What Is AGEA?

The Allen Institute has built a data-driven three-dimensional atlas of the adult C57Bl/6J mouse brain based on the ISH gene expression images of the Allen Mouse Brain Atlas. Only the coronal gene expression data were used to construct this application. The AGEA feature (which stands for Anatomic Gene Expression Atlas) characterizes the multi-scale spatial relationship in the mouse brain as derived from coronal gene expression data without a prior knowledge of classical anatomy. Using the AGEA feature of the Allen Mouse Brain Atlas, you can:

- View and navigate 3-D spatial relationship maps (Correlation mode) and search for genes within the coronal dataset with local regionality (Find Genes) and,
- Explore a transcriptome based spatial organization of the brain (Clusters mode).

To launch this feature, click on the AGEA tab from the Allen Mouse Brain Atlas and you will see the following image.
The upper panel of images are orthogonal views of a 3-D Nissl reference atlas volume. The reference space was divided into 200 µm volumes, or voxels. The cross hairs in the top panel of images select for a seed voxel from which to compare to gene expression profiles to all other voxels in the brain. For more detailed information on how AGEA was constructed please see the AGEA user guide or Ng L, et al. (2009) An anatomic gene expression atlas of the adult mouse brain. *Nature Neuroscience* 12(3): 356-362.

The AGEA Viewer

This section describes the controls for the Correlation and Cluster modes of AGEA.
A. Seed Selector panel: Use this area to select the seed voxel as marked by the red crosshairs. Either click or click and drag to navigate to a different correlation map.

B. Map panel: This area displays the three orthogonal views of the currently selected correlation map in coronal, sagittal and horizontal planes. The red crosshair marks the currently selected voxel. Either click or click and drag to move to a new 3-D location. Note that this volume can be navigated in 3-D for any selected voxel location from A.

1. About/Permalink/Zoom: Clicking on "About" will bring up more information on AGEA, clicking on "Permalink" creates an URL in the address bar that effectively saves information about your current viewing state. This URL can be saved for later access to directly take you back to the current view and color scale settings. Clicking the arrows will toggle between zooming the images to fit in the window and a higher resolution, more zoomed mode. At a fixed zoom level, you may need to use the browser scroll bars to view all the images.

2. Mode Selector: Click on Correlation or Clusters to switch the viewer to different modes.

3. Position: The position of the seed selector crosshairs in millimeters in PIR (+x = posterior, +y = inferior, +z = right) orientation, where the origin is the anterior, superior, left corner of the volume.

4. Lock/Sync: The icon on the left locks the planes shown in the selected expression map B to the same position as the seed map A. Click to toggle the locking behavior. Conversely, click on the right icon to move the seed voxel to the current selected map voxel.

5. Allen Reference Atlas Label (Panel A): The structure or structural grouping from the Allen Reference Atlas to which the seed voxel (indicated by red crosshairs) belongs. Click on the name to open a window with an interactive reference atlas viewer.


7. Position: The position of the seed selector crosshairs in millimeters in PIR (+x = posterior, +y = inferior, +z = right) orientation, where the origin is the anterior, superior, left corner of the volume.
8. **Allen Reference Atlas Label (Panel B):** The structure or structural grouping to which the selected voxel (indicated by red crosshairs) belongs. Click on the name to open a window with an interactive reference atlas viewer.

9. **Correlation:** Value of the correlation at the currently selected voxel with respect to the seed voxel selected in panel A. This shows numerically how well the target voxel is correlated with the seed.

10. **Gene Finder Button:** (in Correlation mode only) Click this icon to find genes with enhanced expression in the voxel targeted by the crosshairs in panel A.

11. **Color scale control:** Use to adjust the false color mapping of the correlation map to threshold the images for regions of higher significance. All voxels with correlation within the select range are rescaled to span the color scale.

12. **Download:** Click to download the currently selected correlation map as raw flat file with numbers saved as floats.

**Using Correlation Mode**

In Correlation mode, the lower panel shows orthogonal views of the selected spatial relationship map. The correlation values in the bottom figures can be interpreted as a measure of average co-expression between two voxels. The higher the correlation value between voxels, the more common it is for genes from the seed voxel to be co-expressed. Higher correlation between two voxels indicates more spatial correlation of expression and thus potentially higher possibility that the spatial regions spanned by the voxels are anatomically related. This may indicate that the voxels compared share common cell types or represent a coherent functional map. The correlation map can also be used to locate coexpressing areas in other brain regions. In the above figure, the map indicates that there is higher co-expression in thalamic structures than in other regions of the brain.

When a seed voxel is selected using the crosshairs in the images from the top panel, you can find correlations between the seed voxel and other voxels in the brain by selecting a distinct voxel in the lower panel using the crosshairs in the images from the lower panel. The correlation between the regions will be displayed below the lower panel (9).

The gene finder search facility is among the most powerful aspects of AGEA’s functionality. It enables users to search an anatomic region of interest for genes within the Allen Mouse Brain Atlas coronal database that exhibit localized enrichment. Clicking the “Find Genes” button returns a list of genes selected by the crosshairs in the upper panel (A). Voxels in the yellow to red range (top third of the range) are considered the local region of interest (target domain) while all voxels above threshold cutoff (all non-dark blue voxels) forms the contrast domain region. These domains are used in the differential search to find the genes that exhibit localized enrichment.

**Gene Finder Search Results**

Clicking the “Find Genes” button will return a list of experiments based on the input search criteria on a separate page. The AGEA correlation map as well as the seed location in PIR orientation (in µm) is illustrated at the top of the page.
Each row of the search results includes the following information.

<table>
<thead>
<tr>
<th>Column</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Add the experiment to your selections by clicking the checkbox</td>
<td>A numeric value representing the fold change between expression of the target domain and the contrast domain</td>
</tr>
<tr>
<td>Fold Change</td>
<td>Click the experiment number to view experimental detail page</td>
</tr>
<tr>
<td>Experiment ID</td>
<td>Click Gene Symbol to return gene metadata</td>
</tr>
<tr>
<td>Gene Symbol</td>
<td>Full gene name (click to see 3-D thumbnail and perform a NeuroBlast search)</td>
</tr>
<tr>
<td>Gene Name</td>
<td>Image from the seed location</td>
</tr>
<tr>
<td>Seed Location</td>
<td></td>
</tr>
</tbody>
</table>

You will have to visually inspect the dataset to ensure the signal that AGEA detected was real and not an artifact of the image collection.

**Clusters Mode**

Selecting Clusters mode switches the lower panels to view a data-driven hierarchical binary tree spatial organization of the brain computed from the AGEA correlation maps. To construct the decomposition, all 61,053 voxels were assigned to the root node of the tree. As we descend the tree, a node is bifurcated into two nodes to achieve maximal dissimilarity between two groups of voxels based on correlation values. The final bi-tree consists of 122,105
nodes with a maximum depth of 54 levels and 61,053 leaf nodes (one for each voxel in the brain). Effective visualization of this large data structure is via an easy-to-use Tree Depth slider mechanism to navigate the bi-tree, providing 3-D context and visualizing the multi-scale partitioning.

In the above example, when the field CA3 is selected from the top panel you can see the relationship of this region to the rest of the hippocampal formation at a tree depth of 10.

The voxels of a node are visualized with a systematic color coding scheme. All voxels of a node are assigned a color based on the 'jet' color scheme where the leaf node with low-order voxels are assigned shades of blue. The colors then run through green, yellow, orange, and finally, higher-order voxels are assigned shades of red.