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CMA Methodology Overview

The CMA methods of analysis comprise general segmentation, cortical parcellation, subcortical parcellation, and white matter parcellation. These methods of analysis subserve *volumetrics* and *human brain mapping*.

Volumetrics
Volumetrics is a science dealing with brain structure measurements as well as algebraic relations that relate these volumes (Caviness, 1999). Because of their comprehensive and quantitative nature, our methods of analysis provide a set of volumes that can be used for statistical analysis of covariance and modeling (Cereb. Cortex, Kennedy, 1998), thus enabling characterization of normative data as well as comparisons with disease data sets.

Human brain mapping
The principle aim of our system provides a basis for brain function and metabolic activity mapping by determining a finite and specific set of quantifiable regions of interest or parcellation units.

The methods of general segmentation, cortical and subcortical parcellation, and white matter parcellation are designed in the context of a *neural systems approach* (namely, the motor system, perceptual (somatosensory, visual, auditory, gustatory, olfactory) systems, nociceptive (pain) system, cognitive (attention, executive, memory, visual/spatial, language) systems, and the affective (limbic) system). These methods may help elucidate basic questions in neuroscience, such as the relationships between cytoarchitectonic fields, cerebral connections, and neural functions.

A Guided Tour of CardViews

CardViews, short for cardinal views, is a program that creates visual images of the brain in the coronal, axial, and sagittal planes and displays them all on the same screen. This makes it easy to cross-reference a point you are not sure about. CardViews is used for segmentation and parcellation of the brain.

Type the following line at the prompt of any workstation in the "Cave":

cardviews 1110 2

The computer will "think" for a moment and then begin to load brain images corresponding to the second scan of subject 1110 in the lab's image database. The program that you are loading is called CardViews. The images that you see are actual magnetic resonance images from a real person. Aligned on the right side of the screen are the CARDinal VIEWS used in general anatomical study: coronal, as if the person is facing you; sagittal, as if you are staring right into their right ear; and axial, looking from the spine toward the top of the head (axial seems like a top-down view, but it's really bottom-up).

The left side of the images in any cardinal view is the right side of the brain.

The way the images were obtained allows CardViews to illustrate any area of the image in the three cardinal planes. Through the use of projection lines, any area can be cross-referenced to help determine sulcal boundaries, extent of gray matter areas, vasculature, nerves, etc. To see a quick illustration of this, single-click with the left mouse button on the rectangles called "auto trans" and "Projection." This will bring up crosshair lines. Now move the pointer to the large central image and double-click the left mouse button on any area of the image. You will see the other views change to show the intersection of the crosshairs in the other two planes. Experiment by double clicking around the central image and watch the other views transform their images. The horizontal line in the central image shows the axial plane. The vertical line shows the sagittal plane.

There are slice numbers in the corner of each view. If you look above the central image you will see the same numbers next to the abbreviations "COR", "SAG", and "AXI." As you double-click around the central coronal image, you'll see the sagittal and axial numbers change to reflect the position of the projection lines.

In general, you will work with brains that have 64 or 128 coronal slices with 256 slices in the sagittal and coronal planes. The slice numbers referenced by the projection lines are listed next to COR, SAG, and AXI. The arrow buttons next to the numbers are another way to change slices. A single-click on any of the smaller images will bring it to the central window.

Single-click with the left mouse button on the word "Quit" at the top left of the CardViews window. This is how you quit the program. Start it up again like you did at the beginning (type "cardviews 1110 2"). Notice that CardViews starts with the middle slice of the coronal plane (in this case slice 32 of a 64-slice brain) and that the slice 128 in both of the other two planes.

You can adjust the brightness and contrast of the screen to enable easier viewing. You will adjust the screen to many different levels of brightness and contrast depending on which structures you are

segmenting. To change the brightness and contrast, click in the central image box with the middle mouse button. Now move the mouse around. You should see the brightness of the image changing. Click the middle mouse button again while in the image box. This will set the image brightness and contrast. Play around with this feature for a bit. Notice that if you click in the lower right hand corner of the image box, and slide the mouse upwards, you will increase the brightness of the screen. If you then slide the mouse to your left, you will decrease the contrast of the screen. Watch how the outside of the brain seems to be larger or smaller depending on the brightness of the screen. Also note how the gray and white matter appear to "bleed" together as the contrast is decreased. After you're done, set the brightness/contrast to a level where you can see the edge of the brain without the white and gray bleeding together. Your cursor will be approximately two-thirds up in the image box, underneath the word AXI.

The exact position of your cursor will vary depending on which computer you are sitting at, so if your cursor isn't here, that's okay!

The Three Cardinal Views

CardViews will display a sulcal line in all three planes. Make sure the "auto trans" button is on by single clicking on it (auto trans allows you to scroll through the three views with the up and down arrows next to the COR, SAG, and AXI slice numbers). If it is active, there will be a thick white line around the box. Move the mouse to the central image and single-click the right mouse button. You will see the words "NAV draw_mode" and something in green just above the central image. Draw a giant "X" across the coronal image: single-click the left mouse button at the top-left of the image and again at the bottom-right; single-click the right mouse button to exit from draw mode; initiate draw mode again by single-clicking the right mouse button; similar to before, left-click once at the top-right corner of the image and once at the bottom-left; then right-click to exit draw mode. If all went well, you should now have a green-colored " X" across the coronal slice.

Click on the sagittal image on the right side of the screen. This will bring the sagittal image to the central image screen. Now click in the up arrow next to the word SAG. You will notice some green dots move. As you continue to press the up arrow, you should notice the dots moving further apart. This is because you are moving laterally to the edge of the brain, and the dots are getting further apart as you approach the side of your "X." Click in the down arrow key to move medially. Eventually you will pass the center of the brain, and move laterally towards the right side of the brain. The dots will start to move further apart as you approach the other end of the "X."

Next click on the axial view to move this view to the center of your screen. Hit the up arrow next to the number by the word "AXI." You will again see the green dots come closer and further apart. These correspond to your "X." This demonstration was to help you understand how the different views are connected to one another, and how sulci lines appear in different views.

When you're done playing, quit CardViews. Then restart the program as you did before. When the NAV screen appears, turn on "auto trans." You are ready for the next section of the tour.

CardViews Four Modes

There are four modes in CardViews: NAVigation, SEGmentation, REView, and Tile Display. The buttons to change modes are just below "Quit." NAV mode is used to draw sulcal lines (for parcellation) and boundary lines that help to determine where one structure ends and another begins. SEG mode is used for segmenting structures and editing outlines. REV mode is used to

label the outlines and check for errors. Tile Display presents you with a larger series of brain slices which allows you to easily follow a structure through multiple slices. It can also be used to draw sulci lines, check for labeling errors, and compare the outlines from different segmentors.

NAV mode

So far you've been playing around in NAV mode. NAV mode is used for parcellation and to assist in segmentation. You will draw and save lines called sulci lines in this mode. Notice the word OVERLAY at the bottom of the screen, under the central image. Next to it is written the path where your sulci files are stored. You don't need to know what this means (that's what computer techs are for) but you should see 1110_2 written somewhere in this line. That indicates that you are working on brain 1110 scan 2. Below this line is the word "Prefix." You will save your sulci lines with your own personal prefix. To do this, first click on the line next to the word "Prefix." Enter your 3 initials, followed by the letter s. For example, if your name is John Frank Brown, you would enter jfbs. Then hit return. When you hit return, you should see the Sulci File line change. Your prefix now appears next to 1110_2. You are now ready to draw sulci lines.

To draw a sulci line, you will follow the same procedure you did previously to draw the "X." First enter draw mode by clicking the third mouse button. Then click where you want the line segment to begin with the first mouse button. Click at another point to draw a segment. Click somewhere else. You should have a line with 2 segments. Continue to play with the drawing feature. After you've finished drawing your line, make sure you hit the third button to exit draw mode and return to base mode. You can re-enter draw mode to reinitiate drawing in a different area.

To get rid of a line you don't like, click on it while you are in base mode. This will turn the line black. Continue to hold your cursor down on the now black line, and drag your cursor outside of the center image box. This will erase that sulci line.

To save your sulci lines, hit the "SAVE sulci" button at the bottom of the screen. Make sure you are in base mode when you hit the "SAVE sulci" button, or your sulci lines will not save. If you want to save more lines, hit "SAVE sulci" again after drawing them. A window will pop up asking if you want to overwrite your existing file. Click on the "Overwrite" button to save your new sulci.

SEG mode

SEG mode is used to segment. To enter SEG mode, click on the "SEG" button under "Quit." If you look at the top box under the three boxes for NAV, SEG, and REV mode, you will see the word SEG on the second line. Next to it is the word base, indicating that you are in base mode. There are many different drawing methods available for use in SEG mode. The method you are using will always appear next to the word SEG.

Underneath the center image block you should see a line that reads OVERLAY. This is similar to the SULCI FILE in NAV mode in that it tells you what brain you are working on. Below that is the prefix line. In SEG mode, your prefix is just your initials (no "s."). For example, if your name is John Frank Brown, your prefix is jfb. Click on the line next to the word "prefix." Enter your initials, and hit return. You should see your initials become part of the line next to OVERLAY.

There are a few differences between NAV mode and SEG mode. One of the most apparent differences is the way projection lines work. Up to this point you've been playing with the projection lines in NAV mode. In SEG mode, they are not as automatic. Click on the projection box to bring up the crosshairs. In NAV mode, you could double click anywhere on the image in the center box to

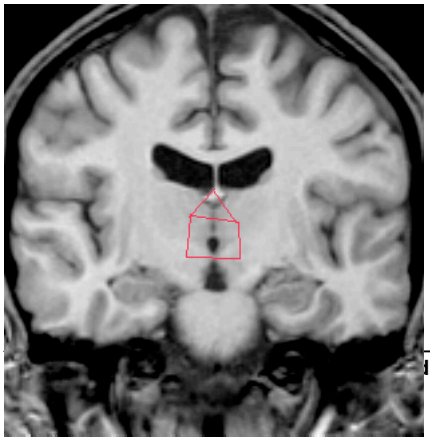
reveal the same place in the sag and axial views. This doesn't work in SEG mode. To move the crosshairs to a specific point, use scroll bars next to the small coronal image in the right side of the screen. Using the knobs on these scroll bars, position the crosshairs to the area you want to investigate. Then hit the "Transform" button next to the SAG and AXI words. This will move the crosshairs to that position in these 2 views. Click the "Projection" button to turn off the projection lines.

The point of SEG mode is to create outlines (also referred to as "otls") that can be used in volumetric analysis. For example, you will create an outline of the amygdala on every slice that has amygdala. This enables statisticians to estimate the amygdala volume for the brain. There are four drawing methods we use to help in creating outlines: the intensity contour, the histogram, drawing, and the optional auto-seg. These are all explained in greater detail in the "General Methods and Tools of Segmentation" section. Anytime you enter into one of these drawing methods, the word "ok" in the upper left box will change, to indicate which method you are in. When you exit that method, that word will return to "base."

The four methods of drawing enable you to trace brain structures. When you are done tracing a particular structure, you extract it. Extracted outlines can be saved, labeled, and are what we use in analysis.

Now we'll try and create a simple outline. We'll do this by drawing. Drawing in seg mode works slightly differently than it does in NAV mode. Place your cursor in the center image box. Click the right mouse button. You'll notice you've switched from "base" to "draw_mode." Hold down the first mouse button and drag it across the screen. You've just drawn a line. Now click the right mouse button again to exit draw mode. Now try to draw a circle. Click the right mouse button again to enter draw mode. Hold down the left mouse button and draw a closed circle; this can be a sloppy circle, just make sure you create some sort of closed shape. Click the right mouse button again to exit draw mode. Now place your cursor inside the circle. Press "e" to extract the outline. You'll notice part of your red circle is now green. Hit the "SAVE" button. Now hit the clear button. How hit the load button. If all went well, your green outline was saved, and loaded, and the red contour disappeared when you hit the clear button; this is because only extracted outlines can be saved.

Notice that there are eight colored boxes in the upper left white box. We'll focus on the first five. These boxes are your contour boxes. There is a small black box in the red box. This means that any contours you create or erase will be red. Using the method described above, draw a red line (make sure to exit draw mode when you are done). Now click on the yellow box. The little black box has moved from the red box to the yellow box. Draw a line. It should be yellow. Now hit "x." The "x" function is used to get rid of all contours of a given color. Your yellow line should be gone, but the red one still remains. Click on the red box. Now hit "x." The red contour should be gone. Play around with the 5 different colored contours. Draw different shapes in different colors, and extract them.



You'll notice that no matter what color you draw a shape in, it will always turn green when you extract it. You can even create shapes out of two different colored contours. Being able to create outlines using many different colored contours makes segmenting easier and faster.

The concept of extracting is a bit tricky to understand. Here is an exercise to try to make it clearer. First hit the clear button to clear the screen. Next draw a house: First draw a square (make sure that there

are no gaps between the four sides). Now draw a triangle roof on top of the square; making sure the ends of the roof touch the top of the box, and that the two slanted sides of the roof intersect.

Place your cursor inside the triangle (roof) and press "e" to extract it. Only the triangle should turn green. Now hit "w," this unextracts the last thing you extracted (in this case the triangle). Place your cursor inside the box and extract it. Only the box is green. Hit "w" to unextract it. Now place your cursor underneath the box. Hit x. You'll notice that your "house" is green: the shape comprised of 3 sides of the box and 2 sides of the triangle was extracted.

The way the extract command works is as follows: The program detects the first contour you drew that is immediately ABOVE your mouse cursor. Then it follows that contour all the way around until the contour ends. So if you are INSIDE an enclosed shape like your box, the program detects the upper part of the box, and then follows the contour all the way around along the inside of the box. When you extract the house from the OUTSIDE, the cursor hits the bottom of the box, and then follows around the outside of the house. In order to create outlines that can be used in analysis, all structures must be extracted from the inside. We often extract things from the outside as a useful tool during segmentation (this will be described in the methods section). However, remember that structures must be extracted from the inside in order to be used in morphometric analysis.

While you are segmenting, the easiest way to move around is to use the "-" and "+" buttons underneath your prefix. This will automatically change your saved outlines as you change slices.

After you have extracted structures on a slice, you must click the "Save" button before moving to the next slice using the "+" and "-" buttons. Otherwise, you will lose your outlines.

REV mode
Click on the REV button. You'll notice the "review panel" pop up in the left corner of the screen. Review mode is used to check and label the brain. There isn't much to play with until you actually have some saved "otls."

Tile Display mode
Click on the tile display button. A large screen will appear. Click on the "GO" button that is about one quarter down from the top of the screen. You should see a whole bunch of brain images. Tile display enables you to see many slices at one time and is used to check the brain, examine tricky areas, and draw sulci lines. Notice the numbers and scroll bars at the top of the screen. These indicate which slices you are on, and allow you to move to different slices. Just a warning... these scroll bars are tricky to use. Click on the TOP scroll bar and drag it all the way to your left. As you did that, the bottom scroll bar also moved left. You should see the number 3 on the top line, and 32 on the bottom line. Click the "GO" button again. You are now looking at slices 3-32. Click on the BOTTOM scroll bar and drag it to your right. When you do this, make sure you do not drag the mouse cursor outside of that left panel (that is, not past the white line that separates the buttons from the brain images). The program will not cooperate with you if you drag your cursor too far. The top line should read 30, and the bottom should read 59. Click GO again. You are looking at slices 30-59.

Click on the box next to the word "zoom" that is located to the left of the GO button. You'll notice the slice numbers next to the scroll bar have changed. Now click on GO. You are looking at six zoomed images. Play around with the scroll bars to move to different slices. Always click GO to transform the

images. If you want to look at the smaller images again, just click the box next to zoom to turn off this feature. And then click GO.

You can look at multiple sagittal or axial images by clicking the sag or axi box underneath the scroll bars. Then click on GO.

As with review mode, there isn't a lot more you can do in tile display without segmenting first.

To return to the main page of CardViews, click on the CARDVWS button.

Pre-processing

Pre-processing is necessary before a brain can undergo segmentation, cortical parcellation, white matter parcellation, or any other form of volumetric analysis. First, the brain must be positionally normalized along the anterior commissure (AC)/posterior commissure (PC) line. Second, the brain should be cropped to rid the image of as much non-brain tissues as possible. Third, if applicable, the brain should undergo bias field correction so that AutoSeg can be used in the segmentation process.

Positional Normalization

Positional normalization places brain images in a single, standard, uniform position that reduces spatial variability. The orientation of the brain position of MRI images varies considerably; mainly due to differences in subject head position during scanning. Volumes of brain structures can not be reliably isolated and compared on unaligned brains because the position of the brain affects image intensity, and in turn, any extracted outlines. To account for this spatial differentiation, all brains are positioned on a three dimensional plane referenced to a plane that bisects the decussations of the anterior (AC) and posterior (PC) commissures, and the interhemispheric fissure at the level of the PC in the coronal plane.

Procedure

-Load CardViews with the brain PID and SCN# you will be normalizing (e.g. 1680_1).

-Look through all the slices of the brain to make sure there are no slices missing, and that there is nothing else obviously wrong.

-After you have looked at the whole brain, close CardViews.

-At your home prompt, type "norm" or “norm140” for 140-slice scans.

-Type in the PID, SCN# in the provided spaces.

-Now click on "128" (in a 128-slice brain; leave unclicked if normalizing a 64-slice brain) or “158” in a 140-slice brain.

-Click on "load 3D".

-Click "auto incremented transfer".

-Now you will need to locate the most anterior slice where AC extends across both hemispheres in the coronal view.

-When satisfied with AC slice, click on the "AC" button and left click on the position of the AC on the coronal slice. A green cross will appear and can be adjusted by clicking on the directional arrows on the side of the image screen. Adjust the arrows until satisfied; then click on "AC" again to accept the position.

-Find the PC: locate the slice where the PC is most anterior in the sag view.

-Do not accept the PC as the bridge between the superior colliculi. However, if you locate this point and then proceed anteriorly, you will locate the PC.

-Click on "PC", double-check the cross, click on "PC" again.

-Click on "MSP" while you are still on the same slice where you set PC. Place the cursor as high as possible in the brain between the two hemispheres to assure accuracy.

-Click on "MSP" again with the cursor in the middle of the brain.

-Click on "Check Views".

-Click "Displayed", this is when you will be checking the brain to make sure it is optimal. When satisfied, click "reslice". This will create a new scan.

-Click "normalized coronal scan".

-Click "create new scan".

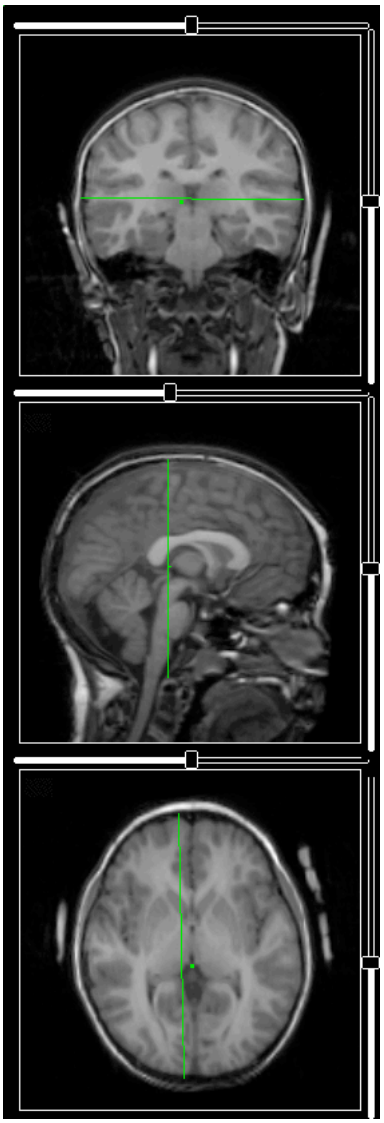
-Click "quit".

Image Cropping

Image cropping creates a smaller scan that focuses on the brain rather than the whole head. For the segmentor, this concentrates the view to just brain, thereby maximizing the number of slices that can be seen with the Tile Display mode in CardViews. Cropping reduces the size of the scan by cutting out slices that don't contain brain and non-brain, and peripheral areas of a slice, such as neck muscle and scalp fat. Specifically, a typical coronal scan will have 128 slices that are 256 pixels wide by 256 pixels high. A cropped scan might only be 117 slices that are 158x165 pixels, meaning several slices were dropped because they were too anterior or posterior to contain brain. Each slice deleted 98 pixels in width and 91 pixels in height. Practically, it reduces computation time because image manipulations are made on the smaller image set instead of the full one. This is why a scan is cropped before using a bias field correction program (e.g. AutoSeg).

To crop a brain, you need to define the maximum length, width, and height of the brain. The cropped brain excludes everything outside of these limits. The golden rule is do not crop-out any brain tissue.

Procedure



-Open the scan in CardViews: "cardviews PID SCN"

-In NAV mode, click "Crop Data" in the lower left of the screen. This brings up a smaller menu called Crop Data.

-Draw three sulci lines across the brain that start and end just slightly beyond the brain exterior. Draw one line in each of the following planes: anterior-posterior in the axial plane ("length"); left-right in the coronal or axial plane ("width"); and inferior-superior in the sagittal plane ("height").

Be sure to use slices that show the maximum extent of the brain in each of the planes. Select an axial slice that shows both the maximum length and width of the brain. Draw both lines on that slice. Lines drawn in the mid-sagittal plane must account for the inferior extent of the brainstem and cerebellar hemispheres as well as the superior extent of the cerebral hemispheres. Lines can be redrawn by removing one or all of them: remove a particular line by clicking on it and dragging it out of the main window; delete all the sulci by choosing the "delete sulci" option in the Crop Data menu.

-After the lines are drawn, click "Crop Data" in the Crop Data menu.

-Scroll through the scan to make sure no brain edges/lobes are clipped. If necessary, click "Uncrop" to redo the lines. Again, delete individual lines by clicking on a line and dragging it out of the window or by clicking "Delete Sulci" to start over.

-Click "Save Settings" to accept the cropping. Then click "Done/Cancel".

Cropping does not make a new scan. CardViews should use the cropped version the next time the scan is loaded. Again, make sure that

the cropping doesn't exclude a brain area. A previously cropped scan can be re-cropped at a later time by choosing "Uncrop" from the Crop Data menu and redefining the limits of the brain.

Bias Field Correction

Bias field correction removes intensity inhomogeneities ("intensity drift") so that brain tissue in one part of an image has the same intensity value as tissue of the same density in another part of the image (or slice). The correction also provides guesses for some intensity transitions (brain exterior, white matter-CSF, gray matter-CSF, gray matter-white matter) that help make general segmentation easier, or at least more consistent from slice to slice (hence the name "AutoSeg"). Unlike cropping, AutoSeg creates a new scan that takes the next available scan number. There are two versions of the AutoSeg program: autoseg2 and autoseg22. Check with your supervisor on which version you should use.

Procedure
-Run the command: "autoseg2 PID SCN"

-Inspect the new scan: "cardviews PID new_SCN." The new scan will generally be the next consecutive scan number. For example, if you run AutoSeg on PID 1345 and SCN 5, the new scan will be SCN 6.

-"shift-a" will bring up the intensity guesses in SEG mode. See the sections on each individual structure to learn how to use AutoSeg to segment. Instructions for the cerebral/cerebellar exteriors, lateral ventricles, and cerebral/cerebellar white matter are included.

AutoSeg maintains a log of all the scans that are bias corrected. This is important if you must delete the scan and CMA database record of a bias corrected scan. Even after they are deleted, running autoseg2 without the '-f' option will create an empty record in the database for the scan that was deleted. The new scan will be the next available scan number. For example: 'autoseg2 1345 5' creates the images and database entry for 1345_6. If the images and database entry are deleted and AutoSeg re-run for 1345_5, the new scan will be 1345_7 and there will be database entries for 1345_6 AND 1345_7. Even aborting the AutoSeg program when it gives the warning message that you are about to overwrite an existing series will create the empty database entry.

If you want to overwrite a bias corrected series, use the '-f' option: "autoseg2 -f PID SCN"

General Brain Segmentation

Segmenting for the first time
The order in which the structures are presented in this manual is the recommended order to segment the brain for the user who has already segmented his/her first brain. This is not, however, the easiest way to teach a new student to segment. If you are segmenting for the first time please follow the following outline:

1) Read the manual as-is until “Detailed Segmentation Instructions.”

2) Read “Cerebral Exteriors,” “Brainstem,” and “Cerebellar Exterior” first in that order.

3) Return to the beginning of the section with “Third Ventricle and Transverse Cerebral Fissure” and read through the rest of the section, skipping the sections already read.

This outline allows the first-time segmentor to master basic segmenting skills while at the same time providing a basis for understanding the brain in MRI images in total.

Order of segmentation
After segmenting your first brain, which teaches you the method, begin segmenting in the order of the “Detailed Segmentation Instructions” section. Keep in mind the following rules:

1) The 3rd ventricle, TCF, and 4th ventricle, must be segmented before the cerebral/cerebellum exteriors and brainstem (where applicable) because they are all midline structures (not split into left and right, extracted as one entity) and will affect the midline exterior lines and extents of the cerebral/cerebellum exteriors. For the same reason the 4th ventricle must be segmented before the brainstem because it serves as a border for the brainstem in certain areas.

2) The exteriors must be segmented before sub-cortical structures can be segmented.

3) The lateral ventricles should be segmented before the basal ganglia because part of the caudate border is determined by the lateral ventricle.

4) The hippocampus and amygdala should be the last subcortical structures segmented on any given slice because many of their borders are determined by the inferior lateral ventricles, and VDC.

5) White matter must be the last thing segmented on any given slice because many of the white matter borders are determined by the sub-cortical structures.

Examples of segmentation techniques
Two popular segmentation techniques are currently used at the CMA. In both techniques, the 3rd ventricle, TCF, 4th ventricle, cerebral exteriors, brainstem, and cerebellar exteriors are segmented on all slices. Then:

1) The user goes through each slice, first segmenting all subcortical structures (lateral ventricles, basal ganglia, thalamus/VDC, and finally inferior lateral ventricle/hippocampus/amygdala), then segmenting the white matter.

2) The user segments the lateral ventricles and basal ganglia on all slices, then segments the thalamus and VDC, and then the inferior lateral ventricle, amygdala, and hippocampus on all slices, and finally segments the white matter on all slices.

Clean outlines
Because of the way CardViews works, structures that are extracted often have stray "dots" that make for messy outlines. This can cause many problems, most notably creating tiny pockets of cerebral cortex in the middle of the brain. For all structures except the cerebral and cerebellar exteriors, structures are extracted from the outside before they are extracted from the inside in order to get rid of these stray dots.

The following procedure has been developed to generate clean outlines:

Extract the structure from the outside (by placing your cursor directly underneath the structure you are extracting). Press "x" to get rid of stray lines. Unextract the structure. Extract the structure from the inside. Hit "x" to get rid of any remaining stray dots. Structures must be extracted from the inside in order to be used in volumetric analysis.

There are certain times when you will not be able to extract an outline from the outside because there are too many stray contours surrounding the interested structure. When this happens, extract the outline from the inside first. Press "x". Then unextract and extract from the outside. Press "x". Unextract and re-extract from the inside to create the final extracted outline. See the section on "extract" in the "General Methods and Tools of Segmentation" for more information on this topic.

Extract outlines once
A common mistake that new users make is to accidentally extract structures multiple times. This can happen when extracting from the inside before unextracting from the outside, by accidentally extracting a structure a second time, or by accidentally hitting the "load" or "recall" button on the SEG window after the brain is already loaded. These are the types of errors you realize after you've made them, and you generally don't forget them once you've made them. Extracting outlines multiple times causes problems. Every time a structure is extracted and subsequently labeled, it is used to create an overall volume for the structure throughout the brain. If you extract an outline more than once, you will artificially inflate the volume of that structure.

Saving
It is very important that you save, and save often when using CardViews. After you have completed segmenting a slice, before moving onto the next slice, hit the "SAVE" button at the bottom of the SEG screen. This will save your outlines. It is generally recommended that you save your outlines after segmenting each structure because occasionally, CardViews crashes.

If you accidentally change slices before you have saved your outlines, return to the slice where you forgot to save. Hit the "recall" button. This should bring up your most recently segmented outlines. Make sure to hit "save" before continuing on.

General Methods and Tools

General segmentation of the human brain involves defining anatomical structures by primary borders, corresponding to signal intensity transitions at brain-CSF or gray-white matter interfaces, or by secondary borders, which are knowledge-based anatomic subdivisions within a gray or white matter field that are not defined unambiguously by signal intensity transitions. [Filipek et al, 1994]

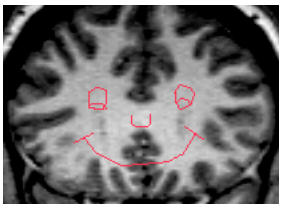
Four methods, which exist on a continuum of user subjectivity and input, help us to define these borders. Several helpful tools supplement these four general methods of segmentation. Combined use of the four general methods, tools, and knowledge of neuroanatomy produce the most efficient and reliable procedure in which to define these primary and secondary borders and therefore segment the human brain.

General Drawing Methods

The endpoint of the four drawing methods is to create an enclosed outline that can be saved and used in morphometric analysis. The four drawing methods create contours which are manipulated by the user into a shape that best represents the structure that is being defined. Once the structure has been satisfactorily represented, the user extracts this shape. The contours that make up the shape turn green, and create an outline that can be saved, labeled, and used in volumetric analysis.

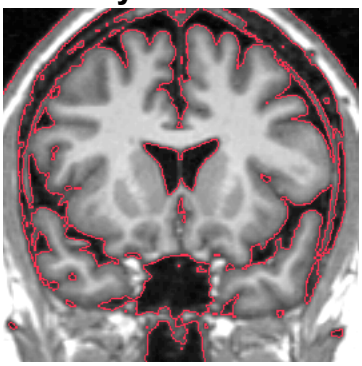
Manual draw method

The first of the four general methods of segmentation is the manual draw method. This method, used in conjunction with the brightness/contrast tool, projections lines, cross-referencing and knowledge of neuroanatomy, allows the user to "eye-ball" and manually draw in borders. This method can be subjective but in certain instances the draw method is the most effective way of defining borders.



The draw mode can be initiated by clicking the right mouse button (while in base mode). Clicking the left mouse button will select the point from which your line will start (represented by the cursor's position) and each subsequent click of this button will create a line between this point and where you moved the cursor. In order to draw a new line in a new area, one must quit the draw mode and reinitiate the draw mode after moving the cursor after to where you wish to resume drawing. Holding the left button and dragging the cursor the desired point can also draw lines. While you are in draw mode, clicking on the middle button will undo the most recently drawn segment.

Intensity contour method



The second method is the intensity contour method, which combines user input with a calculated algorithm. The ultimate decision of the border lies in the eyes of the user. The goal for any contour is to "hug" the exterior of the intended structure so as to include all voxels of the structure but none of the surrounding area.

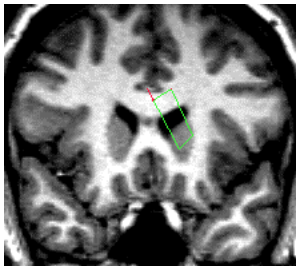
The "c" key of the keyboard activates this function. Clicking with the left mouse button on any voxel of a border will create an intensity contour algorithm, which will give a static contour, or border, throughout the scan based on intensity. The given contour or border can be manipulated incrementally by intensity value, which is referred to as dynamic contouring, by using the right mouse

button and moving the mouse. Dragging the mouse toward you will expand the contour, and pushing the mouse away will tighten the contour. Clicking the right mouse key again will secure the border or outline, and pressing the space bar will close the contour intensity function.

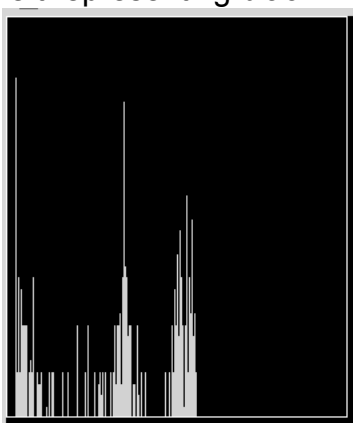
In certain instances more than one contour will be required to accurately define the borders of a certain structure because the surrounding tissue may vary. This is referred to as "piece-wise" contour. In these instances, the pieces of separate contours are connected by the draw function and extracted as one structure. This is described further in the "using multiple contours" section.

Histogram method

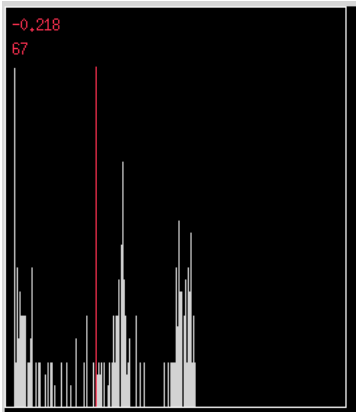
The third method is referred to as the histogram method. This method is less subjective than the intensity contour method but only with clearly defined borders (e.g. between CSF, white and gray matter). Certain subcortical structures lie between white and gray matter intensities therefore rendering the use of histograms as subjective as using the intensity contour method.



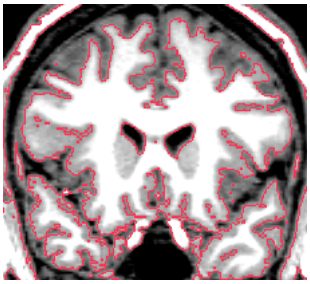
In order to employ the histogram method one must first draw and extract a box (using the draw function), which includes equal parts and the most extreme contrast between the two areas that are being separated by the border. Pressing "shift-f" (with the mouse cursor in an extracted box) will create a new window that contains a histogram on the left side of the screen. The histogram represents the intensity of the voxels within the box. The x-axis represents the intensity (white vs. black) of the voxel, with the right representing white, the middle representing gray, and the left representing black. The y-axis represents the number of voxels that are at a given intensity.



The ideal histogram has only two or three peaks depending on the variety of tissues contained in the box. For example, a box that contains CSF, white matter, and gray matter should produce a histogram with three peaks: one representing the white matter, one the gray, and one the CSF.



A border is defined by averaging the two peaks that represent the two tissues that form the border in question. Clicking on one peak with the middle mouse button and dragging it to the other peak calculates this average. After releasing the mouse button, a red line will appear between the two peaks which represents the intensity exactly halfway between the two most extreme intensities. A contour line that corresponds to this intensity value will also appear. The averaged intensity serves as a reliable border between the two contrasting intensities in question.

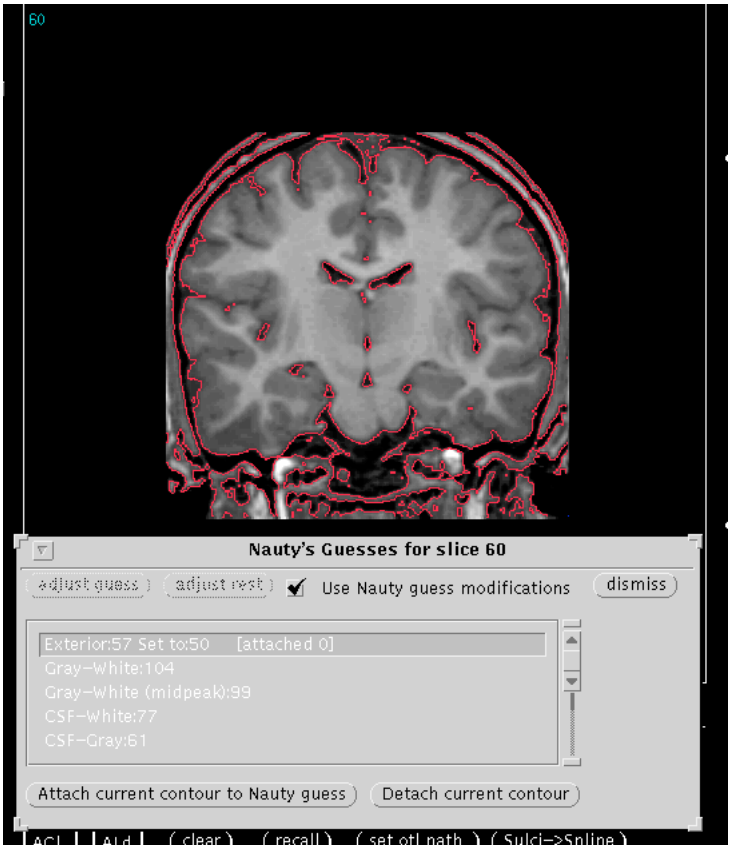


As with intensity contour, it is possible that more than one histogram will be need to define a particular structure. In this instance the two or more given contours should be connected with the draw method and extracted (see "using multiple contours").

If the given intensity is not satisfactory, the red line can be moved by clicking on it with the left mouse button and dragging, this will manipulate the given intensity (dynamic contour).

This histogram can be expanded with the right mouse button. The "s" key returns the histogram to normal viewing size. The histogram window can be cleared with the "clear" button, or closed with the "done" button.

On any given slice, you must clear the histogram window before taking another histogram or the new histogram will be an average of the two histograms.



AutoSeg method

The fourth method is the AutoSeg method. This method is the most user dependent method. It strives to replicate manual segmentation by incorporating both the histogram and intensity contour methods. This method is mostly used for the exteriors, white matter, and lateral ventricles. However, programs are being developed to automatically segment other structures.

To bring up the AutoSeg menu press "shift-a". A window will appear and give you the value the computer thinks should be the intensity value, this will be titled "Nauty's Guesses for slice X".

It may be necessary to change this value according to the user's opinion and this can be done by manually obtaining a contour, selecting the structure in question off of the AutoSeg menu, and clicking on "adjust rest". The words "Set to: X" will appear. "X" is the new value and selecting "adjust rest" will save the value.

After setting the intensity values, AutoSeg can be used to segment by clicking on the structure in question (e.g. exteriors), and manually editing the given contour (filling in holes, excluding non-brain sections, separating hemispheres etc.) The satisfactory outline should be extracted. AutoSeg is turned off by the "dismiss" button.

General erasing methods

There are two functions that are used to erase unwanted contour lines. They vary in the extent of a contour they are able to erase at once.

"x"

The "x" key is used to erase all lines of a given color at once. It is the quickest method of erasing stray lines. After extracting every structure, the "x" key is pressed to rid the image of all stray contours.

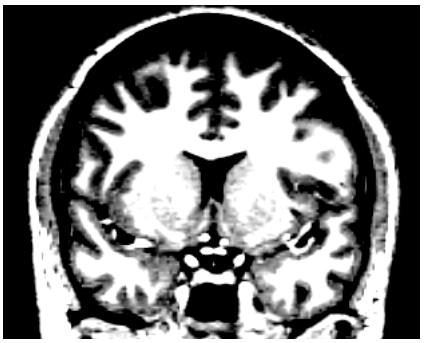
Erase mode
Erase mode is useful in erasing just a tiny portion of a line, or to create outlines using multiple contours (see section below for more information). To initiate erase mode, press "q." Hold down the first mouse button to erase. To exit erase mode, press the space bar.

If you've erased part of a line accidentally, hit "r" to unerase while in erase mode.

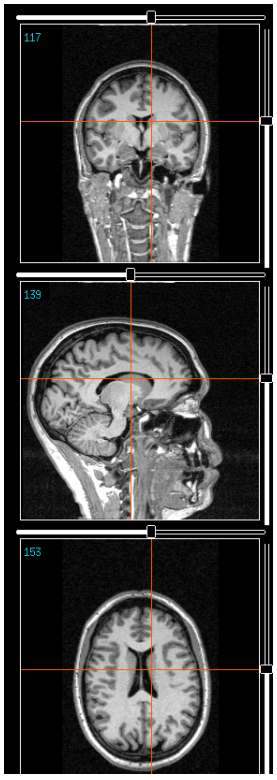
To change the size of the eraser, while in erase mode, press the third mouse button. The erase size will increase from 1 (smallest) to 10 (largest). Pressing the third mouse button while on size 10 will change the eraser back down to size 1.

Tools

Although these methods of segmentation are all fairly reliable methods of defining borders, human brain anatomy is variable and often times not very clear. MRI scan resolution is also not perfect and phenomena such as partial voluming and shadowing do occur frequently to further complicate the borders. In these instances certain tools help us to decide what is brain and what is not, and also to define certain structures. These tools serve to supplement our knowledge of anatomy as well as our methods for defining borders.



Brightness/contrast
The brightness and contrast of the image can be manipulated in order to see divisions between different tissues more clearly and to recognize the true extent of the brain. This is achieved by clicking the middle mouse button and dragging upward to brighten, downward to darken, left to decrease the contrast of an image, and right to increase the contrast.



Cross-referencing and projection lines
In certain instances it may be easier to recognize a border or structure in a view of the brain other than the coronal. On the right side of the CardViews screen there are three windows with three different orientations (coronal, sagittal, and axial) of the brain. These allow for cross-referencing and a 3-D visualization of the brain and its structures.

In order to pinpoint a certain area of the brain in different orientations projection lines are available to mark the location in the three different orientations. Projection lines can be called to the screen by clicking on the projection line button in the left corner. The position of the crossbars can be manipulated in the smaller views of the images on the right of the screen.

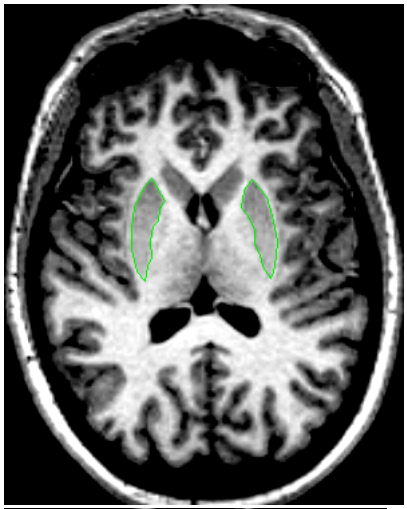
Once the crossbar is positioned in the questionable area, hit the "transform" button next to the slice numbers and the words SAG, and AXI. By transforming the other views, one is able to investigate the corresponding points in the other two planes, enabling you to better identify the point under examination. To

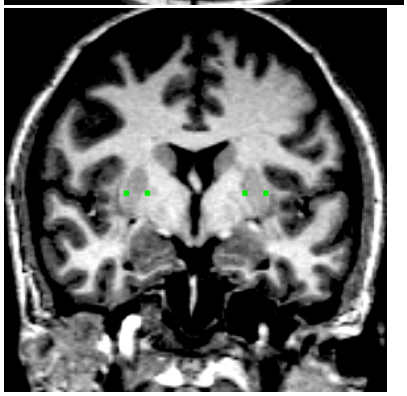
examine a view more closely, click on the smaller views at the right of the screen. The smaller view will then occupy the large screen.

Sulci lines

Drawing sulci lines in NAV mode is another tool useful for defining tricky borders. This tool allows you to draw lines either around structures (e.g. thalamus) or between structures (e.g. hypothalamic fissure) in the sagittal and axial views. Certain boundaries or structures may be more visible or better defined in other views than the coronal. These lines show up as dots in the coronal view and can serve as a useful skeleton for the structure in question or as a point of division. Saved sulci can be recalled in both NAV and SEG mode.

In order to draw sulci you must be in NAV mode. To enter NAV mode, left-click on the "NAV" button in the upper left corner of CardViews. Click on the view (smaller images to the right) you would like to draw the sulci in (cor, sag, axi).





Drawing in NAV mode is much like drawing in SEG except: lines cannot be drawn by dragging the mouse with the left mouse button held down (the click-move-click method must be employed), individual lines can be saved (no need to extract), and lines drawn on a slice are automatically saved (albeit temporarily) without any key strokes when the right mouse button is clicked (while in center window) to exit draw.

In order to change pen colors (sometimes useful for multiple sulci on one slice) press the "s" key and click on desired color, hit the space bar to return to the image.

To erase a whole line, left click and drag the line out of the box. If you want to erase a segment of a line and you have not yet exited the draw function, middle click and the segments will be erased sequentially.

Sulci "reference dots" should appear in the other small views to the right. Sulci, or sulci "reference dots" can be recalled in SEG by hitting the "drw sulc" button. Left clicking in the small coronal view in the upper right hand corner will remove the sulci from the image.

To permanently save sulci lines, make sure your sulci prefix is on the prefix line (usually your prefix with and "s" added), and any previously drawn sulci are loaded. Also make sure you are in base mode. Click with the left mouse button on "Write Sulci" button and left click on the "Overwrite" button. This saves all new lines drawn to the selected sulci file while retaining any previously saved lines.

Additional drawing features

There are other tools available for use while segmenting that make the process, easier, faster, and more reliable.

Extract
Extraction is used to create an enclosed outline that can be saved, labeled, and used in volumetric analysis. However, it can also be used as a tool in segmentation by ridding an outline of stray dots.

In general, Extract is a tool that highlights any contour immediately above the cursor. If you are inside an enclosed structure, it extracts the enclosed outline. If you are underneath a structure, it extracts the outline along the outside of the shape. Once something is extracted it turns green, and all other lines of different colors can be erased.

This tool helps to clean up outlines (get rid of stray lines), ward against double lines (which may take voxels away from the volume of an extracted structure), and ensure that outlines are continuous. For all structures except the cerebral and cerebellar exteriors, structures are extracted from the outside before they are extracted from the inside.

The detailed procedure is as follows: extract the structure from the outside. Press "x" to get rid of stray lines. Unextract the structure. Extract the structure from the inside to create the extracted outline.

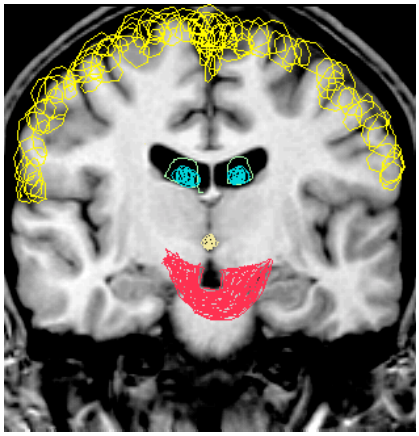
There are certain times when you will not be able to extract an outline from the outside because there are too many stray contours surrounding the interested structure. When this happens, extract the outline from the inside. Press "x". Unextract and extract from the outside. Press "x". Unextract and re-extract from the inside to create the final extracted outline.

Unextracting outlines
To unextract the last outline you extracted, press "w." If you continue to hit w, you will unextract structures in the reverse order in which they were extracted.

To unextract all outlines on a slice, hit "shift-w."

You can also unextract outlines with the drag method. Click on the outline you want to unextract with the left mouse button. Continue to hold down this button. Drag the cursor outside of the image box. This unextracts the outline.

Different colored contour lines



There are five different colors you can use to create outlines. These colors correspond to the five left colored boxes that are located in the upper white box on the SEG screen.

A smaller black box is located in one of these five boxes (red by default). Because the red box is highlighted, anytime you activate a drawing method (manual drawing, intensity contour, histogram, AutoSeg), the contour that appears on the screen will be red. Anytime you active an erasing method ("x", erase), the contour that will be manipulated will be red. If you click on the yellow box, all drawing and erasing will pertain to the yellow lines. This is also true for the other three colored boxes. Using multiple colored contours is helpful in segmenting certain structures (as described below).

Segmenting with multiple contours
Contours of multiple colors can be used to create an outline. This is very helpful when a structure is surrounded by multiple structures with different intensities. Separate lines that represent each side of the structure can be used, and these can be attached together when creating the final outline.

To use this feature, first define one part of the structure under question. This most often requires use of the intensity contour or histogram function. Then, using the erase function (press "q"), clip the ends where the line no longer looks correct. Click on the part of the line that is correct. It will turn white. Then press "v." This will turn the line the color of the next "dump" level. By default, this will be yellow. The dump level refers to the 5 contour color boxes in the top left white box of the SEG screen (to change the dump level to a different color, hit b). Press "x" to get rid of all red contours. Create the next border you need. Clip the ends of the contour where applicable, click on the part of the line you want, and press "v." Then press "x" to get rid of red contours. Repeat this procedure until your structure has been defined. If necessary, connect any gaps in your outline with the draw function. Then extract your outline from the outside. Press "x" to get rid of stray red contours. Then click on the yellow contour box. Press "x" to get rid of stray yellow contours. Click back onto the red contour box. Unextract your outline, and re-extract it from the inside.

Toggle
To toggle between the image and your contours and outlines, hit "shift-r." This is helpful is checking extracted outlines, and in checking to make sure contours are located where you want them to be.

Third Ventricle and Transverse Cerebral Fissure

General Description

Third Ventricle

The third ventricle is located along the most medial part of the diencephalon. From the medial sagittal view, the third ventricle takes on a donut shape in most brains. The third ventricle is connected to the lateral ventricles via the Foramen of Monroe, and the fourth ventricle via the aqueduct. As with all ventricles, the third ventricle is filled with cerebral spinal fluid (CSF) which appears as black on the MRI scan.

The third ventricle is bordered anteriorly by the lamina terminalis. Its inferior border is the ventral diencephalon (VDC), beginning with hypothalamus anteriorly, and moving posterior to include the mammillary bodies, substantia nigra, red nucleus, and subthalamic nuclei. Its lateral border is made up of the hypothalamus and other VDC structures (ventrally) and the thalamus (dorsally). The superior border is the fornix (anteriorly) and then a thin layer of choroid plexus that extends to the posterior border and curves down to create part of the ventral border of the third ventricle. The posterior border also includes the pineal gland. This is best seen at the level of the pineal and suprapineal recesses where the third ventricle appears as a small pocket inside the transverse cerebral fissure. A think layer of choroid plexus borders the ventricle dorsally and laterally. The CSF of the transverse cerebral fissure surrounds this portion of the choroid plexus. Ventrally, the ventricle is bordered by the habenula.

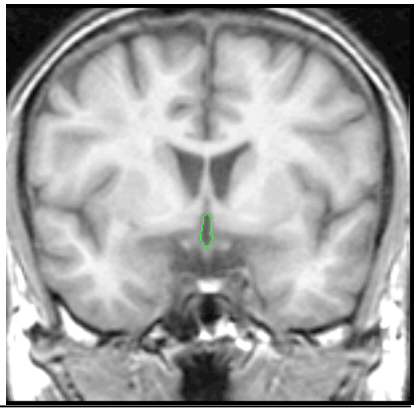
Transverse Cerebral Fissure

The transverse cerebral fissure (TCF) is posterior and superior to the third ventricle, separated by the choroid plexus membrane. It first appears just posterior to the thalamus. Towards its ventral extent, the TCF surrounds the third ventricle laterally. The TCF is bordered dorsally by the fornix and laterally by the thalamus and fornix. The TCF lies outside of the brain exterior and is filled with extraventricular (subarachnoidal) CSF. In some ways, it is an imprecise label because in its posterior extent, what is extracted and labeled as CSF will include TCF and the pineal gland. Though this label is imprecise it is necessary as it ensures we do not include any TCF in the third ventricle.

Procedure

Segmentation

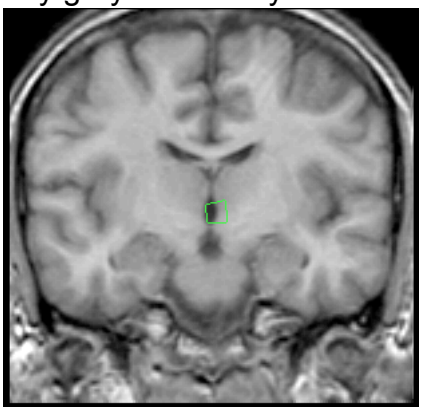
A number of drawing methods are used for the third ventricle and TCF depending on where you are in the brain. A histogram is used to segment the third ventricle and TCF anteriorly. Midway back, both a histogram and manual drawing are necessary to segment these structures. In their posterior ends, the intensity contour method and histogram are needed.



Part I - anterior portion of third ventricle

The third ventricle begins behind the lamina terminalis. This is difficult to see in MRI scans. Approximate the beginning of the third ventricle on the slices between the start of the optic chiasm and anterior commissure. In its anterior most slice, the third ventricle is often nestled within the optic chiasm. Because the optic chiasm is outside of the brain (see section on cerebral exteriors), the third ventricle appears as a teardrop hanging from the middle of the brain. On this slice, it is difficult to generate a histogram, so the intensity contour function is

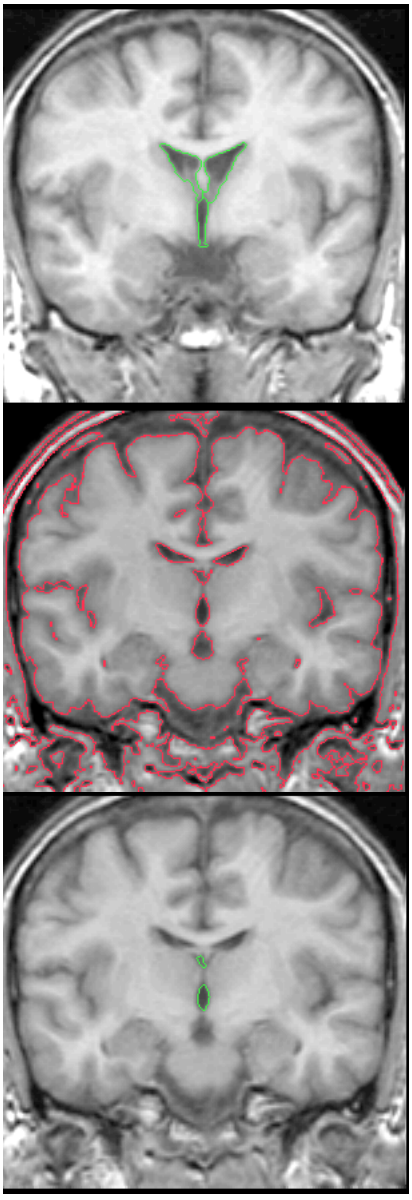
used. Adjust the contour until it fits tightly around the third ventricle, making sure you don't include any gray matter in your outline.



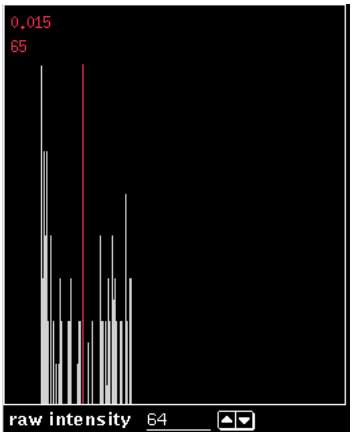
On the second or third slices of third ventricle, it is possible to use the histogram function to generate the third ventricle outline. In this case, you are going to draw a box that contains equal amounts of the CSF from the third ventricle, and gray matter from the thalamus/VDC.

Part II - middle portion of third ventricle and beginning of transverse cerebral fissure

As the third ventricle continues posteriorly, choroid plexus serves as its dorsal border. This becomes particularly significant at the level of the foramen of Monroe. At times your third ventricle histogram will include the foramen of Monroe and part of the lateral ventricles in your outline. Using the draw function, manually edit your outline so the foramen of Monroe is not part of the third ventricle.

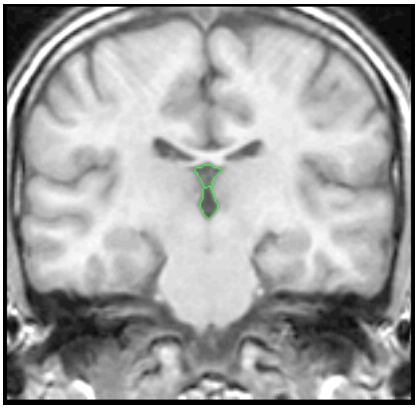


Immediately posterior to this level, the TCF begins and continues posteriorly. As you generate your third ventricle histogram, you should start noticing a small contour superior to the third ventricle that represents the TCF. When this small outline appears, begin extracting the TCF. Because the contour that best fits the third ventricle often underestimates the size of the TCF, a separate histogram should be taken. Include equal amounts of CSF from the TCF and gray thalamic tissue in your box. Use the intensity that lies half way between the peak for the thalamic gray matter and the peak for the CSF inside the TCF when creating your outline.

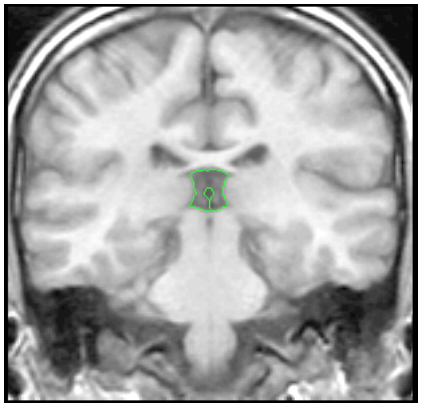


Slices at the anterior level of the TCF contain the interthalamic adhesion, which appears to divide the TCF from the third ventricle. This is due to MRI resolution, which usually does not provide a sharp image of the area dorsal to the interthalamic adhesion. Anatomically, one expects to find some third ventricle above the adhesion that is bordered dorsally by choroid plexus. This is difficult to see in MRI scans. By CMA convention, the contour that is generated superior to the interthalamic adhesion is TCF even though it contains both third ventricle and TCF. Brighten the intensity of the screen. It may be possible to see the choroid plexus that separates these two structures within this "TCF" outline. If it is, manually draw a line just under the choroid plexus.

At the level immediately posterior to the interthalamic adhesion it becomes more difficult to distinguish between the TCF and third ventricle, as the two appear to "fuse." By brightening the screen you should see a thin layer of choroid plexus which divides the two. A single histogram for the third ventricle will provide a contour that will encompass both the third ventricle and TCF, so the two structures must be divided manually. Brighten the screen enough to see the choroid and manually draw a line under it such that the third ventricle and TCF are separated, and extract each independently.



Part III - posterior portion of third ventricle and transverse cerebral fissure



At its posterior-most end, the third ventricle becomes almost completely surrounded by TCF. To see this detail, the screen must again be slightly brightened. It is difficult to derive a histogram that will provide an appropriate contour for the third ventricle alone, so it is best to use the intensity contour function to isolate this small region. Manual editing is often necessary to complete an enclosed area for extraction. Extract the third ventricle first. Then generate an outline for the TCF by creating a histogram between the CSF of the TCF and the thalamic gray matter.

As you move posteriorly. the contour you generate will embody both the TCF and the pineal gland. This is desirable because you do not want to include the pineal gland as brain.

The third ventricle will appear as a "free-floating" structure inside the TCF outline. Extract the third ventricle before you extract the final TCF outline, and be sure to draw a line that connects the third ventricle to the TCF. This is done to exclude the third ventricle from the TCF volume.

Labeling

The third ventricle is extracted and labeled as third ventricle. Because it is outside of the brain, the TCF is labeled as CSF.

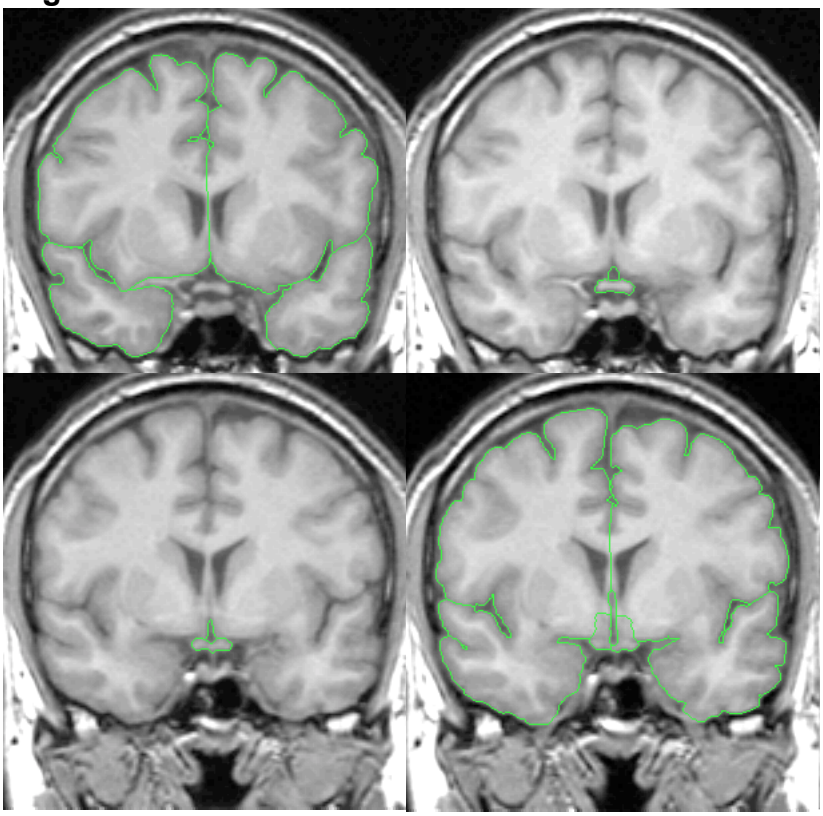
Optic Chiasm

General Description

The optic chiasm contains the crossed and uncrossed white matter fibers of the optic nerves as well as the surrounding gray matter (e.g. the suprachiasmatic nucleus).

Procedure

Segmentation



The outline for the optic chiasm is created using contour lines and manual drawing.

Start segmenting the optic chiasm, from anterior to posterior, on the first slice it becomes the inferior border of the third ventricle. This is in the proximity of the coronal slice containing the anterior commissure.

Create a contour line the surrounds the optic chiasm. Its superior border will include some of the inferior border of the third ventricle. Extract the outline.
Stop segmenting the optic chiasm when it "separates" and becomes the optic tracts.

Labeling

This outline is labeled "optic chiasm."

Fourth Ventricle

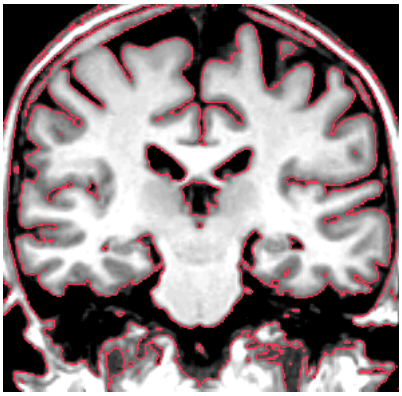
General Description

The fourth ventricle is a, CSF-filled structure located between the brainstem and the cerebellum. Its anterior border is the brainstem. Laterally and posteriorly it is bordered by the cerebellum. Its posterior border (above the cerebellum) is the midbrain tectum (superior and inferior colliculi). We include the cerebral aqueduct as part of the fourth ventricle.

Procedure

Segmentation

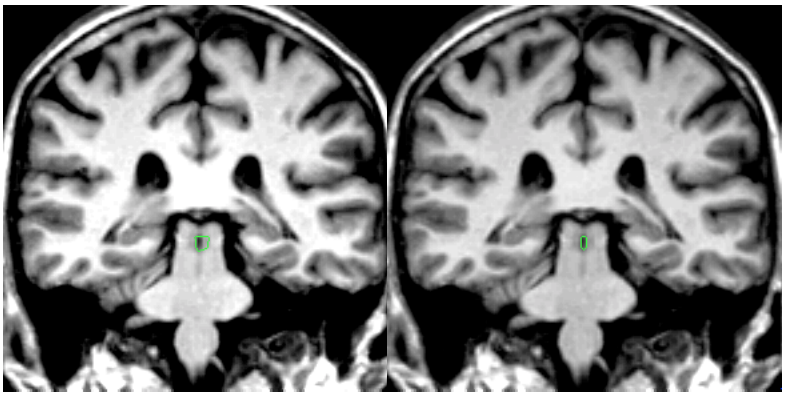
Extraction of the fourth ventricle is ideally done using the histogram method. Depending on which region of the fourth ventricle you are looking at, the box drawn for your histogram will contain CSF from the ventricle and either cerebellar white matter, cerebellum gray matter, brain stem, or some combination thereof. The intensity contour method and manual drawing are also employed.



Part I - cerebral aqueduct

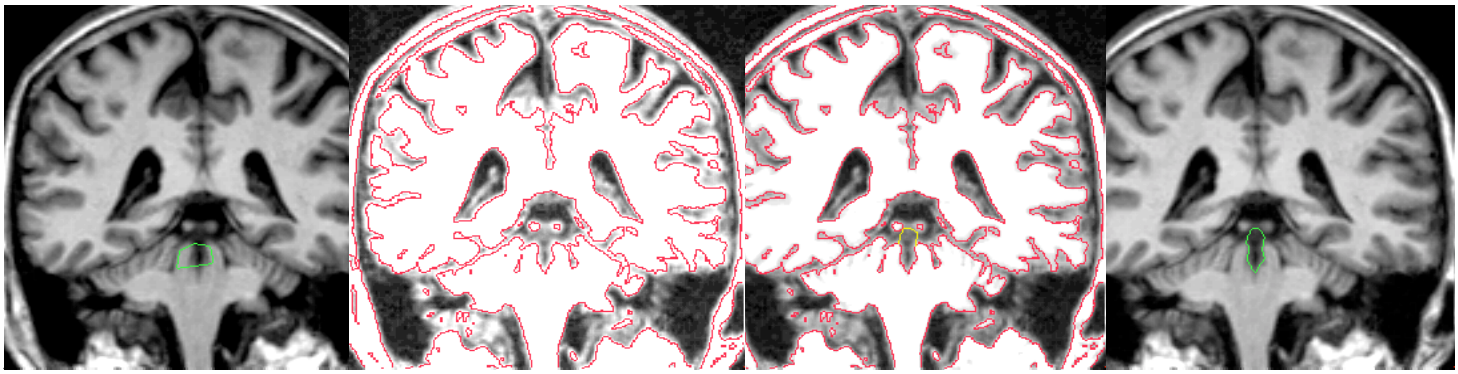
The aqueduct first appears just under the posterior commissure. A histogram should be taken between the CSF of the aqueduct and the brainstem. However, because there is so much partial voluming in this area, the histogram will likely be modified using an intensity contour line. The dorsal border of the fourth ventricle will have to be drawn manually. Continue to use a histogram for the remainder of the aqueduct, modifying as necessary with the intensity contour function.

Part II - fourth ventricle in the brainstem



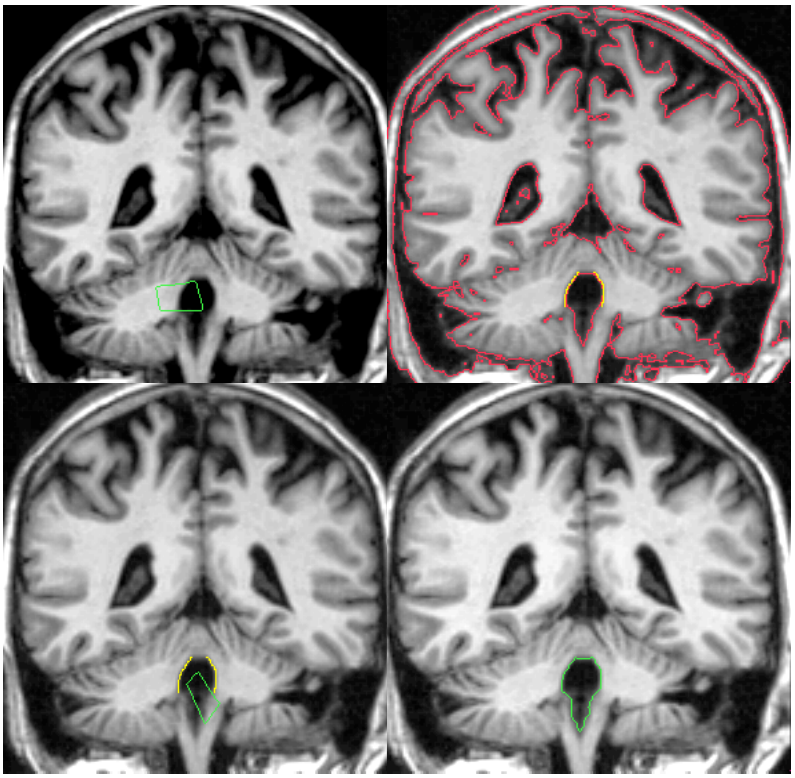
As you move posteriorly, you will begin to see the actual beginning of the fourth ventricle. The small circle that is the aqueduct will begin to elongate. Continue to use the histogram method; draw your box between the CSF of the fourth ventricle and the surrounding brainstem tissue. Modify as necessary with the intensity contour function.

As the fourth ventricle continues posteriorly, it will start to widen. A histogram should be taken between the CSF of the fourth ventricle and the surrounding brainstem tissue. Often this histogram will not yield the dorsal border of the 4th ventricle. Brightening the screen will enable you



to see this border. It should be drawn in using the draw function, and then attached to the contour given by your histogram.

Part III - fourth ventricle in the brainstem and cerebellum

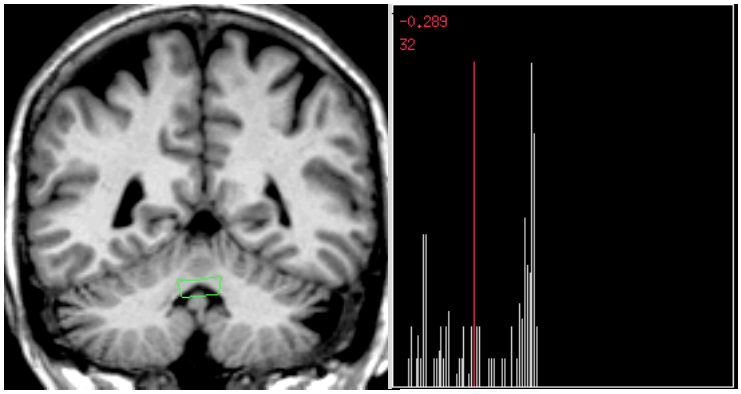


As the 4th ventricle is surrounded by cerebellum white matter, multiple histograms will yield the most accurate fit. Generate a histogram from a box containing CSF of the fourth ventricle and the cerebellum white matter. The only part of the contour that you want is that between the cerebellar white matter and the CSF of the fourth ventricle. Now generate the rest of the outline with the histogram method .

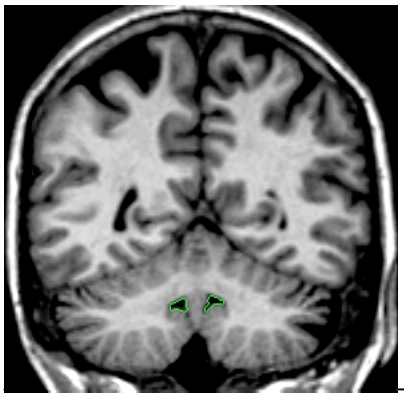
The box for your second histogram should contain equal amounts of CSF from the fourth ventricle and the brainstem. The generated contour will accurately define the border between the fourth ventricle and the brainstem.

Part IV - fourth ventricle in the cerebellum

When the fourth ventricle is no longer surrounded by brainstem, it appears between cerebellum gray and white matter. Two histograms should be used for this outline: one between the CSF and the cerebellum white matter, and the second between the CSF and cerebellum gray matter.



In its most posterior extent, the fourth ventricle will appear as two separate circles in each cerebellar hemisphere. The most accurate means to extract these structures is to do two separate histograms for each cerebellar hemispheres (CSF - white matter; CSF - gray matter). As with the most anterior extend of the 4th ventricle, modifying this estimate with the contour line may be necessary.



Labeling

Both the cerebral aqueduct and fourth ventricle are labeled as "fourth ventricle."

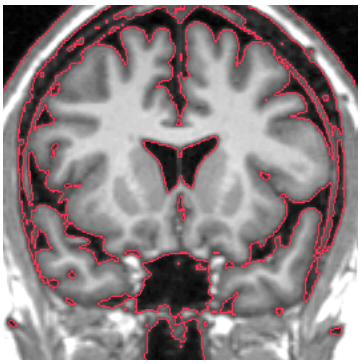
Cerebral Exterior

General Description

The cerebral exterior is the border between the subarachnoid CSF and neural tissue (e.g. the first layer of cortical neurons), and should correspond to the pia mater. Thus, the cerebral exterior separates brain from non-brain, cerebrum from cerebellum, and divides the brain in to its two hemispheres.

Procedure

Segmentation



The exterior is defined using the intensity contour method and manual drawing.

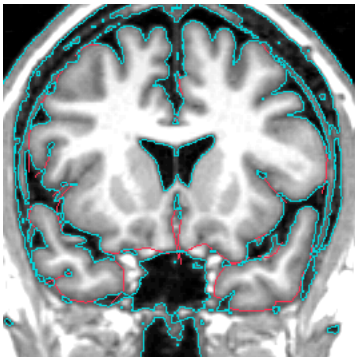
Increase the brightness of the image to verify that you are seeing the actual extent of the cerebral hemispheres. If the white matter begins to bleed into the gray matter, you have gone too far. Create a contour using the intensity contour function that is somewhat larger than the exteriors. Then, adjust the contour until it fits tightly around the hemispheres, making sure you don't exclude any gray matter from your outline.

If when you generated your outline using the contour function you have a small contour inside the brain that represents a sulcus (e.g. the Sylvian fissure), you must connect this small contour with your exterior by tracing along the sulcus in the image.

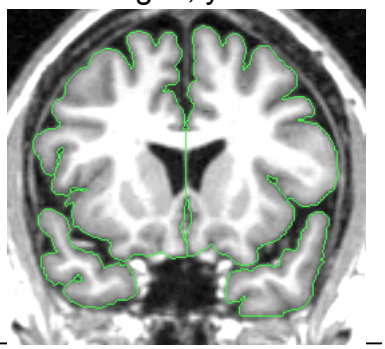
Be sure to draw in the Sylvian fissure when it is present.

The drawing tool also enables you to exclude things that are not brain such as meninges and blood vessels.

To begin editing, return the brightness to normal viewing intensity by decreasing the brightness slightly. Complete your outlines by manually drawing to complete the gaps remaining in your contour outline.



Your outlines should not include anything that is not brain (e.g. dura mater, other meninges, etc.). For our purposes, optic chiasm is considered to be outside of the brain, and therefore excluded from exterior outlines (the optic tract however is included as part of the ventral diencephalon). To determine what is and what isn't brain, it is useful to check the other two views available to you. By transforming the other two images, you are able to investigate the corresponding points in two other planes, enabling you to better identify the point under examination. Once you've determined what is and isn't brain, enter draw mode to make the appropriate corrections on your exterior outline.

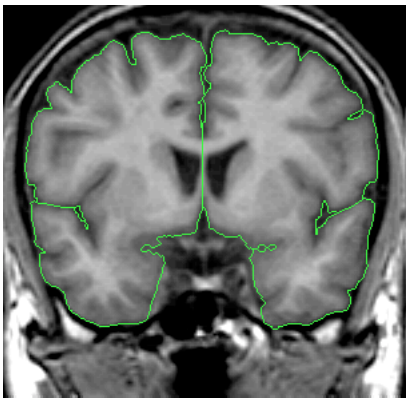


The cerebral hemispheres are extracted independently, and their division is most clear in slices where they are completely separate. When corpus callosum is present, it is necessary to separate the hemispheres by

manually drawing along the midline.

Anteriorly, when the temporal lobes are present but not connected to the frontal lobes, the temporal lobes are extracted separately from the frontal lobes. Thus, you will have four separate outlines that make up the cerebral exteriors.

At the fronto-temporal junction, if the contour encompasses the entire hemisphere but the white matter between the lobes is not continuous, it is necessary to separate the frontal and temporal areas. Each hemisphere and lobe should then be extracted independently.



The exterior line will need to be tighter in some areas. Specifically, make exteriors tighter around the hippocampus and amygdala, so as not to include vessels in that area. Making the exterior line tighter around the hip-amyg area is easiest done after the outline has been extracted and all stray contours have been erased with the x function. Once your outline is complete, hit "shift-r" to temporarily remove the green lines. Reduce the brightness of the screen to adequately see the difference between the brain and the CSF that surrounds the amygdala. Generate a contour line that is tight around this area. Using the erase function make two small holes in the contour you just generated such that the small corner that borders the amygdala is separated from the rest of the contour. Make the line yellow, and clear any extraneous red lines. Recall your previous exterior by pressing "shift-r" again. Connect the yellow contour to the extracted outline with the draw function. When this is done, unextract the outline, and then re-extract the outline such that your tight amygdala border is contained in this new extracted outline. Clear all extraneous lines.

AutoSeg

Setting AutoSeg Parameters

Before you use AutoSeg to segment, you need to set the values for the exteriors.

Start at slice 64 and with a contour line, find the best-fit exterior line. Look at the top of the CardViews screen in the box below the "quit" button. Here you will find the intensity value of the line you have selected. Bring up the AutoSeg menu by typing "shift-a." The AutoSeg window will appear, titled "Nauty's Guesses for slice X" (the X representing whatever slice you are on).

Compare the intensity value of the contour you selected with the intensity value AutoSeg has given for the exterior. If you like the guess AutoSeg has selected, click on the "exterior" button on the AutoSeg window. Then click on the "adjust rest" button at the top of the AutoSeg window.

If you do not like AutoSeg's guess, you can adjust it. First click on the "exterior" button. Then, using a contour line, adjust AutoSeg's contour until it matches with the exterior line you like. Looking at the intensity value provides for an easy way to re-find the line you think fits the exterior best. When you are satisfied with your line, click on "adjust rest." In both cases, after you click on "adjust rest," new words will appear next to "exterior" in the AutoSeg window. You will see the words "set to:" and then a value that represents the intensity of the line. Hitting adjust rest will automatically save the value you have set, even after you quit CardViews.

You will continue setting the values for the exteriors across the entire brain. Your first adjustment is done on the slice halfway between the first and last slices (64 on a 128-slice brain, and 32 on a 64-slice brain). Continue to make the adjustments on by cutting the number of slices in "halves," until you reach the end of the brain. Do this in both directions. For example, on a 64 slice brain, you will set exterior values on slices 63, 62, 60, 56, 48, 32, 16, 8, 4, 2, 1.

On slices where you did not set an exterior value, you will see the words "interpolated to:" and then a value next to the word "exterior". This is the value that AutoSeg has set for this slice. Note that AutoSeg's initial guess remains in the window at all times. In general, AutoSeg's guesses are close to right for the center slices. As you work your way outward anteriorly and posteriorly, your exterior lines will need to be larger. This is a general trend, and by no means a rule to follow.

Segmenting with AutoSeg
To use AutoSeg when segmenting the exteriors, first bring up the AutoSeg window using "shift-a."

Click on the "exterior" button and the appropriate contour line will appear. Complete any manual editing that may be needed. This includes filling in holes, cutting out the optic chiasm, nerves, blood vessels, dura, and other non-brain objects, and separating the brain into separate hemispheres. Remember that when the temporal lobes begin you may have four exterior outlines. Also remember to make the contour line tighter around the amygdala and hippocampus.

You have the option of AutoSeg automatically bringing up the exterior line every time you hit "shift-a." To do this, first select which color you would like the contour to be. Your selection is from one of the 5 possible contour colors as displayed in the box under the "quit" button. Whichever color you select will have a small black box in its center. Now bring up the AutoSeg window. Click on the exterior button. Finally, click on "Attach current contour to Nauty guess." Now whenever you hit "shift-a", the exterior contour will appear in the color of your choice. To end this feature, click on the "Exterior" button, and then click on "Detach current contour."

Labeling
These outlines are labeled as "cerebral exteriors."

Brainstem

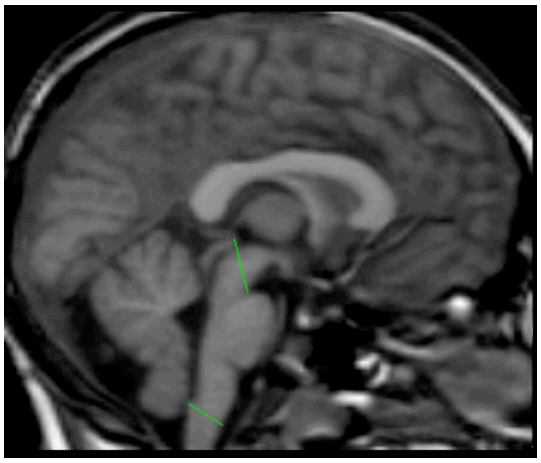
General Description

The brainstem is comprised of three parts called the midbrain (mesencephelon), the pons, and the medulla. The midbrain is the most superior part of the brainstem, continuing behind the pons and down to the medulla. The pons is the more anterior, superior part of the brainstem and the medulla is the more posterior, inferior part of the brainstem, although there is some overlap. The pons is an apple shaped structure, which sits on the anterior side of the more stalk-like medulla. The medulla leads directly into the spinal cord. The posterior border of the brainstem is the cerebellum, although cerebellum and brainstem are present at the same time. The brainstem is bordered superiorly by the diencephalon and inferiorly by the spinal cord. The superior colliculi and inferior colliculi (seen as two bumps on top of the brainstem in more posterior slices) are included as brainstem.

Procedure

Sulci Lines

In order to determine the superior/inferior borders of the brainstem draw two sulci lines in the sagittal view of NAV mode.



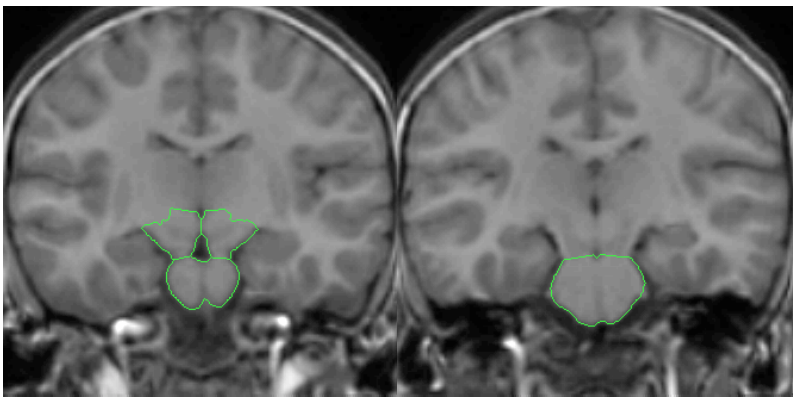
Draw the superior brainstem line as a straight diagonal line extending from the tip of the posterior commissure to the prepontine fissure (sulcus praepontinus) which demarcates ventrally the border between the pons and the midbrain (the most inferior point of the interpeduncular fossa). In coronal sections this line will serve to delineate the superior border of the brainstem from the ventral diencephelon (VDC).

The inferior brainstem line extends from the obex (bottom) of the fourth ventricle across the width of the brainstem to the pyramidal decussation (bottom of the pyramidal tracts). In coronal sections this line will demarcate the inferior border of the brainstem from the spinal cord.

Segmentation

The brainstem is segmented using the contour function as well as manual drawing.

Part I - Anterior portion of the brainstem

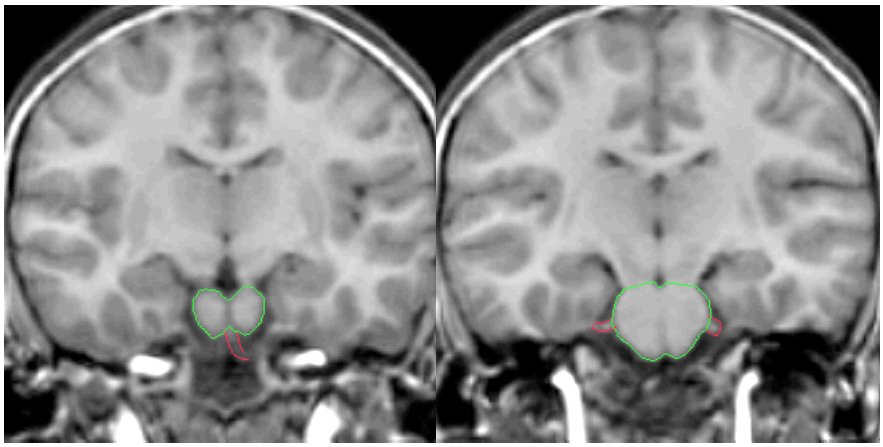


Begin segmenting the brainstem when the first slice containing the brainstem is visible. Create a contour that hugs the extent of the brainstem.

The draw function should be used to connect parts of the contour that are discontinuous, or to exclude non-brainstem parts from the outline. It may be necessary to use the piece-wise contour method (see contour method) on certain areas of the brainstem.

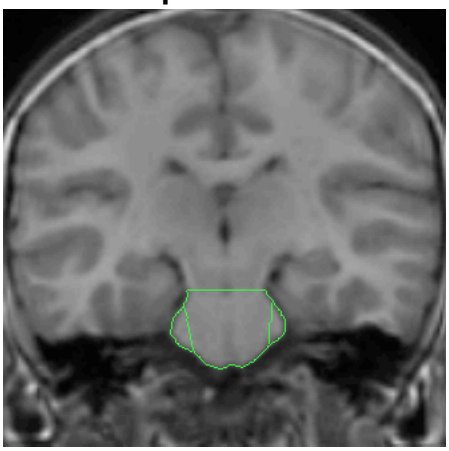
Before your sulci lines are visible, the interpeduncular fossa is used as a reference for the superior border of the brainstem. The interpeduncular fossa is an area of CSF between the cerebral peduncles, where the cerebral peduncles connect with the brainstem. If the border between the peduncles and the brainstem is not visible then use the interpeduncular fossa as the starting point and draw a diagonal border from the fossa to the lateral extent of the brainstem on each side.

The inferior border in the anterior extent (around the pons and beginning of medulla) is visible and can be discerned with the contour function.



Many of the cranial nerves appear in the area of the brainstem. Cranial nerves should be excluded from the brainstem outline. Many arteries or veins also appear around the brainstem, these should be excluded from the outline. For example, the basilar artery appears in the anterior slices of the brainstem and when discernible it is excluded from the outline of the brainstem.

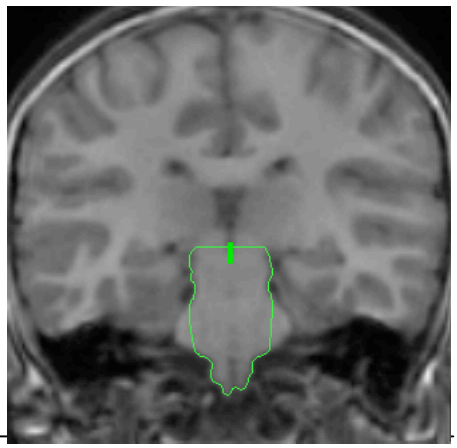
Part II - Superior brainstem line appears



As soon as the superior brainstem line appears, it will be used as the marker for the division between the VDC and the brainstem. The two structures are separated by drawing a line which bisects the vertical sulci line (appearing as a dot), such that everything above it will be labeled VDC and everything below it brainstem.

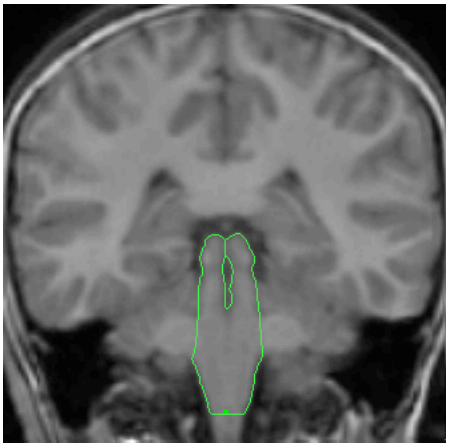
Before the appearance of the cerebellum, there is a dramatic change in the lateral extent of the brainstem. The more anterior of the slices in this region will contain a uniformly colored structure which is extracted and labeled as part of the brainstem (there is no cerebellum present at this point). More posteriorly, one slice before the cerebellum first appears, the lateral extents of the brainstem are segmented separately to exclude the middle cerebellar penducles.

Part III - Cerebellum appears

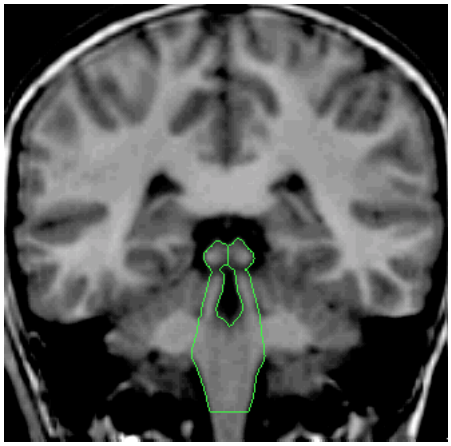


At its anterior extent, the cerebellum shares its medial borders with the brainstem. The lateral extremities of the brainstem are no longer taken as part of the brainstem outline. They are extracted separately as cerebellar exterior and as cerebellar white matter. With the contrast increased it is easy to see the division between cerebellar white matter and brainstem. This division can be manually drawn in or it may be possible to use the contour function. The next slices will have cerebellum present and these lateral extremities will again be extracted as cerebellar white matter.

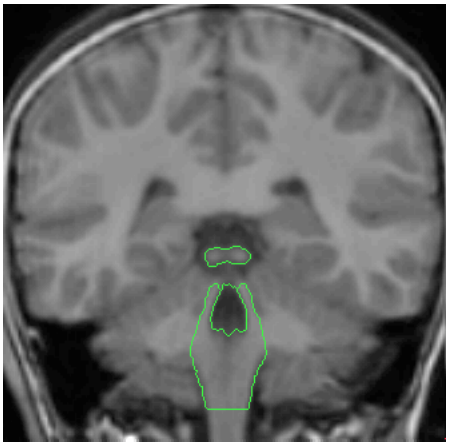
Part IV - The colliculi appear



In the more posterior slices when the superior brainstem line is no longer visible, it is necessary to draw in the superior and interior colliculi manually or with the use of the contour function. There may be a slice or two where the colliculi are not yet attached to the brainstem, in this instance extract them as a separate outline which will still be labeled brainstem.



In this area be careful to exclude the pineal gland from the volume of the brainstem.



The inferior border in the more posterior extent (where the medulla is connected to the spinal cord) depends on the inferior brainstem line drawn for the inferior border of the brainstem. Draw a line that bisects the inferior brainstem line (appearing as a dot). Everything above this line will be brainstem, and everything below it will be spinal cord and considered outside of the brain.

Be sure to always attach the 4th ventricle to the brainstem exterior to exclude it from the volume of the brainstem.

Labeling

The final outline should be labeled "brainstem."

Cerebellar Exterior

General Description

The cerebellum is located anterior to the brainstem and inferior to the cerebrum. Between the cerebellum and the brainstem, and between the left and right hemispheres of the cerebellum is the 4th ventricle. A portion of the cerebellum may even begin to poke through the 4th ventricle as an "island" of gray matter. Although the cerebellum is anterior to the brainstem, the brainstem and cerebellum often show up on the same coronal slices due to the fact that the cerebellum curves around the brainstem. The cerebellum is surrounded by external dura, the transverse sinus, and other non-brain tissue. Because of its location close to the brainstem and the base of the brain, there are many nerves and blood vessels.

Procedure

Segmentation

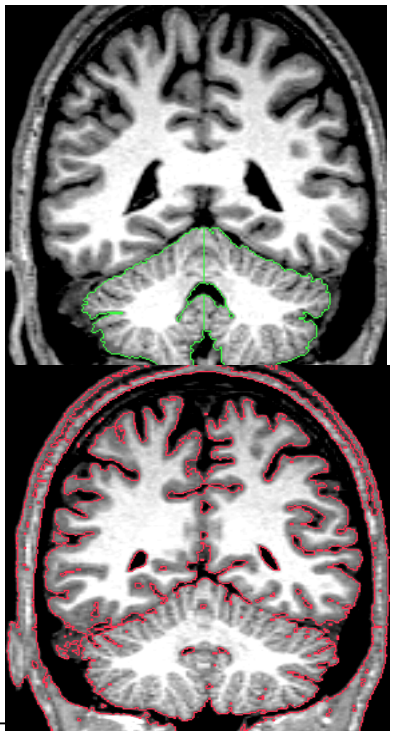
The cerebellum is extracted by using the intensity contour function. The appropriate contour to create the outline is most often the same as was used to create the outline for the cerebral exterior.

In order to see the full extent of the cerebellum it is helpful to increase the brightness of the screen similar to the brightness used to determine the full extent of the cerebral exteriors.

Part I - Brainstem coexists with anterior portion of the cerebellum

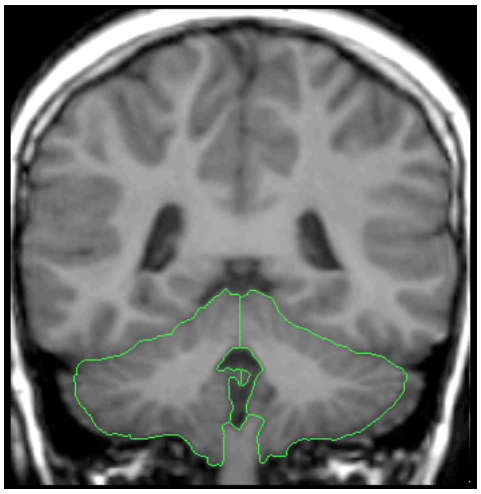
The most anterior slice of cerebellum will contain only white matter and share its medial borders with the brainstem. Generate the cerebellum exterior line with the contour function. To distinguish the cerebellar white matter from the brainstem, increase the darkness of the screen. This division can be manually drawn or it may be possible to use the contour function. Use the draw function to exclude any non-brain artifacts.

Be sure to extract the white matter twice (when no gray matter is present), as it is both cerebellar white matter and the cerebellar exterior at this point.



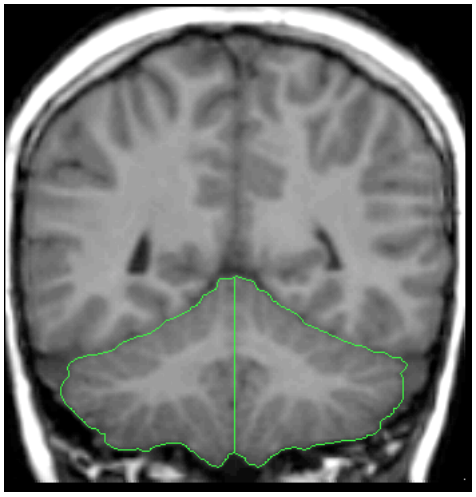
Part II - Fourth ventricle in the cerebellum

A portion of the cerebellum will begin to poke into the 4th ventricle in the most anterior slices. This is easily confirmed in axial slices. Include this "island" in your contour, or create a separate contour for the "island" if it does not connect to the rest of the cerebellum.

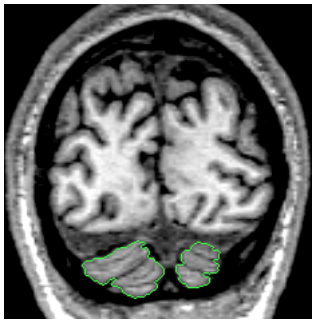


Near the end of the fourth ventricle, it separates into two pieces. Connect each of these pieces to the cerebellum exterior to ensure they are excluded from the cerebellar exterior volume.

Part III - Cerebellum alone



Use the contour function to define the exterior of the cerebellum. Use the draw function to exclude any non-brain tissue, as well as to draw in the midline. Extract the right cerebellum exterior and left cerebellum exterior separately.



Using the projection lines and alternate views is critical in determining what is, and is not, cerebellum. One difficult area is the posterior extreme of the cerebellum, as well as the sinuses that run along the tips of the lateral cerebellum wings in the cerebellar contour. These sinuses will be superior to the cerebellum and may be mistaken for cerebellum.

Labeling

When cerebellum white matter is the only part of the cerebellum present it is extracted twice and labeled as both "cerebellum exterior," and as "cerebellum white matter." This is done so that the white matter is superimposed on an exterior, which is necessary for volumetric analysis with our tools. When both cerebellum white matter and gray matter co-exist, this outline is labeled as "cerebellum exterior."

Lateral Ventricles

General Description

The lateral ventricles are bi-lateral C-shaped structures that extend through all four lobes of the brain. They are filled with cerebral spinal fluid (CSF), and for this reason appear black on the MRI scan. There are five different parts to each lateral ventricle: the anterior horn (in the frontal lobe), the body (in the frontal and parietal lobes), the posterior horn (extending in the occipital lobe), the inferior horn (in the temporal lobes), and the atrium (where the body, inferior horn, and posterior horn meet). For the purposes of segmentation, we consider all parts except for the inferior horn as lateral ventricle. The inferior horn is labeled as "inferior lateral ventricle" and its method of extraction is described elsewhere.

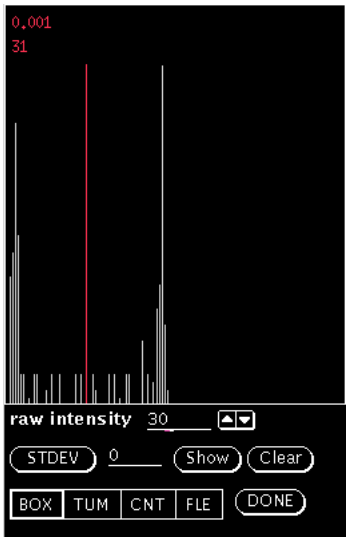
The lateral ventricles are bordered anteriorly by white matter. As you move posteriorly, the lateral wall of the ventricle is bordered by the caudate, and medially by white matter. Moving posteriorly, the lateral ventricles may appear as if they are connected along the midline. They are actually separated by the septum pellucidum. At this point the bottom wall of the lateral ventricle is bordered by thalamus. As you continue to move posteriorly towards the atrium, the thalamus no longer borders the ventricle; hippocampus becomes the medial border. Caudate still comprises the medial border of the ventricle, but it is difficult to visualize on the MRI scan. As you move past the atrium, the lateral ventricle is surrounded by white matter.

Procedure

Segmentation

The histogram method is used to create outlines for the lateral ventricles. One histogram is needed to determine the CSF/white matter border, and another is used to define the CSF/gray matter border. A separate box and histogram should be generated for each ventricle.

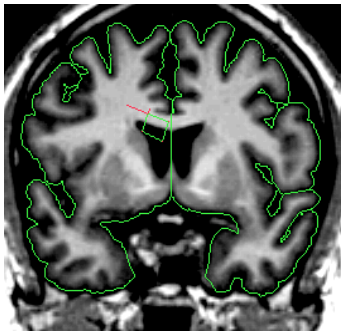
Part I - Anterior portion of lateral ventricles

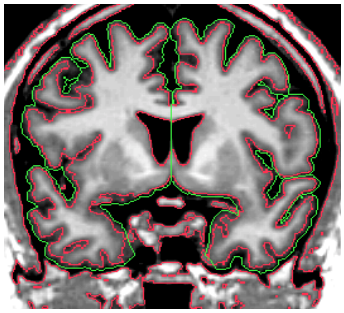


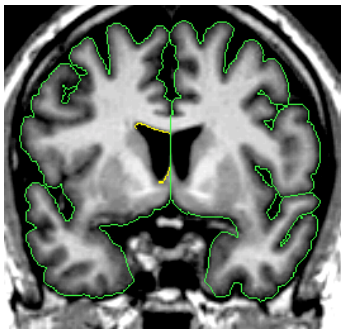
To begin segmenting the lateral ventricles draw a small box that contains CSF from the center of the ventricle and white matter from the corpus callosum. The box should be drawn to include most of the ventricle and an approximately equal amount of bright white matter. Use the darkest CSF and brightest white matter in the box drawn so as a larger contrast is generated (It is usually best, in meeting this goal, to use white matter that is lateral and superior to the ventricle in the histogram box). Generate a histogram and extract the lateral ventricle outline from the outside first, then the inside.

In the extremes of the lateral ventricles' extent, partial voluming of the ventricle and white matter leads to grayish areas, which are neither entirely ventricle nor entirely white matter. If some full-volume lateral ventricle is present, yet you've determined that lateral ventricle exists on the slice in question, use the intensity value (of the histogram line) of the full-volume lateral ventricle on the next or previous slice as a guideline for determining the lateral ventricle borders on the current slice. If no full-volume ventricle is present, draw the border halfway between the peaks of the full-volume ventricle and the full-volume white matter.

Part II - Caudate appears



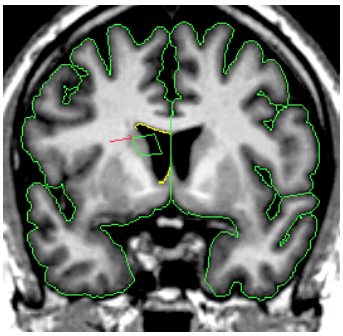




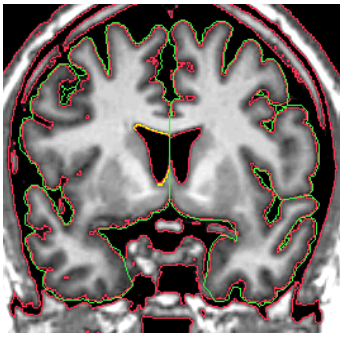
When the caudate is present two histograms are needed to define the two different borders of the ventricle.

Draw the first box and create a contour for the CSF/white matter border.

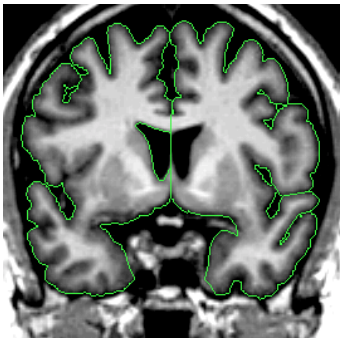
This contour line is not a true representation of the border between the caudate and the CSF, so a separate line will need to be created for that section. Clip the two ends of the CSF/white matter line where the caudate lies. For the purposes of the lateral ventricles, we consider the thalamus white matter, meaning its border with the lateral ventricle is the same contour line as the CSF/white matter line. Use the "v" function to "save" the line.



Now create a line for the CSF/caudate border. Your histogram box should include most of the caudate and an approximately equal sized amount of CSF.



In some cases you may need to manually connect your "saved" contour to the new contour. By convention, in cases in which the caudate is present, include the most inferior extent of the CSF/white matter border as the lateral ventricle border, even if that necessitates drawing a short line from it to the CSF/gray matter border.



Be sure to include your "saved" line when extracting.

The Foramen of Monroe is the passage between the lateral ventricle and the third ventricle. We include it as part of the lateral ventricle, assuming it was included in the contour lines created by your histograms. If not, do not manually draw it in.

Part III - Posterior portion of lateral ventricle

Make sure the lateral ventricle is actually a ventricle, rather than a deep sulcus. To do this, look in other planes using the projection lines. There should be white matter between ventricle and the gray matter. Lateral ventricle can disappear for a few slices, and then re-appear.

In the extremes of the lateral ventricles' extent, partial voluming of the ventricle and white matter leads to grayish areas, which are neither entirely ventricle nor entirely white matter. If no full-volume lateral ventricle is present, yet you've determined that lateral ventricle exists on the slice in question, use the intensity value (of the histogram line) of the full-volume lateral ventricle on the next or previous slice as a guideline for determining the lateral ventricle borders on the current slice. If some full-volume ventricle is present, draw the border halfway between the peaks of the full-volume ventricle and the full-volume white matter.

AutoSeg
Using AutoSeg for the lateral ventricles is very similar to the procedures described above. The added advantage is that you can extract both sides at the same time. You will be using two different AutoSeg variables for the lateral ventricles: CSF-white, and CSF-gray, for the CSF-white matter border, and CSF-caudate border, respectively.

Setting AutoSeg parameters
You should set the AutoSeg values every time there is a major change in the lateral ventricle shape, and borders. Check the most anterior extent, a few slices back where there is little partial voluming, where the caudate first appears, where the putamen begins to appear, where the accumbens disappears, where the caudate and lateral ventricle become shorter, where the caudate disappears, where the lateral ventricle becomes larger again, where the lateral ventricle and inferior lateral ventricle are no longer distinguishable, where the lateral ventricle becomes small again, and where the lateral ventricle disappears and reappears (if applicable). You will compare the CSF-white AutoSeg value with a histogram you have created for the CSF-white matter border. Make sure to check both sides.

If you start to notice that there is a large difference between the CSF-white intensities between the left and right ventricle, you will not be able to use AutoSeg for both sides.

You will also need to check the CSF-gray AutoSeg values against the CSF-caudate histogram. Again, check both sides. You should check this value when the caudate begins, when there is a clear distinction between the caudate and CSF (no partial voluming), where the nucleus accumbens disappears, where the caudate and lateral ventricle become shorter, and where the caudate disappears.

Segmenting with AutoSeg
When the caudate is not present, simply hit the CSF-white AutoSeg button and extract the CSF-white contour AutoSeg gives you. Extract this outline from the outside, then the inside.

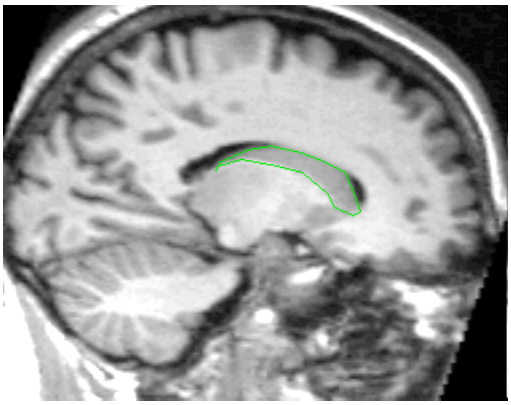
When the caudate is present hit the CSF-white button to reveal this contour. Clip the ends as described above. "Save" the line with the "v" function. Next, hit the CSF-gray button. Extract the lateral ventricle outline being sure to include your "saved" line.

Often you will find that the lateral ventricle on one side begins or ends a few slices before the other side. When this happens, your AutoSeg values are likely to be off. Manually create these outlines as described above.

Labeling
These outlines are labeled as "lateral ventricle."

Caudate

General Description



The caudate is a C-shaped structure with an enlarged head deep in the frontal lobe and an increasingly attenuated body and tail which follow the lateral ventricle around into the temporal lobe. We do not consider the tail of the caudate in the CMA method.

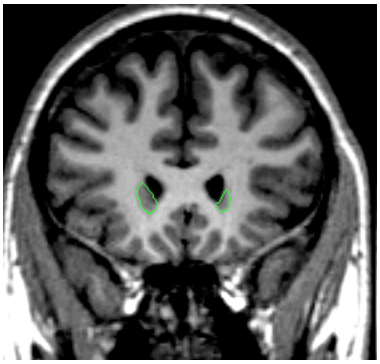
In the coronal view the caudate appears lateral to the lateral ventricles in each hemisphere. The caudate begins very small and reaches its largest extent in the early to mid region and grows smaller as you travel more posterior until it finally disappears. The caudate is bordered inferiorly by white matter, the thalamus (when present), or the nucleus accumbens (when present). The caudate is bordered superiorly by the transverse fibers, or white matter. Laterally, the caudate is bordered by white matter. The medial border is the lateral ventricle.

Procedure

Segmentation

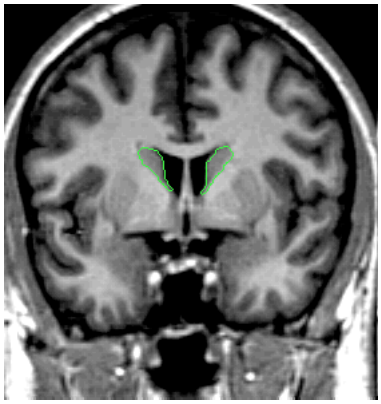
The histogram method is used for creating the caudate outline.

Part I - Anterior portion of the caudate

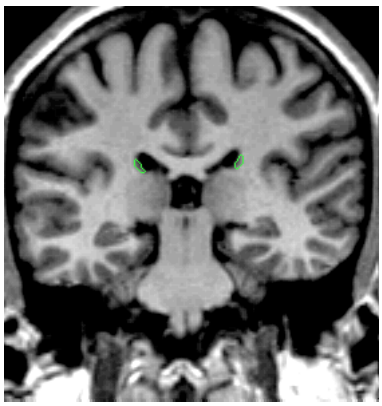


The histogram box should extend from the gray matter at the center most point of the caudate into the surrounding white matter. Approximately equal amounts of both gray matter and white matter should be included in the box.

You may have to manually draw lines to close the caudate against the ventricle before extracting from the inside.



It is important not to include the transverse fibers that run along the superior edge of the caudate. These fibers should be excluded from both caudate and lateral ventricle. We also do not extract the vertical portion or the tail of the caudate.



Anteriorly, there is some partial voluming, so care should be taken not to overestimate the caudate. Projection lines should be used to determine the true extent of the caudate.

Part II - Posterior portion of the caudate

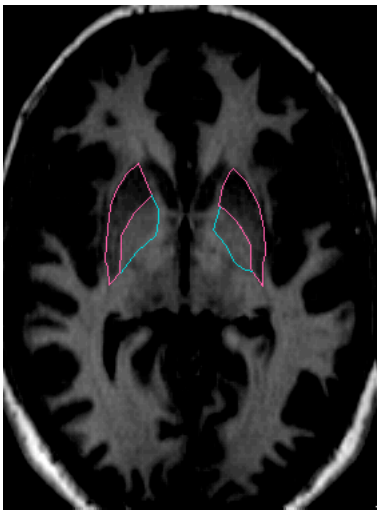
Posteriorly, it is often difficult to get a useful histogram, so intensity contour can be employed.

Labeling

This outline should be labeled as "caudate."

Putamen

General Description



The putamen is a subdivision of the lenticular nucleus (the other division is the pallidum). The lenticular nucleus from the axial view resembles a rounded triangle that is divided into two major sections. The putamen is the lateral part of this triangle.

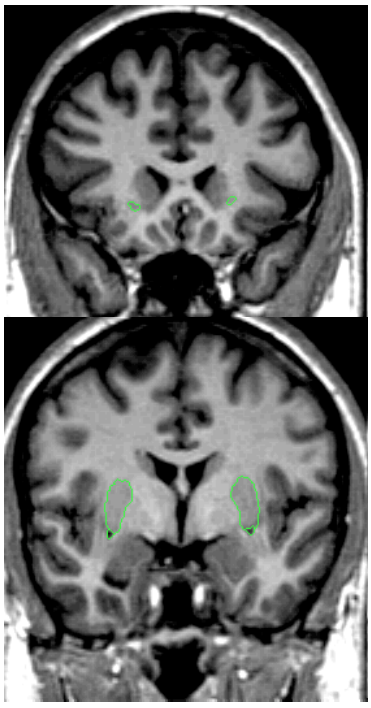
The putamen starts small and ends small in the coronal view. The putamen quickly grows to its greatest size in the middle and in the medial posterior portion it closely resembles a goldfish shape. The putamen lies lateral and partially anterior to the thalamus. It is bordered laterally, superiorly, and inferiorly by white matter.

The putamen is usually bordered medially by the pallidum. When the pallidum is not yet present or has already disappeared the putamen is bordered medially by the internal capsule.

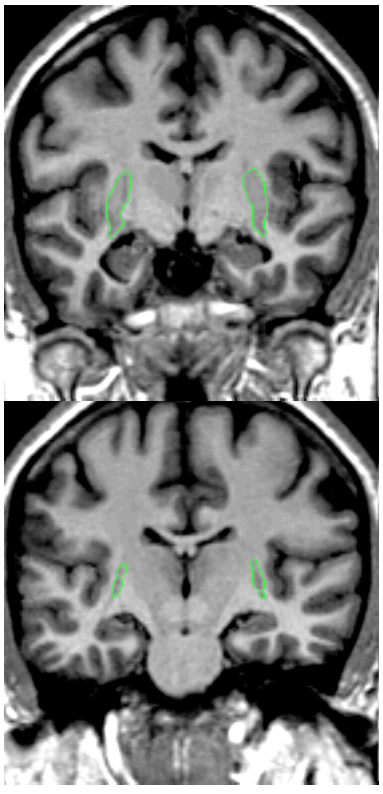
Procedure

Segmentation

The putamen outline is created using the intensity contour function and manual drawing.



Begin by generating a partial outline that you think fits a portion of the putamen, then using the erase function, clip this line where it no longer fits the putamen. Use the "v" function to "save" the line. Create another line with the intensity contour function to generate the rest of the outline. Clip this line where it joins your first line. Use the "v" function again to save this new line. Now extract the completed putamen from the outside, and then extract from the inside (to remove any stray voxels from the volume of the structure).



It is important not to include the claustrum in the putamen; this is the strip of tissue bordering the lateral edge of the putamen.

Often the putamen can be extracted at the same time as the caudate especially in the area where they are connected by the nucleus accumbens. The histogram of the caudate, in many cases, is close to what you want for the putamen as well. After extracting the caudate, before deleting the remaining red lines, you can often go immediately into the intensity contour function and adjust them to fit the putamen. This should be done separately for each hemisphere.

Anteriorly, it is important not to include all of the "fish tail" of the putamen, this is the portion that appears to extend to the amygdala. There should be a strip of white matter between amygdala and putamen. More posteriorly the "fish tail" shape may be more pronounced, not extending to the amygdala, and should be taken as part of the putamen.

There is often a blood vessel near the inferior border of the putamen, this should not be included as part of putamen. The vessel should be extracted separately using a contour line and labeled "vessel." The vessel will serve as at least a portion of the inferior border.

Labeling

The final outline should be labeled as "putamen," and any blood vessels extracted should be labeled as "vessel."

Nucleus Accumbens

General Description

In the area just above the orbital surface of the frontal lobe the head of the caudate appears to be continuous with the anterior part of the putamen. This region of continuity is the referred to as the nucleus accumbens.

The nucleus accumbens is bordered superiorly by the internal capsule, caudate, and putamen. It is bordered inferiorly by white matter, or in its most posterior extent by the subcallosal gyrus. Its medial border is the septal nuclei, and/or the lateral ventricle. Laterally it is bordered by the putamen.

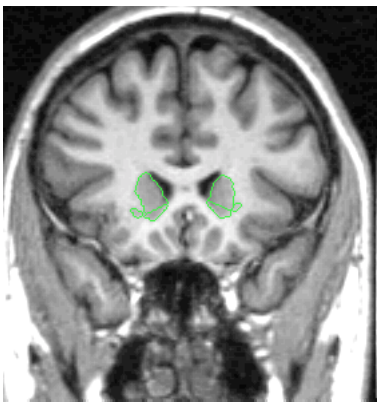
Because the exact borders of the nucleus accumbens are not distinguishable in a standard MRI, the CMA has devised a convention for the segmentation of this structure.

Procedure

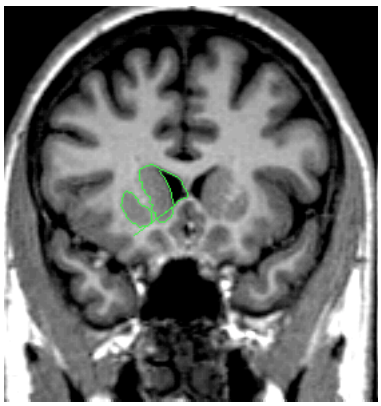
Segmentation

The outline for the nucleus accumbens is created using the intensity contour function. The outline is most often taken at the same time as putamen and/or caudate. The nucleus accumbens is isolated by separating the caudate from the putamen. As a rule, accumbens is not taken if anterior commissure is visible.

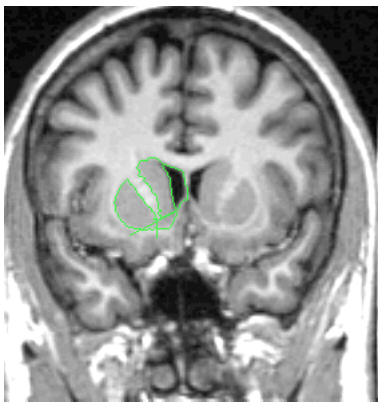
Part I - Caudate and putamen are discontinuous



The most anterior nucleus accumbens is taken in the first slice where both the caudate and putamen are present. When the caudate and putamen are not connected, an oblique line should be drawn from the inferior most tip of the lateral ventricle (where it meets the caudate) to the inferior most, medial tip of the putamen. This line will provide the superior border of the accumbens. The inferior border should be achieved using intensity contour, most often the same line used for the caudate.



Part II - Caudate and putamen are continuous



When caudate and putamen are connected, an oblique line should be drawn from the inferior most tip of the lateral ventricle to the lateral-inferior most tip of the internal capsule (the white matter area between the caudate and putamen). If the white matter tip is an "island" it should be connected with a line to the rest of the internal capsule and the division lines should be drawn with the "island" as the reference (you must also extract this “island” and label as “cerebral white matter.” Adjust the intensity contour to clearly see the full extent of white matter, to its most inferior extent. This line will create the border between the caudate and the accumbens. From this point, a straight, vertical line should be drawn to provide the border between accumbens and putamen. The accumbens should end a slice or two in front of anterior

commissure.

This area can be tricky in terms of order of extraction and method of achieving the conventions. It is helpful to play around to figure out your own style, but we recommend the following procedure: extract caudate and putamen each with their own method and provide a temporary inferior border for each structure. Find borders for nucleus accumbens either with intensity contour or by manual drawing (often useful for the inferior border). Extract all three structures together from the outside, unextract the putamen and caudate, erase (using x) the temporary borders. This will give you a big "U" shape. Isolate the accumbens as described above, then unextract the "U" from the outside and re-extract each structure individually.

The inferior border of the accumbens is defined by axons of the diagonal band; play with intensities to view the differences. The diagonal band divides the accumbens from subcallosal area, be sure not to include subcallosal area in the accumbens.

Labeling
The final outline should be labeled "accumbens area."

Pallidum

General Description

The pallidum is one subdivision of the lenticular nucleus (the other is the putamen). As seen from the axial view, the lenticular nucleus resembles a rounded triangle. The pallidum makes up the smaller medial part of this triangle.

In the coronal view the pallidum resembles a rounded triangle that continues off of the putamen. The pallidum starts anteriorly as a small triangle, reaching its largest extent in its most medial slices, and then dwindles to a small triangle again in the most posterior slices.

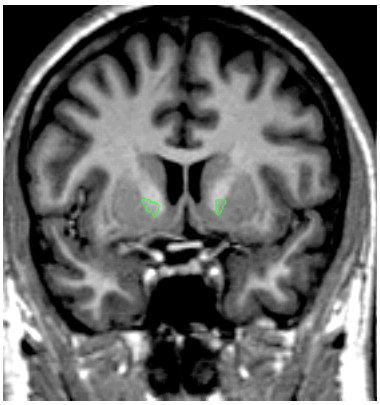
The pallidum is essentially surrounded by white matter except for the lateral border, which is the putamen. The putamen and pallidum are separated at this border by a thin strip of white matter called the lateral medullary lamina. Medially, pallidum is difficult to discern from the surrounding white matter because, although it is gray in terms of its intensity, its darkness is frequently fainter than the putamen.

Procedure

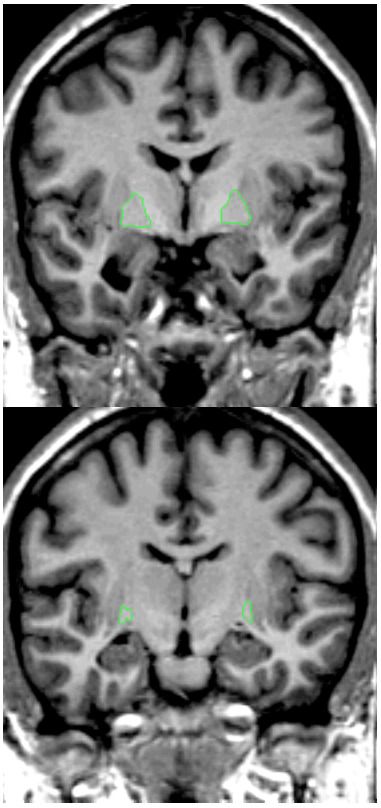
Segmentation

The pallidum should be extracted using the intensity contour function.

Part I - Putamen and pallidum



It is helpful to extract the putamen first, in one hemisphere, then only slightly adjust the contour line to extract the pallidum in the same hemisphere. Since the pallidum and putamen share a border, it is helpful to use the axial view to accurately discern between the two structures. The thin strip of white matter (the lateral medullary lamina) that separates these structures should be extracted as part of pallidum.



Labeling

The final outline should be labeled "pallidum."

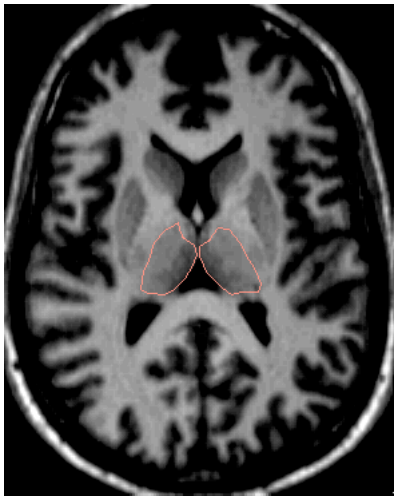
Thalamus

General Description

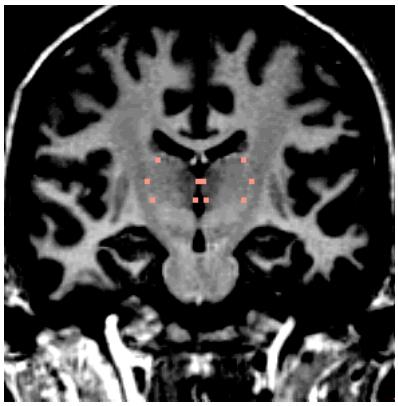
The thalamus is a large egg-shaped nuclear mass present in both hemispheres at the midline. The two thalami are separated medially by the third ventricle, cerebro-spinal fluid (CSF), or the cerebral exterior midline. They are bound laterally by the internal capsule. Each thalamus extends anteriorly to the interventricular foramen (foramen of Monroe), and posteriorly the thalami overlap the midbrain and are bordered by CSF. The inferior border is the hypothalamic fissure, or the hippocampus in the most posterior extent. Superiorly the thalamus extends to the transverse cerebral fissure (TCF), lateral ventricle, white matter, or in the anterior portion, the caudate.

Procedure

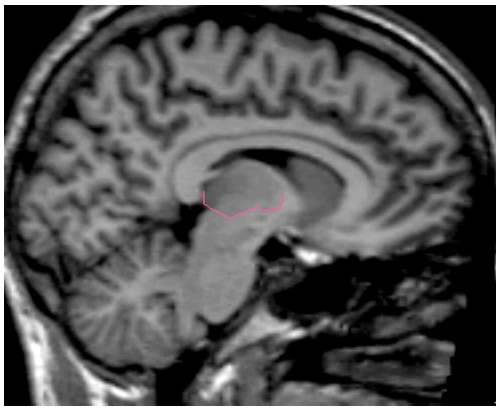
Sulci Lines

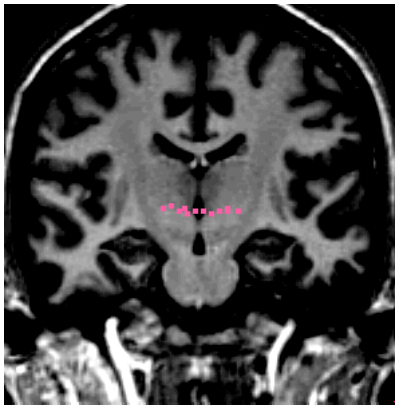


By drawing many sulci lines in the dorsal axial view, the mid axial view and the ventral axial view of the thalamus you can obtain a skeleton of the outline of the thalamus produced by "dots" marking the sagittally drawn sulci in the coronal view. This gives you a guide to find the best fit contour line.



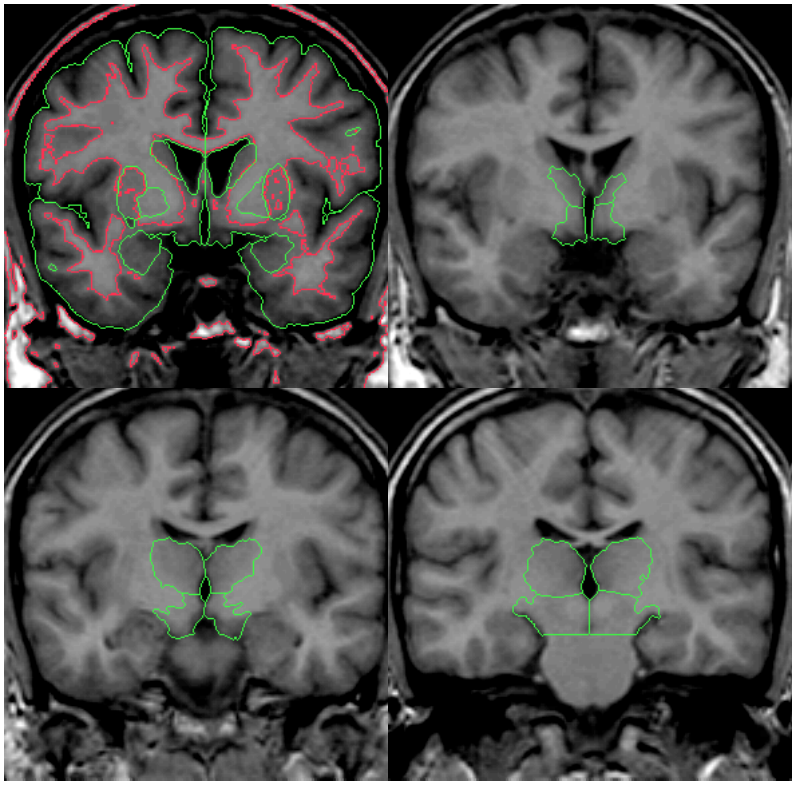
The hypothalamic fissure serves as the inferior border of the thalamus and a dividing line between the thalamus and the ventral diencephalon (VDC). This border is marked by drawing sulci in the medial sagittal views of the thalamus and VDC. The hypothalamic fissure is seen most easily toward the midline in the sagittal view, therefore begin drawing the sulci lines on or close to the most medial sagittal slice. The fissure should be drawn as a line that cups the bottom of the thalamus beginning caudally and moving rostrally as far as possible. It is often advantageous to draw many sulci on consecutive sagittal slices, moving from medial to lateral, in order to see the full extent and curve of the fissure more accurately in the coronal view.





Segmentation
Outlines for the thalamus are created using contour lines as well as manual drawing.

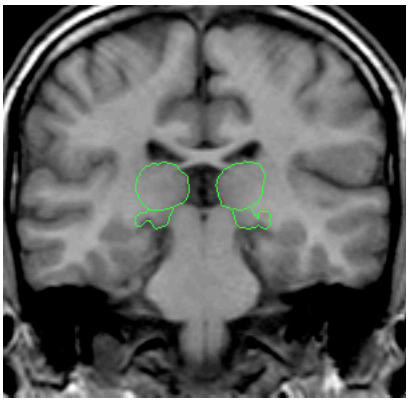
Part I - Anterior portion of the thalamus



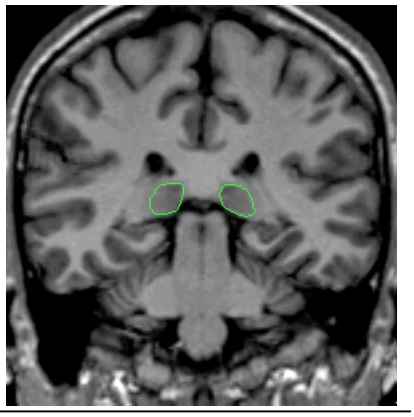
The anterior most extent of the thalamus appears after a few slices of the anterior VDC and is usually just posterior to the anterior commissure. In this region the thalamus appears as a thin sliver that gradually grows bigger and rounder as you move more posterior. The thalamus is bordered medially by the third ventricle. The dorsal border extends superiorly past the dorsal border of the third ventricle but not quite to the lateral ventricle or caudate. As you move more posterior the thalamus begins to touch the lateral ventricle, white matter, and in some cases a little of the caudate.

Use the intensity contour function to obtain an outline that tightly hugs the lateral edge of the thalamus but does not exclude any thalamus. This contour will provide most or all of the lateral border of the thalamus and should meet the superior border provided by the caudate or lateral ventricle. Some manual drawing may be necessary, particularly along the ventro-lateral border. This border should never extend to the pallidum. Projection lines should be used in conjunction with sagittal and axial views when boundaries become less clear.

Part II - Posterior portion of the thalamus



The posterior extent of the thalamus is referred to as the pulvinar. The pulvinar extends posteriorly past the VDC and is located just superior and medial to the hippocampus. In this region the pulvinar is egg shaped and grows smaller as you move to the most posterior extent.



The dorsal medial border must be drawn in order to exclude the fornix and separate the thalamus from the tissue between the ventricles. This is achieved by manually drawing in the border along the foramen of Monroe or, if the foramen of Monroe is not visible, from the bottom corner of the lateral ventricle to the medial cerebral exterior line or third ventricle. The inferior border of the thalamus can be drawn by connecting the "dots" that result from the sagittally drawn sulci lines in the coronal view.

Labeling
This outline is labeled as "thalamus proper."

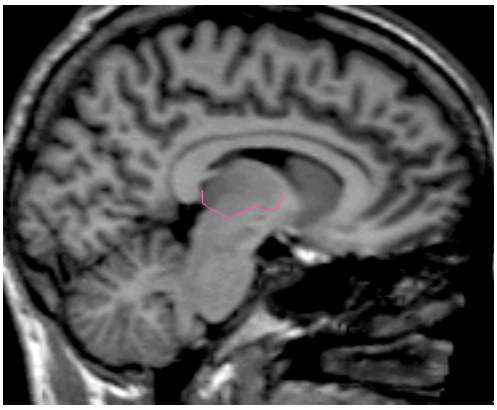
Ventral Diencephalon

General Description

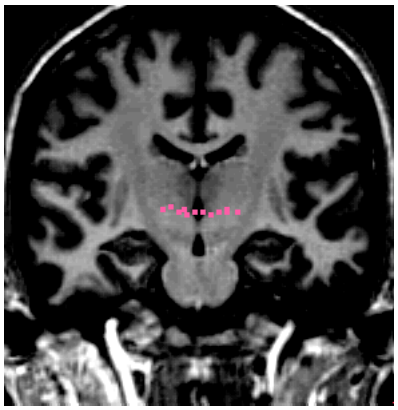
The ventral diencephalon (VDC) is not an anatomical name for a single structure but a name given by the CMA to a group of structures that generally cannot be distinguished from each other with standard MRI images. This "miscellaneous" area includes the hypothalamus, mammillary body, subthalamic nuclei, substantia nigra, red nucleus, lateral geniculate nucleus (LGN), and medial geniculate nucleus (MGN). White matter areas such as the zona incerta, cerebral peduncle (crus cerebri), and the lenticular fasciculus are also included in this area. The optic tract is included in this area in the most anterior extent. Each structure fades in and out of the VDC at different times. Therefore, the VDC greatly varies from slice to slice.

Procedure

Sulci Lines



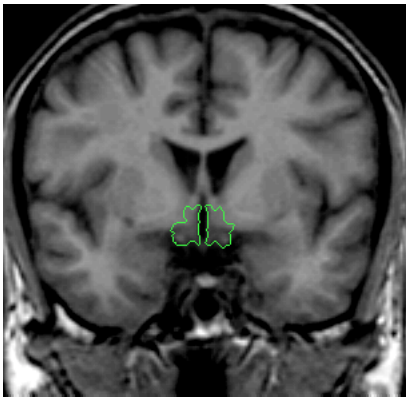
The superior border of the VDC is defined by the hypothalamic fissure, and is the dividing line between the thalamus and the VDC. This border is marked by drawing sulci in the medial sagittal views of the thalamus and VDC. The hypothalamic fissure is seen most easily toward the midline in the sagittal view, therefore begin drawing the sulci lines on or close to the most medial sagittal slice. The fissure should be drawn as a line that cups the bottom of the thalamus beginning caudally and moving rostrally as far as possible. It is often advantageous to draw many sulci on consecutive sagittal slices, moving from medial to lateral, in order to see the full extent and curve of the fissure more accurately in the coronal view.



Segmentation

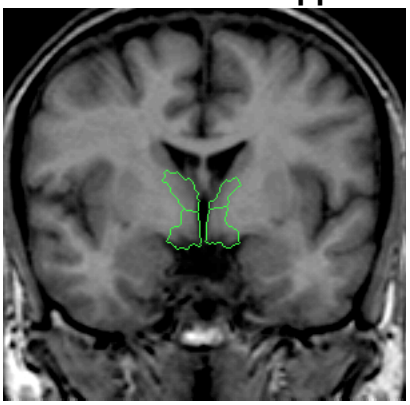
The outline for the VDC is created using contour lines and manual drawing.

Part I - Anterior portion of VDC

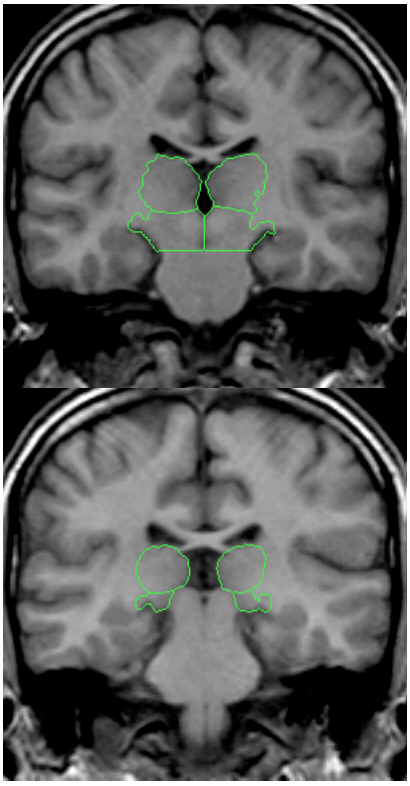


The anterior VDC starts one slice posterior to the anterior commissure and should be defined by an isointensity contour line. The superior border is the hypothalamic fissure, and more posteriorly the thalamus. This contour should exclude the anterior commissure, fornix, or any surrounding white matter and by convention should extend only a little wider than the optic chiasm (when present). The VDC should never extend to the pallidum.

Part II - Thalamus appears



In most cases the same contour line used for the thalamus will work for the lateral borders of the VDC. The contour line should provide most of the lateral border of the VDC, though some manual editing with the draw function may be required. If the best-fit contour shows the internal capsule, or cerebral peduncle cutting dramatically into the VDC manually edit the contour to include that portion of the internal capsule, or cerebral peduncle in the volume of the VDC.



The brainstem will be the inferior border as defined by the sulci line drawn at the top of the brainstem.

The LGN may have to be drawn in manually or defined by a separate contour. The choroidal fissure is the inferior border of the LGN. The most posterior extent of the VDC is the LGN, therefore when the LGN is gone there is no more VDC.

Labeling

This outline is labeled "VentralDC."

Inferior Lateral Ventricles

General Description

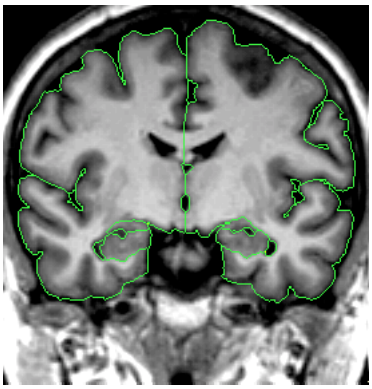
What we refer to as the inferior lateral ventricle (ILV) is actually the temporal horn of the lateral ventricle. This structure is located in the temporal lobe, and throughout its course it will change significantly in shape. The inferior lateral ventricle is extracted so as to exclude it from the hippocampus and amygdala outline; it is not considered a reliably extracted structure that is used for its own morphometric analysis. Most researchers combine the volume of the inferior lateral ventricle with what was previously described as the lateral ventricles when doing morphometric analysis on the lateral ventricles. Because of this, there are many methods that can be used to extract the ILV.

Procedure

Segmentation

The ILV outline is created using either the histogram or intensity contour line methods.

Part I - Anterior portion of the ILV



Anteriorly, the ventricle will appear more as a curved structure that follows the rounded lateral edge of the hippo-amyg area. Moving posteriorly the inferior lateral ventricle will comprise the border between the hippocampus and amygdala.

Create an intensity contour line for the ILV. Your contour line will approximate the value of the lateral ventricles and/or the part of the tightened exterior that borders the hipp/amyg area. Once complete, extract this outline from the outside, and then the inside.

It is also acceptable and more accurate to use a multiple-peaked histogram for the ILV. Draw one box that contains all three of the structures that make up the ILV borders (CSF, white matter, hipp/amyg), and then use the corresponding peaks to create the ILV outline. The first to second peaks will represent the CSF to hipp/amyg (gray) averaged intensity, and the second to third peaks represent the hipp/amyg (gray) to white matter averaged intensity. Use the "v" function to create the outline.

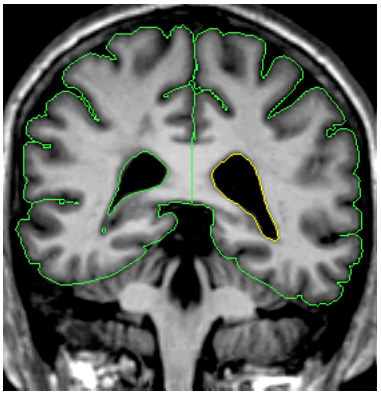
Part II - Medial portion of the ILV

Moving posteriorly, when the amygdala is gone or almost gone, it may be difficult to determine the medial extent of the inferior lateral ventricle. Depending on the brain, it may appear that the inferior lateral ventricle is continuous with the exterior outline. There is in actuality a small membrane called the tele choroides of the lateral ventricle that separates the inferior lateral ventricle from the outside of the brain. Brightening the screen may help to see this thin membrane. If it is not possible to see this border, discretion must be used. The inferior lateral ventricle extends medially to the subiculum, and not past. Once you have made a decision as to the most medial extend of the inferior lateral ventricle, remain consistent with this decision through the posterior course of the brain. As you continue to move more posterior, this will no longer be an issue.

Part III - Posterior portion of the ILV

Toward its posterior endpoint, the ventricle will appear as a small circle that is adjacent to the ventral-lateral corner of the hippocampus. The course of the inferior lateral ventricle is sometimes interrupted, and may be absent for a slice or two but then reappear. Use of the projection lines will

verify the extent of the ventricle at its anterior and posterior limits. At its posterior endpoint, the inferior lateral ventricle will become continuous with the lateral ventricle outline.



Labeling
This outline is labeled as "inferior lateral ventricle."

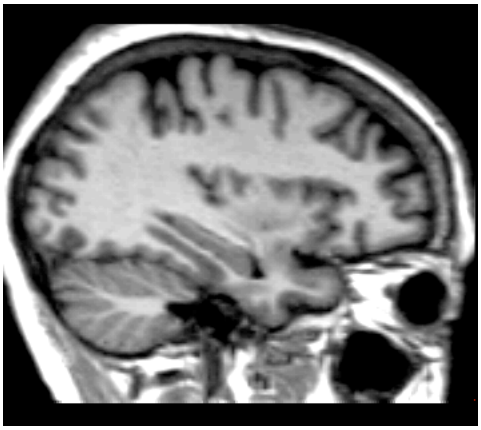
Amygdala

General Description

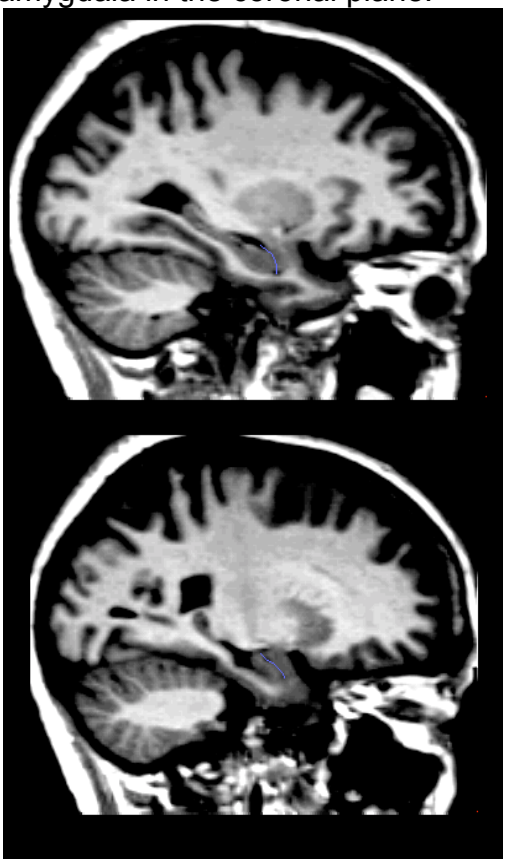
The amygdala is located in the medial temporal lobe. It has a rounded shape and is situated anterior and superior to the hippocampus. Anteriorly, the amygdala borders the entorhinal/perirhinal temporopolar cortex. Superiorly, it borders the basal forebrain and the choroidal fissure. Medially, the amygdala borders the entorhinal cortex (in its anterior most tip) as well as the subarachnoid CSF of the medial temporal surface. Its lateral borders are the temporal horn of the lateral ventricle as well as (more rostrally) the white matter core of the temporal pole. Inferiorly, it borders with the entorhinal cortex (PHa), and more posteriorly, the hippocampus. Its caudal border is the hippocampus.

Procedure

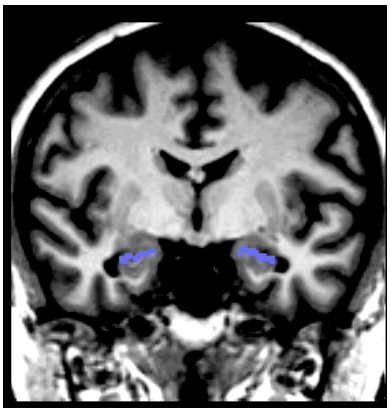
Sulci Lines



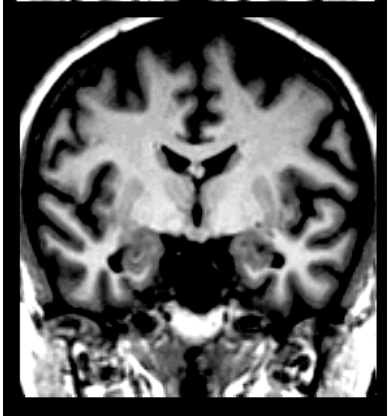
The amygdala is very difficult to see in the coronal view. For this reason, both the sagittal and axial views are very important in understanding the shape of the amygdala. The user should make an effort to learn the anatomy of the amygdala very carefully in these views. Furthermore, sulci lines will prove very useful in determining the borders of the amygdala in the coronal plane.



Sulci lines must be drawn in NAV mode. Use the sagittal view to separate the hippocampus from the amygdala. Start with one side of the brain and move to approximately the most lateral extent of the amygdala. On this slice you will see the hippocampus, a large portion of the inferior lateral ventricle (ILV), and a small gray area that is the amygdala.



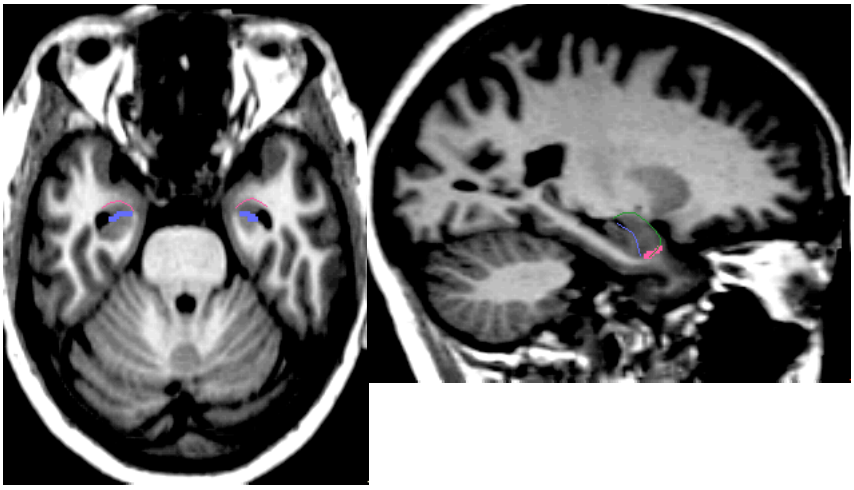
Continue to scroll the sagittal image medially. As you do, the size of the ILV will decrease, and the grayness of the amygdala will become more pronounced. When the ILV is very thin, and the amygdala and hippocampus look as if they will touch soon, draw a sulci line from the superior to the inferior border of the ILV, right in the middle of the ventricle, separating the hippocampus from the amygdala.



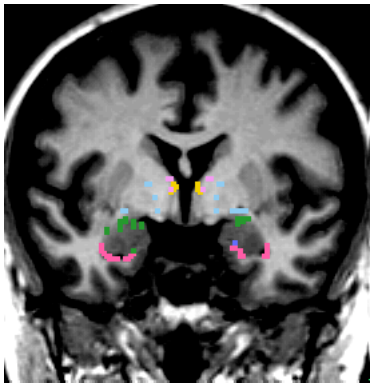
Continue to move medially, and continue to draw sulci lines bisecting the ILV. Draw sulci lines only on slices where you can see the border

between the hippocampus and the amygdala.

As you move more medially, this differentiation will be difficult to see. There are two clues you can use to guide you as you draw these lines. First, the ILV will appear as dark pixels. You will most likely see part of the ILV superior to the hippocampus. You should also see a very tiny dark dot at the inferior-anterior border of the hippocampus. This is part of the ILV, specifically its anterior-most tip. Using the general shape of the ILV you observed in more lateral sagittal views, draw a sulci line from the most superior part of the ILV you can see to its anterior-most tip. A second clue to the division between the hippocampus and amygdala is that the superior border of the hippocampus includes the fimbria. This will appear as a white line just below the ILV. If this can be seen, it can be used as a guide; draw your sulci line along the black pixels that are above this white line.



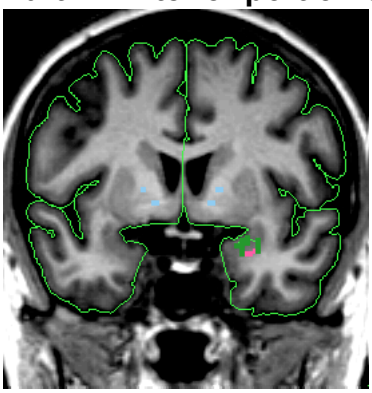
Drawing lines around the amygdala in the axial view (particularly inferiorly) and in the sagittal view (particularly medially) will help with the shape of the anterior amygdala in the coronal view.



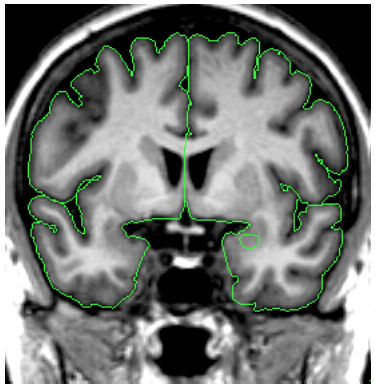
Segmentation

The amygdala is segmented using a contour line and manual editing.

Part I - Anterior portion of the amygdala



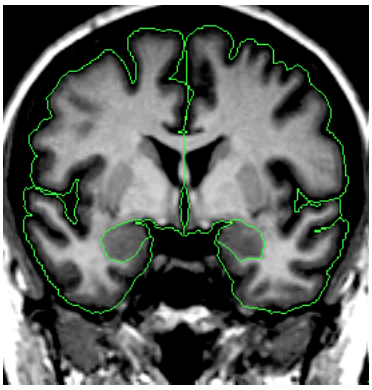
The amygdala begins when the cortex begins to look "puffy." If you've drawn amygdala sulci lines, these will start to appear on your first slice of amygdala. On this slice, check both the sagittal and axial views to make sure there is indeed amygdala on that slice. Using an atlas as a guide to where the first slice of amygdala appears, draw a circle in approximately this area. Then, using projection lines, check both the sagittal and axial views to edit your circle to include only the amygdala. It is most useful to check the middle of your circle to approximate the dorsal-ventral, medial-lateral extent of the amygdala. Then check your medial-lateral approximations in the axial view to make sure you really are only including amygdala in this outline. Note that on this first slice, the amygdala should not touch the cerebral exterior.



The second slice of the amygdala should be segmented using the same methodology. The only difference is that the amygdala may touch the

cerebral exterior on this slice. Also, it may be possible to see the lateral extent of the amygdala on this slice. If that is the case, use a contour line to accurately capture this border of the amygdala.

Part II - Medial portion of the amygdala



As you move posteriorly, the amygdala becomes easier to visualize. Use a contour line to give the general outline of the amygdala, then check the other views available to you to confirm this outline.

When the amygdala is in its full extent, it is fairly easy to see in the coronal view. Remember that the amygdala has a very small wave that crests over the choroidal fissure. Refer to an atlas to see this wave more clearly. Alter the brightness of the screen so that you can adequately see the strip of white matter that separates the amygdala from the cortical areas.

Part III - Hippocampus appears

When the ILV is first visible, it is likely that the hippocampus is present; check for your hippocampal sulci lines as well. To segment the amygdala in this area, use a contour line to define the hip-amyg area. Call up your sulci lines. Depending on the brain, you will see darker pixels that represent the ILV, or lighter pixels that represent the fimbria of the hippocampus (or both) along your sulci lines. If possible, draw a line separating the amygdala from the hippocampus along the dark pixels of ILV. Try increasing the contrast between black and white to better see this division. If after manipulating the brightness/contrast this line is not visible, draw a line that bisects your sulci lines. Then use projection lines to verify that your line really is the division between the hippocampus and the amygdala. When you are satisfied, unextract the hip-amyg outline, and extract the top portion as amygdala.

Part IV - Posterior portion of the amygdala

As you move posteriorly, the amygdala will become smaller as the hippocampal area increases. Continue to follow the procedure outlined above to separate the hippocampus and the amygdala. Continue to take the amygdala until it is no longer visible on the coronal view.

Labeling

Label the most anterior slice of amygdala, which DO NOT touch the hemispheric margin, as "amygdala anterior." Label all subsequent slices of amygdala "amygdala."

Hippocampus

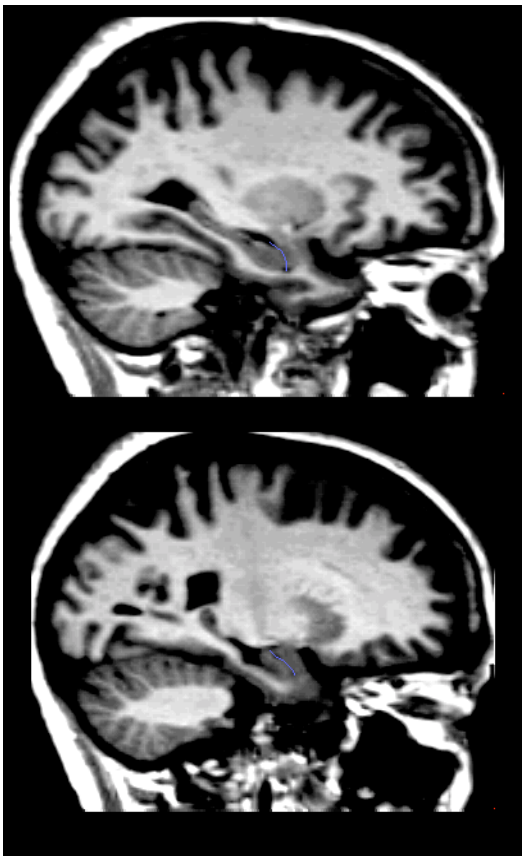
General Description

What we label as hippocampus is by many authors known as the hippocampal formation. It is comprised of the dentate gyrus, the ammonic subfields (CA1,CA2, CA3, CA4), the prosubiculum, and the subiculum. The hippocampus is located in the medial region of the temporal lobe, bulging in the floor of the inferior horn of the lateral ventricle. Anteriorly, and to some extent superiorly, it borders with the amygdaloid nuclear complex (amygdala). Laterally, the hippocampus borders with the temporal horn of the lateral ventricle. The medial border of the hippocampus is mainly with subarachnoid cerebro-spinal fluid (CSF). Inferiorly, the border between the hippocampus and entorhinal cortex (PHa) as well as posterior parahippocampal gyrus (PHp) is demarcated by white matter. The caudal end of the hippocampus is situated under the pulvinar, medial to the trigon of the lateral ventricle, whereas the rest of it is surrounded by white matter, and medially by subarachnoid CSF.

Procedure

The hippocampus is segmented using a contour line and manual editing. In its anterior extent, the hippocampus is very difficult to distinguish from the amygdala. Sulci lines drawn in the sagittal views to separate the amygdala and hippocampus are necessary to segment the hippocampus.

Sulci lines



Sulci lines must be drawn in NAV mode. Use the sagittal view to separate the hippocampus from the amygdala. Start with one side of the brain and move to approximately the most lateral extent of the amygdala. On this slice you will see the hippocampus, a large portion of the inferior lateral ventricle (ILV), and a small gray area that is the amygdala.

Continue to scroll the sagittal image medially. As you do, the size of the ILV will decrease, and the grayness of the amygdala will become more pronounced. When the ILV is very thin, and the amygdala and hippocampus look as if they will touch soon, draw a sulci line from the superior to the inferior border of the ILV, right in the middle of the ventricle, separating the hippocampus from the amygdala.

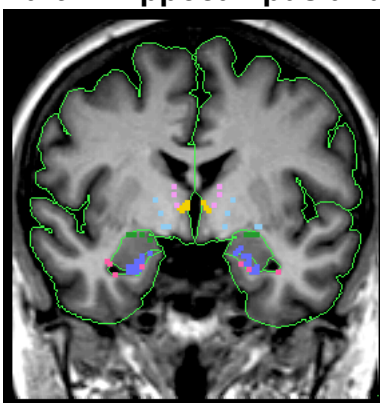
Continue to move medially, and continue to draw sulci lines bisecting the ILV. Draw sulci lines only on slices where you can see the border between the hippocampus and the amygdala.

As you move more medially, this differentiation will be difficult to see. There are two clues you can use to guide you as you draw these lines. First, the ILV will appear as dark pixels. You will most likely see part of the ILV superior to the hippocampus. You should also see a very tiny dark dot at the inferior-anterior border of the hippocampus. This is part of the ILV, specifically its anterior-most tip. Using the general shape of the ILV you observed in more lateral sagittal views, draw a sulci line from the most superior part of the ILV you can see

to its anterior-most tip. A second clue to the division between the hippocampus and amygdala is that the superior border of the hippocampus includes the fimbria. This will appear as a white line just below the ILV. If this can be seen, it can be used as a guide; draw your sulci line along the black pixels that are above this white line.

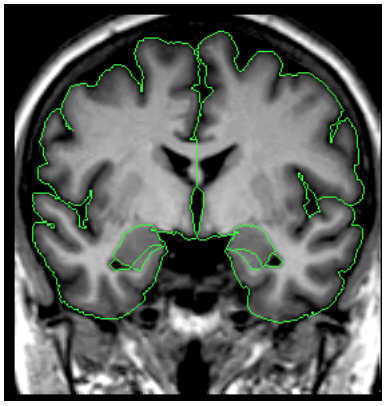
Segmentation

Part I - hippocampus and amygdala coexist



When the temporal horn of the lateral ventricle is first visible, it is likely that the hippocampus is present. Click on the draw sulci button to reveal your sulci lines.

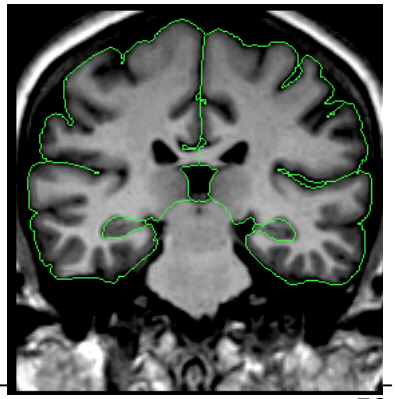
To segment the hippocampus in this area, use a contour line to define the hippocampus-amygdala area. Call up your sulci lines. Depending on the brain, you will see darker pixels that represent the ILV, or brighter pixels that represent the fimbria of the hippocampus (or both) along your sulci lines. If possible, draw a line separating the amygdala from the hippocampus along the dark pixels of ILV. Try increasing the contrast between black and white to better see this division. If after manipulating the brightness/contrast this line is not visible, draw a line that bisects your sulci lines. Then use projection lines to verify that the line really is the division between the hippocampus and the amygdala. When you are satisfied, unextract the hippocampus-amygdala (hipp-amyg) outline, and extract the bottom portion as hippocampus.



At this level, the hippocampus will appear as a small sliver, most likely along the medial border of the hipp-amyg area. To accurately define the medial border of the hippocampus, change the intensity of the screen to brighten the white matter pathway that separates the hippocampus from the cortex. Continue to segment the hippocampus in this manner as you move posteriorly. First use a contour line to define the hipp-amyg area, then edit the borders as necessary (i.e. along the medial border). Use your sulci lines as a guide for the separation between the amygdala and hippocampus. Draw in this border. Unextract the hipp-amyg outline, and re-extract the hippocampus separately. As you move posterior the division between these two takes on a saw-tooth pattern.

Also, the hippocampal area will increase as the amygdala area decreases.

A common error in segmenting the hippocampus is to include partial voluming of the ILV and white matter in your outline. The hippocampus does not extend laterally past the ILV. If part of your outline for the hippocampus does, edit as necessary.



Part II - amygdala disappears

When the amygdala is no longer present, a contour line is needed to outline the hippocampus. Depending on the brain, the inferior and superior borders of the hippocampus may not be adequately captured using one contour line. If this is the case, cut and paste multiple contours using the "v" function as described previously. At this point, the fimbria appear as thin white stripes at the dorsal edge of the hippocampus. You may find that the fimbria are being excluded from the

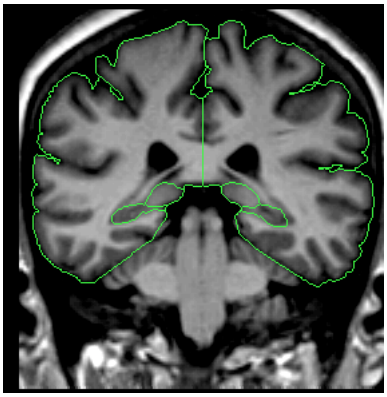
hippocampus when you use the intensity contour function. If necessary, manually draw in the superior border of the hippocampus above the fimbria. Only take fimbria when it's buried within the hippocampus. Do not take the gray matter above the fimbria.

Behind the posterior commissure, do not take fimbria/fornix. Minimize the white matter that you take.

A common error in this area of the hippocampus is to include partial voluming of the ILV in the hippocampus outline. Make sure the hippocampus does not extend lateral or superior to the ILV.

There are pockets of exterior within the hippocampus hat must be excluded from the hippocampus. Attach these pockets to the exterior.

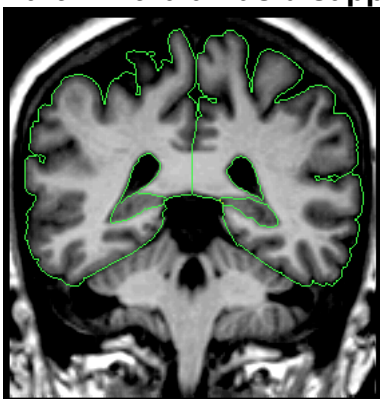
The inferior medial border of the hippocampus, which will include the subiculum, most of the presubiculum, and about a quarter of the parasubiculum, follows the trajectory of the white matter within the parahippocampal gyrus.



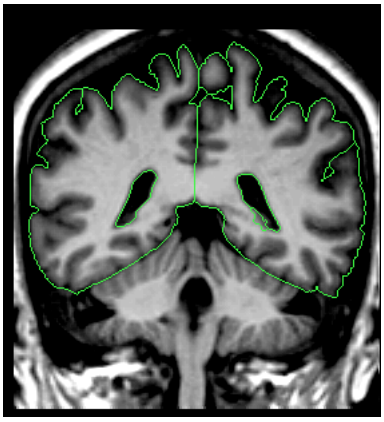
Part III - hippocampus and thalamus coexist

As you continue to move posterior, the hippocampus will start to share a border with the thalamus. To determine what is thalamus and what is hippocampus, projection lines are needed. At about this area, the fimbria are no longer visible, and therefore no white matter strip is included in the hippocampus outline. The hippocampus never wraps above the thalamus. Using the sagittal view is helpful in distinguishing this transition.

Part IV - thalamus disappears



In its posterior extent, the hippocampus will border the lateral ventricle, and will become large after the thalamus is not present anymore.



In its most posterior extent, the hippocampus will border the lateral ventricle laterally, and white matter medially. The posterior hippocampus tucks beneath the belly of the lateral ventricle. If the posterior hippocampus does not tuck beneath the belly of the lateral ventricle, use your projection lines to determine if structure is posterior hippocampus or cingulate.

Extraction
As with all sub-cortical structures, first extract the hippocampus from the outside. Hit "x" to get rid of stray contours. Then extract the hippocampus from the inside.

Labeling
The hippocampus is labeled as "hippocampus" in review mode.

Cerebral White Matter

General Description

The cerebral white matter is comprised of any area of the brain with a high concentration of axons covered in myelin. Because myelin is made of lipid, it has a white appearance in MRI scans. During segmentation the white matter is separated from the cortex and subcortical structures using the histogram function. There is a different white matter parcellation program used to divide the white matter into its constituents.

Procedure

Segmentation

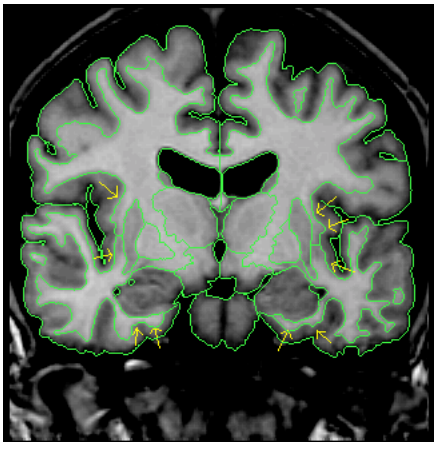
Cerebral white matter is extracted with the histogram method, as well as some manual drawing.

Create a histogram using the entire white matter-containing area as your "histogram box." Select the intensity that falls between the peak that represents cortical gray matter and that which represents white matter. This will generate your white matter contour line. Use a separate histogram for each side of the brain, and for the temporal lobes when they are not connected to the frontal lobes in the anterior portion of the brain. When the white matter is continuous between the temporal and frontal lobes use one histogram for the entire hemisphere.

If the white matter appears continuous on the MRI scan, but your histogram does not give you a continuous outline, manually draw in a strip of white matter between the two lobes.

If the two lobes are connected (share the same exterior outline) the white matter must be continuous.

By convention and by anatomy, cerebral white matter should never extend through the cortical ribbon. Radiologically speaking, an image may be produced in which partial voluming contributes to the illusion that the white matter extends to the edge of the cortex, though anatomically, this does not occur in the normal brain. Manually edit as necessary to ensure the white matter does not touch the cerebral exterior along the edges of the brain. White matter does extend to the medial exterior border where the corpus callosum is located. This should be apparent with your histogram, if this is not apparent, manually edit to include all of the corpus callosum.



Be sure to have continuous white matter lateral to the putamen, and below the hippocampus. Manually draw these borders if necessary.

In slices where the lateral ventricles are completely surrounded within a greater body of white matter, it is necessary to connect the white matter to the ventricle with a straight line before extracting the white matter from the inside. Any other "free-floating" structures should also be connected at this point before extracting the white matter. This convention is followed to make sure that the ventricles, etc., are excluded from the outline for the white matter (and subsequently, its calculated volume).

To be sure that you have extracted only white matter, click on the line believed to be white matter and it will now turn black. Make sure no subcortical structures are included. Having extracted the white matter in SEG mode correctly will prevent you from having to go back to fix any errors later.

In the most anterior and posterior extents of the brain, the histogram you generate for the white matter will look different than it does for the rest of the brain. You will probably see three peaks: one small peak on the right, one large peak in the middle, and one smaller peak on the left. In this instance, create a contour by dragging your mouse from the larger middle peak to the smaller right peak (it is often helpful to expand the histogram with the third mouse button). It may also be the case that the best-fit contour is between the middle peak and the last visible peak on your histogram (no matter what its height). Remember that it is unlikely you will have white matter on the very first and last slices of the brain.

Depending on the scan, you may witness a large extent of "drift." This term means that the intensity of the white matter is variable from one area to the other. For example, the white matter at the top of the image is much brighter than the white matter at the bottom of the image. In such cases, it may be necessary to piece together two white matter histograms. Draw a line between the top and bottom halves of the hemisphere. Extract the top portion and create a histogram. Clip the ends of the resulting contour at the point where it enters the bottom half of the hemisphere. Turn the contour yellow with the "v" function and remove stray lines. Then, using the histogram method, create a contour for the bottom part of the brain. Extract the two contours from the outside, and then the inside. Make sure you've completed all necessary manual editing.

AutoSeg

Setting AutoSeg Parameters

Follow the same rationale as you did with the cerebral exteriors by setting the white matter value in the center of the brain, and working your way towards the ends in halves. In fact, it is often easiest to set AutoSeg values on the same slices you set the cerebral exteriors. You should use the "Gray-White (midpeak)" button when you set the cerebral white matter. Make sure to adjust "Nauty's guesses" for the cerebral white matter AFTER you adjust the cerebellar white matter. See the section on “Setting AutoSeg Parameters” for the cerebellar white matter before proceeding further (the nature of AutoSeg would cause incorrect calculations if the “midpeak” values were set first).

Segmenting with AutoSeg

To segment with AutoSeg, click on the "gray-white (midpeak)" button after you've checked Nauty's guesses. This will bring up the contour for the white matter. Make any necessary manual edits. Extract the outline from the outside. Unextract the outline. Connect any "free-floating" structures to the midline. Then extract the white matter from the inside.

In order to use the AutoSeg values you would have run the bias-correction program on this brain. This means that you should not witness a great deal of drift in the white matter, and that your AutoSeg contours should be correct for both hemispheres, as well as for the upper and lower regions of the brain. However, it is possible that the bias-correction program was not powerful enough to reduce all drift. The most common example of this problem is having a white matter outline that looks correct everywhere except for the temporal lobes. If the temporal lobe is separate, manually segment this area with a histogram. If the temporal lobes are not separate, you will need to modify the outline around the temporal lobes. Draw a line

from the Sylvian fissure to the choroidal fissure. Extract the temporal lobe and create a contour line from the histogram. Save the contour line for the temporal lobe. Then connect it to the AutoSeg value given for the frontal/parietal lobes.

Labeling
Label this outline as "cerebral white matter."

On some slices the fornix is not part of your cerebral white matter outline because the lateral ventricle creates a division between the corpus callosum and the fornix. Extract this small area of fornix and label it as white matter.

Cerebellar White Matter

General Description

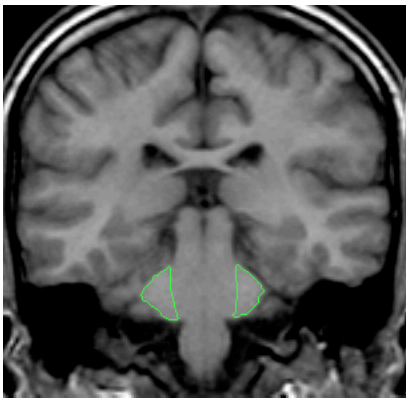
Similar to the cerebrum, the cerebellum has a network of white matter connecting different areas of the cerebellum or connecting the cerebellum to the rest of the brain. The white matter is most dense in the medial anterior areas of the cerebellum and disperses as you go more posterior and lateral. In the most anterior slices the cerebellar white matter is similar to two round masses hanging off of the brainstem, as you move more posterior the balls of white matter begin to have strings of white matter coming off of them. More posterior and in the medial part of the cerebellum the white matter begins to resemble two birds touching beaks at the midline. In the most posterior extent of the cerebellar white matter, the white matter becomes a bunch of white matter streaks that become smaller and more disperse and you move more posteriorly until finally, they disappear. The white matter of the cerebellum does not touch the outside of the exterior of the cerebellum except across the medial border (similar to the corpus callosum in the cerebral white matter).

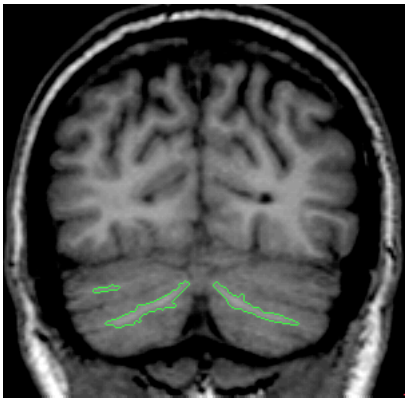
Procedure

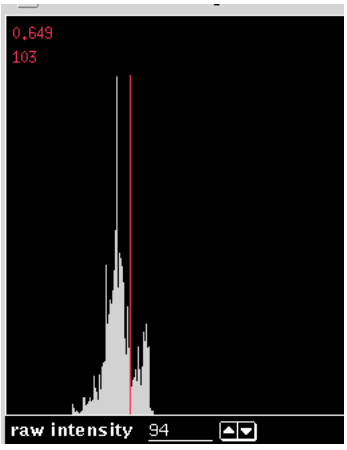
Segmentation

The histogram method is used to give the contour of the cerebellar white matter.

Finding the beginning of cerebellar white matter vs. the brainstem is very tricky because in most cases the white matter shows up before any cerebellar exterior. The CMA convention for this area is as follows: within a two-slice span (128-slice scan) before the appearance of the cerebellum, in the posterior part of the brainstem. There is a dramatic change in the lateral extent of the brainstem, based on color contrast and the appearance of the cerebellum. The more anterior of the two slices will contain a uniformly colored structure which is extracted singularly and labeled brainstem (there is not cerebellar exterior at this point). However, on the more posterior of the two slices, the lateral extremities of the brainstem are no longer taken as part of the brainstem outline. They are extracted separately as cerebellar white matter and again as cerebellar exterior. With the contrast increased it is easy to see the division between cerebellar white matter and brainstem. This division can be manually drawn in or it may be possible to use the contour function. The next slice (moving posteriorly) would have cerebellum exterior present, and these extremities will again be extracted as cerebellar white matter as given by the histogram.



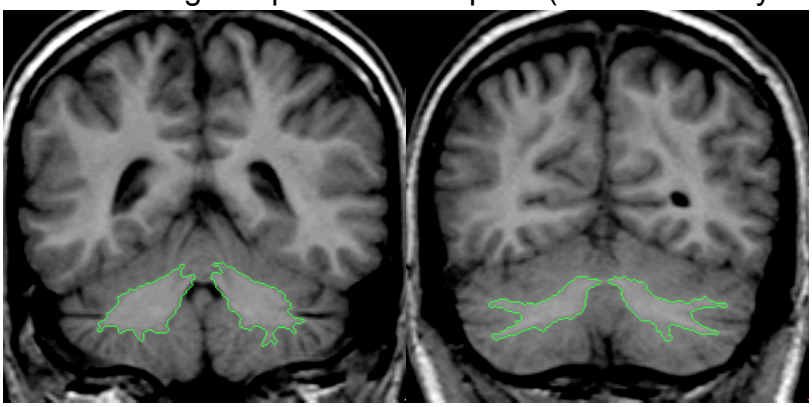


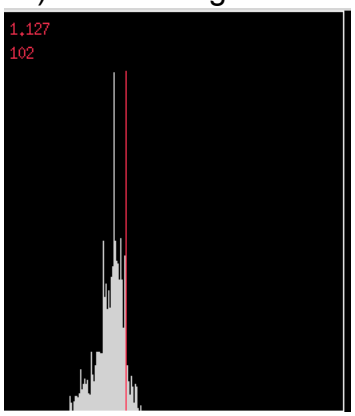


The histogram is taken from the whole area of the cerebellar hemispheres outline. The contour may need some manipulation especially in different areas of the cerebellum. This can be done by right clicking and dragging the averaging line (red line) in the histogram. Averaging the two highest peaks in the histogram should give the right contour.

This works for the most part until you reach the most posterior portion

of the white matter. Often the white matter border in the posterior portion is best given by averaging the middle highest peak with the peak (not necessarily the tallest peak) to the far right.





AutoSeg

Setting AutoSeg Parameters

Begin checking "Nauty's guesses" when the white matter appears as two masses within the cerebellum gray matter. Use the "gray-white" button (NOT the "gray-white (midpeak)" button). Check both sides and adjust rest as necessary. Nauty's guesses should be adjusted every time there is a change in the shape of the white matter: when the balls of white matter begin to have strings of white matter coming off of them, when the white matter begins to resemble two birds touching beaks at the midline, when the white matter becomes a collection of white streaks, and when the white matter ends.

Segmenting with AutoSeg

On the most anterior slices of cerebellum white matter (where it is difficult to distinguish from the brainstem), do not use AutoSeg. To segment the cerebellar white matter using AutoSeg, click on the "gray-white" button after you've checked "Nauty's guesses." Extract the outlines from the outside, then unextract and extract from the inside.

AutoSeg doesn't always interpolate correctly for the cerebellum white matter. If this is the case, it is easy to fix. Move to a slice where AutoSeg appears to have interpolated incorrectly, or where there is no interpolated value. Set the value you want for this slice. All the surrounding slices should then interpolate correctly. To avoid further problems, always set "Nauty's guesses" for the cerebellar white matter BEFORE segmenting the cerebral white matter.

Labeling

This outline should be labeled "cerebellar white matter."

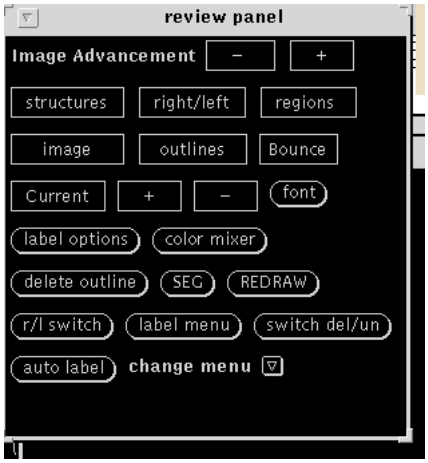
Labeling and Reviewing

After the brain is completely segmented it is necessary to label and review the segmented brain. Labeling of the brain is accomplished in REV (review) mode. This mode allows you to go through all of the structure outlines one by one on each slice, and attach labels to the outlines. REV mode also attaches a colorfill to each outline depending on the label. This produces a "cartoon" version of the brain, which allows for easy label review because every label is a different color.

The colorfilled version of the brain can also be viewed in Tile Display so one can review multiple slices. Another tool for review is the R-L orientation, which is also found in Tile Display. This tool allows one to make sure all structures are labeled with their correct right/left orientation.

Labeling

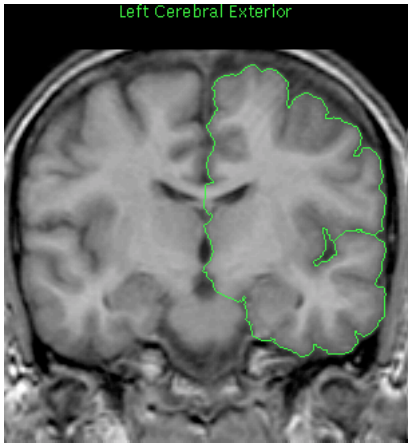
To begin labeling click on the REV button next to the SEG button in CardViews and "review panel" will appear. You can switch back to SEG by clicking on the SEG button again.



Scrolling through slices and structures

Click on the "+" and "-" in the top right corner of the REV menu to scroll through slices. The segmentation lines should appear, if not go back to SEG mode and load your outlines again. It is usually easiest to begin labeling at either the most anterior point of the brain or the most posterior point of the brain. Click on "current", your first outline (the first extracted outline) will be presented individually and will be numbered "0". Each individual structure will be given a number based on the order they were extracted. Use the "+" and "-" next to "current" to scroll through the structures.

Label Menu



Click "label menu" in the window that appears and drag the label menu off to the right of the screen so it is not covering anything you need to see. This menu will give you a list of names to choose among for each structure. Click on the desired label for the individual structure outline in question. The next structure will automatically appear ready to be labeled. You will hear a beep when all of the structures in a slice have been labeled. You must click "save" before you advance to the next slice or your labels will be lost.

If the structure you want to label is not on the menu, click with the right mouse button on the arrow next to "change menu" and change to a menu that suits your needs. If you mis-label, simply click on "-" to go back. Then re-label it.

Multiple outlines for the same structure

Outlines of a structure should only be extracted once, however, mistakes do happen so one should always be aware of repeated outlines. The second time a structure appears, you can click on "delete outline" to erase the extra outline. You can also use "delete outline" if you don't like the way a

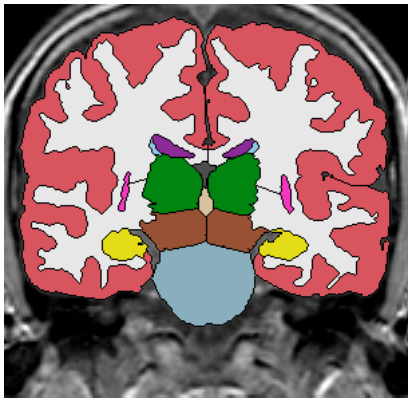
structure was extracted. When you switch back to SEG mode, the "deleted" structure will be outlined in red, ready to be modified and/or re-extracted.

Review

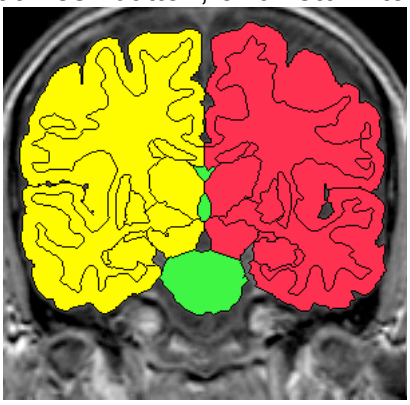
If you are looking at a segmented brain and you quickly want to know what a particular structure is, simply enter review mode and click on the structure in question to find out.

Review labeling in REV mode

Clicking the "structure" button will colorfill your labels. It is helpful to scroll through the colorfilled brain to check labeling. You can return to your outlines by clicking on the "outlines" button, and return to the original image minus segmentation lines by clicking on the "image" button.

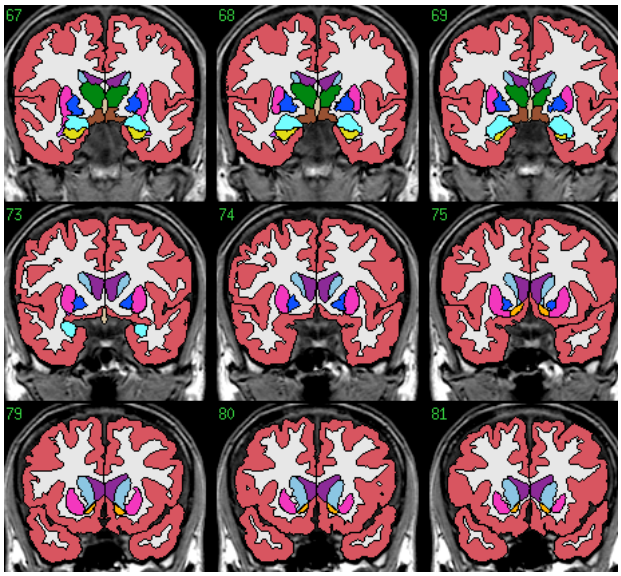


You can also quickly check the R-L orientation of the labeled structures by clicking on the right/left button which will color all structures labeled "left" yellow and those labeled "right" red; the midline structure will be green. If you find a R-L error, click on "r/l switch" to label the correct side.



Colorfill in Tile Display

It is often helpful to review the labeling in Tile Display because you can see multiple slices in one screen. Click on "tile display", to view the brains in a size similar to what your used to, click on "zoom". You can also select the range of slices you want by moving the sliders at the top of the box or by clicking next to them or dragging them. Click on "Go", the range of brains you selected will appear. Now you must type in your prefix on the "prefix" line to load your structures for that brain. Click "Color Fill". Your colorfilled labeled brain will appear.

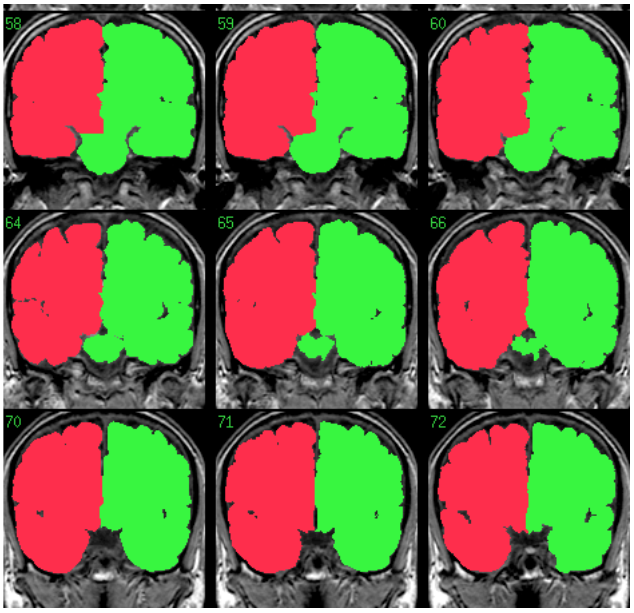


Now look for mistakes, such as mislabeled structures. It is also important to look for small things, such as parts of cortex or white matter that are in the wrong places or missing. If you find a problem go back to SEG mode to fix it. Once you have fixed the structure, you must return to REV mode to re-label what has been changed. Only those structures that have been changed will need to be re-labeled, all others will retain the labels you already assigned to them. It is easy to access the new structure that needs to be labeled by clicking on "-" because the new structure will move to the end of the list because it was the last to be extracted.

Occasionally it will appear that you did not label some structures, or that some are missing altogether, but when you go back to SEG mode, or even REV mode, everything is correct. There is no explanation for this, so if it looks okay in SEG and REV, then it is correct, no matter what Color Fill shows.

R-L Orientation in Tile Display

Directions are the same as for Color Fill, except that you click on "RL" rather than "Color Fill". This will show the left hemisphere in red and the left in green, the midline structures will also be green. Mistakes can be fixed in SEG just like Color Fill, or by clicking on the "r/l switch" button.



Correcting Segmentation Errors

XVol, which was created for volumetric analysis of the brain, is first used to detect errors in segmentation. After those errors have been corrected, a program called Check_otls is used to detect errors no easily found by using XVol. Extract_I is then run to extract the cortical ribbon from a segmented brain, and gives error messages for any problems with the segmentation. The following section describes how to run these processes.

Running XVol to check errors

-At your home prompt, type xvol. Hit return.

-Click on "START." A bigger screen will appear.

-On the right of this bigger screen, click on "CMA Basic", this will unselect the "Don't Sort" box.

-Under the "Set OTL Path", put your cursor in the "PID" box and type in the number of the brain you are checking (this is the patient identification number). You will have to hit return in order for this box to accept the number you typed in. Now type in the scan number of the brain you are checking in the "SCN" box and hit return. Lastly, type in your segmentor initials in the "PRE" box and hit return. Example: 7, 45, bmg

-Click on the "*" button, this will check which slices you have segmented and will automatically insert the range you want.

-Once the range is displayed, click on "from info file"; this will load the voxel dimensions. A small screen will appear, click on the "Load" button, this will load your outlines.

-Now go to the top of the screen and click on the "CONTINUE" button. A new screen will appear.

-Click "graph" under the heading "File: Simple Volume".

-Click on "Continue" again. Another new screen will appear.

-Now click on "Run Volumes". This may take a few minutes. When it is done running, there may be errors and/or messages regarding your outlines indicated by red check marks. To look at what the errors are, click on the "view" button next to the red check mark. When you click on view, the errors or messages will appear on the screen that you originally typed the "xvol" command on. If the screen is not large enough to display all the errors, you will need to press the space bar to view the ones remaining.

-Now, you will need to correct all your errors before you continue. It is a good idea to write these down or print out your errors so you don't have to keep referring back to it once you are in CardViews.

When you label structures in REV, it starts assigning names to structure 0. So, the structure number XVol tells you to fix is one greater than the actual structure with the error. EXAMPLE: if the error message is "Bad fill re-extract structure #13, slice 24" then you need to fix the 12th structure on slice 24.

-Once you have corrected all your errors and looked at all the messages, you can now click on "View Data" on the top of the XVol screen. A new screen will appear and you should click on "graph".

-The graph that appears will be helpful in identifying what structures have double extractions. The bottom axis is the slice number and the side axis is volume. A sharp peak indicates double extractions, or double the volume that you would expect for that structure. Also, a sharp dip indicates that you probably forgot to extract a structure.

-To more easily determine what slice has the error, you can zoom in on the peak by drawing a box around the area of interest using the middle mouse button. This will open a new screen with a close up view of the box you just drew. You will especially need to do this with the number of small volumes at the bottom of the screen. It is difficult to distinguish "what is what" in these small volumes, so just do the best you can in this area. Here again, it is a good idea to write down where your errors are for when you are in CardViews.

-When all your errors have been corrected and the double extractions corrected, you must re-run the volumes in order for the data to be valid. You will return to the screen where you can click on "Run Volumes". This will check all of the data again, should reveal no errors, and the graph should reveal relatively rounded arches with no tall peaks.

-After looking at the graph and fixing all your errors, you can "Exit Program" at the top of the screen to exit XVol.

Running Check_otls

While XVol and can detect some errors there are other types of errors that require a careful review of the outlines to detect.

XVol constructs a graphical display of the volume of each labeled outline across the range of MR slices. With this graph, you're supposed to be able to detect an outline that is extracted more than once because it will appear as a sharp spike (twice the volume) compared to surrounding slices. In my experience, the graph is good for large structures, but it is too difficult to follow the contours of the smaller structures. It also fails to detect occasions when an outline is mislabeled (the graph doesn't "dip" to show the absence of an outline).

Check_otls compares all the outlines in a slice and reports the similar ones.

After running XVol without any errors, type in this command:

check_otls xvol/vol/otl_list

Every instance in which two outlines have the same dimensions within a slice is written to the terminal window. For example, here is part of the Check_otl output from PID 1454, scn 14, and slices 45 and 55:

```
/Data/1454/14/otl/rjm45.otl
   15 Left Cerebellum White Matter          4      4.0     0.003 cm3
   17 Right Cerebellum White Matter         4      4.0     0.003 cm3
/Data/1454/14/otl/rjm55.otl
```

9 Right Cerebellum Exterior	438	52.5	0.162 cm3
19 Right Cerebellum White Matter	438	52.5	0.162 cm3

Outlines 15 and 17 of slice 45 have the same region dimensions, but different hemispheres. If you checked this in CardViews, it would most likely be a case in which two VERY small outlines just happen to have the same volume. Outlines 9 and 19 on slice 55 show two larger regions in the same hemisphere. If you checked this in CardViews, you'd see that it's where the cerebellar peduncles are labeled both 'cerebellum exterior' and 'cerebellar white matter' (half-circles on each side of the brainstem). In this manner, I use CardViews to reconcile every line of output from Check_otls.

So you get the good with the bad: Check_otls gives you the information to find outlines extracted more than once, but it also gives instances of "inconsequential" similarity.

Running Extract_I

-This is just another program used to analyze your segmented brain and detect any errors. This must be done before you can run a comparison of your brain with someone else's.

-Like with XVol, you do not need CardViews open to run extract_I but it is helpful to have it minimized for easy access.

-At your home prompt, type extract_I <PID> <scan> <prefix> <first slice> <last slice>

-Here's an example of what it should look like: extract _I 7 45 bmg 4 118

-Once you hit return, the program may take a few minutes to go through every slice looking for errors. The program is also assigning a new name to each slice as it extracts the cortex. You will be able to follow on the screen what slice it is reading and what slice "I files" are being made. When an error is found, there will be an error message right below the slice it is reading.

-Like with XVol, you will need to return to CardViews to correct any errors that extract_I has found. Again it is helpful to write down the errors or print out that screen before returning to CardViews.

-After correcting any errors, be sure to run extract_I again so that the "I files" will be corrected.

Corpus Callosum Segmentation

Drawing the sulci lines in NAV mode

Sagittal view

-The callosal sulci will be drawn to act as a reference in the segmentation of the corpus callosum. This sulci line will be extended around the whole corpus callosum to provide a reference for the superior, inferior, anterior and posterior borders. When drawing this sulci line, you must make sure to exclude the fornix and to only draw borders where it is absolutely clear there is actually a border of the corpus callosum.

-Draw in as many callosal sulci as you need to see the full extent of the corpus callosum. Usually every three or four slices from the most lateral extent of one side of the corpus callosum to the most lateral extent of the other side will do. You must make sure to pay special attention to the mid-sagittal slices (this is where you should be able to see the corpus callosum in its' entirety).

Coronal view

-You will be able to see your sagittally drawn sulci lines in the cingulate cortex area of the coronal view. You will use these lines as references in your segmentation of the corpus callosum in the coronal view.

Segmentation of the corpus callosum in the coronal view

Splenium of corpus callosum

-In the more caudal/posterior part of the corpus callosum, the splenium, three separate histograms will be used. The first histogram will define the lateral/inferior border of the corpus callosum by defining the CSF/white matter border or the superior/medial borders of the lateral ventricle.

-The second histogram required will define the superior border of the corpus callosum. This histogram will define the white matter/gray matter border of the corpus callosum and the cingulate area of the cortex.

-The third histogram used will depend on whether the thalamus, the hippocampus or the fornix makes up your inferior border. If it is the thalamus or hippocampus a histogram will be used to create this white matter/gray matter border(a different histogram from the cortex/white matter histogram should still be used because cortex and gray subcortical structures may have different intensities). If the inferior border is the fornix, use the reference sulci lines to guide you while you draw in this border.

-Finally, connect the histogram-generated lines to form your corpus callosum. For the lateral/superior border you will have to draw a straight line(usually diagonal) from the lateral-most superior part of the lateral ventricle to the most lateral of the reference sulci lines around the cingulate cortex(be sure not to include any gray matter). For the inferior/lateral border draw a straight line(usually diagonal) from the most inferior point of the lateral ventricle to the most inferior point of the corpus callosum (as shown by reference sulci lines).

-In all slices of the corpus callosum divide it into right/left by drawing a straight midline, and label CC(in the WM segmentation menu).

Middle of corpus callosum

-Only two different histograms (one for the superior border - the cortex/ white matter border, and one for the inferior/ lateral borders - the CSF/ white matter border) will be used for the midsection of the corpus callosum (the area just anterior to the splenium).

-Connect these lines using the same method described above (section A4) in connecting the lateral/superior border of the corpus callosum by using the reference lines. The inferior border will be the superior border of the lateral ventricle, this line will for the most part be continuous but if it is not draw a straight line between the ventricles to exclude the septum and complete the inferior border. This area of the corpus callosum it is usually triangular shaped.

Genu of corpus callosum
- In the most anterior portion of the corpus callosum, the Genu, the same methods described above are used to create the superior and lateral borders (section B1, and A4).

-The inferior border is formed by a histogram of the white matter of the corpus callosum and the gray matter of the inferior cingulate cortex area.

-A straight line is drawn from the most inferior point of the lateral ventricle to the reference sulci lines for the most inferior portion of the corpus callosum in the posterior cingulate area.

-In the most posterior portion of the Genu of the corpus callosum there may be an inferior part of the corpus callosum and a superior part slit by the septum(this is because of the curve of the Genu) pay special attention to your sulci lines in this area to determine where the split occurs and where the corpus callosum is returned to one piece. In the split area follow your sulci lines and draw a straight horizontal line for the inferior border of the superior portion of the corpus callosum and the for the superior border of the inferior portion of the corpus callosum. This will exclude the septum from the corpus callosum volume.

Lesion Segmentation

The segmentation of brain lesions uses special rules, and methods to accurately define lesioned areas.

Procedure

-Lesions must be segmented before any other units are segmented.

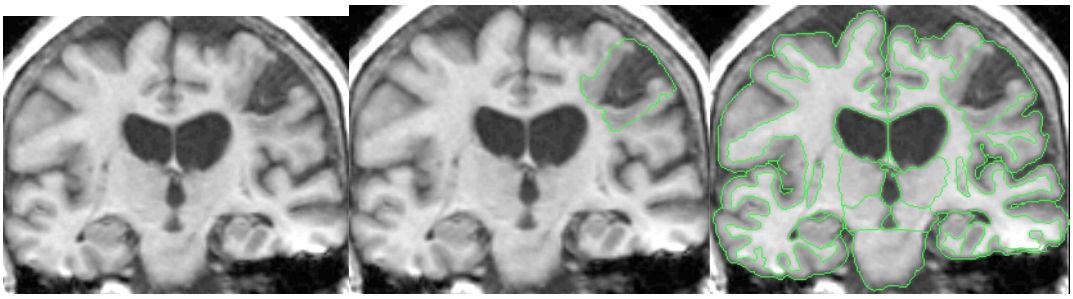
-Scroll through the brain to get an idea of the progression of the lesion.

-Record in your notebook the slices in which the lesion begins and ends.

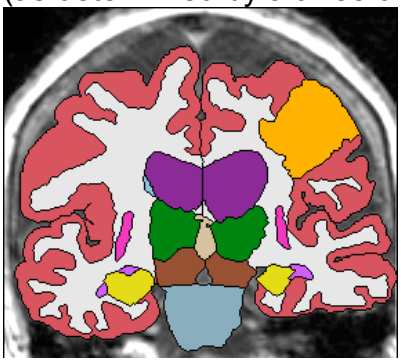
-"Sulci" lines can be drawn in the axial and sagittal views to help in determining the lesions borders in the coronal view.

-Once these lines have been drawn, use the intensity contour method to determine the borders of the lesion. It is important to include the CSF and the lesioned tissue that comprise the lesion area. The lesioned tissue will be at the extreme of the lesions border and will appear darker than surrounding healthy brain tissue. If the lesion is unilateral, you can compare the lesioned area to the contralateral healthy area.

-After the entire lesion has been segmented in all coronal slices in which it appears, the other regions of the brain can be segmented normally with the exception of the cerebral exterior. The cerebral exterior must include the lesion as part of its volume. To accomplish this, use the "clipping technique" (pg. 24, "Segmenting with multiple contours") for lesions that share a border with the exterior. For lesions that do not share a border with the exterior, unextract the lesion, extract the exterior, and then re-extract the lesion.



-When labeling a brain with a single lesion, use the seg.otl label menu and label the segmented lesion as "lesion." If the brain has multiple lesions, use the lesion.otl label menu. Each distinct lesion (as determined by a three dimensional interpretation) must be labeled "lesion 1", "lesion 2", etc.



Generating Volumetric Data with XVol

XVol is a tool used for volumetric analysis of the brain. After you have segmented and/or parcellated the brain, you use XVol to determine the volumes of all the structures you have identified and also to establish a comparison between different brains and between different rators' results of the same brain.

Running XVol for Volumetry

-At your home prompt, type xvol. Hit return.

-Click on "START." A bigger screen will appear.

-On the right of this bigger screen, click on "CMA Basic", this will unselect the "Don't Sort" box.

-Under the "Set OTL Path", put your cursor in the "PID" box and type in the number of the brain you are checking (this is the patient identification number). You will have to hit return in order for this box to accept the number you typed in. Now type in the scan number of the brain you are checking in the "SCN" box and hit return. Lastly, type in your segmentation or parcellation initials in the "PRE" box and hit return. Example: 7, 45, bmg

-Click on the "*" button, this will check which slices you have segmented/parcellated and will automatically insert the range you want.

-Once the range is displayed, click on "from info file"; this will load the voxel dimensions. A small screen will appear, click on the "Load" button, this will load your outlines.

-Now go to the top of the screen and click on the "CONTINUE" button. A new screen will appear.

-Click "graph" under the heading "File: Simple Volume".

-Click on "Continue" again. Another new screen will appear.

-Now click on "Run Volumes". This may take a few minutes. This may take a few minutes, but when it is finished running, any errors will be marked with red check marks like in the simple volume analysis. These errors must be corrected before you can continue. Once corrected, run the comparison from the beginning.

-The data you want to look at will be in a series of four columns. You will need to click on "View Data" and then next to the checked box, click on either "view" or "print". The names of the structures are listed on the far left and the volumes on the right.

Running a volumetric comparison with XVol

-Now you are ready to examine the differences between measurements of different rators of the same brain structures to establish inter-rator reliability.

-Open XVol like you did in the simple volume analysis, by typing "xvol" at you home prompt. Click on "Start".

-Click on "CMA Basic".

-Click on "Comparison."

-Enter your PID, scan and prefix. Below your numbers, enter the PID, scan and prefix of the person you want to run the comparison with.

-Click on "*", then click on "from info file" and "load", just like you did previously.

-If you need to run multiple comparisons at once, click on “add.”

-Enter PID, scan, and prefix of the next person in box #2

-Click on "*", then click on "from info file" and "load", just like you did previously.

-Repeat this process for each person you need to compare to.

-Click on "Continue".

-Under the heading "Set Output" click on "comp_same_ez"

-Click "Run" or "Continue", and then click on "Run Volumes". This may take a few minutes, but when it is finished running, any errors will be marked with red check marks like in the simple volume analysis. These errors must be corrected before you can continue. Once corrected, run the comparison from the beginning.

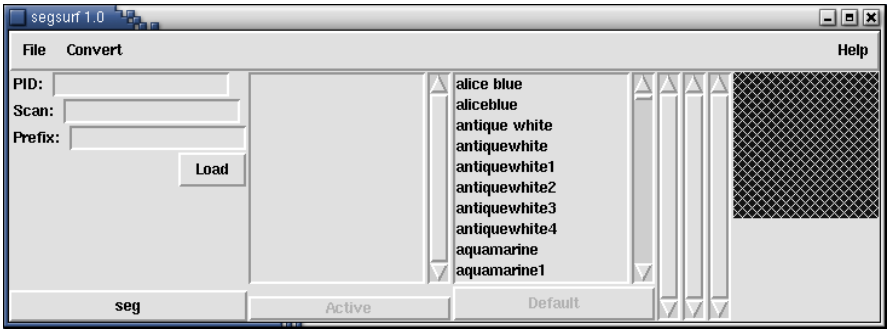
-The data you want to look at will be in a series of four columns. You will need to click on "View Data" and then next to the checked box, click on either "view" or "print". The names of the structures are listed on the far left and the column that you should look at is the far right which represents the percent of overlap between the two sets of data you are comparing.

Creating 3D Models with SegSurf

SegSurf converts CardViews segmentation and/or parcellation files into a format that can be processed and then visualized as a three-dimensional volume. SegSurf must be run on a Linux-based computer running a 24-bit Xserver. The brain to be visualized must be segmented and/or parcellated in CardViews first.

-At a terminal command prompt type: segsurf. Hit return.

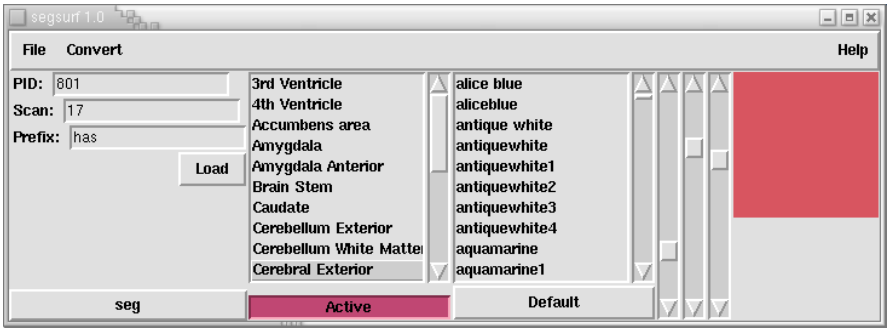
-The SegSurf graphical user interface will open.



In the fields on the left side of the interface, enter the PID, and scan number of the brain you are working with as well as the prefix of the segmentation or parcellation files.

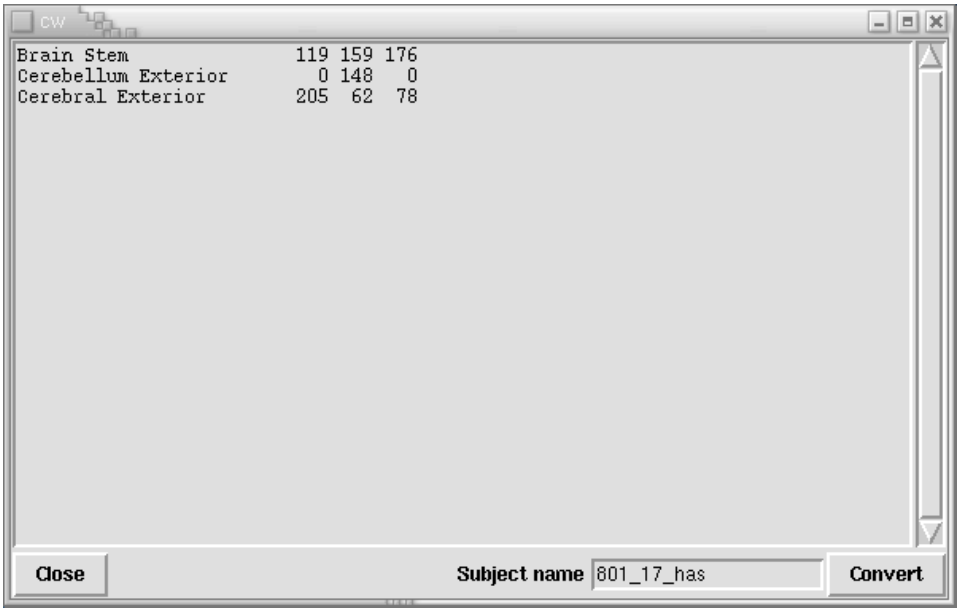
-Click the "seg" button on the bottom left of the interface to open a menu containing segmentation/parcellation label menu files. Select the appropriate label menu file for your segmentation or parcellation.

-In the list above the "Active" button, select a structure to be modeled, and then click the "Active" button. The "Active" button will turn fuchsia and the default color of the modeled structure appears on the far right of the interface. You may select another color from the list above the "Default" button. The new color of the modeled structure replaces the default color. To return to the default color of the structure click "Default". Repeat the above steps for as many structures as you wish to be modeled. To remove a structure you have already selected for modeling, select the structure in the menu and click on "Active". The "Active" button will turn gray.



-Now the CardViews-based data must be set up to be converted to the proper format for 3D modeling. Click on "Convert" in the menu bar and select "Convert…" Another window will appear

with a list of the structures you have selected for modeling, as well as their color values (RGB). If you would like to change the list click “Close” and repeat the steps in the paragraph above.



-In the “Subject name” field, enter the desired subject identifier (we suggest using the PID_SCN_prefix format). Click “Convert” to begin the conversion process. A dialogue box will appear asking if you would like to create the patient identifier you have entered; select “Yes” to continue or “No” to go back and enter a new subject identifier (the program will caution you if the subject identifier already exists). After selecting “Yes” the conversion setup process will automatically run; this will take an ***extremely*** short amount of time. Click “Close” in the conversion window, and then select “File” and “Quit” from the main interface.

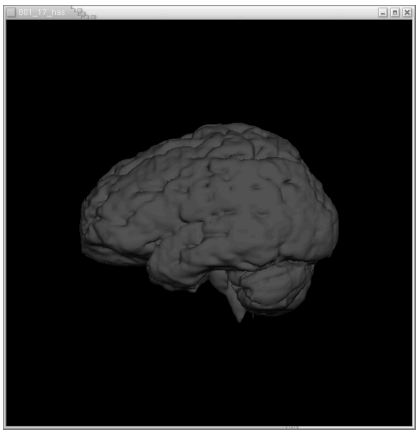
-At the terminal command prompt type: segsurf_convert <subject identifier>. Hit return.

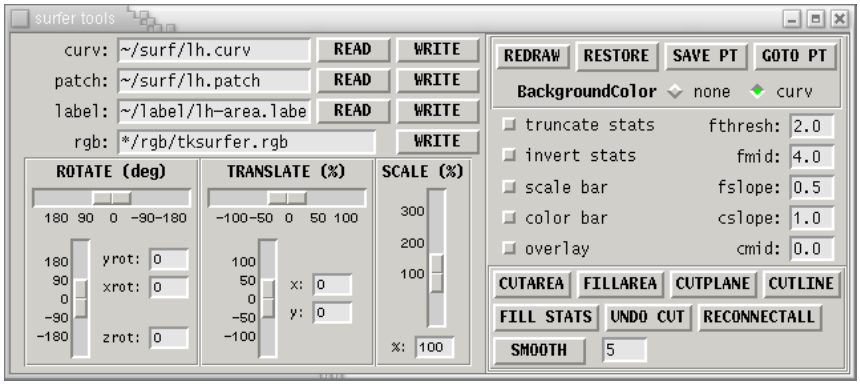
-The conversion process will take some time.

-When the process has completed you will be returned to the terminal command prompt.

-To view your modeled structures type: tksurfer -<subject identifier> lh smoothwm. Hit return.

-Two windows will open, one containing your modeled structures in black and white, and another containing options for viewing the data.

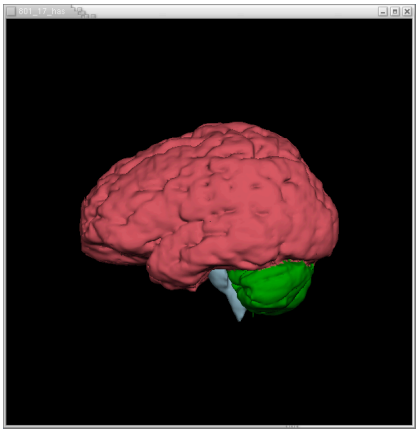




-To paint the colors onto your models, return to your terminal window and find the “%” prompt.

-Type: read_annotations segsurf.annot. Hit return.

-The window containing your modeled structures should now have appropriate colors.



-You can use the slider-bars in the “ROTATE”, “TRANSLATE”, and “SCALE” sections to change the viewing angle, on-screen position, and zoom level respectively. Once you have adjusted the sliders, click “REDRAW” to apply your changes.

General Functions Summary

This summary presents the important buttons in CardViews from right to left.

Default Screen

Quit	quits CardViews
autotrans	all views will transform according to projection lines, as well as slice position in other views
NAV	go to CardViews NAV mode
SEG	go to CardViews SEG mode
REV	go to CardViews REV mode
tile display	go to CardViews tile display, where you can see many images at the same time
Projection	turns the projection lines on or off
COR	gives slice number of coronal image
SAG	gives slice number of sagittal image
AXI	gives slice number of axial image
COR, SAG, AXI arrows	allows you to change the corresponding slice
Transform	changes the slice to the number next to the corresponding position

NAV mode

SULCI FILE	gives path of where sulci files are saved
Prefix	enter the prefix to direct naming of sulci files
LOAD Sulci	loads the sulci for the given prefix
SAVE Sulci	saves sulci for the given prefix
DELETE Sulci	deletes all sulci for the given prefix
CROP Data	starts the cropping program

SEG mode

OVERLAY	indicates where the otl files are saved
Prefix	enter prefix to direct naming of otl files
-	allows you to move posteriorly though the brain
+	allows you to move anteriorly through the brain
SAVE	saves otls for a current slice
load	loads otls for current slice
drw sulc	draws sulci lines for current slice
ACl	automatically clears otls from the former slice when changing to a new slice
ALd	automatically loads otls from the new slice when changing from a previous slice
clear	clears the slice of all otls and contours
recall	loads the version of otls that have been extracted; use this to recall otls when you forget to save otls before moving to a slice

REV mode

- (top of box)	moves the brain posteriorly
+ (top of box)	moves the brain anteriorly
structures	shows the labeled structures in color fill form
right/left	shows which structures are labeled as right/left in cartoon form
image	displays just the MRI image
outlines	displays the outlines; this mode is used when labeling
Current	displays only the current otl to be labeled
+ (middle of box)	advances to the next otl
_ (middle fo box)	advances to the previous otl
delete outline	deletes the otl
r/l switch	changes the labeling of an otl from right to left, or vice versa
label menu	calls up the label menu
change menu	click on the down arrow with the third mouse button to change the label menu

Tile Display

numbers	slice numbers of the first and last images on the screen
scroll bars	used to change the slice numbers that will be seem
box next to cor	if checked, visualizes the coronal images
slice number	reads the middle slice number that is visible
up and down arrows	allows you to change slices on the top incrementally
box next to sag	if checked, visualizes the sagittal image; slice number and arrows used the same as with cor
box next to axi	if checked, visualizes the axial image; slice number and arrows used the same as with cor
prefix	enter your prefix to visualize the otls
box next to zoom	if checked, zooms the images to actual size
GO	transforms the images
CARDVWS	returns the program to the CardViews screen
down arrow next to OTL	hold with the third button to indicate the color the otls should be for the given prefix; enables you to enter up to 5 prefixes/otls at once for comparison purposes
RL	if the otls are loaded, and the brain has been labeled, color-codes what is labeled right and left
COLOR FILL	if otls are loaded, and the brain has been labeled, color-codes what is labeled right and left
arrow next to SULCI	recalls previously drawn sulci lines
arrow next to Change label	when clicked with the right mouse button changes the type of labeling menu, e.g. from cortical parcellation to white matter parcellation labels
arrow next to OSM	click either up or down to increase or decrease the number of save and recall slots
Save	saves the current screen of images, with otls, and sulci if present
RCL	recalls the saved screen (slice numbers and orientation are given on the line to the left)
toggle	allows you to switch back and forth between r/l colorfill and otls.
box next to line	if checked shows previously drawn parc lines
box next to label	if checked pulls up parcellation label menu and pulls up previous labeling
create	Hit this button and right click in desired area on images and a green cross-hair will appear. This works only if the "mark" button is selected
destroy	gets rid of the crosshair
activate	turns the green crosshair red and freezes it, this allows you to create another cross-hair while the red one stays in place
deactivate	turns the red crosshair back to green and allows you to move the green cross-hair around again
cor, sag, and ax buttons	pulls up the slice number next to the orientation in CardViews
color review	recalls extracted outlines if prefix is entered
nodes	pulls up the "node" menu

Nodes Menu

load nodes	loads pulls up previously saved nodes
save nodes	saves nodes
cancel/done	gets rid of the nodes menu

Parcellation menu

Edit parc lines	allows you to go back and edit parc lines
Save extract load	pulls up labeling menu for parcellation
Alt config	changes screen view into a combination of both Tile Display and CardViews
show label	gives actual names of parcellation units on labeled images

Parcellation Labeling Mode

List	show list of labels
Next	scroll through more labels
Reset	return to original labeling list
arrow next to auxlab	right click on the arrow to give a list of labels not on the regular menu, e.g. temporal pole, basal forebrain, and ??? Scroll through and left click on desired label
f	skip a PU in the sequence
b	go back to a PU in the sequence
Hemis	switch hemispheres in labeling sequence
Save	save labels
+ and - buttons	scroll through images

Segmentation Functions Summary

Introduction
There are more buttons and functions that may not be listed here. These are either not useful for our method of segmentation, or are a mystery. Feel free to play around. Unless otherwise specified, "click" refers to a left mouse button click.

FUNCTION	TO USE	OTHER
Extract	While in an enclosed outline, press "e" While underneath an enclosed outline press "e" to extract from the outside.	"w" will unextract the last extracted structures sequentially. You can also unextract outlines by clicking on the outline with the left mouse button and dragging the outline out of the working box. "Shift-w" unextracts all outlines on a slice.
Remove Lines	When you need to get rid of many stray lines press "x." Lines in the selected color will be erased. "Shift-x" will erase ALL lines of all colors on a slice.	
Contour Line	Press "c." With the left mouse button, click on the point where you want the line. Click the right mouse button and drag the mouse to find the best fit contour line. When satisfied, release the right mouse button to set the line. Press "spacebar" to exit.	
Draw Function	Click the right mouse button. Click the left mouse button on point where you want the line to start. Click the left mouse button where you want that section of the line to end. Continue to click with the left mouse button until your line is finished. Click on the right mouse button to exit	To draw in a continuous line, click where you want the line to begin and continue to hold down the left mouse button. When you are done with that line, release the left mouse button. Click the right mouse button to exit draw mode. The middle mouse button will erase the line in the increments in which it was

	draw mode *You must exit draw mode after each line you draw*	drawn. *This only works if you haven't exited the draw mode yet, otherwise use the erase function.* "d" or "f" can be used to change the line color.
Erase Function	Press "q." Click on the point you want to erase with the left mouse button You can either click points to erase with the left mouse button, or hold down the left mouse button. Press "spacebar" to exit.	You can change the eraser size. While in erase mode, click on the right mouse button. The size will range from 1-10. If you want to unerase something you erased, press "r" before exiting erase mode. This will restore all points you just erased.
Toggle Between Outlines and Image	Press "r" to temporarily remove the contours from the screen, leaving all extracted outlines and the image. Press "r" again to return the contours.	Pressing "shift-r" will temporarily remove all lines, press "shift-r" to get the lines back.
Contrast and Brightness	Click the middle mouse button to adjust the contrast and brightness. When you are satisfied, click the middle mouse button again to set it.	Moving the mouse up and down the screen will change the brightness. Moving it across the screen will change the contrast.
Highlight a Line	Click the left mouse button on an unextracted line to highlight the line.	"Shift-c" can be used to delete a highlighted section.
Create a Histogram	Pressing "shift-f" inside an extracted structure (e.g. a box drawn in the area of a structure encompassing the contrast of white and gray matter) will create a histogram.	Clicking with the middle mouse button on one of the highest peaks and dragging it to the other will give a contour line for the structure in question. Clicking with the left mouse button on the red line and dragging, or just clicking where you want the red line to move will allow you to manipulate the contour. Clicking and dragging over an area of the histogram with the right mouse button will zoom in on that area. The histogram can be returned to normal by pressing "s."
Change Color of a	Use erase function "q" to isolate the desired section of the line.	"b" changes "dump level", or the color that pressing "v" will produce.

Line	Left-click on the section to highlight. Press "v" to change contour level/color.	
Extract Only the Outside Contour	Pressing "e" in an enclosed outline with other extracted structures within the outline will extract only the outside contour. (useful for fixing exteriors)	

Atlas of the Segmented Brain