Supplementary Material for Ryan J, Casimo K (2021) A Course-Based Research Experience Using the Allen Brain Map: From Research Question to Poster Session. J Undergrad Neurosci Educ 19(2):A260-A266.

NEUROBIOLOGY 340

TUTORIAL 2: THE MOUSE BRAIN REFERENCE ATLAS

Part I: Investigating the reference atlas

Go to http://portal.brain-map.org and select Reference Atlases from the options available. You can use the drop-down menu at the top of the page or search through the icons for this one:

REFERENCE ATLASES

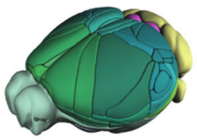
High resolution anatomical reference atlases and histology for mouse and human.

View Atlases →

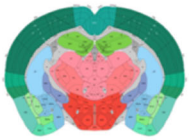
Select the 3D viewer icon for the Mouse Brain Atlas. This opens the Allen Brain Explorer: Beta.

Adult Mouse

These anatomical reference atlases illustrate the adult mouse brain in coronal and sagittal planes of section. They are the spatial framework for datasets such as in situ hybridization, cell projection maps, and in vitro cell characterization.

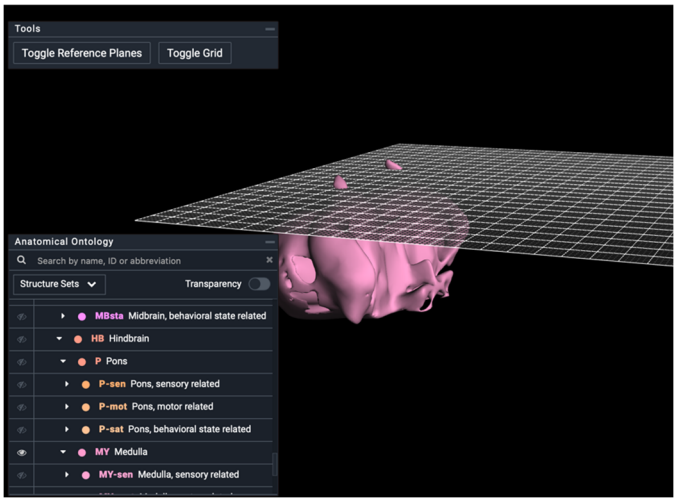


(3D Viewer)



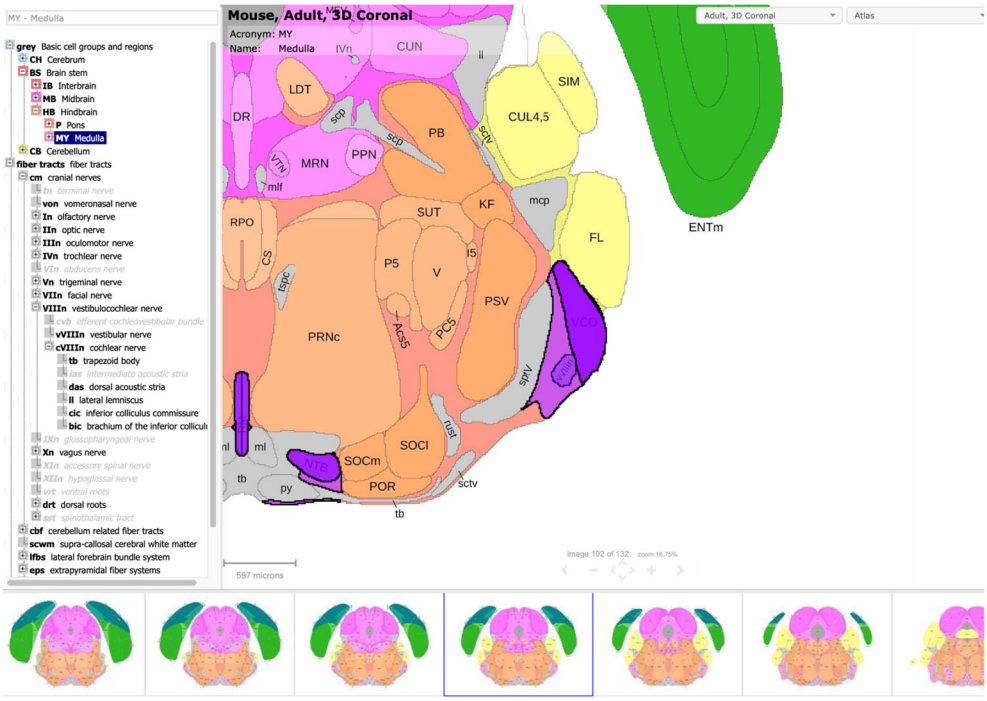
(Coronal Sections)

In the *Anatomical Ontology* pane, click on the eye in the left column next to **Medulla** (MY) to add the medulla to the grid. You can select it from the list or search for Medulla in the *Anatomical Ontology* pane.



You can toggle on/off the horizontal grid and the image planes. Try this to see how they operate.

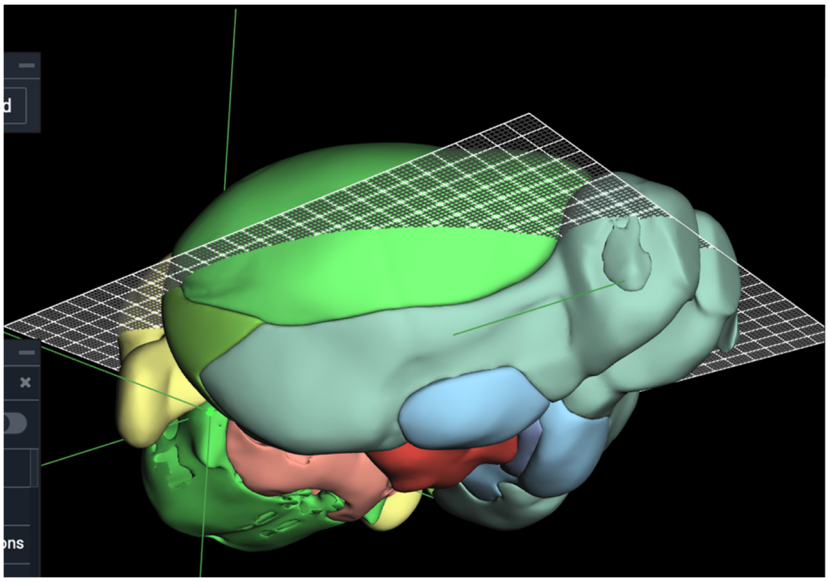
Click on the Medulla to select it (it turns green). Then, in the *Experiment Information* pane, click on the *Source Image* tab and then on the *View in Atlas* button to view the Medulla in a coronal section of the 2D reference atlas. You can zoom in and out in this view using the +/- buttons near the bottom of the atlas image.



Move your mouse over other brain regions and you should see the name of the region pop up. Try clicking on a few of the other brain regions here – you should see the structure turn purple and the same appear in the anatomical ontology list on the left.

Return to the 3D Viewer. Click on the eye next to Pons to add it to the 3D space. Add the following structures one at a time to build a complete 3D brain:

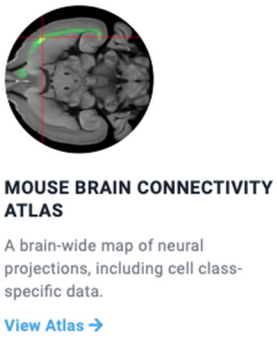
Cerebellum
Midbrain
Hypothalamus
Thalamus
Pallidum
Striatum
Hippocampal formation
Olfactory areas
Isocortex



You have now built up a fairly complete mouse brain. You can make the brain transparent by clicking the transparency toggle in the *Anatomical Ontology* pane. You can also click and drag the brain to move it in 3D space.

Part II: Mouse Brain Connectivity Atlas

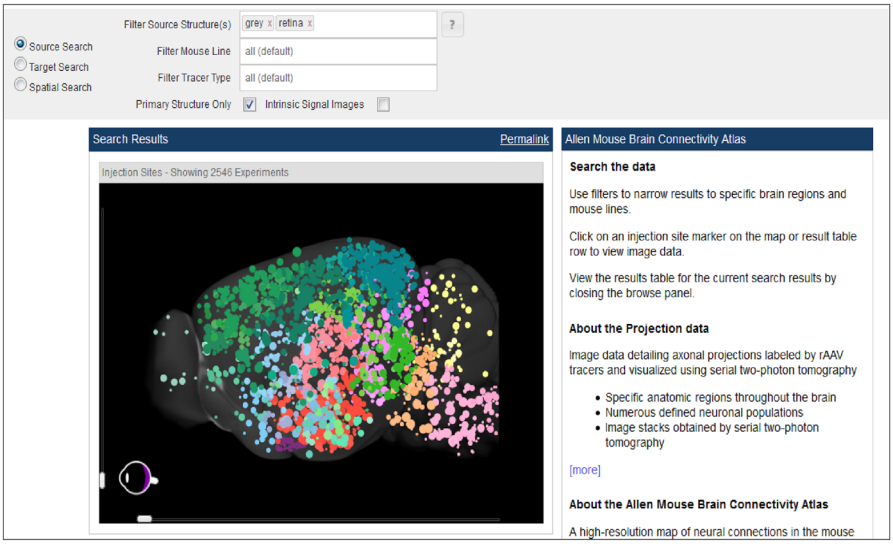
The Allen Mouse Connectivity Atlas is a searchable image database of axonal projections labeled by viral (rAAV) tracers and visualized using serial two-photon tomography. Return to the brain map portal home page and click on the Mouse Brain Connectivity Atlas.



In this dataset, scientists injected a fluorescent tracer into selected regions of the brain, which then spread along all of the axons projecting out of that site. The resulting images let us see all the locations that that brain region projects to. Some experiments also use Cre lines that target specific cell types, so when we look at the projections, we are only looking at projections of certain types of neurons.

There are three ways to search this dataset:
1) Source Search, searches by injection site with additional sub-options to filter by mouse line and tracer type. You can search using the search box or by clicking on the dots on the visualization, each of which represents an injection site.
2) Target Search, searches by both injection site and projection through a target structure of interest.
3) Spatial Search, searches by either a target signal or injection site by selecting a voxel from through which the tracer signal passes.

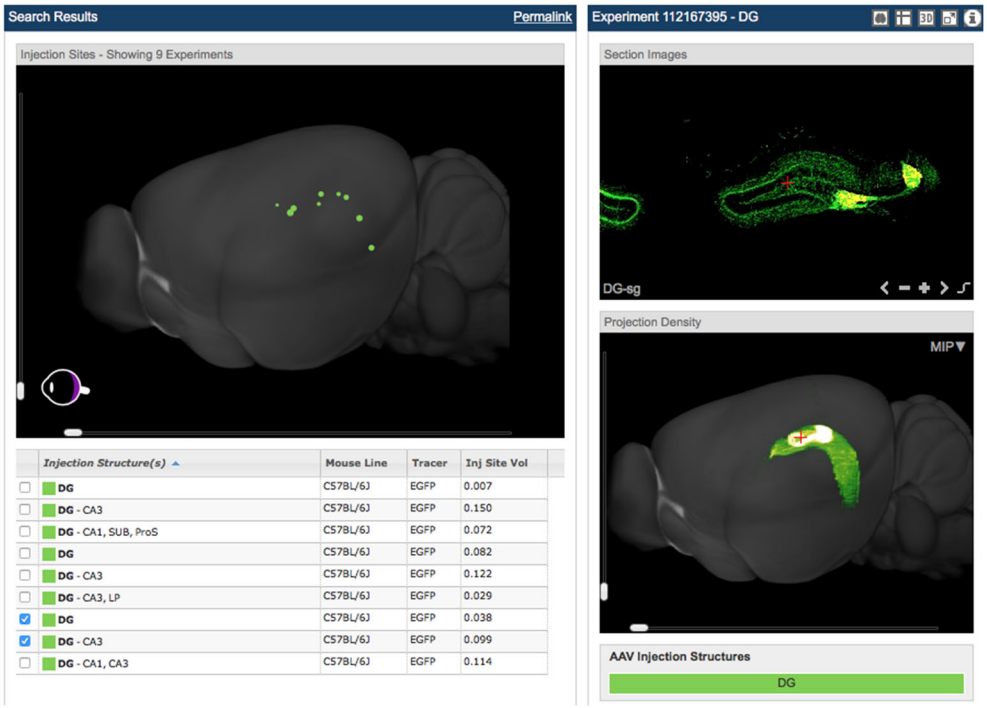
There are example searches in the "Browse the Data" subject box at the bottom of the landing page to demonstrate the various ways in which relevant data can be found.



Using a Source Search

The default view shows all experiments throughout the brain (indicated by "grey" and "retina"). To filter your search by injection site, first remove the “grey” and “retina” by clicking on the x next to their names in the filter source structure box.

Click in the "Filter Source Structure(s)" text box and pick a structure(s) from the ontology list, or type in the structure's abbreviation. For example, enter DG (Dentate Gyrus). In the *Filter Mouse Line* box select "wild" (Wild-type experiments trace all projections from the injection site, Cre line experiments each target a specific cell type). A list of injection sites meeting your criteria appear below the left image (figure below). From the list of available injection experiments select 2 or 3 by clicking on the box to add them to your list.



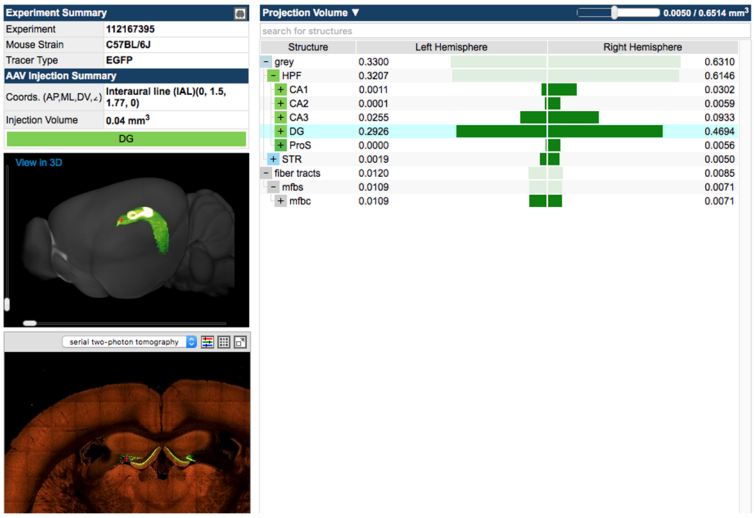
Click on the *View Selections* button on the bottom of the list. This brings up a new pane with the 2-3 selections shown in Individual Image View. Be patient, as this may take some time to load.

Scroll through the slices using the slider bar at the bottom until you see a region with lots of green fluorescence.

Then click on the  icon to synch all your experiments to the same location. Be patient.

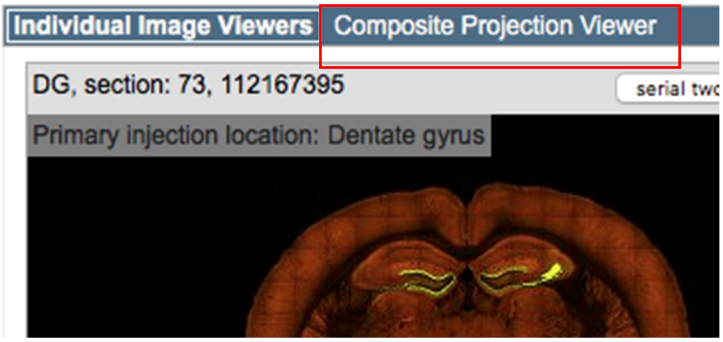
Click on the  icon to bring up a summary page for the experiment.

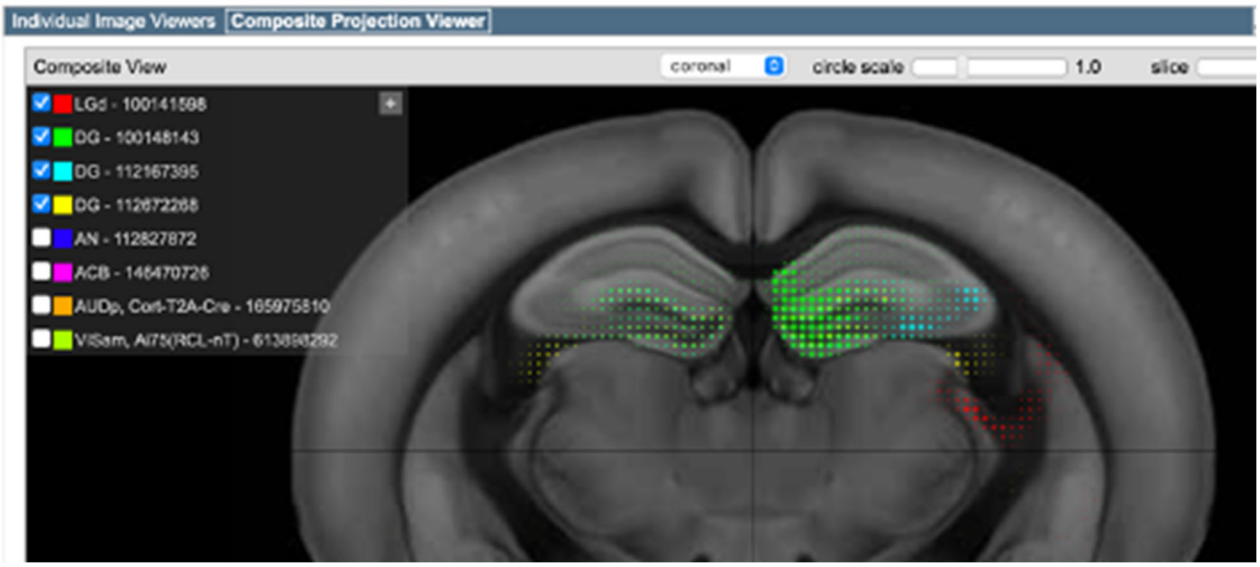
Make a note of the experiment number in the Experiment Summary section. You will use this again in Part III of this tutorial.



Mouse over the histograms to the right to see the red crosshair on the 3Dimage view change to that location. These histograms are measuring how much tracer appears in different target regions. Make notes as to what target regions this source region projects to.

Return to the *Individual Image Viewer*. In the top menu bar click on *Composite Projection Viewer* (figure below).





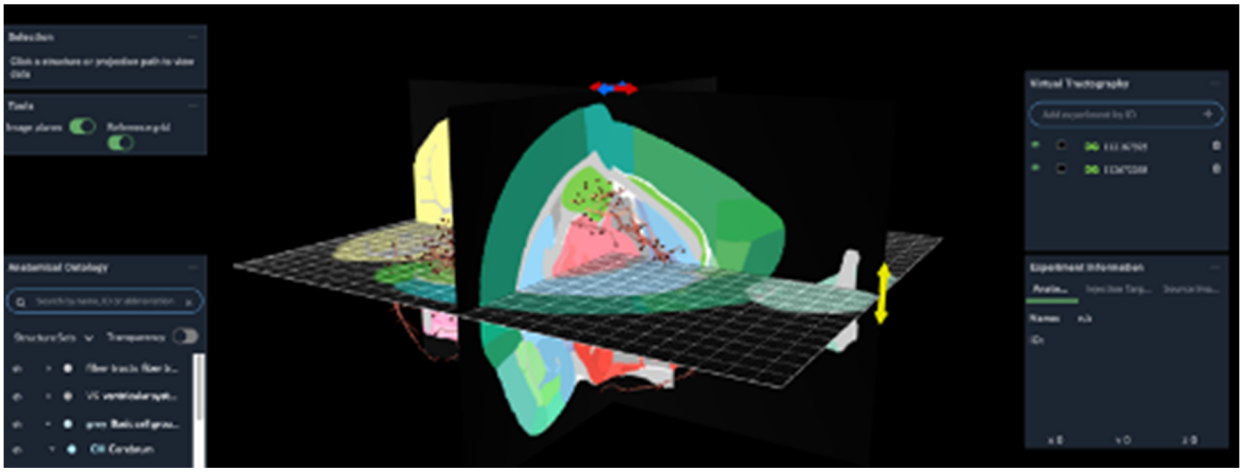
This brings up a composite view of the injection sites indicated with colored spheres.

Click on the small + sign in the upper left next to the list of the experiments to add the image views of your selected experiments to the left side. Along the top of the image, you can select coronal, sagittal or horizontal orientations, increase the diameter of the circles using the *Circle Scale* slider, and move to a new slice using the *Slice slider*.

Part III: Adding a Tract Tracing Experiment

Now we'll use the 3D Brain Explorer tool we covered in Part I of this tutorial to visualize these tracts another way.

Return to the 3D Brain Explorer from the connectivity data by clicking the link in the header of the experiment page, then launching the online 3D Brain Explorer. (This takes you to the same place as the link on the atlas page we used in Part I.) In the Tools pane, turn *Image planes* and *Reference grid* on.



Now, add an experiment from the Allen Mouse Brain Connectivity Database to the 3D brain. Enter the experiment number you recorded in Part II of this tutorial into the *Add experiment by ID* box.

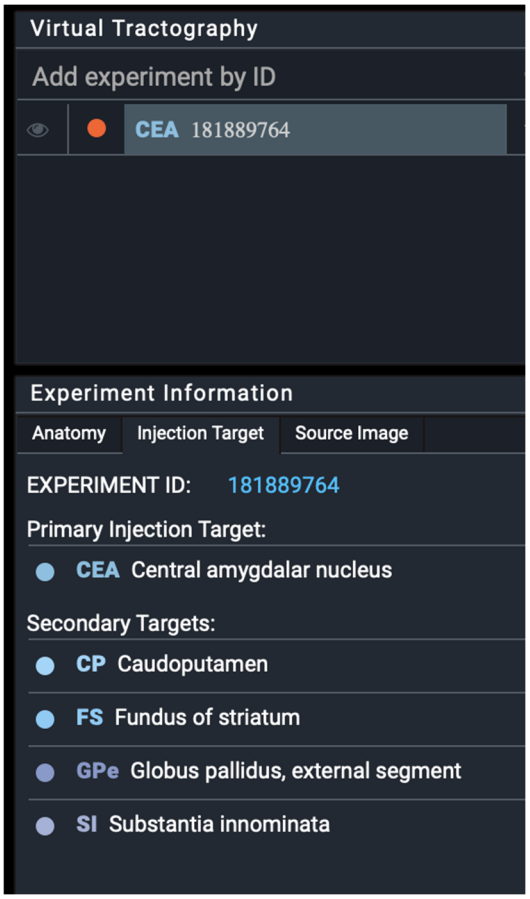
This will add a virtual 3D rendering of the fluorescent data you viewed before to the brain anatomy. This rendering is calculated from the fluorescent images to trace the areas of highest fluorescence as pathways.

If you have not already done so, try turning transparency **on** using the slider in the *Anatomical Ontology* pane.

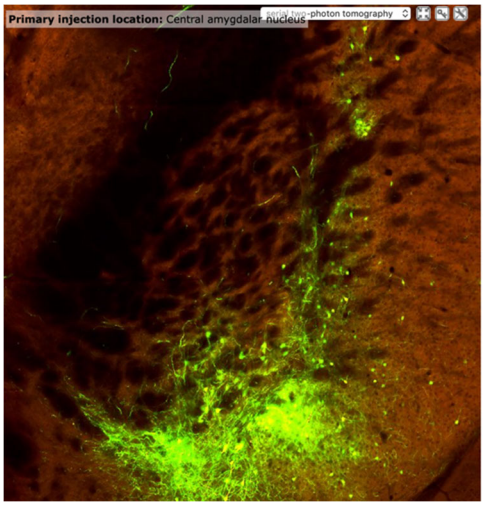
Each computed tract ends in a cube icon indicating the target region. Clicking on any one turns it green and brings up the injection target under the *Experiment Information* pane on the right. You can also view this information in the 2D experiment details, where you obtained the experiment number. In this example, the experiment is #181889764 and the injection target was the Central Amygdalar Nucleus.

Notice that secondary injection targets are also listed. These secondary targets are those that the injection partially affected because it is not perfectly constrained to the main target region.

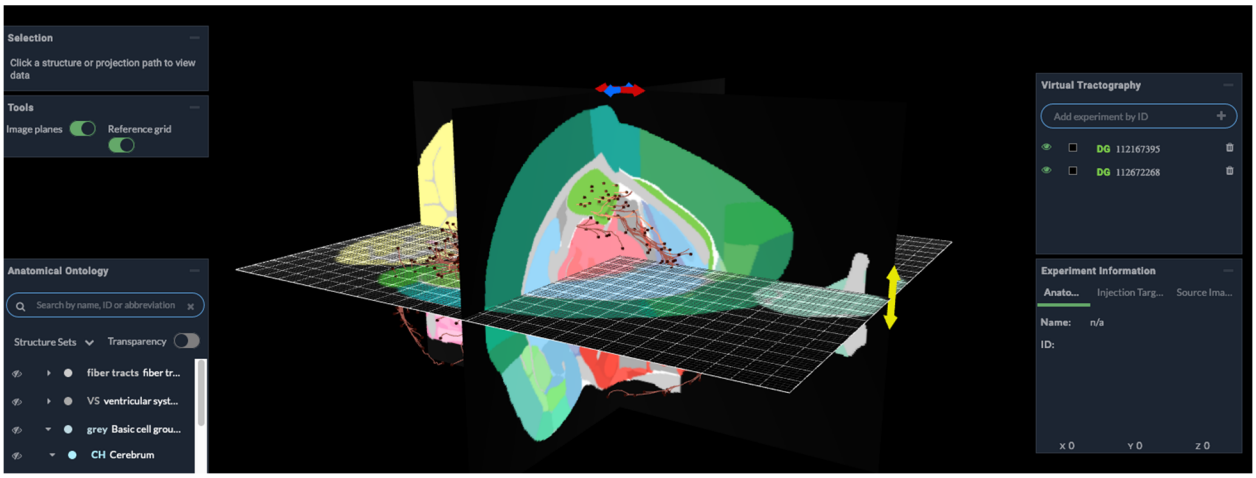
Now, click on the *Source Image* tab. You will see the fluorescent microscopy images tracing projections from the injection site. These are the same images we viewed in Part II of this tutorial.



Click on *View in SIV* to see a high-resolution image of the injection in a new window. (If this button does not appear, try adjusting the size or zoom of your browser window.) Click on the small key icon at the top right of the image to bring up the corresponding region in the reference atlas. Click on the wrench icon to download the image.



Complete questions 1 and 2 on the last page of this tutorial.

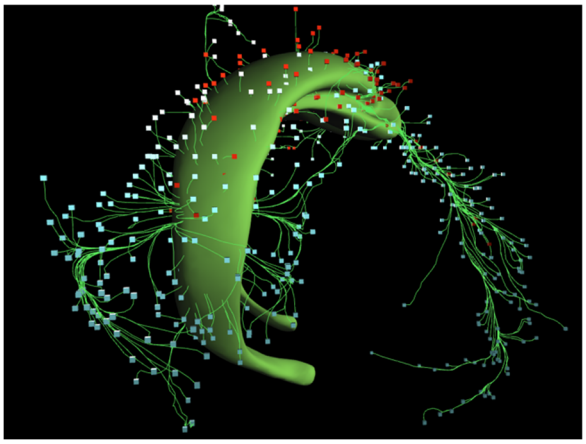


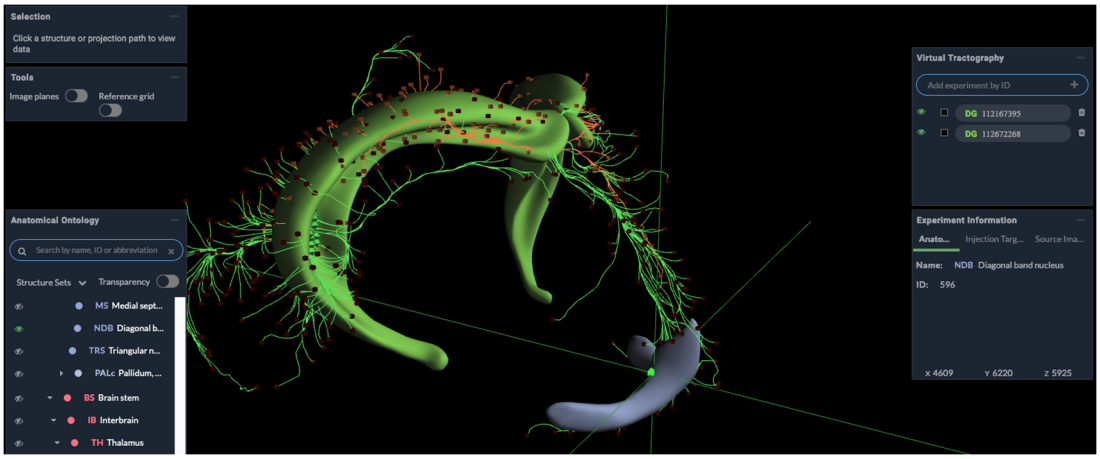
Return to the 3D Brain Explorer window. Toggle both the reference grid and the planes to off.

Enter DG in the *Anatomical Ontology* search box and click on the eye icon next to its name to add that region to the image (figure below).

Each computed tract ends in a cube, representing a computed target. (These are the same computed targets as in the *Experiment Details* page we viewed in Part II.) Click on the boxes at the ends of some of the tracts to bring up the name of those regions in the *Experimental Information* box. Enter those names (i.e., NDB) in the

Anatomical Ontology search and click on the eye icon next to its name to reveal its location and shape (figure below).





Repeat this until all the target structures for this source site are added to the 3D view.

Complete question 3 on the last page of this tutorial.

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Complete these questions/tasks as you work through the tutorial.

(1) What types of research questions can you develop that use these resources?

(2) Select another experiment from the injection experiments. Using images and text, describe what regions are connected.

(3) Use a new Source or Spatial search to explore a new brain region or injection site. Describe your findings in a half page (single spaced) and provide one image (half page) that you think best represents your search.