

General Segmentation using 3D Slicer

Brain segmentation is a process by which the different parts of an MRI image of a brain are separated from each other using outlines and resulting geometric metrics are used to calculate statistically useful measures such as volume, thickness, or surface area. This procedure is typically performed for a number of brains belonging to several experimental groups and can be used to test the hypothesis that specific brain structure from two populations of subjects is different. Segmentation requires a decent knowledge of neuroanatomy, but much of this knowledge can be acquired during the segmentation process.

Basics of segmentation.

3D Slicer is a powerful tool to evaluate MRI images. It is a constantly evolving program that contains a large number of modules to serve different types of users. Several of the modules are important for our purposes. Many of the most common modules and visualization are displayed at the top of the screen in a horizontal toolbar. Different groups of commands are separated by vertical lines. Starting on the left, the first three commands (Load, Load DICOM, and Save) are for file-handling. The second set includes a search function, a module drop-down menu, and navigation keys similar to those found on a web browser. This second set will allow you to toggle back and forth between modules. The next set of icons are the most common modules (core modules). This group is followed by buttons that control how many and which windows are displayed at one time, markups on the image, and a button to take screenshots.

1. *Loading files*

To begin, you will load three image sets. The first is the T1 weighted image set (usually with a *restore* somewhere in the file name), and the second is a T2 weighted image set. The 'T' refers to the relaxation time that is measured. T1 refers to the longitudinal relaxation time and T2 refers to the transverse relaxation time it takes for protons to return to equilibrium after the radiofrequency pulse. For our purposes, images acquired with a T1 weight accentuates certain tissue properties, while T2-weighted images represent different tissue properties. Both are useful for visualizing and drawing boundaries, although the T1 weighted images are conventionally used for segmentation.

The last image set is a combination of the T1 and T2 image set – this is referred to as the T1divT2 image set because the T1 signal is divided by the T2 signal. This transformation has been reported to accentuate the myelin of white matter, and it is also useful for visualizing certain boundaries.

After loading these three image sets into Slicer, you will need to adjust the T1divT2 image set since the transformation process produces values that are out of range. Under the simple filters module, go to IntensityWindowingImageFilter, and select the upper limit. This threshold should be around 10 or so, but check after transformation to make sure none of the voxels are out of range.

To start, load both a T1 and a T2 for a specific case. This presumes an appropriate image is downloaded in a folder on the relevant folder on your computer, that it is has been intensity-corrected and spatially normalized. Click on the load button, and navigate to the T1 image and open it. Make sure you are looking at 4-up on the window visualization drop down from the toolbar. After a brief loading window, this should bring up three images, one for each plane. Go to the segment editor module. Using the menu on the left, create a new segmentation, and name it according to the case and the segmentation you are about to perform. For example, for general segmentation of Case 398860, name the segmentation Case3997760Gen. For the master volume, select the T1 series. You may change this if you want to segment based on the T2 or the T1divT2 image, but it should save in a common volume.

Go to the Volumes module and write down the contrast and brightness values (known here as window (W) and level (L). Also, note the threshold values. Record these values in an excel spreadsheet. This spreadsheet is also good for any observations you may have about the brain. Also, it is useful to uncheck the interpolation box – this will allow you to see the real unsmoothed data.

The images should be intensity bias corrected, but you may have to readjust the orientation of the brain according to Talairach space. This space is defined by a horizontal plane between the anterior and posterior commissure, and a vertical corresponding to the midline. If the name of the file includes an underscored suffix such as ‘_acpc_’, this operation is already done. If not, Slicer has an app for that. It is called ACPC Transform, and it can be found as one of the first modules on the module pulldown menu. Select ACPC Line, then navigate to the anterior commissure. Select the ‘Fiducial’ button and click on the middle of the commissure along the midline. Then navigate to the posterior commissure and click on the middle of this structure in the midsagittal plane along the midline. Go back to the module, select midline, then click as many midline points as you want. Go through the brain and click on regions you know to be on the midline – the falx cerebri is a good thing to place a dot on (for instance). Be cautious, however, it is normal for the occipital pole to occasionally deviate across the midline, so it is useful to keep your markers to the mid portion of the brain.

When you are done placing the midline fiducial points, go to the module and name the transform (e.g., Case398860Gen.acpc). You will now use this transform to change each of the three image sets. Go to the Resample Scalar/Vector/DWI Volume module. Select the T1 in the ‘Input Volume’. Create a new volume in the ‘Output Volume’ selector, and under this pull-down menu, rename it something like T1.acpc. In the ‘Transform Parameters’ section, click on the transform you previously generated under ‘Transform Node’. Then click ‘Apply’. This will generate an image spatially normalized to the ACPC line. Perform the same steps for the T2 and T1divT2 image sets. Now you will segment these transformed images, not the originals.

2. General Segmentation

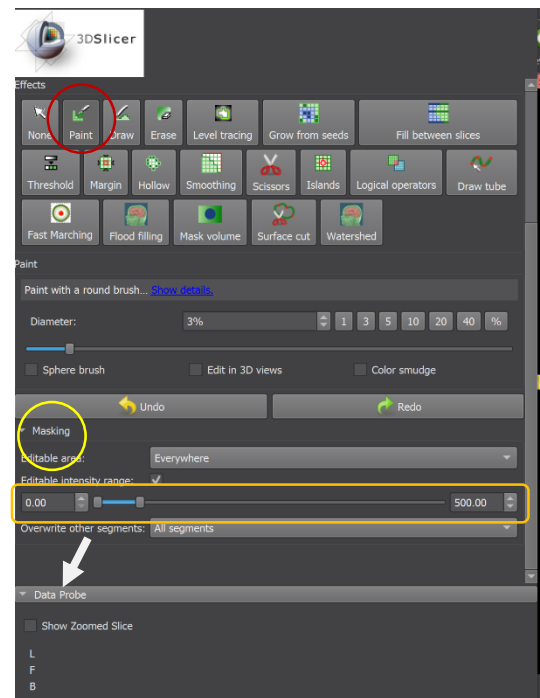
Go to the segment editor, click on add. In the table underneath, a name will appear, called segment 1. To the left is an eye, which makes the segment visible or not visible. Next to that is the color and the name. If you double click on the name, you can rename the segment identity. If you click on the color box, a second window will pop up. Here you can again click on the color to change it, and rename things. However, this is also where you can select names and colors that have already been entered into the system. On the left should be a 'General Segmentation' entry. When you click on this, a series of preloaded structures will pop up. If you do not see this, let someone know. Load left and right lateral ventricles by selecting. After exiting this window, click on the Left lateral ventricle in the main window. Go to a coronal view of the rostral part of the ventricle, where it is relatively nicely sized (slide the bar at the top of the image window around 2/3 of the total distance on the right). The interior of the ventricle should appear quite dark on the T1 image.

Radiological Imaging. Brain orientation is standardized according to a radiological convention; namely, that you are viewing the brain from the perspective of standing at the foot of the bed of a patient. Viewed this way, the left side of the patient is on the right of the screen. A good way to remind yourself is 'Left and 'Right' post-its on your monitor.

The fastest and most accurate way to segment each ventricle is to use the paint tool (Figure 1, red circle) in conjunction with the editable intensity range under the masking sub-tab (yellow circle). What this will do is allow you to paint voxels that fall within a certain range of intensities (yellow box). To find that range, you need to get a sense of which voxels should be included in the ventricle, and which should be included as part of the surrounding white or grey matter. One way to do this is, select the pointer tab (labeled as 'None' under Effects in Figure 1), and point the cursor at different voxels. The ventricle, on a T1 image, should have low values, and you should see the corresponding intensity values in the lower left-hand part of the window, under Data Probe (Figure 1, white arrow; the number will appear on the far left of B). Use this tool to get a sense of where the border is. Then set the lower editable intensity range to -0, and the upper limit to what you have decided (orange box). Then select a relatively large diameter paint tool, and paint the border. Only the voxels in the range should be selected. Do this for one slice, for both gray and white matter, and then zoom out to evaluate. If you see voxels that are dark and excluded from your ventricle outline, think about increasing your threshold. If you see voxels

in the ventricle parcellation that you think are light gray and should be included in gray matter, reduce your threshold. Play around with this just a bit.

But, there is a better way. If you click the threshold button (first icon in the second row in Figure 1, but it changes location based on the window size so you may have to search for it – red circle in Figure 2), you will observe that the whole image starts to slowly flicker. More accurately, an image overlay will cyclically become more and less transparent. This overlay is tied to the intensity values that appear in a bar under threshold range (yellow box in Figure 2). This bar is adjustable. So, similar to what you did in the above paragraph, you can set the upper and lower bounds of the editable intensity range and see it in real time for whatever slice or slice orientation you care to view. This is much better because you can generate a threshold and see the implications for the whole structure, rather than determine it by looking at representation voxels. Even better, when you have figured it out, then simply click on the 'Use for Masking' button, and the range is automatically imported into the paint masking section. Before you set the threshold, make sure you look at different images of the brain (coronal, sagittal, axial) to make sure you getting exactly what you need (not too much, not too little). Make sure that for each brain region, you write down (in the excel spreadsheet) the threshold range so that it can be reproduced if needed.



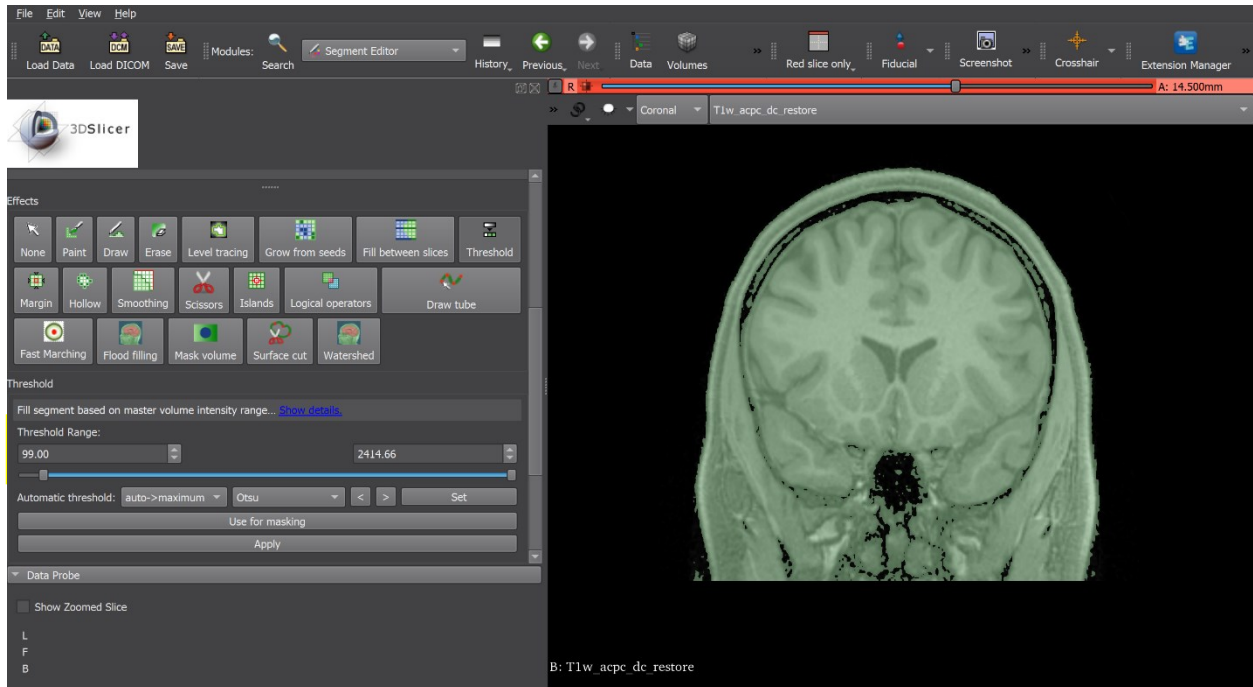


Figure 2 Using Threshold to determine editable intensity range.

At this point, it is helpful to change some of the viewing settings of the segments. Go to the Segmentations module (found either in the large toolbar at the top, or via a shortcut from the segment editor module under the 'master volume' and to the right). Under display, uncheck slice fill. Then go to advanced (located immediately below the slide bars) and increase the slice intersection thickness to 3px, or whatever you would like. Now go back to segment editor.

Now it is time to segment. Go to the most rostral section of the lateral ventricle, and then start painting. Use a relatively big tool. As you progress caudally, the medial border of the ventricle will change from white matter to a small membrane (the septum pellucidum, double yellow arrows, Figure 3) which separates one ventricle (red arrow in Figure 3) from the other. You'll want to make sure you toe this line and not accidentally label voxels in the other ventricle. You will also want to keep an eye out for other dark regions you may inadvertently label with your large brush – the black between the hemispheres above and below are common. If you reliably get these extra voxels, click the erase tool and get rid of them, or press 'z' to undo. If this is happening a lot, consider reducing the size of the brush (under the paint tool settings on the left, or just hit the 'minus' key).

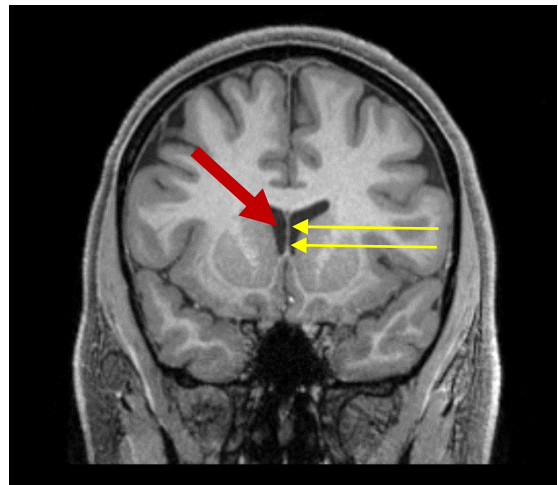


Figure 3: Lateral Ventricle and Septum Pellucidum

When you are painting, it may help to check the 'Sphere Brush' under the settings (Green circle, Figure 4). This will give you a 3D brush and will paint in front and in back of the section you are selecting. When you use this tool and as you go through the coronal sections, you will find that many of the voxels are already labeled when you get there. This is nice and may save some work. Keep in mind, however, that as you proceed, you may label voxels that fall within the intensity criteria, but are outside the region you are interested in labeling. In these cases, you will need to erase those voxels, which takes some time. Also, keep in mind that erase also uses the sphere brush, and so when you erase with this checked, it will erase voxels in the slices in front and in back of the current one, and may undo some of the work you just did. So, keep an eye out for aberrant voxels when you use this approach. It may seem that the sphere brush may be too much work, rather than just going through the images carefully once per section. However, if you use the sphere brush on a structure that goes from smaller to larger (and not in the reverse direction), it can be used easily and without fear of extra-structural labeling.

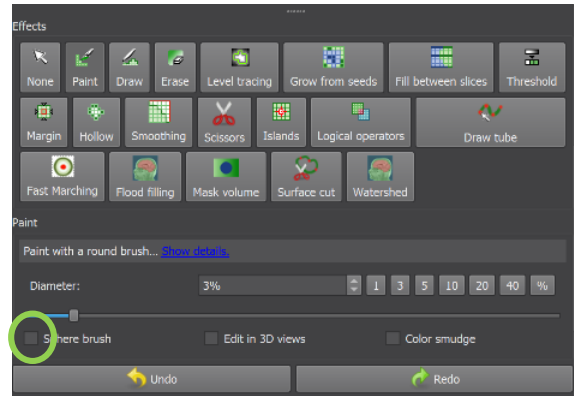


Figure 4: Sphere brush

A few hints on outlining.

1. Do not change the contrast or brightness willy-nilly. If you inadvertently do, go to Volumes and reset the values you recorded at the beginning. There are steps for parcellation that may benefit from changing the contrast or the brightness – feel free to change the contrast if it helps you to see things a bit better and aids in parcellation, but remember to go back to the original settings.
2. It is helpful to zoom in to see some voxels. However, high magnification segmentation can be misleading. Be sure to zoom out and check your work when you are done with a section.
3. Mistakes are bound to be made but do your best to be precise and complete sections the first time. Going back has the potential to be time consuming and frustrating. You will minimize time and errors by trying to make sure that the boundaries are correct the first time around.
4. Hotkeys are very useful. The keyboard number keys correspond to selecting and deselecting different tools. For instance, pressing number 1 will select the paint tool, and pressing it again will return it to a cursor. Similarly, the draw tool corresponds to number 2, and the erase to number 3. The 'z' key is also important – pressing 'z' automatically undoes the last command. Slicer has a memory, so you can hit this several times and it will undo several of your last commands. You will develop your own technique. I like to use my right hand on a mouse (or use a pen) to segment and use the mouse wheel to move through the sections. I place my left ring finger on the '1' key, the middle finger on

the '2' key, and the index finger on the '3' key. I curl the thumb underneath to put it on the z key.

The ventricles are somewhat elaborated structures and are said to exist in the sagittal plane as a C-shape. In this initial segmentation, concentrate only on the part in the front of the brain. There are two things to be aware of as you go from rostral to caudal. First, each lateral ventricle communicates with a small third ventricle, which is located below (inferior) the ventricles on the midline. There is a small canal on either side, called the foramen of Monro, that is the connection (red arrows, Figure 5, left). We consider this foramen to be part of the lateral ventricle parcellation, and the cigar-shaped third ventricle to be along the midline. Second, it is important to pay attention to the borders of the ventricles as you move caudally. About halfway through the brain, the lateral ventricles are flattened horizontal structures (Figure 5, center). Each ventricle is bounded medially by a little mustache – in the T1 images, it is white. This is the fornix – one on either side (Figure 5, center, yellow arrows). It appears to begin superiorly, and progress inferiorly and flare out. Your segmentation should not go medial to this structure, or go below it.

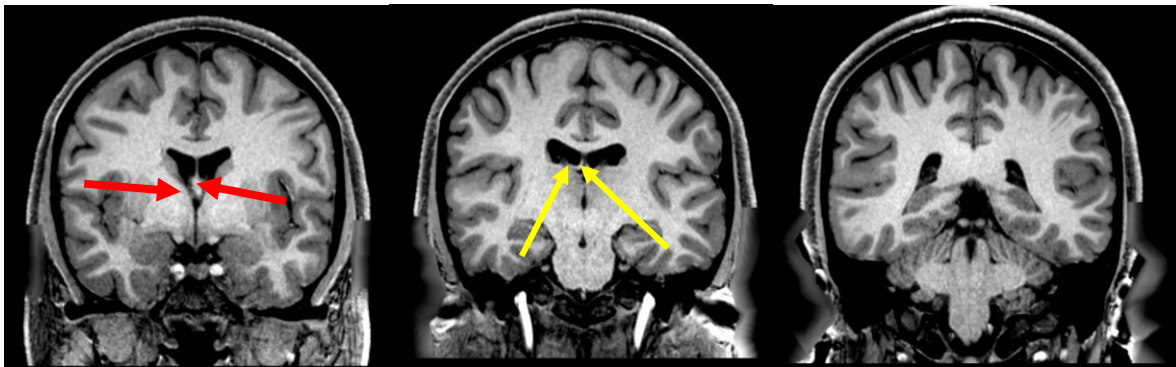


Figure 5: Lateral Ventricle

A final consideration is that the ventricle extends from the frontal part of the brain, then loops down into the temporal lobe to make a C. Before it does this, there is a large dilation in the ventricle (the atrium) from which the temporal extension of the ventricle (called the temporal, or inferior horn) emerges (Figure 5, right). The atrium also has a variable posterior extension that comes from it – this is the posterior (or occipital) horn. In many cases, this appears to end, but be sure to keep looking at slices caudally because sometimes the ventricle opening is merely compressed and reappears more caudally. An axial view is helpful for seeing the full ventricular extent.

For now, do not segment the inferior horn. We will do this separately when we start to think about structures lining the inferior horn (the hippocampus and the amygdala).

After you go through the ventricle, it is time to go back and check your work. You will do this with every structure, but the ventricles deserve a bit of extra caution. There are likely to be small aberrant, unlabeled voxels within the ventricular segmentation. These need to be closed. These voxels are likely to be part of the choroid plexus that are a bit more intense than the

threshold you placed. For our purposes, these voxels are included in the lateral ventricle segmentation. As you review, make sure there are no labeled voxels outside of the ventricle, as often happens underneath the fornix in the mid portion of the ventricle.

At this point, make sure you are in the four-pane viewing mode. If this isn't already done, go up in the segment editor window and click on "Show 3D." This will reveal the 3D ventricle in the 3D window, and you can marvel. In this window, click to rotate and look at all aspects. This is a good place to check whether there are any inconsistencies, aberrant voxels you may have missed, etc.



Figure 6: 3D View

Rotating and zooming around the image may leave the 3D model out of the plane or out of the window. To get it back, go to the toolbar on top of the window (Figure 6, red arrow). There should be an arrow, a number (1), and a crosshair. Click the crosshair to center. Then click on the arrow. There are a lot of commands here, but the most useful (for taking pictures) is the axes with the letters on them (S,P,R,L,A,I). Click on a letter, and the 3D model will snap to that. If you like it, feel free to take a picture. On the big tool bar, click screenshot. Select 3D view, and name it. IMPORTANT. This just takes the picture but it does not save it. To do this, you will have to go to the left and click Save. A dialog box will open. Unclick everything except the picture, select the folder, and you now have your very own ventricle picture.

The Third Ventricle

The lateral ventricles are nice and big, and outlining gives you some experience with high contrast structures. Now let's turn to the third ventricle. This ventricle has several appearances, depending on the image plane. It is located right along the midline in the center of the brain, and it extends from the optic chiasm anteriorly to the midbrain posteriorly. Go to segment editor and add a new segment. Name it 3rd Ventricle.

1. Anterior. In the coronal slice view, locate the anterior commissure (AC – figure 7, blue arrow). Directly underneath the AC on the midline is the third ventricle. Using the paint tool with the intensity range set at the same levels used for the lateral ventricles, outline it. The use of this intensity range will allow you to label the entire structure in one fell swoop, so long as the extent of the paint circle does not extend past the lower border of the brain. If you accidentally extend past this boundary and label some voxels below the brain, you can correct it. Either hit z on your keyboard to undo your outlining or hit 3 (the undo hotkey) and erase those pesky voxels. Then switch back to the paint tool. Once you have outlined the 3rd ventricle, move rostrally. The optic tracts, which are

lateral and lower than the 3rd ventricle, will now merge underneath the ventricle to form the optic chiasm. You should be able to see the boundaries, but feel free to adjust the brightness and contrast to make it more visible should you have any difficulty spotting it. To do this, select the arrow under 'Effects' on the segment editor, or alternatively, if you are using the paint tool already, hit the number 1 again. This will deselect the paint tool and reselect to the arrow. Now go to the brain image, and click and drag. Dragging to the left and right will increase and decrease the image contrast, respectively, while dragging up and down will decrease and increase the brightness. At the next rostral level, the optic chiasm will disconnect from the brain, and above it will be a teardrop-shaped space continuous with what you previously outlined (Figure 7, yellow outline). Label this as a part of the third ventricle. With another section or two rostrally, this space will contract and end. Go back to the AC level. Above the AC, you may see a small hole, which corresponds to the lateral ventricle (toggle back and forth between slides to convince yourself). Outline this small space – it will not be present too far in front of the AC, and will be contiguous with the third ventricle caudally.

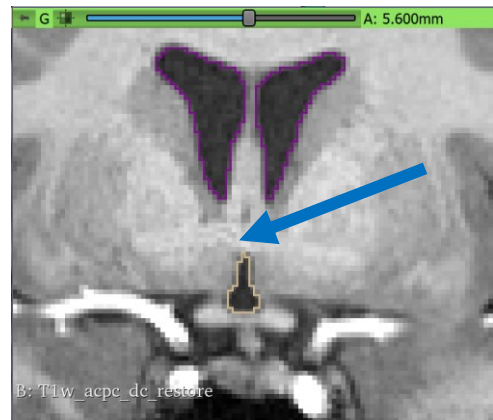


Figure 7: Rostral Third Ventricle

2. Mid-portion. Now you will work your way to the back of the brain. Immediately behind (caudal to) the AC level, you will find the foramina of Monro (n.b., foramen is latin for aperture, foramina is the plural and there is one on either side). These apertures are where the lateral connect with the third ventricle. In figure 8, they are observed lateral to the fornix (purple outlined spaces). Make sure that when you segment the 3rd ventricle, you do not extend into these foramina because they operationally belong to the lateral ventricle segments. The 3rd ventricle should appear here as a fusiform or cigar-shaped structure (Figure 8, yellow outline), rising to the fornix (the paired white matter bundles at the top of the yellow outline in Figure 8) and relatively invariant in width. After the level of the foramen of Monro, the ventricle will constrict. Using the paint tool with the intensity range, you will be able to outline the ventricle with one click and then move quickly caudally.

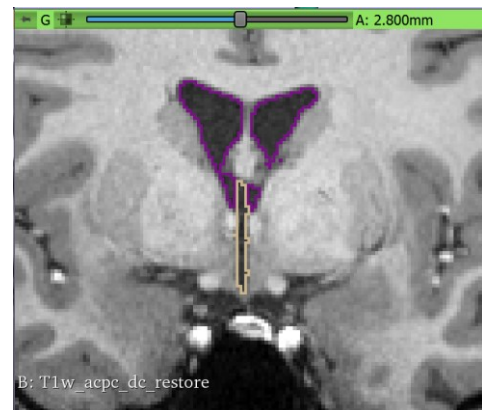


Figure 8: Foramina of Monro

At this point, we need to introduce an additional structure that requires separation from the ventricle. This space, called the transverse cerebral fissure (TCF), is also filled with CSF, and

while it is not directly connected to the third ventricle, it shares a border with the structure that is often indistinct. The TCF will become fairly large as you move further caudally, but here it is present along the midline under and between two fiber tracts called the fornices (singular: fornix; Figure 9, green arrow). In actuality, this region contains part of the third ventricle, as well as the TCF. However, we cannot resolve the difference, and given the small size of the third ventricle here, we operationally define this space as belonging exclusively to the TCF. That being said, in some brains (especially those with larger ventricles), a separation can be observed between the TCF and the lower 3rd ventricle, as a line of thin choroid plexus. This third ventricle space should be confirmed by tracing it forward and backward and seeing it directly connect with the main extent of the third ventricle.

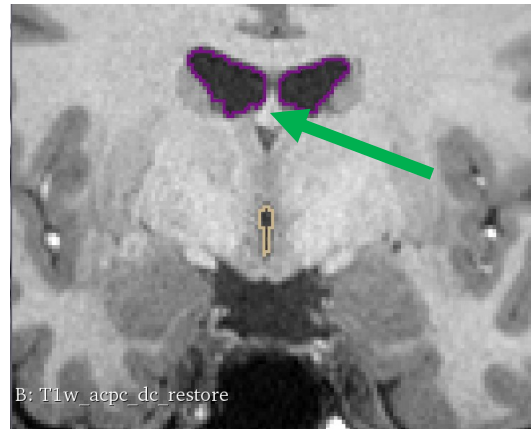


Figure 9: Transverse Cerebral Fissure

3. Caudal portion. More caudally, the third ventricle will open up. At this point, you will observe two round structures underneath the fornix. These are the internal cerebral veins. Underneath these structures, by adjusting the brightness/contrast, you should see a membrane that tents up with the apex of the tent up and on the midline. This is the border between the TCF superiorly and the third ventricle inferiorly. Label the space below this tent with the paint or the draw tool. It helps to toggle to the adjacent brain sections using the arrow keys or the mouse wheel to appreciate this boundary.

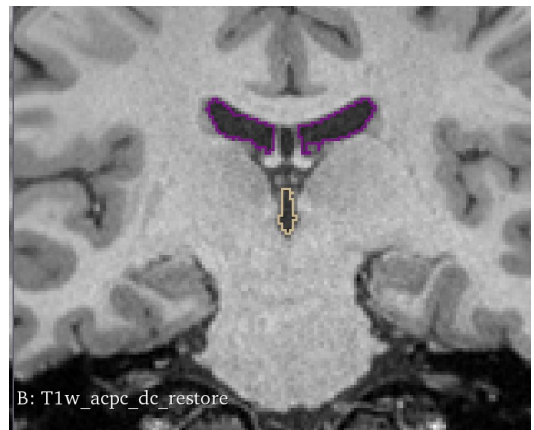


Figure 10: Caudal 3rd Ventricle

You will notice that after a few sections, the third ventricle breaks up into two components by the introduction of a white matter tract (called the posterior commissure) going across the midline. Once you identify the posterior commissure, the ventricular space underneath it is now called the cerebral aqueduct (of Sylvius). This space communicates the CSF of the third ventricle to the fourth ventricle and is considered a component of the fourth ventricle. We will discuss this in the next section, but for now, make sure you do not include this space in your third ventricle segmentation.

What to do the with third ventricle above the posterior commissure? Well, it turns out that the third ventricle sends out two small extensions, one that goes above the posterior commissure as the pineal recess, invaginating the stalk of the pineal gland, and one on top of the pineal gland known as the suprapineal recess. Both of these recesses will appear as disconnected circles in the coronal sections. Increase the brightness and use the T2 to help you examine them. Also, employ the mid-sagittal views to show the continuity.

The Fourth Ventricle

Using the paint tool with the intensity range, outline the cerebral aqueduct on the coronal section and move to the next posterior image. It is useful to start in the coronal plane and then switch to axial once you have completed at least a few segments in the coronal plane. It is also useful to quickly view the fourth ventricle in a sagittal view before finishing this segmentation. Keep in mind that one potential issue is the degree to which the spaces between the cerebellar folds are included as ventricle. Following several of them throughout multiple views will clarify which are connected to the ventricle, and which are not. Use the axial view to inform your decision.

Final comments on Ventricular segmentation:

You will notice that in some brains, the initial part of the fourth ventricle or parts of the third ventricle are fairly small and not labeled by the threshold you have selected due to partial voluming of the surrounding structure. To overcome this, change the threshold so you generate a cohesive segment. Make sure you change it to the lowest threshold that will give you such a complete structure. This is also applicable in some brains in which the dorsal part of the lateral ventricle over the caudate becomes too thin to resolve – in the 3D view, this will appear as holes in the structure. Finally, the connection of the posterior horn with the atrium of the ventricle may be disconnected. Here, the spaces may not be confluent. Change the brightness slightly to convince yourself one way or the other.

The Nucleus Accumbens

Now that the ventricles are complete, we can start segmenting gray matter structures. We will begin with a structure called the nucleus accumbens. To segment this area, we need to understand more about the anatomy of two other regions: the caudate and the putamen. If you examine the lateral ventricle in a section anterior (rostral) to the foramina of Monro, you will find a gray matter structure adjacent to it on its lateral border – this is the caudate ('C' in Figure 11). At the front of the brain, it is quite large and it makes up almost all of the lateral border of the ventricle. As you progress caudally, it will get smaller and maintain its general position. If you move back to the rostral part of the ventricle where the caudate is large, you will see a stippling of white matter on the lateral aspect of the caudate. This white matter tract

is incomplete and separated by thin fingers of gray matter that extend from the caudate laterally through this white matter tract. This tract is called the internal capsule and it contains axons the cerebral cortex sends to the brainstem and spinal cord (Figure 11, 'IC'). There is a gray matter nucleus lateral to the internal capsule called the putamen (Figure 11, 'p'). The gray matter extensions that travel through the internal capsule connect the caudate to the putamen. Rostrally, the putamen is puny, but it will grow in size as you proceed caudally.

You will notice that the caudate and the putamen meet underneath the internal capsule. This area is a separate region called the nucleus accumbens. We have to outline it first because its location uses an operational definition based on morphological landmarks, rather than one based on intensity or visible borders. Segmenting this structure first makes subsequent segmentation of the caudate and putamen much easier.

To start with, add two segments in the Segment Editor Module - Nucleus Accumbens Left and Nucleus Accumbens Right.

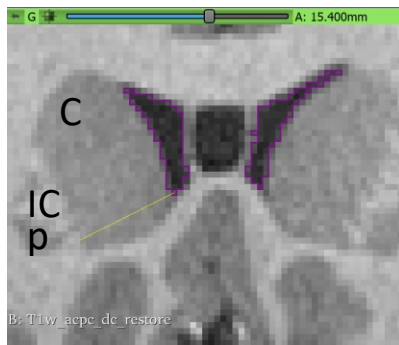
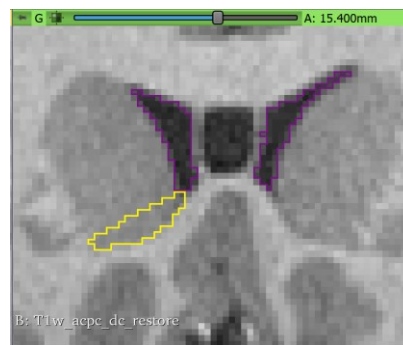


Figure 11 Nucleus Accumbens



We will use the draw tool. Find the first section where the internal capsule / putamen appears (Figure 11). Click on the inferior and lateral point of the lateral ventricle and draw a straight line to the inferior and medial point of the putamen. Do not click a second time just yet,

but just notice where the line crosses the internal capsule. Move the mouse to where this line crosses the internal capsule. Click and you should have a line that goes from the lateral ventricle to end in the gray matter right before (medial to) the internal capsule (figure 11, left). Then use multiple clicks (or click and drag) to follow the inferior gray-white border medially and then dorsally to return to the point from which you originated (figure 11, right). Then right click to accept it. If you have some time and are feeling fancy, you can figure out the intensity range

for the white matter, and enter it as an upper bound on the intensity bound masking. This will help your contour with the underlying white matter, but it may take less time to do it manually.

Do the same thing for the next caudal segment. You will notice that the putamen is getting larger. As you continue to go caudally, you will notice that the internal capsule will disconnect from the inferior white matter, and here the caudate and the putamen will be in continuity (Figure 12). When this happens, place your line from the inferior and lateral border of the lateral ventricle to the midpoint of the internal capsule. Then draw a line straight down to the white matter, then follow the white/gray matter interface medially to the origin.

Continue using this approach until two images before the appearance of the anterior commissure, at which point the nucleus accumbens will end. Keep in mind that as you make your way caudally, the nice coherent white matter will begin to look a bit more grey. It will be lighter than the gray matter, but please do not label it. Keep the distance between the brain surface and the label as in previous sections. If in doubt, use the arrows to toggle between images to convince yourself of a smooth medial and inferior border.

The Caudate

Now that you have outlined the nucleus accumbens, you can move on to the caudate. We will again use the paint tool with the intensity range. For this range, put the lower bound as the upper bound you set for the ventricle. For the upper bound, use the threshold tool. When determining the upper bound border, you want to insure you do not include the internal capsule in this segmentation. At the same time, the caudate is fairly heterogeneous and may contain some voxels that are nearly as light as those found in the internal capsule. To this end, you may anticipate finding some voxels that remain unlabeled within the caudate as you segment. Since these voxels will technically be inside the caudate volume, you will need to go back and manually segment them. Some people may find that it is easier and faster to maximize the upper limit and carefully go along the border with the paint tool. In our experience, there are a limited number of sections that will contain the aberrant voxels, and it is easy and fast enough to quickly go back. Regardless, make sure you record your range on the spreadsheet.

Importantly, go to the masking drop down menu and make sure you select 'None' in the overwrite menu. Also, make sure 'Outside All Segments' are selected in the Editable Area drop-down menu. This makes sure that when you start to outline the caudate, you will not paint voxels that are dedicated to the ventricle or otherwise overwrite any other outline. Practically,

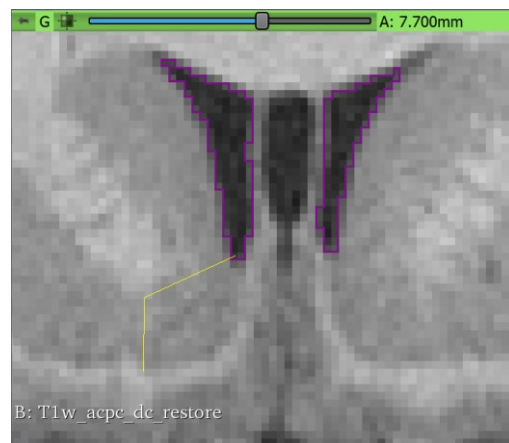


Figure 12 Nucleus Accumbens, caudally

this also means you can be less precise when you are painting boundaries with already established volumes.

Some people like to start segmenting the caudate from the axial perspective (there are fewer sections and so it goes slightly faster), and then finish the trickier inferior margin in the coronal sections. Others prefer to do one take, and start from the rostral part of the caudate, working back. Either of the aforementioned approaches is acceptable. As you do more segmentations, you will find a method of drawing the caudate that is most efficient and accurate for you.

Whichever way you choose, be aware that the caudate has several parts. The portion in front of the foramen of Monro is the head of the caudate. It is bounded medially and dorsally by the lateral ventricle, laterally by the internal capsule, and ventrally by its border with the nucleus accumbens. When the anterior commissure (AC) appears, the ventral portion of the caudate oftentimes narrows and contacts the top of the AC. This region is technically called the bed nucleus of the stria terminalis, but we include it in the caudate. Once we move caudally to the foramen of Monro, the caudate gets pushed to the lateral and inferior border of the lateral ventricle. Here it is called the body of the caudate, and it will diminish in size as you proceed caudally. After the foramen of Monro, the thalamus comes to dominate the central part of the brain and displaces the caudate dorsally and laterally.

You will notice that the lateral ventricle is typically more flattened at this stage. When the thalamus disappears at a more caudal stage, the ventricle enlarges ventrally. What is really happening is that the ventricle curves around the back of the thalamus. This is called the atrium of the ventricle, as described above. The caudate also wraps around on its way to the temporal lobe, and when the ventricle opens up, you can identify the continuity with the caudate you have previously been labeling. Label the caudate as it progresses ventrally, but be careful not to label white matter or choroid plexus. At this point, we will stop with the caudate. It does continue into the temporal horn of the lateral ventricle as the tail (on the roof, no less), but we it is difficult to identify.

The Putamen

The putamen is next. You can start with the same intensity range you used in the caudate, but the larger number of myelinated fibers that randomly pass through the putamen (particularly caudal to the foramen of Monro) makes this approach somewhat ineffective. For this region, use a moderately-sized paint tool, uncheck editable intensity range, and use the paint tool to label the putamen. The putamen is as long as the caudate, and as you progress caudally, you should make the paint tool smaller. An alternate approach is to use the draw tool, with a pen or a mouse. The pen is probably the fastest.

The Globus Pallidus

The globus pallidus is a structure located between the putamen and the internal capsule. It is roughly triangular in shape when observed in the coronal plane, and is nicely viewed in the sections caudal to the anterior commissure. Since there is a great deal of white matter traveling through the globus pallidus, it can be difficult to pick out which portions of the white matter belong to the globus pallidus and which belong to the internal capsule. To begin, pick several sections around the anterior commissure where the globus pallidus is very clear. Then segment it by applying the paint tool without using the intensity range. Next, switch to a sagittal view, and adjust the contrast and brightness to better see the structure. From here, complete the borders, being mindful of the tricky inferior border in particular.

In some brains, the globus pallidus takes up iron. This makes it very easy to identify in the T2-weighted image, so use the T2 as necessary to guide your borders. Remember that the basis of the segmentation is the T1, so the final border should be generated on the T1. Also, understand that the T2 will have additional structures that are darkly labeled and adjacent to the globus pallidus (i.e., substantia nigra, red nucleus, white matter), so if you use the T2 as the initial step, make sure you carefully review the borders on the T1 when you finalize the globus pallidus segmentation.

The Brainstem

The brainstem is a collection of regions that include the medulla, the pons, and the midbrain. This structure is operationally defined by two planes. The first plane separates the brainstem from the thalamus and the ventral diencephalon (VDC). To draw this line, find the midsagittal plane. Using the draw tool, draw a line from the posterior commissure (PC; Figure 13 green arrow) to the prepontine recess (Figure 13, upper, lower end of the thin yellow line). It is useful to set the intensity bounds higher than the margin you set for segmenting CSF so you automatically select brain matter. Make sure that the PC is on the right-hand side of your line. Move around the pons and the medulla with the draw tool. Pause for a moment and look at the brainstem. It has a long axis, and a dip in the fourth ventricle at its lowest point (called the obex; blue arrow). You will want to make a line perpendicular to the long axis of the brainstem at the level of the obex as part of your outline. Next, move along the third ventricle. Cross the superior medullary velum (red arrow) to include the inferior and superior colliculus. The pineal gland is right above the superior colliculus. You will want to go between the pineal and the

superior colliculus to complete your outline. It should look similar to the lower panel of Figure 13. Now close the line.

Go to the coronal view. As you scroll from rostral to caudal through the mid-portion of the brain, you will observe a line in the middle of the brain. Since you drew an outline in the sagittal plane, viewing the brain in the orthogonal coronal plane will represent that plane by a line. Scroll through the brain in the coronal view with the Four-up View so you can see the relationship. Moving your cursor with the shift key held down (or even better, selecting the cross-hair button on the big tool bar) will help you appreciate the relationship between the image planes.

The brainstem will have different appearances as you go from rostral to caudal in the coronal view. In the most rostral sections, the line you placed will be short, and the structure will be disconnected from the brain (Figure 14). The vertical line will identify the brainstem, and we will now use the draw tool to outline the structure. Note that nerves and blood vessels will be connected to or close to the structure, and that these should not be labeled. For example, you can see such a structure coming out of the ventral midline in Figure 14 (red arrow).

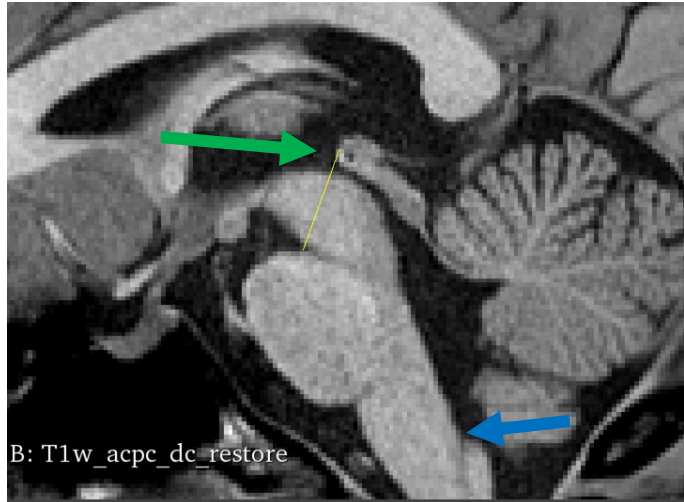


Figure 13: Midsagittal Brainstem

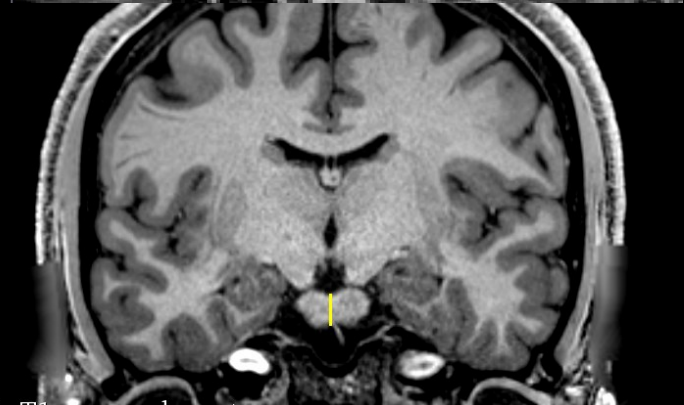


Figure 24: Brainstem Coronal View

As the sections proceed caudally, the labeled brainstem becomes larger and connects to the brain. Your line around the brainstem should be consistent with the previous outline, and should separate the thumb-like structures from the brainstem (Figure 15).

As you proceed even further caudally, the line will get longer. When the brainstem initially connects to the rest of the brain, the line spans two spaces. While scrolling towards the back of the brain, the uppermost space (the interpeduncular fossa) will disappear and the line will

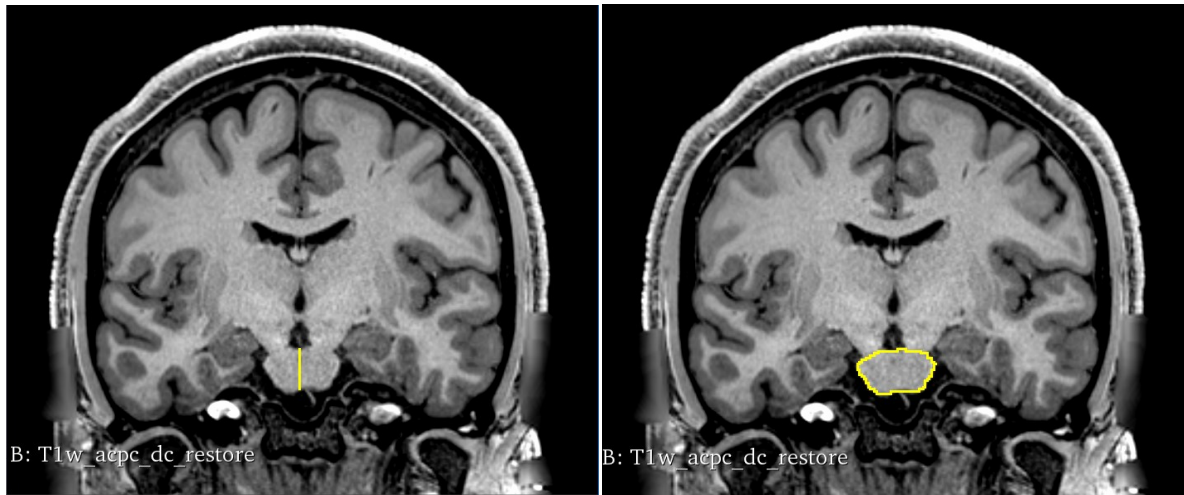


Figure 15: Brainstem Coronal View - more caudally

move dorsally (Figure 16). At this level, use the draw tool to draw a horizontal line at the top of the vertical line, and use this as the dorsal margin of your outline. Continue using the draw tool to draw a contour around the brainstem, and close the outline. Remember to avoid blood vessels and nerves.

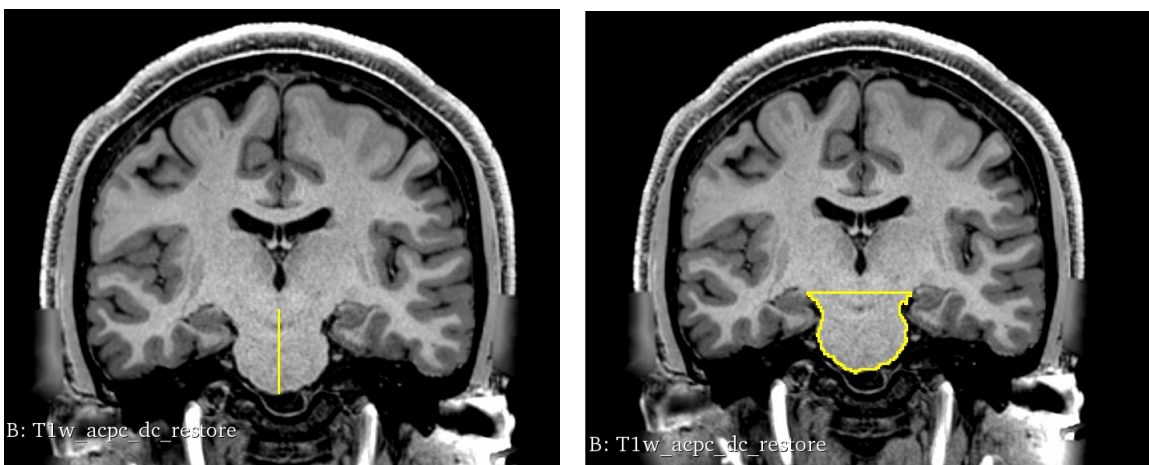


Figure 16: Brainstem, even more caudally

Pause at this section, and scroll ahead to observe the middle cerebellar peduncles (MCP; orange arrows, Figure 17, left). These structures are lateral bumps on the brainstem and are excluded from the brainstem outline per se. Find a section where you can clearly view the peduncles (Figure 17). Note that the vertical line we draw to label the brainstem goes through the entire structure. Using the draw tool, draw a line through each one of the peduncles that is parallel to this vertical fiducial brainstem line. Continue using the draw tool to follow the contour of the brainstem to the other side, then draw another vertical line parallel to the fiducial line. This should exclude the MCPs on each side. Also notice that there is a hole (the cerebral aqueduct) in the middle of the brainstem. In your segmentations this will be labeled as 'fourth ventricle.' It is unlabeled in these sections for clarity.

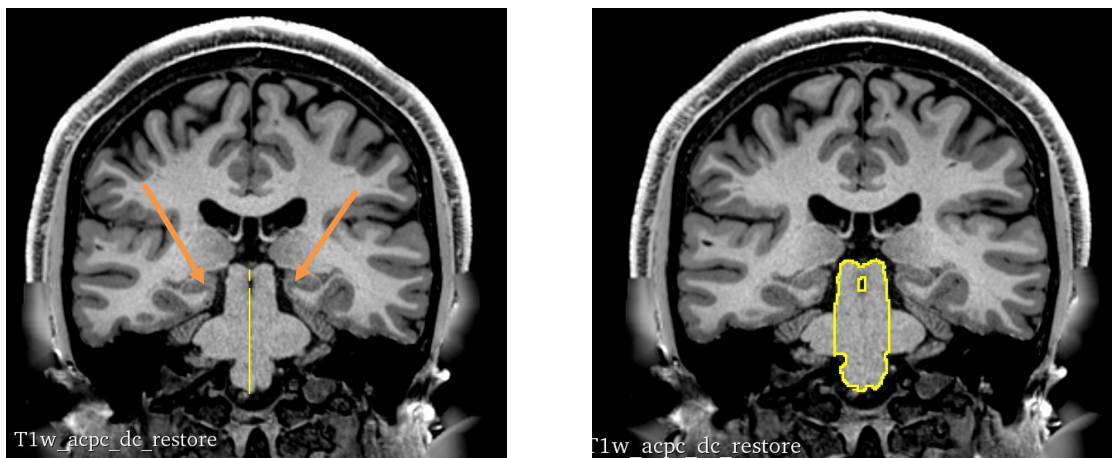


Figure 17: Middle Cerebellar Peduncles and the Brainstem

At point, you have segmented the rostral sections of the brainstem, and jumped forward to segment the brainstem at the level of the MCPs. Now you will close the gap. Move forward one section from the brainstem image you just segmented. Segment the brainstem just like you did previously. To the best of your ability, keep the vertical lines between the brainstem and the MCPs constant in location. As you proceed rostrally, you will notice the MCPs begin to shrink until they are quite small (Figure 18). You will also notice that the brainstem rejoins the rest of the brain again. It is here that you may have to angle the dorsal border away from the horizontal (Figure 18).

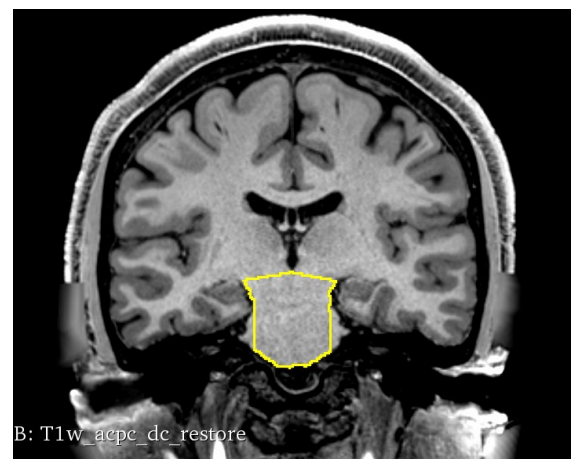


Figure 18: Small Middle Cerebellar Peduncles

Once you have reached the previous brainstem segments and closed the gap, you will have to finish the brainstem caudal to the MCPs. Go back to the first MCP section you segmented and proceed caudally. As you did before, use parallel lines through the MCPs to separate them from the brainstem. Again, try to be constant in placing the positions of the vertical lines that separate the MCP from the brainstem. On the top (dorsal aspect) of the slice, you will begin to notice that the fourth ventricle has increased in size (Figure 19). There are two little, joined



Figure 19: Caudal Brainstem with Inferior Colliculi

bumps (the inferior colliculi) at the top of the fourth ventricle. As you proceed caudally, these two bumps will become disconnected from the brainstem, but you should still outline them. At this stage, the vertical brainstem fiducial line is starting to get shorter at its inferior aspect, and ends within the substance of the brainstem (Figure 19). When it does disconnect from the surface of the brainstem, draw a horizontal line at the base to separate the brainstem. This will also happen with the top of the vertical line. This means that you are almost done with the brainstem.

The Thalamus

As indicated above, the thalamus is a large egg-shaped structure that is immediately caudal to the foramen of Monro. There are two thalami – they each end on the midline and are separated by the third ventricle. Due to the small size of the ventricle and partial voluming effects between gray matter and CSF, this separation is not always apparent. To that end, the line between the left and right thalami should be along the midline.

The thalamus is separated from the hypothalamus by a groove called the hypothalamic sulcus. Use the Four Up view to see the sulcus in a sagittal and a coronal view at the same time (e.g., green line, and green arrow; Figure 20).

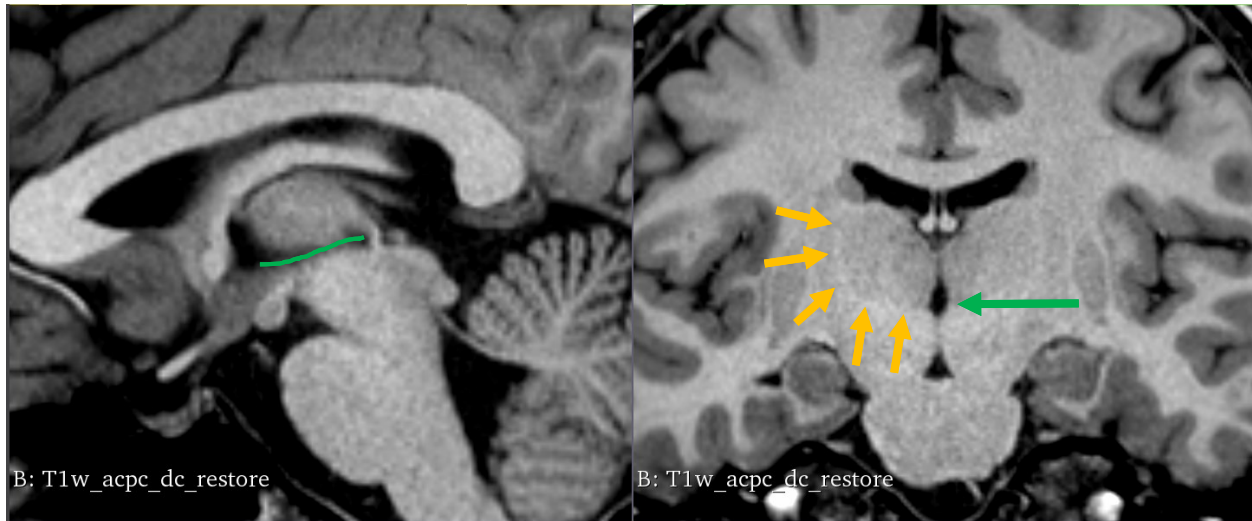


Figure 20: Thalamus

On the coronal view (Figure 20, right), notice the third ventricle. See the most lateral extension of the ventricle indicated by the tip of the green arrow. You will notice that if you follow this tip laterally, it coincides with a white-gray border that progresses dorsally (orange arrow tips). The interior of this line is the thalamus.

In the above view, the borders are clear. This is not always going to be the case. Depending on the brain, you may find you have to adjust the brightness/contrast settings to observe the borders. Also use the T2 image to help define the borders.

In general, the most difficult portion of the thalamus is the lateral border. This is because the lateral aspect contains many of the axons (white matter) that enter and leave the thalamus. This admixture of white and gray matter creates a poorly resolved boundary. A good approach is to identify a border that is very clear at the level of the mid thalamus (e.g., Figure 20, right), and then move forward and back to adjacent sections. This border will move very little from section to section. The inferior border of the thalamus will always be a horizontal line that begins at the hypothalamic sulcus and the medial border will be along the third ventricle.

Once you are comfortable with the mid-levels, turn your attention caudally. The caudal border of the thalamus is at the level of the midbrain, and appears disconnected (Figure 21, red arrow). Notice the white matter tract on the left (lateral to) the thalamus indicated by the red arrow. is the fornix. On this brain, the thalamus on the right side of the image (not labeled) is not continuous with the white matter. This side is a little more rostral. Here, you can also identify a reasonable lateral border, but beware! That linear gray matter structure (blue) is the caudate as it wraps around, so the border is medial to that line. Make sure that when you draw the border, you are not actually drawing the caudate.

As the you proceed rostrally from a section such as Section 21, notice that the hypothalamic sulcus appears. You now have your inferior border, and the lateral border should be somewhat sharp (Figure 22, orange arrow tips).

As you move rostrally, you'll see that the thalamus shrinks (Figure 23). The prominence of the hypothalamic sulcus may be a bit hard to appreciate, but in these cases, take the midpoint of the third ventricle as a guide. You should still be able to divine the border. Note in Figure 23 that the thalamus of the left hand side of the image is smaller than the right. As you move rostrally, the thalamus will continue to contract until it ends.

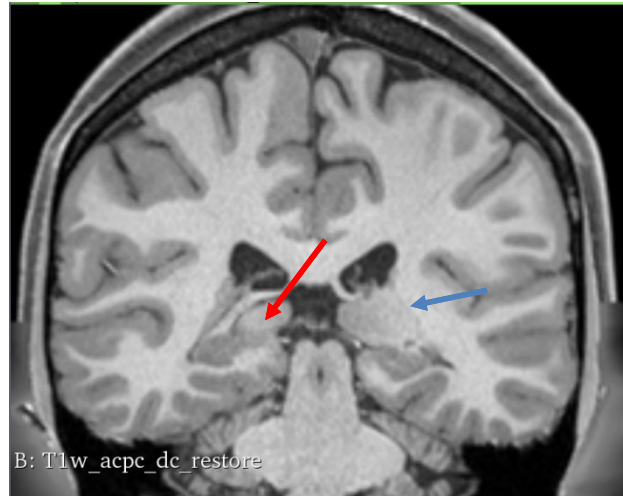


Figure 21: Caudal Thalamus

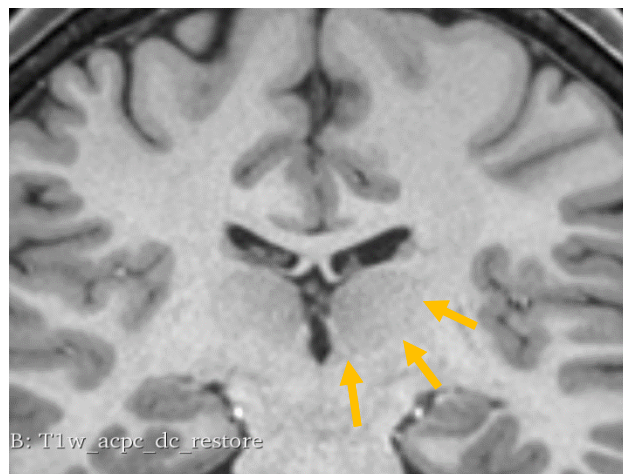


Figure 22: Caudal-ish thalamus

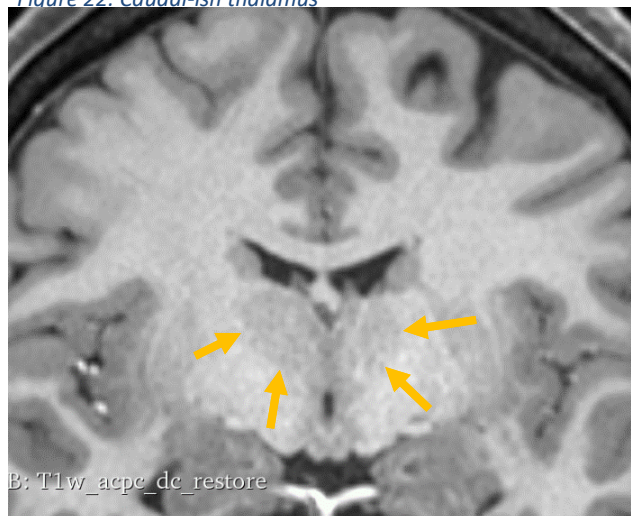


Figure 23: Rostral Thalamus

Again, some thalami are harder than others. Here are a few hints:

1. The T2 image set and the T1/T2 set may come in handy in this situation. Remain mindful that the T1, T2, and T1/T2 sets should be used interchangeably to determine the optimal border. Also remember that the T2 and T1/T2 images will have sections where the white matter may have a similar intensity to that of the gray matter. It is best to start with a mid-thalamic section in which the lateral border is very clear. Outline the thalamus, and then move rostrally or caudally, doing the same. Frequently refer back to the previously labeled gradient, as well as between image sets to ensure you have correctly identified and outlined the border. It is also useful to examine the axial view to further check your work.
2. Don't be afraid to ask for help. The first time through, take some time to struggle, but only to a healthy and reasonable extent. For all structures, and particularly for this one, the more you do, the better you will get.

The Ventral Diencephalic (VDC) Region

This region includes a number of structures in the ventral diencephalon, including the hypothalamus, subthalamus, some white matter structures, and some rostral midbrain structures. In many respects, this is a catch-all region. The superior border of this area is the hypothalamic sulcus, which separates the VDC from the thalamus, which means that for most of this segment, much of its superior border will be the same as the inferior border of the thalamus.

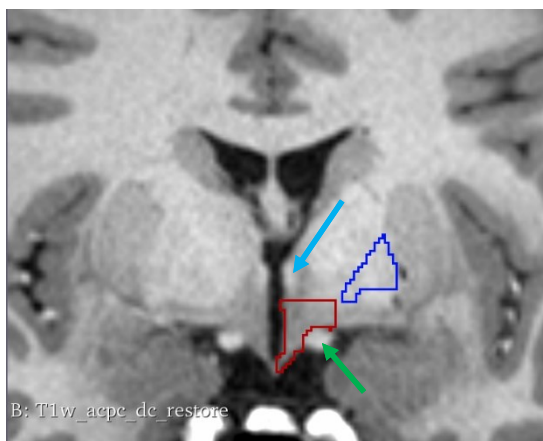


Figure 24: Rostral VDC

Start in the section immediately caudal to the anterior commissure. At this level, the thalamus has yet to appear and the hypothalamic sulcus is usually not yet present. The globus pallidus is quite small (depicted in blue outline in Figure 24). If there is no hypothalamic sulcus, start along the midline under the white matter of the fornix (blue arrow). Follow the third ventricle to the base of the brain, crossing the ventral aspect. Go laterally, and follow the brain. You'll notice a large white ovoid structure (green arrow). This is the optic tract. Do not include this, but instead draw a line at its dorsal aspect.

Once at the lateral aspect of the optic tract, draw a line dorsally (making sure you don't hit the globus pallidus), and return to the origin. Your final outline should be similar to that seen in red in Figure 24.

As you progress caudally, the thalamus will appear (green outline, Figure 25) and you can use the inferior border of the thalamus as the superior border for the VDC. Make sure that overwrite is turned off in the masking section so you don't overwrite the thalamus as you segment. In thalamic sections, the lateral border of the VDC should be approximately the same as the thalamus. With that in mind, make sure that the lateral VDC border does not touch the globus pallidus.

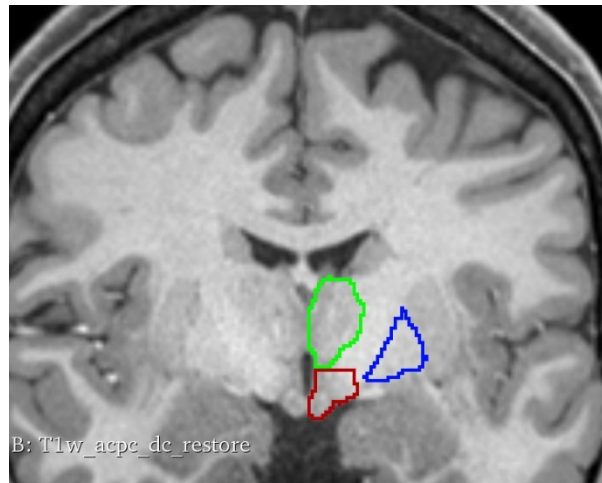


Figure 26: VDC, more caudally

As you progress even more caudally, the VDC will shrink considerably and will come to be interposed between the thalamus (green) and the brainstem (yellow; see Figure 26). Keep an eye out for a structure called the lateral geniculate nucleus of the thalamus (LGN), which appears superior and medial to the hippocampus (gray matter indicated by red arrow in Figure 27). Since the VDC includes the LGN, include it in your segmentation. Depending on the case, the LGN may in fact be discontinuous from the VDC and appear as an island that is disconnected from the rest of the VDC.

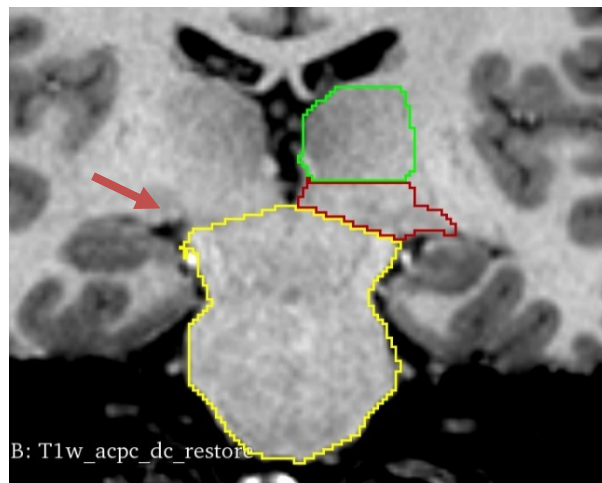


Figure 27: VDC, even more caudally

The Hippocampus

The hippocampus is a gorgeous structure located in the medial aspect of the temporal lobe. It is bordered by the white matter of the temporal lobe and by the inferior horn of the lateral ventricle. It is somewhat difficult to conceptualize on coronal sections, so parasagittal sections are optimal for viewing and segmenting. A great way to think of the hippocampus is that it intrudes on the inferior horn of the lateral ventricle. As you get better at segmentation, you will likely start segmenting the inferior horn along with the rest of the lateral ventricle. For the purposes of this guide, however, it seemed most appropriate to discuss the inferior horn as we talk about the hippocampus.

Interlude: The inferior horn of the lateral ventricle

Return to the lateral ventricle segment, and recall the intensity limits you previously employed. Next, go to the sagittal view and proceed from the lateral edge of the brain towards the center. As you do, you will see a black space appear that is in the center of the temporal lobe of the cerebral cortex (upper part of Figure 28). You will typically see a nicely defined space at the lower and upper portions (tip of the orange arrows, Figure 28). Between them is the hippocampus (tip of the red arrow). As you proceed medially (Figure 28, middle), the hippocampus will butt up against the white matter. The lower space will stay the same, but the upper space will enlarge as it becomes the atrium (we have seen this before). The atrium of the ventricle will continue to enlarge, and the lower tip will shrink. This hippocampus changes shape a little, but maintains its position.

There is one other thing to think about. Consider the hippocampus: there are layers of white matter within, and a thin but definite covering of white matter that separates the hippocampus from the ventricular space. In front of and dorsal to the hippocampus is an additional structure, the amygdala (green arrow). This structure does not have a white matter encapsulation as does the hippocampus, but instead appears as a blob. Importantly, there is part of the ventricle that appears between them.

Proceed to segment the inferior horn as a separate structure than the lateral ventricles in these sections, but don't go more medially.

There are three main issues with the inferior horn we need to think about before going any further, as follows:

1. In many brains, the inferior horn is quite small and due to partial voluming effects, it is unresolvable, even at the resolution we use. As a result, the inferior horn 3D render may contain patches rather than a cohesive whole. Start with the threshold you used for the lateral ventricles, but keep in mind you may need to reduce it to produce a cohesive structure.

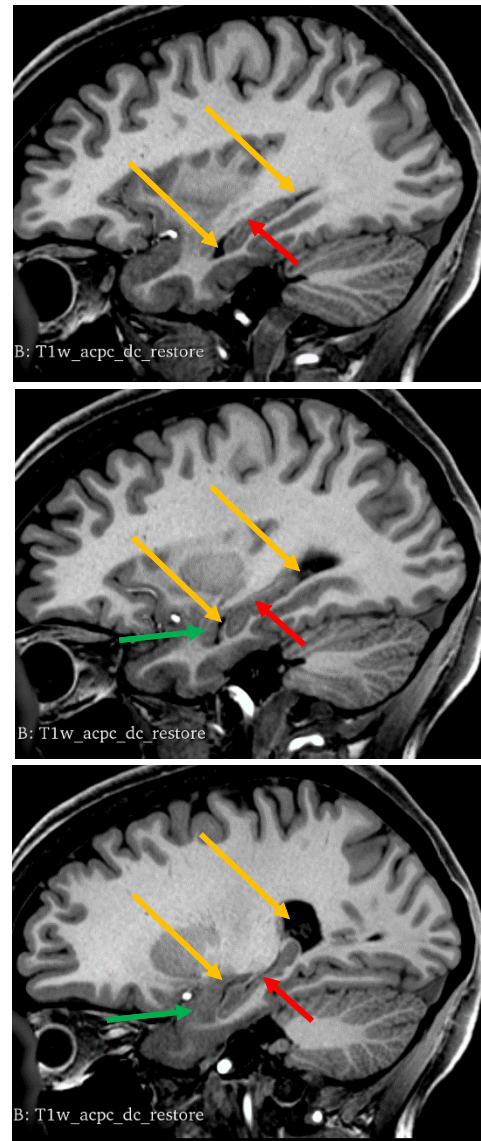


Figure 28: Inferior horn of the lateral ventricle

2. The hippocampus tends to have visible holes in it (e.g., blood vessels) that may not explicitly communicate with the ventricle. Some of them do, some are blind, and some communicate with the subarachnoid space outside of the brain. When you segment, make sure you follow any questionable spaces to convince yourself it is ventricle.
3. The subarachnoid space that surrounds the brain is not directly contiguous with the inferior horn of the lateral ventricle, although in some brain, it appears to be. For example, observe the inferior horn of the lateral ventricle, on the right side of the image in Figure 29. You may believe that the tips of the green arrow and the red arrow are both in the inferior horn. You would not be correct. The green arrow is, but the red arrow is not. There is a membrane that extends from the fornix that separates the ventricular space (green arrow tip) from the subarachnoid space (red arrow tip). This membrane is too small to see, so use the fornix as a proxy. In many cases, the spaces are adequately separated.



Figure 29: Coronal View of inferior horn of the lateral Ventricle

Now that you have a sense of the potential issues, go to the coronal sections and start where you see the tip. Oftentimes, you will see a scalloped outline or multiple cross-sectional space profiles, as seen in Figure 30 (orange arrows; both are ventricle). Segment and follow these spaces backward, keeping continuity in mind, until you reach the atrium, where the outline you are working on will merge with the previously segmented outline.



Figure 30: Tip of Inferior horn of lateral ventricle

Back to the hippocampus...

You will need to employ the sagittal series and the coronal series. Start with the sagittal series, as in figure 28. Segment the gray matter, but not the white matter that serves as the boundary to the structure. Here is a good place to select an intensity range that will exclude the CSF (ventricular space) and the white matter. Once you feel comfortable in the sagittal view, move to the coronal view. Review Figure 20, and notice that at the tip of the ventricle, the hippocampus is beneath the spaces of the lateral ventricle. Make sure your segmentation outlines do not go above the ventricle (that would be the province of the amygdala). Confirm

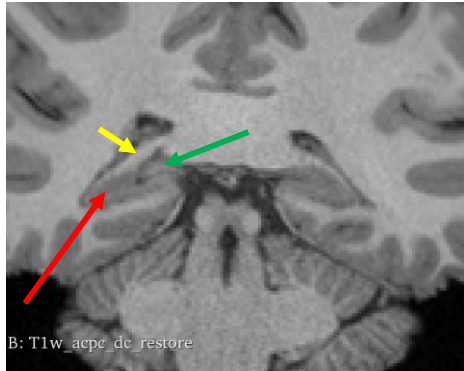


Figure 31: The caudal hippocampus

your outline on the coronal series as you move caudally. The end of the hippocampus (Figure 31; red arrow) is immediately below the caudal end of the thalamus (Figure 31, green arrow). It is here you will also see a major efferent fiber tract of the hippocampus emerging (the fornix; yellow))

The amygdala

The amygdala is named for its shape (almond). It is a collection of nuclei located at the tip of the inferior horn. The nuclei are not individually discernable, so the entire amygdala is blob-like in appearance. Start at the tip of the inferior horn of the lateral ventricle, in a section similar to that illustrated on the right hand side of Figure 32. The structure underneath and medial to the black spaces is the hippocampus. The structure above the ventricle is a small initial sliver of the amygdala (red outline; Figure 32). Notice that the right side of the image does not contain the amygdala. Once you move rostrally, the ventricle and hippocampus disappear, and all you have in this position is the amygdala.

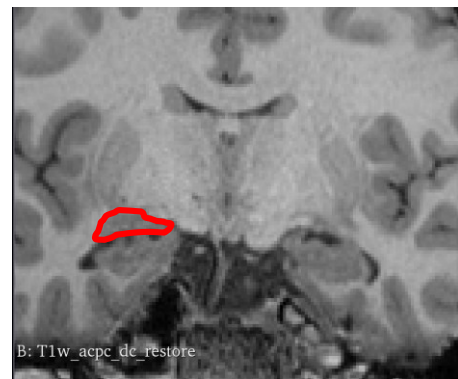


Figure 32: The Rostral Hippocampus and Amygdala

However, there is a region where there is limited or no ventricular space, but the hippocampus and amygdala are both present. Figure 33 is an example of such a section. On the left hand side, the lateral ventricle is more or confined to a little triangle on the lateral aspect of the medial temporal lobe. There are two structures indicated: the tip of the red arrow is within the amygdala and the tip of the green arrow is within the directly subjacent hippocampus. The hippocampus is identifiable because it is encircled by white matter, which makes its separation from the amygdala possible.

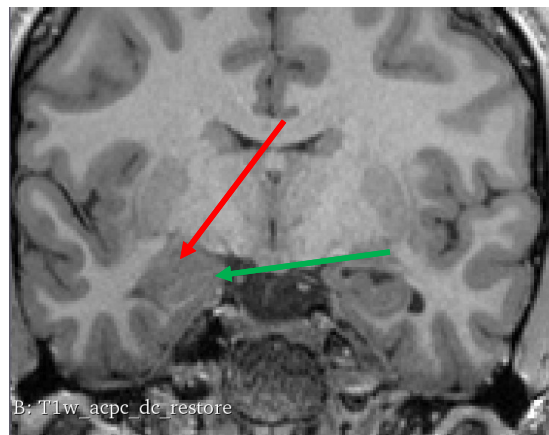


Figure 33: The rostral end of the hippocampus

Move forward, and you should see the hippocampus disappear, leaving only the amygdala. At this point, observe that the amygdala appears to be continuous with the cerebral cortex. Draw the border with the cortex when the cerebral cortex bends medially and dorsally (orange arrow tip, Figure 34). Follow this forward a few slices. Be sure to play with the contrast to visualize the white matter medial to the amygdala. The anterior border of the amygdala is difficult. It is here where amygdala becomes buried by a layer of cortex wrapping around the temporal lobe (red dotted line). Deep to that layer is a bit of grey matter that will increase in size from rostral to caudal. In Figure 35, the cortex around the temporal lobe is labeled with a red dotted line, and the rostral beginning of the amygdala is noted at the tip of the arrow. In caudal sections, this will grow and obliterate the overlying cortex to become the size of the structure you have outlined caudally.



Figure 34: Mid amygdala

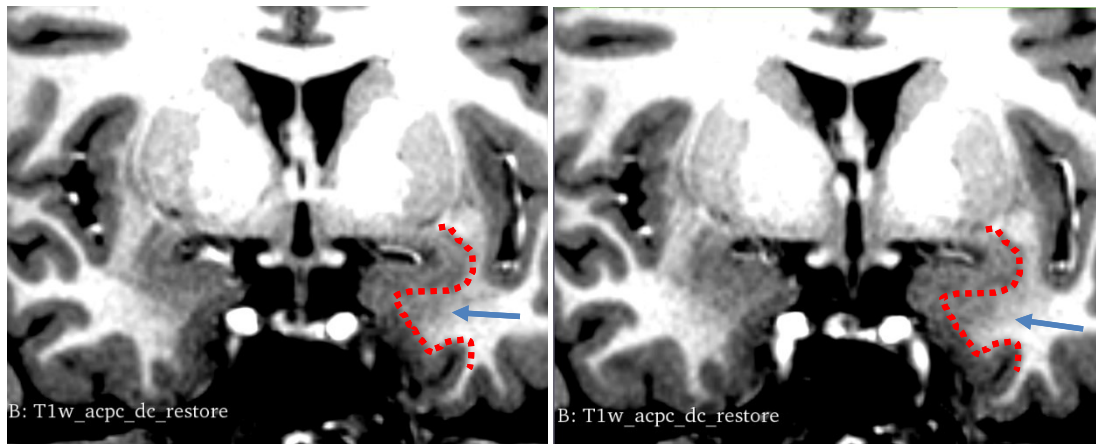


Figure 35: Anterior Amygdala