Johns Hopkins University

Introduction to Connectomics

Comparing Addicted Brains

Author:
Brandon Lim
Frank Bu
Frank Miao
Kavya Tumkur

Professor:
William Gray Roncal

January 16, 2018

1 Scientific Question

Our goal is to investigating the effects of opiate abuse on synapses within the hypothalamus of mice, for future implications of addictions in humans. Our expectation is to see less synapses (low synapses density) in addicted brains.

2 Hardware + Facilities

Our research requires the following:

- Transmission electron microscope: needed to analyze brain matter at a microscopic, synaptic level. There exist previous studies on how drugs affect the brain on a macro level; however, our research is concerned with the microscopic level.
- Ultramicrotome: used to cut mouse brain samples into thin sections for detailed analysis with the transmission electron microscope.
- Diamond cutter: required to cut brain samples in ultramicrotome.
- Mice: initial test subject. Used due to their similar anatomy to humans and inexpensiveness.
- Opioid: drug to be tested. Chosen due to the controversy surrounding epidemic of opioid painkiller abuse in the United States.
- Computing: for collection and analysis of data. For this project we will use 27 Titan Graphic Processing Units (GPUs). Despite their name, Titan GPUs are regularly used for AI and machine learning applications.
- Lab Space: facility needed to store lab equipment and conduct experiments. For the sake of storing as many mice as needed, in a very controlled environment, a well-kept, large space would be needed.

3 Data Collection Processes

Mice will be split into 5 groups. The first group will have 80 mice and the other four groups will have 180 mice each.

- First group will be control group,
- Second group will be given opiates daily for half a year. Within this second group, the mice will be split into 3 subgroups. The first subgroup will be given a small dosage of opiate, the second subgroup a small dosage, and the third a large dosage.
- Third group will group will be given opiates daily for a year. We will have 3 subgroups within this group, similar to what we did for the second group.
- Fourth group will be given opiates daily for a year and a half. We will have 3 subgroups within this group, similar to what we did for the second and third groups.
- Fifth group will be given opiates daily for two years bodies. We will have 3 subgroups within this group, similar to what we did for the second, third, and fourth groups.

In each group, only half will be dissected. This is to account for any accidental deaths, or possible outlying mice. Therefore, we will only use 400 mice brains for the rest of the experiment.

Since the mouse brain is 1 cubic centimeter, the hypothalamus of the mouse is approximately 2 cubic millimeters. We intend to create a connectome for each mouse's hypothalamus, which will produce 800 cubic millimeters of data. After the allotted time for each group, the mice will be dissected to extract the hypothalamus. The hypothalamus was chosen, due to the many motivation and pleasure associated functions it controls. Then we will slice the hypothalamus into sheets of roughly 1 micrometer using an ultramicrotome. These sheets will be further cut in to ultra-thin sheets using a diamond cutter.

Transmission electron microscopy will be performed on these sheets. We decided to use transmission electron microscopy (TEM) instead of scanning electron microscopy (SEM), because from our research we found that SEM yields images with coarser spatial resolution than TEM does. Since the hypothalamus of the mouse is so small, we think the image quality should be as precise and detailed as possible. TEM also allows faster imaging than SEM due to the fact that the sheets used for TEM are thinner than those used for SEM (though cutting these ultra-thin sheets for TEM can take a long time).

4 Information Extraction Processes

- Use VESICLE (Volumetric Evaluation of synaptic Interfaces using computer Vision at Large Scale) to obtain a detailed mapping of mouse's brain. VESICLE is an approach for mammalian synapses detection with high accuracy. The main functionality of the approach is to capture unique features of synapses (dark, fuzzy voxels that forms an ellipsoidal collection) using deep learning classifier and random forest classifier.

- Use the automated images-to-graphs pipeline to convert EM images to brain graph, which helps us better understand the connection. This fully automated pipeline will convert image data acquired from EM to brain graph, which gives us a clean map consisted of nodes and edges. From this graph, we can focus on the connectivity properties. This will gives us further analysis because we are able to see the connectivity of each node directly.

- Statistical Analysis: By using the random forest classifier (because of its scalability advantage), we are able to detect and map all found synapses in a 3D volume. We will divide the hypothalamus into regions, and then compare the synaptic densities of each mouse group by their specific region. We will identify regions that show a strong correlation between synaptic density inclusion of the word "graphic", Titan GPUs are regularly used for AI and machine learning applications.

- Lab Space: facility needed to store lab equipment and conduct experiments. For the sake of storing as many mice as needed, in a very controlled environment, a well-kept, large space would be needed.

5 Data Sharing

To upload data, we choose Amazon Snowball, which is designed for petabyte data transportation. In terms of data upload and data storage, to enable further research into the effects of opioids on brain matter in the microscopic level, analyzed data will be available on an Amazon Web Services (AWS) S3 Server. Other than the potential for collaboration, other advantages of using an AWS server for this project include its flexibility and delegates server maintenance and security to a well-regarded third party.

6 Cost

- Buying 800 adolescent mice will cost $12,000
- It takes roughly $1 per day to take care of one mouse, including food and water, so taking care of the mice over the span of the experiment will cost about $1,000,000
- The median salary of a clinical laboratory technician is about $39,000 per year. We will hire 30 clinical laboratory technicians to give the different mice groups the drug treatments. Since the mice will be kept in the laboratory for up to 2 years, paying these 30 employees for 2 years will cost about $2,340,000.
- The transmission electron microscope will cost $1,000,000
- The cost of 80 mg of OxyContin is about $6 (when legally purchased). Therefore 80 grams will cost about $60,000.
- 1 cubic millimeter of data needs 2 petabytes of storage. It costs about $10 million to store 1 cubic milimenter, or 2 petabytes, of data. Therefore, to store 800 cubic millimeters of data it will cost about $8,000,000,000.
- The cost of renting a well kept facility is roughly $80 per square foot per month. With an estimated need of 1000 square feet for this experiment, a budget of $5,000,000 will be allocated for renting a lab space.
- To head the project, we will hire a team of 10 expert researchers, each with a stipend of $300,000 a year. In total, this will cost $9,000,000 for the 3 years that we expect this experiment to take place for.
- All of this amounts to a total cost slightly less than $9 billion, which fits our budget.

7 Feasibility

We believe this experiment is feasible. It makes sense to first run a test using mice, and only extend this experiment to humans if a significant association between drug usage and synaptic density is discovered, since human drug-addicted brains donated to research are harder to come across. We also came in close to $9 billion, giving us $1 billion of wiggle room in case something in our experiment goes awry. This experiment is also set to take about 3 years, which means it won't exceed the 5 year limit.

8 References

Bureau of Labor Statistics. "Medical and Clinical Laboratory Technologists and Technicians : Occupational Outlook Handbook." U.S. Bureau of Labor Statistics, U.S. Bureau of Labor Statistics, 15 Dec. 2017,
http://bit.ly/2mFgDmP

Catlin, Wil. "Boston Continues to Lure Large Corporate Tenants." Boston Office Spaces, Boston Reality Advisors, 18 Sept. 2017,
blog.bostonofficespaces.com/category/lab-space-in-cambridge/.

Filbey, Francesca M. "An Introduction to 'The Addiction Connectome: Brain Connectivity in Drug and Alcohol Addiction.'" Taylor and Francis, 7 November 2013.
www.tandfonline.com/doi/abs/10.3109/00952990.2013.856661.

Gray Roncal W., Pekala M., Kaynig-Fittkau V., Kleissas D. M., Vogelstein J. T., Pfister H., et al. (2015). VESICLE: volumetric evaluation of synaptic inferfaces using computer vision at large scale. BMVC (in press).

Gray Roncal W, Kleissas DM, Vogelstein JT, Manavalan P, Lillaney K, Pekala M, Burns R, Vogelstein RJ, Priebe CE, Chevillet MA, et al. (2015) An automated images-to-graphs framework for high resolution connectomics. Front Neuroinform 9:20. doi:10.3389/fninf.2015.000

Grueter, Brad A, et al. "Integrating Synaptic Plasticity and Striatal Circuit Function in Addiction." Current Opinion in Neurobiology, Elsevier Current Trends, 12 Oct. 2011,
www.sciencedirect.com/science/article/pii/S095943881100153X.

"Hypothalamus Anatomy." Overview, Gross Anatomy, Microscopic Anatomy, 11 Dec. 2017, emedicine.medscape.com/article/1949061-overview.

Silvestri, Ludovico, Leonardo Sacconi, and Francesco Saverio Pavone. " The Connectomics Challenge ." Functional Neurology 28.3 (2013): 167 – 173 . Print.