Exploring the Hemibrain

Team Tiny Brain/Not Fly Scientists

Programming and Systems for Data Analysis

CS5010

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Final Project Write Up

Introduction:

What happens when you combine biologically inclined students with non-biology students? You end up trying to find a dataset that is beginner friendly, understandable, and interesting enough that it can keep someone's attention. So I ask, do you want to learn about the fruit fly brain? One of the most common questions is simply, "Why a fruit fly?". It is with this question that we hoped to find some broad and general answers in our data set.

It took some time and many data sets to narrow down and a data set that the team could agree on. Due to a team member day to day work we were able to agree on using the hemibrain dataset provided by Howard Hughes Medical Institute's Janelia Research Campus. It was in this process that two members, Chelsea and Matt, discovered they worked for the same employer (HHMI). Chelsea's work directly involves the dataset we chose while Matt is involved in headquarters software development. Once we decided on our final dataset we hoped to find meaningful data from the expansive data set. Since no one on the team is a fly scientist this resulted in the team taking an exploratory data analysis approach. Our final approach was guided by the idea of presenting this data in a digestible format for non-biologists which was inspired by our non biology members.

Data Source/Cleaning:

Our data set of choice is that of the hemibrain of the *Drosophila Melanogaster* (Fruit Fly). This data set belongs to a female adult fly brain. The data set was obtained via use of the Janelia Research Campus Neuprint database which can be accessed via their web interface and the Neuprint-Python API which was used for the majority of the project. The data set is a connectomic data set containing electron microscopy imaging data of all of the right side, and portions of the left side neurons, their connections (synapses), and region of interest data (neuropils and tracts) of the hemibrain. While the hemibrain is not the whole brain, it contains many large ROI's, including sub regions, used frequently in fly research such as the Central Complex (CX) and the Mushroom Body (MB). The significance of the data set is more towards the general area of connectomics. It is a small scale, yet complex, model for applying connectomic methods to larger data sets such as mammals like the mouse and ultimately the human brain. In a more practical application, currently there are multiple labs at Janelia and abroad that use the data set to perform research on circuits and connections involved in behaviors such as memory, navigation, and reproduction behaviors.

This data set was chosen for a few reasons. One of the main reasons comes down to the amount of data provided in an accessible manner through Neuprint. While the provided data set is not of the full brain it is still considered to be the most complete connectomic data set for a brain to date. Another reason was due to the familiarity one of our group members has with using the data set since it is a product of that group members day-to-day work. A third reason this data set was chosen was due to the ability to perform exploratory data analysis on such an expensive and well documented set of data.

Data Cleaning on our data set was conducted primarily to reduce the size of the data and keep the most relevant information for analysis. One example came when analyzing two different versions of the data set for ROI completeness. The oldest (V.1.0.1) data set had two ROI's (AL-DC3(R) and FB-column3) for synaptic sites that were listed as infinity. In order to prevent these values from affecting summary statistics these ROI's were removed from the data set. Additional cleaning was conducted on the full hemibrain neuron list. Firstly, the data file was exported in a JSON file which was converted into an easily understood data frame. For the purposes of this project we filtered out all no status, orphan, left side, leaves, and unimportant bodies leaving us only Traced and Roughly Traced bodies. This reduced the original list from 186,684 bodies to approximately 20,000 bodies for analysis. Additional cleaning was

conducted to remove columns that did not provide relevant information to our analysis such as soma size and soma location.

Experimental design:

The general approach the group took when looking at this data set was an exploratory one. We did not have a central research question due to our exploratory approach which gave us the freedom to explore any interests that each member had. One of the main goals developed was conducting a validation on the database. We were interested in confirming easily identifiable attributes, as defined by literature outside of the dataset. Queries were developed to make sure that the attributes matched what was provided in the dataset. From this line of research an additional foray into Graph Theory was introduced into the project for connectivity. The exploration of connectome properties through graph theory has wide applications in neuroscience (Vecchio 2017). Measurement of node centrality through multiple measures can help elucidate structure function (Sporns 2018). Centrality measures are usually positively correlated in graphs, but the hierarchical structure and "rich club" organization of neural connectomes means different measures of centrality may be substantially different (Sporns 2018). A very introductory graph theoretical analysis using the networkX library was decided on for this project. Focus was shifted to the Janelia pre-computed cross-ROI connectivity data after computing slightly different connectivity data for 20 ROIs took the server over 20 hours. Estimated time to return the full set of data for this analysis is weeks. Exploration of whether the conclusions differ when considering only connectivity to neurons within a brain region that also output to that brain region will have to wait for someone with more computational resources to devote to the problem. We can see why Janelia has cached pre-computed values on the server. Focus was on producing pleasing graph visuals and summaries of some basic node properties in order to present to an audience of our classmates in an interesting way.

In order to obtain connectivity data in a usable form for graph analysis, we created pivot table data frames with ROI-ROI connectivities and weights of connections. Given a list of ROIs, we wrote functionality to add each ROI to a networkx graph object and iterate over the pandas dataframe to obtain edges and edge weight from the corresponding nonzero entries in the pivot table. In order to simplify our analysis, we built this functionality into a class and a few user-defined functions in connections_suite.py. In order to have a null to compare to, we generated an Erdos-Enyi random graph with the same number of nodes and average connectivity of the Drosophila brain. The analysis is presented in more detail in the connectivity iPynb and in the conclusions section of this report.

Separate from this original goal some of members also decided to conduct summary statistics and visualizations on different aspects of the data such as neurons and ROIs. This was pursued since it would be a much more easily digestible set of information to present to a non biology/neuroscience audience. It would also provide a general overview of what the data set provides. We were able to extract neuron information as a result of the database structure. The hemibrain database is built on neo4j which is a graph database management system that functions using nodes to represent neurons (Figure 1). This did require team members to learn some Cypher, a SQL-esque structured language for querying property graphs, in order to appropriately pull desired data through neuprint-python scripts. Once the desired data was pulled the team relied on the pandas library to conduct appropriate data analysis and summary statistics. Additionally, other neuprint-python calls were used as established and defined in the corresponding dataset documentation. The built in calls were primarily used for visualizations, however some members did integrate the calls for their analysis as they felt comfortable.

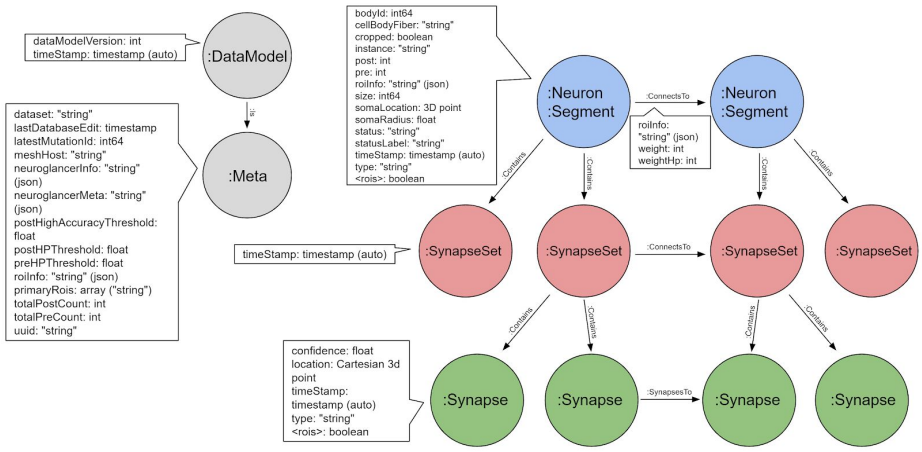

Figure 1: Property Graph for Neuprint

Summary Statistics:

Neurons:

There are 20,026 completed neurons on the right side of the hemibrain. Each neuron has its unique body ID assigned, and passes through ROIs, the region of interest. The average number of ROIs a neuron passes through is 10, in a range between 1 and 117. The neurons that have the most and least number of ROIs pass through are Body ID 1418618235 and 356131764, respectively.

ROIs:

There are 226 unique ROIs on the right side of the hemibrain. The average number of ROIs passed through per neuron is 918, and the region VLNP(R) has the most neurons pass through, which is 11,314.

Pre/Post Synapses:

There are 281 pre-synapses/T-Bars and 857 postsynapse/PSD per neuron on average. The minimum and maximum numbers of T-Bars are 0 and 17,628, respectively, while the minimum and maximum numbers of PSD are 6 and 127,148, respectively. The scatter plot of the neurons for pre versus post synapses is displayed below, that is showing there are more post than presynapses in a neuron.

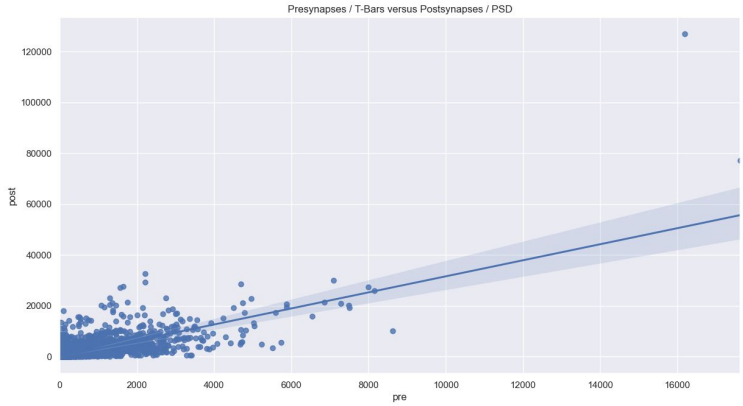

Figure 2: Plotting presynaptic counts against postsynaptic counts. Note: The scaling of pre (x-axis) is 2,000 and post (y-axis) is 20,000

Pre/Post Synapses in ROIs:

We were also interested in synapses in ROIs, because we thought the behaviour of fruit fly was governed by the connectivity of neural circuits. The information of synapses in ROIs would give us more insight into connectivities among ROIs. The statistical summary of Pre and Post synapses in primary ROIs was acquired by using functions of groupby and describe. The box plots of both were generated by using seaborn catplot function with boxplot option. On average, post-synapse count per ROI was 1315, while pre-synapse was 405. Clearly, the post synapse number in ROI was much higher than pre synapse number, which was consistent with single neuron property and scientific discovery in drosophila.

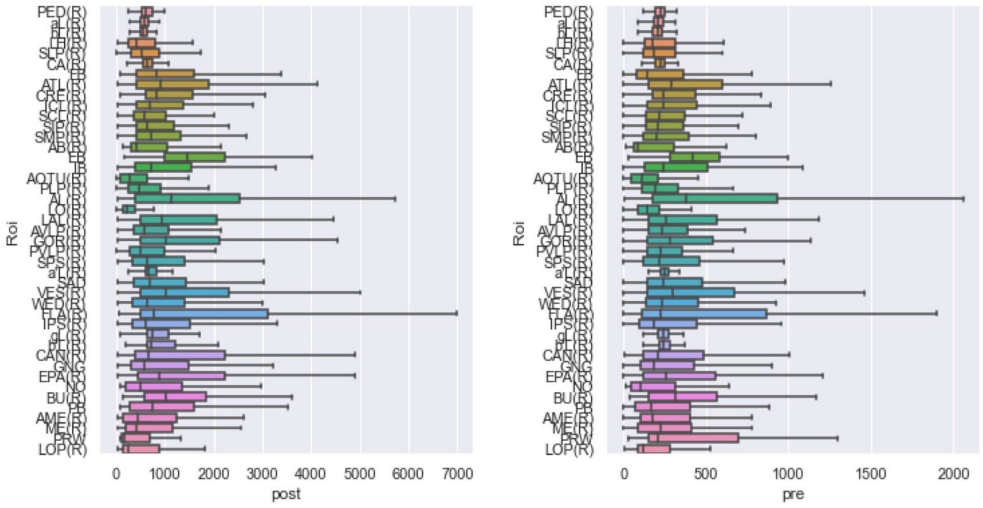

Figure 3. Post/ Pre synapses counts in ROIs. The left side shown post synapses, average count 1315.The right side shown pre synapses, average count 405.

MBONs:

MBONs (mushroom body output neurons) are one of the interesting neuron types that are well characterized and involved in memory and learning. Essentially they are the output for integrated sensory info and play a critical role in learning and memory formation. There are 64 MBONs on the right side of the hemibrain. The average number of ROIs that each MBON passes through is 23, while the average number of ROIs for all right side neurons is 10. The average numbers of pre-synapses and post-synapses are 890 and 10,307, respectively. These numbers are also greater than the average numbers for all neurons.

Other Neuron Statistics:

We also queried the data with other properties, like cell types, and neuron counts per ROI and length of neurons in ROIs. Because of incompleteness of the dataset, we got around 5,000 cell types from 20,000 neurons, some of the cell types only have one neuron. So we had to manually combine cell types based on their abbreviations. In total, we created 9 cell types, and put the rest into a large category "other". So far we don't know any biological meaning of neuron size and counts per ROI. This data might be meaningful in the future with more scientific discoveries in drosophila.

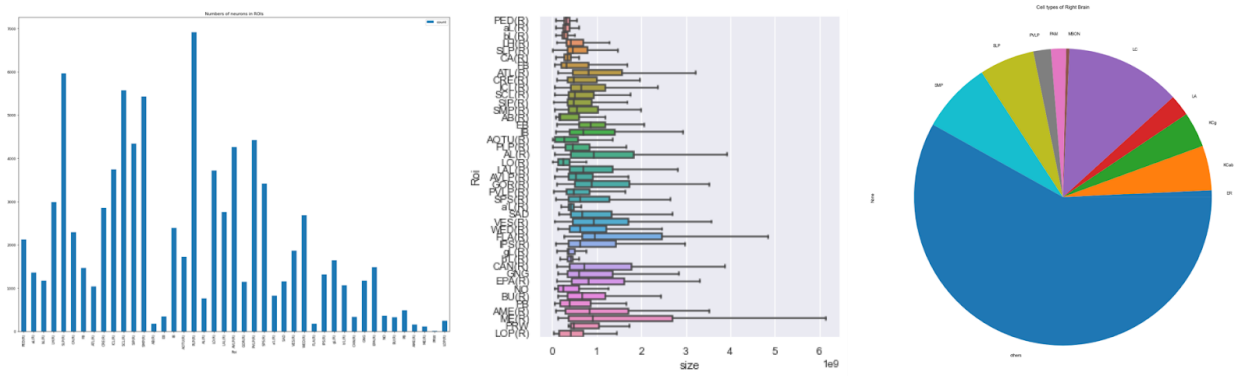

Figure 4. Statistical summary of neuron count, neuron size in ROIs. And a pie chart showing cell types in the dataset.

Version Comparisons:

We compared the two released versions of the hemibrain to compare summary statistics across time. The initial data set(V.1.0.1) was released in January 2020 and the latest was released
in June 2020. Accounting for first hand knowledge from Chelsea, between the two version releases approximately 6 months of work was completed by human proofreaders which lead to us comparing completion statistics across versions. Additionally, 79 ROIs were added to the latest version. Through our comparisons we found that as a result of the added ROIs, the biggest change between data sets was the mean pre synaptic and postsynaptic sites. The mean count decreased across both pre and post sites in the latest version. Excluding the mean pre and post synaptic counts, all other descriptive statistics remained relatively the same (Figure 3).

When considering data set completeness the focus shifted on the percent completeness of the synaptic sites on each neuron. Between data sets, the difference in mean percent postsynaptic sites increased by 3.22 % and the mean presynaptic sites increased by 0.02767%. These results fall in line with how synaptic sites function. In general, a neuron will have more postsynaptic sites than presynaptic sites. To confirm we ran the ratio of post:pre and found that between the two data sets the ratio value remained relatively the same. In the old data set the ratio value for post: pre was 6.65 while in the latest it was 6.59. Lastly, we compared the least and most completed ROIs between versions. We found that the most completed ROI, EBr3am, remained the same while the least completed ROI did change from EPA(L) to FLA(R). In conclusion, these numbers confirm continued work on behalf of Janelia as completion has increased without any major concerns or unexplained outliers between versions.

```
Latest Dataset
         p_presyn         t_pre   p_postsyn        t_post
count  229.000000  2.290000e+02  229.000000  2.290000e+02
mean    93.442736  9.165484e+04   60.196853  6.044246e+05
std      3.332198  2.164123e+05   20.172745  1.555558e+06
min     81.192053  5.100000e+01   20.558988  1.950000e+02
25%     91.983696  5.226000e+03   44.004432  2.437500e+04
50%     93.942688  1.252800e+04   63.723538  5.465600e+04
75%     95.710166  6.143800e+04   74.094809  3.371190e+05
max     99.854581  1.861182e+06   94.226570  1.356352e+07
--------------------------------------------------------------
Oldest Dataset
         p_presyn         t_pre   p_postsyn        t_post
count  150.000000  1.500000e+02  150.000000  1.500000e+02
mean    93.415063  1.343923e+05   56.973805  8.942202e+05
std      3.603109  2.573065e+05   24.110162  1.859341e+06
min     81.575311  4.040000e+02   18.794567  2.403000e+03
25%     91.769063  5.898000e+03   32.603004  2.993750e+04
50%     93.964872  3.668650e+04   58.965348  1.664785e+05
75%     96.042271  1.325665e+05   81.910875  8.148918e+05
max     99.854581  1.861218e+06   93.917381  1.357282e+07
```

Figure 5: Descriptive statistics obtained via df.describe() for each of the data set versions for comparison.

Data Visualization:

For data visualization, we identified three levels of interest. First, we sought to represent what the low-level data was telling us at a biological level. For this, we defined the skeleton class to transform raw numbers about neuron connectivity to a human-interpretable representation of those connections in 2D space. Then, we sought to describe global attributes of the dataset using traditional plots and tables, eg the box and whisker plots. Finally, we sought to represent the high level connectivity of the drosophila brain. For this we used heat maps and spring representations of graphs.

Spring representations were chosen due to their relative intuitiveness and friendliness to display in image form. Each spring representation of connectivity color coded nodes based on a centrality parameter of interest to highlight nodes of interest. Edge color coding based on connection weight was considered and ultimately discarded as more distracting than helpful for the purposes of a short presentation. Graphs generated through our connector class are presented in a separate ipynb titled connection analysis in order to display them in a more user-friendly format. Histograms of the centrality parameters also accompany these graphs to give an idea of the distribution of connectivity characteristics in the brain. We also included a 3d rendering the pathway defined by betweenness centrality in the drosophila brain from an external database. This is in order to place the pathway in the context of the rest of the brain, which extends beyond the dataset.

The data for the heatmap visualization portion of our summary statistics was pulled from the neuprint-python API call fetch_roi_connectivity, which returned a Pandas Dataframe with four columns: to_roi, from_roi, count, and weight. We then used a pivot table to expand the to_roi and from_roi columns into a matrix, with each cell representing a different combination of to_roi and from_roi, and filled the value of each cell with the weight column value. This data was then fed into the sns library heatmap function to produce the following heatmap plot:

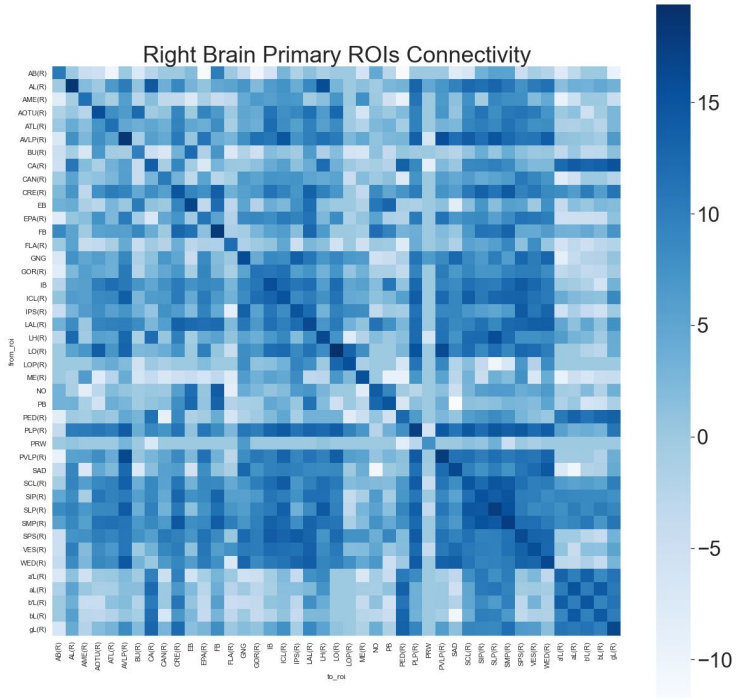

Figure 6. Heatmap of ROI connectivity

Skeleton Graph

The other major visualization we decided to create was a combination of a scatterplot of synapse connections overlaid onto a skeleton graph of the neurons connected to those synapses. This provides users with a fantastic way to visualize how different ROIs and cells types are distributed throughout the brain.

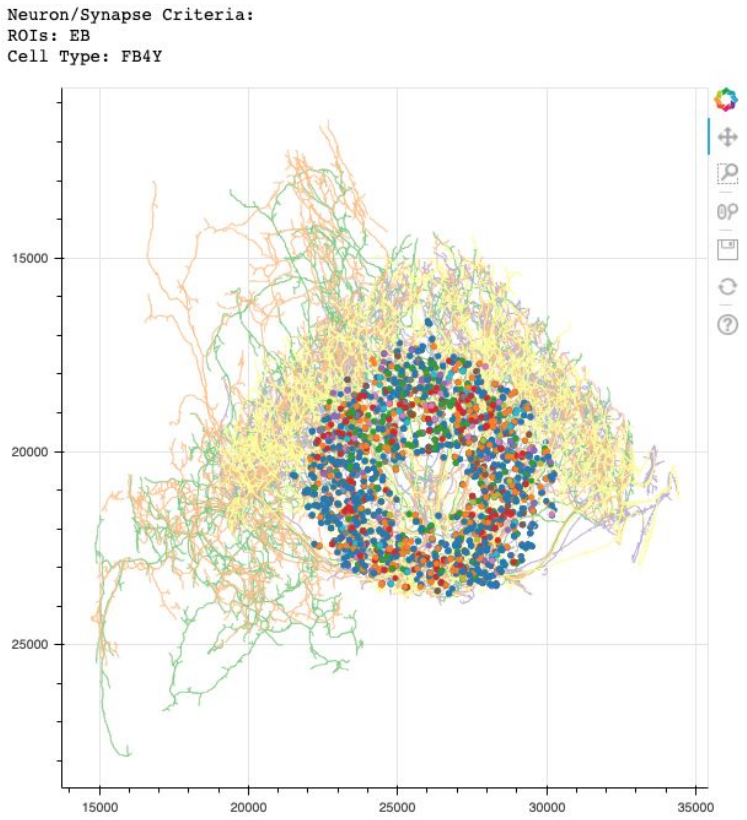

Figure 7. Skeleton Graph for FB4Y cell type neurons and synapses in the EB ROI

For this portion of the project, we created a SkeletonGraph class that accepts a "cell type" string and a list of ROIs as parameters. When run in a Jupyter notebook (which is required for the bokeh plotting library used for the skeleton graphs), the class then automatically generates and returns a scatter plot of the t-bar synapse connections, and the associated neurons overlaid on a skeleton graph plot of the neurons.

This class made heavy use of the neuprint-python API. It utilized the fetch_synapses call to return the set of synapses that met the inputted cell type and ROI criteria. It then used the fetch_synapse_connections call to fetch the related synapse connection point DataFrame. It also used the fetch_neurons call to fetch neuron information for neurons associated with the initial synapses returned by our query criteria. The class then made use of a utility function in the neuprint-python library called merge_neuron_properties to merge the neuron coordinate information DataFrame with the connections DataFrame, so that we could perform certain operations like group connection points by color. Finally, the SkeletonGraph class used the fetch_skeleton API call to fetch neuron skeleton coordinate information as a DataFrame that could then be plotted using the bokeh plotting library.

The class could then be easily imported and utilized in other Jupyter notebooks, which allowed us to quickly produce a number of interesting visualizations for different ROI and cell type combinations.

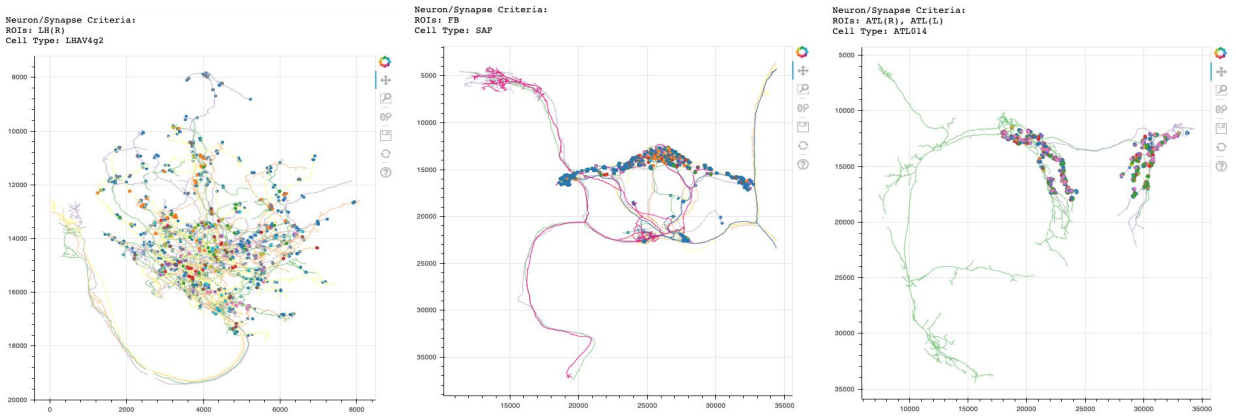

Figure 8. Skeleton Graphs for various cell type and ROI combinations

Validation and unit testing:

Testing the entire input space for validation of these tools is not practical. There is a problem of 'known unknowns.' There are large portions of the drosophila brain that are not well understood and are included in this dataset. While it is possible to verify that output from these sections is syntactically correct, errors in the program or the database leading to inaccurate conclusions are difficult to ascertain as we cannot be certain of expected output. We choose to focus on several well-studied and highly stereotyped regions of the drosophila brain. We verify that our tools show a high degree of agreement with published studies of the drosophila brain.

Three relatively unique unit tests were defined due to the nature of our dataset. We could be sure that we were receiving data in the correct format, but incorrect data in response to a query is not obvious. Much of the dataset represents uncharted territory and none of us are experts in the fly brain. We settled on three consensus properties of the drosophila brain to assess via query to ensure that our queries matched the properties of the Drosophila brain that we do know about. There is a singular neuron that innervates every kenyon type cell in the drosophila brain named the APL (Amin 2020). We structured a unit test that queries the database for all of the downstream partners of APL and for all Kenyon type cells, then checks to see that each Kenyon type cell is represented in the dataframe of neurons downstream of APL by checking for their bodyID as the key. There is also a subtype of neuron called the ring neuron, that localizes into several distinct adjacent regions. The targets of these regions are mutually exclusive. We check the targets of the ring Neurons in EBr3 and EBr1 to ensure they do not overlap (Scheffer 2020). Lastly, we know that the projection between the antennal lobe and the lateral horn should be enriched for a type of neuron called an MBON (Aso 2014). We define a unit test that passes if the neurons that connect the two lobes have a higher proportion of MBONs than either of the two lobes. These are relatively strange unit tests, but necessary in our view as the data in this dataset is not very human readable. Without having any basis for what to expect out of this data, these help us parse for logical errors in our core query syntax.

The regions were chosen for their degree of stereotyping (consistency across different flies, degree of characterization, and general interestingness. For example, the test case involving the projections from the antennal lobe to the lateral horn is an area of active research with results that are consistent with the literature. The antennal lobe is responsible for receiving olfactory stimuli in drosophila and the lateral horn is responsible for behavior modification in response to these stimuli (Shizumi 2017). Published data indicates that there should be a substantial population of MBONs (mushroom body output neurons) that project from the antennal lobe to the lateral horn. 7 of 48 of the connections spanning these two regions are MBONs, roughly 12%. This is a drastic enrichment compared to .2-2% MBON content in either region. These 7 projections represent 24% of the total MBON input/output of the antennal lobe and a whopping 64% of the MBON input/output for the lateral horn. This is consistent with the emerging role

of the lateral horn in learned behavior instead of simply 'innate' behavior (Lerner 2020). It is also consistent with published input/output ratios (Lerner 2020).

We also define unit tests to ensure that each of the connector objects attributes is initialized correctly and is updated appropriately by each method. We decline to unit test the networkX library as it is an established and well-used library. A few helper functions are defined in the connections_suite.py and they are tested using pre-generated networkx canonical graphs with known results.

We unit-tested our codes for neuron query, neuron data generation, data conversion, and neuron data analysis to make sure these functions can fulfill our goals. Most of these functions were defined in Neuron_fetch.py, A separated Unit_test.py was created to test these functions. The following table shows how we did the tests, and a screen capture shows the test results. There were two functions running data conversion. Because the size of the data was big, the conversion would take a relatively long time to complete.

	Function Name	Description	Input	output	How to test	Test result
1	make_unwanted_columns_list	remove unwanted columns based on string provided	List, string	List	assertEqual(),	pass
2	left_or_right_brain	assign a boolean value to neurons to mark from right or left brain	Data frame	Data frame	pd_testing.assert_frame_equal()	pass
3	connection_setup	Setup connection with "NeuPrint" website	None	client	assertIsNotNone(), return client object is not non	pass
4	Neuron_filter	Remove unwanted neuron data	Data frame	Data frame	assertNotEqual(), len(return df) not equal old df	pass
5	fetch_primaryROI	From NeuPrint fetch "Primary ROI"	None	Data frame	assertIsNotNone(), return df is not none	pass
6	fetch_neurons	From NeuPrint fetch customized query	None	Data frame	assertNotEqual(), len(return df) not 0	pass
7	Dict2df	convert dictionary dataset to dataframe	None	Boolean	assertEqual(), True successfully run the code	pass

Table 1. Unit test list from neuron date generation, and data analysis

We defined a separate class of unit tests for the SkeletonGraph class that focused mostly on functionality unique to that class. As a basic sanity check, two unit tests were run to ensure that the input parameters were initialized correctly. We then ran unit tests for different combinations of invalid queries, checking to see if inputting both an invalid cell type and an invalid ROI would result in the validQuery property being set to False. We also ran unit tests to make sure that these invalid queries would return the correct error message when the test script tried to generate the skeleton graph. Another unit test was set up to ensure that valid queries returned the correct number of items. Finally, a unit test was added to check that inputting a valid query and then generating the skeleton plot from the query would result in the plot attribute on our class being set to be a bokeh figure plot.

Figure 9. Screen capture of unit test results

Final Conclusions:

Hear us out on this, you should care how Drosophila change their behavior in response to smell. The lateral horn is responsible for so called 'innate' behaviors thought to be hardwired into the brain. However, it is also responsible for learning (Lerner 2020). Drosophila are challenging previously widely held notions about 'hardwired behavior'. In fact, while both humans and drosophila exhibit a number of stereotyped behaviors from birth, these behaviors are modifiable in both organisms (Schwartz 2017). The drosophila hemibrain data helps show us the mechanism behind this observation. The wiring is in the same place. The mechanisms that modify behavior in response to stimuli and enable learning can act on the circuits involved in innate behavior. This indicates that there is very little that we do not have the capacity to either learn or unlearn.

The dataset we examine is a fixed graph (with constant updates), but a living brain is a dynamic graph. Synapses can develop or atrophy over, connections may be formed or pruned as an organism lives and learns. By implementing some relatively simple graph theoretical measures on data with single neuron resolution, we defined a pathway of interest for information modulation and pass through in the drosophila lateral protocerebrum. Here it is visualized below in the virtual fly brain. Compare it to the figure from Jai et al in 2010, showing GFP expression in FRU expressing neurons known to modulate courtship behavior in drosophila. Our purely graph theory based model constructs something very similar to an in-vivo circuit defined by neurons that express FRU and are thought to be involved in the control of complex mating behaviors (Jai 2010). It is potentially possible to identify biologically meaningful circuits based purely on the mathematical analysis of structural data.

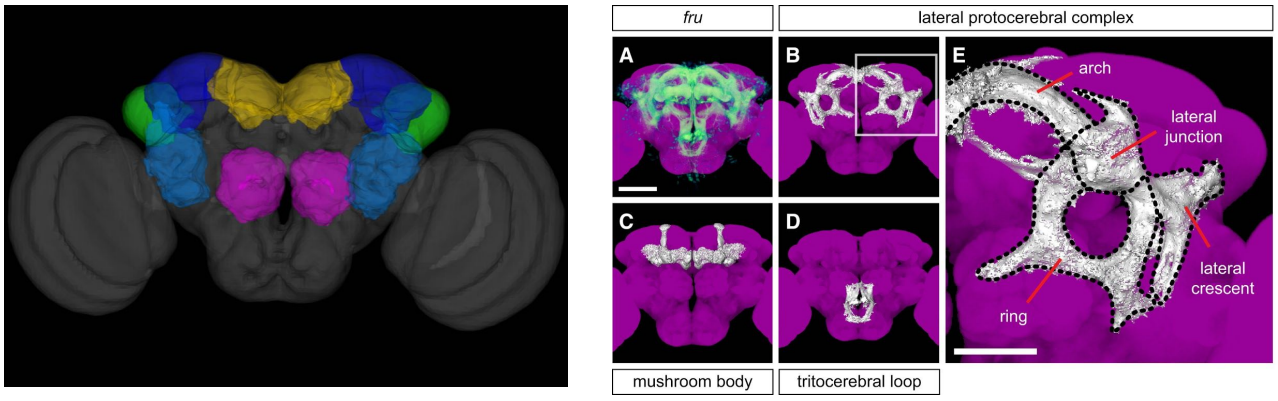

Figure 10. Comparison of high betweenness regions to in-vivo network controlling complex behavior from Jai 2010.

We also find that the average out degree of neurons greatly exceeds their in degree. This has potential implications for signal amplification in the brain, indicating that the firing of a single neuron has the potential to cause a cascade of neuron activation and global changes in brain activity. This paired with our finding that single neurons tend to bridge many ROIs on average and have the capacity to form many thousands of input and output connections also raises the degree of complexity in this lower order brain. Clearly there is some hierarchy among neurons and some act as integration points for a wide degree of

information with complex regulatory outputs. As painstaking as it may sound, searches for complex regulatory networks and cliques at the single-neuron level are in order. In fact, this is being done by the authors of the dataset to some effect (Scheffer 2020).

These findings have substantive applications for how we understand the brain and treat complex neurological and behavioral disorders. Identifying the substrates of behavioral circuits would allow us to quantify their degree of change in disease states. Given that the brain is plastic, this represents an opportunity to determine the degree of change likely needed to alleviate symptoms of a disease state. It is well understood that commonly used drugs to treat mental disorders, such as antidepressants, work through synapse remodeling (Castren 2013). A full understanding of these disease states and their effective treatment may rest on the construction and analysis of datasets like this one.

Lastly, we conclude that the Neuprint database is a safe playground for beginning data scientists. While it may take some setup to get a machine ready to explore the database it is well documented and fairly straightforward to navigate. There is no need to have a deep understanding of the biological underpinnings to understand the relationships pulled from the data. The underlying properties of how the database was created and how to pull information from it can be applied to larger and non-biological dataframes. The fact that it provides some pre-computed values and free computational resources on the Janelia servers allows an entry level exploration of complex networks without prohibitive barriers to entry.

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