

Amygdala

Manual Tracing Methods

In use at:

NeuroImage Analysis Lab
University of North Carolina
Autism & Fragile X Research

Original Protocol developed by:

Center for Neuroscience and the M.I.N.D. Institute
University of California Davis

AMYGDALA TRACING: Introduction & Images:

- The images used in the following methods are T1 gray level images, aligned along the long axis of the hippocampus. Prior to applying these tracing methods, the original T1 images are aligned along the hippocampal axis and resliced to isotropic voxels (1.01562 mm³) using the BRAINS2 software package (University of Iowa).
- At UNC, tracing is done with the IRIS/SNAP program. For coronal images from BRAINS2, the IRIS/SNAP file orientation is **RIP**. In IRIS/SNAP, voxels covered by 50% or more of the polygon you draw are included in the volume measurement.
- Note that in IRIS/SNAP you will trace the right amygdala on the left side of the screen and the left amygdala on the right side of the screen.

Overview of Steps:

1. Trace the amygdala in the coronal plane.
2. Check tracing in the axial plane and exclude the putamen.
3. Check tracing in the sagittal plane and define the rostral extent of the amygdala.

Coronal tracing method:

- Find the most caudal section of the amygdala as it appears dorsal to the inferior horn of the lateral ventricle and hippocampus, and lateral to the optic tract. Begin by tracing the amygdala from the dorso-lateral extent of the optic tract.
- In caudal sections, the putamen forms the lateral border of the amygdala. If this border is seen, extend a line from the dorsolateral extent of the optic tract directly lateral (horizontal) to the amygdala/putamen border. If this border is difficult to see, extend the horizontal line laterally to the white matter. You can further define and exclude the putamen in the horizontal / transverse view (*described later*).
- Continue tracing the amygdala by following either the putamen/amygdala border, along the white matter, or (if all else fails) a line directly ventral (vertical) until the lateral ventricle is reached.
- The ventral border of the amygdala is initially formed by the lateral ventricle, then more rostrally by the hippocampus. The ventro-medial portion of the amygdala extends just ventral to the optic tract. More ventral, this border is formed by the amygdala-hippocampal transition area (which is included as part of the hippocampus). If this border is ambiguous, a division may be defined by a line perpendicular to the optic tract. More dorsal, the border is formed by the medial surface of the brain. Therefore, continue tracing the ventral surface of the amygdala along the ventricle, then amygdala-hippocampal transition area, medial surface of the brain, to the starting point at the dorso-lateral extent of the optic tract.
- Continue tracing the amygdala in more rostral sections as described above. When the medial surface of the brain extends further lateral than the optic tract, use the dorso-lateral extent of the medial surface as the dorsal border of the amygdala. Draw a straight horizontal line laterally from this point to the white matter. At this point, the putamen is no longer present and the lateral border is formed by white matter. Follow the curve of the white matter to the lateral ventricle.
- Further rostral, the hippocampus forms the ventral border of the amygdala, divided by a thin section of white matter (alveus). At this level, the amygdala-hippocampal transition area will no longer be present. Instead, the ventral border of the amygdala will be fairly horizontal (from the lateral border to the medial surface of the brain at the semiannular sulcus).
- If the border between the amygdala and hippocampus is difficult to find, look for the dorsomedial point of the lateral ventricle—if the ventricle is curving in medially, it will point to the alveus.

- As the hippocampus recedes medially in more rostral sections, the entorhinal cortex begins to form the dorso-medial border of the amygdala. The most dorsomedial point of the amygdala is at the semiannular sulcus on the medial surface of the brain.
- Look for white matter to separate amygdala from entorhinal cortex medially. If this is difficult to see, then find the most medial point of the white matter (ventral to the amygdala) and draw a straight-line dorso-medially to the semiannular sulcus.
- As the hippocampus and lateral ventricle disappear along the ventral border of the amygdala, white matter primarily forms the dorsal, lateral, and ventral borders. The medial border is formed by the entorhinal cortex and the dorsomedial border of the amygdala will be formed by the medial surface of the brain. Be careful to exclude vessels, which appear bright white in the image.
- As you continue to trace the amygdala in rostral sections, the medial surface of the brain will extend further lateral (joining the lateral sulcus) to separate the temporal lobe from the rest of the brain. At this point, the dorsal border of the amygdala is defined by the surface of the brain.
- Continue tracing the amygdala until it is indistinguishable. You can be generous here since the rostral border of the amygdala will be trimmed in the sagittal view.

Horizontal (transverse) view trimming:

- Begin at the most dorsal section of the amygdala and progress ventrally. The putamen may be found as an elongated tail extended caudally from the caudal portion of the amygdala. It may also appear a slightly darker gray. To exclude the putamen, follow the white matter along the lateral border of the amygdala as it extends caudally. Continue to draw a straight (medial-caudal diagonal) line, through the putamen, to the white matter on the medial side of the putamen just lateral to the thalamus. Further ventral, this line may terminate at the medial surface of the brain.
- Before continuing, review the rest of the tracing through the transverse sections and delete any scattered points. Do not bother smoothing out lines, as they will then appear jagged in the coronal view.

Sagittal view trimming:

- Begin by reviewing the amygdala on the most medial section you traced. To determine the rostral extent of the amygdala, follow the natural curvature of the ventral surface along the white matter as it extends rostrally. If possible, continue to follow the white matter to the

surface of the brain. If not, then follow the natural curvature from the most rostral tip of the white matter to the medial surface.

- Also, check the amygdala-hippocampal border in the sagittal view. The division between the amygdala and hippocampus appears as a diagonal line (approximately 45° dorso-caudal to ventro-rostral) from the dorso-caudal tip of the amygdala to the ventro-rostral tip of the hippocampus (often marked by a small portion of the lateral ventricle). This may be checked again after tracing the hippocampus.
- Review the rest of the trace through the sagittal sections and delete any scattered points. Do not bother smoothing out lines, as they will then appear jagged in the coronal view.

Final check:

Review amygdala tracing in the coronal view before calculating volume measurements.

Summary Information

Tool used: IRIS/SNAP manual tracing tool (UNC).

Approximate time to complete one case is 1.5 to 2.0 hours

This time includes tracing and thorough review of segmentation.

UNC NeuroImage Analysis Lab reliability statistics for pediatric amygdala segmentations

Intra-rater for primary rater (average right and left) = 0.90

Average inter-rater ICC (two raters, average right and left) = 0.78

Average inter-site ICC (two raters, average right and left) adult images = 0.92

	Right Amygdala	Left Amygdala	Average Right/Left
Intra-rater (rater A)	0.91	0.89	0.90
Intra-rater (rater B)	0.85	0.53	0.69
Average intra-rater	0.88	0.71	0.80
Inter-rater (ICC) for A & B	0.86	0.69	0.78
Inter-site (with UC Davis)	0.94	0.89	0.92

References:

Schumann, C.M., Hamstra, J., Goodlin-Jones, B.L., Lotspeich, L.J., Kwon, H., Buonocore, M.H., Lammers, C.R., Reiss, A.L., & Amaral, D.G. (2004). The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *The Journal of Neuroscience*, 24 (28), 6392-6401.

UC Davis, Center for Neuroscience Amygdala Tracing Protocol.

Completed pediatric amygdala traces using IRIS/SNAP (right amygdala in green; left in blue).

