EADC-ADNI HARMONIZED PROTOCOL FOR MANUAL HIPPOCAMPAL SEGMENTATION:
USER MANUAL

Appendix II

1. Introduction

This document provides instructions to perform manual segmentation of the hippocampus on magnetic resonance images (MRI), as defined by the EADC-ADNI Harmonized Protocol for Hippocampal Segmentation (HarP) and described elsewhere [1]. Anatomical boundaries are described in detail with reference to pertinent publications relating to the hippocampus [2,3] and the human brain [4]. Reference information relative to the entorhinal/perirhinal cortex was taken from [5]. Additional material is available at www.hippocampal-protocol.net. Individual morphological variability may correspond to differences in the described landmarks. In this manual, some possible anatomical variants were considered, however the tracer should always check the anatomy of the segmented structures using an atlas of the human brain to ascertain the correct anatomical identification.

We suggest to reference the HarP by both the definition [1] and validation papers [6] whenever possible. Validation versus pathology may also be referenced [7].

1.3 Abbreviations

MRI	Magnetic Resonance Imaging/Image
GM	Gray Matter
WM	White Matter
CSF	Cerebro-Spinal Fluid
HarP	Harmonized Protocol
SU	Segmentation Unit
AC	Anterior Commissure
PC	Posterior Commissure
EADC	European Alzheimer's Disease Consortium
ADNI	Alzheimer's Disease Neuroimaging Initiative
3D	Three-dimensional

2. Segmentation procedures

2.1 Image Orientation

The orientation of MRIs is determined on the sagittal view, by the line connecting the anterior and posterior commissures of the brain (AC-PC line) [8]. The coronal slices used for segmentation are resliced orthogonal to this plane.

2.2 Direction of segmentation

The segmentation is described and should be performed from rostral to caudal, on coronal slices.

2.3 3D navigation

The HarP requires the use of visualization tools allowing 3D navigation. For many landmarks, the morphological details visible in the coronal slice are not sufficient to determine whether the tissue belongs to the hippocampus. To perform accurate segmentation, the axial and sagittal planes must be checked frequently, and according to the advice reported in this HarP user manual.

3 Segmentation landmarks

The partition of the hippocampus into head, body and tail was not matter of decision of the Delphi panel. Since this kind of partition is useful to help describe the anatomical landmarks of this protocol, we use it as commonly found in current literature on the hippocampus.

Here, we consider as hippocampal head the most caudal portion of the hippocampus, as long as it appears as a folded structure in the sagittal view, or as a double-layer structure in the coronal view.

The level of the body includes, in rostro-caudal direction, the first slice where the hippocampus appears as a single, unfolded structure on the sagittal view and as a single- rather than double-layered structure in the coronal view.

The level of the tail includes the last portion of the hippocampus, starting approximately at the level where the colliculi can first be visualized in the coronal view.

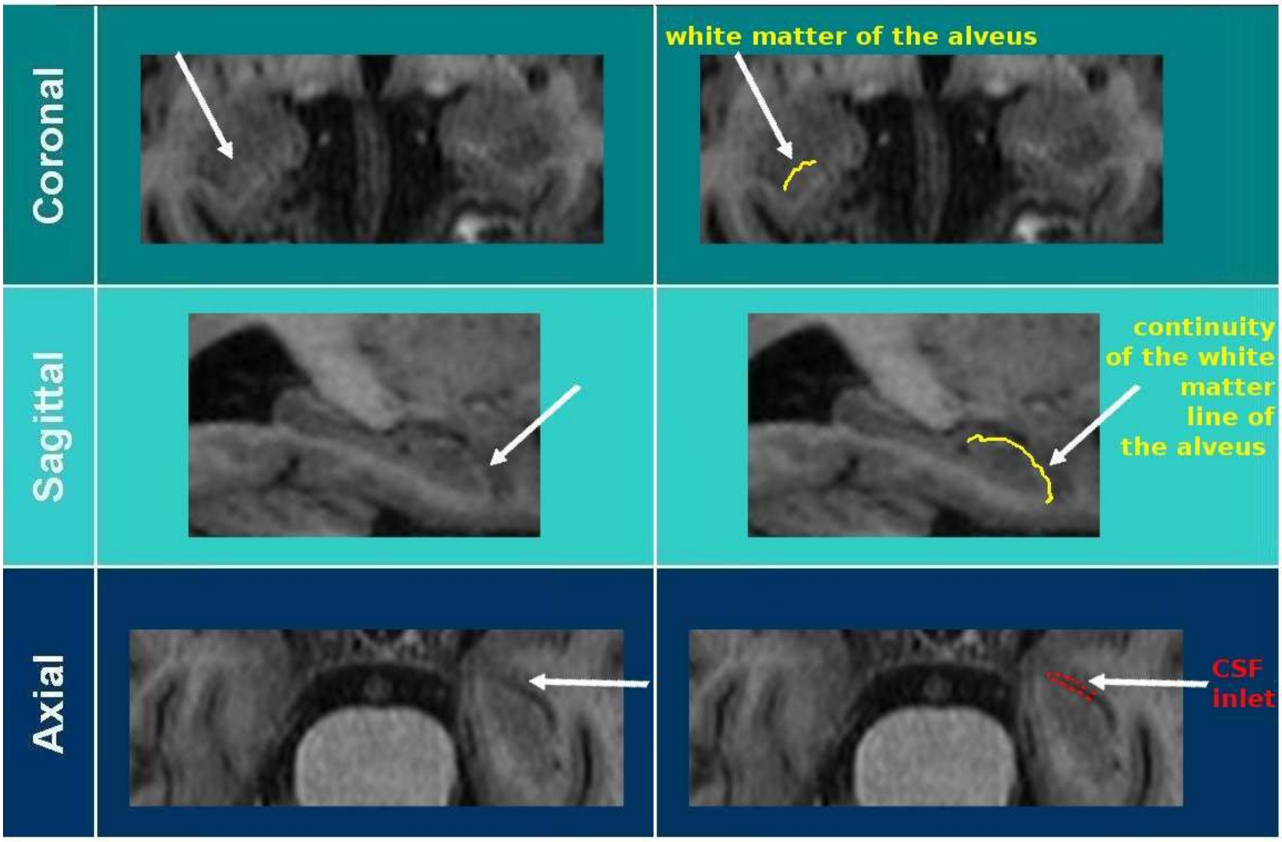
3.1 Most rostral slice

The most rostral slice where the hippocampus is segmented is the first slice where some hippocampal tissue is visible below the amygdala in the coronal view, and after checking in the 3D (axial and sagittal planes). The very first tissue that can be detected with the most accurate 3D navigation is the alveus, a thin WM layer that covers the GM of the hippocampal head. The alveus may not be clearly visible in the coronal plane in some slices – depending on signal to noise and contrast properties of a particular MRI scan. Therefore, the segmentation of hippocampal tissue on the most rostral slices needs 3D navigation to discriminate the continuous hyperintense line of the alveus on the dorsal border of the hippocampus in the sagittal view (Figure 1). An inlet of CSF can be discriminated between the hippocampal head and the amygdala in the sagittal and axial planes (Figure 1, arrows).

The GM located below the alveus, and confirmed to belong to the hippocampus from the sagittal views and axial views, is included in the segmentation. All of the GM located above the alveus is excluded as belonging to the amygdala. The alveus is included in the segmentation. Sometimes the first slice only shows the WM of the alveus: if this can be distinguished with confidence, using both the coronal and sagittal planes, this WM should be included in the segmentation as the very first slice.

Please note that, especially in lower field strength images, intensity inhomogeneities in this region may be mistaken as the alveus, but actually belong to amygdalar tissue. Gray scale intensity differences alone do not distiguish which voxels belong to the hippocampus or t so the amygdala. For this reason, checking in 3D is always necessary. In the coronal view, the digitations of the hippocampal head at this level make an undulating dorsal boundary. However, the sagittal and axial views usually show smoother boundaries, and clearer contours of the alveus and the CSF, that aids the anatomical identification of tissue.

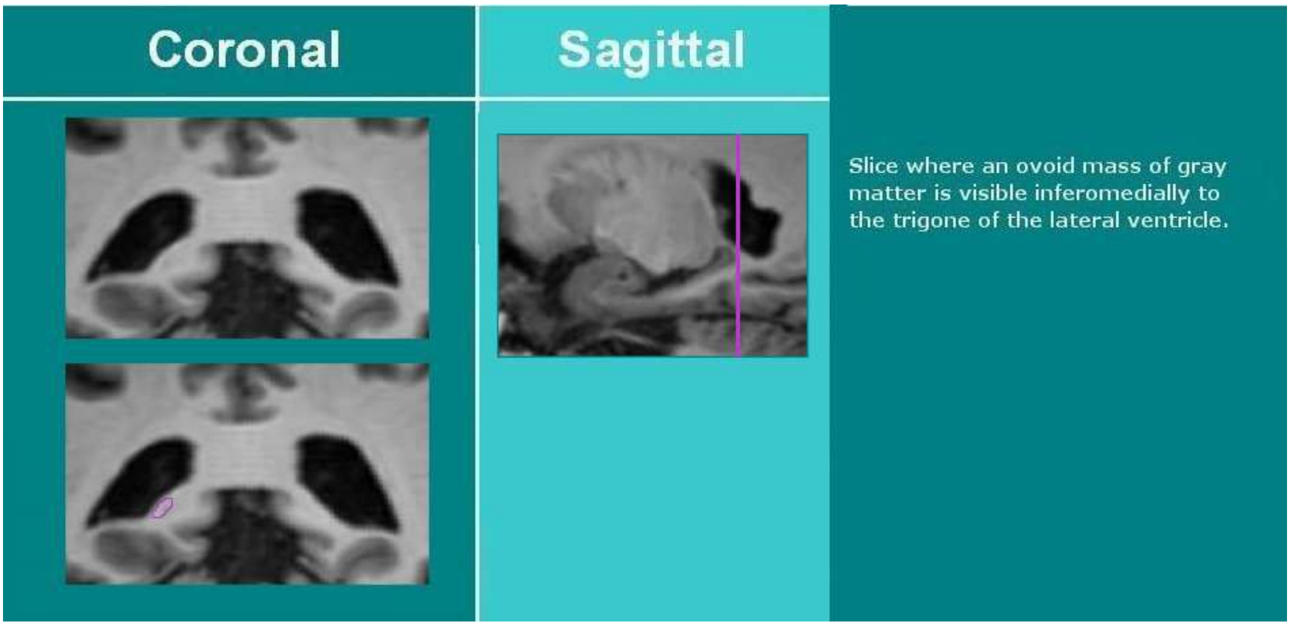
Figure 1. Identification of the most rostral hippocampal tissue.
Coronal view: first slice where hippocampal tissue can be detected and segmented. This may consist of the alveus WM only. Sagittal and Axial views: Segmentation of the most rostral coronal slice must be ascertained in 3D, where the continuity of the WM line of the alveus can be seen in the sagittal view, and where the CSF inlet separating the hippocampal head from the amygdala can be clearly seen in the axial view (arrows; in the right panel the target is segmented in yellow to highlight the exact structure pointed by the arrow).



3.2 Most caudal slice

The most caudal slice where the hippocampus is segmented is the last one, in the rostro-caudal direction, where a small ovoid GM mass is visible inferomedially to the trigone of the lateral ventricle (Figure 2).

Figure 2. Last coronal slice where the hippocampus can be detected and segmented. The vertical line in the sagittal view corresponds to the level shown in the coronal view. Based on the criteria of inclusion of the hippocampal WM (alveus and fimbria, see section 3.4.4), the WM adjacent to the hippocampal GM included in the segmentation must also be included.



3.3 Ventral boundary

The ventral boundary of the hippocampus is defined by the WM of the parahippocampal gyrus throughout the whole structure.

3.4 Dorsal boundary

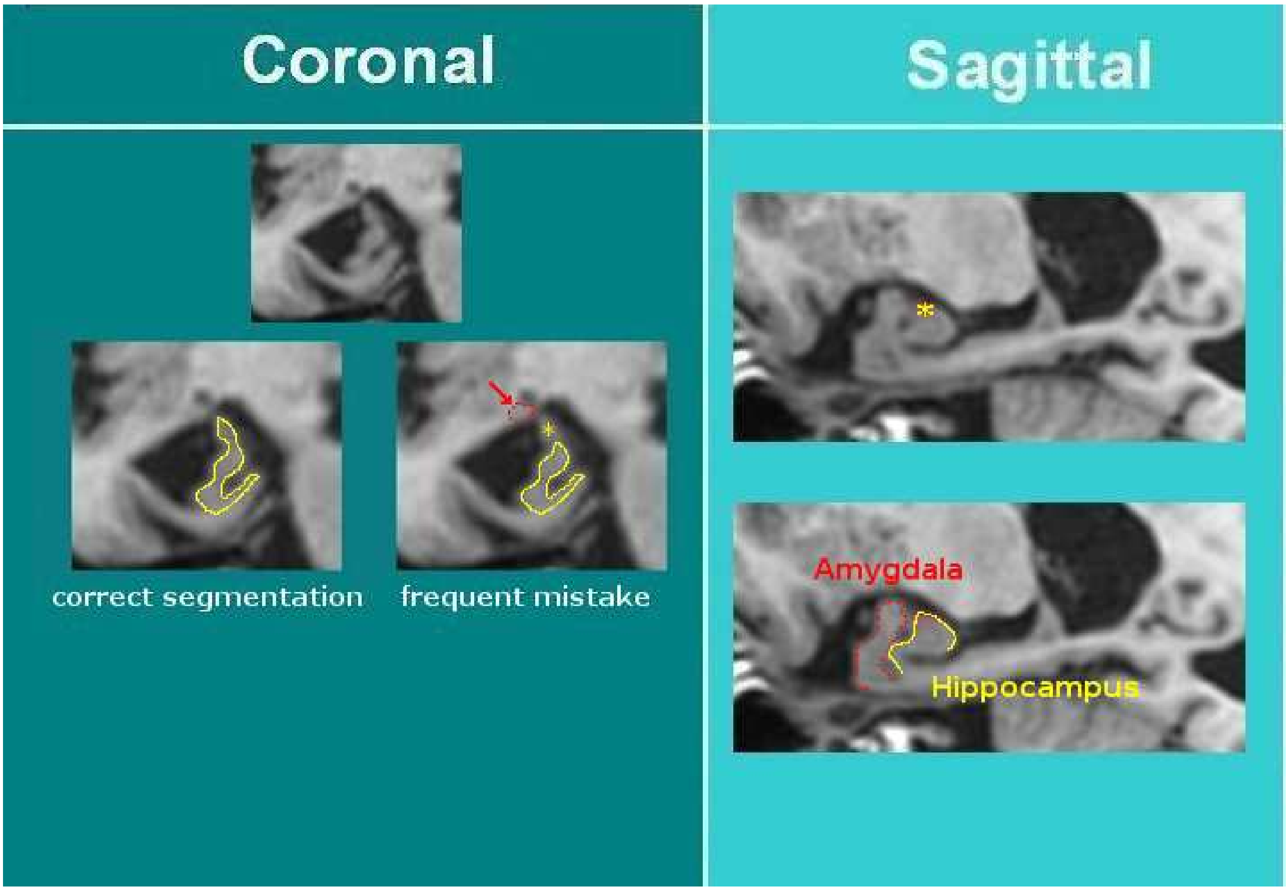
Depending on the level (head, body, tail), on individual morphology, and on the image quality, the dorsal boundary of the hippocampus is defined by the most dorsal boundary of the hippocampal GM bounded by CSF, or by the most dorsal boundary of the alveus and fimbria (to be included in segmentation, see section 3.4.4), whenever present and visible.

3.4.1 Dorsal boundary at the level of the head

In the most rostral slices, the neighboring tissue is the GM of the amygdala.

Determining the dorsal boundary is aided by using 3D navigation. In particular, the sagittal view enables identification of the folded shape of the hippocampal head (Figure 3, sagittal view). Both the sagittal and the axial views show the CSF separating the hippocampal head from the amygdala, and provide a better visualization of the alveus.

Figure 3. Separation of the hippocampal head (yellow segmentation on the left) from the amygdala (red dotted segmentations) at the level of the vertical digitation of the hippocampus. The yellow asterisks denote the vertical digitation, that must be included in the segmentation. The sagittal view shows the clearer separation of the hippocampal head from the amygdala. The separation between the two structures is very clear due to the visible folded shape of the hippocampal head and the CSF inlet separating it from the amygdalar tissue.



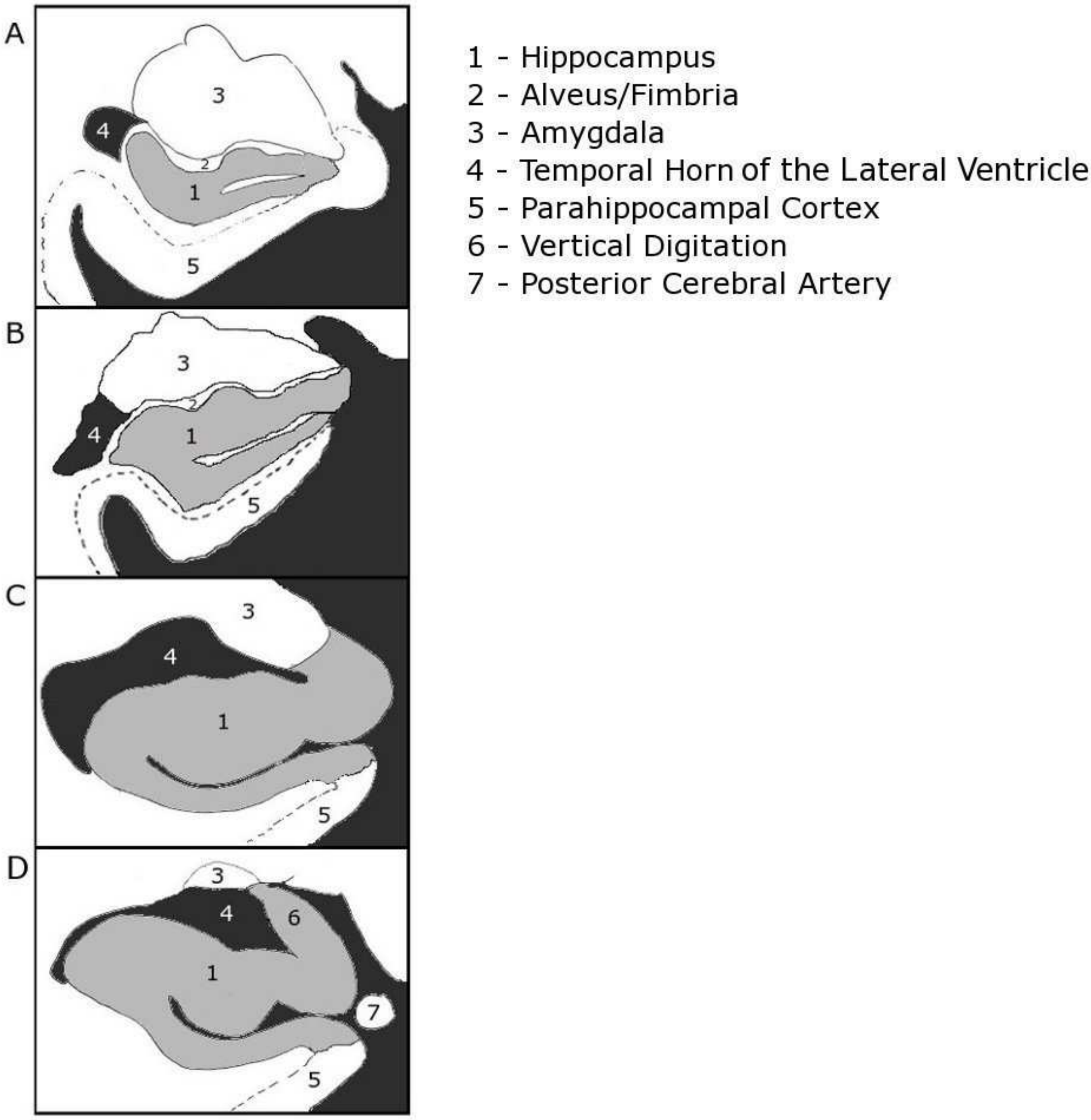
The boundary between the hippocampal head and the amygdala is an issue not only for the most rostral hippocampal tissue, but for many slices along the hippocampal head.

When the hippocampal head is not separated from the amygdala (Figure 4a and 4b), the dorsal boundary consists of the WM of the alveus/fimbria (to be included in the segmentation).

When the CSF inlet from the lateral boundary with the lateral ventricle (see Figure 4: “4 – Temporal horn of the lateral ventricle”) extends medially, separating the hippocampus from the amygdala, the dorsal border is partly defined by this interface with the CSF of the temporal horn of the lateral ventricle. At this level, the amygdala extends into a vertical-oblique band of tissue containing its most caudal accessory basal and cortical nuclei (Figure 4c. See also [2] at page 135: Figure 7.5 b). This oblique band belonging to the amygdala must be excluded from the segmentation. At this level, therefore, the dorso-medial border of the segmentation consists of the border with these last nuclei of the amygdala. In this region, it is usually not possible to detect voxel intensity differences helping boundary discrimination, therefore this boundary must be inferred through very careful examination of the involved structures using the 3D visualization and relevant atlases (Figure 4; [2] at page 131: Figure 7.4 b, 135: Figure 7.5 b, 139: Figure 7.6 b). It must be underlined that this is a very frequent source of mistakes, deserving great attention for correct anatomical segmentation.

In more caudal slices of the head, when the amygdala appears as progressively more buried in the above temporal stem above, (Figure 4d, see also red arrow in Figure 3), the dorso-medial hippocampal tissue consists of the end of the vertical digitation of the hippocampus, that must be fully included in the segmentation (Figures 3 and 4d). Please note also that the apical tissue of these last slices of the hippocampal head (where the amygdala is buried in the temporal stem, as 3 in Figure 4d) is a frequent source of mistakes: in these slices, all of the apical tissue of the vertical digitation must be included in the segmentation (see “correct segmentation” in Figure 3), as confirmed by the sagittal view during 3D navigation. At this level, the neighbouring region to be excluded from segmentation is only the CSF, and the small boundary with the amygdala, running continuously with the profile of the temporal stem.

Figure 4. Neighbouring structures at the level of the hippocampal head.



3.4.2 Dorsal boundary at the level of the body and tail

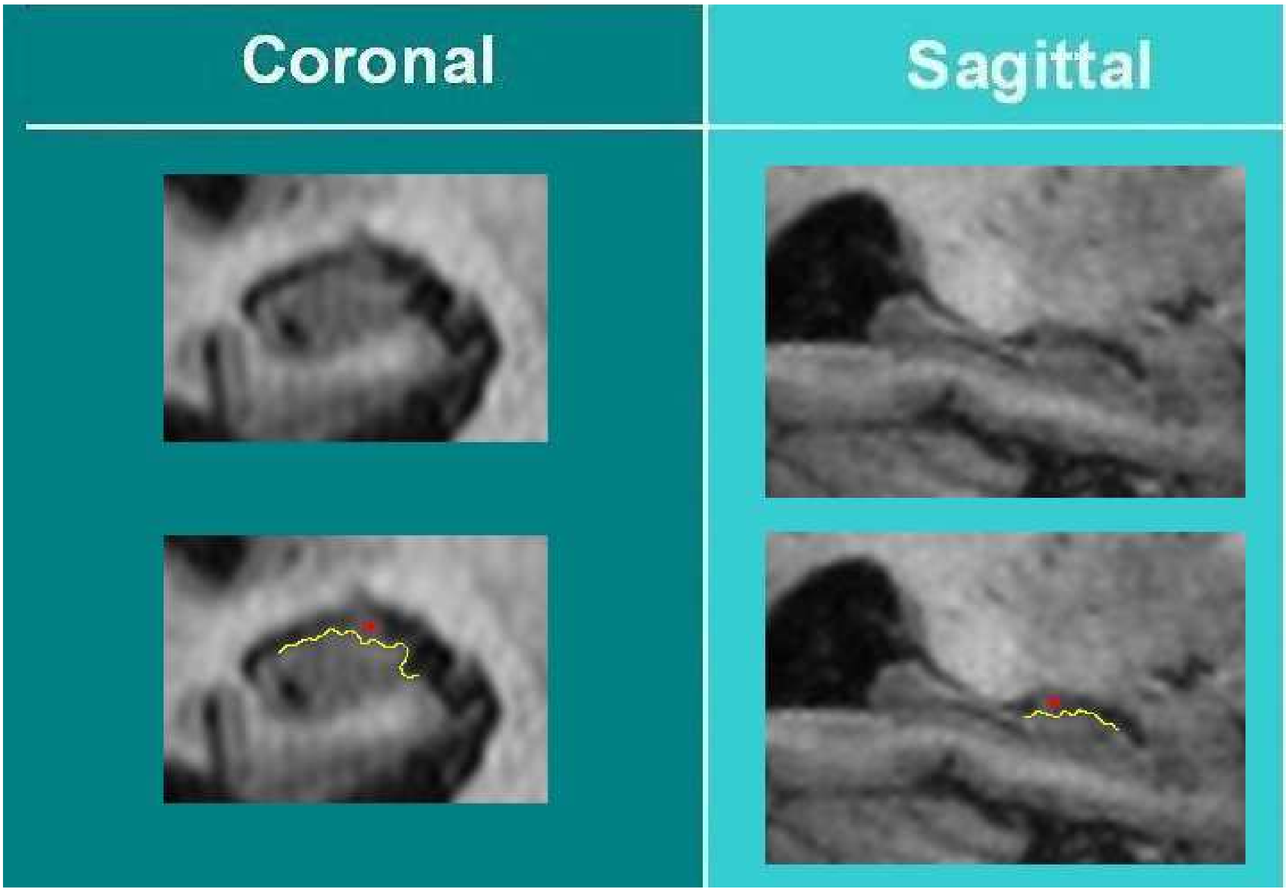
At the level of the body and tail, the dorsal border of the hippocampus is the interface of hippocampal tissue (GM, or alveus/fimbria in the dorsolateral aspect) with the neighbouring CSF.

3.4.3 Exclusion of the choroid plexus

At the level of the hippocampal body and tail, the choroid plexus of the ventricle extends along the dorsal aspect of the hippocampus. The choroid plexus consists of capillaries, separated from the ventricles by choroid epithelial cells, which filter liquids from the blood to generate CSF. It is found in temporal horn of the lateral ventricles, and must be excluded from the hippocampal segmentation. Distinguishing the hippocampal tissue from the choroid plexus may be difficult since the two structures are characterized by a similar gray intensity on MRI. However, the choroid plexus appears less dense than the hippocampus, looking similar to an inflorescence. The alveus/fimbria can usually be visualized at this level, allowing the segmentation of the dorsal border, and the exclusion of the choroid plexus located above.

When the alveus/fimbria is not visible, and the choroid plexus is dense and therefore visually similar to hippocampal GM or WM, it can be recognized through 3D navigation as being detached from the hippocampus along its length (red asterisk in Figure 5).

Figure 5. Choroid plexus in coronal and sagittal views.



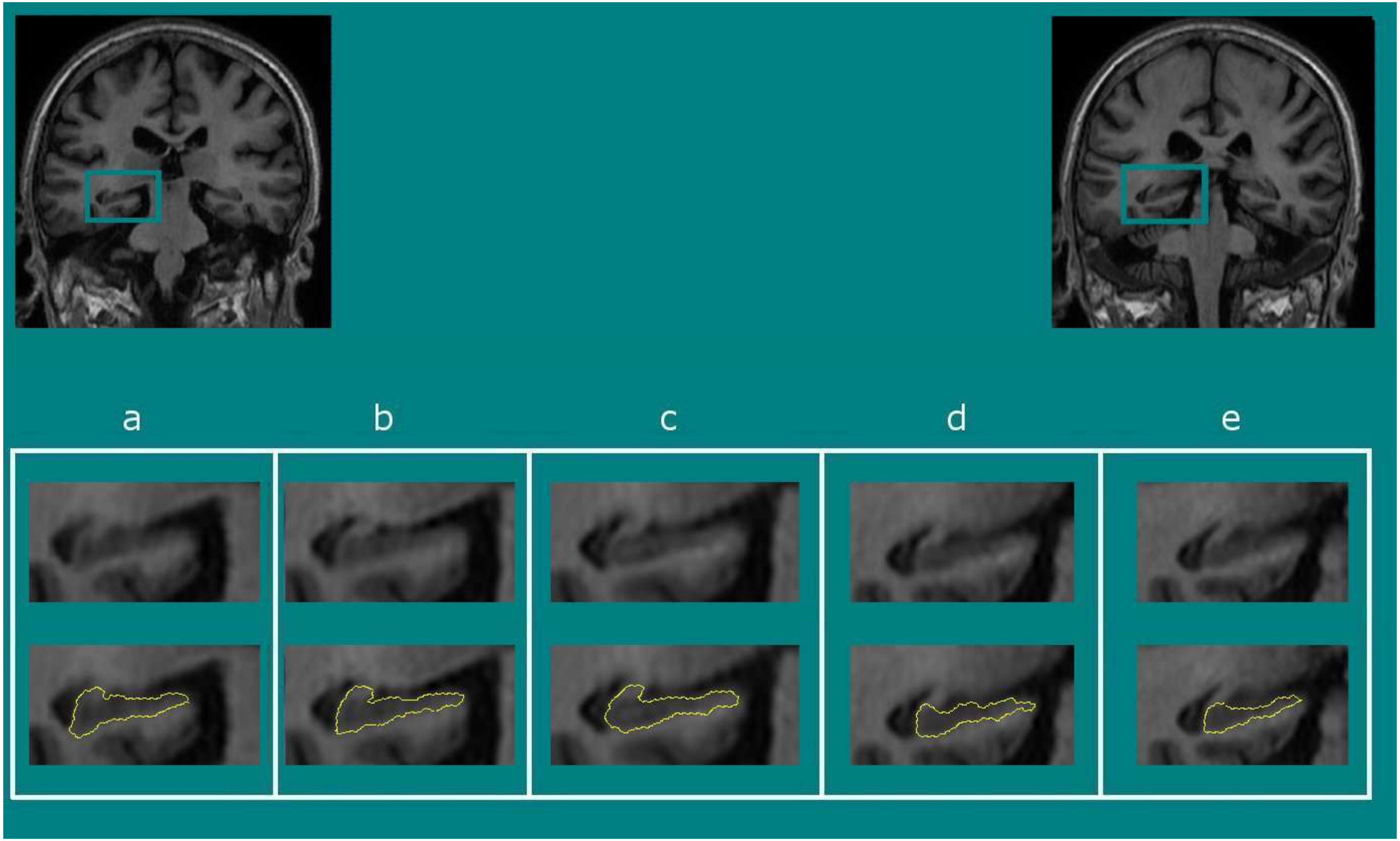
3.4.4 Inclusion of the alveus/fimbria

The alveus-fimbria-fornix pathway is one of the major conduits for subcortical afferent and efferent connections. The fibers of the alveus/fimbria are continuous with the fornix; these structures take different names based on their location.

The ventricular surface of the hippocampus is covered by a thin layer of myelinated fibers called “alveus”. More caudally, the fibers of the alveus extend obliquely, from lateral to medial, on the hippocampal surface, collecting in the thicker bundle called “fimbria”. The fornix is the continuation of this bundle, detaching from the hippocampus to reach the target subcortical structures. At the post-commissural level, as these WM fibers depart from the hippocampus, they are named “crus” and “column of fornix” [3] ({{552 Andersen,P. 2006}} , page 47; [2], Figures 7.15 d-e, 7.16 d-e, 7.17 d-e, 7.18 c).

At the level of the crus of fornix, the dorsal boundary of the hippocampal segmentation is defined by the WM of the crus of the fornix and/or by the visible interface with the CSF of the temporal horn of the lateral ventricle, of subarachnoid space of the transverse cerebral fissure, or of the choroidal fissure. The alveus/fimbria WM to be included in the segmentation is that where it appears attached to the hippocampal GM. When the fornix is visible in the coronal view, the WM directly attached to the GM, and running parallel to the parahippocampal WM located at the ventral border of the hippocampus, must be included (Figure 6 a, b, c). The WM continuing lateral to the hippocampal body, and attached to the GM, must also be included in the segmentation.
More caudally,when this WM starts detaching from the hippocampal body and diverging from the plane parallel to the parahippocampal WM (Figure 6 d, e), it is classified as fornix and must be excluded from the segmentation. The fornix is clearly detached from the hippocampal GM, and borders the CSF, or other cerebral structures, on both sides.

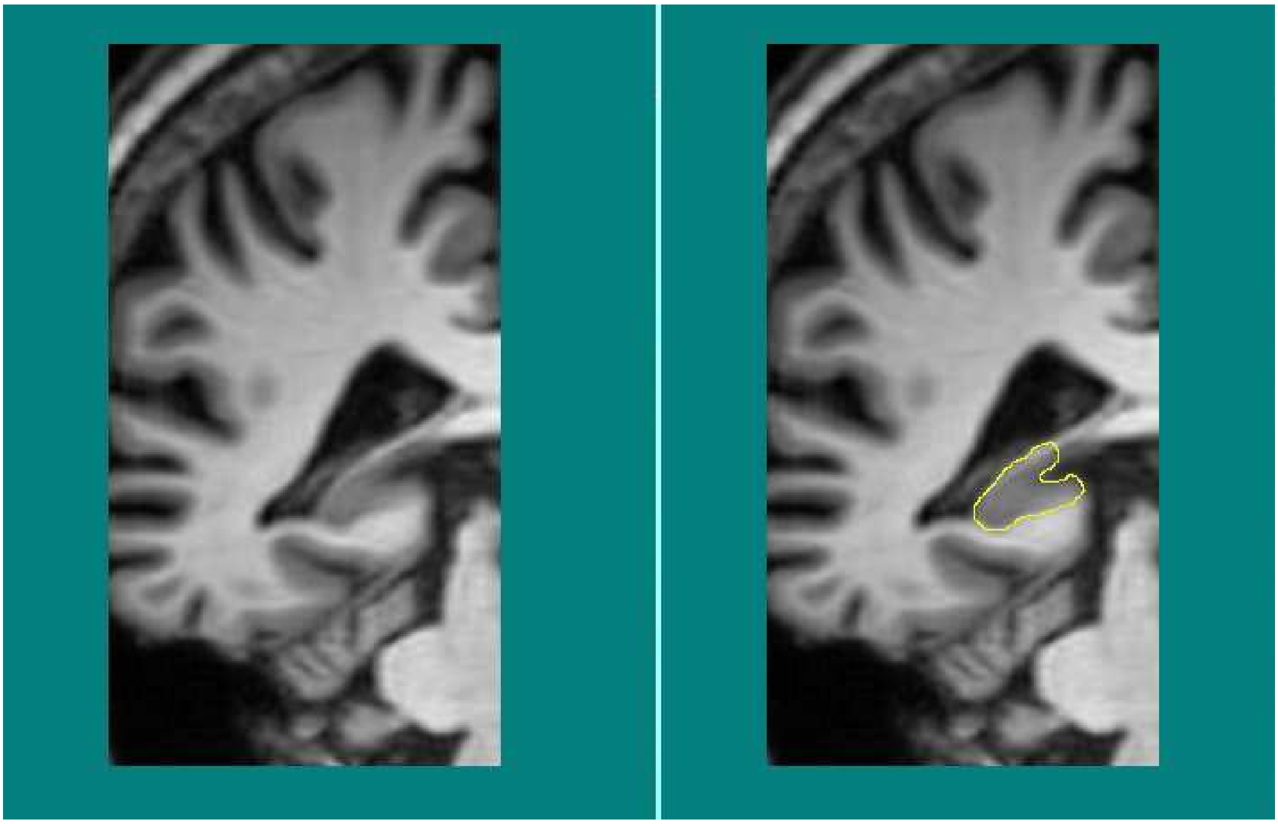
Figure 6. Slices at the transition from the fimbria (a, b, c: to be included in the segmentation) to the fornix (d, e: to be excluded from segmentation).



Note that slices caudal to the appearance of the fornix in its full length still show hippocampal WM (alveus/fimbria) at the dorso-lateral aspect, adjacent to the lateral ventricle. This must be included in the segmentation.

Based on these criteria, wide portions of fimbria, in caudal slices, are included in the segmentation, since they are attached to the hippocampal tail (Figure 7; See also Figure 2).

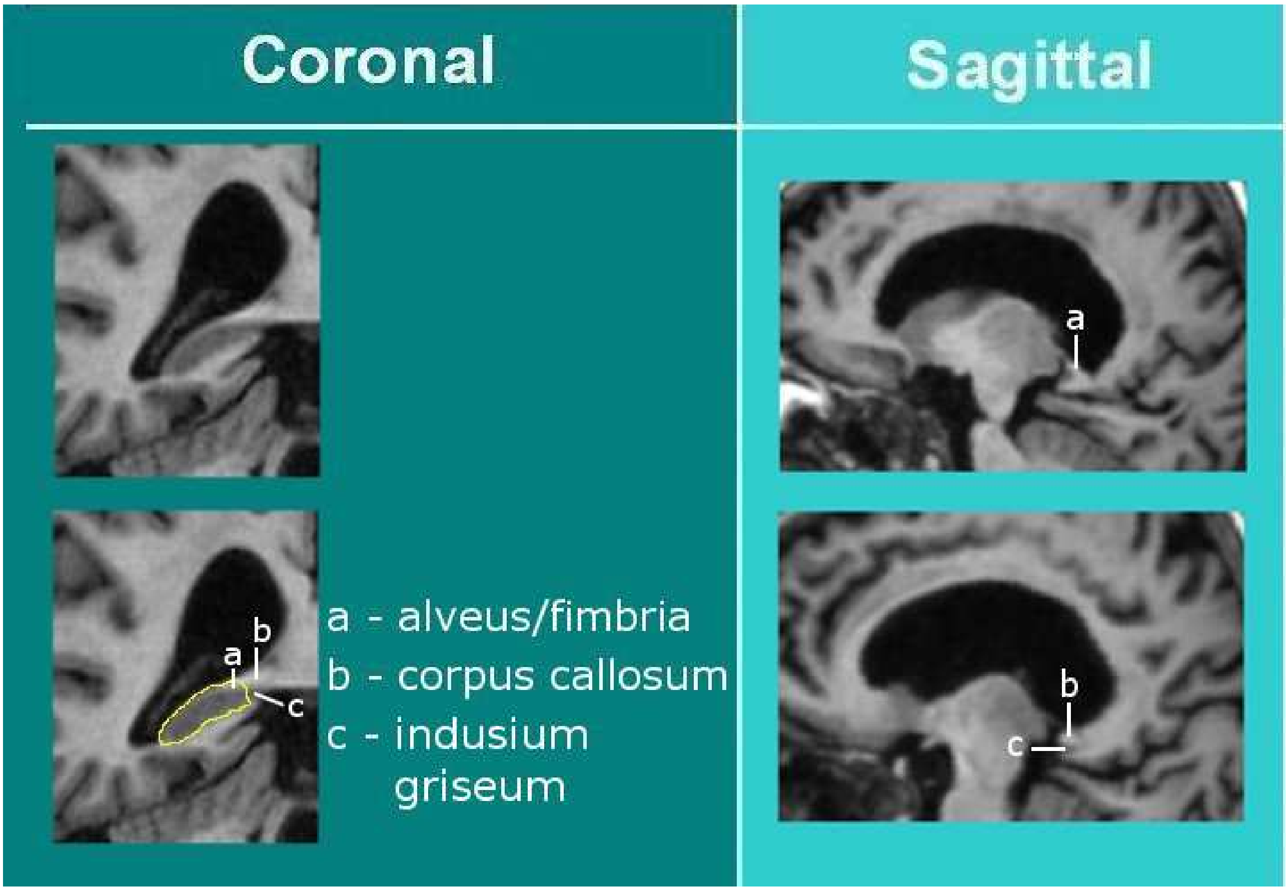
Figure 7. Alveus and fimbria WM, as it appears in slices caudal to the appearance of the fornix. The right panel provides an example of correct segmentation, including the alveus and fimbria (WM attached to hippocampal GM, and parallel to the parahippocampal WM border), and excluding the fornix (detached from the gray voxels that were attributed to hippocampal tissue by the tracer).



Only when the WM bundle is not adjacent to tissue considered as hippocampal GM, and is bordered by CSF, or is adjacent to other structures, is this WM considered as fornix and is not included in the segmentation.

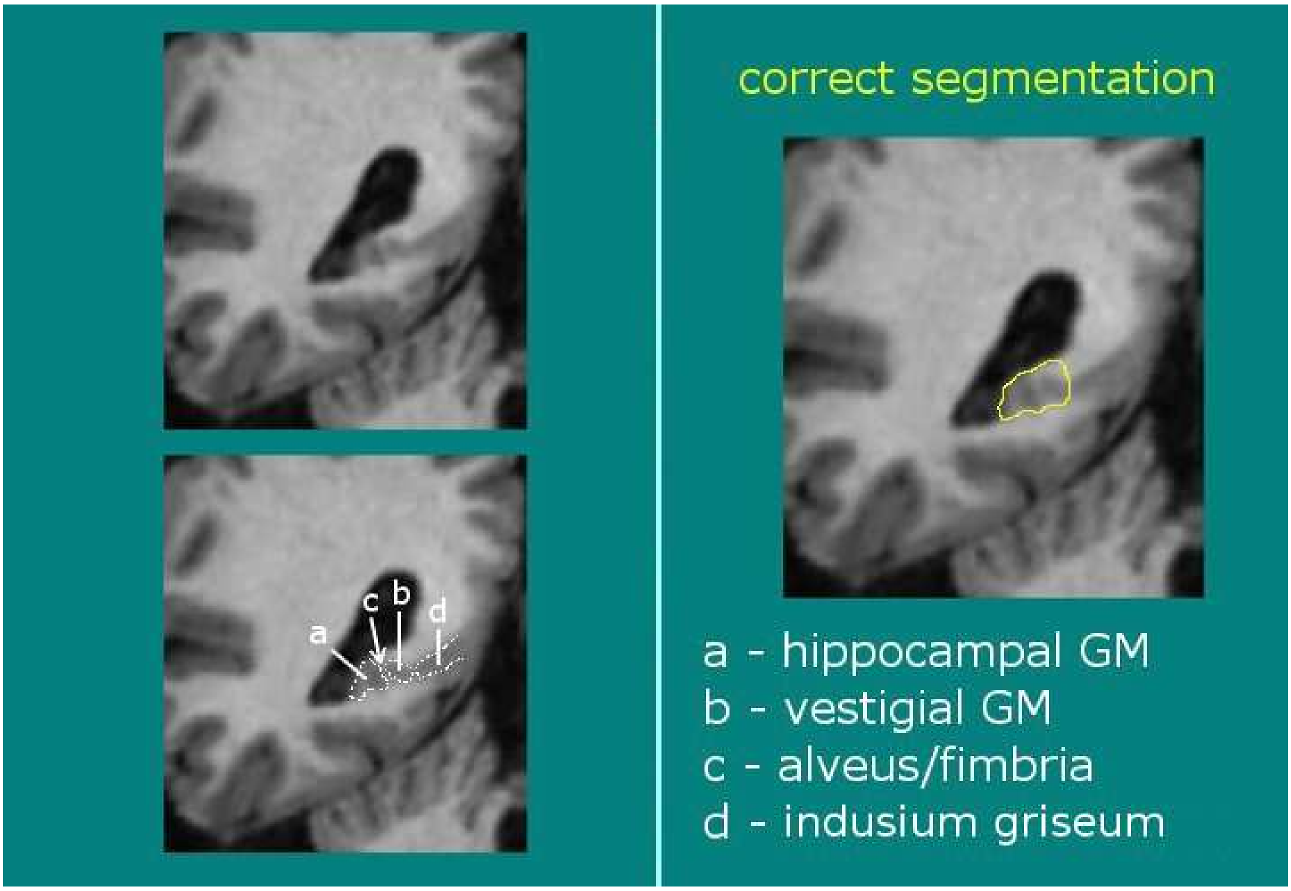
When the WM of the fimbria becomes rather thick, the sagittal view shows clearly whether it is adjacent to the hippocampus and is still fimbria (i.e., must be included in the segmentation, as “a” in Figure 8) or whether this WM is joining the corpus callosum and is adjacent to structures other than the hippocampus (i.e., must be excluded, as “b” in Figure 8). This boundary is located at the same level with the boundary between the hippocampal GM and the indusium griseum (supracallosal gyrus, or gyrus epicallosum). Therefore, checking the sagittal view at this level helps the decision making for both the inclusion of the WM and the boundaries of the medial hippocampal GM.

Figure 8. Caudal slice at the level of the boundary with the indusium griseum.



At the most caudal level, it is possible that some hippocampal GM adjacent to the lateral ventricle (a in Figure 9) is separated from other hippocampal GM (b in Figure 9) by the WM of the alveus/fimbria (c in Figure 9), that appears here as located in between the hippocampal GM in the coronal slice. All of this tissue (lateral GM, fimbria and medial hippocampal GM) must be included in the segmentation, with only the exclusion of the most medial GM (d in Figure 9) that is normally considered indusium griseum (check with 3D navigation, as illustrated in Figure 8). The accurate segmentation of these cases is shown in Figure 9, right panel. This is a source of frequent mistakes when the 3D navigation is not used for the definition of the exact boundaries of the hippocampus. For this reason it is important to consider that the fimbria as it appears in the coronal plane should not be considered as a boundary for the hippocampal GM in these cases but, rather, the boundaries must entirely be defined using 3D navigation, and the fimbria in between must be included in the segmentation.

Figure 9. Tissue attribution and correct segmentation of a caudal slice where the hippocampus and the fimbria are visualized as alternate layers on the coronal plane.



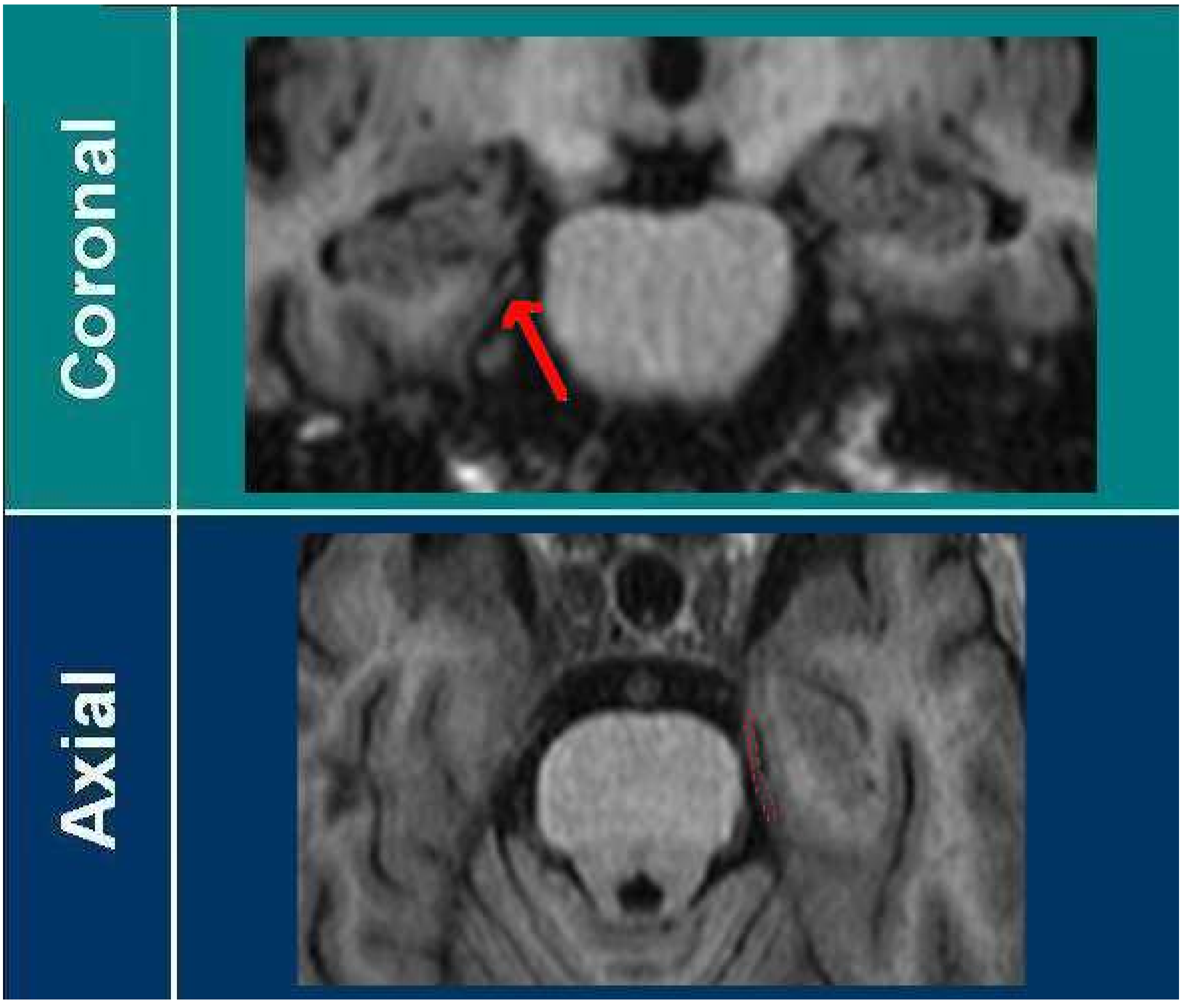
3.5 Medial boundary

The medial boundary of the hippocampus is defined in different ways depending on the rostro-caudal position.

In general, attention should be paid to exclude meninges/tentorium cerebelli (Figure 10), the posterior cerebral artery (7 in Figure 4d), or other vessels. These structures often appear of the same gray intensity as the hippocampal GM, and are often located very close, or even adjacent, to the medial border of the hippocampal body.

As to the medial tissue located caudally, considered to be “vestigial” and excluded by some segmentation protocols, this is instead fully included in this protocol.

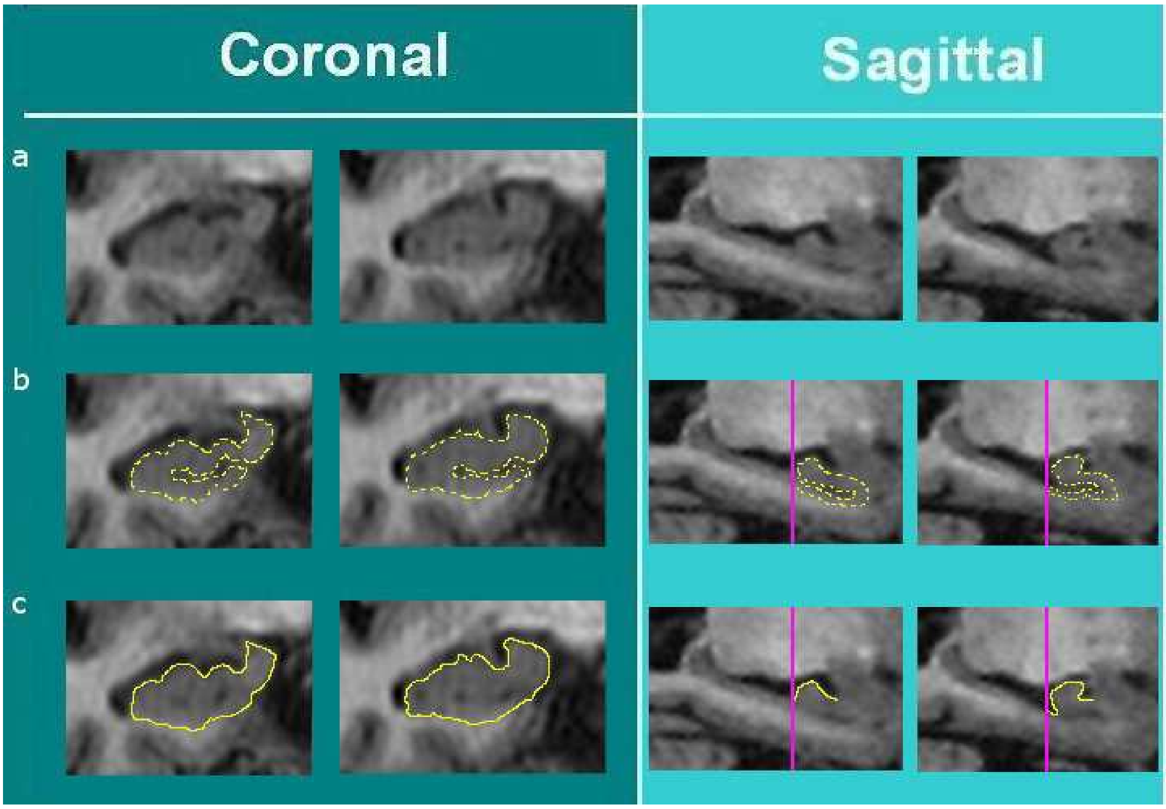
Figure 10. Tentorium cerebelli in the coronal and axial views. This structure must be discriminated and excluded from segmentation.



3.5.1 Medial boundary at the level of the head

The head level includes all of the rostral slices, up to those where the hippocampus can be seen as a folded structure on the sagittal view, or as a double-level structure in the coronal view (Figure 11b) (level of the uncal apex).

Figure 11. Hippocampal head at the level of the uncal apex. The dotted segmentation (b) denotes the folded structure of the hippocampus at this level. The continuous segmentation (c) corresponds to proper tracing based on the HarP.

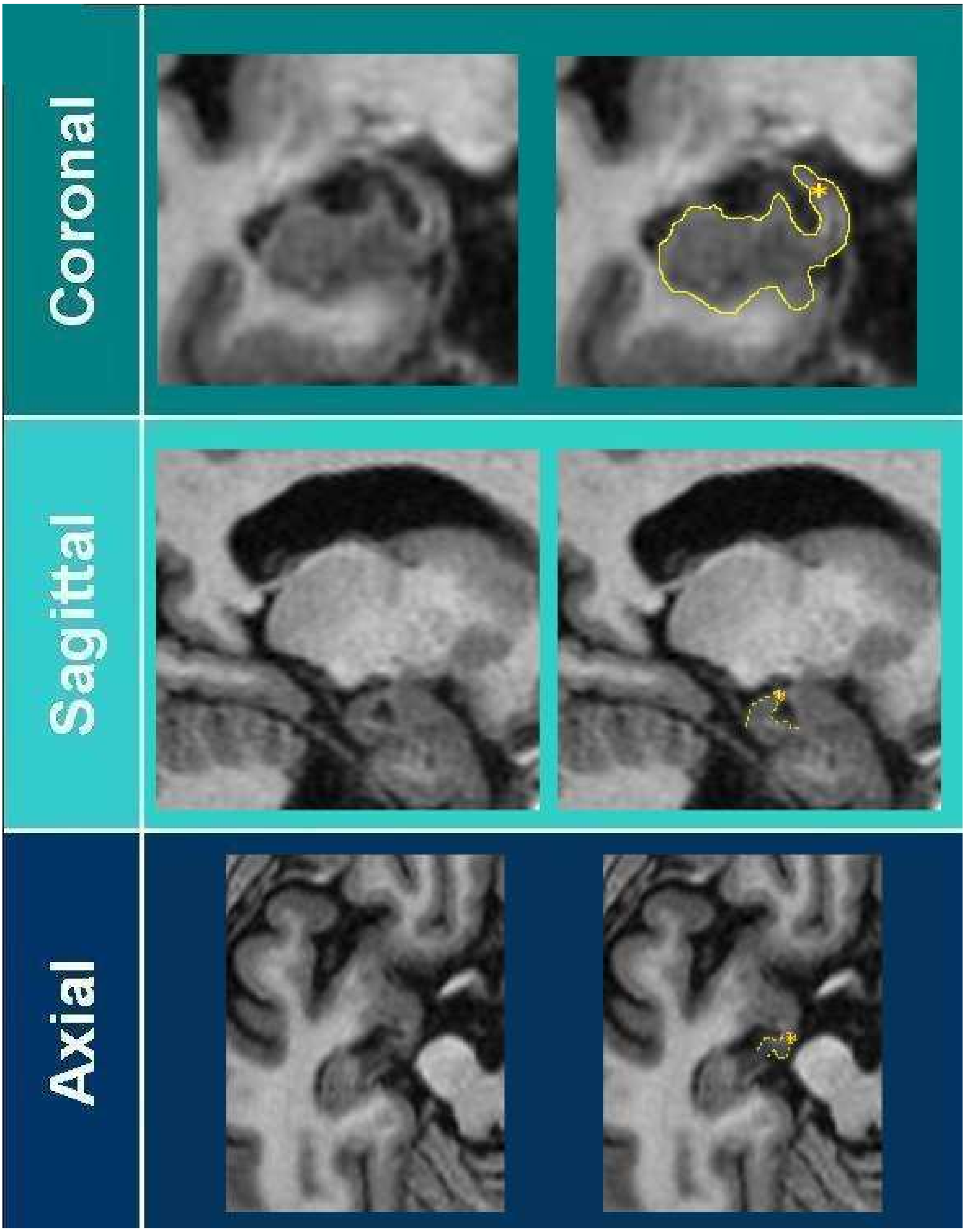


In the most rostral slice of the hippocampal head, the medial boundary can consist of the border with the GM of the amygdala, or of the parahippocampal cortex/entorhinal cortex of the ambient gyrus (see Figure 4a; [5]), or of parahippocampal WM, depending on morphological variability in subjects. The exact boundary can be ascertained with the help of the 3D navigation, showing the complete outlines of the alveus on the rostral and dorsal aspects of the hippocampus, as apparent in the axial and sagittal views respectively. At this level (see Figure 4a) the most medial GM of the ambient gyrus is composed of entorhinal cortex; [5], [2]: pages 131-134, Figures 7.4 b-d), and should be excluded from the segmentation. The segmentation must include only the tissue located beneath the alveus, located lateral to the ambient gyrus. This level can be easily checked in 3D, observing the continuity of the hyperintense line of the alveus (see figure 1, sagittal view), and the continuity of the boundary defined by the CSF located between the amygdala and the hippocampal head (see Figure 1, axial view).

In the subsequent slices, proceeding in the rostro-caudal direction, the ventromedial part of the gyrus ambiens is composed of subiculum and must be included in the segmentation (Figure 4b). At this level, the medial border of the hippocampus reaches the CSF of the ambient cistern (Figure 4b). In more caudal slices of the hippocampal head, the medial GM of the hippocampus borders the CSF of the ambient cistern (Figures 4c-d and 11). At this level, in the axial view the tissue to be included in the segmentation lies caudal to the alveus and to the CSF inlet separating the hippocampal head and amygdala.

At the level of the hippocampal head where the amygdala is clearly separated from the hippocampus by the CSF, the vertical digitation (asterisk in Figure 3 and 12; 6 in Figure 4d) must be included in the segmentation, until the level at which it connects to the amygdala, as visible in the sagittal or axial views (Figure 12).

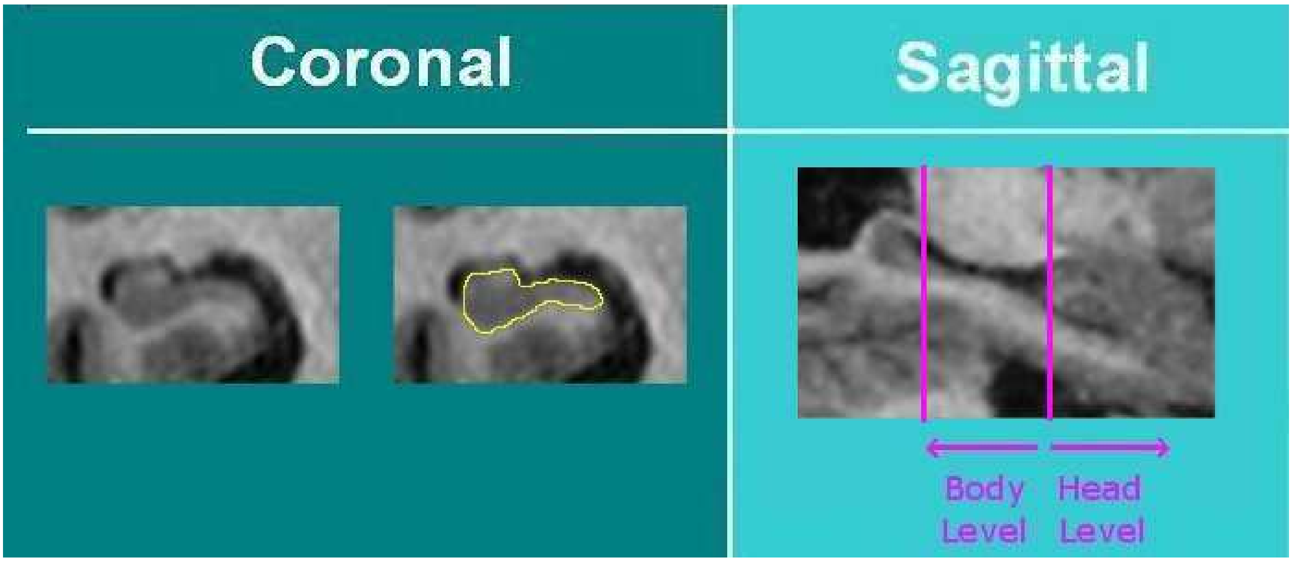
Figure 12. Inclusion of the vertical digitation (asterisk) in the segmentation. This figure allows to appreciate the sagittal and axial visualizations of the hippocampal tissue to be included in the segmentation.



3.5.2 *Medial boundary at the level of the body*

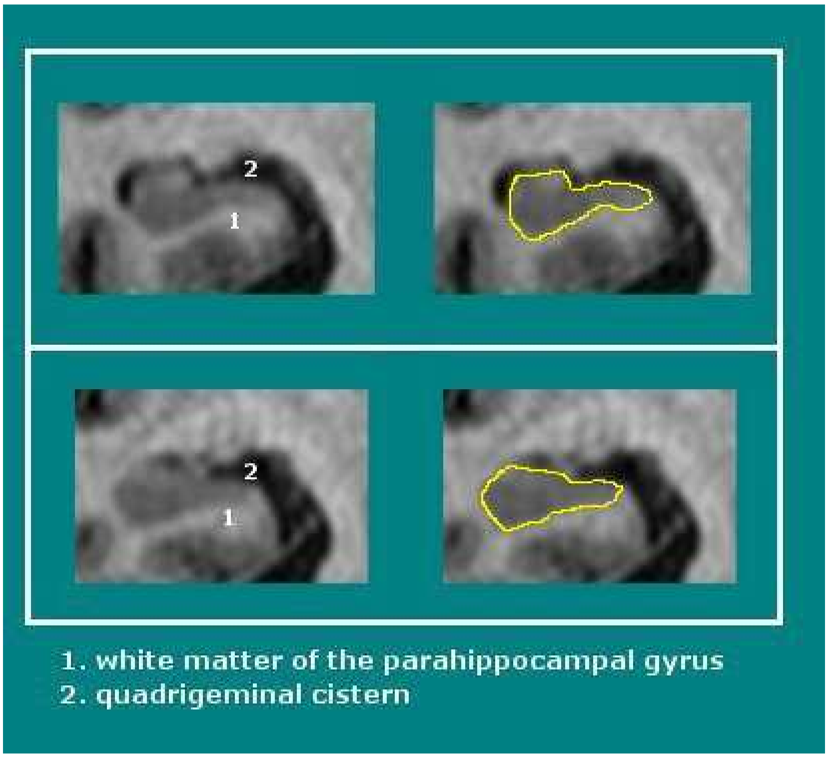
The level of the body includes, in rostro-caudal direction, the first slice where the hippocampus appears as a single- rather than double-layered structure in the coronal view, and as a single, unfolded structure on the sagittal view (Figure 13).

Figure 13. Hippocampal body.



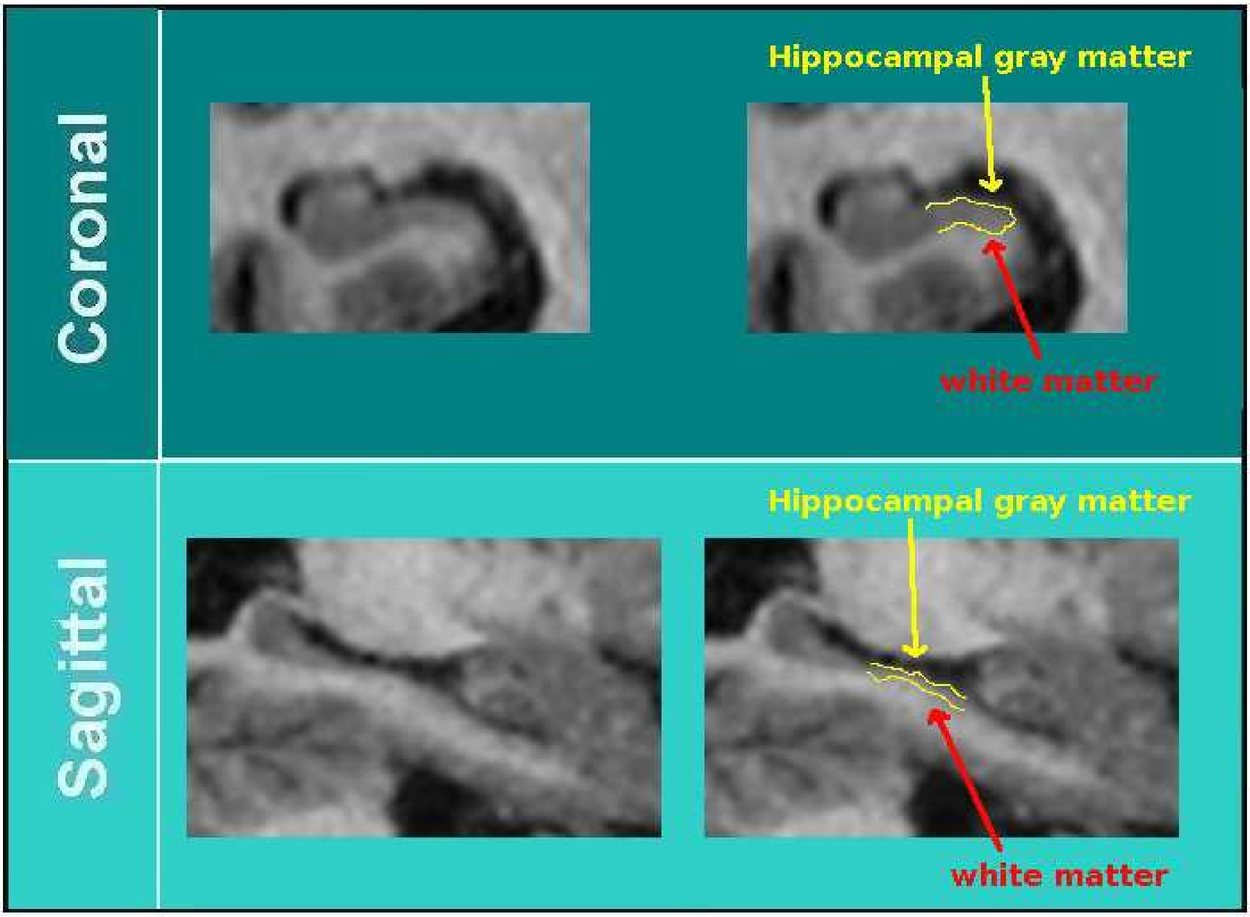
At this level, the hippocampal GM borders the CSF on the dorsomedial aspect, and must be separated from the adjacent entorhinal or perirhinal cortex on the ventromedial aspect. At this level, it is difficult to identify from the MRI whether the GM tissue belongs to the hippocampus or to the adjacent structures [5]. However, the segmentation of the medial boundary of the hippocampus based on visible details that provide a continuity in the shape of these structures proved to be reliably segmented within and across tracers [9,10]. Therefore, the medial border of the body must be segmented according to the Morphology criterion as also described in the Protocol of SUs in [9]. This criterion consists of the tracing of an irregular line continuing from the visible interface with the WM of the parahippocampal gyrus, to the ventro-medial aspect based on the continuity of the boundary as detected from morphological details and GM intensity (Figure 14).

Figure 14. Medial boundary of the hippocampal body segmented based on the visible morphology.



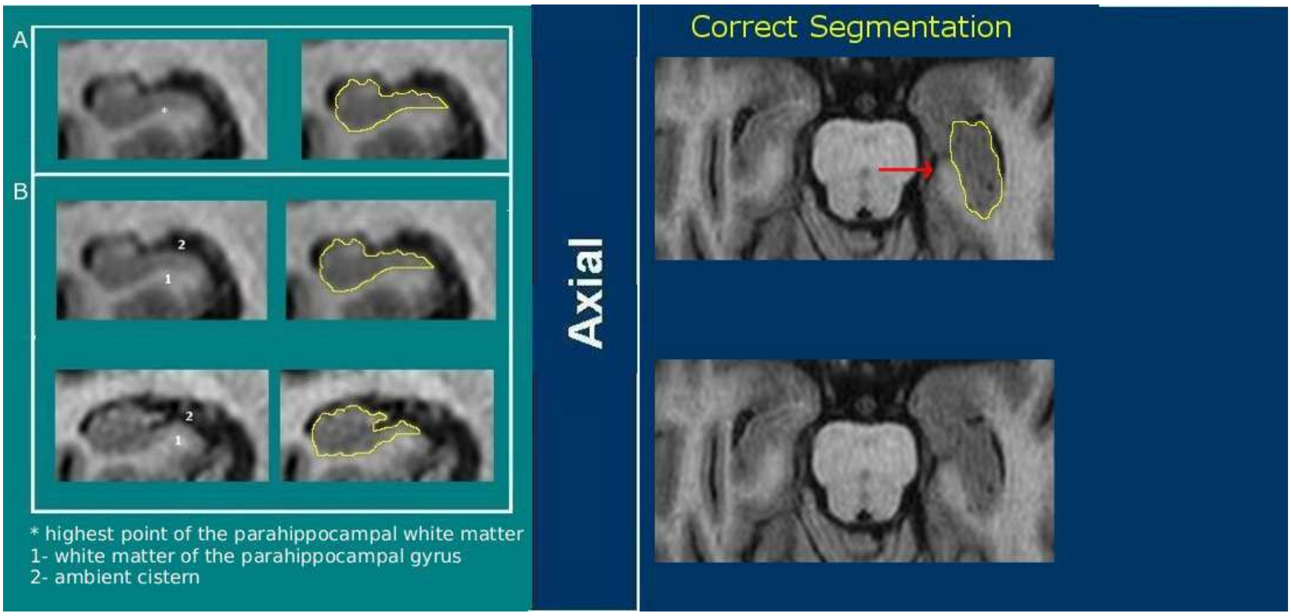
The segmentation of the medial border with the morphology criterion can be aided by the sagittal view. If the medial tissue of the subiculum in the coronal slice corresponds, in the sagittal view, on a continuous longitudinal line of gray tissue, running adjacent to the continuous line of the below parahippocampal WM, along most of the hippocampal length, that GM must usually be included according to the Morphology criterion (Figure 15).

Figure 15. Segmentation of the medial boundary of the hippocampal body according to the morphology criterion.



In those slices where there are no visible morphological details allowing the separation of the hippocampal GM from the entorhinal cortex, an arbitrary horizontal line can be used [9]. This alternative and second choice criterion relates only to those specific slices where morphological details of all possible 3D views do not help segmentation, and not to all slices of the segmented hippocampus. The Horizontal line criterion consists of a horizontal line connecting the highest point of the parahippocampal WM (see * in Figure 16A), medially, to the ambient cistern (see 2 in Figure 16B) [9]. The 3D navigation helps to check whether the horizontal line was correctly drawn. The most medial tissue segmented on the coronal plane must be checked on the axial view: at this level, if the tissue falls beyond the thick parahippocampal WM (red arrow in Figure 16) rather than lying within the hippocampal sulcus (Figure 16 right panel), the horizontal line was not properly located in the coronal segmentation (please note that instead, when the morphological criterion is adopted, the segmented tissue can properly be visualized on the other bank of the parahippocampal WM region in the axial view, beside the cistern).

Figure 16. Checking the correct location of the medial hippocampal tissue segmented with the horizontal line criterion (II choice criterion for the segmentation of the medial border of the body). The medial hippocampal tissue, segmented with the horizontal line criterion on the coronal view, may in some cases be visualized on the other bank of the parahippocampal white matter in the axial view (red arrow). This does not happen when the morphology criterion is adopted



3.5.3 Medial boundary at the level of the tail

The level of the tail starts approximately where the inferior and superior colliculi are jointly visualized in the coronal view. The medial boundary of the tail consists of the border with the WM of the parahippocampal gyrus and/or the CSF of the quadrigeminal or perimesencephalic cistern. The GM of the Andrea Retzius and of the fasciolar gyri [2] often known as "vestigial tissue", is fully included in this protocol. No hippocampal tissue must be excluded for the whole extent of the hippocampal tail, and the only medial/ventromedial boundaries are those described in this section. At the very caudal level, the ventromedial boundary consists of the GM belonging to the isthmus. The ventro-medial separation of the hippocampal tissue from the GM of the isthmus in this case relies on GM signal, morphological details from 3D navigation (Figures 17 and 18), and comparison with brain atlases. Hippocampal tissue ends at the level of the calcarine sulcus (reference mark "4" in Figure 18).

Figure 17. Caudal slice at the level where hippocampal GM borders the isthmus.

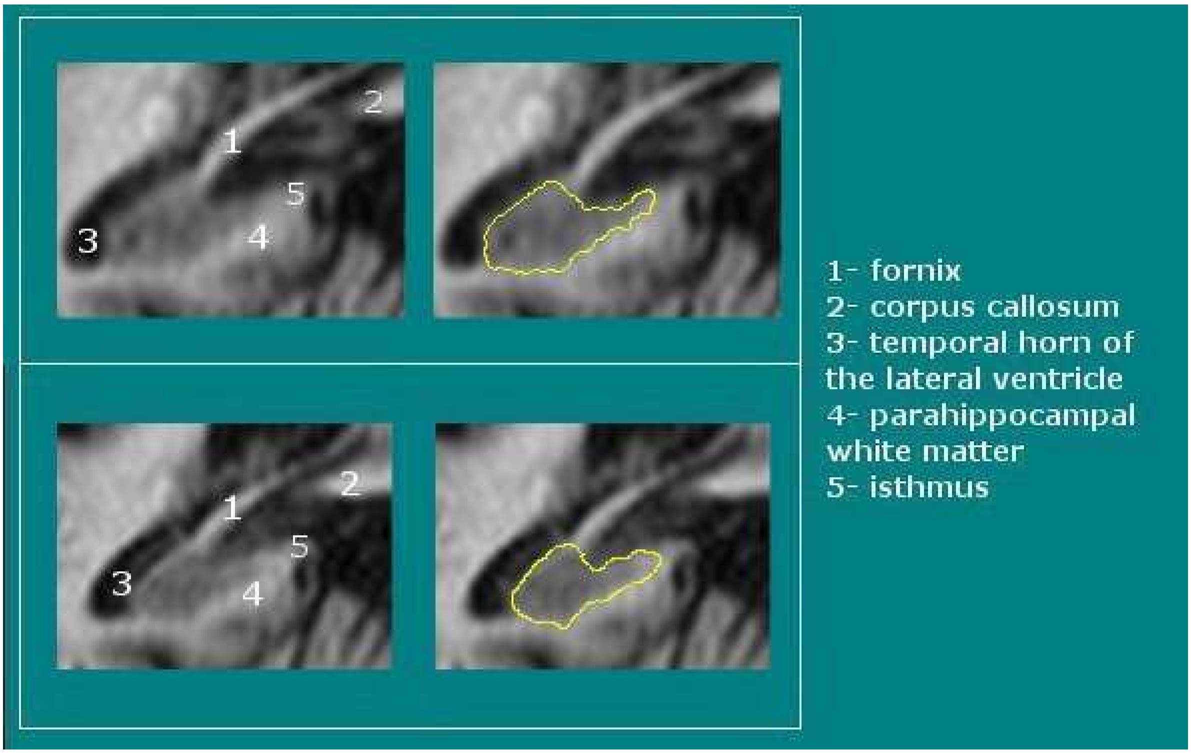
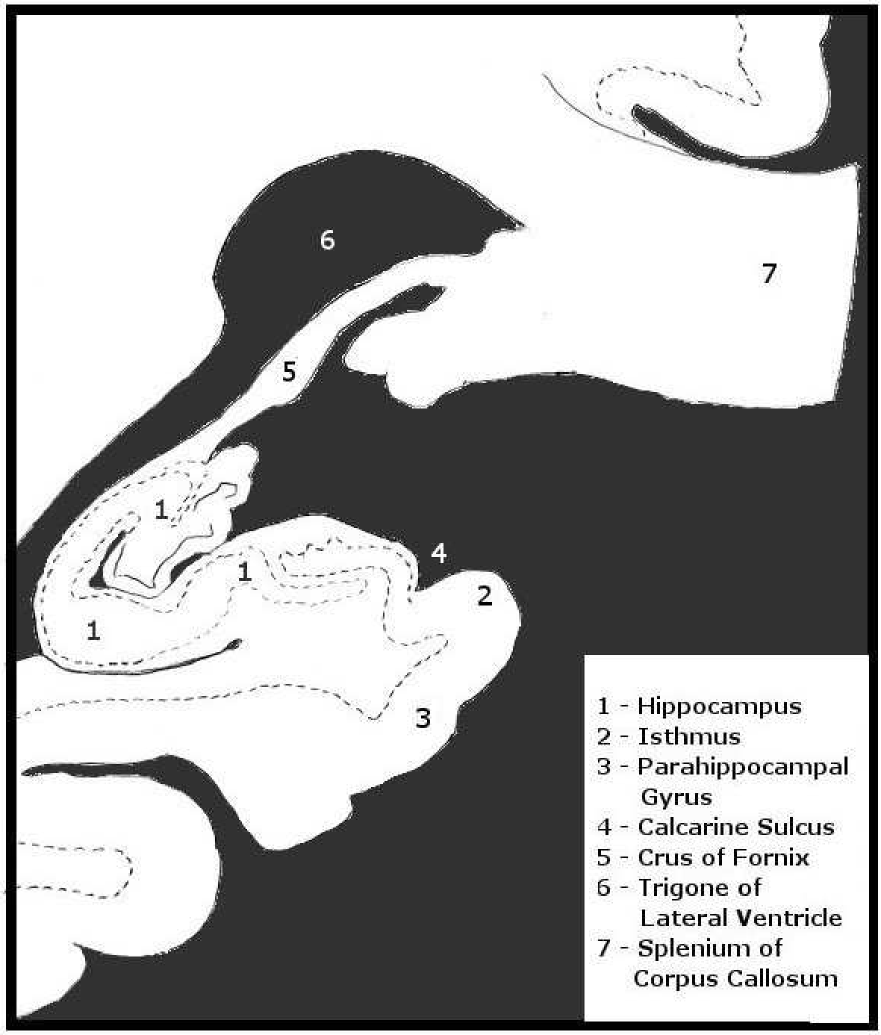
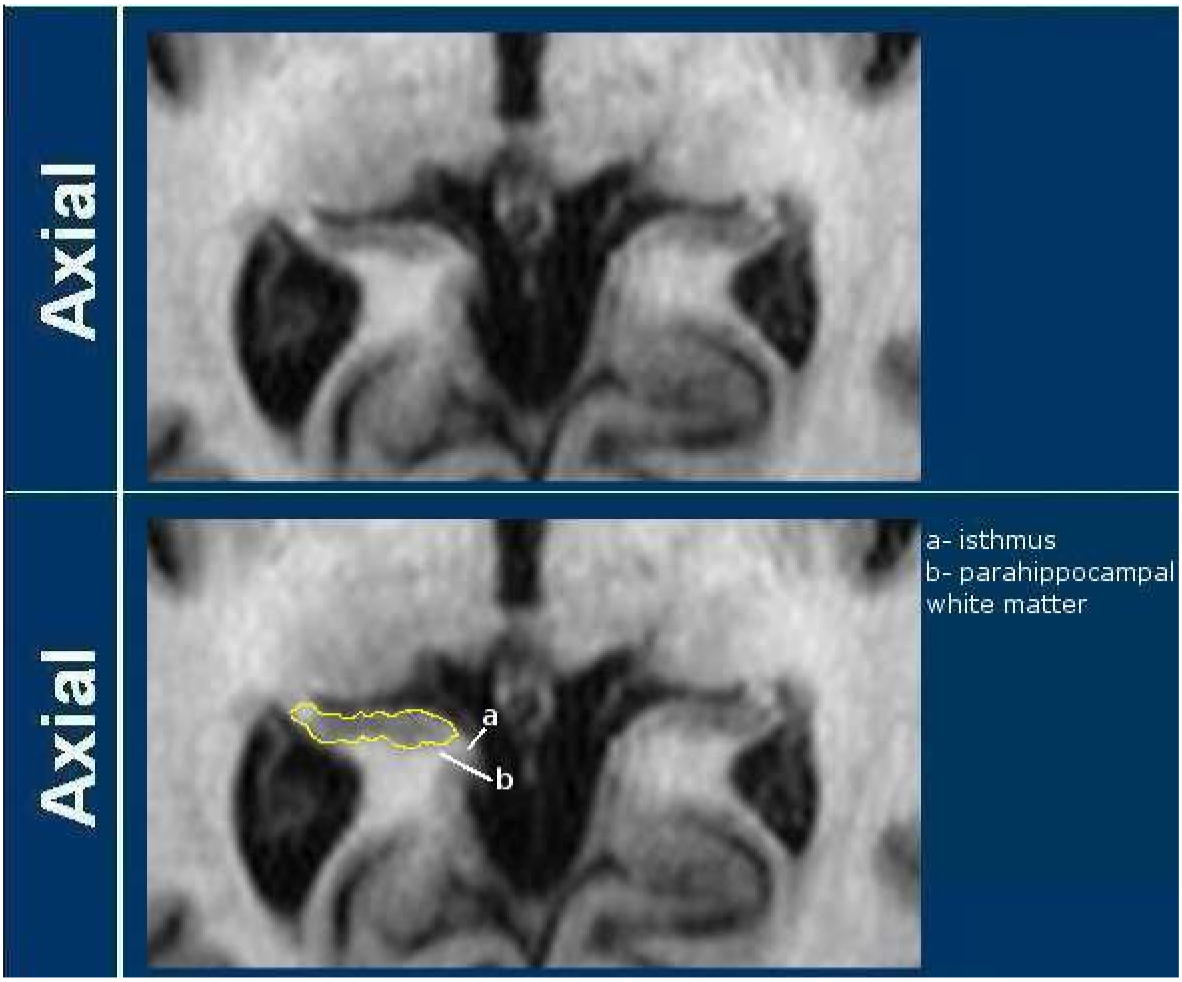


Figure 18. Representation of hippocampal and neighbouring structures at the level where hippocampal GM borders the isthmus.



On the axial view, the isthmus (a in Figure 19) can be recognized as being located on the most medial bank of the thick WM of the parahippocampal gyrus (Figure 19), facing the quadrigeminal cistern, and separated from the hippocampus.

Figure 19. Localization of the isthmus on the axial view.



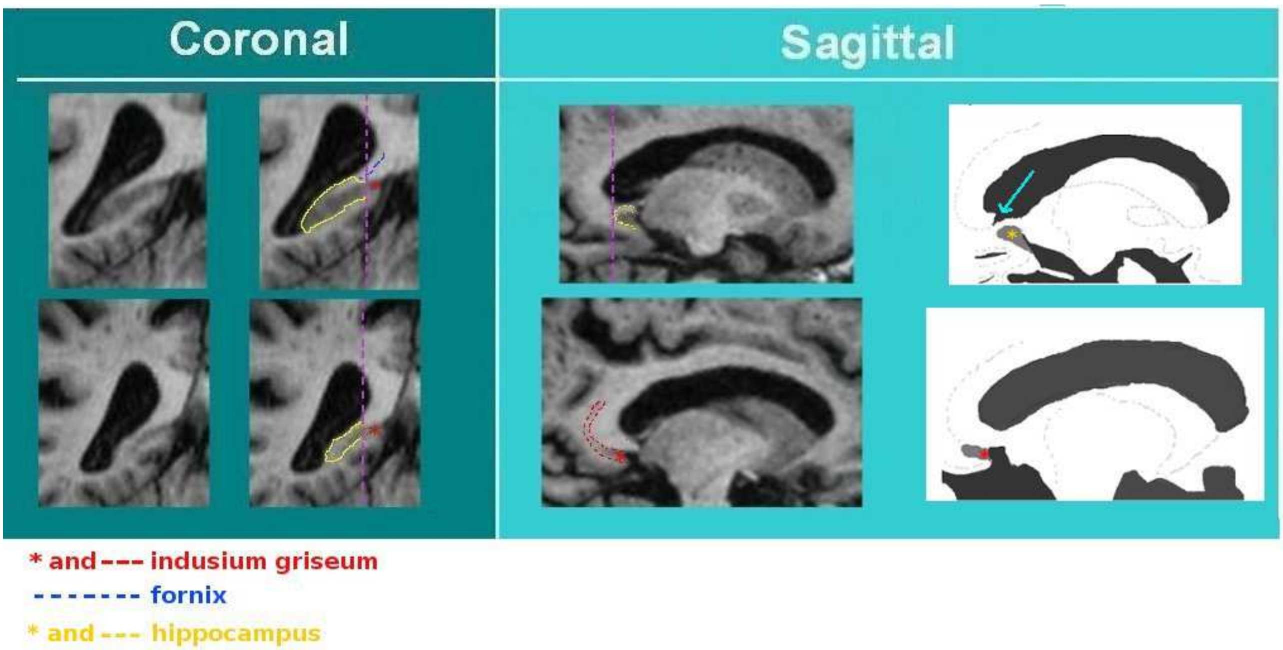
At the most caudal level, when the section of the splenium is located above the hippocampus, the medial hippocampal tissue borders the cortex of the indusium griseum. The boundary can be identified with the help of 3D navigation, and specifically by the sagittal view. When the sagittal view shows that the GM continues in a thin gyrus along the corpus callosum, the corresponding GM should not be included in the segmentation on the coronal slice, since this thin GM line curving around the splenium is the indusium griseum (or supracallosal gyrus; Figure 20; [11]). On the sagittal view, the separation (see arrow in Figure 20, right panel) of the fimbria (to be included in the segmentation) from the corpus callosum (to be excluded) by the CSF space is a very clear landmark helping to discriminate the hippocampal GM, located below the fimbria, from non-hippocampal GM, located below the corpus callosum.

On the coronal view, at the level of the boundary between the hippocampus and the indusium griseum, the dorso-lateral border of the WM, where the fimbria/fornix joins the corpus callosum, shows a change of slope. The boundary with the indusium griseum lies approximately beneath this change of slope (pink dotted line in figure 20), i.e. below the beginning of the corpus callosum. Here, the dorsal WM thickens greatly in proximity of the corpus callosum. This thicker WM consists of a dorsal layer of fornix (blue dotted line in Figure 20), covering the dorsolateral aspect of the corpus callosum, wedging itself between the hippocampus and the fornix. These structures can be discriminated very clearly in the anatomical illustration in [2] (page 159, Figure 7.10). The entirety of this WM region is, of course, excluded from the segmentation, and the below GM must be checked on the sagittal view to ascertain the boundary with the indusium griseum.

Ventrolaterally to this wedge shape, the hippocampal WM fiber tract is attached to the hippocampus, and thus is considered fimbria (i.e., it must be included in the segmentation; Figure 20, top row).

At this level, the boundary of the medial hippocampal GM with the indusium griseum must be segmented according to the same Morphology criterion described for the medial border of the hippocampal body. However, the morphological details consist in the information that can be obtained mainly from the sagittal view, since usually there are little or no differences in gray intensity corresponding to the boundaries between the hippocampus and indusium griseum.

Figure 20. Slice visualizing the hippocampal tail at the level where it borders the indusium griseum.
The separation of the fimbria from the corpus callosum by CSF helps the discrimination of the hippocampal GM from the indusium griseum (pink dotted line on the sagittal view, and turquoise arrow on the right cartoon). This separation can be more pronounced depending on the slice and on individual morphology.



3.6 Lateral boundary

If visible, the lateral boundary is defined by the alveus and fimbria, to be included in the segmentation. The border to be excluded from segmentation usually consists of the hypointense voxels of the CSF of the temporal horn of the lateral ventricle. In the most rostral slices, the lateral boundary may still consist of amygdalar nuclei, depending on individual morphology. In subjects with no or very little atrophy, the temporal horns may be very small, and the lateral boundary may consist of the WM of the temporal lobe.

4 Other segmentation criteria

4.1 Exclusion of internal CSF pools

The CSF pools within the hippocampus must be excluded from the segmentation when the tracer is sufficiently sure that the hypointense voxels correspond to CSF, and not to partial volume effect. This needs to be ascertained through the connection of the hypointense voxels with the CSF located external to the hippocampus, or with other hypointense voxels in rostro-caudal direction, even on a small number of slices. Connection in rostro-caudal direction is ascertained using the 3D navigation and scrolling contiguous slices. Internal CSF pools should be excluded from segmentation also when they relate to individual differences in the morphology of the hippocampal fissure. According to this definition, sulcal cavities (or cysts) are also to be excluded.

4.2 Scantily visible structures

The hippocampal tissue must be segmented only when it is visible on MRI, and not based on *a priori* knowledge of hippocampal morphology. However, when not visible, as it may happen in very atrophic brains, *a priori* knowledge of hippocampal morphology must be used to carry out additional checks to ascertain whether any hippocampal tissue can be detected in regions where it is expected. For example, in some cases the subiculum is so atrophic that only with searching it can be visualized, adjusting image contrast and monitor settings specifically for that purpose (Figure 21). If, after a specific search guided by anatomical knowledge, thin portions of tissue can be detected, these should be segmented with a thin trace, trying not to overestimate its volume. If no tissue can be detected even after careful search, the segmentation should not complete the hippocampal shape based on *a priori* knowledge. Rather, the segmentation should only include the tissue that is actually visible after all possible checks.

Figure 21. MRI of a very atrophic patient where the subiculum can be identified after careful check based on *a priori* knowledge of hippocampal morphology.

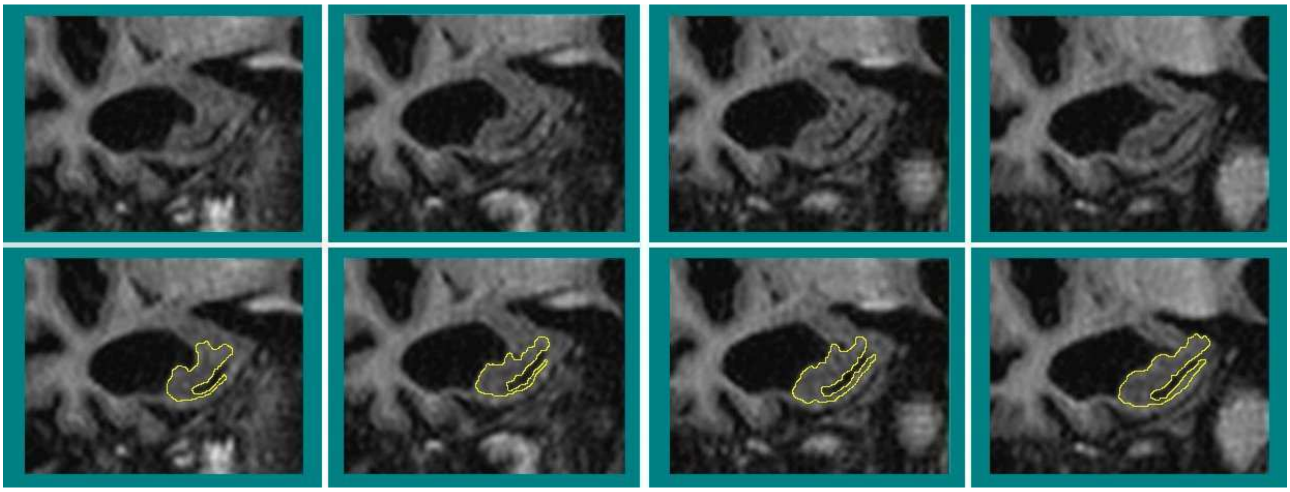
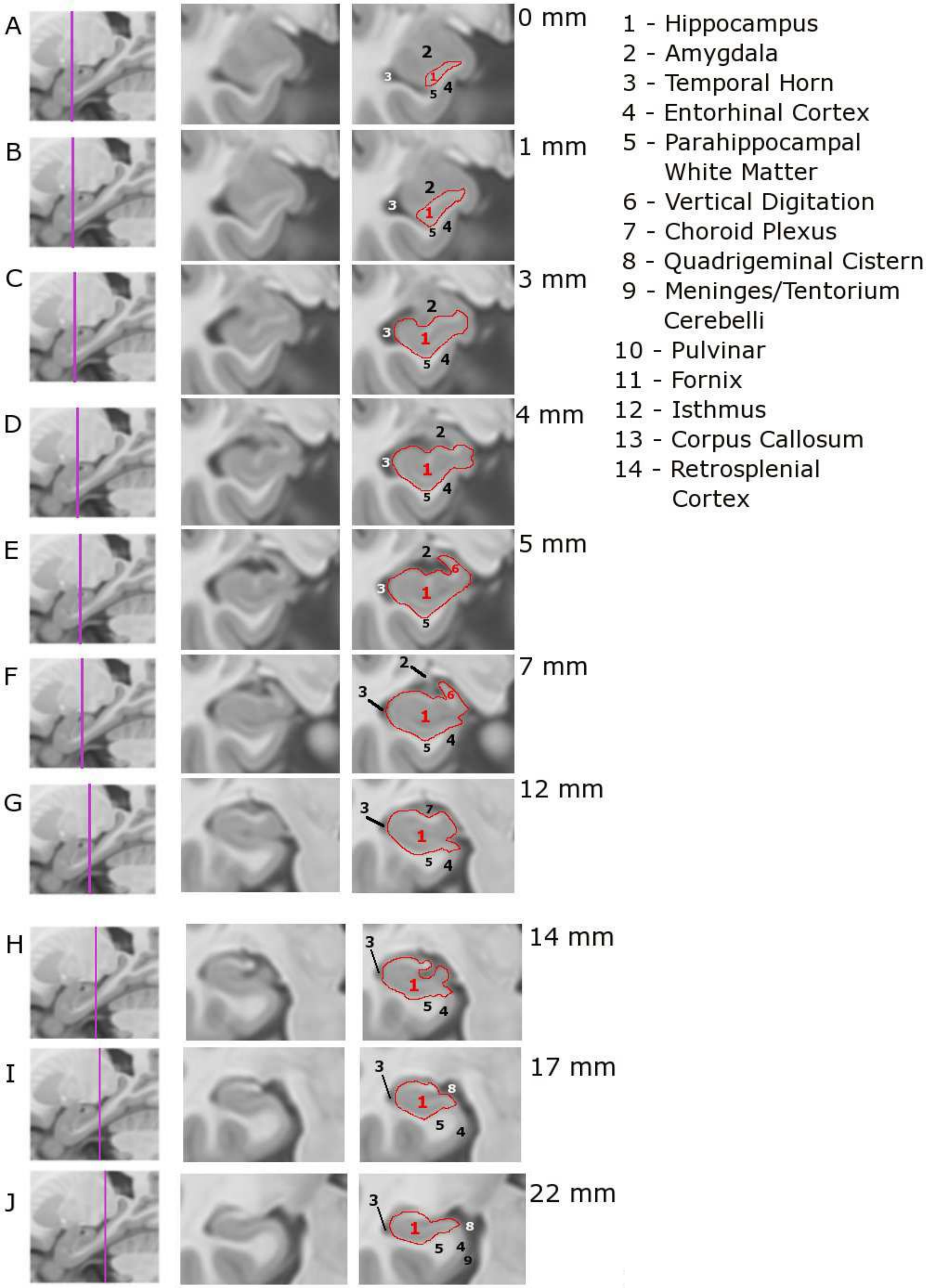


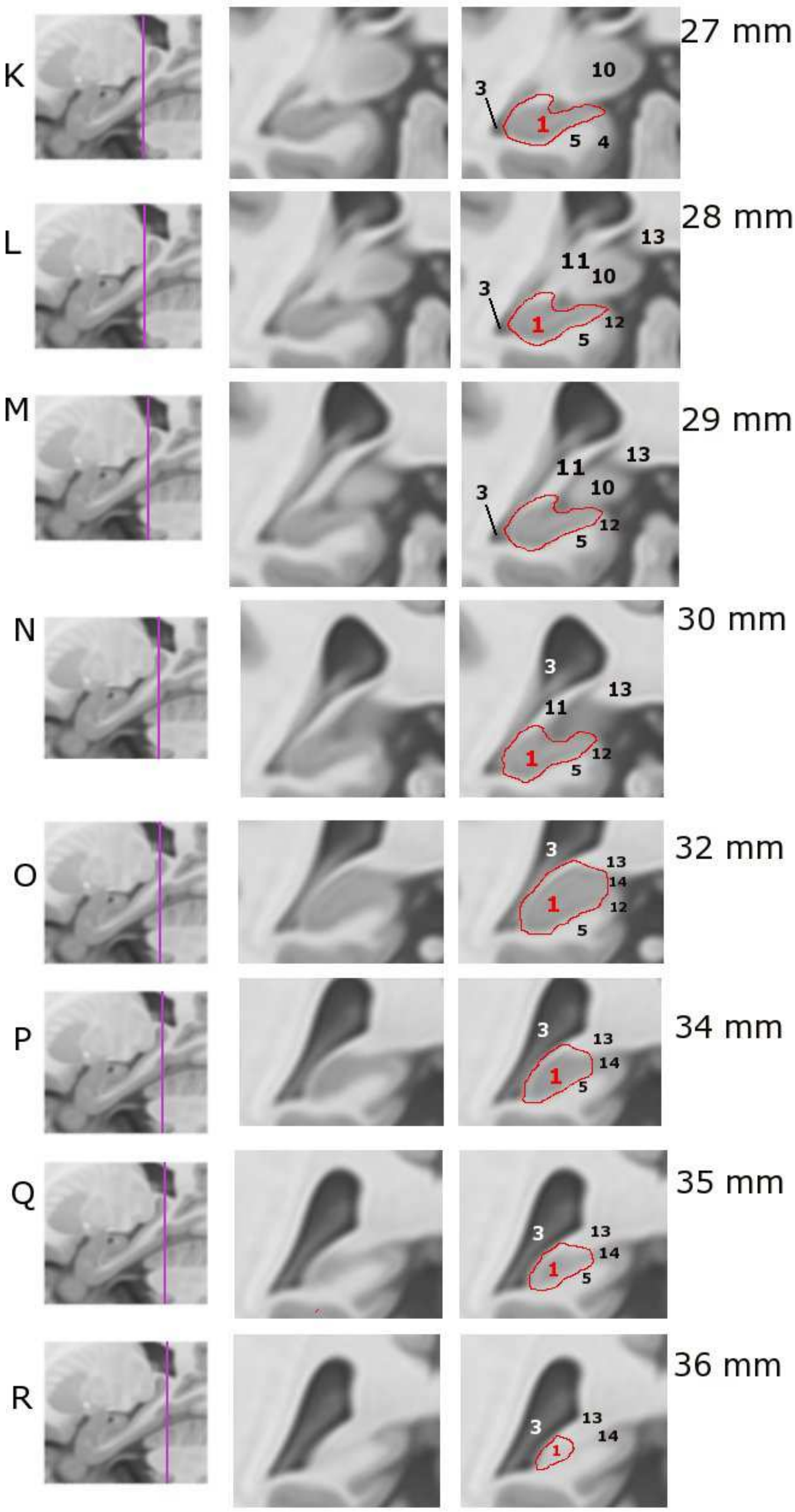
Table. Summary of landmarks of the EADC-ADNI HarP. This table provides an overview of landmarks. Careful reading of the text is required for appropriate segmentation.

	Most Rostral Slice	Most Caudal Slice	Dorsal Boundary	Ventral Boundary	Medial Boundary	Lateral Boundary
Head	Where the hippocampus or the alveus first appears below the amygdala. The alveus is included in segmentation, CSF is not included	Last slices where the hippocampus appears as a folded or double layer structure in coronal and sagittal views	Amygdala	WM of the parahippocampal gyrus	Amygdala/entorhinal cortex in the gyrus ambiens; CSF	Amygdala (in the most rostral slices); CSF; WM of the temporal lobe in trophic subjects. The alveus is included in the segmentation.
Body	Where the hippocampus appears as a single (rather than double) layered structure in coronal and sagittal views	(approximately) where the inferior and superior colliculi are jointly visualized in coronal view	CSF	WM of the parahippocampal gyrus	Line delineating the outer contour of the subiculum bordering the entorhinal or perirhinal cortex; or -Second Choice- horizontal line from the highest medial point of the parahippocampal WM to the cistern; CSF	Alveus/fimbria (if visible; to be included in the segmentation); CSF; WM of the temporal lobe in trophic subjects
Tail	(approximately) where the inferior and superior colliculi are jointly visualized in coronal view	Where a small GM mass is visible inferomedially to the trigone of the lateral ventricle	CSF; (alveus/ fimbria included in the segmentation up to the separation from the fornix)	WM of the parahippocampal gyrus; (for a small ventromedial portion: isthmus)	CSF; parahippocampal WM; isthmus; indusium griseum	CSF (Alveus/fimbria included in segmentation)

Please note that subdivisions into head, body and tail are provided based on current use in literature and aimed to facilitate communication regarding landmarks. They do not relate to consensual definitions of the Delphi panel [1].

Figure 22. Example of hippocampal segmentation based on the HarP on the ICBM 152 template. Almost all 1 mm slices are shown.





1 - Hippocampus
2 - Amygdala
3 - Temporal Horn
4 - Entorhinal Cortex
5 - Parahippocampal White Matter
6 - Vertical Digitation
7 - Choroid Plexus
8 - Quadrigeminal Cistern
9 - Meninges/Tentorium Cerebelli
10 - Pulvinar
11 - Fornix
12 - Isthmus
13 - Corpus Callosum
14 - Retrosplenial Cortex

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