IMPACT: Invoking Measures to Piece Autism Connectomics Together

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1 Introduction

Affecting 3.5 million people around the world, autism is a developmental disorder that often affects a person's ability to communicate and socially interact with others. [1] Over the course of research on autism, there has been a long history for the search for knowledge on the biological connection between the amygdala and autism. [2] While research in the past has compared the behavior of people with autism to people without the disorder, current research lacks information on the connectome of the amygdala for those with autism. Because autism is often considered "a disorder of brain connectivity," understanding the neuropathology of the brain influenced by autism is the next step in understanding and eventually finding treatments for the autistic.

As far as general wiring goes, the brains of neurotypical individuals are fairly standardized. This is not the case for those diagnosed with Autism Spectrum Disorder (ASD). Some studies have found that ASD brains have higher connectivity, while conflicting studies have concluded that they have fewer connections. Our study will be able to reconcile those opposing results, as it has found that ASD brains are not only wired differently from neurotypical brains, but have a number of idiosyncrasies when compared to one another as well. This could help explain behaviors at different points on the autism spectrum [14]. Previous research has shown that there are less neurological connections in the brain of an autistic person [3]. Current efforts in autistic research and the Autism Brain Imaging Data Exchange are aimed at enhancing our understanding of the connectomes found in brains that suffer from autism [4]. Because it is hypothesized that there are abnormal neural connections in autism spectrum - and research has already indicated this on the macroscale - IMPACT will attempt to map the connectome of the amygdala of a brain with autism spectrum disorder. This mapping would facilitate our understanding of the amygdala and its role in autism, as well as facilitate some identification of neurobiological subgroups and sex differences in autism [4].

Question: How do the connectomes of the amygdala differ between those with autism and those without?

2 Methodology

To research the relationship between the amygdala and autism spectrum disorder, we will conduct gene editing and high-resolution imaging. Because no techniques have currently been observed that allow us to map the connectome of a human, this research will begin at a smaller scale: mice. A mouse was chosen because of the extended knowledge and data already observed and recorded with regards to its connectome [5] Half of the mice will be genetically modified using CRISPR to induce autism [6]. By knocking out the CHD8 gene, we will be able to test on autistic mice [7] After inducing autism in the brain, they will be allowed to mature. The mice would have to be tested and observations will be recorded with respect to how they are behaving. Then, brain tissue from the amygdala will be extracted from each mouse and imaged using the Talos TEM. The data will be collected, processed and stored as described in the proposal below. By extracting the information from these images, we will be closer to mapping the connectome of mice with autism, comparing and understanding the relationship between the amygdala and autism, and ultimately looking for differences between autistic and non-autistic brains.

3 Hardware + Facilities

3.1 Talos F200C TEM for Life Sciences [11]

Since this is an electron microscope, this technology is a worthwhile investment. It creates 3Dl images of molecules and cells, which is perfect for the experiments at hand. It offers a range of applications, is easy to use by scientists, processes images fast by reducing the obstacles in the process, and provides users with models to further scientific discovery. Only one is required for the experiment since it has such capabilities and since it is so expensive. To obtain images of the amygdala of mice with and without autism, such an electron microscope with precision and magnification is required. Minute differences between autistic mice and non-autistic mice will have to be noticable, and this electron microscope is ideal for specific results.

3.2 AberSAN ZXP4 [12]

This technology is incredible because it has unlimited storage opportunity. In addition, this hardware specializes in storing software-based big data. Hence, it is perfect for the kind of data that the experiments that will be conducted will yield. The data from the electron microscope as well as the processed CIFTI files can be stored here with as many backups as needed since the storage is expandable. The unlimited storage also accounts for the multitudes of experiments that can occur; even if there are more conducted than anticipated, this hardware is capable of handling the data.

4 Data Collection Processes

CIFTI is a file type composed of a matrix of values and a CIFTI XML which describes the matrix of values [13]. According to the CIFTI working group, "each dimension of the matrix uses one of the several possible mapping types." This format was developed and utilized for the Human Connectome Project, which means that CIFTI files are a common and standard file type for brain mapping. The need for such an image processing file type was recognized to analyze large amounts of data from MRI and tractography. Once the brain maps have been completed by the software, the information can be stored in the ZXP4. Since the hardware has unlimited storage capabilities, there should be no problem with large file sizes. Additionally, more storage can be purchased if necessary. With this, the data from the electron microscope and the CIFTI files will all be stored and can be used for the subsequent steps of the experiments.

5 Information Extraction Processes

There are two major technical considerations we need – converting images obtained from electron microscopy into connectome graphs and comparing graphs of different amygdalas to one another.

To convert images to graphs, we can use the image-to-graphs framework from Roncal et al. [8]. This process first does segmentation - finding neurons and synapses in the images. We find neurons in the image using machine learning; one option is the Gala library [9]. Synapses in the image are also found using machine learning (random forest classifier). Then neurons and synapses have to be associated with one another; Roncal et al. suggests doing this by finding "neuron labels (i.e., graph nodes) that overlap most frequently with the labeled voxels from each synapse object". With this information we can generate the connectome graphs where neurons correspond to nodes i the graph while synapses correspond to edges connecting two neurons together.

Overall, we will be graphing many amygdalas and we need to be able to compare the amygdalas of healthy mice to mutant mice with autism. We can do this based both on overall number of synapses (in autistic amygdalas we predict there to be more) and based on differences in neural circuits between the two.

By mapping multiple mouse brains in both control and experimental groups, we can try to ensure that there is a significant difference between the two groups; however, because mouse amygdalas have not been mapped often before, it is unknown how much variation there may be in synapse number and neural circuitry between amygdalas of different healthy specimens.

6 Data Upload

6.1 Open Connectome Project [10]

Modern science is faced with complexities of dazzling proportions. Yet, only a small number of people interested in these goals are actually able to make significant contributions, often due to lack of access. With the open source project, we can upload their image data with one-click, and it will be incorporated into the database and processed automatically. Other researchers can then run analyses on their data, compare it with the datasets available, and offer amendments.

6.2 ABIDE [4]

Data from the ABIDE repository has been used by researchers spanning a broad range of scientists, disciplines and countries to inform our understanding of the neural bases of autism, as well as to promote biomarker discovery and innovation of imaging analyses methodologies.ABIDE I mainly focussed on the feasibility and utility of MRI data. However, the complexity of any connectome, along with the substantial heterogeneity of Autism Spectrum Disorder (ASD), highlight the need for even larger and better quality samples. ABIDE II was established to further promote discovery science on the brain connectome in ASD. To date, ABIDE II has gathered over 1000 additional datasets with greater phenotypic characterization, mainly those of ASD and associated symptoms. This data has been openly released to the scientific community. According to HIPAA guidelines, 1000 Functional Connectomes Project, and INDI protocols, all datasets are anonymous, with no protected health information included.

7 Data Storage

AWS Import/Export Functionality

Standard Storage	Standard
First 50 TB / month	$0.023 per GB
Next 450 TB / month	$0.022 per GB
Over 500 TB / month	$0.021 per GB
PUT, COPY, POST, or LIST requests	$0.005 per 1,000 requests
GET and all other requests	$0.004 per 10,000 requests

8 Cost

Item	Unit Cost	Quantity	Total Cost
Principal Investigators	\$350,000/year	5 years, 20 people	\$35,000,000
Co-Investigator	\$150,000/year	5 years, 100 people	\$75,000,000
Research Assistants	\$80,000/year	5 years, 1200 people	\$480,000,000
Computer Scientists	\$145,000/year	5 years, 200 people	\$145,000,000
Non-Technical Employees	\$50,000/year	5 years, 150 people	\$37,500,000
Lawyers	\$400,000/year	10 people	\$4,000,000
Control Mouse Embryos	\$2,000/embryo	10,000 mice	\$20,000,000
Genetically Modified Mice	\$5,000/embryo	10,000 mice	\$50,000,000
Plasmids	\$300	12000 mice	\$3,600,000
Artificial Cerebrospinal Fluid	\$135/25mL	100 mice/day	\$49,275,000
Thermocycler	\$50,000	20	\$1,000,000
Centrifuge	\$520,000	20	\$11,000,000
Talos L120C TEM	\$1,000,000	10	\$10,000,000
TEM Service Contract	\$250,000/year	5 years, 10 TEMs	\$12,500,000
Consumables	\$60,000/year	5 years, 10 TEMs	\$3,000,000
AberSAN ZXP4 storage unit	\$197,605	2	\$395,210
Expandable to 7.2PB of Raw Data Storage	\$300,000	1	\$300,000
Storage per Month	\$0.021/GB/Month	5 years, 4 PB	\$25,200,000
Tianhe Super Computer	\$400,000,000	2 computers	\$800,000,000
Maintenance of Computer	\$40,000,000/year	5 years, 2 computers	\$160,000,000
21.5-inch Mac	\$2,000	300	\$600,000
Dell 34 UltraSharp Curved Monitor	\$1,000	300	\$300,000
Electricity	\$5,000,000/year	5 years	\$25,000,000
Natural Gas	\$1,000,000/year	5 years	\$5,000,000
Food	\$6,860/year/person	5 years, 1490 people	\$51,107,000
Building	-	-	\$2,000,000,000
Miscllaneous: Transportation, Books etc.	\$25,000,000	5 years	\$125,000,000
Lab Equipment	-	-	\$50,000,000
Software Licenses	\$1,000/software	5 programs, 1490 people (est)	\$7,450,000
Miscellaneous Equipment	\$5,000,000	5 years	\$25,000,000
Human Trials - Brains	\$5,000	1,000 brains	\$500,000
Neurosurgeons	\$600,000/year	5 years, 10 people	\$30,000,000
Doctors	\$300,000/year	5 years, 50 people	\$75,000,000
Sales Tax	9%	-	\$186,542,750
Property Tax	2.5%	5 years	\$280,700,000
Grand Total			\$6,232,447.0

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