MEDIAL TEMPORAL LOBE SEGMENTATION GUIDE

A PROTOCOL TRACING GREY MATTER IN T2-WEIGHTED MR IMAGES

Olsen-Amaral-Palombo Protocol

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CONTENTS

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1 GETTING STARTED WITH SEGMENTATION

In this section, we will start off by surveying the goals of medial temporal lobe segmentation and introducing the necessary tools for manual segmentation.

GOALS OF SEGMENTATION

The medial temporal lobes (MTL) are composed of several regions of interest (ROI) for perception and memory research, including the rhinal cortex, hippocampus, and parahippocampal cortex. To understand the relationship between grey matter volume in these MTL regions and specific cognitive processes, we need to determine the boundaries of these regions. This is achieved through the manual segmentation, or tracing, of MTL regions. Here, we will introduce segmentation based on the Olsen-Amaral-Palombo (OAP) protocol.

TOOLS REQUIRED FOR SEGMENTATION

What software is used in this segmentation guide?

ITK-SNAP (Yushkevich et al., 2006) is used as the primary segmentation software in this guide. We use version 3.8.0 in this guide (download link here).

What tools do I need to follow this segmentation guide?

We recommend using a drawing pad, tablet or a stylus pen for segmentation. A mouse can also be used, however. We recommend the link here.

Which anatomical plane is used for segmentation?

Segmentation is done on the coronal view, which allows for the best visualization of the ROIs. The axial and sagittal views are useful when differentiating between sulci in the brain.

What type of scan do I use to segment under this protocol?

The OAP protocol segments on T2-weighted images. T1 scans are used for anatomical reference and should be co-registered for alignment prior to starting segmentation. To learn more about high-resolution co-registration, we recommend this tutorial, which uses ANTs registration (Advanced Normalization Tools; to learn more see here).

What other tools will I need for segmentation?

You will also need a spreadsheet file for segmentation notes. This will include all your ROIs for each subject along with helpful notes about landmarks in the brain. Download our template here.

2 USING ITK-SNAP FOR SEGMENTATION

In this section, we review how to open images for segmentation and set up your workspace.

STEP 1: DOWNLOADING ITK-SNAP

First, download ITK-SNAP 3.8.0 (click here for download link). It is strongly recommended that you install the latest version of ITK-SNAP for segmentation.

STEP 2: OPENING T2-WEIGHTED IMAGES

In ITK-SNAP, select *File* from the top menu ribbon, then *Open Main Image* to open the T2 image for one subject. After selecting the file (.nii/.nii.gz), a pop-up window will open. Select *Next* and then *Finish* to open the T2. You should see four panels, with a sagittal, axial, and coronal view, plus an empty panel for 3D rendering.

Figure 2.1: Opening main T2-weighted image in ITK-SNAP.

STEP 3: OPENING T1 IMAGES FOR STRUCTURAL REFERENCE

Next, open the T1 for the same subject as a reference. Select *File*, then *New ITK-Snap Window*. In the new window, click *File* and then *Open Main Image* to select the T1 file (.nii/.nii.gz). If the T1 and T2 are properly co-registered, the scans should automatically align in the same space.

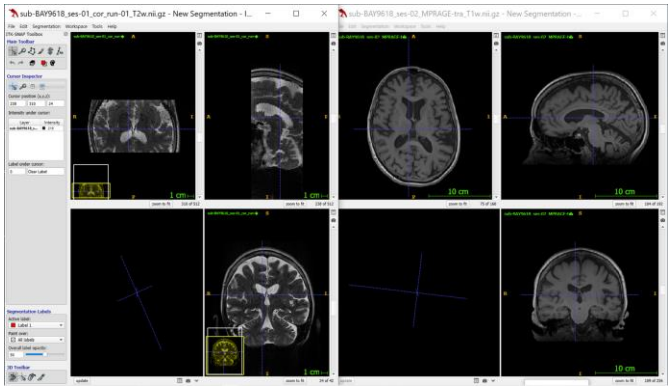


Figure 2.2: T2- and T1-weighted images open in two separate windows.

STEP 4: SWITCHING BETWEEN VIEWS

Since segmentation is done primarily on the coronal view of the T2-weighted image, you can easily switch between views (coronal, axial, and sagittal) by clicking *Edit* in the top menu ribbon and then *Views* in the dropdown menu to select *Next Display Layout* (for shortcuts in ITK-Snap, see http://www.itksnap.org/pmwiki/uploads/Documentation/snap_shortcuts_v3.pdf).

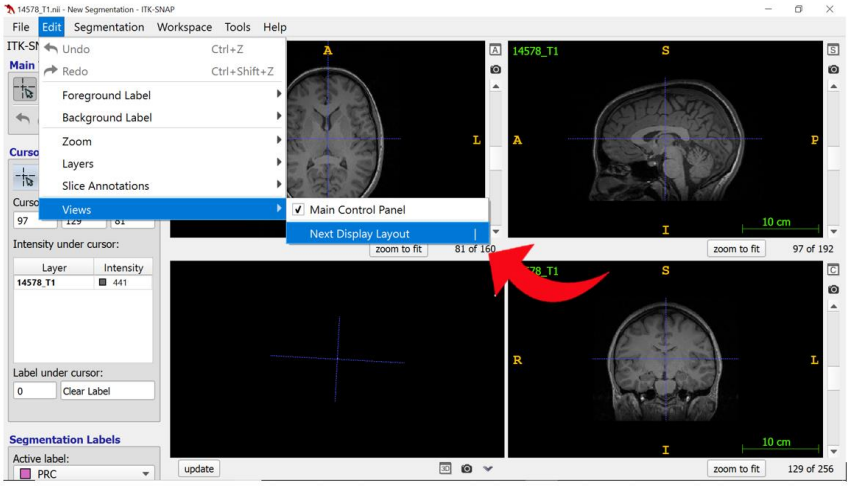


Figure 2.3: Changing views in ITK-SNAP

STEP 5: OPENING LABEL EDITOR

On the T2-weighted image ITK-SNAP window, select *Segmentation* on the top menu ribbon and click on *Label Editor* in the dropdown menu to change your segmentation labels. A pop-up window will open called *Segmentation Label Editor*.

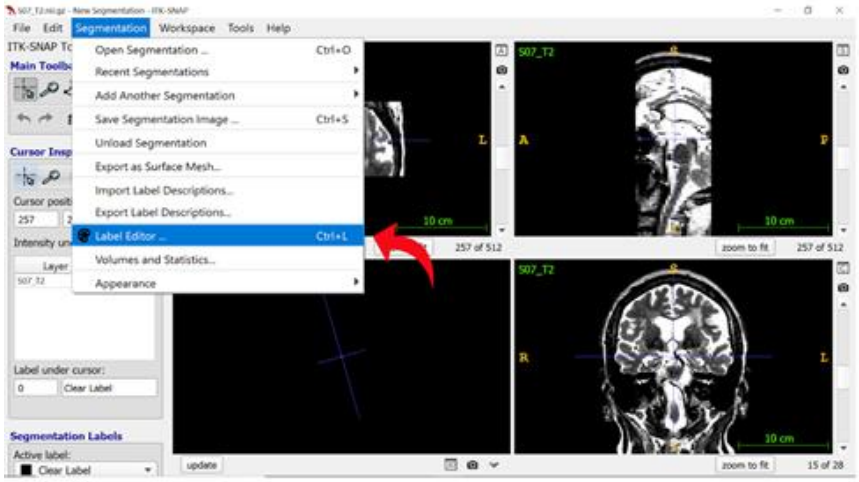


Figure 2.4: Opening the label editor in ITK-SNAP

STEP 6: EDITING LABELS

Add the appropriate labels for MTL regions by typing each region's name in the *Description* field, then selecting the colour for that region. You can learn more about naming conventions and the standard segmentation colours in the OAP protocol in **Labels, Naming Conventions, and Contrasts**.

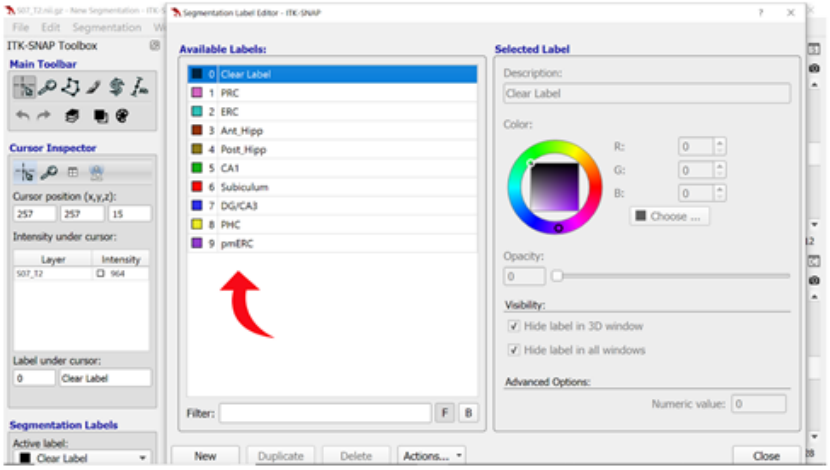


Figure 2.5: Editing labels for segmentation using ITK-SNAP

STEP 7: EXPORTING LABELS

To make segmentation easier each time you re-open ITK-SNAP, you can export and save your custom labels, which you can then later re-import. Select *Actions…* at the bottom of the pop-up window and then choose *Export Label Descriptions* from the dropdown menu. Save these labels as a text file (.txt). In future sessions using ITK-SNAP, you can simply select *Segmentation* from the top menu ribbon and then *Import Label Descriptions…* to re-import this file.

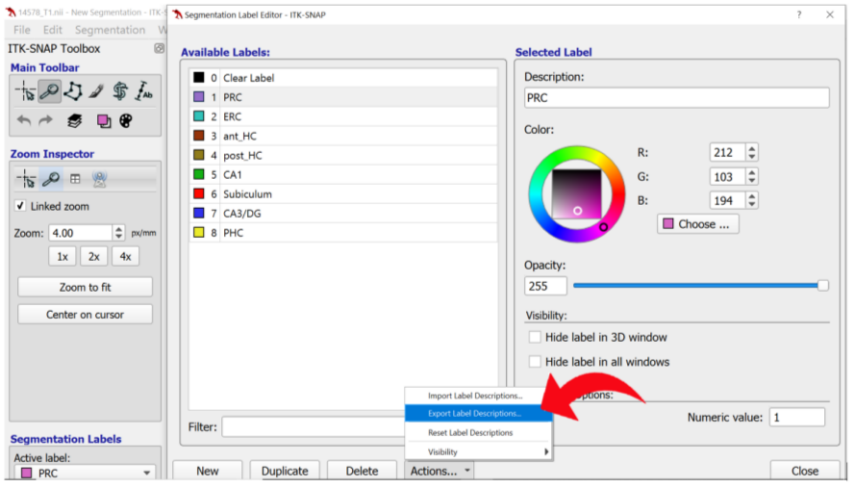


Figure 2.6: Exporting segmentation labels for later sessions

STEP 8: CHANGING IMAGE CONTRAST

To optimize the differentiation of gray matter from white matter in the T2-weighted scan, we can change the contrast levels. Select *Tools* in the top menu ribbon and click *Image Contrast*, then *Contrast Adjustment*.

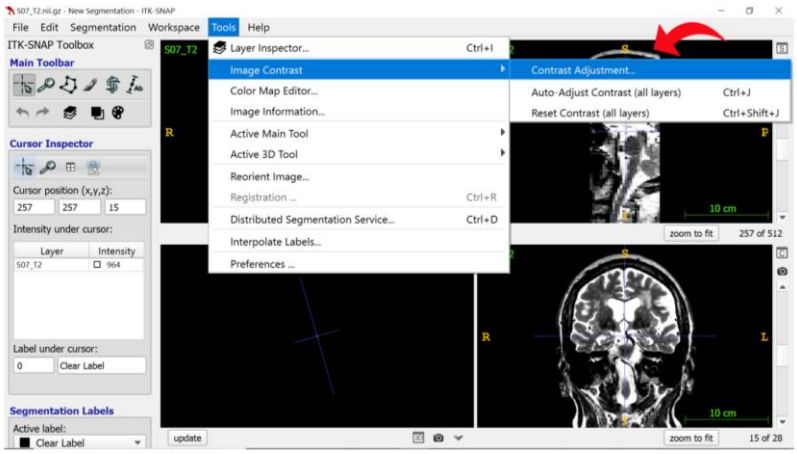


Figure 2.7: The dropdown menu under *Tools* allows you to change the image contrast.

STEP 9: CHOOSING IMAGE CONTRASTS

In the pop-up window called *Image Layer Inspector*, you may need to adjust your image contrast to better see certain boundaries. We recommend a minimum contrast at approximately 200 and a maximum contrast at approximately 800 to 900. Be sure to record your contrast selection for each brain in your segmentation notes.

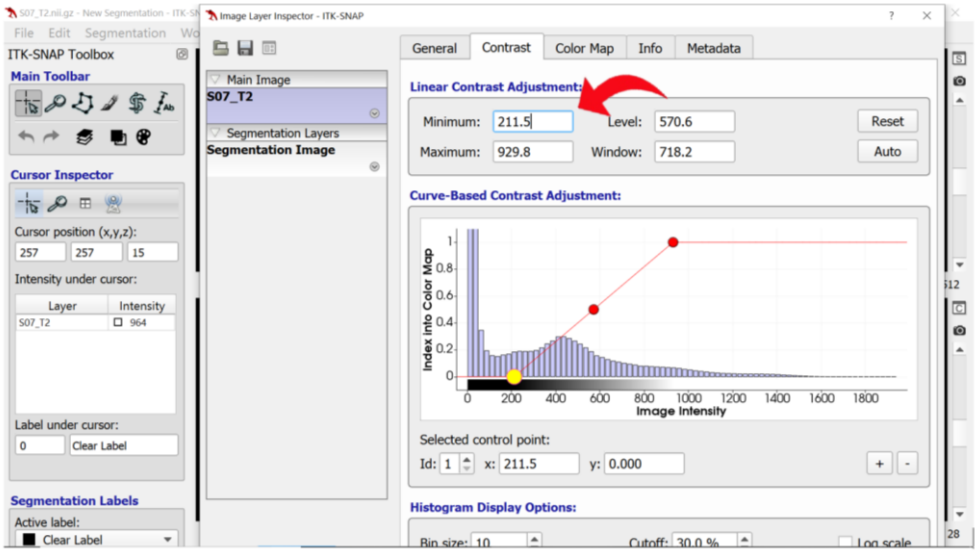


Figure 2.8: Adjusting image contrasts in ITK-SNAP.

STEP 10: ORIENTATION IN ITK-SNAP

Lastly, before starting segmentation, you should get familiar with the layout of ITK-SNAP. The left side of the screen shows the right hemisphere of the brain, and vice versa. Small letters *R* (right) and *L* (left) represent the hemispheres on each side of the coronal view.

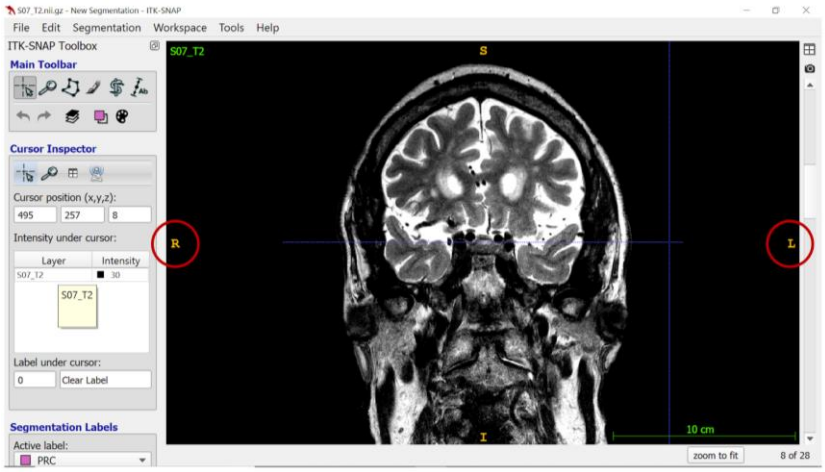


Figure 2.9: The right hemisphere is on the left side of the screen (and vice versa).

At the bottom right-hand corner of the ITK-SNAP window, you can see the total number of slices, as well as which slice you are currently on. The slice number should increase as you scroll from anterior to posterior in the brain. Make sure to record the total number of slices in your segmentation notes spreadsheet (see **Getting Started with Segmentation** for more information on the spreadsheet).

Figure 2.10: The number of slices in the brain scan is shown on the bottom right-hand corner.

STEP 11: TRACING TOOLS AND SAVING SEGMENTATIONS

As a final step, let's review the toolbox on the left-hand side panel. See the figure below (Fig. 2.11) for information on how to use the main toolbar.

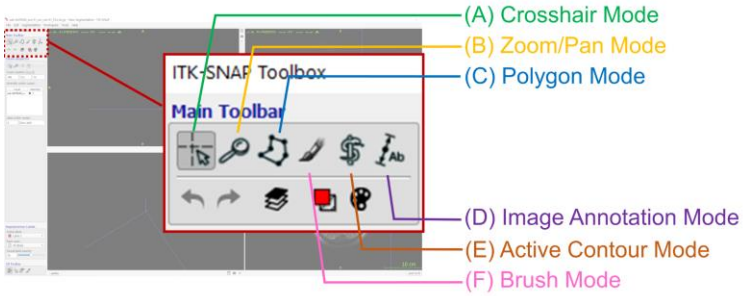


Figure 2.11: The menu options in ITK-SNAP. Full descriptions of annotated tools (A - F) are in Table 1.

TOOL MODE	DESCRIPTION
(A) Crosshair	See cursor coordinates and move crosshairs to a specific position
(B) Zoom/Pan	Adjust zoom on display and centre zoom on crosshair position
(C) Polygon	Useful outline tool to create shape outlines for tracing
(D) Image Annotation	Measure a region for segmentation depth rules or tag a slice
(E) Active Contour	Used for automatic segmentation (not featured in this manual)
(F) Brush	Used frequently for tracing, allowing freehand drawing on voxels

Table 2A: Menu options from Figure 2.11 described by their function.

At the bottom of the toolbox, you can adjust the active segmentation label, its opacity, and whether you want to trace over all voxels or only specific labels.

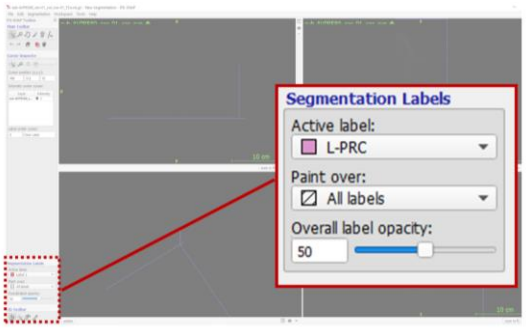


Figure 2.12: The bottom part of the toolbox adjusts the segmentation label you are using and its opacity.

Finally, to save a segmentation, select *Segmentation* from the top menu ribbon and then *Save Segmentation Image…* in the dropdown menu.

3 LABELS, NAMING CONVENTIONS, AND CONTRASTS

ORDER OF TRACING

Draw one ROI in all slices at a time for one hemisphere of the MTL, after you understand the lay of the land (see **Getting Started with Segmentation**). Move to the other hemisphere only after you have completed all ROIs in the first side. If you are only segmenting hippocampal subfields, then you can skip the PRC and ERC steps to proceed right to the hippocampal head. Otherwise, for the whole MTL, we recommend the following order per hemisphere:

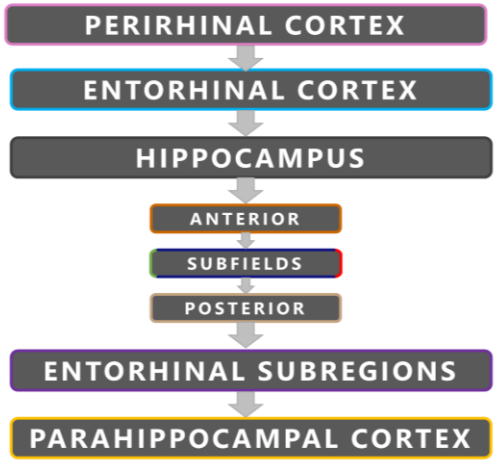


Figure 3.1: Order of segmentation in the medial temporal lobes.

NAMING CONVENTIONS AND LABELS

Naming conventions should include the prefix L- or R- for the hemisphere. If you have more than one segmenter or rater per subject, make sure that these labels and colour values are consistent. See the table below for our recommended labels:

REGION OF INTEREST	LABEL	COLOUR	HEX
Perirhinal Cortex	PRC	Pink	#f791d8
Entorhinal Cortex	ERC	Cyan	#00ccfc
Anterior Head	Ant_Hipp	Orange	#e26213
Posterior Hippocampus	Post_Hipp	Copper	#c3a37f
Hippocampal CA1	CA1	Green	#7fc92d
Hippocampal Subiculum	Sub	Red	#ff0000
Hippocampal CA3 + Dentate Gyrus	CA3/DG	Navy Blue	#3915e9
Parahippocampal Cortex	PHC	Yellow	#fff900
Posteromedial Entorhinal Cortex	pmERC	Purple	#994cd3

Table 3: Labelling and colour conventions for the OAP protocol.

CONTRASTS

The contrast is set to optimize the differentiation of gray matter from white matter. We recommend a minimum contrast of 200, and a maximum of 800 to 900 on the T2-weighted image. The contrast should be consistent across raters. Generally, you can keep the same contrast throughout all slices of a brain, though you may adjust to better see a structure. For all structures segmented, make sure you record the minimum and maximum contrast in the segmentation notes spreadsheet.

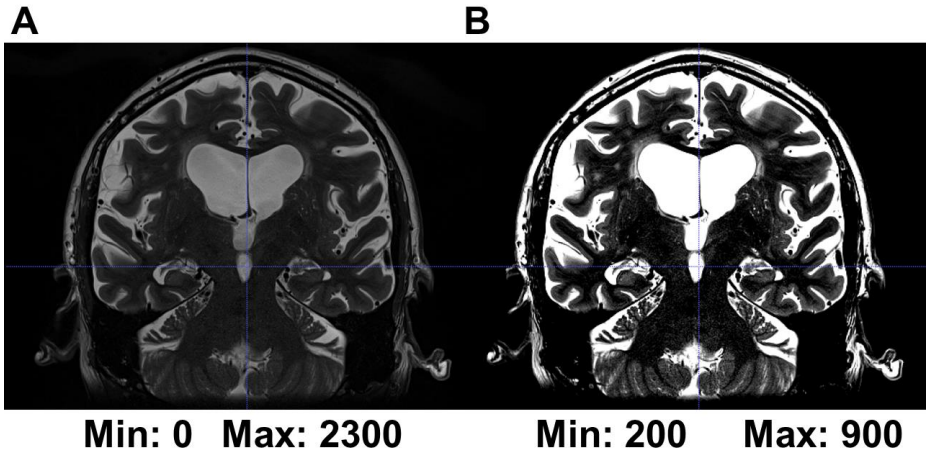


Figure 3.2: (A) Structures are harder to see in a T2-weighted image with default contrast. (B) Structures are clearer in a T2-weighted image when the minimum and maximum contrasts have been adjusted to 200 and 900, respectively.

VOXEL RULES

Cerebral spinal fluid (CSF) will appear on the T2-weighted scan as white voxels. When CSF in the collateral sulcus is greater than 1 voxel, draw around it. When CSF is 0 or 1 voxels wide, include it into the collateral sulcus structure. The voxel rule should also be followed when considering including CSF regions in other regions in the hippocampus. Furthermore, when considering whether to include the lateral border of the ERC (where the ERC climbs up the bank of the CS to meet the PRC), you should also follow the voxel rule and only include the border if it is 1 voxel thick or less.

4 LAY OF THE LAND: MEDIAL TEMPORAL LOBES LANDMARKS

With your segmentation notes spreadsheet open in a separate window, start by identifying the landmarks outlined in the following section. This process will allow you to get a “feel” for your particular subject's anatomy before you actually start segmenting. You will make notes about these landmarks and which slice you identify them in your spreadsheet. It is recommended that you move in an anterior-posterior direction when identifying these landmarks. It is critical that you follow the order outlined in this section, as certain earlier decisions on landmarks will inform later ones. Screenshots with examples are included to help you. Please note, however, that you will need to read the rules for each landmark carefully as your T2 images will certainly vary greatly (see **Variability in Landmarks** for more information).

LANDMARK 1: FIRST SLICE CONTAINING THE COLLATERAL SULCUS

The first slice of the MTL is the first slice in your image set where you can clearly see the collateral sulcus (CS). This is the most anterior slice. The grey matter ribbon only consists of the perirhinal cortex. The depth of the CS determines the medial and lateral borders of the perirhinal cortex. It is important to identify the CS first. In your spreadsheet, note down the first and most anterior slice that you can identify the CS. Take a look at the example below:

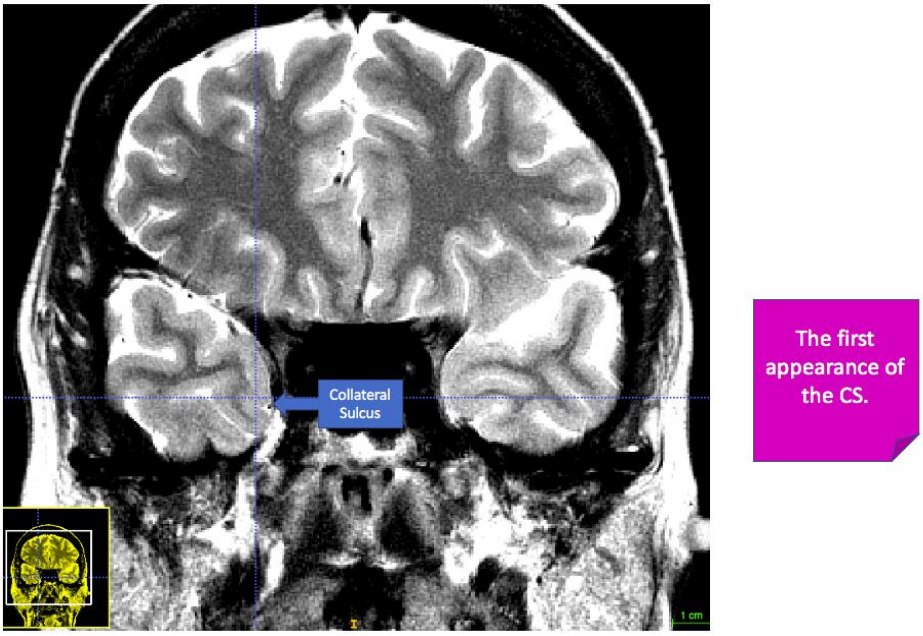


Figure 4.1: The first appearance of the collateral sulcus (CS) in a T2-weighted MR image.

Careful - in some brains it is easy to confuse a prominent rhinal sulcus (RS) with the CS in early anterior slices. To learn more, see the Collateral Sulcus in **Segmenting Regions of Interest in the Medial Temporal Lobes.**

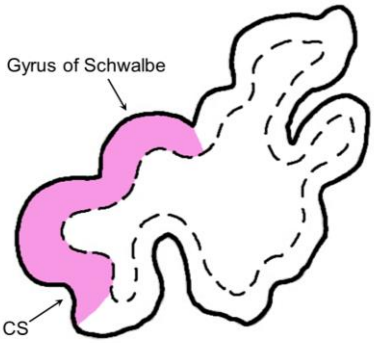


Figure 4.2: According to Insausti et al. (1998), the first appearance of the CS marks the transition between the temporopolar cortex (not included in the OAP protocol) and the perirhinal cortex (shown here in pink). Image adapted from Insausti et al. (1998). CS = collateral sulcus

LANDMARK 2: THE FRONTAL-TEMPORAL JUNCTION/LIMEN INSULAE

This key landmark will determine where you start drawing the entorhinal cortex (ERC). To find this landmark, look for the frontal-temporal junction (FTJ)/limen insulae. The slice in which there is a clear band of white matter that joins the frontal lobe to the temporal lobe is the slice in which the FTJ/limen insulae is indicated. It is sometimes easier to visualize this landmark on a T1-weighted scan.

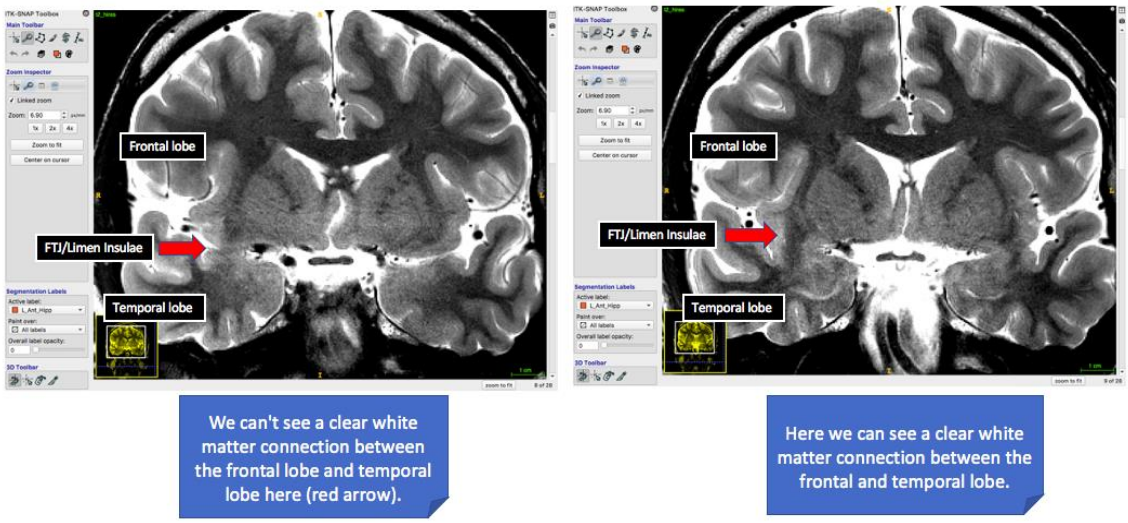


Figure 4.3: The two examples here compare a slice (T2-weighted) in which the limen insulae grey matter is visible in the **left image**; however, there is no clear white matter connection between the temporal and frontal lobe yet, versus in **right image** there is a clear connection between the white

matter of the frontal and temporal lobe. This is a clear indication of the presence of the FTJ/limen insula, which will determine the delineation of the entorhinal cortex. See the next image below for another example.

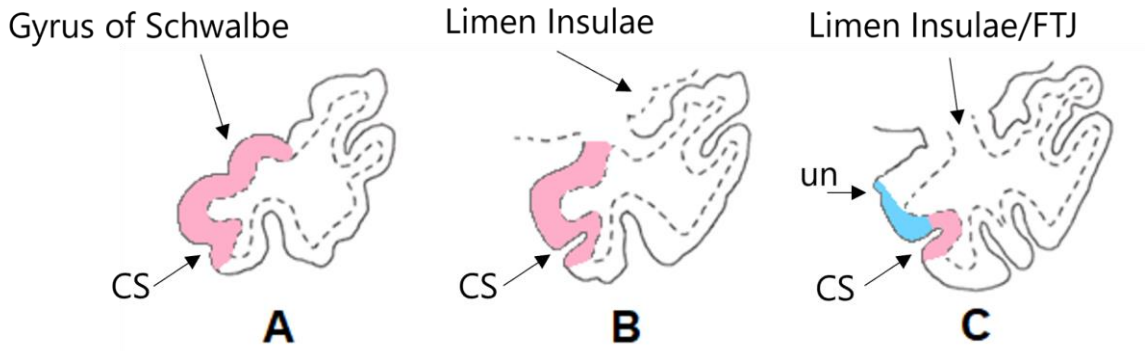


Figure 4.4: Adapted from Insausti et al. (1998). (A) The emergence of CS and gyrus of Schwalbe (with PRC depicted in pink), (B) Moving posteriorly, the limen insula is now present but the white matter connection between the frontal and temporal lobe is not clear, so we only continue to trace the PRC. (C) There is a clear connection between the white matter of the frontal lobe and temporal lobe. This is the slice in which you draw the ERC (depicted in blue) from the PRC up to the uncal notch (un). This is also the slice in which you make a note in your spreadsheet for the presence of the FTJ/limen insula. Boundaries based on Kivisaari et al. (2013), see **Helpful Additional Resources for Further Reading**.

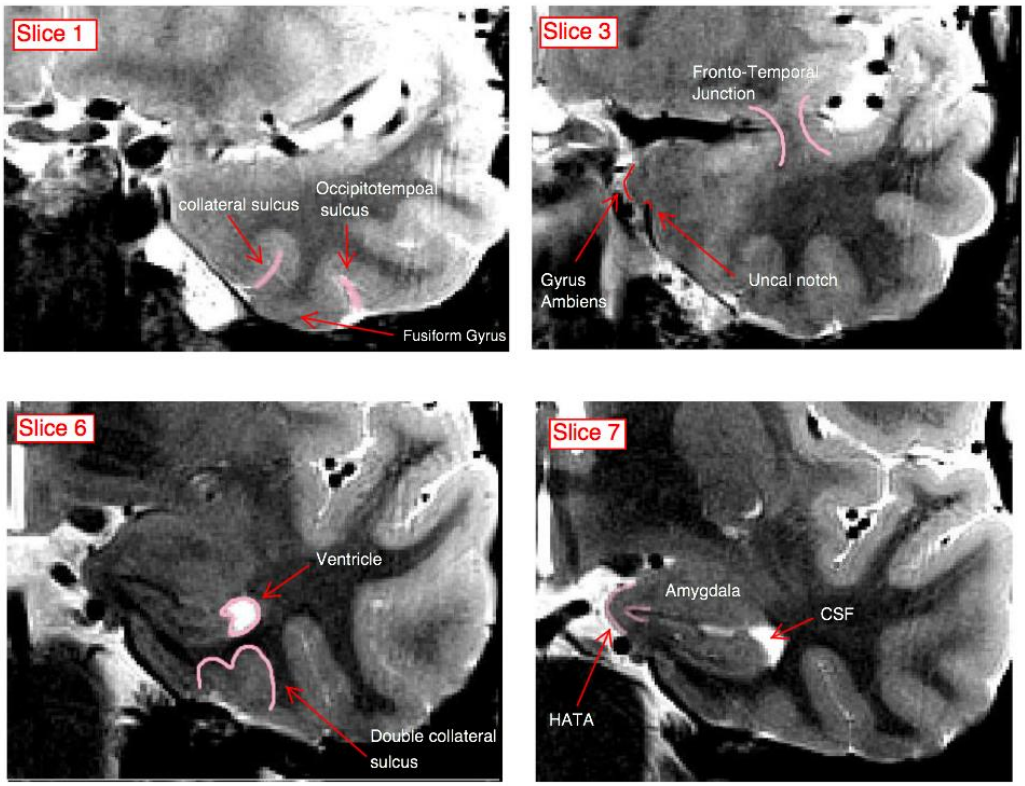


Figure 4.5: This image brings together the first two landmarks (CS and FTJ/limen insulae) as described above. As you move from anterior to posterior, the CS may change from a single to double CS.

LANDMARK 3: THE FIRST SLICE CONTAINING VISIBLE HIPPOCAMPAL HEAD

Next, you will need to look for the hippocampal head. To find this landmark:

A. In its first appearance, the hippocampal head will probably look like a “bean” shape
B. The amygdala is located superior and the ventricle is lateral to the hippocampal head
C. Ambient gyrus appears in the same slice as the appearance of the hippocampal head

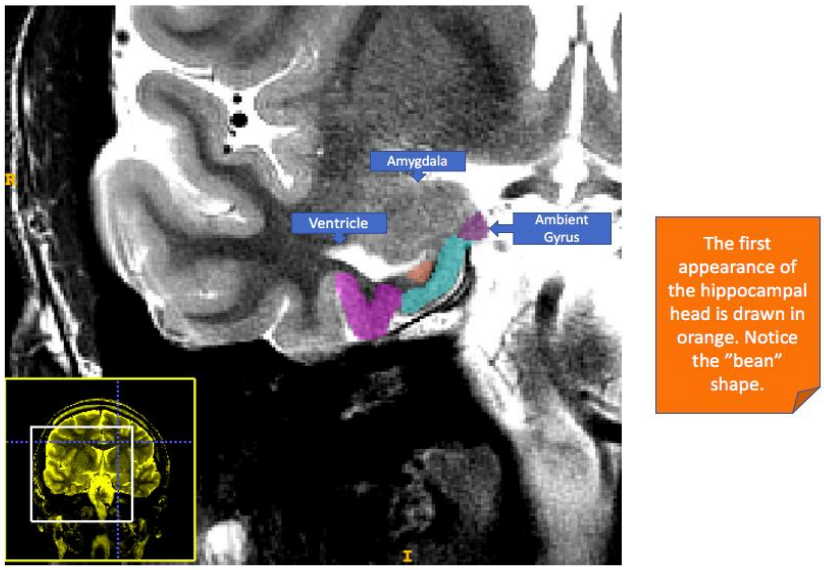


Figure 4.6: This image depicts the first appearance of the hippocampal head (shown in orange). Notice the ventricle laterally, and the ambient gyrus medially.

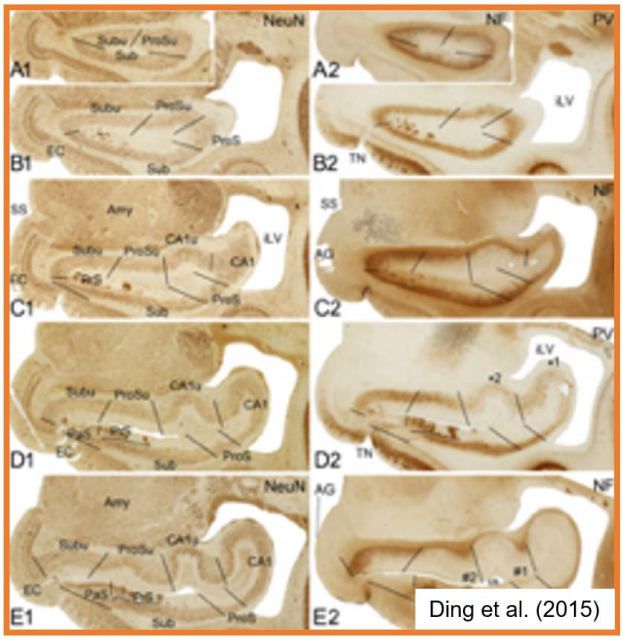


Figure 4.7: This image will help you determine the shape of the hippocampal head in the brain you are segmenting. The example shown is adapted from Ding et al. (2015). Notice how the head shape can resemble a “bean” (A1, A2, B1, B2) or more like the hippocampal body (C1, C2).

LANDMARK 4: THE FIRST SLICE CONTAINING DENTATE GYRUS

After identifying the hippocampal head on 2-3 slices (depending on the brain you are segmenting and the quality of the T2 scan) you will start to see subfields of the hippocampus. At this point, the hippocampus will look thicker than previous slices and the superior digitations of the hippocampus will have smoothed out. This is the first slice of the dentate gyrus (DG) and, by extension, other subfields of the hippocampus. Finally, a darker C-shaped band should be visible, separating hippocampal cornu ammonis area 1 (CA1) from DG. Note that in the OAP protocol, we do not distinguish between DG and cornu ammonis area 3 (CA3).

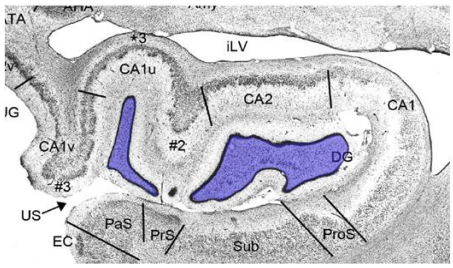


Figure 4.8: The image above, adapted from Ding et al. (2015), will help you with identifying dentate gyrus (highlighted in blue).

LANDMARK 5: THE LAST SLICE CONTAINING THE UNCUS

The last slice of the uncus in the image below would be the second box from the left. You should note here that this EC/PRC to PHC transition is valid for 2-3mm thick slices. For thinner slices, there will be more slices in between the uncal apex and the start of the PHC (Pruessner et al. (2000) suggests it starts 5mm posterior to the uncal apex).

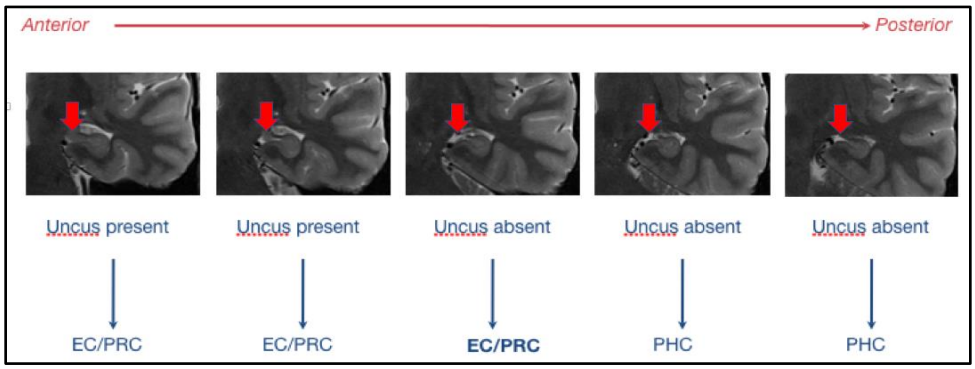


Figure 4.9: Anterior to posterior cortical transition showing the final slice containing the uncus. After one slice where the uncus is absent, you can start tracing the PHC, and the ERC/PRC disappears. Image adapted from: Carr, V.A. (2013), *Variability in collateral sulcus anatomy: The challenge of reliably segmenting medial temporal lobe cortices.* Hippocampal Subfield Segmentation Summit, Davis: Oral presentation.

LANDMARK 6: THE LAST APPEARANCE OF THE COLLICULI

The last clear appearance of the colliculi is the final slice where we segment the hippocampal subfields. After this slice, the hippocampus transitions to the tail segment.

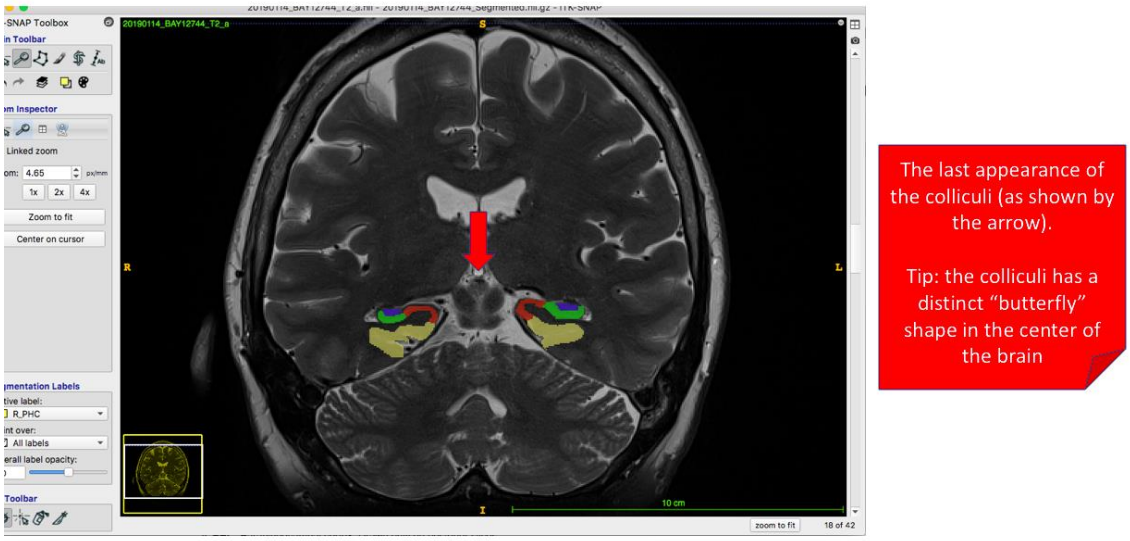


Figure 4.10: The final appearance of the colliculi, which resemble a "butterfly" shape in the centre of the brain.

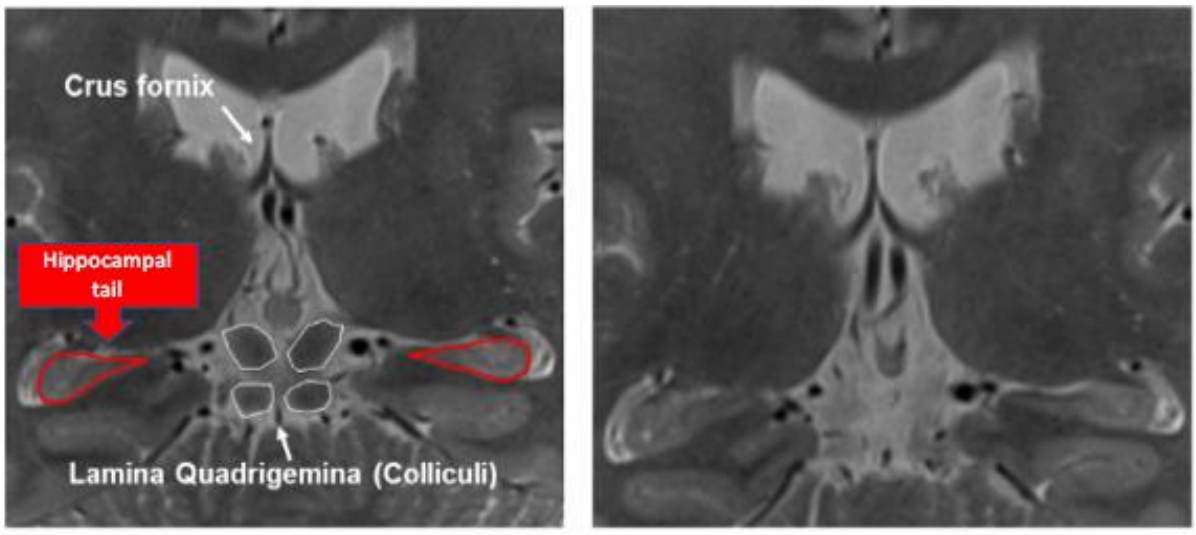


Figure 4.11: On the left, the final posterior slice of the hippocampal body, containing the colliculi, crus fornix, and the "tear drop" shape of the hippocampal body. On the right, the colliculi are no longer visible, making the first slice of the hippocampal tail.

LANDMARK 7: THE LAST SLICE WHERE THE HIPPOCAMPAL TAIL IS VISIBLE

The last slice of the MTL is the slice in your image set where you can clearly see the grey matter portion of the hippocampus tail. After the last slice of the MTL the bright CSF laterally to the hippocampus will clearly sweep up and meet up with the more superior ventricle.

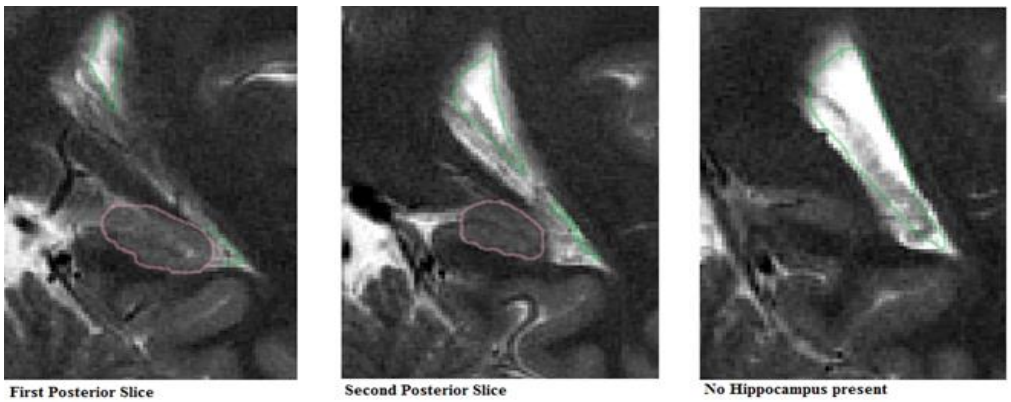


Figure 4.12: The "sweeping" of CSF towards the superior ventricle means that the hippocampal tail is no longer present in posterior slices.

Helpful Information Before You Start Segmenting

Anterior - Posterior MTL Boundary

Certain cortical structures are found only in anterior MTL, whereas others are found only in posterior MTL. Specifically, the entorhinal cortex (ERC) and perirhinal cortex (PRC) are drawn only on anterior slices, and the parahippocampal cortex (PHC) is drawn only on posterior slices. Hippocampal ROIs are drawn on both anterior and posterior slices, but the anterior head and tail will only appear in the anterior or posterior portions, respectively.

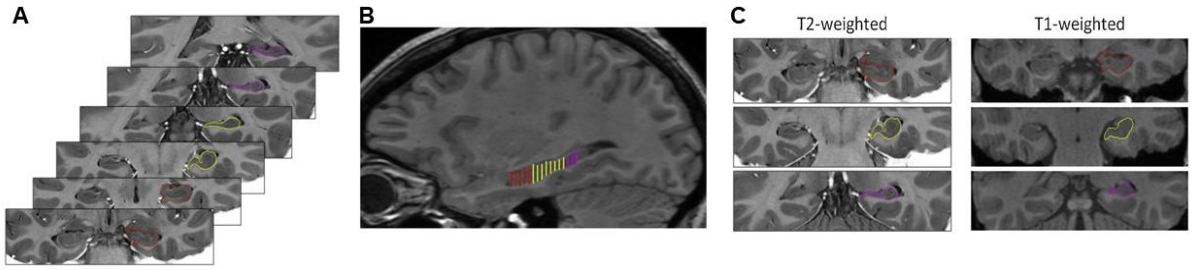


Figure 4.13: The hippocampus is segmented into the head (anterior, red), body (yellow), and tail (posterior, purple). The body of the hippocampus is further segmented into subregions (not shown here). (A) Coronal slices showing progression from head to tail of the hippocampus. (B) Sagittal slice showing progression from head to tail of the hippocampus. (C) Head, body, and tail of the hippocampus shown on T2- vs. T1-weighted coronal slices. Image from Daugherty et al. (2015).

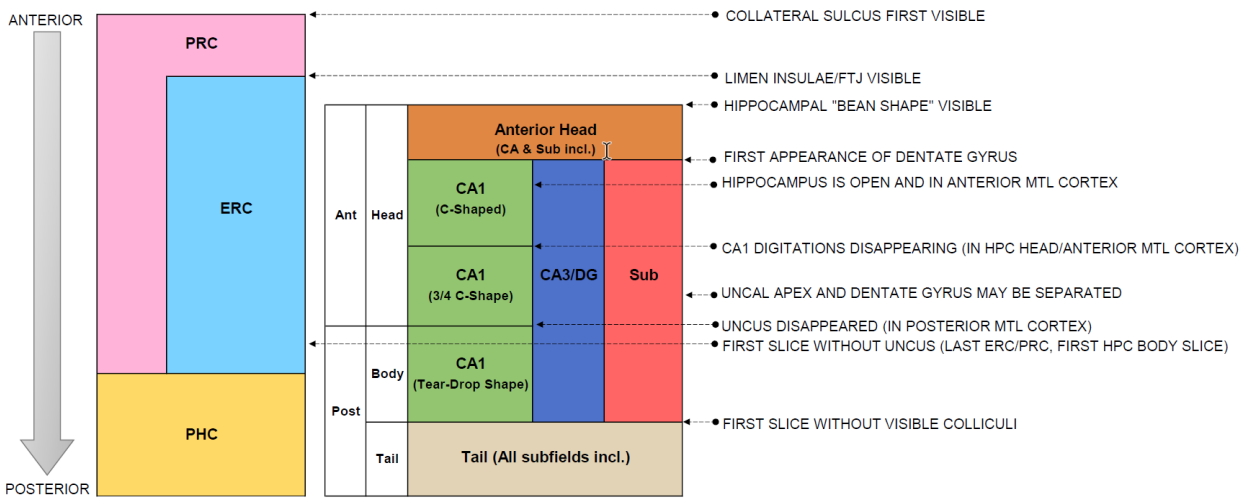


Figure 4.14: Putting all the MTL cortices and hippocampal subfields together for segmentation.

Anterior Slices

Anterior cortices will be drawn starting on your first slice of the MTL (as defined by Landmark 1 in **Lay of the Land: Medial Temporal Lobes Landmarks** above) and will continue to be drawn on slices exhibiting an "open" hippocampal border. The first slice with a "closed" hippocampal border (created by CSF superior and medial to the HPC) will be the final anterior slice. A closed HPC can also be defined as the first slice with the disappearance of the uncus.

Posterior Slices

Posterior cortices will be drawn on the remainder of slices identified as encompassing the MTL (i.e., until the most posterior slice of MTL as defined by Landmark 7 in **Lay of the Land: Medial Temporal Lobes Landmarks** above). Remember, the first slice of the posterior cortices will appear one slice after the disappearance of the uncus. See image below for an open versus closed HPC.

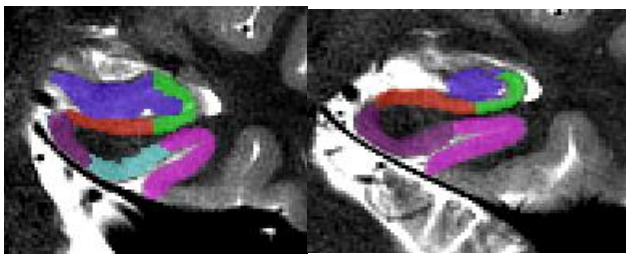
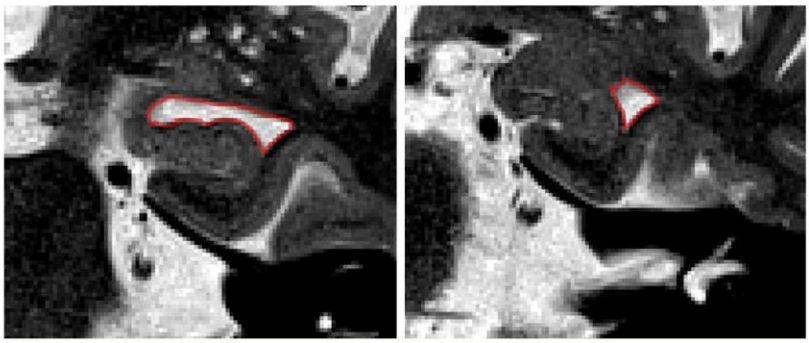


Figure 4.15: (Left) Open hippocampus in anterior slices. (Right) Closed hippocampus in posterior slices.



TIP:

If you flip back and forth going in both directions (posteriorly and anteriorly), you will see a band of bright CSF superior (outlined in red) to the hippocampus.

Anterior slices: CSF band is open medially (left)
Posterior slices: CSF band is closed (right)

Figure 4.16: CSF band superior to the HPC in anterior vs. posterior slices of the MTL. The reason for the "open" border in anterior slices is the presence of the gyrus intralimbicus adjacent to the HPC.

5 SEGMENTING THE MEDIAL TEMPORAL LOBES

Perirhinal Cortex

The perirhinal cortex (PRC) is drawn in **pink** on all anterior slices. The first slice of PRC is drawn on the first slice in which the collateral sulcus (CS) is present. This is the most anterior slice of the MTL. In slices prior to the limen insulae/FTJ, the superior border is determined by the gyrus of Schwalbe (Figure 5.1). In slices after the limen insulae, the lateral border is determined by the depth of the CS (Figure 5.2).

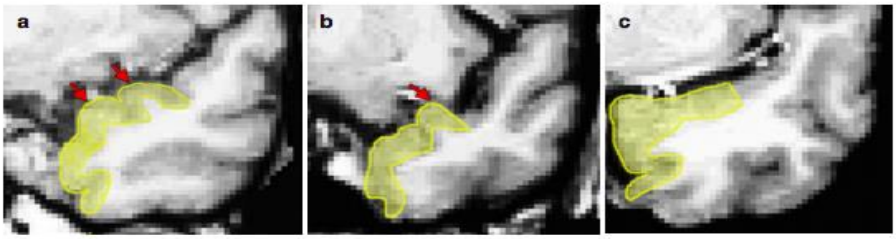


Figure 5.1: Image adapted from Kivisaari et al. (2013) showing the superior-lateral border of the PRC, based on the presence of the gyrus of Schwalbe. (a) Two gyri of Schwalbe are visible (red arrows) so the superior-lateral PRC borer is drawn to the most lateral fundus. Do not include a bump that goes beyond the midpoint of the MTL as a gyrus of Schwalbe. (b) One gyrus of Schwalbe is visible, so the superior border is drawn to the fundus of the gyrus. (c) No gyri are visible, and the superior aspect of the temporal pole appears flat, so the superior-lateral border is drawn to the midpoint of the entire superior portion of the MTL.

If you are having trouble determining the midpoint of the MTL (i.e., the superior-lateral PRC border prior to the limen insulae/FTJ), you can flip forward through the slices until you find the FTJ/limen insulae. On slices before that one (i.e., more posteriorly), the superior-lateral border of the PRC should not extend past where the FTJ appears in more anterior slices.

After the limen insulae/FTJ appears, the medial border of the PRC is determined by the depth of the CS. See Figure 5.2 and Table 5A below for a visual of the CS depth rules.

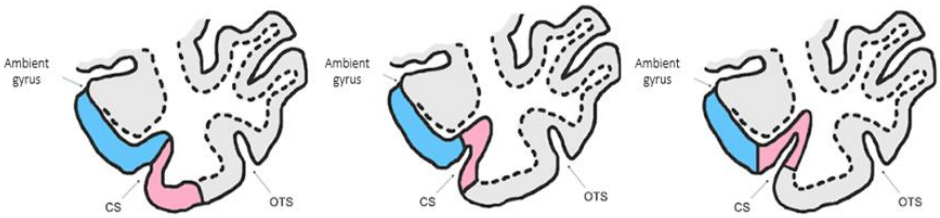


Figure 5.2: These examples of the collateral sulcus are adapted from Insausti et al. (1998): CS = collateral sulcus, OTS = occipitotemporal sulcus, blue = entorhinal cortex, pink = perirhinal cortex.

Collateral Sulcus

The depth of the collateral sulcus (CS) determines where the medial and lateral border of your PRC. The depth rule only applies to PRC, as shown in Table 5A below (but does not apply to PHC). However, the double CS rule **does** apply to the PHC as well.

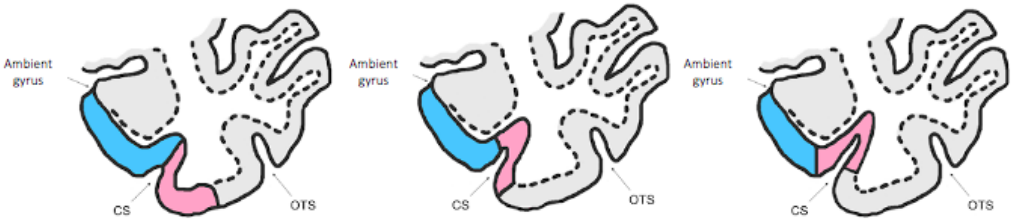
	SHALLOW	REGULAR	DEEP
MEASUREMENT	<1 cm	1 – 1.5 cm	>1.5 cm
FREQUENCY	~16%	~82%	~2%
LATERAL EXTENT	Half-way across fusiform gyrus	Lateral "elbow" of CS	Half-way up lateral side of CS
MEDIAL EXTENT	Fundus (tip) of CS	Half-way up the medial side CS	Medial "elbow" of CS
EXAMPLE			

Table 5A: The following table will help you determine the depth of the collateral sulcus in the brain you are segmenting. Note that the images above depict the left hemisphere of the brain. However, the same rules apply for the right hemisphere. Examples are adapted from Insausti et al. (1998; see **Helpful Additional Resources for Further Reading**).

In the case that there is a double CS, draw the medial edge to the fundus of the more medial CS and draw the lateral to the fundus of the more lateral CS. An estimated 25% to 35% of subjects will have a double CS (Insausti et al., 1998; Pruessner et al., 2002). Not all slices in one

subject may be a double CS. In the figure below, B and C demonstrate an interrupted and a branched CS.

Figure 5.3: The variation seen in the collateral sulcus. The collateral sulcus can be uninterrupted, non-side-branched as in the first image, interrupted as in the second image and side branched as in the third image. (Perirhinal cortex = pink, Entorhinal cortex = blue).

Note that PRC bifurcation rules still apply before the limen insulae/FTJ - so if the CS bifurcates, draw to the fundus of the more lateral sulcus. However, the medial boundary rule (depth rule) would not apply for the medial sulcus at this point. So, if you are drawing the CS as a double before the limen insulae/FTJ, include the PRC all the way to the superior lateral extent of the gyrus of Schwalbe (but, see **Variability in Landmarks** for examples where the PRC may be interrupted by an irregular CS or deep rhinal sulcus before the limen insulae/FTJ).

Entorhinal Cortex

The entorhinal cortex (ERC) is drawn in **light blue** on all anterior slices. The first slice containing the ERC will be the first slice where the limen insulae/FTJ is present. There are two distinct ways to draw the ERC: in slices before the HPC anterior head, and in slices after the start of the HPC.

In the slices before the anterior head:

The medial extent is drawn along the grey matter ribbon to the natural taper point in the bisection of the apex of the most medial point of parahippocampal gyrus (see **Glossary of Key Terms**). The lateral edge is drawn to the edge of the PRC (see image below).

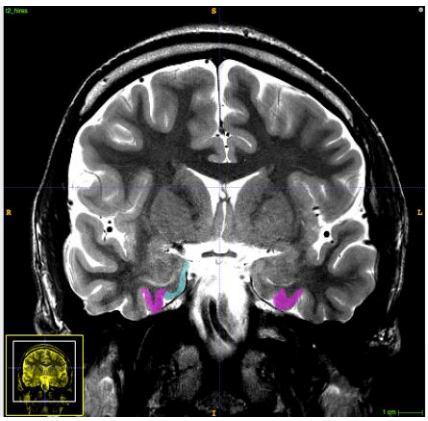


Figure 5.4: Segmentation of the entorhinal cortex (blue) in slices before the anterior head of the HPC, with the lateral edge drawn to the edge of the PRC (pink).

In the slices after the start of the hippocampus:

The medial extent is drawn along the grey matter ribbon to the bisection of the hippocampal fissure. In more posterior slices, this will meet up with the subiculum. The lateral edge is drawn to the edge of PRC (see image below).

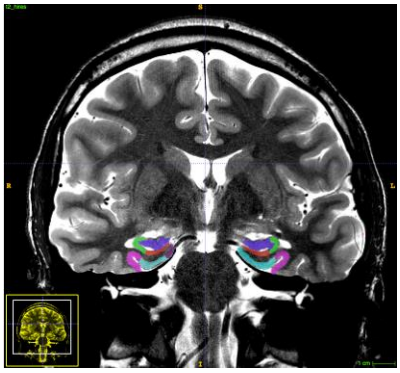


Figure 5.5: Segmentation of the entorhinal cortex (blue) in slices after the start of the hippocampus, with the lateral edge drawn to the edge of the PRC (pink)

Parahippocampal Cortex

The parahippocampal cortex (PHC) is drawn in **yellow**, on the posterior slices only. The PRC follows the grey matter ribbon connecting the subiculum to the collateral sulcus. Start drawing PHC on the second slice the uncus is absent (on the same slice where you start drawing CA1 phase 3). The medial extent is therefore the edge of the subiculum, and the lateral edge is drawn to the elbow of the collateral sulcus (collateral sulcus depth rules do not apply in the posterior hippocampus).

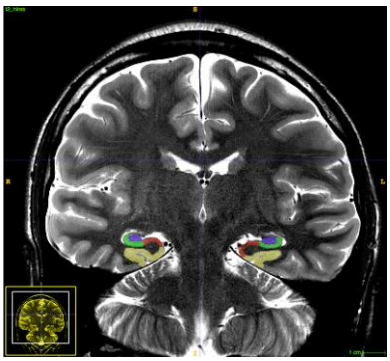


Figure 5.6: Segmentation of the parahippocampal cortex (yellow) in posterior slices of the MTL cortex.

If there is a double sulcus and the medial sulci and the lateral sulci originate from the same collateral sulcus, then follow the bifurcation rules. If the medial and lateral sulci do not come from the same collateral sulcus or do not merge in subsequent slices, then only draw the lateral sulci and do not include the medial sulci in the ROI at all. If a small medial sulcus appears (usually before the appearance of the calcarine sulcus) that later merges with the collateral sulcus (the lateral sulcus in this case), then include both in the ROI. Always draw the medial sulcus as a whole when the collateral bifurcates (unlike PRC bifurcation rules). In the case there is a calcarine sulcus in the posterior regions of the MTL, draw the subiculum to its natural taper point, and include only the collateral sulcus in the parahippocampal cortex.

6 SEGMENTING HIPPOCAMPAL SUBFIELDS

In the OAP protocol, the hippocampus (HPC) is segmented into the anterior (head), the body (with subfields), and the posterior (tail) regions. The figure below should help you with visualizing the way that subfields (i.e., subiculum, CA1, and DG+CA3) are arranged along the horizontal long axis of the HPC.

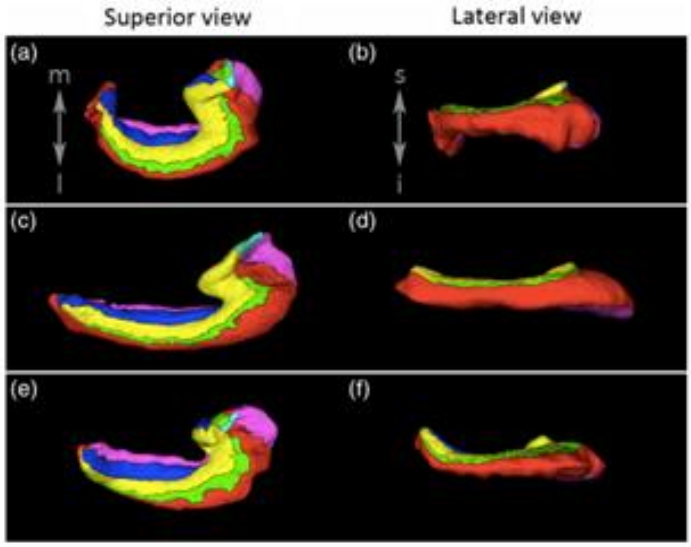


Figure 6.1: Illustration of the curvature of the tail in three hippocampi. In the top row (a, b) the tail shows a strong curve in the medial direction. In the middle row (c, d) the tail shows very little curving in either direction. In the third row (e, f) the tail shows limited curving in the medial direction, and stronger curving in the superior direction visible in the lateral view. Image adapted from de Flores et al. (2020).

Anterior Head

The anterior head (which is also sometimes referred to as the hippocampal head) is defined by the part of the HPC that falls anterior to the first appearance of the dentate gyrus. The OAP protocol does not differentiate this ROI into the various subfields. The most posterior part of this ROI resembles Figure 23.15.C in Insausti & Amaral's (2004) book, chapter 23, which we have reproduced here below. A key feature to look for is the "bumps" or "digitations" on the superior portion of the HPC without a very clear stratum radiatum lacunosum moleculare (SRLM). It is important to note that the digitations are not always completely visible.

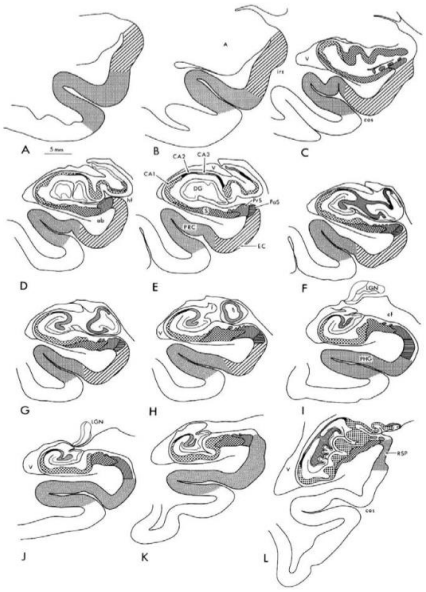


Figure 6.2: Reproduced image from Insausti & Amaral (2004; chapter 23) of the anterior head progressing to the body.

Keep in mind that in images with 2-3mm slice thickness the anterior head usually spans 3 coronal slices, sometimes 2 and rarely 1. In higher resolution images, there can be more than 3 slices of anterior head. The anterior head will fall in the predetermined lateral, superior, and inferior borders. If it is touching the amygdala, however, the medial extent will be drawn half way up the hippocampal-amygdala transition area (HATA). Only draw this ROI until morphology of the HPC includes evidence of the DG in the center of the HPC, and a visible C-shape on the lateral edge, at which point you can start segmenting subfields. It is highly recommended that you look at the Ding et al. (2015) paper (see **Helpful Additional Resources for Further Reading**) when learning how to draw the HPC head.

Figure 6.3: Detailed images of the hippocampal head (anterior head).

Posterior Hippocampus

The posterior HPC (sometimes referred to as the hippocampal tail) is where the posterior thalamus and caudate meet up with the HPC. At this point along the long-axis, the subfields can no longer be reliably segmented. In some brains, there will be "bumps" that can be visualized on the inferior portion of the body (compared to the hippocampal head that had them on the superior portion). The last slice containing the colliculi (butterfly shape in the centre of the brain) is the last slice the hippocampal subfields are drawn (see Landmark 6 in **Lay of the Land: Medial Temporal Lobes Landmarks** above). Start drawing the posterior HPC on the first slice that the colliculi are no longer present.

On 3mm thick slices, the posterior HPC usually spans 2 slices, sometimes 1 and rarely 3. However, on thinner slices you will likely have up to 5 slices with the posterior HPC present. The posterior slice will have a clear difference of the grey matter region between it and the slice one posterior to it (see Landmark 7 in **Lay of the Land: Medial Temporal Lobes Landmarks** above). This will also be the last slice of the MTL, where the bright CSF laterally to the HPC will "sweep up" to meet up with the more superior ventricle. This image below will help you find the final slice of the hippocampal body before you start segmenting the posterior HPC:

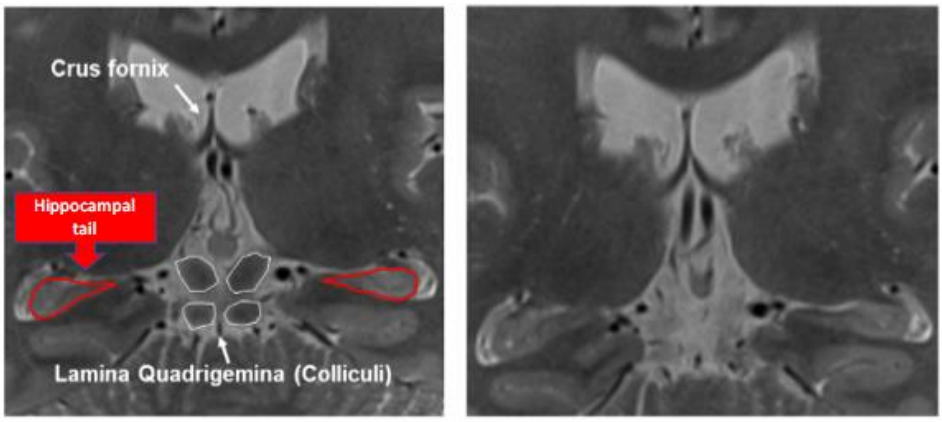


Figure 6.4: Left: Final posterior slice of the hippocampal body displaying the colliculi, crus fornix, and "tear drop" shape of the hippocampal body. **Right:** The colliculi are no longer visible, and therefore the image is considered to be the first slice of the hippocampal tail (or posterior hippocampus).

The posterior HPC is drawn in copper (see **Labels, Naming Conventions, and Contrasts** above for more information).

Hippocampal CA1

After segmenting the anterior head (and identifying slices of the posterior HPC), move onto the subfields in the hippocampal body, starting with CA1. The most anterior sections of CA1 are drawn when the HPC starts to look like a "generic" HPC and there is a definitive C shape in the lateral portion of the body. This will coincide with the first slice of the appearance of the dentate gyrus (DG+CA3). The CA1 is drawn in **green** (see **Labels, Naming Conventions, and Contrasts** above). CA1 will always be guided by the border created by the SRLM and will include the SRLM. If the SRLM is not clear, extend the thickness of the SUB into the thickness of the CA1. There are 3 phases of CA1 that require different methods of drawing boundaries. See the 3 phases below:

CA1 Phase 1: The HPC is "open" and in anterior MTL (i.e., uncus is present):

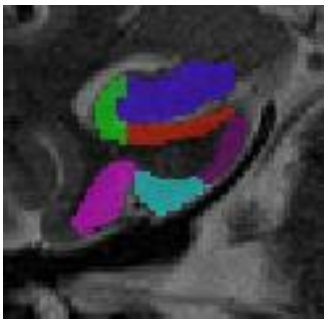


Figure 6.5: CA1 Phase 1, where the superior-medial extent of the CA1 is defined by going half way up the 1st bump, when there are 2 or fewer "bumps"/digitations. If there are 3+ digitations, use the "one third rule" for the superior-medial boundary (one third refers to the width of the HPC, so from the most medial part to the most lateral along the horizontal plane following the angle of the body). The inferior-medial extent is defined by the imaginary line drawn straight down from the superior border of CA1. This will give a resulting "C" shape.

CA1 Phase 2: The uncus is starting to disappear/disappeared and in anterior MTL:

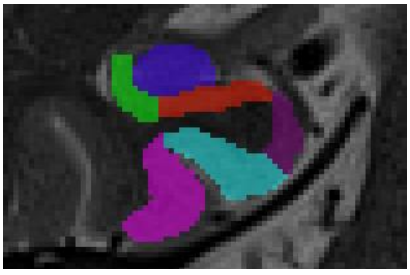


Figure 6.6: CA1 Phase 2, where the superior-medial extent of the CA1 is ¾ up the "C" shape. The inferior-medial extent is the bisection of the hippocampal body including the CA1 along with CA3+DG.

CA1 Phase 3: When the uncus has disappeared and the MTL is in the posterior slices:

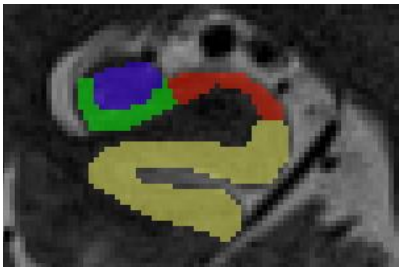


Figure 6.7: CA1 Phase 3, beginning on the second slice the uncus is absent. The superior-medial extent is ¾ up the C shape. The inferior medial-extent is drawn to the very medial tip of CA3+DG. It is a "teardrop" shape and the most medial potion might be easy to miss. At this phase, you should get a "3-point intersection" between the CA1, Sub, and CA3+DG at the medial edge of CA1.

Subiculum

The subiculum (Sub) is drawn on all slices in which the hippocampus can be divided into its subregions. The subiculum is drawn in **red** (see **Labels, Naming Conventions, and Contrasts**). The lateral extent of the Sub will always be marked by the inferior-medial portion of CA1. There are two different rules for drawing the subiculum and they depend on whether you are in anterior or posterior slices.

Anterior (CA1 is in phase 1):

The inferior-medial border will then always be drawn to the bisection of the elbow that connects to the ERC (to the hippocampal fissure).

Posterior (second slice the uncus is absent, CA1 is in phase 2-3):

The inferior-medial portion will always be drawn ½ way down the isthmus (see below for helpful tips on how to find the halfway point). The isthmus is measured from the end of CA1 to the beginning of the collateral sulcus (CS).

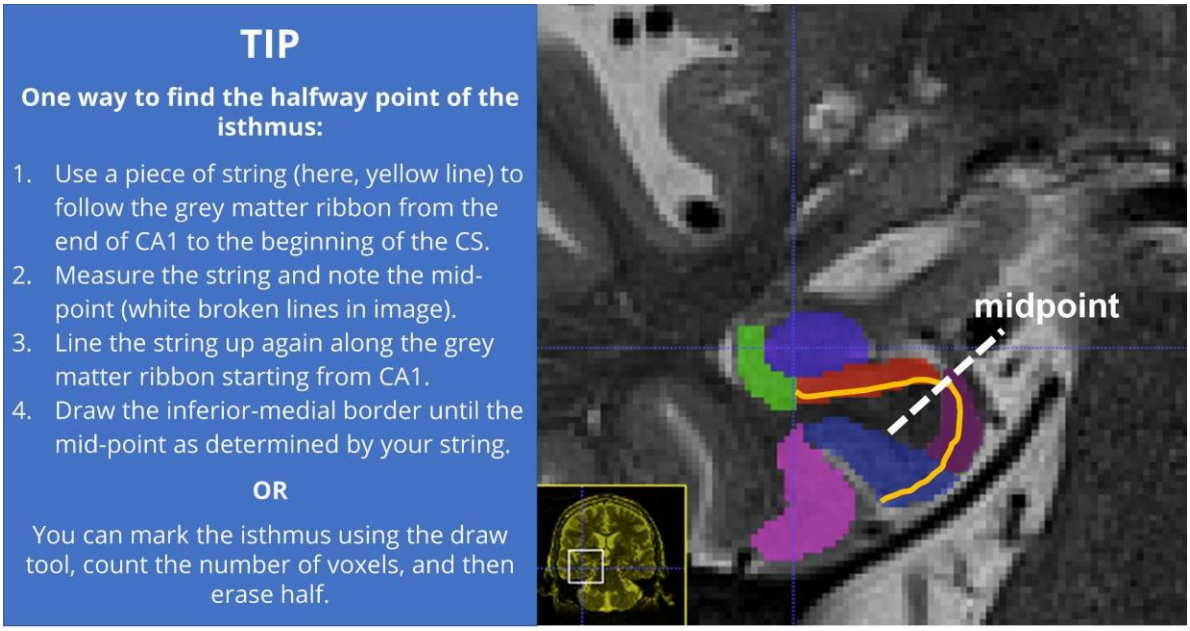


Figure 6.8: How to find the halfway point of the isthmus.

DG+CA3

The dentate gyrus (DG) and CA3 are drawn in as one ROI. DG+CA3 is drawn in **blue** (see **Labels, Naming Conventions, and Contrasts**). It is drawn on all slices that the hippocampal subfields are defined. Simply follow the grey matter region defined laterally by CA1 and superiorly by the Sub. Superiorly, CA3/DG will be determined by a strip of bright CSF, or in more anterior regions it will border the amygdala. This region will also be bordered by the temporal horn of the lateral ventricle.

When defining the region, it is important to not include the white matter (alveus and fimbria) on the superior edge. Keep in mind that this region also typically resembles a tear drop shape in the posterior slices. In the case where the uncal apex is present, trace out both the uncal apex and the lateral body of the HPC.

The medial portion is defined as the closing of the tear-drop share of the HPC. In anterior slices where the HPC is “open” white matter will surround the medial portion.

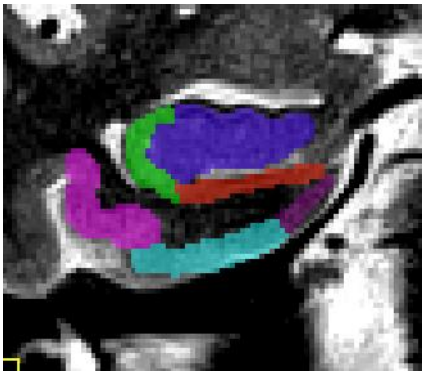


Figure 6.9: Do not include the white matter (alveus and fimbria) on the superior edge of DG and CA1.

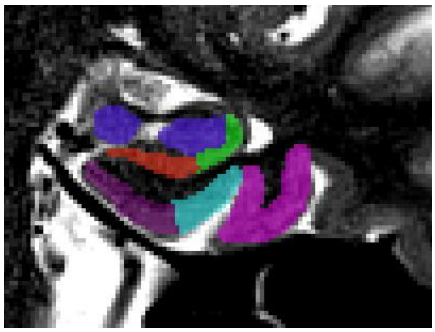


Figure 6.10: In the case where the uncal apex is present, trace out both the uncal apex and the lateral body of the HPC (depicted in navy blue).

7 SEGMENTING SUBREGIONS OF THE ENTORHINAL CORTEX

Segmentation of subregions of the entorhinal cortex (ERC) is accomplished by tracing the posterior-medial ERC (pmERC) from the initial segmentation of the complete ERC. The pmERC is traced in **purple**.

ITK-Snap instructions for segmenting ERC into sub-regions

Click the Polygon tool in the Main Toolbar on the left panel of the ITK-SNAP window. Then, in the Segmentation Labels box in the left panel below, choose pmERC as your *Active label* and ERC as your *Paint over* label. Note you do not need another label called alERC as whatever is not pmERC is alERC. Now you can draw a circle around the area of ERC you want to be pmERC and click accept.

Rules for segmenting the ERC into sub-regions

The following rules will help you identify the alERC from the pmERC:

1. When the amygdala is present and the hippocampal head has not appeared yet, ERC is fully covered by alERC (i.e., do not label pmERC).
2. Approximately 2 mm after the appearance of the hippocampal head, draw the superior boundary of the pmERC to match the superior boundary of the ERC. The inferior boundary of the pmERC is at the uncal notch (see **Helpful Additional Resources for Further Reading,** Maass et al., 2015). This rule assumes that the hippocampus is still "enclosed" in white matter (i.e., the head looks like a digitized bean or "lazy boy" shape).
   - In the case that the pmERC starts on a slice where the uncal notch has not yet appeared (i.e., in cases where the hippocampal head starts before the limen insulae/FTJ), draw pmERC to the medial ¼ of the ERC (see **Variability in Landmarks** section for more information about this case).
3. Moving posteriorly (in subsequent slices), when the hippocampal sulcus is present, and lower bank of the anterior head (i.e. subiculum, which does not yet get its own label) is contiguous with ERC, draw the inferior pmERC boundary halfway between width of previous slice and next slice. Note that the hippocampal subfields are not yet segmented on this slice

of MTL. More specifically, draw the inferior boundary of pmERC up to about an average midway point from how far up it was drawn in the previous slice and how far up it will be drawn on the subsequent slice.

4. Moving more posteriorly, the pmERC covers ⅓ of ERC when the first slice of DG appears.
5. Next, draw pmERC covering ½ of ERC at the hippocampal slice that is 2/3 away from the start of the hippocampal head to the last slice of the ERC.
6. For the last slice that contains uncus (i.e., last slice of hippocampal head), ERC is covered by ¾ pmERC. To review terminology of the hippocampus (e.g., hippocampal head), see **Figure 4.14** in **Lay of the Land: Medial Temporal Lobe Landmarks**.
7. In the final slice where no uncus is present, ERC is fully covered by pmERC.

8 VARIABILITY IN LANDMARKS

Rhinal Sulcus:

Variability in the depth of the rhinal sulcus (RS) within anterior slices of the temporal lobes has been noted previously (Hanke, 1997; Xie et al., 2017). However, most investigations into the structure of the perirhinal cortex (PRC, i.e., Brodmann areas 35 and 36) either do not describe slices before the appearance of the hippocampal head (Insausti et al., 1998; Taylor & Probst, 2008), do not distinguish the RS from the CS (Bonisha et al., 2004), or do not encounter any brains with significant variation of the RS before the appearance of the entorhinal cortex (Augistinack et al., 2013).

Ding and colleagues (2009) found that when a deep RS exists, anatomical area 35 is primarily found in the fundus and the lateral/anterior bank of the RS. In a follow-up study, Ding & Van Hoesen (2010) note that in brains with a deep RS which merges with the CS in anterior slices, area 35 is located in the medial bank of the CS, and area 36 occupies the fundus and lateral bank of the anterior CS.

From these findings reported in Ding and colleagues (2009; 2010) we recommend the following for segmenting early anterior PRC slices:

If a visible (deep) rhinal sulcus is present in early anterior slices such that it interrupts the extension of the PRC up to the Gyrus of Schwalbe from the CS, use the bifurcation rules. That is, when tracing the PRC before the limen insulae you should consider the rhinal sulcus and collateral sulcus as a double sulcus. Draw from the superior border of the Gyrus of Schwalbe to the fundus of the more lateral sulcus (i.e., CS).

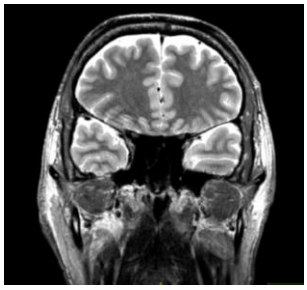


Figure 8.1: In rare cases, a very deep RS may join with the superior temporal sulcus and interrupt the extension of the PRC up to the Gyrus of Schwalbe. Here, the fundus of the RS is not visible. In these slices, only trace around the CS. In subsequent slices, if the RS has a visible fundus, continue to apply bifurcation rules (or, if the RS disappears, continue to trace the PRC as usual).

Other cases of variability:

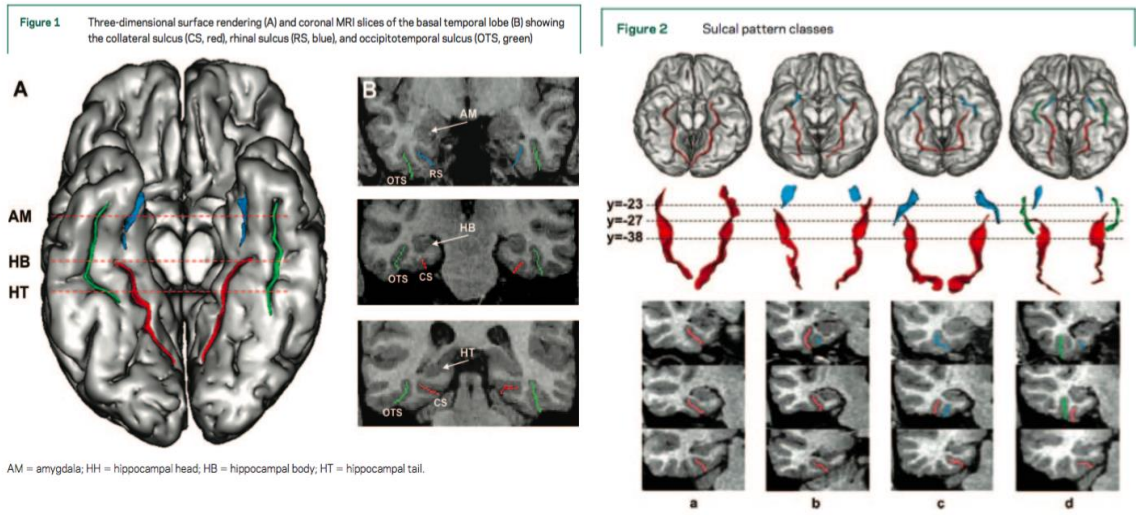


Figure 8.2: This image (left) depicts a coronal slice of the basal temporal lobe. In Boxes A and B, the collateral sulcus is shown in red, the rhinal sulcus is shown in blue, and the OTS is shown in green (Kim et al., 2008). Different sulcal pattern classes are shown in the figure on the right (Kim et al., 2008): (A) Type 1: one-branch CS connected with RS; (B) Type 2: two-branch CS connected with OTS in its posterior portion; (C) Type 3: two-branch CS having connection between RS and OTS in its anterior portion; (D) Type 4: three-branch CS with no connection between the sulci.

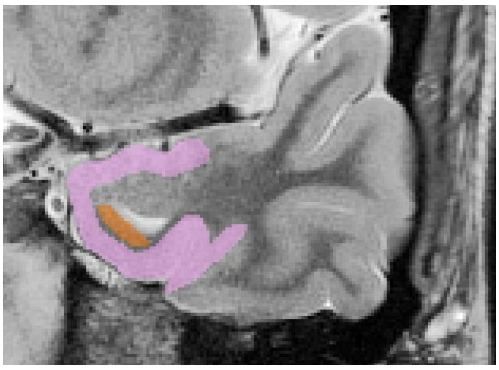


Figure 8.3: This is an example of a younger adult brain where the hippocampal head appears before the limen insulae.

9 COMPUTING VOLUMES AND STATISTICS

Once you have a segmentation, it is important to get some information about it. For this, there is a volumes and statistics option in ITK-Snap that allows you to see the volume corresponding to each label in your segmentation as well as intensity statistics for each label.

STEP 1

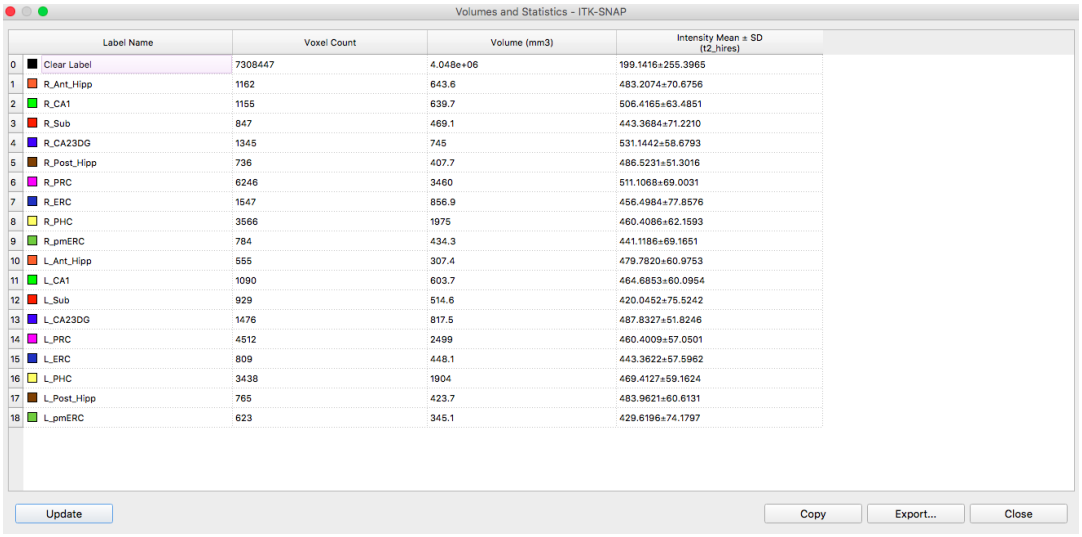
Open image and corresponding segmentation.

STEP 2

In the task bar, select **Segmentation -> Volumes & Statistics**

Here, you will see information about:

- Number of voxels that belong to the structure
- Volume of the structure (in cubic millimeters)
- Mean image intensity inside the structure
- Standard deviation of the image intensity in the structure



STEP 3

Select **Export** and specify a filename with a **.txt** extension and click **OK.**

STEP 4

Import text file into a spreadsheet application for analysis.

For analysis, it is important to note that you cannot use raw structural volumes in your models. This is because you need to account for total estimated intracranial volume (ICV).

To do this, refer to the methodology section of Raz et al. (2015). You may correct for ICV via a linear equation: Volumeadj = Volumerawi - b(ICVi - Mean ICV), where Volumeadj is the adjusted regional volume, Volumerawi is the original volume for an individual, b is the slope of the ROI volume regressed on ICV, and Mean ICV is the sample mean of ICV (Raz et al., 2015).

10 GLOSSARY OF KEY TERMS

TERM	DEFINITION
Alveus	A thin layer of medullary nerve fibers on the ventricular surface of the hippocampus. OAP protocol does not include this region in any ROI.
Amygdala	Collection of nuclei involved in memory, decision making and emotional reactions. Not segmented in this protocol. Located anterior/superior to the hippocampal head. Can be visualized in the sagittal view to help identify the most anterior slice of the hippocampus.
Artifacts	Distortions in MR images that are not native to the actual structure. Can be due to various reasons (e.g., subject motion or normal cardiac function).
Calcarine sulcus	Sulcus where the primary visual cortex is concentrated in the occipital lobe (posteriorly) and retrosplenial cortex is located more anteriorly. Not segmented in this protocol, but can be seen in the coronal view in posterior slices of the hippocampus. How anteriorly it lies depends on the individual's anatomy, but usually comes in inferior to the hippocampal tail in the most medial part of the parahippocampal gyrus.
Cerebral spinal fluid (CSF)	Clear colourless liquid found in the brain and spine. Shows up as bright white on T2 images and black in T1 images.
Collateral sulcus	Sulcus running anteriorly to posteriorly of MTL. Can disappear at times, or be shallow, regular or deep in size, and can also be a double sulcus. Best visualized in the axial view in T1 image.
Cornus ammonis (CA) 1-4	Subfields of the hippocampus. CA_1 is drawn as a different section from CA_2, CA_3, and CA_4.CA_4 is not recognized by all neuroanatomy labs as a distinct region from the "hilus" region of the dentate gyrus.
Dentate Gyrus (DG)	A subregion of the hippocampus involved in the formation of new episodic memories and neurogenesis. Drawn as one region of interest along with CA_2, CA_3 and CA_4.

Elbow	Informal term used for the region where two structures meet at a definitive bend.
Fimbria	A prominent band of white matter along the medial edge of the hippocampus. OAP protocol does not include this region in any ROI.
Fornix	The fornix, named for its archlike configuration, is formed from the fimbria, which is the fringelike medial continuation of the alveus that sits on the superior surface of the hippocampus just below the ependymal lining on the floor of the temporal horn of the lateral ventricles. OAP protocol does not include this region in any ROI.
Hippocampal fissure	Also called, “Vestigial hippocampal fissure” or “Hippocampal sulcus”. Area which lies in the “open” region (space in between different tissues) which lies in the medial hippocampus, superior to the subiculum and inferior to the uncus (in the head).
Entorhinal cortex (ERC)	Interface between the hippocampus and neocortex. Drawn only in the anterior portions of the MTL.
Fronto-Temporal Junction/Limen insulae	The region of grey matter that joins the frontal lobe to the temporal lobe. The first slice of the fronto-temporal junction is also the first slice that the ERC is also drawn.
Fusiform gyrus	Gyrus connecting the collateral sulcus and occipitotemporal/inferotemporal sulcus.
Gyrus ambiens	Most medial portion of the uncus. Gyrus ambiens is a landmark that demarcates the most superior aspect of the ERC.
Gyrus intralimbicus	Most posteromedial gyrus of the hippocampus and most posterior portion of the uncus. When the gyrus intralimbicus is present, it gives the hippocampal head the distinctive “open” or “island” shape.
Gyrus of Schwalbe	A gyrus that marks the superior lateral point of the PRC anterior to the limen insulae. Can exist as two gyri, one gyrus or not be present at all. When Gyrus of Schwalbe is either not present or unidentifiable, the midpoint of the superior grey matter ribbon is used as the superior-lateral boundary of the PRC (see Fig. 5.1).

Hippocampal-amygdala transition area (HATA)	The strip of grey matter that connects the amygdala and the hippocampus. Bisection of this region gives the superior boundary of the more anterior portion of the hippocampus. This region often has a mixture of cell types making it hard to classify as a particular subfield.
Hippocampus	Involved in declarative memory formation and storage of autobiographical memory. Includes the CA_{1-4}, dentate gyrus, , and subiculum.
Hippocampal sulcus/Uncal sulcus	Separates the uncus from the adjacent parahippocampal gyrus. Portion of hippocampal fissure lying ventral to uncus. (i.e., uncal notch). One of the boundaries for segmenting ERC. (see Duvernoy (2005) Chapter 7.1 p. 145-147)
Isthmus	Geographical term used here to describe the narrow strip of grey matter that connects the hippocampus to the MTL cortices. Contains subiculum, ERC, PRC, and PHC.
Medial Temporal Lobe (MTL)	Involved in episodic and semantic memory. Includes the amygdala, brainstem, hippocampus and hippocampal region (perirhinal, parahippocampal, entorhinal neocortical regions).
Occipitotemporal sulcus (OTS)	Also referred to as the Inferotemporal sulcus (ITS). Sulcus that is lateral to collateral sulcus. Can appear as a double and is not drawn as part of the MTL.
Parahippocampal cortex (PHC)	Posterior MTL cortex. Involved in memory encoding, retrieval, scene processing (e.g., “parahippocampal place area”).
Rhinal sulcus	From Augustinack et al. (2013): "we define the rhinal sulcus as completely separate from the collateral sulcus (Braak and Braak, 1992; Ono, 1990; Suzuki and Amaral, 1994a; Van Hoesen, 1995; Van Hoesen et al., 2000) and do not ascribe to the rhinal sulcus being the anterior part of the collateral (Hanke, 1997). The sulcal boundaries for the entorhinal and perirhinal cortices can be elaborate, but in the most simple terms, a rhinal sulcus borders anteriorly and the collateral sulcus borders laterally.” See “Variability” section. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3508349/
Perirhinal cortex (PRC)	Involved in visual perception and memory. Drawn only in the anterior slices of the MTL.

ROI	Region of interest.
Stratum radiatum lacunosum moleculare (SRLM)	Stratum radiatum, stratum lacunosum, and stratum molecular layers. Represents the border between CA_1/subiculum and DG/CA2/3. The SRLM is included in the CA1 and subiculum ROIs and excluded from the DG/CA2/3 ROI.
Subiculum	Major output structure of the hippocampus. Bordered medially by the ERC and laterally by CA_1.
Sulcus semiannularis (ssa)	Sulcus that borders the gyrus ambiens and marks the superior-medial border of ERC. Hard to see on MR images, so is no longer used as a boundary in this segmentation protocol.
Uncus	Medial grey matter structure that is part of the hippocampus and contains a mixture of subfields. Used as an anatomical landmark to differentiate between the anterior and posterior hippocampus.
Ventricles	Cavities in the brain that are filled with CSF. Show up as bright white patches on T2 images.
Vestigial Hippocampal Fissure	With atrophy, can appear as white due to CSF, adjacent to the hypointense band (i.e. SRLM) separating DG from CA regions.

11 HELPFUL ADDITIONAL RESOURCES FOR FURTHER READING

Read the resources below to familiarize yourself with the anatomy, function and current segmentation procedures of the MTL.

A) Duvernoy, H. M. (2005). *The human hippocampus: Functional anatomy, vascularization and serial sections with MRI* (3rd ed.). New York: Springer-Verlag.

This resource contains useful information about MR images and the anatomy of the hippocampus. Chapter 4 overviews hippocampal anatomy, while chapter 7 describes the sectional anatomy of the hippocampus and surrounding structures in MRI.

B) Insausti, R., Juottonen, K., Soininen, H., Insausti, A. M., Partanen, K., Vainio, P., ...Pitkanen, A. (1998). MR Volumetric Analysis of the Human Entorhinal, Perirhinal and Temporopolar Cortices. *American Journal of Neuroradiology*, *19*(4), 659-671.

*A good segmentation protocol. NB: We do not use the term temporopolar cortices in this current protocol. *Click on citation for full PDF.*

C) Amaral, R. & Insausti, R. (2004). Hippocampal formation. In J. K. Mai & G. Paxinos (Eds.), *The Human Nervous System* (3rd ed.).

Chapter 24: Hippocampal Formation contains useful subfield figures for segmentation.

D) Mueller, S. G., Chao, L. L., Berman, B. & Weiner, M. W. (2011). Evidence for functional specialization of hippocampal subfields detected by MR subfield volumetry on high resolution at 4T. *Neuroimage*, *56*(3), 851-857.

*This resource outlines functions of the medial temporal lobe ROIs. Figure 1a contains a segmentation example for an older adult participant. *Click on citation for full PDF.*

E) Kivisaari, S. L., Probst, A., & Taylor, K. L. (2013). The perirhinal, entorhinal and parahippocampal cortices and hippocampus: An overview of functional anatomy and protocol for their segmentation in MR images. In S. Ulmer & O. Jansen (Eds.), *fMRI: Basics and Clinical Applications*. Berlin: Springer.

This resource provides a detailed description of the segmentation procedures of the MTL cortices. Section 19.4 describes the anatomy of the MTL overall, with section 19.4.2 describing a

segmentation protocol for the MTL. Figure 19.3 highlights the Gyrus of Schwalbe (a border for the segmentation of perirhinal cortex), referred to in this protocol.

F) Pruessner et al. (2000). Volumetry of hippocampus and amygdala using high-resolution MRI and three-dimensional analysis software: minimizing the discrepancies between laboratories. *Cerebral Cortex*, *10*(4), 433-442.

*This paper outlines a protocol to divide the whole hippocampus into the head, body and tail on T1 images. Useful as a reference when alternating between T1 and T2-weighted images to make a decision on boundaries. *Click on citation for full PDF.*

G) Pruessner et al. (2002). Volumetry of temporopolar, perirhinal, entorhinal and parahippocampal cortex from high-resolution MRI images: comparing the variability of the collateral sulcus. *Cerebral Cortex, 12*(12), 1342-1352.

*This paper is a follow-up to the one listed above, with details on dividing the MTL on a T1 image. *Click on citation for full PDF.*

H) Daugherty, A. M., Yu, Q., Flinn, R., & Ofen, N. (2015). A reliable and valid method for manual demarcation of hippocampal head, body, and tail. *International Journal of Developmental Neuroscience*, *41*, 115-122.

I) de Flores, R., Berron, D., Ding, S. L., Ittyerah, R., Pluta, J. B., Xie, L., ... & Wisse, L. E. (2020). Characterization of hippocampal subfields using ex vivo MRI and histology data: Lessons for in vivo segmentation. *Hippocampus*, *30*(6), 545-564.

J) Raz, N., Daugherty, A. M., Bender, A. R., Dahle, C. L., & Land, S. (2015). Volume of the hippocampal subfields in healthy adults: differential associations with age and a pro-inflammatory genetic variant. *Brain Structure and Function*, *220*(5), 2663-2674.

K) Maass, A., Berron, D., Libby, L. A., Ranganath, C., & Düzel, E. (2015). Functional subregions of the human entorhinal cortex. *Elife*, *4*, e06426.

L) Kim, H., Bernasconi, N., Bernhardt, B., Colliot, O., & Bernasconi, A. (2008). Basal temporal sulcal morphology in healthy controls and patients with temporal lobe epilepsy. *Neurology*, *70*(22 Part 2), 2159-2165.