

# Exploring Pathways in the Brain

In this lesson, students learn about neuroanatomy, gene expression in the brain, and how brain regions are linked through neural projections.

## Resource structure

This resource is divided into three sections, which grow progressively more challenging and scientifically sophisticated and build on each other.

Less advanced students may be assigned only Section 1 or Sections 1 & 2, depending on their level and the needs of your course. The sections build on each other, so an instructor teaching an advanced college class that would use section 3 would use the full resource.

In the first two sections, students explore neuroanatomical atlases and the connections between gene expression and brain region functions. In the third and final section, students are given targeted guidance and inspiration to design their own experiment that explores connectivity between brain regions.

## Recommended grade level

Section 1: introductory-level college neuroscience students, highly motivated high school students

Section 2: introductory to intermediate college neuroscience students

Section 3: intermediate to advanced college neuroscience students, graduate students

## Learning goals

Section 1:

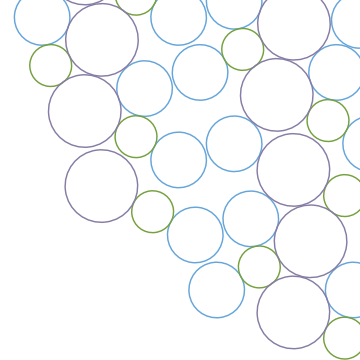
- Students will be able to identify brain regions on an atlas in different planar views.
- Students will be able to explain the relationship between brain regions and their specialized functions.

Section 2:

- Students will learn to interpret gene expression data from ISH experiments.
- Students will be able to explain the link the specialized function of brain regions to patterns of gene expression.

Section 3:

- Students will be able to describe the method of projection tracing, its applications, and limitations of the method.
- Students will be able to interpret previously collected connectivity data.
- Students will explore applications of connectivity data to genetic diseases and other conditions.
- Students will design their own experiment to explore connectivity in the mouse brain.



## Student knowledge

Students should already have a basic understanding of:

- Central dogma of molecular biology, the relationship between genes and proteins
- Neurons as the primary unit of computation and cognition in the brain

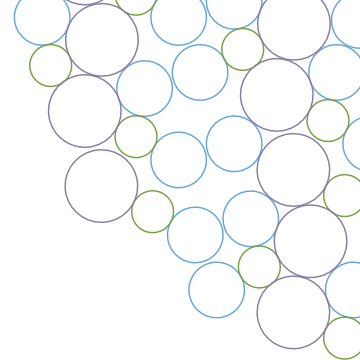
Students do not already need knowledge of:

- Neuroanatomy and functional specialization
- Any neuroanatomy, brain region names, or how to read a brain atlas
- How to interpret gene expression data (in the form of *in situ* hybridization or RNA sequencing)
- The role of connectivity and neural projections in brain function
- How to collect and interpret connectivity data in the form of projection tracing

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Teachers are welcome to adapt the lesson to suit their classes and curriculum, but may not share modified lessons. If you develop your own lesson plan using Allen Institute resources, we invite you to share your experience with us at [communications@alleninstitute.org](mailto:communications@alleninstitute.org).



# Outline for teachers

## Section 1

Suitable for introductory-level college neuroscience and for some very advanced and motivated high school students, also used as a prelude to the more challenging sections 2 and 3

- Lesson introduces the idea of functional specialization of brain regions
- Students visit reference atlases and find pre-selected brain regions, get oriented to neuroanatomy, and annotate blank anatomy with location of selected regions
- Students look up general functions of selected brain regions
- Students use tree of brain regions to describe relationship between brain regions and subregions
- Includes a worksheet with free response data interpretation questions

## Section 2

Suitable for introductory to intermediate college students, and also used as prelude to the more challenging section 3

- Students look up expression of 2-3 genes (either of interest to other parts of the curriculum or from pre-selected list) in Allen Mouse Brain Atlas
- Students answer free response questions very generally connecting relative gene expression/protein functions to brain region function

## Section 3

Suitable for intermediate to advanced college and graduate students

- Students read a selected scientific paper explaining Allen Mouse Brain Connectivity Atlas
- Students complete mini-experiment:
  - Option 1: identify all known targets for a given source and interpret in light of function
  - Option 2: Compare Cre lines projections vs. wild type injection-traced projections for a given region (focus on cortical sources)
- Students answer free response questions interpreting data collected
- Students design their own experiment that expands on the assigned mini-experiment



## Introduction for instructors

The Allen Institute is a nonprofit biomedical research institute located in Seattle, Washington. Our four divisions – Allen Institute for Brain Science, Allen Institute for Cell Science, Allen Institute for Immunology, and The Paul G Allen Frontiers Group – are dedicated to answering some of the biggest questions in bioscience and accelerating research worldwide. We share all of our data and research findings with the scientific community and general public. Launched in 2003 by founder Paul G. Allen, the Allen Institute is supported by government, foundation, and private funds to enable its projects. The Allen Institute for Brain Science creates large-scale, open datasets that address fundamental questions about the brain's components and functions. These datasets and other tools form the Allen Brain Map, and are publicly available online at [brain-map.org](http://brain-map.org).

### Key neuroscience concepts underlying this lesson

- All human brains have the same general organizational patterns, as do all mouse brains – variation occurs on the level of single cells and their connections.
- The brain can be divided into regions, which have functional specializations such as V1 in the occipital lobe (primary visual processing), precentral gyrus (issuing motor commands to the body), or hippocampus (memory processing, navigation).
- Gene expression varies across the brain and expression of a specific gene can sometimes be linked to the function of a specific region.
- Different regions of the brain share physical connections, and some information on brain function can be inferred from the presence and characteristics of connectivity.
- Modern neuroscience requires scientists to integrate information from different levels – from single cells to entire brain regions containing hundreds of millions of neurons. At each level, scientists also have to integrate different types of data, such as electrophysiological records, 3D images, behavioral data, and genetics.

This curriculum uses two datasets from the Allen Brain Map at [brain-map.org](http://brain-map.org): the Allen Mouse Brain Atlas and the Allen Mouse Brain Connectivity Atlas.

### Allen Mouse Brain Atlas

- The Allen Mouse Brain Atlas is a comprehensive review of gene expression across the healthy adult mouse brain.
- Over 20,000 genes' expression levels were surveyed across the whole mouse brain.
- Gene expression is recorded with in situ hybridization (ISH), a process that results in images that show where a gene was detected. For each gene, the slices are located 200um apart and span one full hemisphere of the brain.
- The ISH data images are accompanied by an annotated, illustrated, whole-brain reference atlas showing the positions of different brain regions. In this lesson, students use the reference atlas for both the human and mouse adult brains.



## Allen Mouse Brain Connectivity Atlas

- The Allen Mouse Brain Connectivity Atlas is a high-resolution map of axons in the mouse brain developed from stereotaxic injections of fluorescent viral tracers showing projections from source regions (site of the injection) to target regions throughout the brain.
- The fluorescent tracers enable the identification of whole-brain axonal projections.
- The atlas includes data from wild-type mice and from transgenic mice genetically engineered to target specific cell types (Cre lines).
- The atlas consists of image data, quantification of image data, and other visualization tools.

## Open research questions and applications for resources

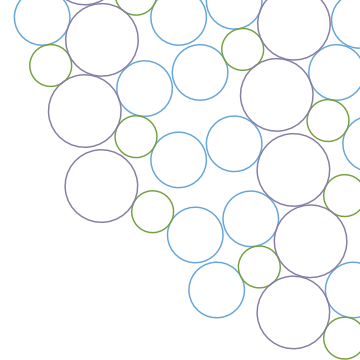
These two datasets, along with the rest of the Allen Brain Atlas resources, can be used to address a wide variety of open questions in neuroscience. Allen Institute staff and other scientists around the world conduct research using the data we collect. Some of the broad open questions are addressed in the students' homework reading, Five unsolved mysteries about the brain. Additional articles about breaking discoveries, the process of research, and more are available here and may be of interest for advanced students to pursue further reading. Core to the mission of the Allen Institute is our open sharing of data worldwide, and thousands of scientists use our resources in their research every day. Notable projects where other research teams have used these datasets have included:

- Data Stories: A Tiny Brain Structure with an Outsized Role in Neurological Disorders showcases several uses of the original Allen Mouse Brain Atlas, the first project of the Allen Institute for Brain Science ([alleninstitute.org/what-we-do/brain-science/news-press/articles/tiny-brain-structure-outsized-role-neurological-disorders](https://alleninstitute.org/what-we-do/brain-science/news-press/articles/tiny-brain-structure-outsized-role-neurological-disorders))
- Data Stories: Understanding STING highlights research on the impact of infectious diseases on the brain ([alleninstitute.org/what-we-do/brain-science/news-press/articles/data-stories-understanding-sting](https://alleninstitute.org/what-we-do/brain-science/news-press/articles/data-stories-understanding-sting))

The breadth and depth of the Allen Brain Atlas provide opportunities for advanced students to pursue additional projects, such as independent science fair projects, multi-week class experiments, and computer science-oriented projects. Data Stories: Decoding the Brain features a high school student who used the Allen Brain Observatory for an independent science project.

Teachers who use this curriculum or other Allen Brain Atlas resources in their classrooms, or who have students who pursue advanced independent projects, are welcome to share their experiences with us. Please contact us at [communications@alleninstitute.org](mailto:communications@alleninstitute.org).

The remainder of this packet consists of worksheets for students.



# Exploring Pathways in the Brain

## Section 1: Exploring Anatomy

### Introduction and goals for students:

- In this assignment you'll find brain regions on a reference anatomical atlas, look up their functions, and think about how brain regions specialize and what that means for brain function.
- You'll learn how to navigate a brain atlas in three dimensions.
- You'll also compare the brain regions you learn about between humans and mice.

Read the article Five Unsolved Mysteries of the Brain: [alleninstitute.org/what-we-do/brain-science/news-press/articles/5-unsolved-mysteries-about-brain](https://alleninstitute.org/what-we-do/brain-science/news-press/articles/5-unsolved-mysteries-about-brain).

Reflection question: What surprised you in the article? What do scientists know more about in the brain than you expected? What do scientists know less about?

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**Instructions:** Visit [atlas.brain-map.org](https://atlas.brain-map.org). In the Mouse Brain section, select Sagittal 2011 from the icons on the right. The sagittal plane refers to slicing the brain lengthwise, from front to back. In these images, the front of the head is on the left side of the screen.

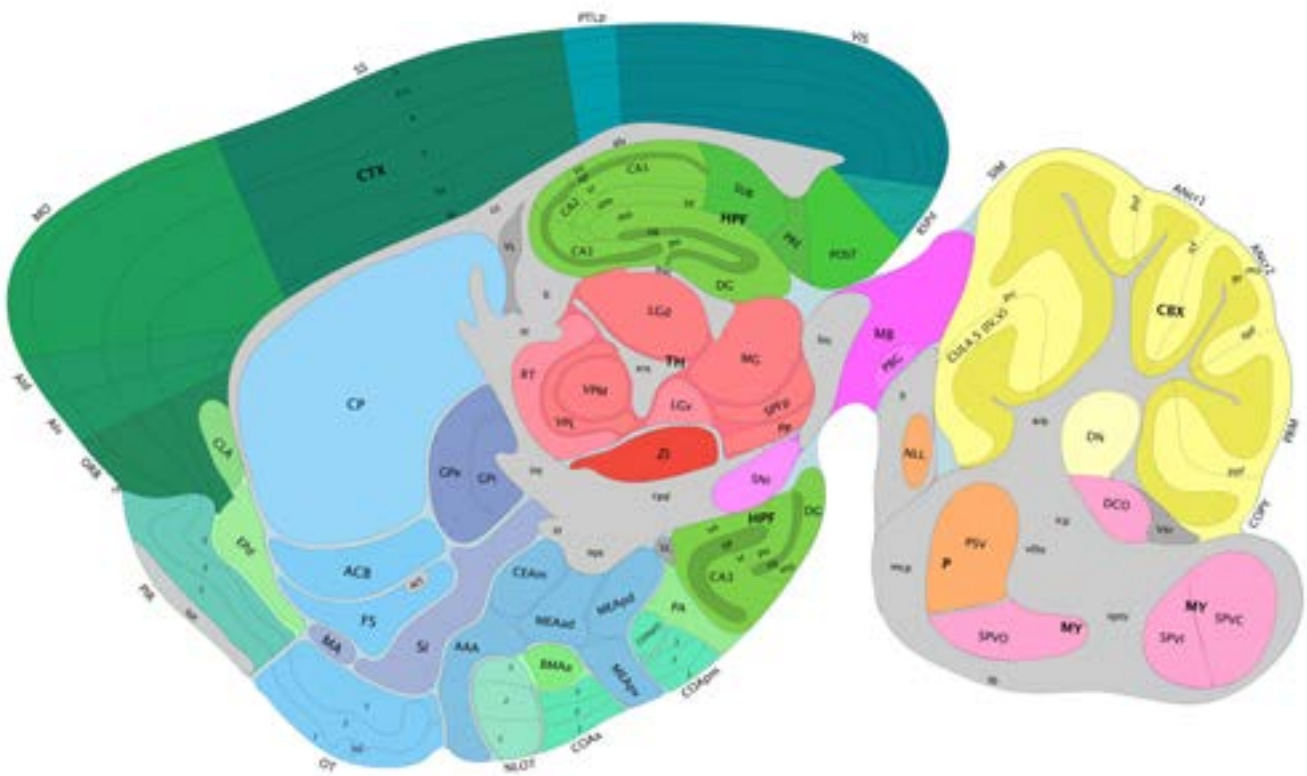
The images are arranged in the navigation strip below the full-size image in order from the lateral edge, closest to the side of the head, to the midpoint of the brain, where the hemisphere meet. At the bottom of each image are +/- signs to zoom into the image and <> arrows to navigate to adjacent slices. The colors indicate major brain regions. You will see that the atlas is labeled P56 - this indicates the age of the mouse (an adult).

You will look at sagittal slices 9, 14, and 21. The numbers are at the bottom of the slice and count up from the lateral (towards the side of the head) to medial (towards the middle of the brain) direction.



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- Visual areas (labeled VIS)
- Primary motor area
- Nucleus accumbens
- Hippocampal formation
- Pons



## Slice 9

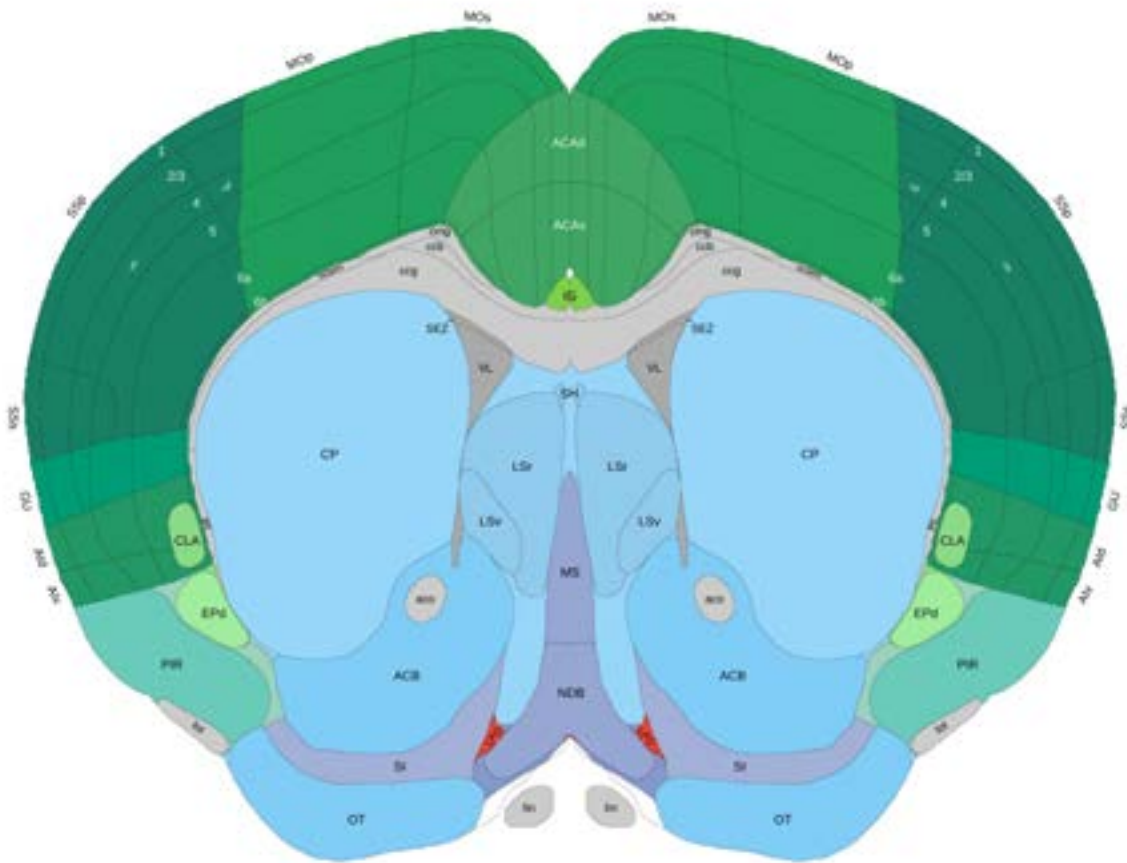




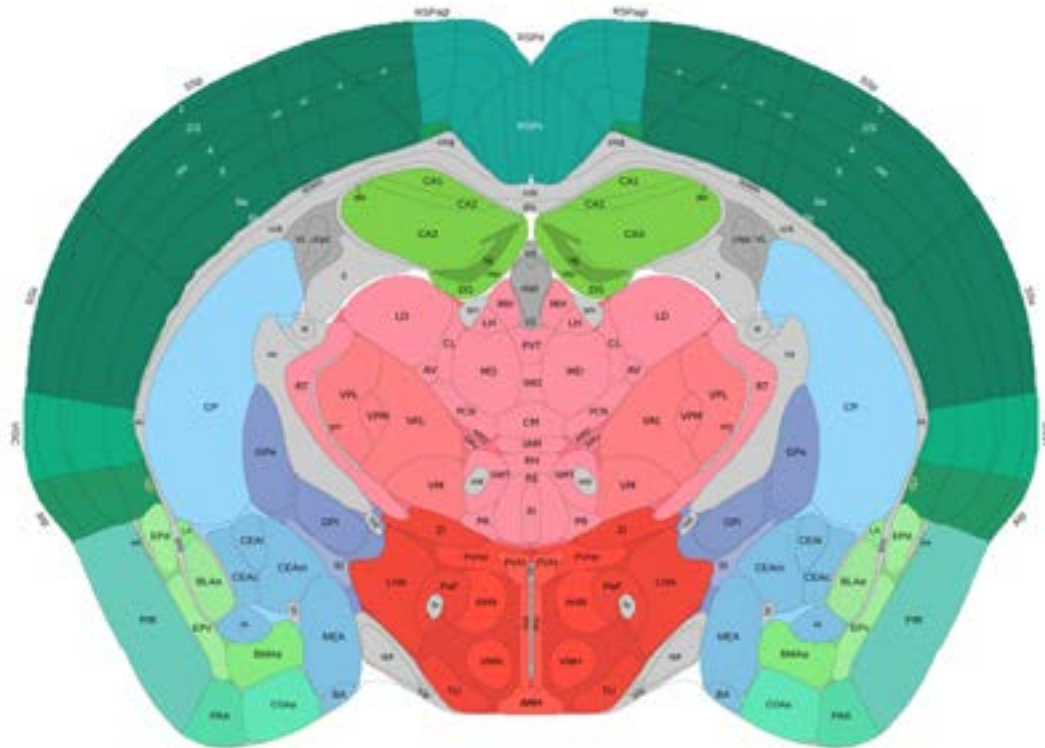


**Instructions:** Go back to the [atlas.brain-map.org](https://atlas.brain-map.org) main page and select Coronal. The coronal plane refers to slicing the brain vertically from front to back, so the slices are parallel to the face. These slices of the brain atlas show the same brain as the sagittal atlas you just looked at, just sliced in a different direction.

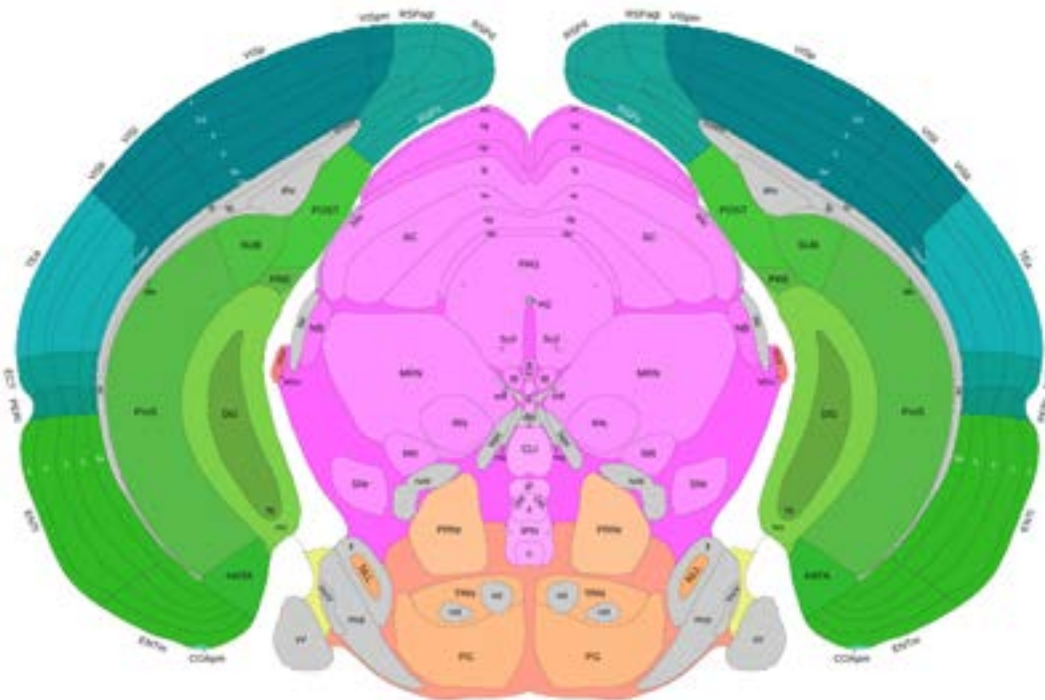
Choose any one of the brain regions you found on the sagittal slices and look for it on coronal slices numbers 47, 65, 91, and 114. The color-coding is the same as the sagittal atlas. Your region will not appear in all four coronal slices!



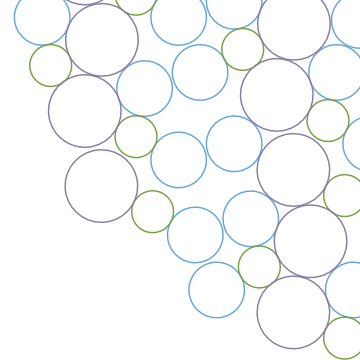
**Slice 47**



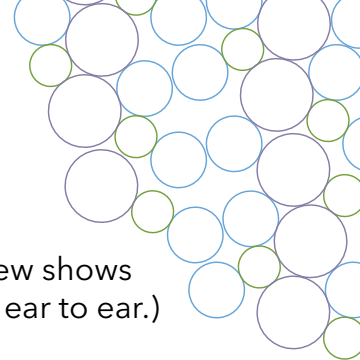
Slice 65



Slice 91



## Slice 114



Why is it helpful to look at the brain in different planes? (Reminder: the sagittal view shows the brain sliced from the front of the brain to the back, and the coronal view from ear to ear.)

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Pick one of your brain regions, or choose another major brain region like the isocortex or cerebellum. List all of the larger regions it is part of, and all of the sub-regions that are part of it, if it has any. You can find this information using the tree on the left side of the atlas, which shows how the brain regions relate to each other. How does this help you get oriented in space and anatomy? Describe your experience.

Brain region: \_\_\_\_\_

Larger regions this area is part of: \_\_\_\_\_

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Sub- regions that are part of this area: \_\_\_\_\_

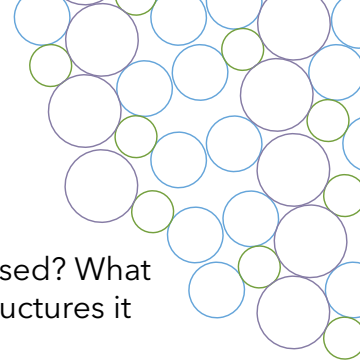
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Observations: \_\_\_\_\_

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Were you surprised by how many different structures the brain has? Or not surprised? What did you expect to see when you started exploring the brain, for the number of structures it has and how they are defined?

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Every brain region has a specialized role or roles. These roles range from decision-making and planning to visual image processing to sleep regulation, and they depend on the inputs the brain region receives from sensory organs and other brain regions.

Pick one of the brain regions you found on the atlas and research its function in more detail online. A good source for students for this kind of information is the Brain Facts 3D brain interactive: [brainfacts.org/3d-brain](http://brainfacts.org/3d-brain).

Brain region: \_\_\_\_\_

Function: \_\_\_\_\_

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If that brain region, and only that region, was damaged from illness or injury, what do you predict the effect would be on the mouse's function?

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Pick two of your brain regions. Go back to [atlas.brain-map.org](https://atlas.brain-map.org) and select the atlas for Human (Brodmann). The two different versions of this atlas use slightly different methods for labeling brain regions, but we are going to use the Brodmann atlas for this activity. (The human atlas is only available in coronal view.)

For each brain region, comparing between the human and mouse brain: What do you notice about differences between the anatomical position, size, or other features of your first brain region between the human and mouse? Think also about differences in behavior and cognition between humans and mice.

Brain region #1: \_\_\_\_\_

Observations: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

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\_\_\_\_\_

Brain region #2: \_\_\_\_\_

Observations: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_





# Exploring Pathways in the Brain

## Section 2: Integrating Gene Expression and Anatomy

### Introduction and goals for students:

- Brain regions have different functional roles. In this assignment, you'll explore the relationship between the function of a brain region and its gene expression profile.
- You'll learn about in situ hybridization and how to interpret ISH data.
- You'll also have an optional opportunity to compare your findings between humans and mice.

Read highlights from the paper accompanying the Allen Mouse Brain Atlas, "Genome-wide atlas of gene expression in the adult mouse brain" ([nature.com/articles/nature05453](https://www.nature.com/articles/nature05453), doi:10.1038/nature05453). Focus on the methods section, which explains the methodology, and Figures 3 and 6-9, which demonstrate interpretation of the data that you will use.

**Instructions:** Visit [mouse.brain-map.org](https://mouse.brain-map.org). You will search in the atlas for three genes of your choice from the list below. Your instructor may direct you to look up other or additional genes.

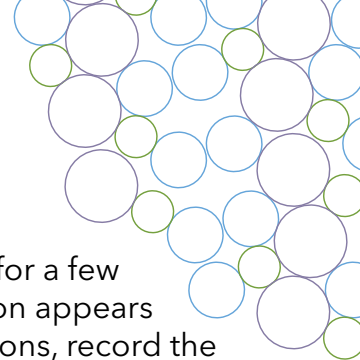
Select three of these genes, or write down the genes your instructor provides you:

- Htr1b
- Drd1
- Drd2
- Cux2
- Rorb
- Prnp

Enter your gene name in the search box at the top of the page. More than one experiment will likely appear in the results list after your search. These experiments use slightly different parameters, and some are in coronal slices and some in sagittal slices. Choose one of the experiments in sagittal slices by clicking on the gene symbol or experiment number of that row.

A new page will let you scroll through the sagittal slices of the brain showing expression of that gene. These are the same sagittal slices you looked at in the illustrated atlas before. You can also use the small Brain Explorer box to the left of the slice images to see the expression projected in 3D and use the slider to rotate it. To learn more about how the website and how the data was collected and processed, click the Help button in the page header.

Now use the NIH Gene database ([ncbi.nlm.nih.gov/gene](https://ncbi.nlm.nih.gov/gene)) to look up the function of your genes. Enter your gene name into the search box and select your gene from the list. (This database includes genes from other species, so make sure you select human!) What protein does it encode? What is its role? This information should appear in the "Summary" section of the gene entry. Fill in your answers in the table below.

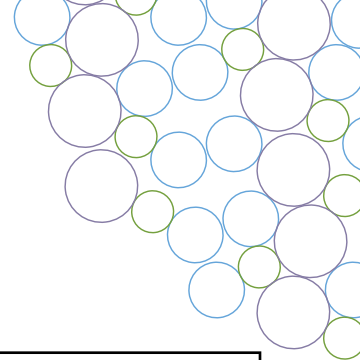


Allen Institute scientists have calculated the relative expression level of the gene for a few major regions based on the intensity and density of the ISH signal. This information appears in the form of a bar chart below the brain images. From those pre-computed regions, record the region of the brain that had the highest expression of each of your genes using the table below. If you hover over a bar in the chart, it will write out the brain region's name in full at the top of the chart. There may be more than one region with similar expression levels – if so, record the few highest.

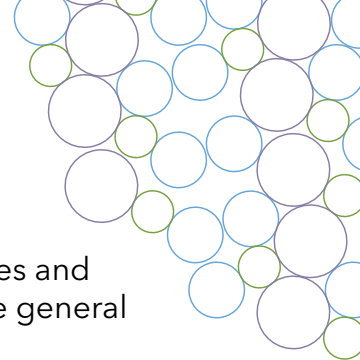
Take note of the pattern of expression across the regions with precalculated expression. Is the gene expressed fairly equally across the whole brain? Is there one region, or a few regions, with much higher expression than all others? Is distribution fairly mixed, with some having high expression, some low, and some in between? Note your observations in the table below.

*It is important to know that the signal amplification process used in creating these images means that the numerical values reported are not absolute. You cannot infer an exact ratio for the expression level of two genes from the intensity levels reported.*

Record your data in the table on the next page.



| Gene name | Protein encoded | Role of protein | Regions with highest gene expression (list) | Overall pattern of expression (describe) |
|-----------|-----------------|-----------------|---|--|
|           |                 |                 |   |  |
|           |                 |                 |   |  |
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In the previous portion of this worksheet, you looked up the function of your genes and found a region or regions where that gene had high expression. Now, look up the general function of that brain region.

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Discuss the relationship between the function of the gene/protein it expresses and the function of the brain region. How are they connected? This will require some additional research - we recommend the NIH Gene Database ([ncbi.nlm.nih.gov/gene](https://ncbi.nlm.nih.gov/gene)) for gene and protein information and the Brain Facts 3D brain interactive ([brainfacts.org/3d-brain](https://brainfacts.org/3d-brain)) for functional information.

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Many genes have similar, low levels of expression across the brain. How do you interpret this finding?

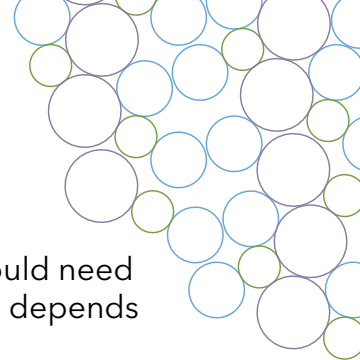
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What other brain regions or sensory systems do you predict your brain region would need to work with or rely on to complete its tasks? For example, the primary visual area depends on receiving input from the eyes.

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Mice are frequently used in scientific research to test effects we think might appear in humans, but results in mice do not always correspond directly to findings in humans.

Thinking about mouse and human anatomy or gene expression, describe at least two reasons why mouse and human results in a similar experiment may differ. (Hint: Think about genes, the anatomy of corresponding brain regions, and the size of the brains.)

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How could you use gene expression data to design an experiment in mice that will more accurately reflect human biology? (Hint: Think about comparing gene expression data in humans and mice.)

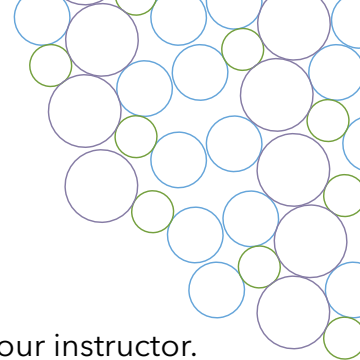
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### Challenge assignment #1:

Complete this part of the assignment for an added challenge, or as directed by your instructor.

The slice positions of the reference atlas used in Section 1 of this exercise closely correspond to the slices in the gene expression atlas at [mouse.brain-map.org](https://mouse.brain-map.org). For a challenge, look at the in situ hybridization (ISH) slices and find an area of tissue that has interesting expression patterns (very high, very low, very different to immediate neighbors, etc.). The precalculated expression levels are for relatively large regions (such as the whole isocortex).

To do this, you will want to compare the ISH data to the annotated reference atlas. At the top right of the ISH image, click the button that looks like a box with an arrow pointing out of it. In the pop-up that appears, click the button that looks like a key to bring up a side-by-side view of the ISH data and the annotated anatomy.

Brain region you identified: \_\_\_\_\_

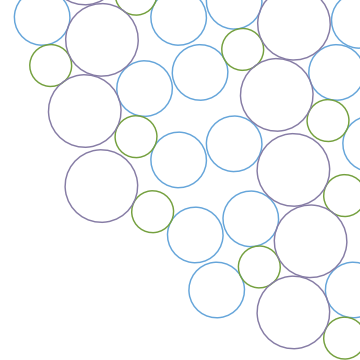
### Challenge assignment #2:

Complete this part of the assignment for an added challenge, or as directed by your instructor.

Go explore the Allen Human Brain Atlas ([human.brain-map.org](https://human.brain-map.org)). Most of the data was collected with RNA microarray instead of ISH, but it also shows relative expression levels of all genes in the whole human brain. A microarray uses probes that attach to RNA transcripts that are attached to a chip, and extracted RNA from one whole brain region at a time is applied to the chip to measure how much is present. The expression levels are shown as the amount of transcript present per brain region instead of as images, like the ISH data.

You can explore the Allen Human Brain Atlas however you want. One way to explore is to go back to the table you filled out with the genes and their expression patterns in the mouse brain, and look up the expression pattern of one of those genes in the human brain.





# Exploring Pathways in the Brain

## Section 3: Exploring Brain Connectivity

**Introduction and goals for students:**

- In this assignment, you'll explore how brain regions are connected to each other via individual neurons.
- You'll learn how connectivity can be measured and how to interpret connectivity data.
- You'll complete a short experiment using connectivity data and discuss your findings.
- You'll design your own experiment using the type of data you just learned how to interpret.

Read highlights from Oh et al. (2014). A mesoscale connectome of the mouse brain. Nature. [pubmed.ncbi.nlm.nih.gov/24695228/](https://pubmed.ncbi.nlm.nih.gov/24695228/), doi: 10.1038/nature13186. Focus on the methods section and Figures 1 and 2, which explain how the database was created and show some example projection patterns. Optional: Read this news article describing an expansion and update to the database, also reviewing some of the methods used: [alleninstitute.org/what-we-do/brain-science/news-press/press-releases/new-high-resolution-map-how-brain-wired](https://alleninstitute.org/what-we-do/brain-science/news-press/press-releases/new-high-resolution-map-how-brain-wired).

Explain how the projection data was collected for the wild type injection tracing data.

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Explain how the projection data was collected for the Cre line data. How is it the same as the method used in the wild type mice? How is it different?

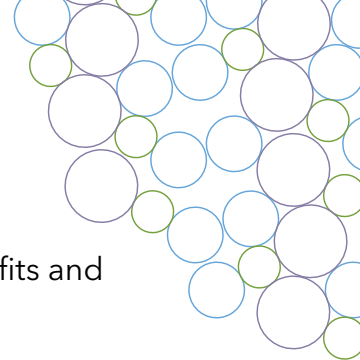
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Contrast these two variations on the projection tracing method: Explain the benefits and limitations of each.

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Explain why both injection tracing, both with and without using Cre lines, can't be used in humans.

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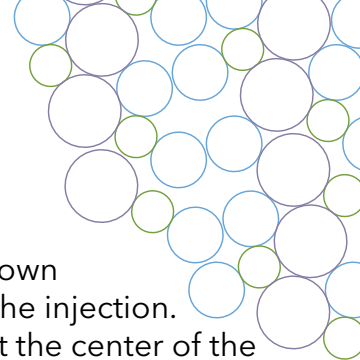
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**Instructions:** Go to [connectivity.brain-map.org](https://connectivity.brain-map.org). Each point on the mouse brain shown represents one injection site and notes the primary brain region associated with the injection. (You may need to select a specific source structure from the list below the brain at the center of the page in order for these points to show up.) If you click on any point, additional data will appear in the sidebar showing the projections from that injection.

For injection experiments only: note which brain regions are included in each injection site. No injection site will completely fill a specified brain region without also slightly spilling into neighboring regions. Many injections cover more than one brain region, but the region that contains most of the injection site is considered the “primary” source for the traced pathways.

Explore a few experiments to get a sense of the data. Make sure you look at a few experiments from Cre lines and a few wild type injection experiments.

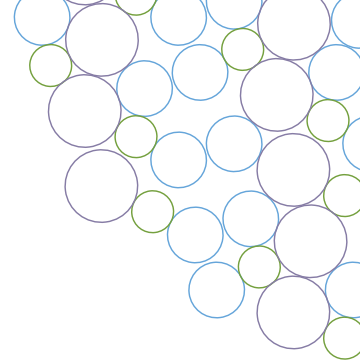
At the top of the sidebar, click the “i” button to bring up more detailed information about the results from that injection experiment, including a graph showing calculated projection volume (larger values = more projection from that source to this target).

To further visualize the projections, copy the experiment number from the top of the sidebar or the experiment detail page, then click the “Brain Explorer” button in the top navigation bar of the page and launch the beta 3D Allen Brain Explorer page. Turn on “Basic cell groups and regions” by clicking the eye icon and turn on transparency, then paste your experiment number into the “Add experiment by ID” box. You’ll visualize the projection of your cell in 3D to help you get oriented further.

Note that the data shown on the website is not thresholded – all signal detected, no matter how small in volume or low density, is shown. You can adjust the threshold with the slider bar at the top right of the experiment detail page. As you explore the data, consider how much signal needs to be present for an area to be considered a true target for that source region, and how your interpretation might change if the injection included more than one source region. The Allen Institute scientists often set the threshold to  $\log(\text{volume}) < -1.5$  to avoid false positives, so we’ll use that for this experiment. Set the minimum volume for display using the slider at the top of the experiment detail page.

**Experiment:** Choose and complete one of these two virtual experiments. For an added challenge, complete both experiments.

- Option 1: Identify all known target regions for a given source region and interpret your findings in light of the functions of those regions.
- Option 2: Choose any one region and look at the source injections in that region. Find which Cre line or lines are associated with sources in that region. Compare the projection patterns of the Cre line(s) against the wild type injection-traced projections. Are the projections from the Cre lines different from the projections from the wild type experiments?
  - Based on the characteristics of the Cre lines, we recommend focusing on cortical source regions. The largest number of Cre lines are associated with cortical sources.



## Both experiment options:

Your source region:

General function of your source region:

## Data collection for experiment option 1:

Create a table for your data collection in your lab notebook or on scratch paper with these columns:

- Target regions
- Target region function
- Interpretation

In each row of the table, list target regions (that meet the threshold) and their functions, then interpret each connection between the source and target in light of the regions' functions.

## Data collection for experiment option 2:

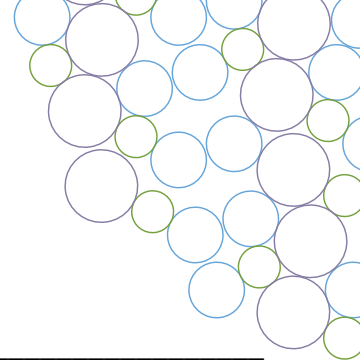
Create a table for your data collection in your lab notebook or on scratch paper with these columns:

- Cre lines
- Target regions - Cre lines
- Target regions functions - Cre lines
- Target regions - wild-type

In each row of the table, list the Cre lines that have injection experiments in that source. For each Cre line, find all target regions (that meet the threshold) and identify their functions. After you have finished analyzing data from all Cre lines with injection sites in that brain region, make another row of the table for the injection tracing experiments in wild-types. Find all of the wild-type injection tracings from the same source region, find all target regions (that meet the threshold) and identify their functions.

Compare the projection patterns for the Cre line and wild-type experiments for your source region. How are they similar? How do they differ? What do you think drives differences you observe? Describe your observations separately from the table.

Submit your data table to your instructor as directed.



Why is it useful to study connectivity?

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This dataset uses a method of measuring connectivity that cannot be used in the human brain. What is an application to the study of human brains do you see from this data?

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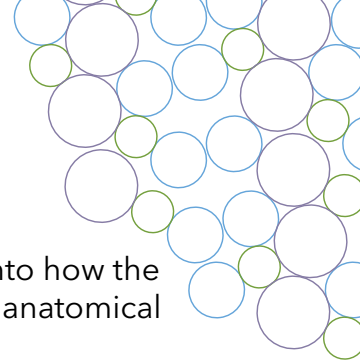
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What are two examples of insights anatomical connectivity patterns can give us into how the human brain works? What is an example of something that you cannot infer from anatomical connectivity data alone?

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This dataset tracks individual neurons throughout the brain. What other features of the brain and neurons could you study the connectivity of, in anatomy or function, and at various scales? You may want to do some research on various methods used to study connectivity to answer this.

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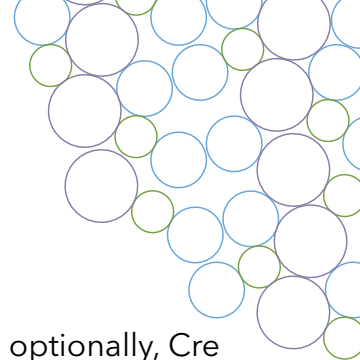
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## Design your own experiment

Design own experiment that investigates connectivity using injection tracing and, optionally, Cre line methods.

**Research question:**

**Method(s) used.** You may want to include other methods you have learned about in classes in addition to injection tracing and potentially Cre lines:

**Data you would collect:**

**How you would interpret the data:**

**Expected results:**

For an added challenge, investigate technology that can be used to study connectivity in humans and design an experiment to be done in humans that uses that technology.