

This supplement contains a qualitative comparison of the Mai-Paxinos atlas, the Allen atlas and our atlas *NextBrain*. Each slice in our comparison (one per page) correspond to one of ten selected sections from the digital version of the Mai-Paxinos atlas, which is publicly available at: <https://www.thehumanbrain.info/brain/sections.php>

These were originally published as part of the book:

JK Mai, et al. "Atlas of the Human Brain", 3rd edition, Academic Press (Elsevier), 2008.

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On the left of each page, we show the labels from the Mai-Paxinos atlas. On the right, we show the labels of the approximately equivalent of the Allen reference brain, freely available for non-commercial use at:

<http://atlas.brain-map.org>

These are also presented in the journal article:

SL Ding, et al. "Comprehensive cellular-resolution atlas of the adult human brain." Journal of comparative neurology 524.16 (2016): 3127-3481.

In the middle of each page, we show an approximately corresponding coronal slice of *NextBrain* (with the boundaries between the most likely regions overlaid in red). We note that there are anatomical differences due to:

- The slightly different orientation of their sections, compared with *NextBrain*.
- The probabilistic nature of *NextBrain* - which is particularly noticeable in cortical regions.
- Anatomical variability between the single specimens that the atlases are based on – while ours is an average.

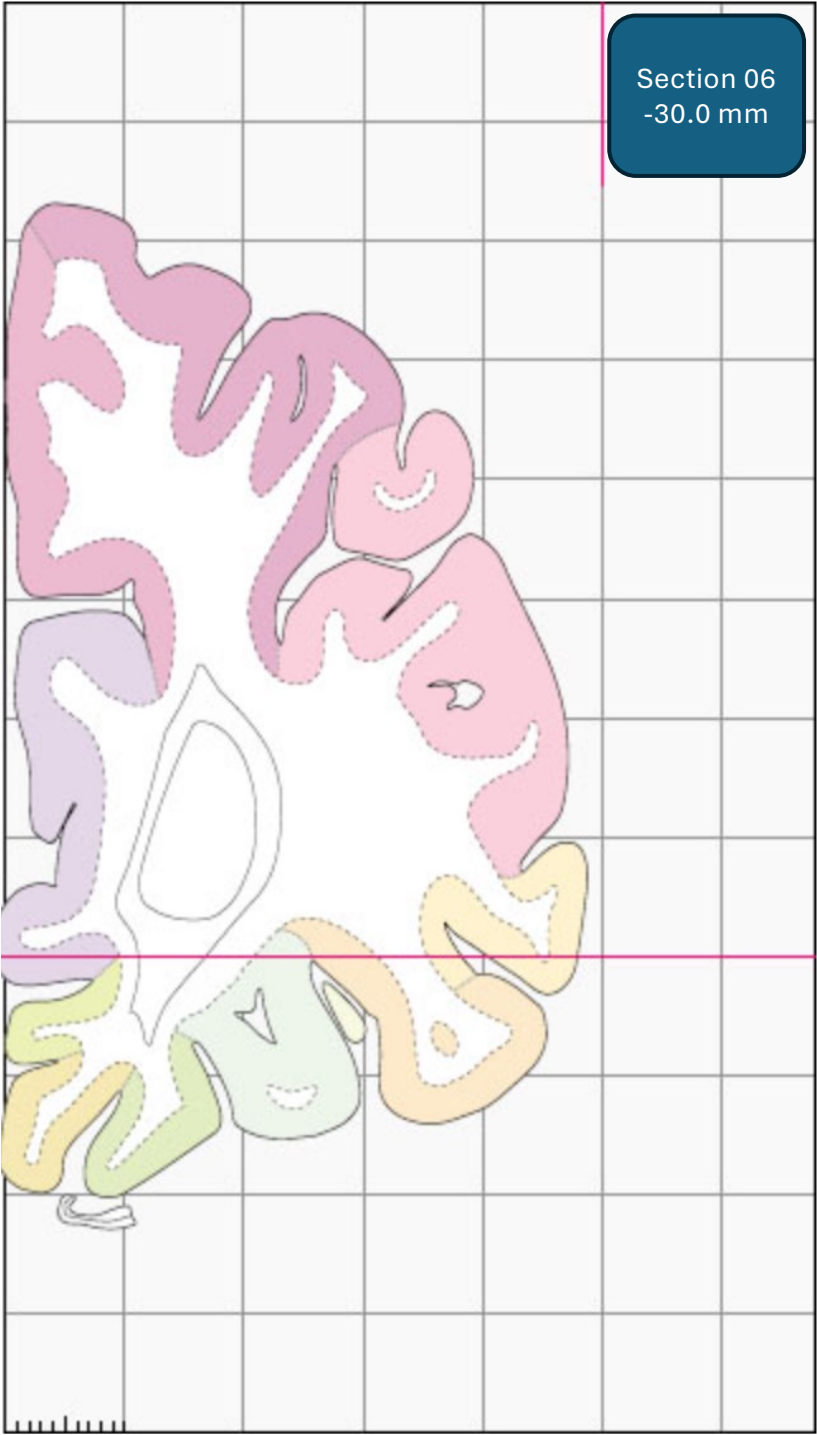
In general, the agreement between *NextBrain* and the two atlases is good, especially for the outer boundaries of the main structures, and for the borders between the grey matter and white matter and/or the cerebrospinal fluid. This is particularly clear in the striatum, the hippocampus, the amygdala and the thalamus. The main differences lie in:

- The delineation of sub-structures within the main cortical regions. For example, the superior frontal gyrus is labelled as a whole in both Allen and *NextBrain*, rather than separating its medial and lateral part as done in the Mai-Paxinos (e.g., Slices 1-2 below). Similarly, the insula is labelled as a whole in both the Mai-Paxinos atlas and *NextBrain*, rather than into its agranular and dysgranular subcomponents as in the Allen atlas (see Slices 2-3 below). We note that *NextBrain* directly takes the cortical parcellation from FreeSurfer.
- The annotation of the smaller nuclei within the subcortical regions. For example, the nucleus accumbens as a whole in *NextBrain*, rather than its lateral, medial and central part in the Mai-Paxinos (see Slice 2 below). We emphasise that these differences are present *even between the Mai-Paxinos and Allen*, and they are mainly due to the forced choice of applying arbitrary anatomical criteria for poor visualization and insufficient contrast in smaller regions, and to different anatomical definitions used by anatomists.
- Increased thickness of some regions (particularly cortical) in *NextBrain* when taking the maximum-probability labels, due to the probabilistic nature of the atlas.

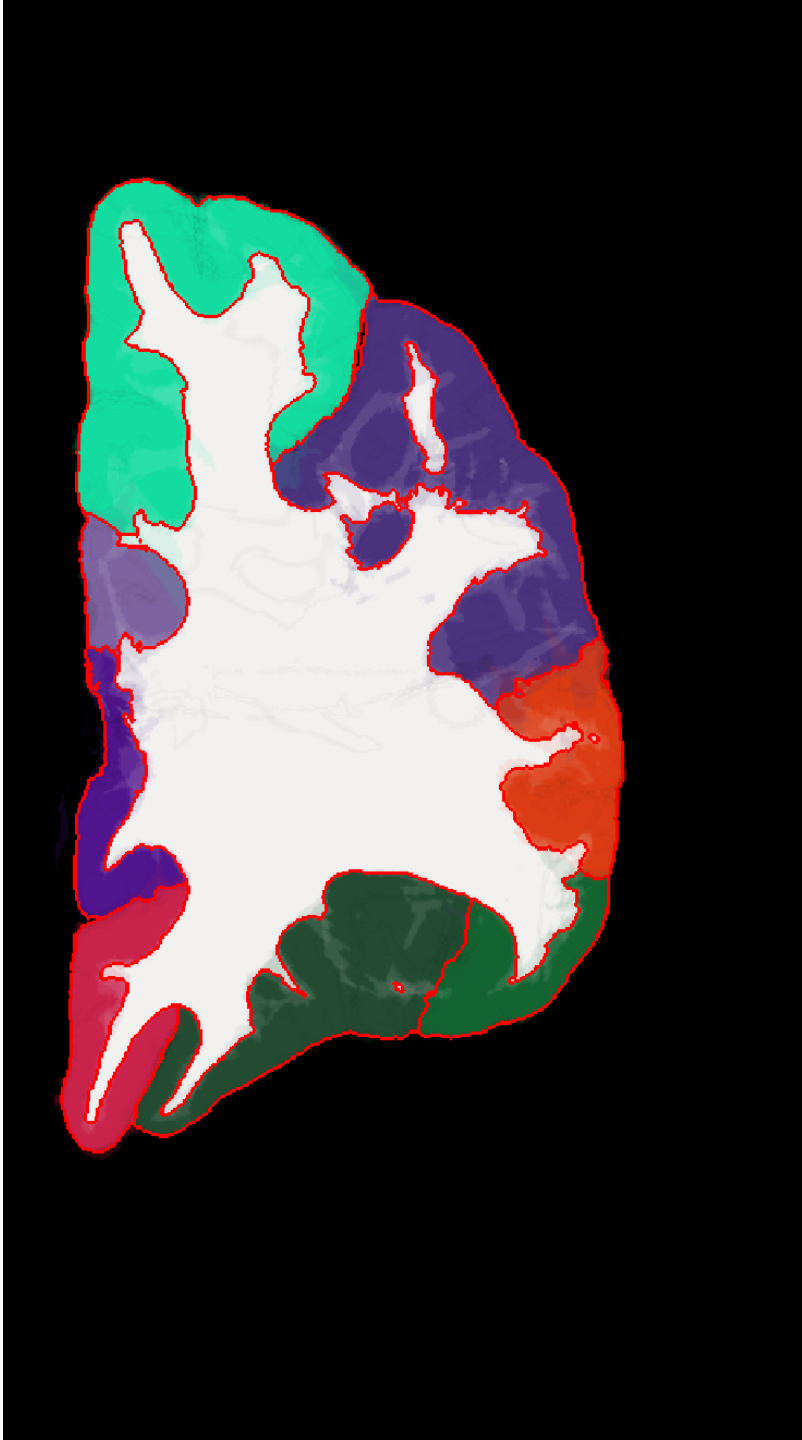
Some further comments about specific brain regions:

- The smaller subnuclei of the hypothalamus are difficult to delineate and accurately replicate (see, e.g., differences between Mai-Paxinos and Allen in Slice 3). *NextBrain* is fairly close to Allen in this area. We also note that larger subregions (anterior-inferior, anterior-superior, tubular-inferior, tubular-superior, and posterior with the mammillary bodies) can be reliably segmented from in vivo MRI ("Automated segmentation of the hypothalamus and associated subunits in brain MRI." *Neuroimage* 223, 2020: 117287).
- Although the outer boundaries of the hippocampus match between the atlases, there are some differences in the anatomical delineation when it comes to the subfields and internal boundaries. For example, in our atlas the CA3 region includes part of the CA2, and the CA4 the molecular layer of the dentate gyrus, rather than separating them into individual components (see, e.g., Slices 6-7 below).
- The overall boundaries and division of the amygdala into its broad subnuclei is generally consistent across the three atlases. One of the main differences between *NextBrain* and the other atlases is the more detailed subdivision of the main nuclei in Mai-Paxinos and Allen. While an extended segmentation of the larger nuclei (e.g., lateral, basal, accessory basal) would be certainly feasible in the future, subdividing smaller nuclei (e.g., medial into rostral and caudal subdivision; cortical into anterior and posterior) will always be challenging to robustly delineate. Again, we emphasise that there are clear differences between the Mai-Paxinos and Allen atlases in these subdivisions, particularly for smaller regions of interest (see, e.g., Slice 4 below).
- The thalamus has some clear differences between atlases, due to the complexity of visually identifying reliable boundaries between its subnuclei. However, the main subdivisions (e.g., pulvinar or mediodorsal regions) are consistent across the three atlases (see, e.g., Slice 6 below).

Mai & Paxinos (labels)



NextBrain (probabilistic labels)



Allen reference brain (labels)

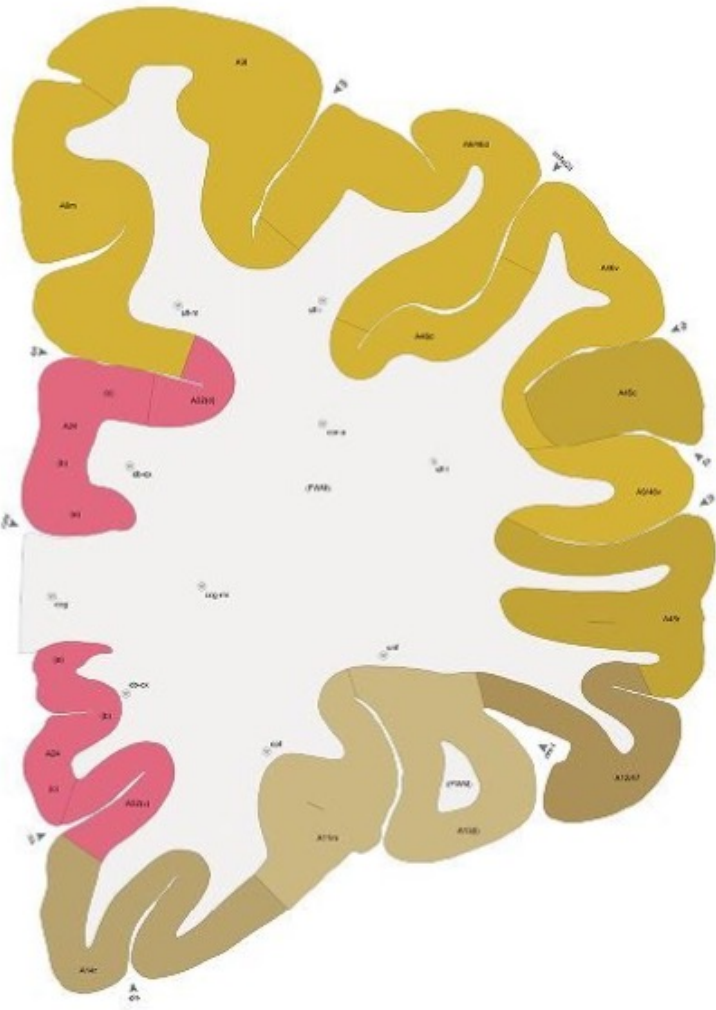
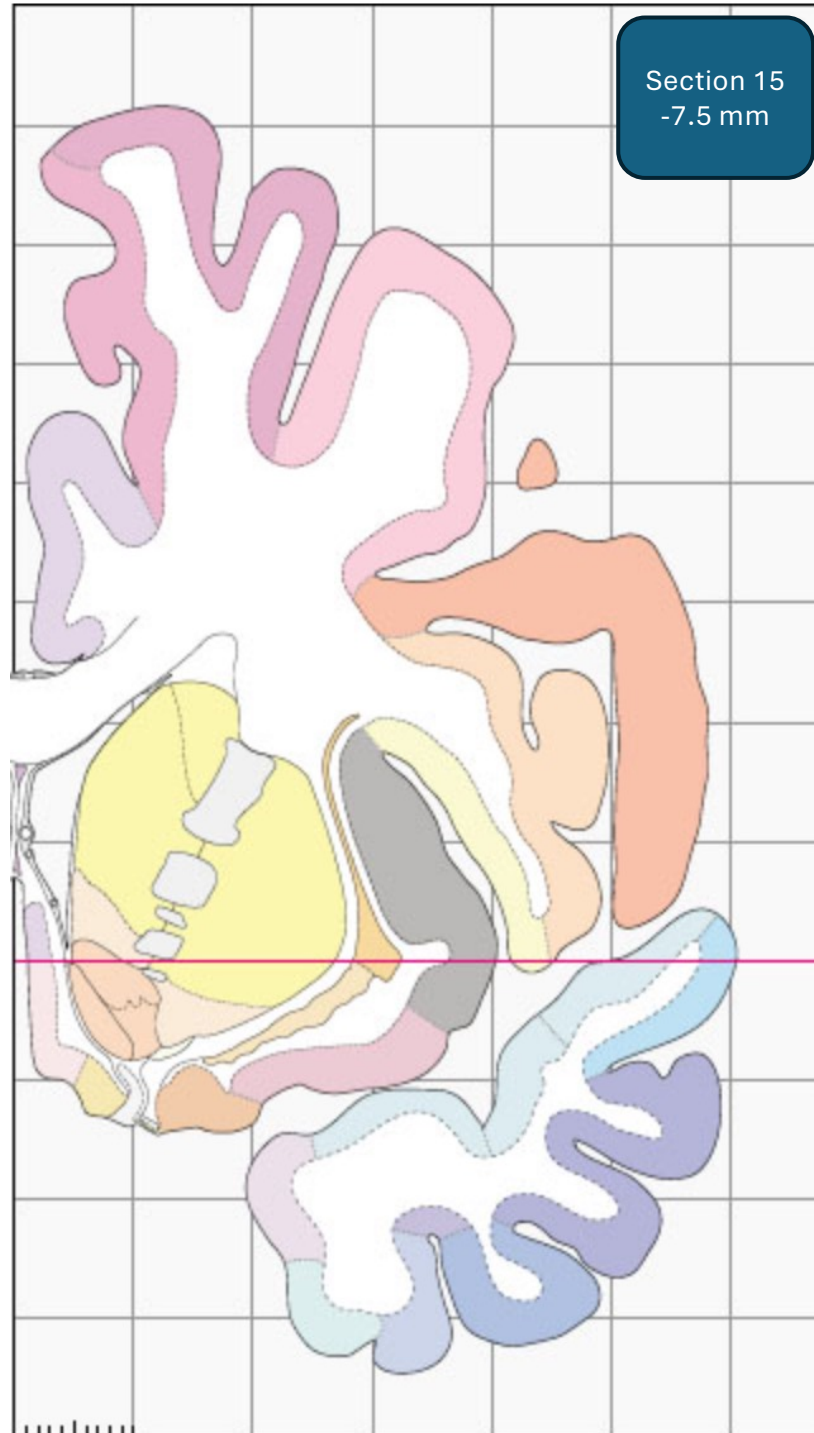


Image 12

SLICE 1

Mai & Paxinos (labels)



NextBrain (probabilistic labels)



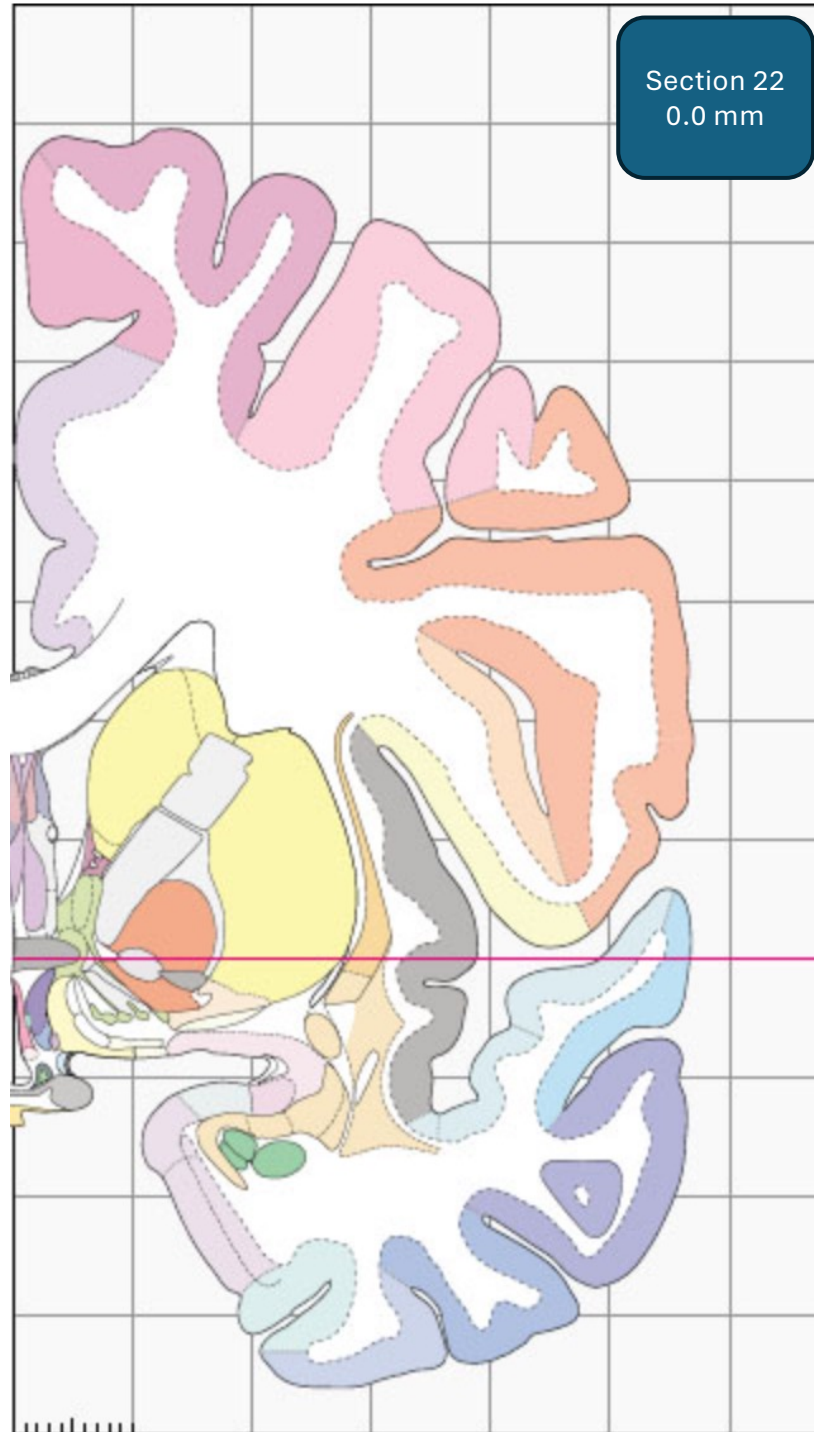
Allen reference brain (labels)



Image 18

SLICE 2

Mai & Paxinos (labels)



NextBrain (probabilistic labels)



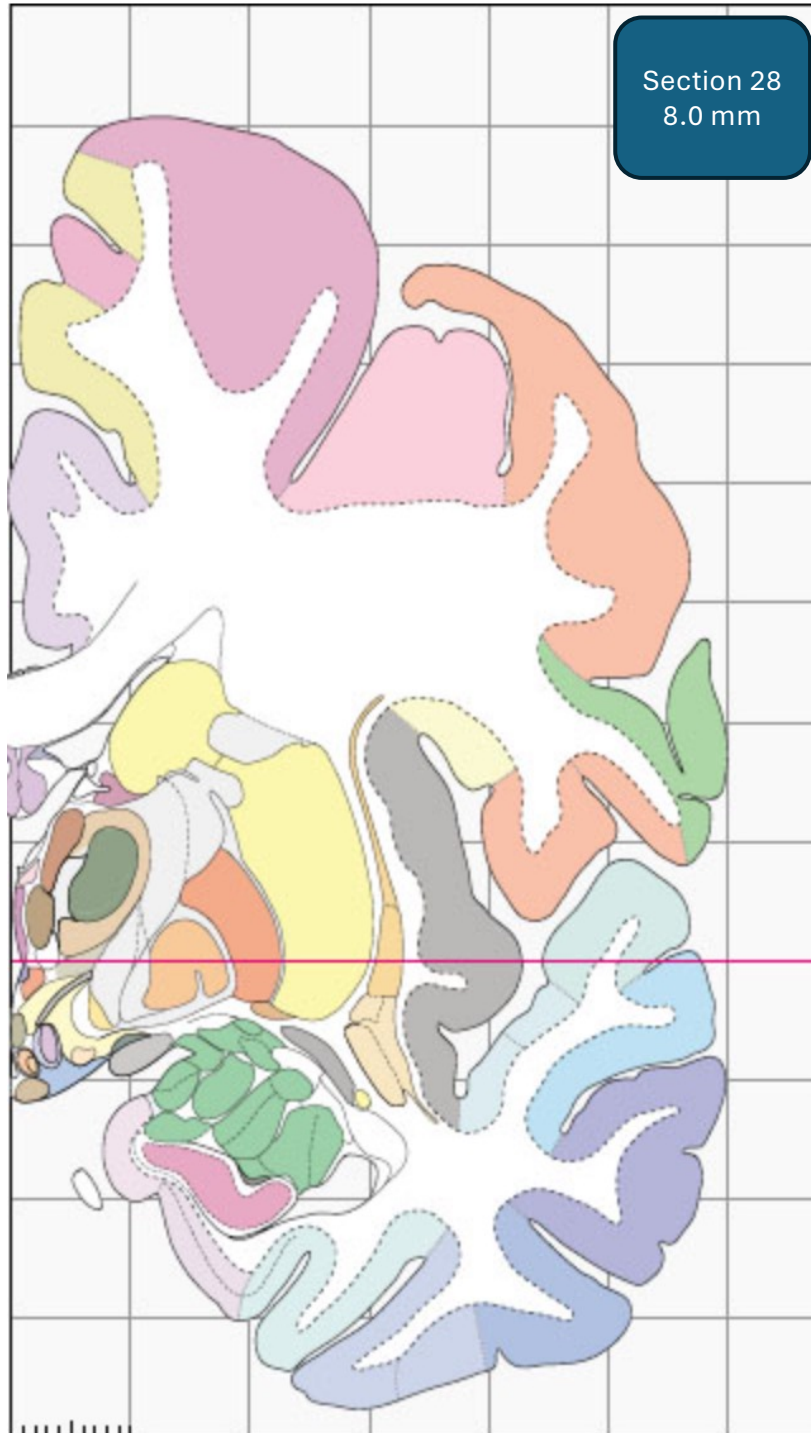
Allen reference brain (labels)



Image 24

SLICE 3

Mai & Paxinos (labels)



NextBrain (probabilistic labels)

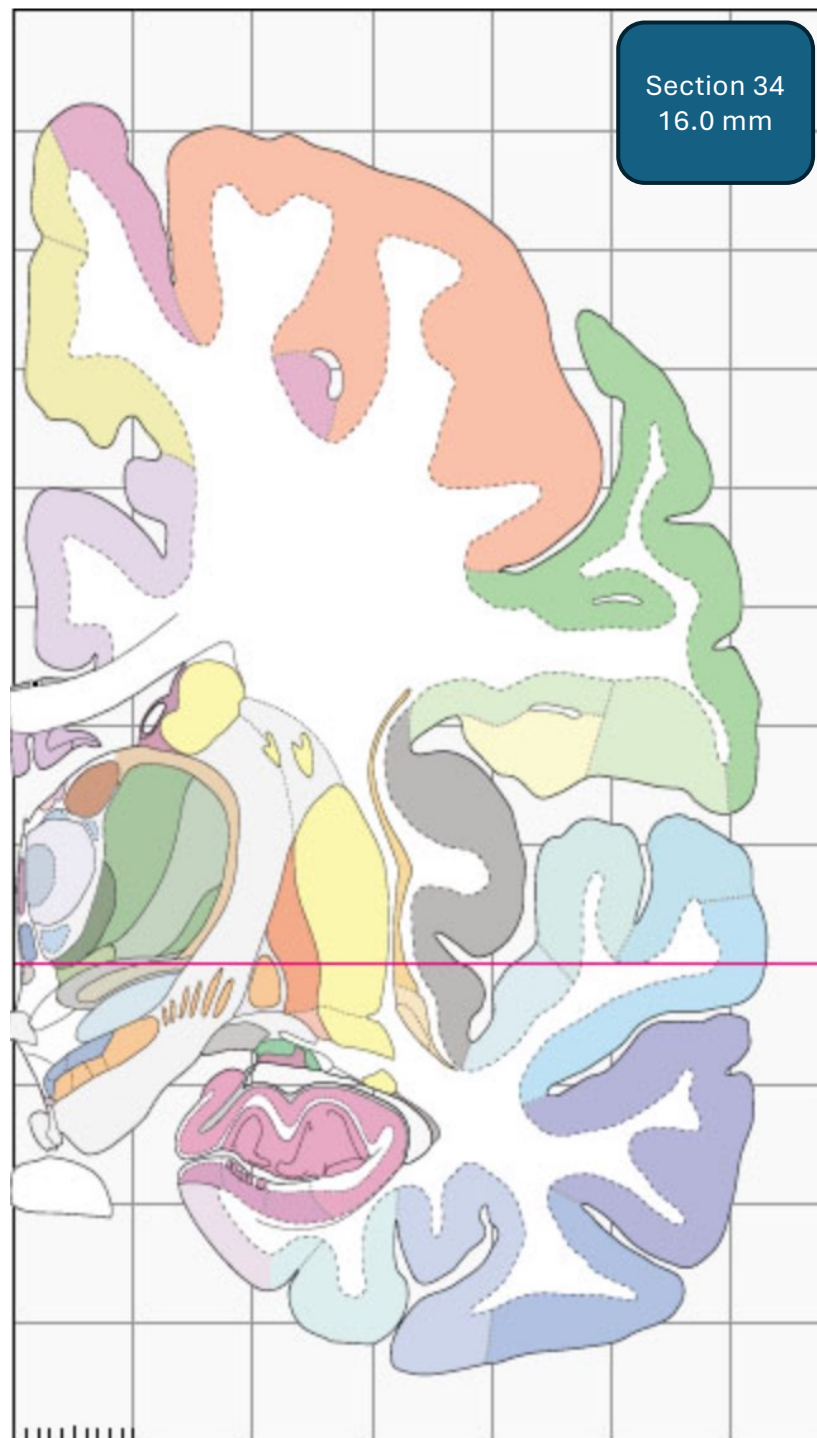


Allen reference brain (labels)



SLICE 4

Mai & Paxinos (labels)



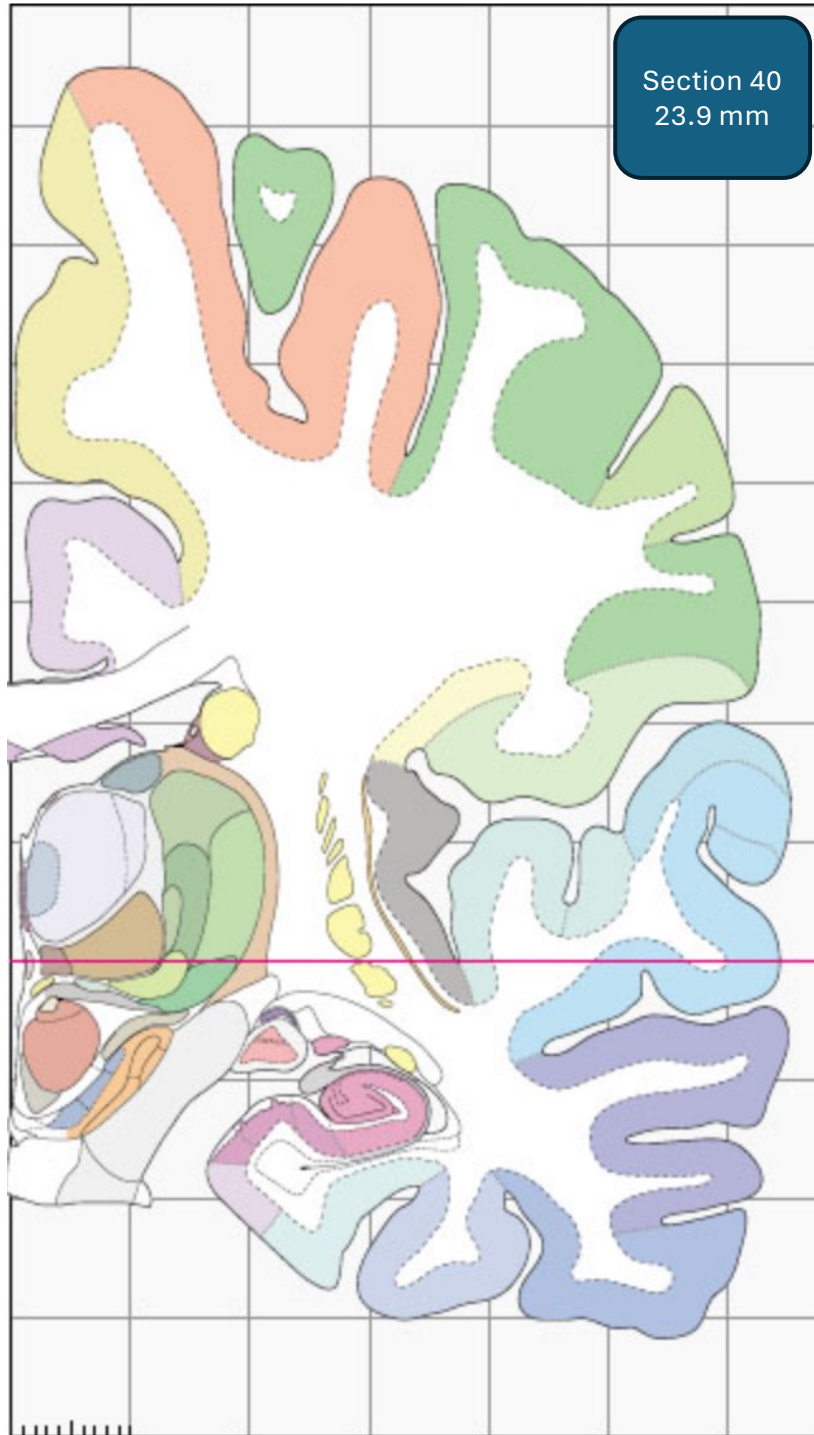
NextBrain (probabilistic labels)



Allen reference brain (labels)



Mai & Paxinos (labels)



NextBrain (probabilistic labels)



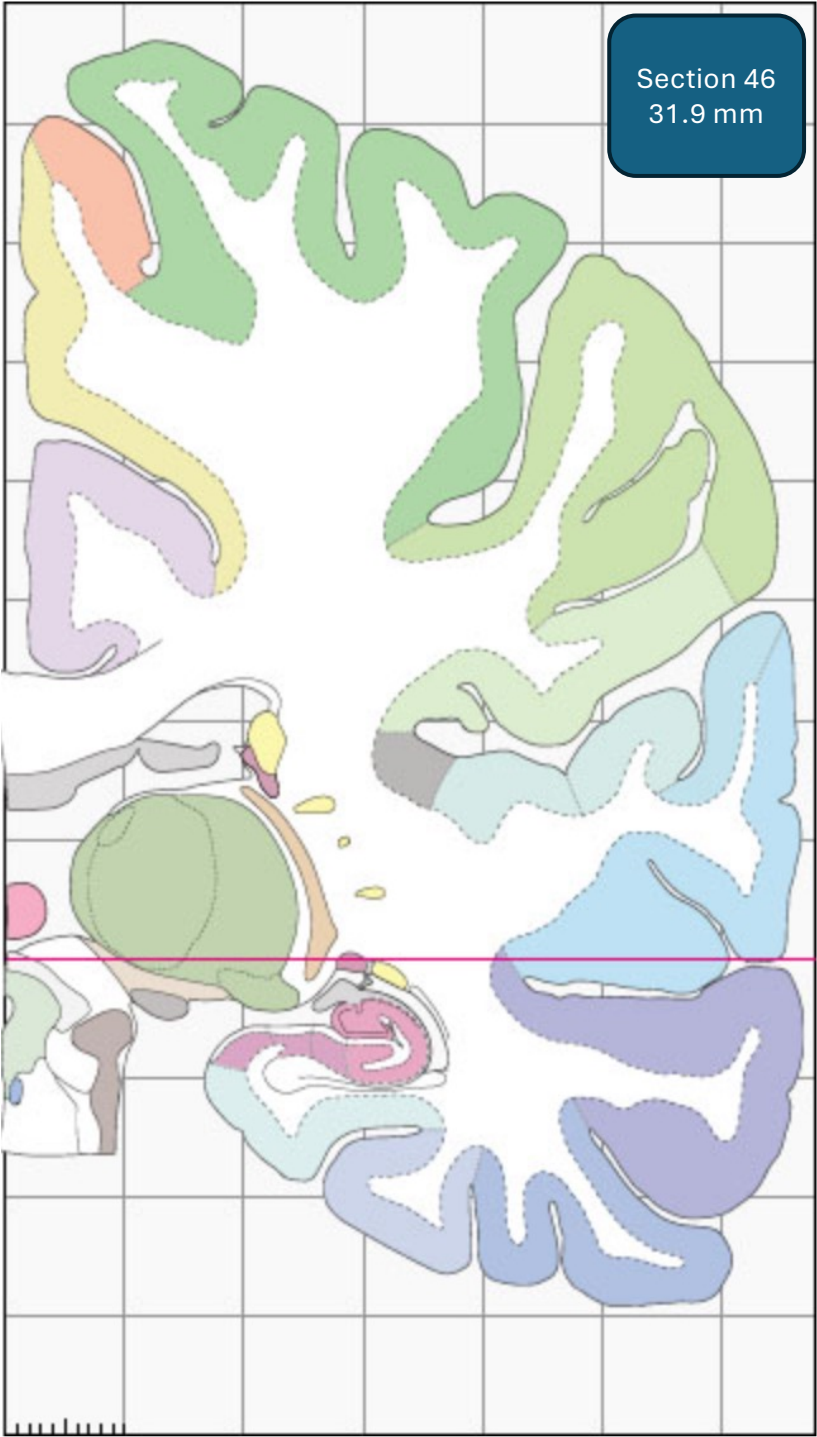
Allen reference brain (labels)



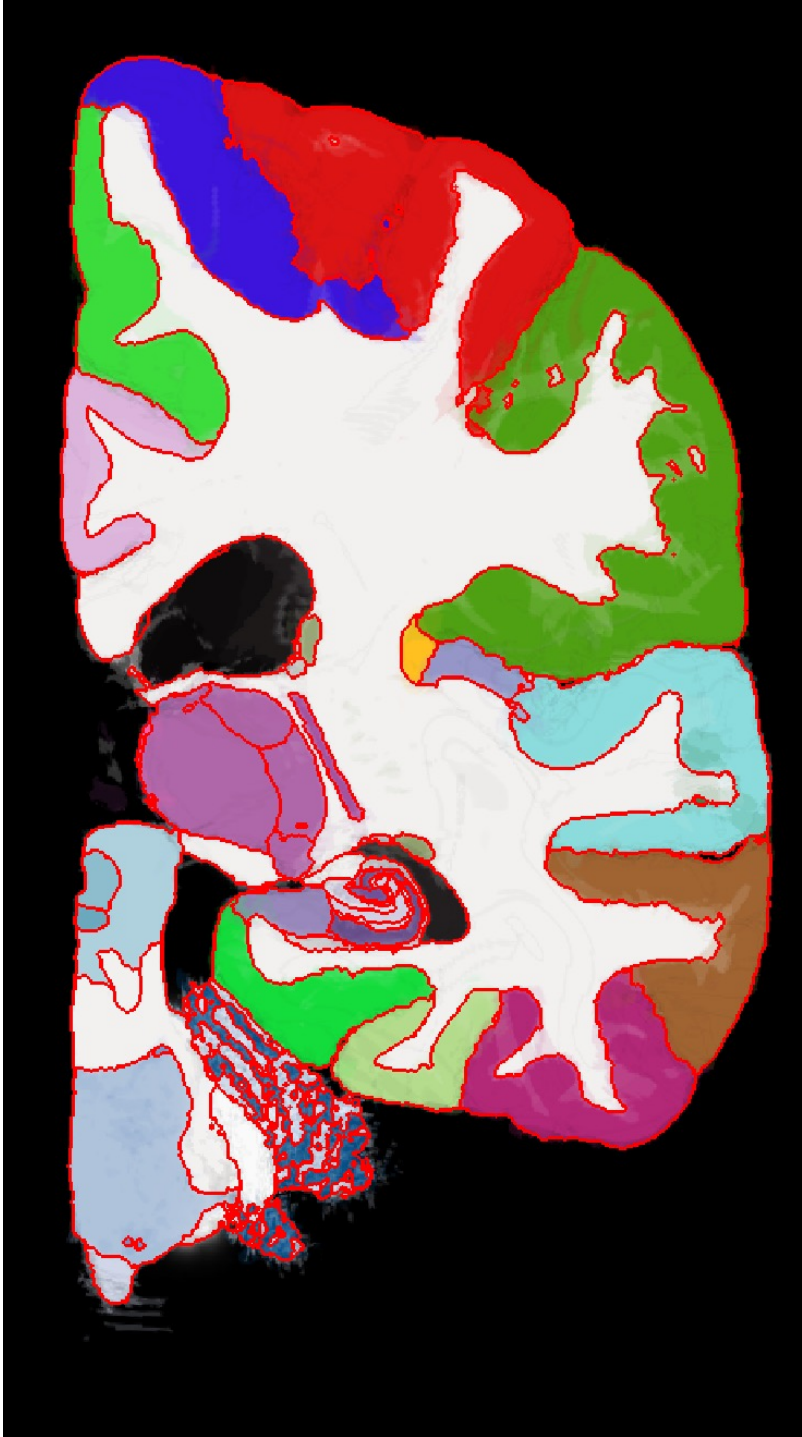
Image 53

SLICE 6

Mai & Paxinos (labels)



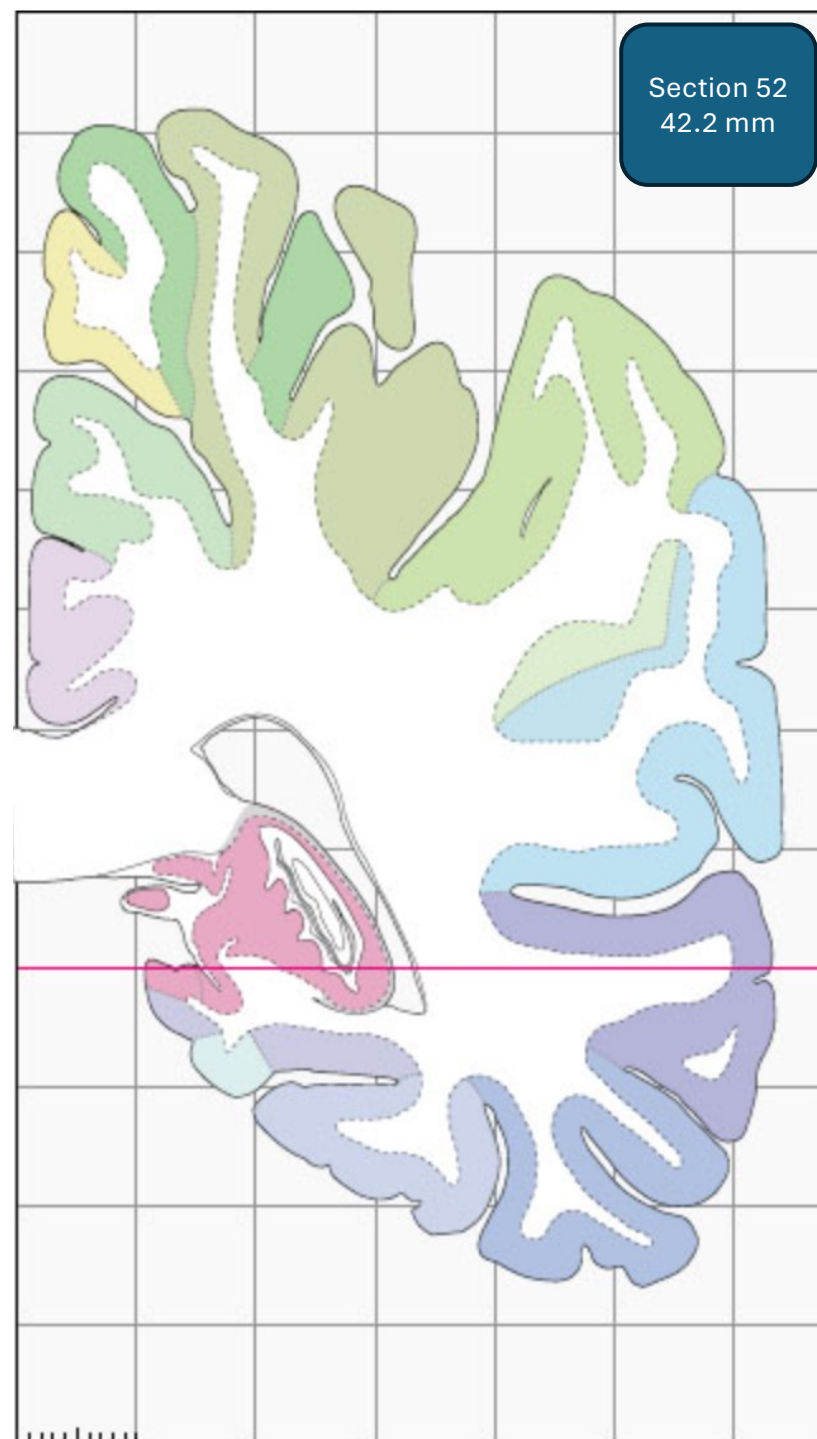
NextBrain (probabilistic labels)



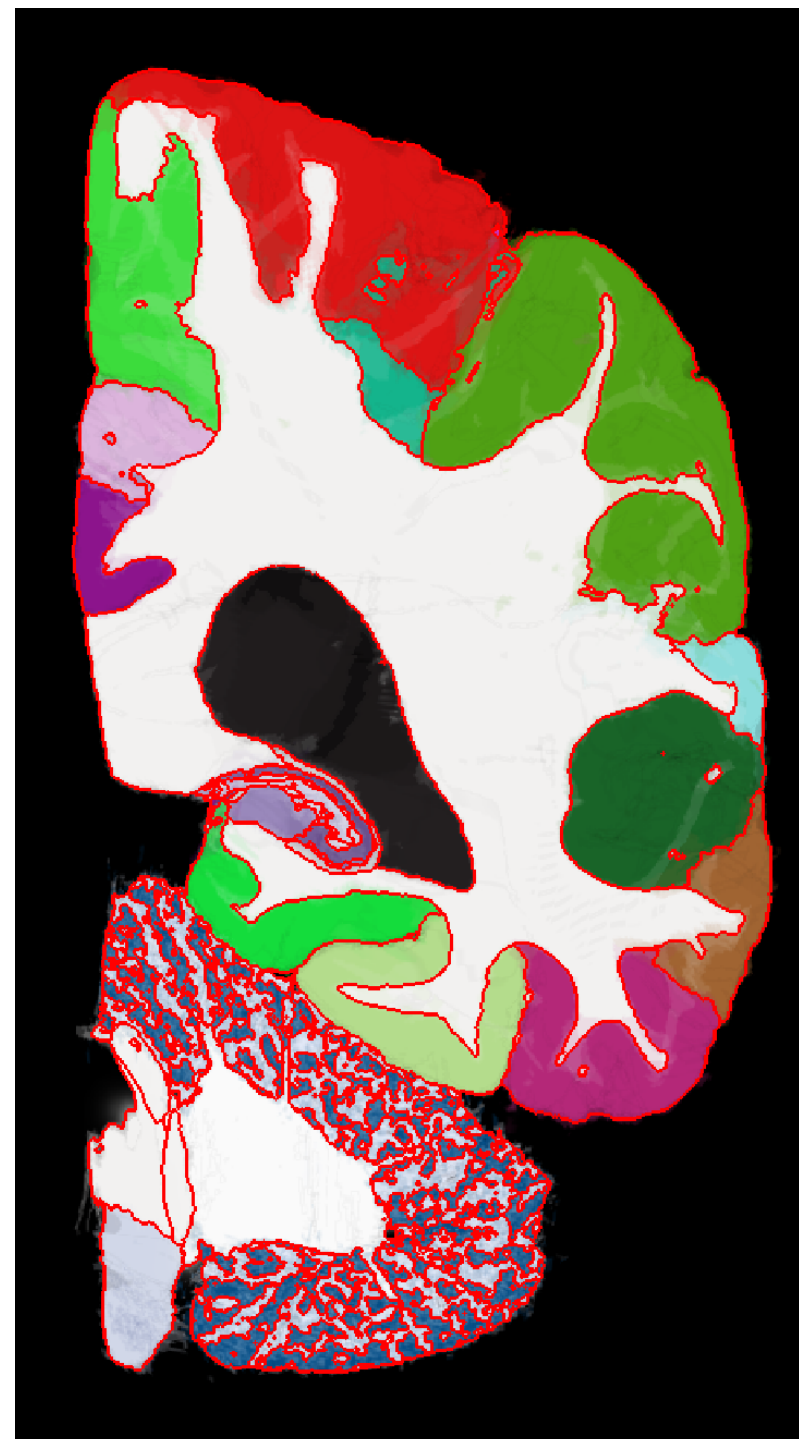
Allen reference brain (labels)



Mai & Paxinos (labels)



NextBrain (probabilistic labels)

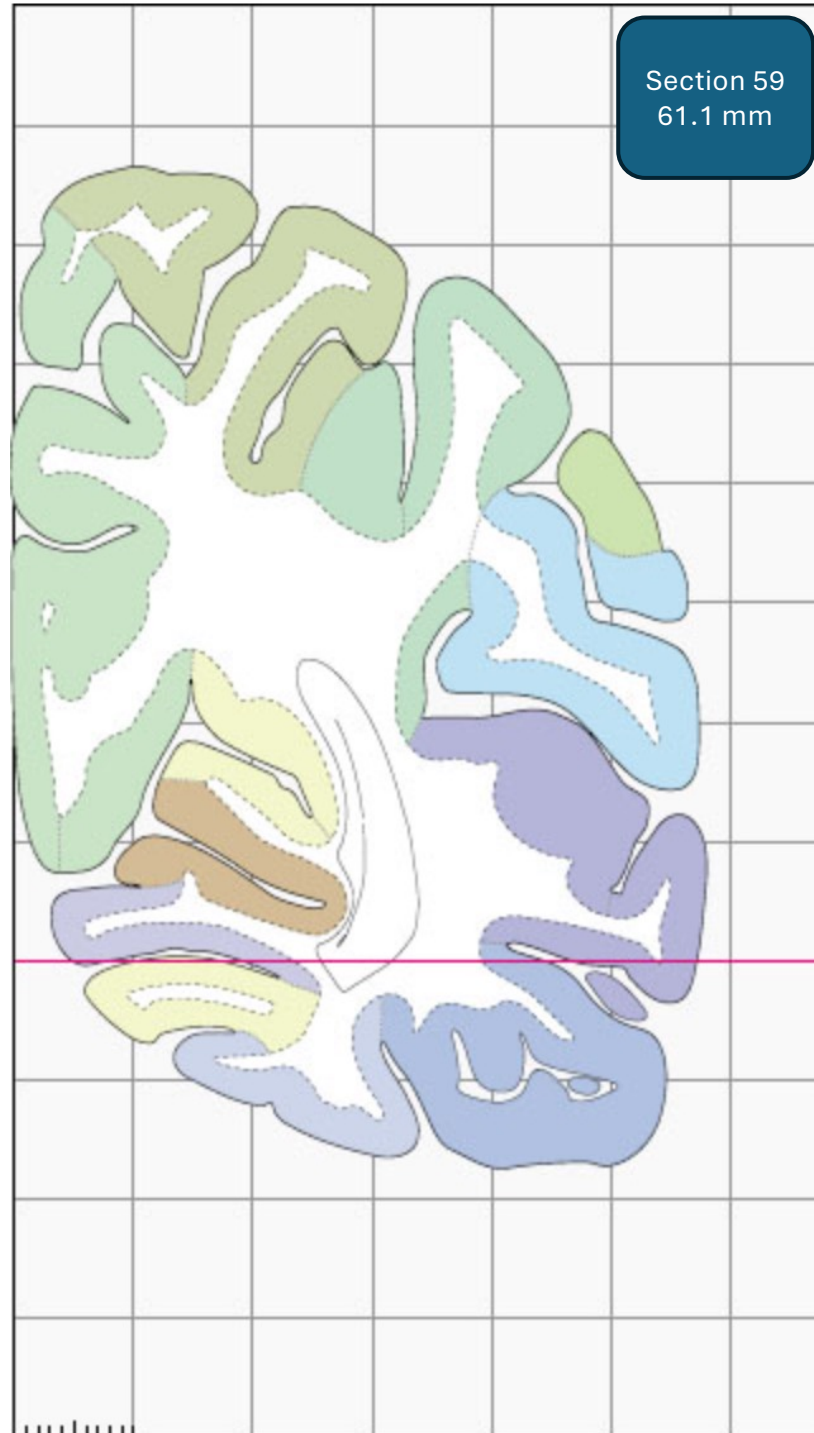


Allen reference brain (labels)

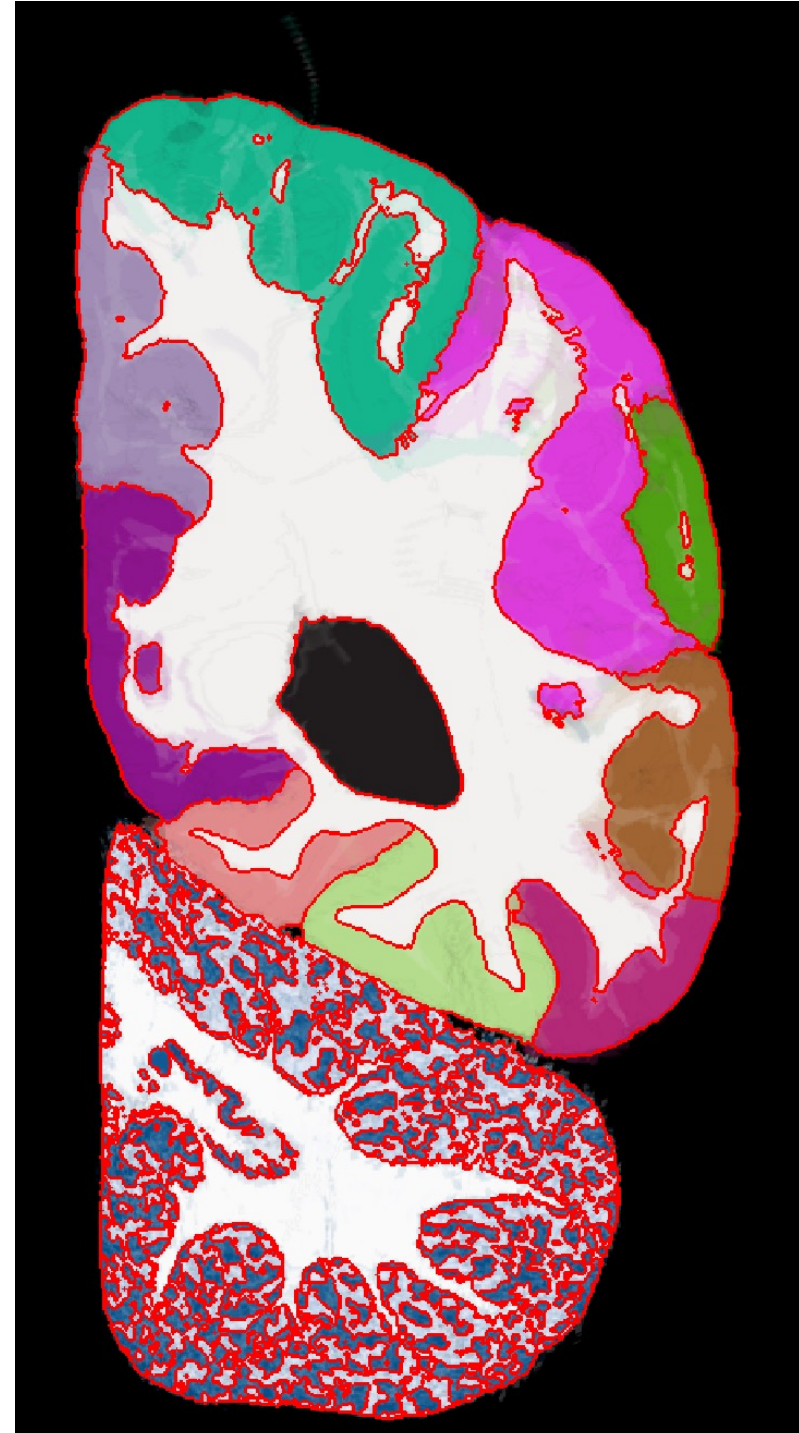


SLICE 8

Mai & Paxinos (labels)



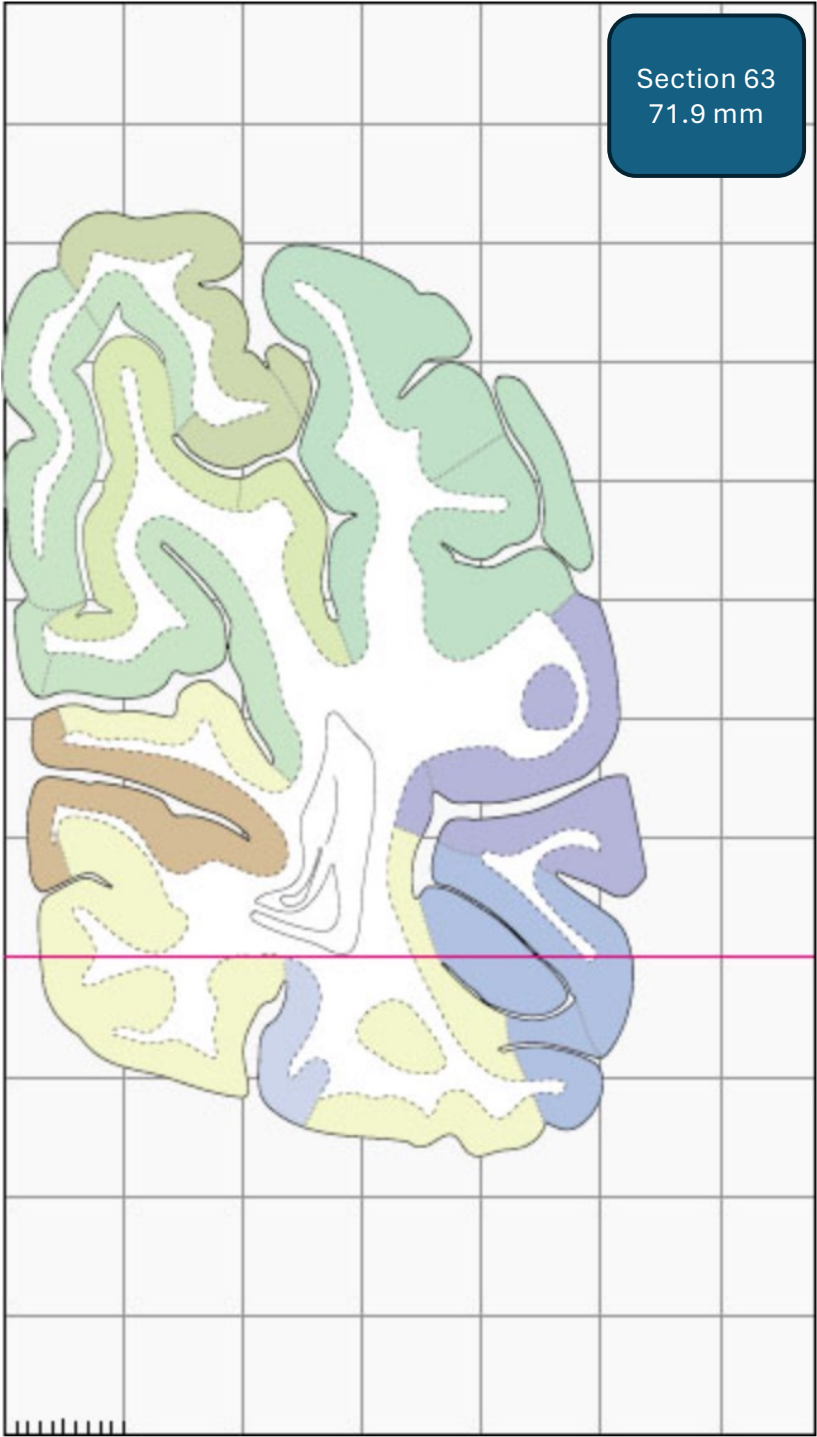
NextBrain (probabilistic labels)



Allen reference brain (labels)



Mai & Paxinos (labels)



NextBrain (probabilistic labels)



Allen reference brain (labels)

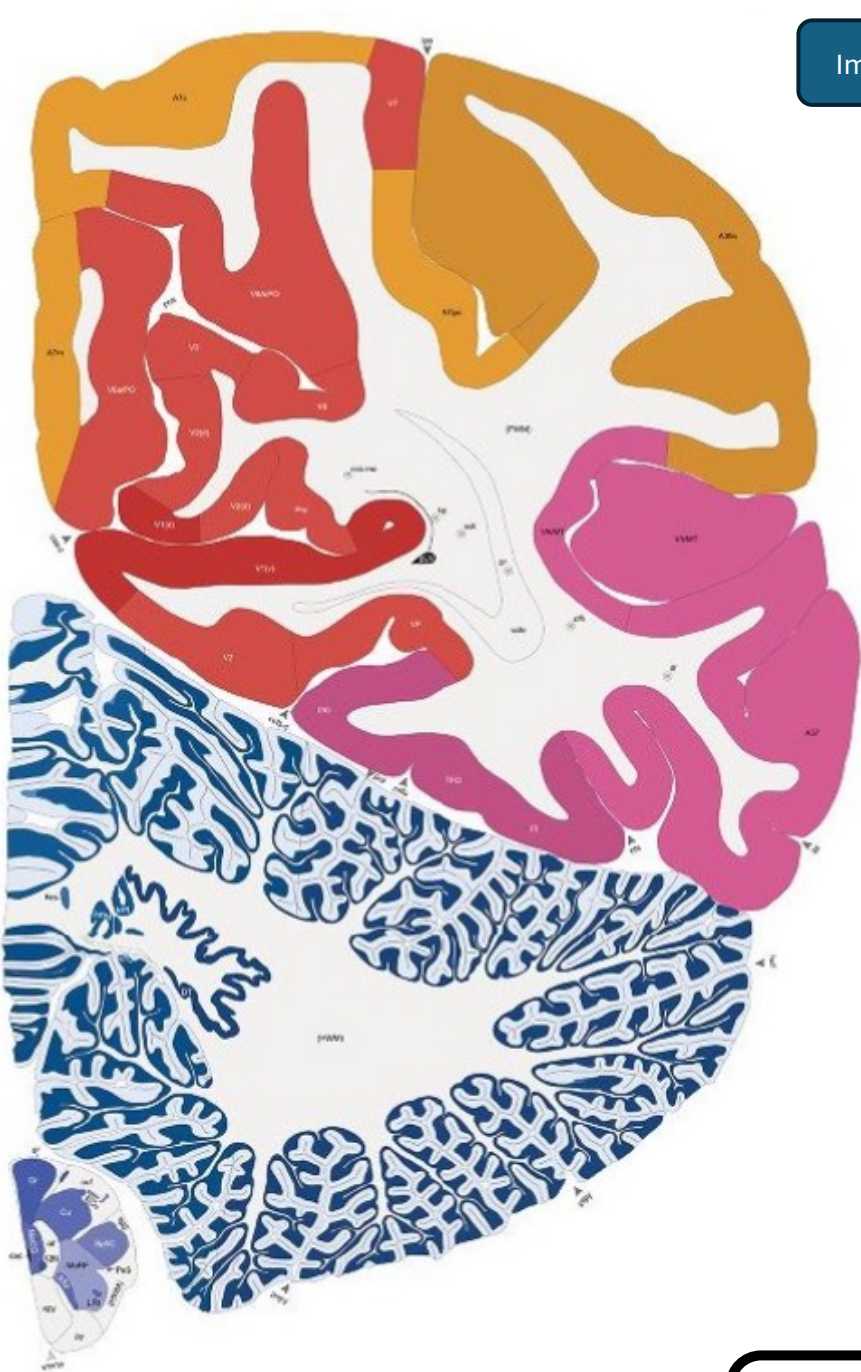


Image 88