

I declare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification except as specified.

(*Bruce Robert Muller*)

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Chapter 1

Introduction

Drosophila is a genus of fruit fly which many researchers use as a model organism for their research. There exists a vast amount of neural, genetic and structural data related to the *Drosophila* brain [7, 22, 24, 40, 25]. It is important to keep creating tools for visualising fly brain data, in order to help researchers new to the brain learn the complex structure, and for experienced researchers to better understand the data they are working with [25, 28]. Scientific knowledge is sometimes added to by combing two seemingly disparate areas, and the aim of this project is to combine structural fly brain data with the well known process of 3D printing [25].

The structural anatomy of the fly brain is particularly complex, and consists of 41 individual and non-overlapping regions, which can be viewed on computer models as two dimensional slices (see Figure 2.1) [22]. Each of these regions have been uniquely named and formed into an ontological tree structure consisting of regions and subregions [22, 15].

3D printing is the general process of fabricating an object in a layer by layer fashion [18, 25]. Many different techniques exist and some of these are briefly discussed in Section 3.7.1. Once solely used within the realms of industry and research laboratories, 3D printing has now become widely used by both organisations and hobbyists alike, with affordable machines being used for printing anything from food to self-replicating robots [18].

The goal of this project is to create a physical model of the fly brain using 3D printing technology. We hypothesise that a 3D printed model of the fly brain would enable a useful tool for researchers, compared to a purely visual computer model; engaging the sense of touch [28, 21].

Our motivation is broken down into:

- Investigating the viability of combining fly brain anatomical data with 3D printing to create a physical model.
- Discovering problems and limitations of creating such a model within the time frame of an MSc project.
- Evaluating the usefulness of a model to *Drosophila* researchers.

The result of this work produced a closefitting model consisting of 54 separate parts as illustrated in Figures 3.21, 3.24 and 3.25, which was presented to six *Drosophila* researchers in a qualitative evaluation. Participants found the model engaging and increased their awareness of fly brain anatomical complexity. Further, most participants commented that the tactile engagement would be useful to aid learning, particularly for students new to fly brain anatomy. Compared to viewing anatomy via two or three dimensional computer models, results strongly suggest a physical model would provide a platform for faster and more accurate learning of fly brain anatomy.

The final model shows clear feasibility of creating data driven models of *Drosophila* bioinformatics. Limitations exist in the computational modelling process and physical implementation. Firstly, converting the original volumetric data to surface meshes which can be additively fabricated is imperfect, and we suggest future work on reducing or entirely eliminating this error (see Section 3.1). Secondly, the detail to which we can create a model which requires preprocessing is limited by how much detail our design software is capable of computationally handling. Thirdly, the model is limited by the 0.2 mm resolution of the printers used for this project.

In Chapter 2 we provide background on the fly brain, 3D printing, design software, outline a few example applications, and convey the general design vision for the project. Chapter 3 details the work undertaken in creating a regional fly brain model. The qualitative evaluation of the model is presented in Chapter 4 with an outline of limitations encountered, and we finish with some concluding remarks in Chapter 5.

Chapter 2

Background

This chapter provides background information for the work undertaken and evaluation of creating a 3D printed model of the fly brain. Section 2.1 provides some detail on the fly brain and its complexites. Section 2.2 discusses examples of where 3D printing has previously been used to create models useful for certain applications. Section 2.3 introduces additive fabrication with its various methods. Section 2.4 introduces the design vision for this thesis, of constructing a physical model of the fly brain.

It should be noted that significant portions of this chapter references work from the Informatics Research Proposal which led up to proposing the project described by this thesis and is referenced where appropriate [25].

2.1 The Fly Brain

There exists comprehensive data for the structural composition of the *Drosophila* brain which is freely available online using the Virtual Fly Brain (VFB) data hub [22]. Figure 2.1 illustrates a 2D slice through the fly brain where you can see the various regions as depicted by the VFB ontology viewer [22]. Each of these of these regions is commonly referred to as a neuropil, where each region has clearly defined boundaries (non-overlapping) and unique nomenclature [15].

The data used for this project is split into 41 separate regions where 29 of these consists of two bodies, symmetrical about the centre of the brain (e.g. the left and right Optical Lobe regions); and 12 consist of single central bodies spanning the central midplane [22]. Although, the fly brain is symmetrical in general, it should be noted that there exist slight variations in structure from the left and right half of the brain, as our data originates from a real fly.

These data files represent the structural data of the fly brain in terms of 2D slices which combine together to form volumes. The data itself was recorded by Arnim Jenett (Janelia Research Campus), Kazunori Shinomiya and Kei Ito (Tokyo University) [22]. The recording is of an adult female brain which used a confocal scanning microscope and a synaptic marker nc82 [22, 27].

Altogether these regions contain roughly 100,000 neurons, of which about 16,000 have been mapped and recorded (available via the VFB database) [7]. It is worth noting that while these neurons project into the mentioned neuropil domains, the neuron cell bodies are located within an external structure surrounding the brain surface [7]. Figure 2.2 illustrates an example neuronal projections and their structure, accessed from the VFB hub.

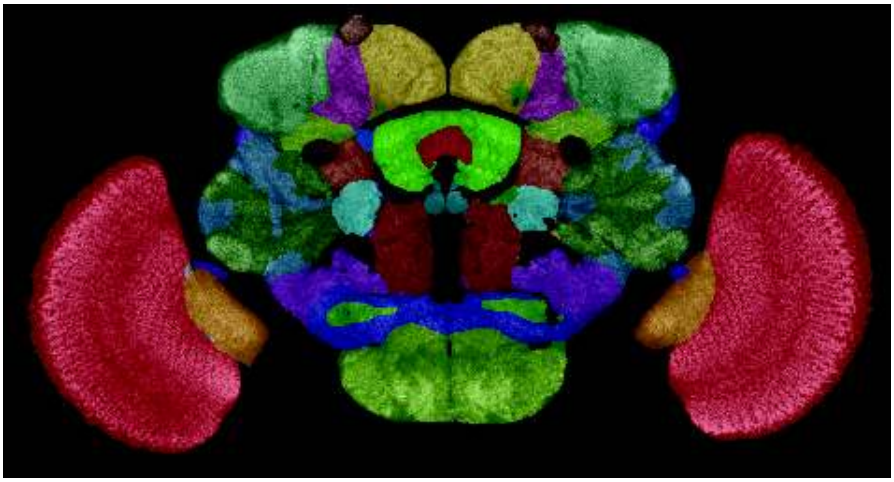

Figure 2.1: This image illustrates a 2D slice of the *Drosophila* brain where various regions are depicted with different colours (taken from the VFB ontology viewer [22]). For example, the light red represnts the Medulla of the the left and right Optical Lobes, and the light green at the centre of the slice represents the Fan Shaped Body.

Using the VFB ontology viewer we can view detailed information concerning each of the 41 major brain regions, such as:

- Neurons which pass through the region in question.
- Neurons with synaptic terminals in that region.
- Nerves which innervate regions.
- Search by clonal unit or gene expression in a region.
- Synonyms and relationships with other regions or neuropil domains.

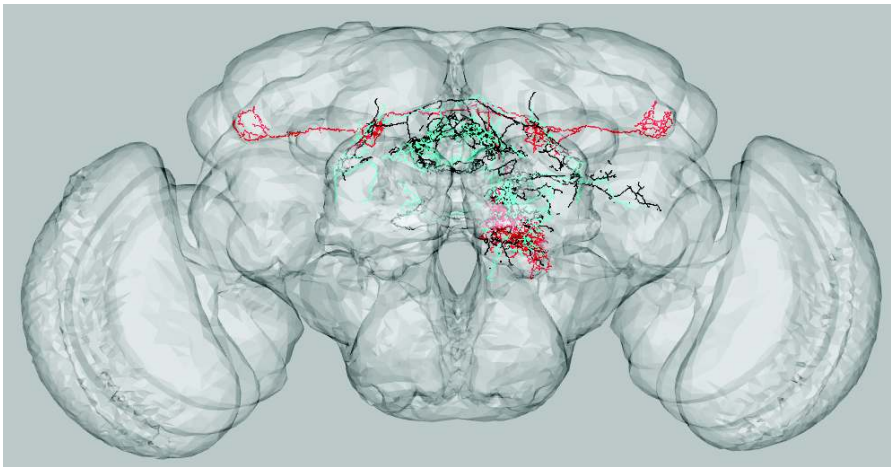

Figure 2.2: Neural data is available from the VFB hub and many different neurons can be displayed with the various regions they innervate [22]. Each colour in this image represents an individual neuron.

One of the most studied brain regions in the fly is the Mushroom Body, which consists of 4 subregions: the Medial Lobe, Pedunculus, Calyx and Vertical Lobe (see Figures 3.10 and 3.15). Another well studied section is the Central Complex, consisting of: the Ellipsoid Body, Fan Shaped Body, Nodulus and Protocerebral Bridge. Also worth noting are the two Optical Lobes consisting of: the Medulla and Accessory Medulla, Lobula Plate, and Lobula (see Figures 3.10 and 3.15).

Previous work shows that the complexity of the neural interconnections between regions makes it harder to understand the processes leading to their development [40]. The Mushroom Body and Central Complex are well studied regions, where the surrounding neuropils are seen largely to be unorganised in comparison [16]. Creating a physical model of the fly brain aims at making the structural anatomy clearer to researchers, focusing attention on less studied areas, and laying the ground work for potentially more detailed models where neural connectivity information can be integrated.

The fly brain contains complicatedly shaped regions and interfaces between them; a physical model can help researchers not only learn the anatomy, but formulate future research directions, and note features which may not be obvious from a computer model. It is worth stressing that the data used in this project comes from a real individual fly brain, and is not, like in some teaching aids, a fabricated dataset. As we will see in Section 2.2, there are examples of using 3D printed models in aiding both education of complex anatomy and research of other areas.

2.2 Applications of a Physical Model

This section outlines some example applications of 3D printing to physical models, which can often be split into outreach, education and research uses.

Often 3D printed models can be used as a means of raising funds, and engaging the interest of people such as young students at schools and outreach events [33, 25]. Figure 2.3 (left) illustrates a 3D printed model of Mars where many surface features are integrated [14, 25]. Capabilities of 3D printing include creating intricate and eye-catching shapes with multiple colours, which makes it an attractive option for outreach to the general public or for showcasing spatially complex parts of research. For example, Figure 2.3 (right) illustrates a model created from a mathematical algorithm by Professor Neri Oxman [18, 25].

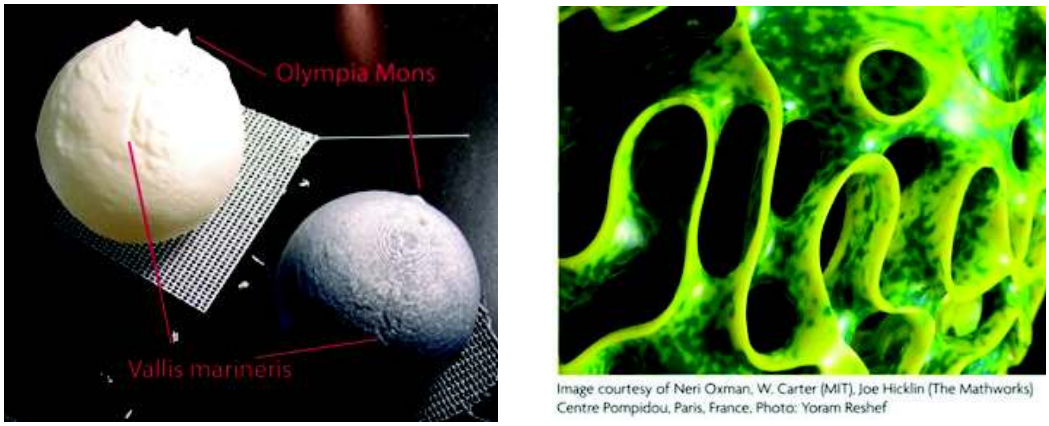

Figure 2.3: Left: This 3D printed model of Mars illustrates the capacity of 3D printing to make eye catching displays with salient details (taken from Horowitz *et al.* 2014 [14, 25]). Right: 3D printing can also be used to fabricate mathematically defined shapes which would be extremely hard to create with traditional methods of fabrication (taken from Lipson *et al.* 2013 [18, 25]).

Additively fabricated models have been used extensively for conveying biological information. For example, surgeons have used a 3D printed model of an actual tumour entwined around healthy lung tissue (see Figure 2.4 (left)); providing clear benefits to a surgeon's preparation [26, 25]. Additionally 3D printing has been used to create models from scans of a human skull to assist surgical planning or fitting of cranial implants [41]. It was suggested during the course of this project (see Chapter 4) that similarly a physical model of the fly brain could be used to aid fly brain removal and dissection.

Companies *Miramodus* and *Biologic Models* create very detailed models of molecules such as proteins, where various chemical properties can be conveyed in both structure and colour [25, 2, 3]. Figure 2.4 (right) depicts a model of the protein Thermolysin by *Miramodus*, created using 3D printing, further illustrating the educational use of this technology [3].

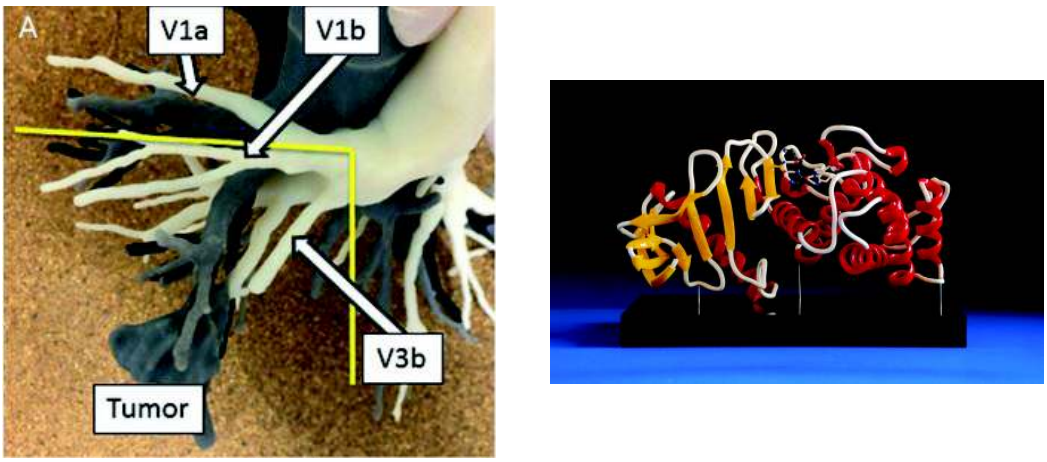

Figure 2.4: Left: This illustrates a 3D printed model of a real tumour (grey) with healthy lung tissue (white) which is useful for assisting surgical operations (taken from Nakada *et al.* 2014 [26, 25]). Right: 3D printing has been used to create detailed models of molecules for education. This figure shows protein Thermolysin created by modelling company *Miramodus* (taken from *Miramodus* website [3, 25]).

Traditional methods of learning are often based upon using textbooks and computer models, as these forms are easily accessible and producible. Similarly, the fly brain structure is learned via research papers, 2D diagrams, and 2D/3D computer models (as with the VFB ontology viewer depicted in Figure 2.1) [22].

Research has shown that tactile interaction with a physical model allows for significantly improved learning for complex anatomy [28, 41]. Work by Preece *et al.* has shown that while a 2D screen provides easily accessible learning, the danger is that students with poorer ability to visualise spatially may become cognitively overloaded [25, 28].

These studies experimentally compared learning the anatomy of an equine foot using traditional methods (text books, computer models) compared to a physical model, and found that engaging multiple senses generally resulted in improved learning [25, 28]. Students were split into three experimental groups: those learning on the physcial model, textbooks and computer model. Moreover, it is interesting to note the scores

between the two traditional test groups (textbook and computer model) were not only significantly lower than the physical model, but very similar. Figure 2.5 (left) illustrates the physical model created in the study by Preece *et al*.

Like the fly brain model constructed from this project (see Section 3.7), the equine model is also fabricated from rapid prototyping techniques and held together in several parts using magnets [28]. The fly brain also contains complex anatomy, and learning or visualising the structure would be greatly benefited by a physical model where each region can be manipulated individually and combined to form the whole brain.

Figure 2.5 (right) depicts a 3D printed model of a neuron created from real data [30]. The model had to be preprocessed using surface mesh software (*MeshLab* and *MeshMixer*) before it was ready to be printed; and a similar process was required for printing the fly brain.

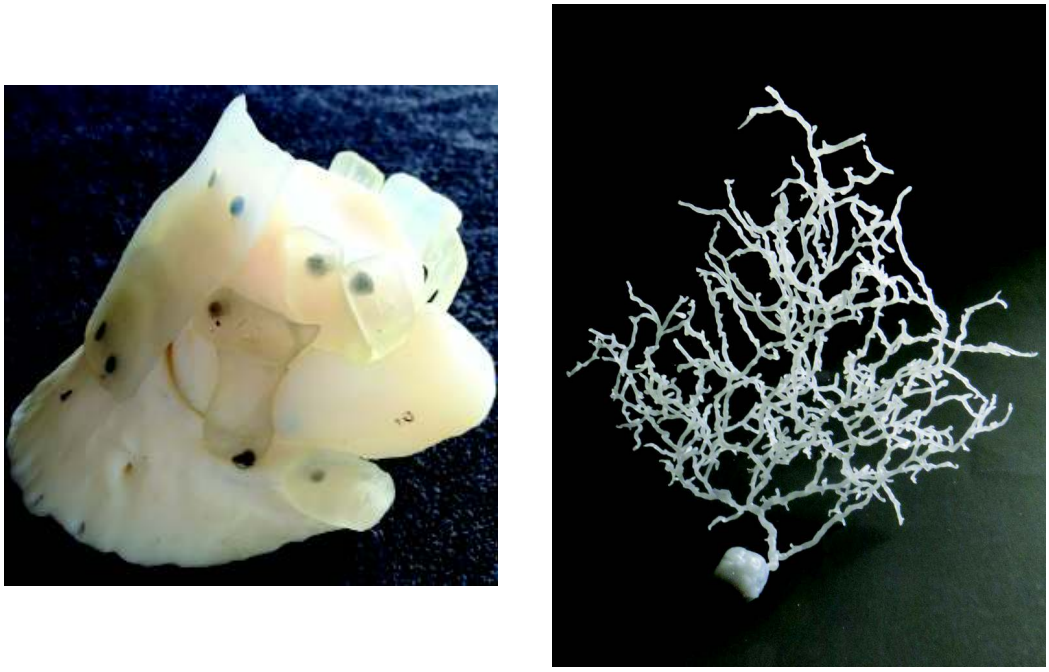

Figure 2.5: Left: Physical models help students to learn better by engaging their sense of touch. This model of a horse foot was created with 3D printing in a study to see if a physical model improved medical students learning complex anatomy. The model here is held together with magnets so it can be disassembled and reassembled, a key concept for out modelling the fly brain (taken from Preece *et al*. 2013 [28]). Right: This image shows a 3D printed model of a real neuron. The original data was scaled up in dimensions and preprocessed with software (*MeshLab/MeshMixer*) to tidy it up for 3D printing; a similar process is followed by this project in creating the fly brain model (taken from Robinson *et al*. 2014 [30]).

Furthermore, 3D printing has been used to create models specifically as research tools. Two examples are: 3D printing a model of a vascular system to help verify computational models of blood flow; and 3D printing mathematically defined shapes to help in understanding gravitational potential wells [20, 38, 25].

2.3 3D Printing

Also known as additive manufacturing/fabrication and rapid prototyping, 3D printing has been around in some form or another since the 1980s [13]. Originally conceived and developed by Charles Hull, 3D printing was used for creating plastic objects from photopolymers which had very long production times [13]. In 1986 Hull would move on to develop the STL (stereolithography) file type which bridged the gap between computer modelling software and fabricating a physical object with a 3D printer [13].

The STL format consists of representing the surface of a 3D object in terms of a triangular mesh (see Figure 2.8 where such a mesh is visible) [13]. Each triangle is defined by three vertices, and the spatial coordinates of each of these vertices are stored within the STL file [13]. Often, Computer Aided Design (CAD) software is utilised to design a 3D object and export as an STL file for 3D printing [13]. Since we are working with real fly brain data, we will first be forming our data into a CAD compatible form, processing our model (see Sections 3.2 to 3.5) and finally exporting as an STL file for 3D printing.

Once the desired STL files are obtained for 3D printing, specialised software is usually used to convert the STL file into a sliced form, sometimes known as a G-file [13]. This sliced format is what the 3D printer understands, and allows it to form a 3D object in a layer by layer process [13].

Work done by Wang *et al*. outlines an updated method for producing an STL file from computer tomography (CT) medical scans [37]. Wang *et al*. highlight the fact that analysing 2D medical images is slow and time consuming, and suggest producing a 3D physical model helps solve this time constrained problem; parts may be 3D printed within a matter of minutes to hours, allowing tactile iteration and analysis on a fast schedule [37].

Numerous 3D printing methods exist, which can print in a wide range of resolutions and materials. Fused Deposition Modelling (FDM) is commonly used for its simplicity and reliability; and involves layering down drops of thermoplastic which quickly link to surrounding material [25, 13, 29]. Various material can be amalga-

mated with a powerful laser to form a 3D object in a process known as Selective Laser Sintering (SLS); or with Sterolithography (SLA) which uses an Ultra Violet laser to cure photopolymers together [25, 29]. These techniques are more capable of providing a finer resolution than FDM but with vastly increased cost. There exist other manufacturing methods such as various Inkjet techniques and Laminated Object Manufacturing (LOM) but these will not be directly relevant for the work undertaken in this project, where we only utilised FDM printers [13].

The most common 3D printing materials used today are polylactic acid (PLA) and acrylonitrile butadiene styrene (ABS) [13]. For this project we used printers which were optimised for PLA, which is a biodegradable thermoplastic. Although primarily using PLA, we also used a form of thermoplastic polyurethane (TPU) which is commercially known as *Ninjaflex* by creator company *Ninjatek*. The TPU material allowed parts to be printed flexibly which was useful for fitting parts into the final fly brain model; whereas PLA parts form as non-flexible solids.

This project used two printers at the Fabrication Lab of the University of Edinburgh (Institute for Micro and Nano Systems) [34]. These were the *Wanhao* and *CreateBot* FDM printers, illustrated in Figure 2.6.

2.4 Design Vision and Approach

We wanted to create a 3D physical model which reflected the complex anatomy of the fly brain with the various neighbouring regions, where researchers could properly visualise and manipulate these individual regions. Put simply, our design vision was to create a 3D puzzle of the fly brain consisting of the many regions available individually from the VFB hub (e.g. those regions depicted in Figure 2.1).

Our aim was for these regions to be recognisable, and easily taken apart and put back together again in a fluid and modular fashion. For this reason, we envisioned using cylindrical magnets for cohering the regions at their various interfaces. A significant part of the model making work would involve using CAD software to bring the regional data together, and create the holes for placing magnets into.

The only previous work found in making a 3D physical model of the fly brain was implemented by Nicolas Aimon [5]. In this work the whole brain was 3D printed in a transparent material and optical fibres used to create a variable-luminescent lamp to reflect data-driven neural activity within the fly brain (see Figure 2.7). This project used computer graphics software *Blender* (www.blender.org) to help eliminate empty

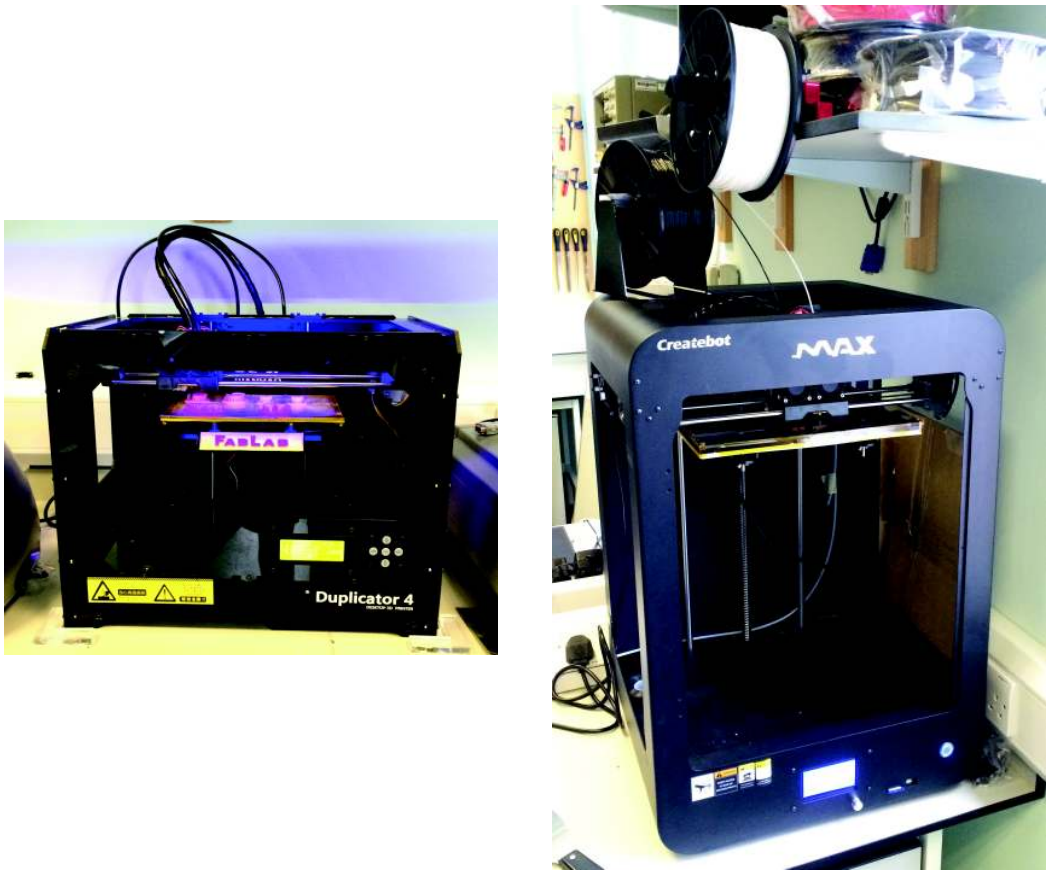

Figure 2.6: This figure shows the two 3D printers used for creating the fly brain model depicted in Figure 3.21. Left: The *Duplicator 4* printer by *Wanhao* was used for most of the parts. Right: The *Createbot* set up for dual printing, used for printing parts in two colours.

spaces within the fly brain data which were causing printing abnormalities [25, 5].

In this project, STL surfaces were generated from an *ImageJ* plugin (*3DViewer*) which uses the marching cubes algorithm [31, 19].

2.4.1 Autodesk Inventor

Bio Inspired Design (BID) is used to create tools such as prosthetics, dentures and even robotics [10, 12, 23]. We wanted CAD software capable of handling detailed STL meshes for our fly brain regions, and needed to process these meshes as solid bodies, and operate tasks such as creating the numerous holes for magnet placement. We chose to use *Autodesk Inventor* (referred to as just *Inventor* from here) for processing brain regions into a fitted model. *Inventor* is very versatile and robust, containing all of the required tools for designing a model [1].

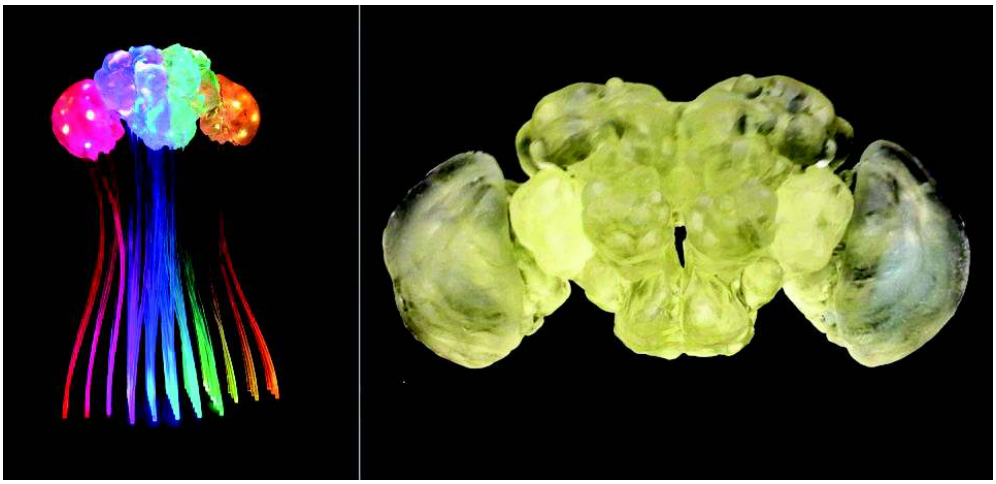

Figure 2.7: The only previous work we found in 3D printing a fly brain model is that by Nicolas Aimon who engineered a fibre optic based lamp [5]. The lamp illuminates based on neural data recorded from an actual fly brain based on rough locations within the brain.

Typically *Inventor* is used for creating industrial objects such as water pumps, but has been used in a multitude of application domains, including within BID [23, 9, 36]. *Inventor* has also been used to help physically model CT scans of a human skull, where an FDM 3D printer was used [41]. It is difficult to find many examples of where *Inventor* has been used to manipulate or model real biological data.

An *Inventor* plugin (*Meshenabler*) was installed which allowed the STL files to be converted into a solid body format (called an *Inventor* part) which allowed features such as holes to be made on surfaces. See Figure 3.12 which illustrates a typical extrusion which can be made on an *Inventor* part. Additionally, *Inventor* can be used to create any number of features, such as smoothing edges, combining parts together, splitting parts and eliminating interference between separate parts which overlap. Also, *Inventor* is very good for modelling and visualising many parts in a single assembly where the colour can be manually set for individual parts.

Inventor parts are saved in an *ipt* file format, and can be combined and manipulated in the *ipt* modelling environment. *Inventor* also provides a different environment called an *assembly*, where multiple *ipt* parts are imported and saved in the *iam* file format. The *ipt* environment is usually used for manipulating parts individually whereas the *assembly* environment is optimised for operations between parts, and provides a somewhat different tool set for this purpose. Figure 2.8 illustrates the *assembly* environment where an individual part has been selected, and the other part becomes transparent for

visualisation purposes. Figure 2.8 depicts a test phase being modelled (see Figure 3.11) where you can see various holes have been extruded into the Lobula Plate part.

Once the solid bodies have been processed accordingly, *Inventor* can be used to export the parts into an STL file for 3D printing. It should be stressed that the model features, such as the magnet placements, are created computationally before anything is physically fabricated. During fabrication, our features are printed directly as a part of the model parts.

In summary, *Inventor* was used for bringing the model parts together, implementing preprocessing steps and creating magnet placements.

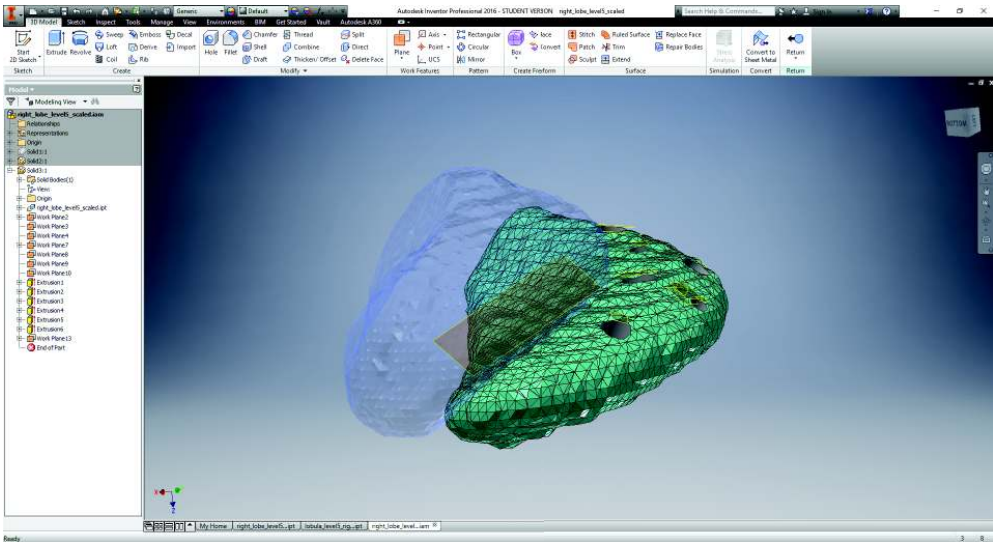

Figure 2.8: Illustrating the *Inventor* assembly environment with Lobula (transparent) and Lobula Plate (blue) fly brain regions. The yellow squares are *workplanes* used to make holes within the model (see Section 3.5).

Chapter 3

Methods

This chapter outlines the practical work undertaken in creating the final fly brain model (see Figure 3.21). It includes the computational design and implementation, and the actual physical implementation. The description roughly follows a chronological timeline of events for the work flow leading to the final construction.

Firstly, significant work was required in organising the regional fly brain data, converting the raw files representing volumes into surface meshes, and importing the data into *Inventor*; which is described in Section 3.1. Secondly, the scale of the final model needed to be decided and all of the regions scaled accordingly, as described in Section 3.2. Thirdly, Section 3.3 describes the mirroring options, decisions and implementations which were made towards making the final model symmetrical. Fourthly, the computer model for the assembly of over 50 fly brain regions required processing, as described in Section 3.4. Fifthly, Section 3.5 describes the three test models which were fabricated, their findings, and creating the numerous holes for magnet placement within the computer model. Next we describe fly brain regions which presented problems, the possible solutions considered, and justify those which were chosen in Section 3.6. Finally, we describe the process and results of 3D printing all of the parts, cleaning them up, and attaching magnets in Section 3.7. The final model is also presented in Section 3.7.2.

At each stage we describe difficulties, suggest potential solutions, and justify the solution finally implemented. Chapter 4 includes a description of alternatives and potential improvements given the work undertaken and its evaluation.

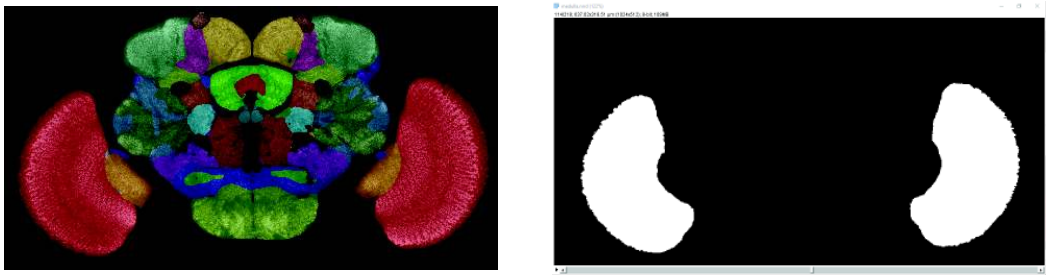

Figure 3.1: This image shows the same 2D slice of fly brain regions as in Figure 2.1 (left) but in comparison with viewing the Medulla individual region file with *Fiji* (right). This allowed STL meshes to be created with the correct region names.

3.1 Initial Work

Initially, we formed our design vision as described in Section 2.4. We considered constructing an additional model which integrating neural circuitry data available online (see Figure 2.2), but after initial discussions we decided there was insufficient time available for this implementation. Consequently the neural circuitry idea is left as potential future work and we decided to focus on creating the regional model, and assessing its usefulness to researchers. Simply put, our design vision is to create a 3D puzzle of as many of the separate fly brain regions as possible.

The raw data we started from is in the form of 41 separate Nearly Raw Raster Data (NRRD) files accessed from the VFB website. The NRRD file format is created for representing high dimensional scientific data and its visualisation [4]. Each of the 41 NRRD files represents a particular region in the fly brain as represented on the VFB ontology viewer [22]. Figure 3.1 (left) illustrates some of these regions in various colours using the VFB interface. Note that these regions and their NRRD files may represent either a single volume spanning the central plane of the fly brain (midplane of symmetry) or two volumes, one on either side of this plane. These NRRD files are named arbitrarily by number (e.g. Domain11, Domain12) and initial work involved naming these files according to their biological names as described by the VFB ontology (e.g. Medulla, Lobula). This involved viewing each NRRD file individually (using software tool *Fiji*) and matching up by manual visual inspection with the VFB viewer (see Figure 3.1). Table A.1 in Appendix A outlines the one-to-one map of numerical domain number to biological name for future reference (valid as of 10/06/16).

Next we needed a way to transform these region files (as NRRD slices) into surface mesh STL files such that they can be imported into *Inventor* for model design process-

ing. Using the *3D Viewer* plugin for *ImageJ/Fiji* (created by Schmid *et al.*), we were able to easily convert each NRRD file into an STL surface mesh file [31]. The *3D Viewer* plugin allows visualisation of the data in three main forms: volumetric, isosurfaces and orthoslice sets [31]. The form relevant for STL meshes are the isosurfaces and the plugin allows for the creation of the surface mesh with integer levels of detail, called "resampling" levels (parameter with *3D Viewer/Fiji*). The higher levels of detail (where level 1 is higher than 5) resulted in a surface mesh with a greater number of triangular faces representing the surface than a lower level. We wanted to retain as high a level of detail as possible on the surface meshes for each region. The problem here is that *Inventor* processes very slowly (and is sometimes unstable) on files which are too big and with surfaces with too many triangular faces; detail had to be sacrificed for *Inventor* performance. By testing *Inventor* performance at various levels of detail it was found that a resampling level of 5 was sufficient. Whilst *Inventor* did struggle (freezing, crashing, very long file saving times) at times with large file sizes when modelling all of the fly brain regions as a whole on resampling level, it was found to be manageable. Using a higher level of detail would have been unmanageable given the time available for this project.

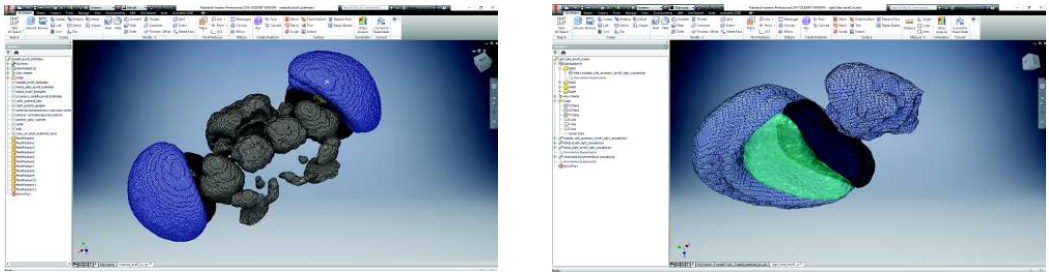

Figure 3.2: Illustrating *Inventor* screenshots of building the fly brain model. Regions can be displayed in various colours and transparency for visual clarity. The left image shows modelling of regions (Medulla in blue, Accessory Medulla in yellow, Lobula in black, and some central regions in grey/white). The right image depicts modelling of the right Optical Lobe with its connection to the central fly brain body (Medulla/Ventrolateral Protocerebrum parts in blue, Lobula Plate in Turquoise, and Lobula in transparent black). The Optical Lobes are somewhat loosely connected to the main body so it was important to create a strong magnetic connection here.

Subsequently, 41 STL files were created using this level of detail and the STL files containing two volumes were further split into individual files for separate brain halves. There exist 12 single brain regions which span the midplane of the fly brain and

29 double brain regions which are symmetrical across the midplane. See Figure 3.17 for an illustration of the right half of the fly brain with the midplane visible. It should be emphasised at this point that the left and right halves of the brain are not precisely symmetrical as there are slight variations in the surface features (given the data comes from a real fly brain). After spending a significant length of time learning how to use *Inventor*, the STL files were imported and converted to solid bodies, which may be edited by *Inventor* in creating features such as holes. The solid bodies were created using an *Inventor* plugin called *Mesh Enabler* [1]. Once in this solid body form, each region was saved as an *Inventor* part (an .ipt file extension) for subsequent processing.

Figure 3.2 shows what parts of the model look like within *Inventor*, where each part can be individually manipulated.

3.2 Scaling the Model

After importing each region into *Inventor*, the default scale provided by the STL files was approximately 1542 cm in the computer model's longest dimension (from one Optic Lobe to the other, see Figure 3.2 (left)), which we will refer to as the width from this point onwards. Although the model width could have been in the range of 50 - 60 cm given the printing volume of our 3D printer, we decided that an ideal width would be 30 cm for the purposes of easily handling the model. Using 30 cm as the width, we scaled all of the fly brain regions accordingly; that is we scaled all regions down by a factor of approximately 30cm/1542cm = 0.0195. Importing each unscaled *Inventor* part (.ipt file), the scale can be fixed, by using the *Invnentor* "derive" tool. This involved deriving, scaling and saving each fly brain region individually as a new *Inventor* part, making sure the biological names are preserved across this process.

3.3 Mirroring the Data

It was decided to make the final model symmetrical across a central plane (see Figure 3.3), to allow a coherent model where it was easy to identify corresponding parts from the left and right halves of the fly brain. Additionally, it would make creating the numerous magnet placements much simpler as the holes need only be created for one half of the fly brain, and then mirrored for the other half. Here the right side of the brain is defined as the half which would be on the right belonging to the fly, as depicted

in Figure 3.17. The right side was arbitrarily chosen as the half of the brain to be modelled, the other half of the brain data was discarded.

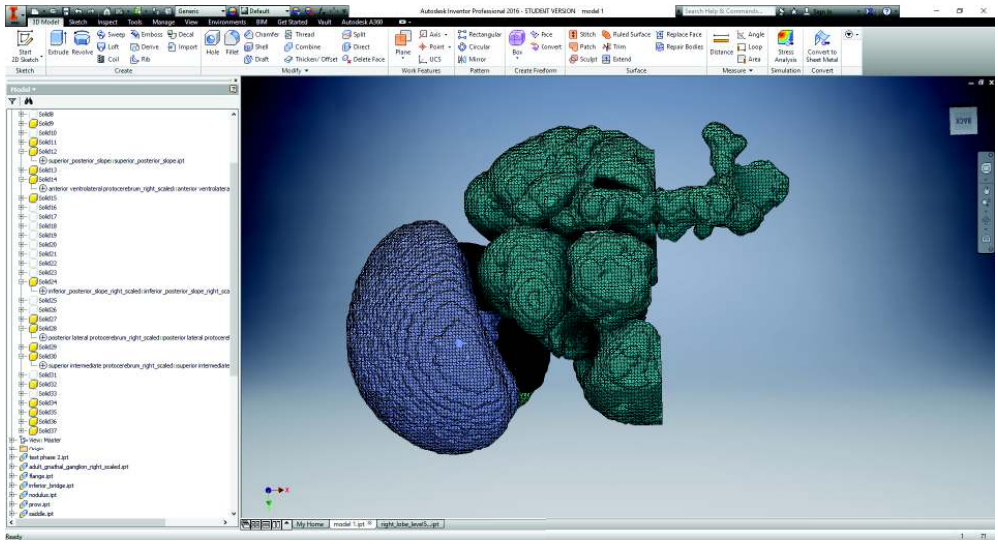

Figure 3.3: This figure illustrates the right half of the fly brain with the Mushroom Body region reflected in the midplane to form both halves. The Mushroom Body was used to define the midplane, allowing a symmetrical model to be created. The left half of the original fly brain data was not used for modelling.

Mirroring the right half involves deciding upon a plane to cut the data in half to duplicate the data. There are 12 brain regions which span across the middle of the brain. Each of these middle regions can be used to define a midplane based upon that particular region's centre. Because we are handling real data, we have a choice between 12 midplanes which are all within a few millimetres (model scale) of each other. Three of these planes are illustrated in Figure 3.4. Whilst the data could potentially be split using multiple of these mirror planes, it was decided this may cause fitting issues at a later stage. The only alternative was either to take an average between these planes or choose one in particular.

The Mushroom Body is one of the most heavily studied regions within the fly brain and a prominent structure within the model. For this reason, the Medial Lobe of the adult Mushroom Body (the topmost region in Figure 3.4) was chosen to define the midplane (the first plane from the left in Figure 3.4). The Medial Lobe of the adult Mushroom Body also defines this plane clearly as there is a precise mirroring point where the two halves of the data come together at a reduced number of vertices.

Figure 3.5 illustrates the middle regions. As can be seen, the left half of the brain was discarded and the middle regions split in half according to the chosen mirror

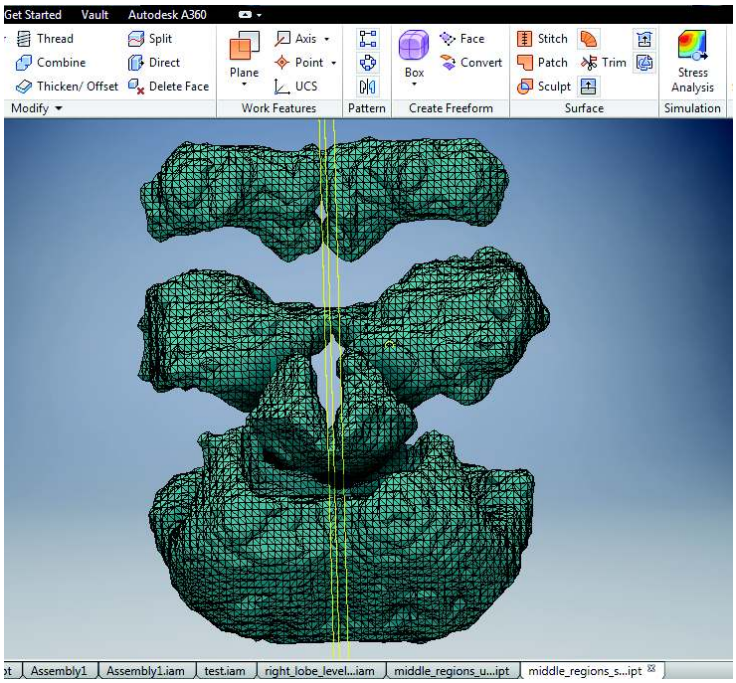

Figure 3.4: The figure depicts four of the central regions prior to discarding the left half of the brain data. This screenshot shows three planes (yellow, observed edgewise) considered for splitting half of the brain, and are defined by the various regions spanning the midplane. We decided to use the plane (left most plane in this image) defined by the Medial Lobe of the Mushroom Body (topmost region) for the midplane, used to mirror the right half of the brain anatomy. Note how the original left and right brain data is slightly unsymmetrical.

plane using *Inventor* tools. Note, the problem with splitting in this way means a slight amount of data is arguably lost in the discarded halves of the middle regions (as different centres can be defined for each region).

3.4 Resolving Interference Between Regions

The primary problem identified in creating a regional fly brain model is that each of the regions overlap very slightly between neighbouring regions. Figure 3.5 clearly illustrates this overlap between neighbours. The original data (in its sliced volumetric form) does not overlap, but the marching cubes algorithm, used by the *3D Viewer* plugin of *Fiji* to form 3D surfaces of these volumes, causes each volume to become slightly larger than they ought to be. The *3D Viewer* plugin allows an integer thresholding value to be set which is used by the algorithm to form the surface meshes. We tried

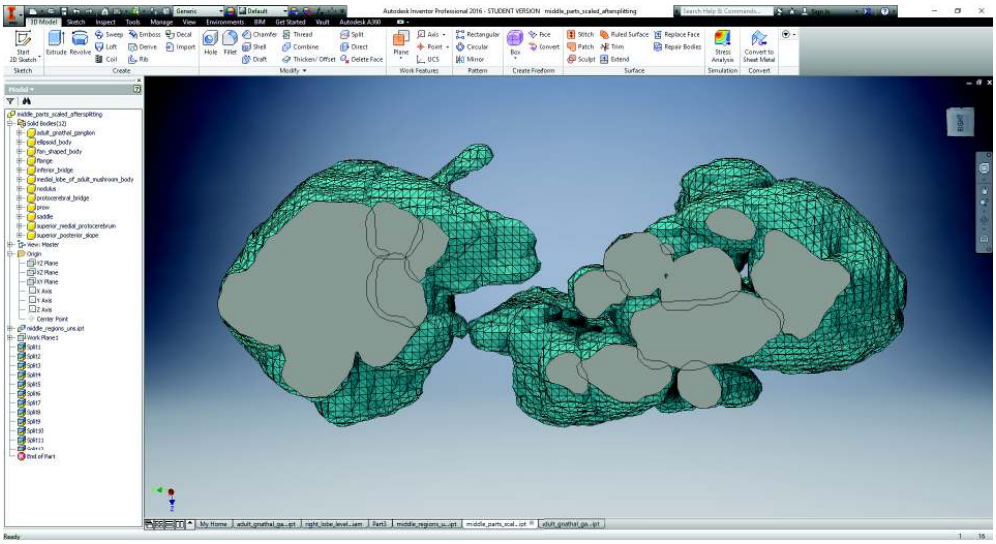

Figure 3.5: Illustrating the regions which span the midplane of the fly brain for the right half of the brain. The grey areas are where the midplane has sliced through the model, revealing a cross-section through these right-half brain regions. Empty spaces are mostly filled with regions which do not span the midplane. Note also how the cross-section reveals the regions overlapping slightly upon their neighbours.

experimenting with this value but without resolving the overlap between surfaces. A default value of 50 was used for the *3D Viewer* threshold value for the final model data. Further work could be done in experimenting with different algorithms and parameters to resolve this issue at the source.

Figure 3.6 shows an example of the overlapping which occurs between regions; you can see how the border of the Lobula and Lobula Plate overlap into each other. While it is possible that these overlaps would not cause much problem with some regions (e.g. it was acceptable within the Optic Lobe test print of Section 3.5) it was likely to cause fitting problems in more complicated areas such as the Central Complex; and it was desirable to remedy these overlaps to provide a well fitted model. Two methods were identified as potential solutions.

Firstly, each region could be slightly translated in 3D space such that the neighbouring regions would not overlap. Figure 3.7 (left) illustrates a method in which *Inventor* can rigidly move a region along a specified axis. Starting from a central region within the interior of the brain, one could gradually move apart each overlap (e.g. from the Mushroom Body). The problem with this method is its time consuming nature, requiring a great deal of precision, and alterations would have knock on affects with potentially many other regions. Additionally, the final model may display unwanted

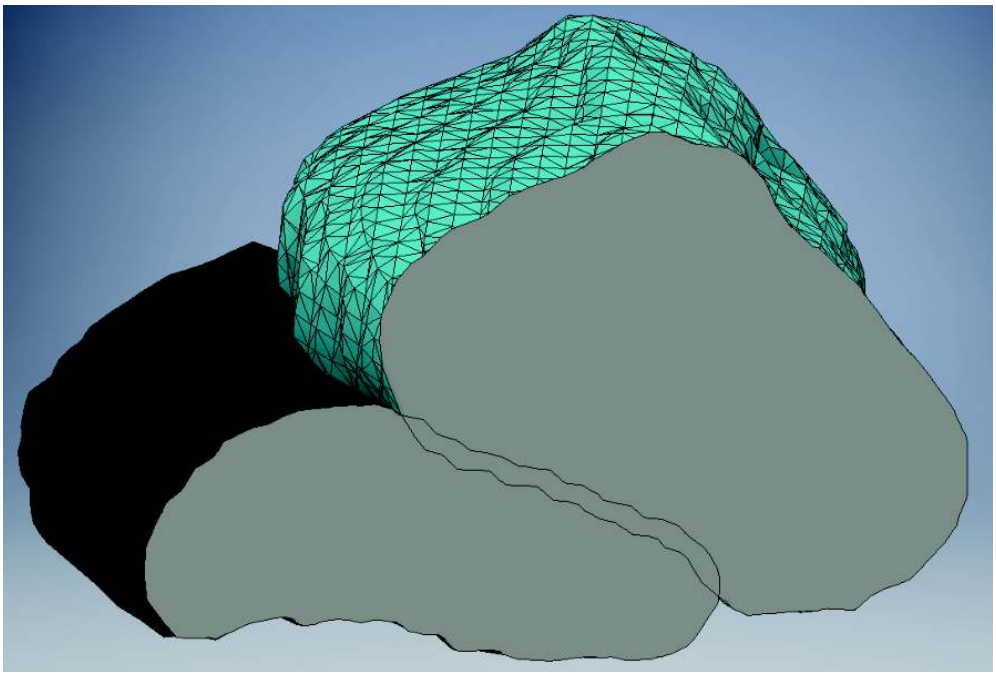

Figure 3.6: Depicting the Lobula (blue) and Lobula Plate (black) which overlap as viewed with *Inventor*. These two regions have been sliced through their centres for visualising the overlap, as with Figure 3.5.

gaps in places where in fact there should be none. The second option was chosen for its usefulness in resolving the overlaps in a manageable way.

This second option considered involved leaving every region exactly where it was in the original data; preserving the relative positioning between regions. After researching *Inventor* functionalities, it was discovered that the overlaps could be resolved directly by using one overlapping region to cut into (or sculpt) the other overlapping region.

For example, one can define the surface of the Lobula (white mesh in Figure 3.7 (right)) as an *Inventor* feature and use this to detect and discard the overlap with the Lobula Plate (defined as a solid body, pink mesh in Figure 3.7 (right)). As in the example depicted in Figure 3.7 (right), we have three options for processing each overlap between two neighbouring regions (region 1 and region 2):

- Option 1: Eliminate the overlap in region 1 using the surface mesh of region 2.
- Option 2: Eliminate the overlap in region 2 using the surface mesh of region 1.
- Option 3: Eliminate the overlap entirely from both regions.

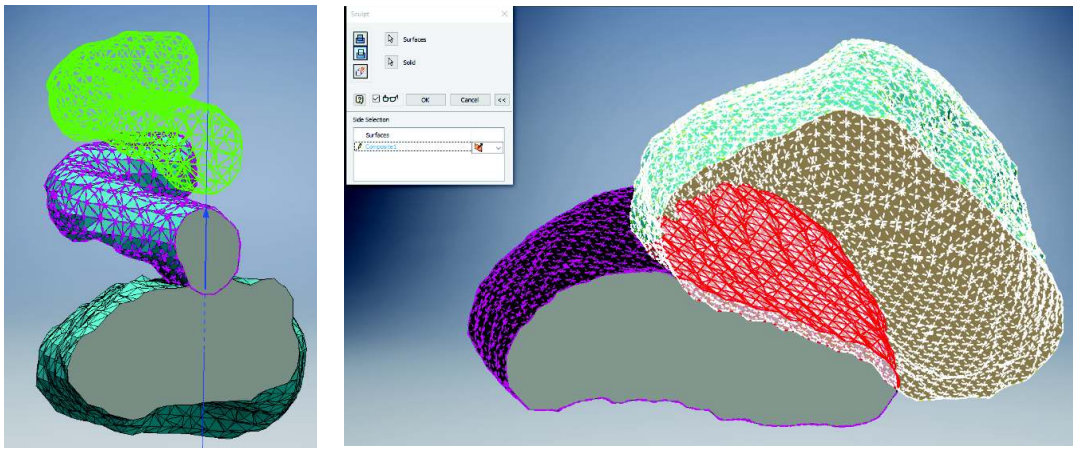

Figure 3.7: Left: Regions can be translated along a particular axis within *Inventor*. This image illustrates moving the Protocerebral Bridge (green/purple) in a direction chosen to be away from the Fan Shaped Body (unhighlighted). Right: Overlapping regions can be detected and processed without moving them. This image illustrates the Lobula (white/blue), the Lobula Plate (black/purple) and the interference between them (red).

The effects of these three options are depicted in Figure 3.8; options 1,2 and 3 are depicted clockwise from the top left image respectively. The first two options result in a close fitting model, while the third one would result in a model with numerous gaps between regions. Figure 3.8 (bottom) shows how a gap would be created between two example regions with the third option; that is the result of discarding the interference depicted in red from Figure 3.7 (right). Creating these gaps does not accurately represent the original data, and may result in fitting issues given the numerous empty spaces which would be made between most of the brain regions. For these reasons, the first two options were chosen for subsequent processing.

An overlap existed between every two regions which neighboured each other and sometimes these were significant interfaces (see Figure 3.9 (right)) and other times very small (e.g. between the Bulb and Fan Shaped Body).

Figure 3.9 (left) illustrates how some regions (blue) wrap very closely and intricately around other bodies such as the Mushroom Body (white). Consequently, resolving all of the overlaps was a very time consuming task with a total of 133 overlaps individually resolved in the final model. Resolving each overlap required deciding which region to cut into, as described in the two initial options above. These decisions were made by considering the resulting surfaces (see Figure 3.8 (top right)) and which would be easier for extruding a potential magnetic connection into (flatter surfaces are

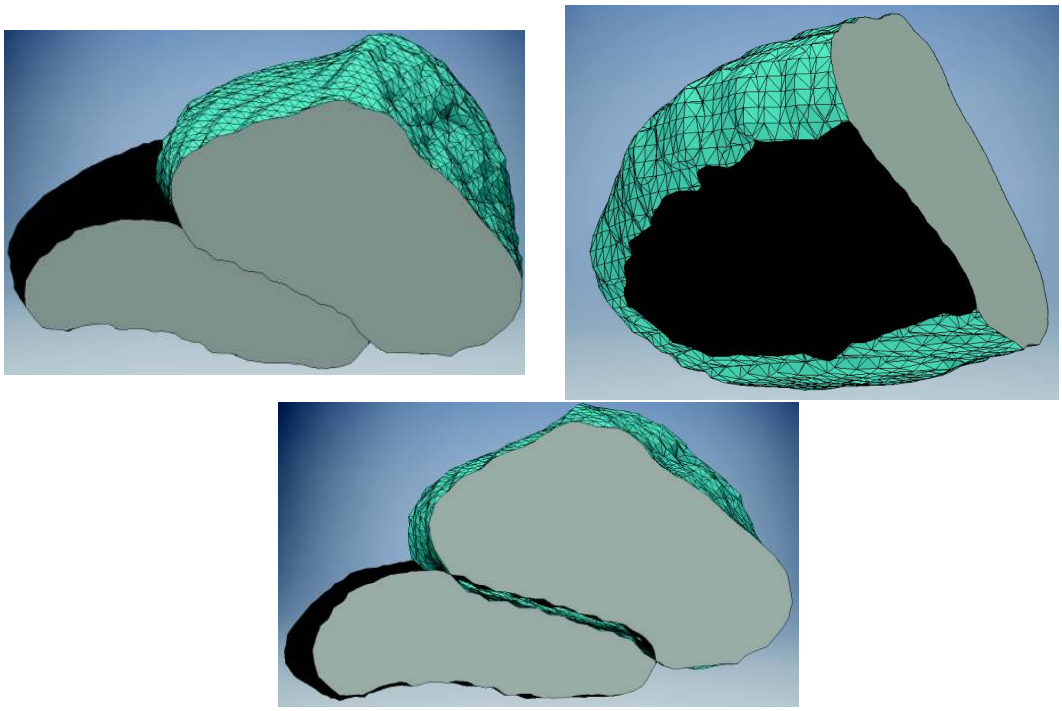

Figure 3.8: Here we illustrate the three options available in resolving overlaps in three images. Two brain regions are used for this example. Top left: The Lobula (blue) cutting into the Lobula Plate (black). Top right: The Lobula with a cut (black) from the Lobula Plate (not visible). Bottom: Entirely removing the overlap from both regions.

easier than bumpy surfaces). Additionally we tried to balance the number of cuts into a single region over all regions as far as possible, so as not to alter a single region far more than another region.

Inventor contains a tool (*Interference Detector*) which was useful for detecting interferences between regions and to confirm interferences had been resolved. The *Interference Detector* tool also calculates the precise volume of overlap. Figure 3.9 (right) illustrates the use of this tool in detecting overlaps (red) between the Mushroom Bodies and the surrounding neighbours; clearly illustrating the need to resolve these interferences to create a model which fits together properly.

Figure 3.10 illustrates resolving overlaps with the 4 regions of the Mushroom Body, Ellipsoid Body, Fan Shaped Body and Protocerebral Bridge. These were printed in a test model (see Section 3.5 and Figure 3.11 (bottom)) and the results show it fits together very well.

Note that in processing these overlaps we have sacrificed some data by sculpting into various regions, for the benefit of a model which fits properly for its use as a research or learning tool. As you can see in Figure 3.8 (top right), resolving the inter-

ferences in this way does create notches or sharp edges in some regions but this is a compromise we make for creating a flush fitting model.

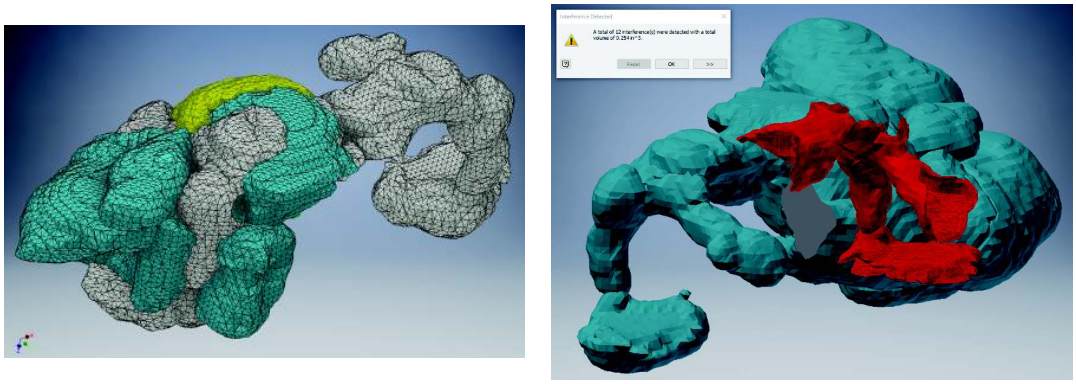

Figure 3.9: Regions around the Mushroom Body are particularly difficult to physically model due to the intricate interfaces between surrounding regions, which wrap around each other. Left: Mushroom Bodies (white) and surrounding regions (blue/yellow). Surrounding regions include the Crepine, Optic Tubercle, Superior and Inferior Clamp. Right: The overlap between the Mushroom Body and neighbouring regions can can be visualised using *Inventor*, depicted here in red.

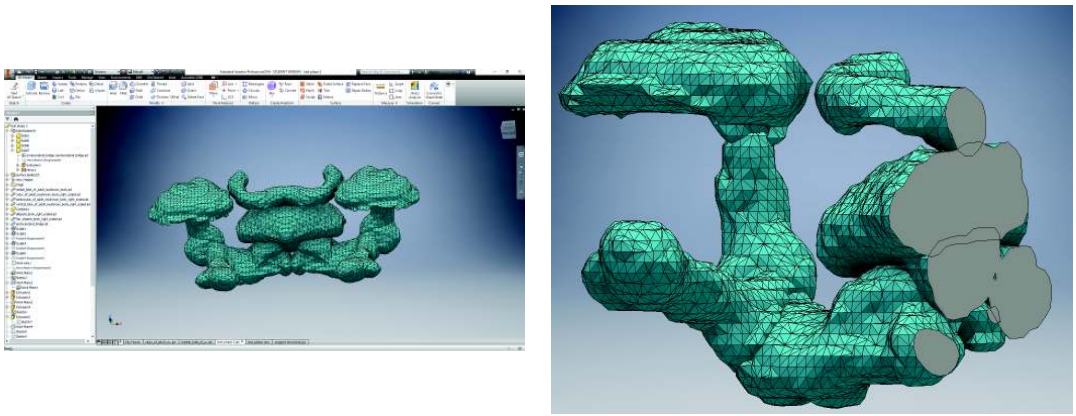

Figure 3.10: These images illustrate *Inventor* modelling for the Mushroom Body and Central Complex together. Left: Central Complex regions displayed with *Inventor*. Right: Midplane cross-section of the Central Complex depicting overlaps.

3.5 Test Phases and Creating Extrusions

Three test phases were implemented in which models were 3D printed; these are illustrated in Figure 3.11. The first phase involved fabricating the right Optical Lobe

regions (Lobula, Lobula Plate, and Medulla), where the Accessory Medulla was combined with the Medulla for convenience. The purpose of this phase was to observe any issues in printing the parts and to test various sizes of holes for the magnet placements. These holes were engineered within *Inventor* with differing levels of depth and width, and included a small amount of leeway for the magnets to fit into the holes. The resulting model showed that a leeway of at least 0.1 mm was needed as most of the magnets did not fit into the engineered holes. Otherwise, no printing problems were detected from this test phase.

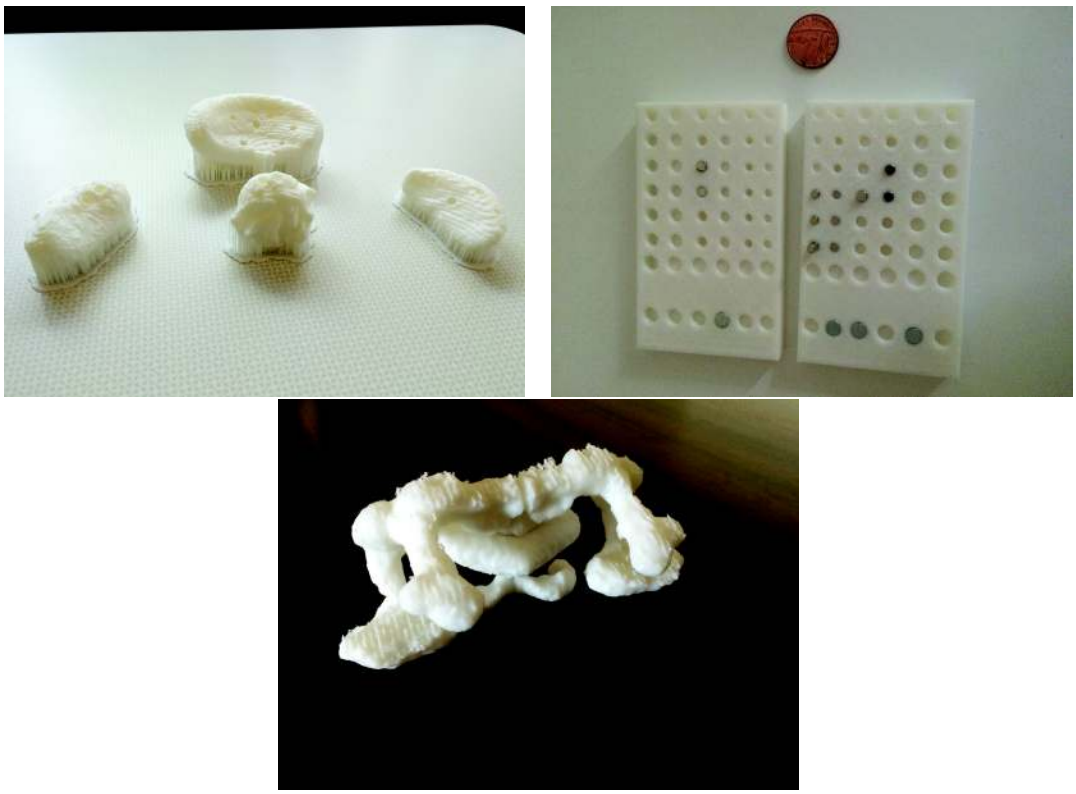

Figure 3.11: Top left: Test 1 involved fabricating the regions (Lobula, Lobula Plate, Medulla) of the right Optic Lobe with test holes. Top right: Test 2 consisted of testing various hole size for magnet placement. Bottom: Test 3 consisted of constructing the Central Complex and Mushroom Body regions.

The second phase involved designing and printing a rectangular block of holes with increasing width and varying depth in order to test exactly what hole size worked. Figure 3.11 (top right) illustrates this model with some test magnets in place. The model was designed using *Inventor*, and tested magnets of 3, 4, 5 and 6 mm diameter. The hole width range was 0.0 - 0.5 mm using an increment of 0.1 mm. Depths were tested in blocks of three holes, for depths of 2, 3, 4 and 5 mm for each of the magnet

diameters.

The results show that generally a hole leeway diameter size of 0.3 - 0.4 mm is required for a good fit. Occasionally the magnet is too loose, and superglue was found to work well in these cases. The particular glue which worked well for this project was *Loctite Powerflex Superglue*, widely available from most hardware stores, which bonds well with PLA and the Neodymium magnets used.

The third test phase involved fabricating some of the central brain regions which are liable to cause more fitting issues and investigating the effectiveness of resolving overlaps. The model is depicted in Figure 3.11 (bottom). The results show that a hole leeway of less than 0.4 mm is not always enough and that the overlap solution described in the previous section works effectively. Once all of these overlaps were resolved, the computer model was closefitting and ready for creating magnet holes.

A part file environment was used to resolve all of the interferences within a single *Inventor* file. Subsequently, an assembly file was created with all of these resolved parts as the assembly environment makes placing holes between regions much easier than a part environment due to its assembly oriented tool set.

Placing a hole involved creating a 2D workplane between two regions being connected (see Figure 3.12), sketching an appropriately sized circle on this plane, and then extruding a cylindrical hole from this circular cross-section into both bodies.

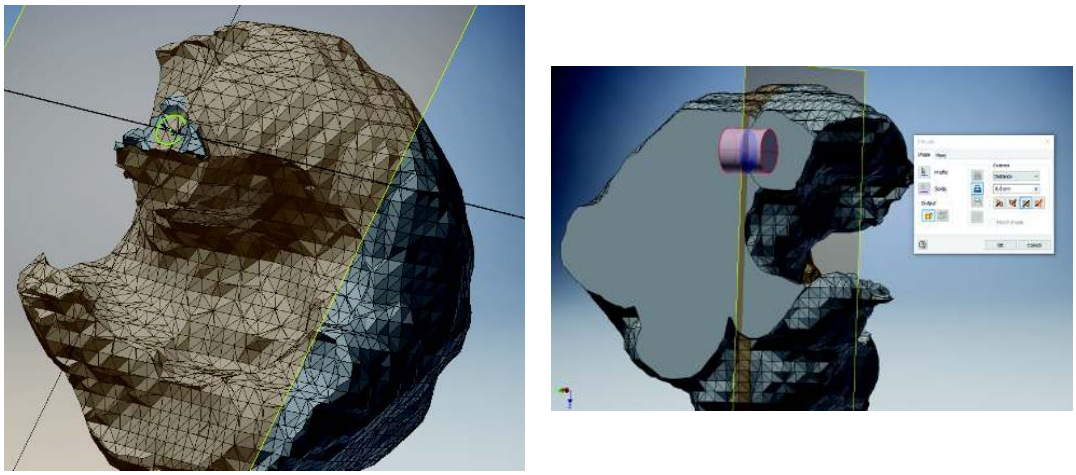

Figure 3.12: Holes are created within the computer modelling by drawing 2D sketches of circles and expanding these in both directions to form cylindrical spaces where magnets can be placed. Left: Creating a workplane and 2D sketch. Right: Creating an extrusion from a 2D sketch to form empty space within the model for magnets.

Much of this work involved trial and error at getting a suitable hole which would

hold the magnet in place. Mostly common sense was used in these placements and deciding what dimension of magnet to use and thus the dimensions of the holes. Care had to be taken not to overlap between holes. This was a time consuming task as around 120 holes (one half of the fly brain) were created for magnet placements to ensure there is sufficient connectivity to hold the model together. The task was broken down into placing holes between outer regions, where stronger magnets were used, and internal regions, where weaker magnets were used. In particular, sufficient connection needed to be ensured between the Optic Lobes and the central body of the fly brain.

Once all of the holes were created, the regions were duplicated in the midplane. *Inventor* is capable of mirroring holes as well as bodies, and this was used for the regions spanning the midplane, but at times mirroring with *Inventor* was a very slow and unstable process. Free software *Netfabb* was used to help perform the final mirroring tasks. Both *Inventor* and *Netfabb* were used to export each region as an STL for the final model.

The magnet dimensions used in the final model were: 6 x 3 mm, 5 x 3 mm, 4 x 3 mm, 3 x 3 mm and 4 x 5 mm (diameter x depth).

Combined Region Name	Subregions Combined
Region 1	Medulla Accessory Medulla
Region 2	Anterior Ventrolateral Protocerebrum Posterior Ventrolateral Protocerebrum
Region 3	Superior Intermediate Protocerebrum Superior Lateral Protocerebrum
Region 4	Saddle Antennal Mechanosensory and Motor Center
Region 5	Medial Lobe of Adult Mushroom Body Vertical Lobe of Adult Mushroom Body Pedunculus of Adult Mushroom Body Calyx of Adult Mushroom Body

Table 3.1: Regions which were combined into a single region for 3D printing.

3.6 Problematic Regions

A number of regions were identified which were best combined, as described by Table 3.1 and we summarise the reasons for these.

Region 1 (as defined in the above table) was created because the Accessory Medulla is a very small part, and it was more stable to combine it with the Medulla. Region 2 comprises the only parts which connect easily with the Optical Lobes and combining them made this connection stronger and with less chances of connectivity and stability issues. Region 3 was made to make connecting the model together easier with the Mushroom Bodies. Region 4 contains two parts which would be very difficult to fit into one another in a conventional way. The Antennal Mechanosensory and Motor Center (AMMC) would have to pass through a natural hole in the Saddle which may be unfeasible using solid material and makes a model much harder to construct. See Figure 3.13 for an illustration of these parts separately, which shows how difficult these parts are to physically model. Region 5 constitute the Mushroom Body, which is a well studied and important area to fly brain research. Consequently, it was decided that it would be best to illustrate these regions as a single part, which also makes building a model more feasible as many of the other regions border this centrally located Mushroom Body (see Figure 3.9).

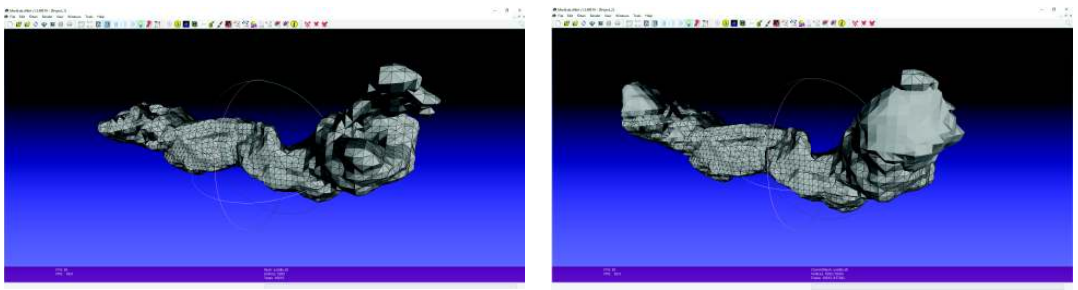

Figure 3.13: Some regions presented difficulty in modelling as separate physical parts due to their shape which would make assembly difficult with solid parts. Left: The Saddle region without the AMMC. Right: The Saddle region with the AMMC (without grids). Viewed using *MeshLab*.

As a compromise to printing all the subregions in Table 3.1 separately, the combined regions 2-5 were printed using 2 colours for illustrating the subregions on each part. Figure 3.14 illustrates these regions. Dual printing required a 3D printer with two nozzles, and we used the *CreateBot* printer for these parts (see Figure 2.6 (right)). All other parts were printed using the *Wanhao* printer depicted in Figure 2.6 (left).

In order to obtain these dual coloured parts we needed the preprocessed (with overlap cuts and holes) STL files for the individual subregions comprising each dual printed part. This required using the sculpting technique to resolve overlaps (as described in Section 3.4); the surface of the combined region was used to sculpt the original (unprocessed) subregions to obtain the required separate STL files. These dual printing results also help to showcase the capabilities of 3D printing in combination with the fly brain.

Figure 3.14: Some regions were printed in two colours (from left to right): Mushroom Body Parts, left/right Anterior/Posterior Ventrolateral Protocerebrum Parts, left/right Superior Intermediate/Lateral Protocerebrum Parts, and the Saddle/AMMC Parts.

Another capability of 3D printing is being able to print not only in various colours but in various materials. Up to this point we have printed solely using an FDM printer on PLA material, but it is also possible to print in a flexible material known as ninjaflex, which is a unique kind of thermoplastic polyurethane (TPU). See the *NinjaTek* website for further details, including technical specifications. Using ninjaflex (1.75 mm diameter) we printed various parts (Crepine, Saddle/AMMC, and Medial Protocerebrum) as illustrated in Figure 3.16.

The two Crepine regions were created in TPU to allow them to wrap around the Medial Lobe portion of the Mushroom Body. Using *Inventor*, a thin cut was made along one side of the Crepine to allow the flexible material to be wrapped around the Mushroom Body at the appropriate place. Small 3 x 3 mm holes were placed within this artificial cut of the Crepine to attempt a stable magnetic connection. Figure 3.15 shows the Crepine both as a computer model with *Inventor* (left) and the final parts wrapped around the Mushroom Body (right).

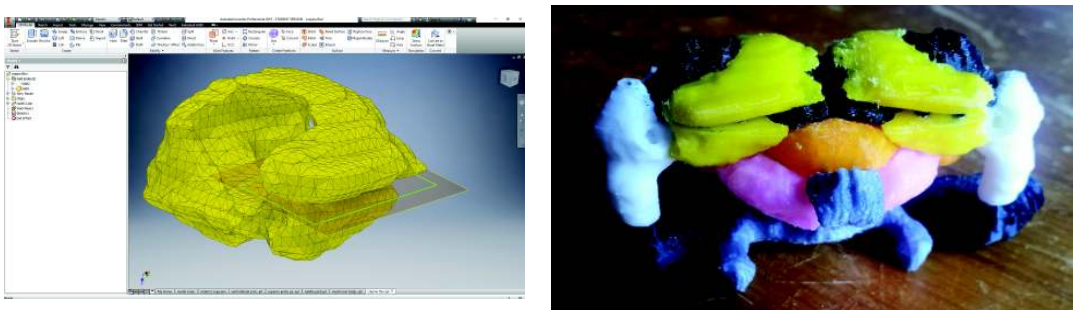

Figure 3.15: The flexible material was very useful for 3D printing regions which could not be fitted into place using a solid material. The Crepine (depicted here in yellow) was fabricated in flexible material to allow it to wrap around the Mushroom Body. Left: Inventor modelling of the Crepine, where we have sliced a rectangular section out of one half and used a workplane to create small magnet placements within the cut. Right: Crepine parts (yellow) wrapped around the Mushroom Body dual print part (black/white). The right image also shows Central Complex regions attached: Bulbs and Ellipsoids Body (orange), Nodulus and Protocerebral Bridge (upper and lower respectively, silver), and the Fan Shaped Body (pink).

During construction of the final model (see Section 3.7.2) we found some regions did either not physically fit into their proper positions due to solid material blocking their amalgamation, or caused too tight a fit for inclusion of all regions. The former was true of the Saddle/AMMC and Medial Protocerebrum parts, while the latter was true of the Mushroom Body. To resolve these fitting problems, the aforementioned regions were printed in TPU to allow them to flex around obstructions. Figure 3.16 illustrates these regions separately and in their proper positions respectively. The final model employed a solid Superior Medial Protocerebrum with a flexible Mushroom Body to allow for the most complete representation.

Finally, note that some of the data and processing operations resulted in very small artifacts which had to be manually removed. Examples of these include removing a small single vertex connection between the Antennal regions, and a small separate entity of the Crepine region which was disconnected from the main body. The small operation at the Antennal regions and Crepine was cleaned up using *Meshlab*, including the use of the *Fill Hole* tool within the same program.

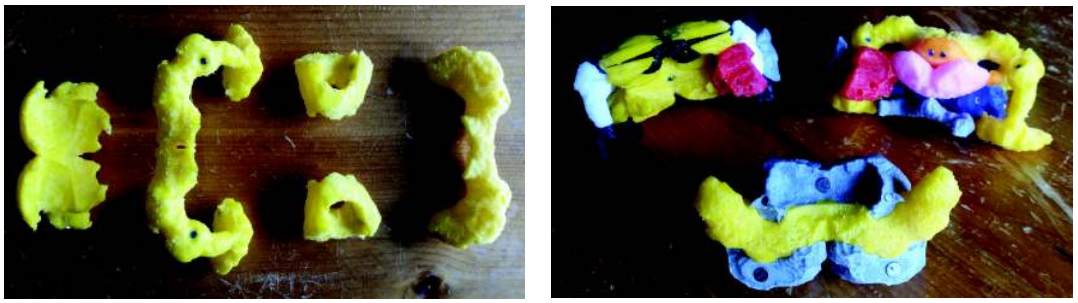

Figure 3.16: This figure shows various flexible parts which were 3D printed (yellow) to allow a close fitting model. Left: Separate flexible regions (Superior Medial Protocerebrum, Mushroom Body, Left/right Crepine regions, Saddle/AMMC (left to right). Right: Flexible regions combined with some of their neighbours where we use both solid and flexible Mushroom Bodies.

3.7 Printing and Building

Once all of the preprocessing of the data was complete, each region was exported as an STL for both halves of the fly brain. The following sections outline the printing and building phase respectively.

3.7.1 Printing

The 3D printer primarily used for fabricating each region was the *Wanhao* printer as illustrated in Figure 2.6 (left). *Inventor* was used to help decide the colours to use for fabricating each region (see Figure 3.17). We had available a total of 10 different colours for printing: white, black, blue, green, red, yellow, orange, pink, purple and silver. All of these colours were already available to use in the Fabrication Lab at the University of Edinburgh, apart from pink, purple and silver, which we ordered from our supplier (http://www.printme3d.com/). Where possible, regions were allocated to colours such that no two neighbouring regions were the same colour. The STL meshes for the final preprocessed model constituted a total of over 400,000 triangular faces.

The price of the PLA and the Ninjaflex/TPU material (both 1.75 mm diameter) was 18/kg and 84/kg respectively as ordered from our supplier at the time of this writing. The final model used approximately 0.55 kg of PLA and 0.07 kg of TPU, including the support materials, which amounts to within 20 pounds worth of material.

The *Wanhao* 3D printer uses the *MakerBot* software to prepare files for printing (http://www.makerbot.com/). *MakerBot* allows STL files to be imported and orientated

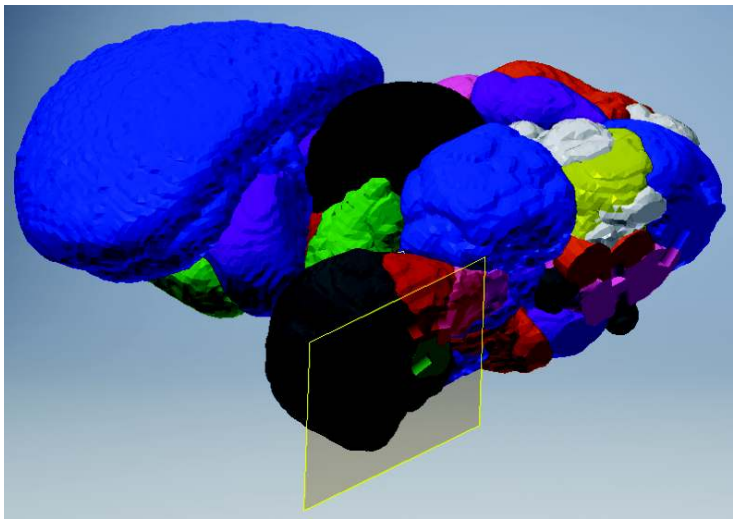

Figure 3.17: This figure shows a screenshot of the final preprocessed model within *Inventor*. This is the right half of the fly brain where the rectangular yellow plane represents the midplane where we can mirror our model. Some of the magnet placements can be seen in the midplane cross-section but most are not visible from the exterior of the model. Also, *Inventor* helped deciding on a colour scheme for the model.

on a virtual 3D printing bed as depicted in Figure 3.18 (left). *MakerBot* imports STL files, allows for manual positioning on the printing bed, processes the bodies for 3D printing and exports the job in a format which the 3D printer understands (the *Wanhao* printer uses the x3g format). Processing the bodies includes adding rafts and support structures to each of the solid bodies, as required.

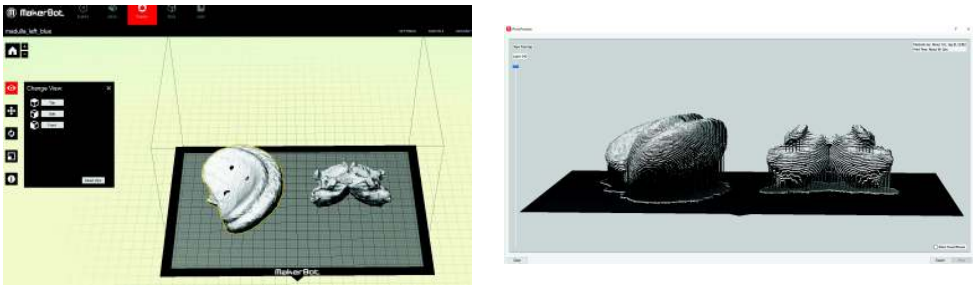

Figure 3.18: Using *MakerBot* to prepare parts for printing. Parts can be orientated on a virtual printing bed (left) and previewed with support structures (right).

A raft is a thin layer constructed at the base of a part, before anything else is printed, and the bodies (and supports) are printed on top of it (see Figure 3.11 (top left)). Primarily rafts are used for ABS (we use PLA or TPU) to help adhesion to the printing bed, but also allow for more stable printing with a stronger foundation to make for a

successful print.

Support structures are added to each body automatically by the software on previewing or exporting your print job, and consist of thin bridges of material (the same material as your object) to assist the printer's layer by layer construction. Fabricating basic objects with surfaces that do not contain overhangs relative to the printing bed, can be printed without support material. An example of an object not requiring supports is the second test model (see Figure 3.11 (top right)) which contains no overhanging surfaces as printed from the bottom up. On the other hand, almost all of the fly brain regions contain sloping surfaces which require supports to print correctly (see Figures 3.11 (top left) and 3.18 (right)).

Care had to be taken with orientating each of the brain regions such that supports were minimised in the holes for magnet placements, to make cleaning up each section easier. Additionally, it should be noted that, where possible, it is best to orientate your bodies such that supports are not present on concave surfaces, where removal of support material is harder. A printing file was created for each colour, carefully orientating each region individually. Figure 3.18 illustrates the virtual *MakerBot* environment for preparing your printing job and the preview of the output which includes the support raft and support structures.

Note, the printing settings can be altered from within *MakerBot* to alter features such as nozzle temperature, printing speed and percentage of infill. The percentage infill is the fraction of the body which makes up solid material. In our case we chose 20 % infill which means that 20 % of the interior volume of printed bodies consists of latticed scaffolding and 80 % of the interior volume is hollow. An infill of 20 % was chosen as it has provided accurate results from previous fabrications. The settings were kept consistent with default values apart from those outlined in Table 3.2.

Setting	Value
Extruder Temperature Left	0 Celcius
Platform Temperature	60 Celcius
Infill Density	20 %
Infill Pattern	Diamond (fast)
Number of Shells	3
Support Angle	48 Degrees

Table 3.2: *MakerBot* settings which were altered from default.

It is worth mentioning we had the possibility of using a soluble support printer which prints bodies in ABS material. This printer has the advantage of supports which dissolve away, which would make cleaning up the printed brain regions significantly easier. It was decided to use the *Wanhao* 3D printer as the soluble support machine would cost between 500 - 700 pounds to fabricate the model in materials, whereas we spent within 100 pounds on materials using the cheaper option. Given more time for the project, it would be interesting to investigate printing fly brain parts using different 3D printing methods, such as SLA and SLS.

Figures 3.19, 3.14 and 3.16 illustrate parts printed in standard PLA, dual printing PLA, and flexible TPU respectively.

Figure 3.19: Building the model required manually removing and polishing away 3D printer support structure. Left: Some of the solid PLA parts before support structure removal. Right: The *Rotacraft Variable Speed Mini Rotary Tool Kit* used to polish away support material (taken from the Halfords website).

3.7.2 Building the Model

Constructing the model from the 3D printed parts consisted of two phases: cleaning up each part and attaching magnets. As described in Section 3.6, there were a few regions (i.e. Saddle/AMMC, Mushroom Body, and Superior Medial Protocerebrum) which caused fitting issues along this process and were resolved by replacing these regions with flexible counterparts.

Cleaning up each part required removing support structures and rafts. Most of the support material is easily removed by hand or with pliers, but much of it remains close to the surface and inside magnet holes which needs to be removed for the model to fit properly. An electric powertool was used extensively for doing this precise polishing: the *Rotacraft Variable Speed Mini Rotary Tool Kit* (see Figure 3.19 (right)). This kit was invaluable given its many accessories for precisely polishing away the support

material in tight spaces, and was also used for expanding holes slightly in cases where the magnet did not fit into place. Figure 3.20 (right) shows a comparison between polished and unpolished parts.

The final model contained over 200 holes for magnets and most of these were utilised to form a model which holds together well. Attaching magnets involved carefully gluing magnets into place such that the correct polarities lined up. Care was also taken not to place repulsive magnet ends close to each other within the interior of parts; this way magnets help each other within single parts to stay in place, instead of working against each other. For this purpose, each magnet was marked with ink to keep track of magnetic polarities. As mentioned previously, *Loctite Powerflex Superglue* was found to be effective for adhering the Neodymium magnets to the PLA or TPU. These rare earth magnets can be cheaply purchased from many online retailers. See Figure 3.20 for a closeup of the magnetic placements. It was important to use protective gear (plastic gloves, and glasses) during these polishing and gluing tasks.

The final model consisted of 54 separate parts and is illustrated in Figure 3.21. The model uses a Flexible Mushroom Body and Saddle/AMMC to allow every part of the data to fit within the whole model. Using a solid Mushroom Body prevented all of the parts from fitting together (e.g. the Superior Clamp parts). For the most part, the model holds together very well, with a few small exceptions where parts pull away slightly from their intended connections. Given the intricate and close fitting nature of the data, it is unsurprising that certain sections do not perfectly connect.

Figure 3.22 (left) shows the model with the Optic Lobes disconnected from the central body. Figure 3.22 (right) shows the back section of the central body, with the Mushroom Body and some of its surrounding structures. Figure 3.23 shows frontal sections of the central body to illustrate how the various parts fit together with small regions (e.g. the Epaulette and Cantle parts) concealed by surrounding parts. Figure 3.24 illustrates how the model can be broken down into various sections. Figure 3.25 shows the model in its completely dismantled state.

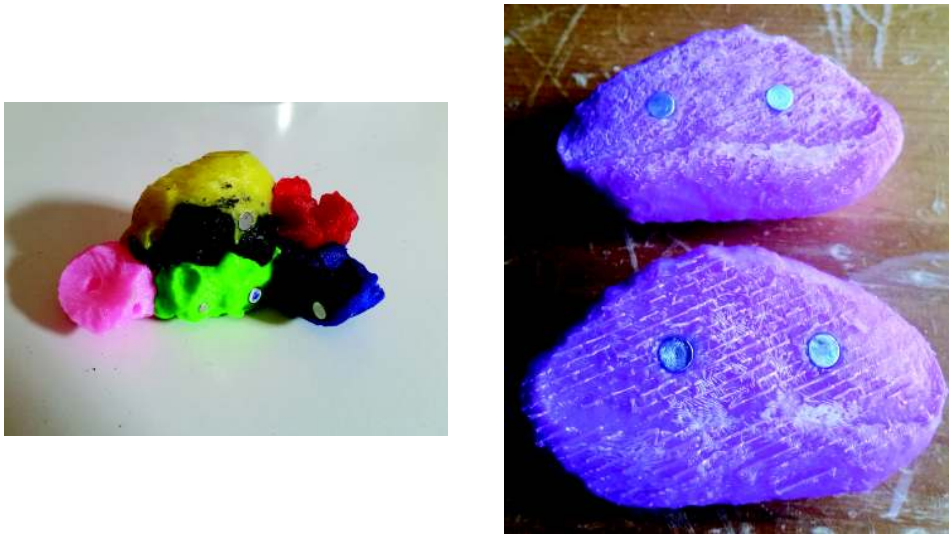

Figure 3.20: These images show parts from the final model. Left: Closeup of parts where you can see the various magnet placements. Right: Lobula parts with polished (top) and unpolished (bottom) surface for comparison.

Figure 3.21: These images illustrate the final fly brain model in its completed form as viewed from above (left) and below (right).

Figure 3.22: The final model contains 54 detachable parts representing 36 distinct brain regions. Left: Optic Lobes detached from the main body. Right: The aft section of the model with the various neighbours of the Mushroom Body (yellow). The Crepine parts are also yellow, as illustrated in Figure 3.15.

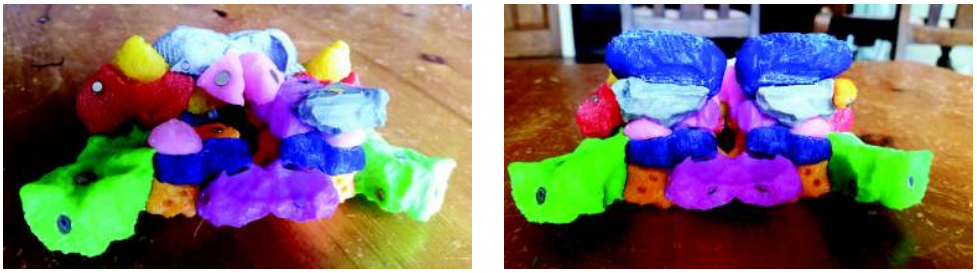

Figure 3.23: These figures depict the font section from the final model with various regions detached. The model is close fitting and reflects the original data very well for teaching purposes. The modular nature of the model works well.

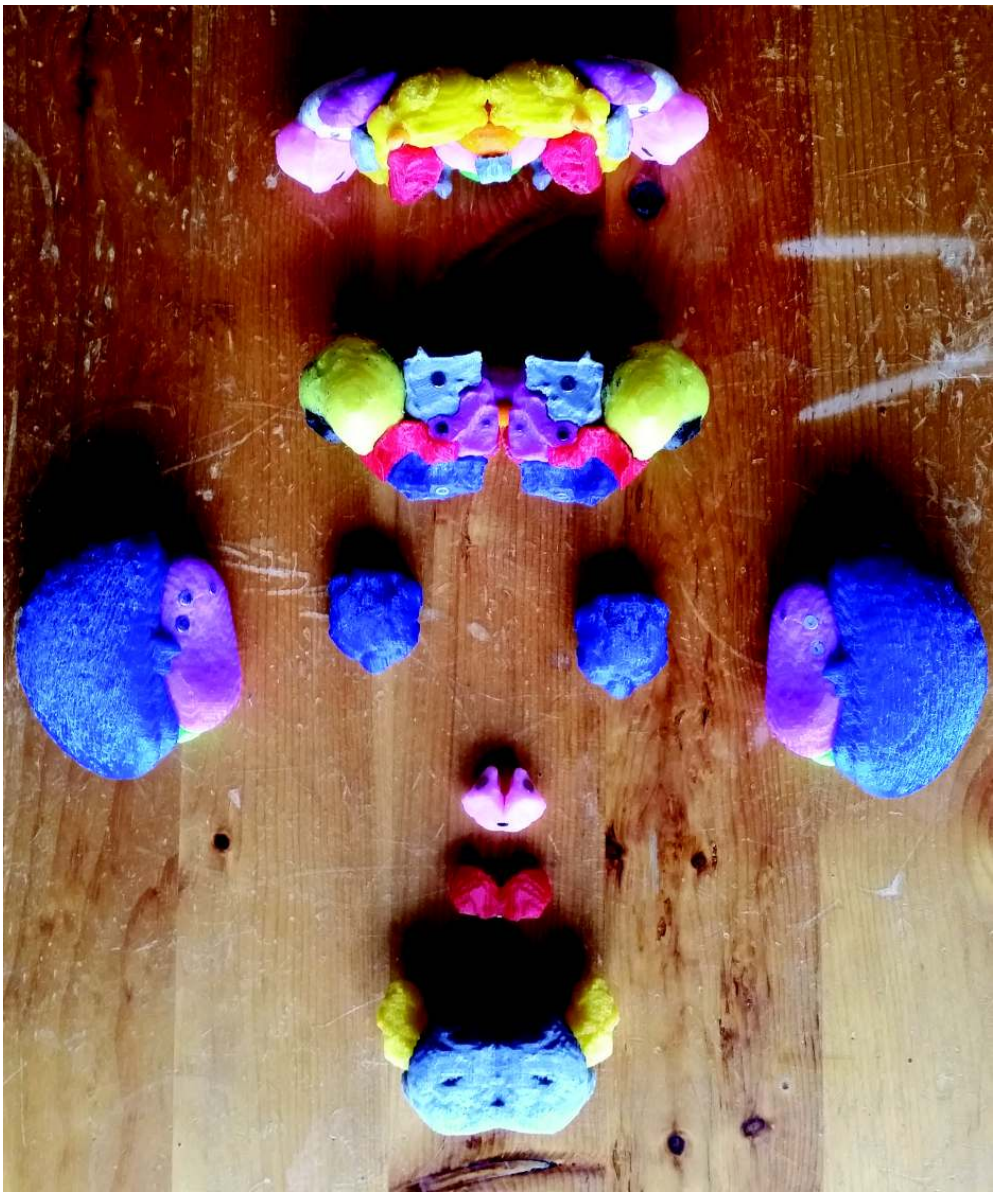

Figure 3.24: The final model breaks down into sections effortlessly and parts easily re-assemble to cohere in an easy way which promotes engagement and tactile interaction.

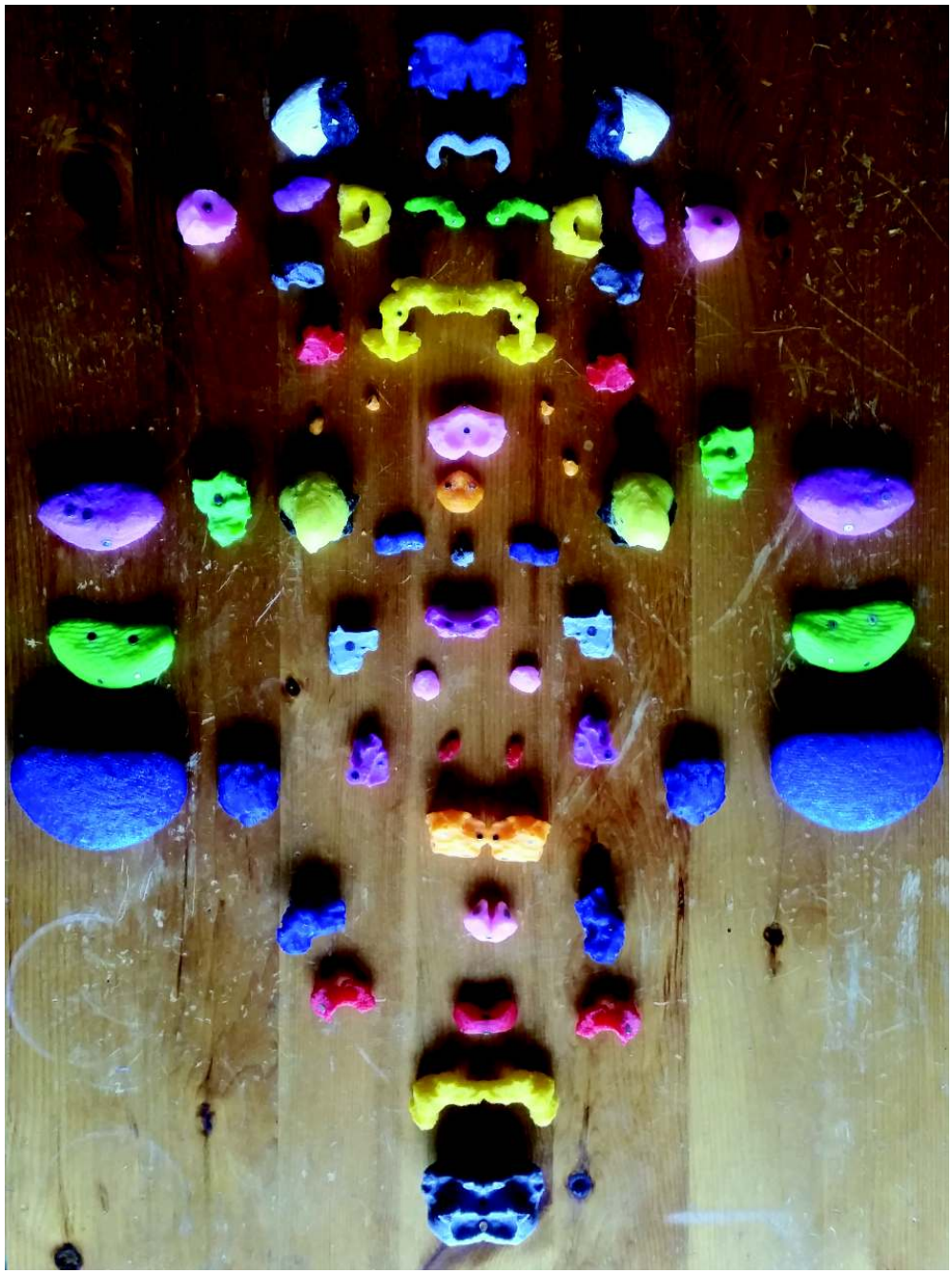

Figure 3.25: This figure illustrates the final model completely broken into its 54 individual parts (roughly placed relative to assembly order).

Chapter 4

Evaluation

The model created as depicted in the previous chapter was made with a high level of detail and precision, which reflects well on the original data. The aim of this MSc project was to evaluate its potential as a tool for researchers working with *Drosophila* or similar species, and to discover the problems, capabilities and limitations of constructing a 3D printed model of a regionalised fly brain. As described in Section 3.4 a slight altering of interfaces between regions was necessary to produce a model which fits together properly, and as such provides a useful tool. At the point of finalising the model's construction, we wanted to evaluate the model based on its utility to researchers who work with *Drosophila*.

As described in Section 2.2, numerous 3D printed models have been created for various purposes, from outreach and education, to research tools. We wanted to discover the usefulness of the fly brain model primarily as a learning and research tool. For this purpose, we brought together six researchers who work with *Drosophila* for an evaluation session to interact with the model and our qualitative analysis of the model is largely based on their judgement and engagement.

The evaluation session took place on the afternoon of the 8th of August 2016, at the Hugh Robson building of the University of Edinburgh's George Square campus. The participants' research areas were either directly associated with the fly brain or in an area directly related to fly bioinformatics, and thus were good candidates for this evaluation. The general format for the session consisted of presenting the project and its goals to the participants, allowing considerable time for their interaction with the model (as a group and individually), and finally asking each participant questions about the model individually.

The participants were very interested and engaged with the model as can be seen

in Figure 4.1. The interactions illustrated clear utility in at least capturing user imagination and curiosity about the structure of the fly brain.

Figure 4.1: Participants were presented with the complete fly brain model (left) and interacted with broken down sections (right) for evaluating the usefulness of the model.

Each participant was asked questions to judge the usefulness of the model as a research or learning tool. These questions were primarily broken down into:

- Is there anything in this model which looks wrong to you, or might cause a problem as a research or learning tool?
- In your opinion, would this model help researchers new to the fly brain learn the regional structure, and if so, what features of the model do you think assists this learning?
- After interacting with the model, are there any biological features you have noticed which you did not previously know?
- After interacting with the model, do you think it would be useful in formulating research questions, or can you already think of any yourself?
- Thinking of your own research, is there anything that would be particularly useful for you to have modelled in this way?

Occasionally the participants found it difficult to answer a question as they did not feel sufficiently familiar with specifics of the fly brain. Additionally, these questions shared a common thread and sometimes resulted in similar answers being posited for multiple questions. The following discussion will take points which arose from each of these questions, combining responses from participants.

4.1 Qualitative Evaluation Discussion

What stood out the most was how the participants found engaging their physical senses in tactile interaction very useful. They liked being able to take regions apart and put them back together again; and commented on how this physical interaction helped not only with engaging their interest but in understanding how the various parts fitted together, making the anatomy more intuitive. Most commented on this interaction being a significant advantage over observing 3D structures on a computer screen. This result is in agreement with previous research on using physical models to convey complex anatomy; being able to see internal surfaces and how they spatially relate to each other is very useful [28]. The model was able to really engage with the participants, capturing their interest, curiosity and imagination.

The next point to mention is their increased state of awareness. Many comments were made of how the model had increased their awareness of the complexity of the fly brain. Some found it surprising that there were so many regions bordering each other within the anatomy and said they had a better awareness of where regions fitted into place from interacting with the model. The three dimensionality of the model helped users to appreciate that many regions are concealed within the interior brain. Additionally, participants found they had become more aware of the smallest regions of the brain (e.g. the bulb and gall), which are easy to miss from computer models (see Figure 4.2). Participants were not previously aware of the intricate shapes of the bounding regions, and were intrigued by features such as regions which seem to wrap around neighbours. Additionally, one participant noted that the model had made them aware of the flatness of the fly brain. Furthermore, it was commented that the model is useful for highlighting two regions which neighbour each other, where one has known function and the other no known function. The model has clear utility in making people more aware of the subtleties in shape and anatomical structure given the comments which were made.

A number of suggestions were made concerning areas the model could be improved upon or caused confusion. Participants expressed that some kind of instruction or code would be useful in putting parts together; knowing what goes where, while further comments were made that forcing users to construct the model by trial and error without instructions allowed for more complete learning of the anatomical structure, which is less likely to be forgotten. Most participants recognised popularly studied regions such as the Mushroom Bodies and Antennal Lobes, or obvious features such as the

Figure 4.2: Illustrating some of the smaller regions of the model: Cantle (red), Nodulus (silver right), right Gall (orange left), and both Bulb parts (orange) either side of the Fan Shaped Body (pink).

Optic Lobes, but did not possess detailed recognition of less studied regions. This is quite common amongst researchers who work in various sections of fly anatomy. It would be useful to include names or labels (e.g. acronyms) on the parts themselves to aid recognition and learning, and also to include some way of easily differentiating which half of the brain a region belongs to.

Some participants were keen to start forming connections between parts by trial and error and spatial knowledge, while others felt somewhat reluctant due to the detail and complexity of the model. It was commented that some of the smallest parts may be better combined with others to reduce the complexity and risk of confusion. It may be more useful to create multiple models with differing levels of detail; combining parts to form a more general model, which is less complicated.

Another suggested that a less bold colouring scheme would improve the model. In general, the many colours were seen as a useful feature and helped with picking out neighbours. Participants commented that someone colour blind might have trouble separating some of the bordering colours, such as red and green, although one of the participants was colour blind themselves and had no problem separating regions by colour.

The 3D printing process required horizontal stretches of support material which had to be polished down to the surface. Sometimes it was difficult to tell apart the support structure remnants from actual model data. Also, the dual colour parts exhibited abnormalities in places, where there existed specks of the wrong colour on the surface, which may cause slight confusion (see Figure 3.14). Overall these are minor points and the model was seen by participants as very high in detail and quality. The scale of the model was also commented as good, and should not be any smaller given the tiny size of some parts.

Additionally, the model exhibits empty spaces in some small volumes between regions. Many of these are in reality filled with biomaterial which is technically not a part of the brain and not included from the original data. The question is how to indicate that these spaces are not completely void within the model. One possibility would be to print these spaces as a part of another region but using a unique colour that is unassociated with any brain region.

All participants agreed that the main area of use for the current model would be as a learning tool, particularly for those unfamiliar with the brain. Additionally, it was pointed out the model would be very useful for workshops and for outreach purposes, where it helps to capture the imagination, curiosity and attention of your target audience. Everyone agrees that the model is valuable for learning fly brain anatomy quickly and that physically interacting with the model helps to form clearer memory of anatomy details than that knowledge gained from a computer model. Many brain regions, such as the Mushroom Body (see Figure 3.14), have distinctive shapes which the model helps to remember clearly.

One of the participants expressed that it would be a very useful learning tool for students entering fly brain related research groups. The model would certainly help students or researchers new to the brain realise the complexity of the anatomy in a very real and solid way, and enable faster and more concrete learning of the anatomical structure.

A learning tool is the most obvious application for this kind of model, with respect to how the whole fits together and what the shapes look like. Participants noted parts of the brain where there were horizontal lines separating regions (see Figure 4.3), and suggested that the model may be useful in terms of spurring researchers to investigate features like these. Furthermore, the straight lines suggest there may exist limitations in the way regions have been defined.

A key point which arose from two of the participants is that of assisting fly brain

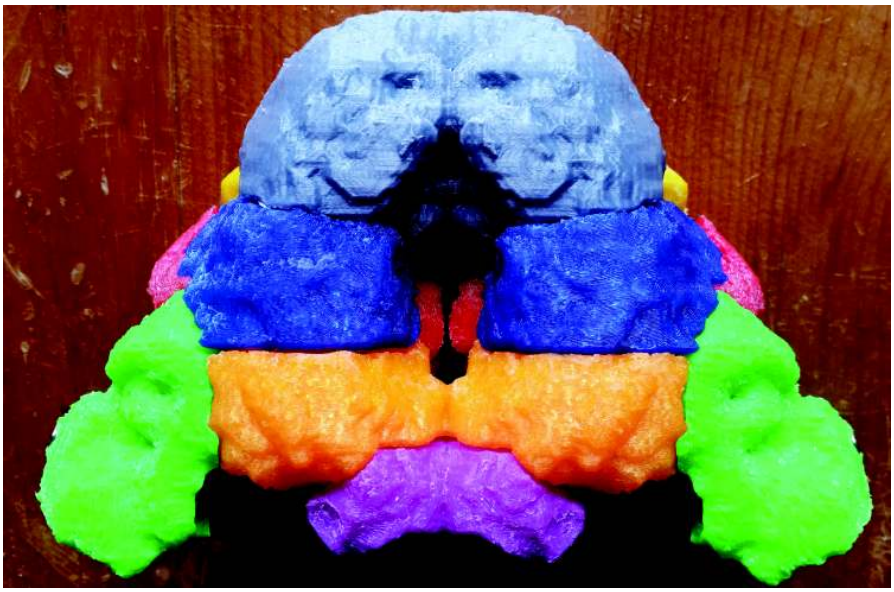

Figure 4.3: Frontal section of the final model viewed from the bottom: some regions exhibit remarkably horizontal interfaces.

dissections. A standard format is advised for dissecting the fly brain from its head capsule, which requires detailed precision and patience [39]. Often new fly brain researchers find it difficult when it comes to brain dissection, and a physical model like the one constructed would help in teaching tried and tested dissection methods in a students' surgical preparation [39]. It would also help in recognising the shapes of regions during a dissection, providing a rough guide. As described in Section 2.2, 3D printed models of intricate biological data have previously been used to aid surgeons in preparing for an operation, and a similar usefulness applies to dissecting the fly brain. In this case, also modelling the material surrounding the fly brain would be useful, as these constructs usually must be removed in fly brain dissections.

The current model seems useful in terms of aiding a researcher's spatial imagination within their thinking process, which could potentially help them towards thinking about their own research directions. The model does certainly help to increase the awareness of the fly brain structural complexity, and one can argue that this heightened awareness leads to aiding research output [20].

A number of potential research applications for this kind of physical model were suggested. Many fly brain researchers work with the vast collection of neuronal connections, and comment that a model integrating this data would be potentially of more use as a research tool. Many fly brain researchers have difficulty visualising spatially the neural connections present from region to region, and therefore this data would be

of more use to aid visualisation as a physical research tool (see Figure 2.2). A model integrating neural circuitry could be used to help understand the connections between different parts and their interaction.

Furthermore, another noted feature of the model was how the shapes of the brain regions do not seem randomly formed. It asks the questions: how are the neurons forming these regions finding their way? What process allows one region to wrap around another?

Signalling molecules aid neuron development and navigation, but there are also physical constraints applied by the parts surrounding the fly brain as well as the brain regions themselves [32]. While the 3D model constructed form this project will help researchers think spatially about the various regions, it may be worth attempting to model the physical constraints on neuronal development in a physical model, if indeed one can integrate neural circuitry sufficiently into such a construction.

One participant commented that physical models of this kind would be useful for comparing between insect species. For example, some insects (e.g. bees) display foraging behaviour and require a good map of the external world, whereas others require less function in terms of learning and memory; using a 3D model would be useful in comparing between the relevant brain regions in these functions, across species. As function varies with species, the models would be useful in investigating the variation in size and shape of the regions associated with the studied function. Another suggestion was to use this kind of modelling to print structures associated with *Drosophila* larvae which many researchers work with; and even comparing the different stages of development from larvae to adult fly.

Another potential application for this modelling approach was suggested: comparing size and shape of the brain regions which express structural mutations. Structural abnormalities could potentially be associated to different kinds of fly strains with mutations that may affect the most ideal form of genetic assessment to be performed. Further, certain fly brain regions change shape on discarding certain neuron classes and a physical model could help to understand the underlying processes.

Two of the participants suggested this physical kind of modelling could be useful applied to cilia or flagella, which are composed of many intricate structures. Cilia or flagella involve arm like structures formed from dynein, a protein which converts chemical energy into mechanical, and is used for both internal and external cellular transport and movement [11]. It is not always clear how these structures fit together and interact between one another, and a physical model may help towards visualising

how different dynein motor molecules integrate and interact. One participant noted that there now exists some very detailed data from electron tomography of cilia, so this may be a viable avenue of using physical modelling. Moreover, often robotic movement has been biologically inspired and dynein motor related model research could potentially be of use to researchers working towards creating soft robotic systems.

Overall, the participants of this evaluation found the model very useful for increasing their awareness of the brain complexity, and the anatomical structure of the various regions and how they integrate as a whole. Although the main utility of the model was envisaged as a learning tool, potential applications were suggested for further models integrating data from multiple species or even neural connectivity, which would be of more use from a research point of view.

4.2 Capabilities and Limitations

Evaluation participants were impressed with the detail of the model and how well all the regions connect together, which goes far in illustrating the viability of using 3D printing to create valuable *Drosophila* based learning or research tools. The FDM printer used for making the model utilised a resolution of 0.2 mm which was more than sufficient for creating a good quality model. Whilst 0.2 mm was enough resolution for models built in mind as a teaching tool, it seems likely that a higher resolution might be required for integrating neural circuitry cleanly into a model, depending on the desired scale. Although species such as the Squid are known to possess axon widths in the range of 0.5 mm - 1.0 mm, mostly axon widths are within micrometre scales [8]. While 3D printers exist which can print down to around 20 micrometres, these are less common and generally much more expensive to use than the basic FDM printer employed for this project [17].

Around 100 pounds was spent on ordering 3.5 kg of PLA/TPU materials in varying colours, although roughly 0.6 kg of material was used in constructing the fly brain model. Approximately 90 pounds was spent on materials and tools for creating the model (e.g. polishing tools, magnets and glue). We considered using a soluble support printer which would have resulted in a smoother finish but the costs were in the range of 500-700 pounds.

As described in Chapter 3, overlapping meshes of each region slightly limited the accuracy of the model. Moreover, the detail of each region mesh was limited by the number of faces which *Inventor* could reasonably process. A detail level of 5 was

found to work best for keeping *Inventor* stable whilst retaining a high level of detail from the original data, with over 400,000 faces in the final computer model.

Certain regions were merged to form a single part in the model as described in Section 3.6. This enabled us to showcase the capability of fabricating parts in two colours, so that users may still see where regions interface. Although this limited the functionality slightly, it also simplified the complexity of the model.

In constructing the model, certain regions (see Section 3.6), were discovered not to physically fit into their intended place. The easiest solution was to print in a flexible material (see Figure 3.16), such that parts could be flexed around obstructions and into their proper positions.

Chapter 5

Conclusions

Historically physical models have been time consuming to design and produce but with the advent of 3D printing and robust CAD software, the process is now much faster. Objects can be processed for printing using numerous CAD tools, and printed within hours of conception. The increasing popularity of 3D printing by scientists and hobbyists alike has greatly reduced the financial cost of 3D printing which was previously only feasible for established companies [18]. 3D printing allows models to be produced quickly and alterations can easily be made which has a clear advantage over traditional methods of first creating molds for parts. Additionally, we can print in various materials which allows further freedom in the design process.

Physical models are often overlooked in favour of traditional teaching and research tools, such as textbooks and computer modelling (see Section 2.2). Nature is abundant with biologically complex data, such as the structure and interaction of protein molecules, DNA/RNA structure, cilia and flagella, human organ anatomy, and neuroanatomy to name just a few. While computer models are very useful, they disregard a vital aspect of human learning with tactile interaction. Studies have shown that engaging the sense of touch enables improved learning in terms of retention, accuracy and speed; and especially helps those with less than average spatial processing abilities [28, 21].

Another example of complex structural data is that of the *Drosophila* brain, which consists of 41 clearly defined and non-overlapping regions [22]. The goal of this project was to investigate the viability of creating a 3D printed regionalised model of the fly brain, and whether it is useful to researchers working with *Drosophila*. As described in Chapter 3 and illustrated in Figures 3.21, 3.24 and 3.25, such a model was created within roughly two months of the time available to this project.

Work involved converting the volumetric brain regions into an STL format, using the *3DViwer* plugin of *ImageJ* which uses a thresholded marching cubes algorithm [19, 31]. The main problem encountered was this conversion was not entirely precise, and generated surfaces which overlapped their neighbours slightly. This was countered by using CAD software to correct these overlaps directly, but further work could investigate avoiding these overlaps entirely. Experimenting further with the thresholding value used by the marching cubes algorithm may be worthwhile or possibly using a variation of the algorithm.

The fly brain STL files chosen for final modelling (see Section 3.1) were very detailed and it was interesting to note the capability of *Inventor* in processing this data, which although an extreme example of its application, was roboust and stable enough to handle the required processing.

We aimed to produce a model which balanced between utility (regions which fitted together properly) and reflection of the original data. The model was presented to several *Drosophila* researchers for evaluating the usefulness of the model in a qualitative manner (see Chapter 4). Results indicate that the model would primarily be useful as a learning tool for both experienced and new researchers of the fly brain. Tactile interaction with the model helped significantly with engaging a user's interaction with the brain structure. Evaluation participants agree that a physical model helps users to remember the anatomy more permanently and in more detail than would be the case of learning from computer models. Furthermore, the model would be useful in aiding fly brain dissections.

The final model was created with over 100 cylindrical magnets for adhering regions together. The 3D printing process was limited in terms of a 0.2 mm resolution used for this project, and altogether materials and tools came to within 200 pounds. In particular, leeway had to be modelled into the circular magnet placement holes.

Given more time for this project, we suggest laser scanning parts of the model to provide a computer model which can be compared to the original data, and which also provides a reasonable baseline for future physical models made in a similar fashion.

In our case, it appears that 3D printing is somewhat less accurate when it comes to printing circular features, than is the case with straight features likely due to the grid constrained movement of the 3D printing gantry [41]. Further work should investigate whether this limitation is unique to FDM printers or not. Qualitative discussion with *Drosophila* researchers indicated various improvements which could be implemented in a future model, from construction instructions and part labels, to making a model

with less parts for learning.

Although the model's main use lies with teaching, other areas of use were suggested from interspecies comparison, comparing structural mutations and modelling the interaction between dynein motor molecules in cilia or flagella. It should be noted that much more data is available with regards to the *Drosophila* brain and surrounding anatomy, such as protein expression patterns and neural connectivity [22]. Further work could explore integrating such elements into an additively fabricated model, and use a transparent material for maximising the visualisation of neural tracts. In particular, researchers find it difficult to visualise the neural connectivity of certain classes of neuron which innervate multiple brain regions, and a model illustrating this could be helpful to aid visualisation. We suggest developing an automated process where neural data could be quickly fabricated into a physical model, and hope this project has laid some groundwork towards such an endeavour. Moreover, future work could investigate the possibility of automating the design process outlined by this project in fabricating physical models.

Further work could involve researching a better colour scheme for the model. One possibility is to use the LAB colour space for improved visual clarity between regions [35].

Additionally, further work could investigate printing methods (e.g. SLA) capable of higher resolutions, and that would be more useful as a research tool. The *Photonic Professional GT* 3D printer by *Nanoscribe* is capable of sub-micrometre resolution, which is within scale of a typical axon width [8, 6]. Although such equipment (the *Photonic Professional GT* currently values at around 200,000 pounds) is expensive, potentially this technology could become more affordable in time. It is interesting to note that if we take the width of the fly brain to be approximately 0.6 mm, the final model depicted in figure 3.21 is roughly (300/0.6 mm) 500 times larger in scale. At this scale a typical axon width is around 0.5 mm, and certainly within the modelling capabilities of affordable 3D printers.

Appendix A

Appendix 1: NRRD Filename Mapping

File Domain Number	Biological Domain Name
2	Accessory Medulla (Part of Medulla)
3	Lobula
4	Nodulus
5	Bulb
6	Protocerebral Bridge
7	Lateral Horn
8	Lateral Accessory Lobe
9	Saddle
10	Cantle
11	Antennal Mechanosensory and Motor Center
12	Inferior Clamp
13	Vest
14	Inferior Bridge
15	Antler
16	Crepine
17	Pedunculus of Adult Mushroom Body
18	Vertical Lobe of Adult Mushroom Body
19	Medial Lobe of Adult Mushroom Body
20	Flange
22	Lobula Plate
23	Ellipsoid Body

24	Adult Antennal Lobe
25	Medulla
26	Fan Shaped Body
27	Superior Lateral Protocerebrum
28	Superior Intermediate Protocerebrum
29	Superior Medial Protocerebrum
30	Anterior Ventrolateral Protocerebrum
31	Posterior Ventrolateral Protocerebrum
32	Wedge
33	Posterior Lateral Protocerebrum
34	Anterior Optic Tubercle
35	Gorget
36	Calyx of Adult Mushroom Body
37	Superior Posterior Slope
38	Inferior Posterior Slope
39	Superior Clamp
40	Epaulette
49	Adult Gnathal Ganglion
50	Prow
51	Gall (Part of Lateral Accessory Lobe)

Table A.1: Nrrd regional file mapping for biological names.

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