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Survey of Protocols for the Manual Segmentation of the Hippocampus: Preparatory Steps Towards a Joint EADC-ADNI Harmonized Protocol

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Abstract

Manual segmentation from magnetic resonance imaging (MR) is the gold standard for evaluating hippocampal atrophy in Alzheimer's disease (AD). Nonetheless, different segmentation protocols provide up to 2.5-fold volume differences. Here we surveyed the most frequently used segmentation protocols in the AD literature as a preliminary step for international harmonization. The anatomical landmarks (anteriormost and posteriormost slices, superior, inferior, medial, and lateral borders) were identified from 12 published protocols for hippocampal manual segmentation ([Abbreviation] first author, publication year: [B] Bartzokis, 1998; [C] Convit, 1997; [dTM] deToledo-Morrell, 2004; [H] Haller, 1997; [J] Jack, 1994; [K] Killiany, 1993; [L] Lehericy, 1994; [M] Malykhin, 2007; [Pa] Pantel, 2000; [Pr] Pruessner, 2000; [S] Soininen, 1994; [W] Watson, 1992). The hippocampi of one healthy control and one AD patient taken from the 1.5T MR ADNI database were segmented by a single rater according to each protocol. The accuracy of the protocols' interpretation and translation into practice was checked with lead authors of protocols through individual interactive web conferences. Semantically harmonized landmarks and

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differences were then extracted, regarding: (a) the posteriormost slice, protocol [B] being the most restrictive, and [H, M, Pa, Pr, S] the most inclusive; (b) inclusion [C, dTM, J, L, M, Pr, W] or exclusion [B, H, K, Pa, S] of alveus/fimbria; (c) separation from the parahippocampal gyrus, [C] being the most restrictive, [B, dTM, H, J, Pa, S] the most inclusive. There were no substantial differences in the definition of the anteriormost slice. This survey will allow us to operationalize differences among protocols into tracing units, measure their impact on the repeatability and diagnostic accuracy of manual hippocampal segmentation, and finally develop a harmonized protocol.

Keywords

Hippocampus; manual segmentation protocol; harmonization; anatomical landmark; Alzheimer's disease; manual tracing; medial temporal lobes; atrophy; degeneration; MRI

INTRODUCTION

Hippocampal volumetry is a marker sensitive to disease state and progression in Alzheimer's disease (AD). The proposal for revised diagnostic criteria [1] posits that, even in the preclinical stages of the disease, the presence of hippocampal atrophy on magnetic resonance imaging (MR) is a marker suggestive of AD, the others being temporo-parietal hypometabolism on FDG PET, abnormal CSF tau and Abeta42 proteins, cerebral amyloidosis on molecular PET imaging. Currently, hippocampal volumetry is included as a secondary outcome measure in several clinical trials of disease modifying drugs to support the claim of disease modification [2].

Manual outlining on MR images by trained raters is presently the most accurate, validated and used procedure to measure hippocampal volumes [3–6], and the gold standard for the validation of automated segmentation algorithms [7–12]. However, a large number of protocols for the manual segmentation of the hippocampus is available and adopted in different fields of neuroscience research, including those investigating a variety of psychiatric and neurodegenerative conditions [13, 14]. These segmentation protocols differ in their definition of anatomical boundaries and tracing procedures, thus originating hippocampal volume estimates that cannot be straightforwardly compared. Indeed, the mean volume for a normal hippocampus can range from 2 to 5.3 cm^3 [14] across laboratories worldwide. Even if individual differences in head size are taken into account, this range is far too broad to accept hippocampal volumetry as a valid marker for any neurological condition. Although these differences are in part caused by heterogeneities in image acquisition and preprocessing, heterogeneities in the landmark definitions are major contributors. Heterogeneity was found in the definition of the most rostral and most caudal slices, in the criteria for inclusion or exclusion of hippocampal white matter (alveus and fimbria), in the definition of boundary lines with adjacent anatomical structures [13, 14].

This heterogeneity prevents a direct comparison of the outcome of different studies, and slows down the transfer of the marker from the research laboratory to the clinical setting. Standard operational procedures (SOPs) are clearly required for manual hippocampal volumetry to be transferred to routine diagnostic settings and gain status as a surrogate outcome in clinical trials for disease modifying drugs. SOPs will promote the large use of hippocampal atrophy measurements for the early diagnosis of AD and allow the comparison of the effect of different drugs in clinical trials. Moreover, the automated approaches need to be validated using a gold standard, for a given clinical population. SOPs may represent the gold standard for the many automated algorithms aiming to extract hippocampal volume with minimal or no human input that are presently under development [15, 16].

The aim of this study is to survey a selection of the most popular protocols for hippocampal segmentation used in AD research, in order to extract commonalities and differences. Importantly, we sought explicit input from protocol authors to check for proper understanding of their work. This survey is the first step of a larger project aiming to develop an internationally harmonized protocol for hippocampal segmentation.

MATERIALS AND METHODS

In this work we surveyed the landmark definitions provided for the manual segmentation of the hippocampus from MR images. A pilot survey was first carried out on 5 protocols belonging to the repertoire routinely used at LENITEM as tracing criteria, or for study purposes. The study design was then extended to a wider set of protocols including all of the most commonly used ones within the AD literature (see '*Selection of segmentation protocols*' below). Hippocampal manual segmentation was performed by a single tracer on the left hippocampus of two subjects based on each protocol (see '*Selection of scans for the prototypical tracings*', '*Image processing*', and '*Prototypical tracings*' sections below), and tracings were then checked for correctness with the lead authors of the protocols ('*Authors' check*') to obtain certified segmentations. We then extracted differences among protocols through the semantic harmonization of terms (see '*Extraction of similarities and differences*' below).

Selection of segmentation protocols

The pilot survey was carried out on five protocols routinely used in our laboratory for learning and as tracing criteria [17–21]. In the experimental phase, the selection was expanded to include all the most widely cited protocols in the AD literature (Table 1).

The protocols mentioned in the two available reviews on manual segmentation of the hippocampus were examined first [13,14]. Original protocols were drawn from the recent paper by Konrad and colleagues [13] and the review by Geuze and colleagues [14]. The review by Konrad and colleagues' included 71 protocols for hippocampal segmentation. Of these, 50 protocols provided an original description of landmarks for segmentation and were retained in the present survey, while the remaining 21 redirected the reader to previously published protocols and, thus, were excluded from this survey. All but one [22] of the protocols reviewed by Geuze and colleagues were included among these 50; the one that was not [22] was retained in the present survey.

Two of the 5 protocols of the pilot study [19,20] were not among those reviewed by Konrad and colleagues or Geuze and colleagues, and were retained in the present survey.

In order to ascertain whether the 53 protocols retained in the present survey included all of the most cited protocols for hippocampal segmentation in the AD literature, we repeated the same search carried out by Konrad, using the key-words "Alzheimer*", "AD" or "dementia" instead of Konrad's "depression", "major depression" or "unipolar depression". This search lead to the inclusion of 3 more protocols [23–25] with a high rate of citations in the AD literature (Table 1), leading to a total of 56 protocols retained in the present survey.

To be selected for harmonization, protocols had to satisfy five criteria, considered in the following hierarchical order: i) Number of citations in the AD literature greater than 40. Literature citations of the 56 protocols were identified using the ISI Web of Science portal, and the keywords "(Alzheimer* OR dementia) AND hippo*" were used to compute citations only within the AD literature (Table 1, "Citation in the AD literature"). Papers with less than 40 citations on December 31, 2009 were excluded; ii) at least the head and body of the hippocampus were included in the segmentation; iii) adjacent structures such as the

amygdala, the choroid plexus, and major portions of the parahippocampal cortex were excluded from the segmentation; iv) three-dimensional (3D) T1-weighted MR sequences with slice thickness smaller or equal to 3 mm were used; v) MR scans were acquired using a scanner with field strength greater than 1 Tesla; vi) the availability of a lead author to perform a one hour web-conference was required, to check the correctness of the tracings according to the specific protocol (Table 1, "Compliance with selection criteria").

Finally, we included 12 protocols (Table 1, First author, publication year, in alphabetical order): [B] Bartzokis, 1998 [21]; [C] Convit, 1997 [26]; [dTM] deToledo-Morrell, 2004 [23]; [H] Haller, 1997 [27]; [J] Jack, 1994 [17]; [K] Killiany, 1993 [24]; [L] Lehericy, 1994 [25]; [M] Malykhin, 2007 [20]; [Pa] Pantel, 2000 [19]; [Pr] Pruessner, 2000 [18]; [S] Soininen, 1994 [28]; [W] Watson, 1992 [29].

Selection of scans for the prototypical tracings

Tracings of the left hippocampus were carried out on images of one healthy control and one AD patient taken from the3DT1-weighted structural ADNI dataset [74], following the landmarks of each of the 12 protocols. The accuracy of the application of the protocols as described in the manuscripts was checked with the pertinent lead author.

The AD patient (ID: 021 S 0642) was selected from those with moderate to severe medial temporal atrophy (MTA) (score of 3 on Scheltens's visual rating scale, ranging from 0 to 4) [75], was 85 years old, had MMSE score of 25/30, and Clinical Dementia Rating (CDR) of 1. The control (ID: 023 S 0058) was chosen for having minimum atrophy (score of 1 on Scheltens's visual rating scale). The control was 70 years of age, had a MMSE score of 30/30, a CDR of 0 [75].

Image processing

A combination of several freely available tools was used to prepare the raw MR ADNI images for manual segmentation. DICOM images were converted to Analyze/NIFTI format using the MRIcron software (V.8.0, http://www.cabiatl.com/mricro/). Prior to prototypical tracing, the 3D image was manually reoriented based on the requirement of the corresponding protocol. Seven of the protocols considered in this study required the reorientation of the image to the long axis of the hippocampus, 5 required aligning the image to the line which passes through the anterior and posterior commissures of the brain (AC-PC line). These reorientation steps were performed using the 3D-Slicer software (V.3.2, http://www.slicer.org/). All MR images were analyzed in native space.

Prototypical tracings

A single tracer (RG) segmented the left hippocampus of the two subjects according to each of the 12 protocols, after reorienting the image as required by each protocol. The intra-rater (0.94) and inter-rater (0.89) correlation coefficients of the tracer were computed previously, on a sample of 20 healthy controls, not including the ones examined for this study, and in comparison to another expert tracer within the laboratory [76]. The protocol used for computing the correlation coefficients was that by Pruessner and colleagues [18]. Tracings were performed using Multitracer (http://air.bmap.ucla.edu/MultiTracer/) developed at the Laboratory Of NeuroImaging (LONI) at UCLA (Los Angeles, USA), allowing simultaneous 3D navigation in the axial and sagittal planes (Fig. 1). Hippocampal boundaries based on each protocol were traced on approximately 20 1.2 mm thick coronal slices.

Authors' check

Hippocampal tracings were certified as compliant with the original protocols using a three-stage check procedure: check with the lead author of the protocol, trace editing, and final check.

A Power Point presentation was provided to the lead author showing native and segmented slices of the two sample subjects. These slices were also paired with pictures of corresponding histological cuts, and text notes were added to describe tracings, landmarks and questions on unclear issues. Power Point presentations begun with a survey table, summarizing the explicit landmarks for that protocol, the main included and excluded structures, and other possibly specific key features of each protocol (Power Point presentations can be accessed at http://www.hippocampal-protocol.net/SOPs/investigatedprotocols.html). The lead author was asked to correct the survey table, examine the tracings, and take note of the unclear points that he/she would be asked to discuss subsequently. The appropriateness of our understanding of criteria was verified with the lead author at both the semantic and the practical levels, by means of individual teleconferences (TCs). TCs were carried out with a web-seminar system that allowed all participants to share the same desktop view, scroll the slides, control the cursor and carry out tracings on the same image viewed by all concurrently. TCs were recorded (available upon request), to ensure information availability over time. The tracings were verified, and unclear points were elucidated, by working on the power point presentations and with the use of Multitracer when necessary. It should be noted that the correction stage included the update to advances in tracing method, such as the use of 3D visualization tools, or other changes occurring in the tracing methods over time. After the TC, the tracings were edited according to the author's input and resent to the author for further check. This procedure was iteratively repeated until the author was satisfied that tracings had been performed according to his/her original description. These presentations, including the final summary table and the final "certified" tracings, are available at http://www.hippocampal-protocol.net/SOPs/investigatedprotocols.html.

Extraction of similarities and differences

A compendium of the survey tables of all protocols was created, by reporting the features for each segmentation criterion as it was certified by the lead authors (http://www.hippocampal-protocol.net/SOPs/LINKPAGE/anatomical-landmarks-certified-12.xls). A semantic harmonization of the features was necessary in order to ensure the correct comparison among these heterogeneous criteria, since the very same landmark was indeed named and described using different words in different protocols (http://www.hippocampal-protocol.net/SOPs/LINK PAGE/harmonized-anatomical-la ndmarks-12.xls). This phase of the survey can also be considered as the first step in the operationalization of the differences among the examined protocols. By operationalization, we mean the process of reducing the variability among protocols into a finite number of differences, sufficiently well defined to lend themselves to quantitative investigation. In practice, the wide heterogeneity in landmarks would be reduced into a finite number of elementary tracing units, which could be measured and tested.

The harmonized definitions for each landmark were chosen by two of us (MB and RG) after having checked our comprehension in the teleconferences, based on the ability of definitions to unequivocally identify the landmark, and assuming that tracing would be carried out on coronal slices from rostral to caudal.

After the semantic harmonization, the extraction of differences among the author-certified protocols was carried out for each of the examined portion of the hippocampus where

different criteria could be applied, i.e., most anterior and most posterior slices, superior border (i.e., inclusion or exclusion of alveus and fimbria), and medial border at the level of the body. Each different criterion adopted for each of these portions denotes a different definition of landmarks, which outline specific regions of hippocampal tissue. This means that, based on these criteria, well defined regions of hippocampal tissue can be isolated, and included or excluded in the segmentation of the hippocampus on MR images, depending on the adopted protocol. Therefore, this phase was also the basis for the operationalization of differences among protocols into a limited number of concrete and elementary segmentation units, to allow quantitative investigation in the next stage of this project.

The semantic harmonization and the extraction of similarities and differences also implied a hierarchy-based selection. Landmarks internal (e.g., alveus) or adjacent (e.g., CSF, parahippocampal white matter) to the hippocampus were considered as having higher value than those external to the hippocampus (i.e., pulvinar), and, whenever possible, were chosen for the definition and first operationalization stage, since these are invariant to the plane of orientation of the 3D MR images.

RESULTS

Protocols were uniform as to magnetic field strength of MR (1.5T) and reproducibility values. Moreover, they were uniform on use of 3D visualization, and in the exclusion of non-hippocampal tissue (amygdala, choroid plexus). Instead, differences could be detected in the direction of segmentation (i.e., rostral to caudal), as well as in the population used for validating the procedure (Table 2).

The survey tables for each protocol are available at: www.hippocampal-protocol.net. As illustrated in the summary survey table, following semantic harmonization of landmarks (http://www.hippocampal-protocolnet/SOPs/LINK PAGE/harmonized-anatomical-landmarks-12.xls.), differences between the protocols that most likely had an impact on the volumetric estimates concerned heterogeneities in the definition of (a) the orientation of the images; (b) the most posterior slice; (c) the superior border; (d) the separation from the parahippocampal gyrus at the level of the subiculum, in the hippocampal body (Table 3). Heterogeneities in the definition of (e) the most anterior slice are not shown in Table 3 for reasons that will be explained below.

a) Plane of tracing. Seven of the protocols reoriented the images along the long axis of the hippocampus [17, 21, 23, 25, 26, 28, 29], and 5 used images oriented along the AC-PC line [18–20, 24, 27]. Among these, 3 are the most recently published protocols [18–20], consistently with a trend to taking advantage of the greater availability of AC-PC automatic registration algorithms [77–80], that minimize and facilitate human work in the preprocessing stages.

b) Definition of the most posterior slice. Important differences characterize the protocols as to the definition of the most posterior slice where hippocampal tissue is segmented. The most restrictive protocol stops tracing at the level where both the inferior and superior colliculi are visible (Table 3; Fig. 2A). Less restrictive protocols stop when the crus or both crura of the fornices are seen in full profile, these two criteria differing often by one single slice (Fig. 2B). The less restrictive ones trace as long as they can detect hippocampal gray matter on the coronal slices (Fig. 2C), the only difference among these consisting in the attempt to exclude gray matter belonging to the vestigial hippocampal tissue of the Andrea Retzius and fasciolar gyri (see point *c*, *Definition of the superior border*).

c) Definition of the superior border. The definition of the superior border concerns the inclusion or exclusion of hippocampal white matter (i.e., alveus and fimbria) along the

structure and, for most caudal slices, the inclusion or exclusion of the vestigial gray matter (i.e., fasciolar gyrus and Andrea Retzius gyrus) that extends dorso-medially (Fig. 3, last column).

Seven of the protocols included alveus and fimbria in the segmentation (Fig. 3, bottom line), and five excluded these white matter layers (Table 3; Fig. 3, 3rd line), at least whenever visible.

As to the vestigial gray matter located on the most caudal portion of the hippocampus, arbitrary linear demarcations are adopted to separate the uppermost tissue belonging to the fasciolar and Andrea Retzius gyri [Pr] (Fig. 3, last column), or a superior portion is limited, by excluding a little layer of gray matter below the cingulate gyrus and the isthmus of the cingulum [M]. As in the case of the most anterior slice, the separation from vestigial gray matter may benefit from the 3D visualization allowed by recent software.

d) Definition of the medial border. The definition of the medial border was not problematic for the head and tail. Instead, its definition differed through the protocols for the level of the body, where the subicular region of the hippocampus joins the entorhinal cortex within the parahippocampal gray matter.

Five different methods can be found across the 12 protocols to separate the hippocampal body from the adjacent parahippocampal gray matter (Table 3; Fig. 4). Arbitrary linear demarcations are adopted by most of them. The most restrictive protocol draws a vertical line from the most medial point of the cornu Ammonis (CA) gray matter, on the dorsal aspect of the hippocampus, down to the parahippocampal white matter (Fig. 4A). Less restrictive protocols use oblique lines with different angles. These oblique lines are drawn from the lowest point of the parahippocampal white matter, and proceed medially to the liquor of the cistern, with an angle of 45°, or with a similar angle as the parahippocampal white matter below (Fig. 4B). Three protocols used a horizontal line connecting the highest point in the medial parahippocampal white matter to the CSF (Fig. 4C). Finally, three protocols segment the hippocampal gray matter relying on the visible morphology determined by the white matter shape and possible gray matter signal (Fig. 4D).

e) Definition of the most anterior slice. Heterogeneities in the definition of the boundary with the amygdala were found (http://www.hippocampal-protocol.net/SOPs/LINK PAGE/anatomical-landmarks-cer tified-12.xls), but these can be overcome due to the currently available software allowing 3D navigation (Fig. 1). This common approach allows direct visualization of the exact cursor location and of the tracing in different planes simultaneously; i.e., within the hippocampal head, within the amygdala, or in any neighboring or boundary region, the cursor can be seen from orthogonal visualization planes, where the spatial relationships among neighboring structures can be seen in different perspectives, thus providing additional complementary information. This possibility to visualize in 3D the position of the cursor allows to disambiguate whether the gray matter belongs to the hippocampus or to the amygdala in the most anterior slice. Thus, for this slice, the definition of anatomical landmarks in the coronal plane, which may differ among protocols, and which is less reliable than the direct visualization in 3D, can be considered less relevant. The consequence of this approach was that the only difference that applied to the segmentation of the hippocampal head consisted in the inclusion or exclusion of the hippocampal white matter (alveus/fimbria) when visible, i.e. in the definition of its superior border (see point *c*).

DISCUSSION

In this work, we have extracted similarities and differences among 12 protocols for hippocampal segmentation widely used in the field of Alzheimer's disease, in order to capture the source of volume variability that can be ascribed to heterogeneity in landmark definition across protocols. This extraction is the basis for an operationalization procedure aiming to achieve a finite number of well-defined units representing differences among protocols, that enable the gathering of quantitative information on these differences. Quantitative investigation will assess their impact on re-trace reliability, and on the volume differences due to AD. This and other information will help a panel of experts to make evidence-based decisions about the specific features that should be included in a harmonized protocol, using a Delphi procedure, within an international project that is currently ongoing ("A harmonized protocol for hippocampal volumetry: an EADC-ADNI effort", Alzheimer's Association funding number 174022, www.hippocampal-protocol.net).

Heterogeneities across protocols

The main differences between protocols concerned the definition of the most caudal slice, of the medial border at the level of the hippocampal body, and the inclusion or exclusion of the alveus and fimbria. The definition of the most rostral slice was considered to be no longer relevant as current software packages for 3D visualization can clarify the anatomical localization of the cursor on the MR image. It is possible that this tool may also solve the separation of the vestigial fasciolar and Andrea-Retzius gyri. There is consensus that their exclusion from proper hippocampal tissue should be recommended, and care in excluding them was indeed used by two protocols [M, Pr]. Most of the protocols [B, C, dTM, J, K, L, S, W] did not trace any hippocampal tissue located caudal to the slice where the crus or both crura of the fornices could be seen in full profile. This led to an *a priori* exclusion of the vestigial hippocampal tissue, but also to sacrificing a large portion of the hippocampal tail, which may convey relevant information about AD [76].

Different arbitrary linear demarcations were used to trace the medial border, to separate hippocampal tissue from the rest of the parahippocampal gyrus, at the level of the subiculum. No anatomical details have ever been certified as valid landmarks for the tracing of this boundary [13]. The next stage of the project will evaluate whether arbitrary linear demarcations are more reliable than tracings based on the visual morphology apparent on the MR images.

Results in the context of the literature

Two reviews on protocols for hippocampal segmentation were previously carried out. The first [14], examining most cited protocols up to December 2003, provided an extensive description of heterogeneities in all components of tracing protocols, including image acquisition parameters, pre-processing procedures, landmark definitions, and provided recommendations to carry out optimal hippocampal segmentation. The second [13] focused mostly on landmark definitions. Although we extracted protocols from these reviews, we evaluated a much lower number of reports than those examined in the review by Konrad. Nonetheless, our results are similar to this larger review. Across the 71 protocols examined, Konrad found heterogeneities in the definition of the most anterior and most posterior slices, of the in feromedial border of hippocampal body, in the inclusion or exclusion of the alveus and fimbria, and on the use of arbitrary linear demarcations when anatomical detail provided by the imaging sequence was unclear [13]. All these sources of variance were also detected among the 12 protocols examined in our study. This increases the confidence that, although our study was carried out on a limited number of protocols, they were representative of the variability of landmarks present in the literature on hippocampal segmentation, without

missing anatomical landmark specifications that may affect a harmonization project. Furthermore, we obtained the authors' certification of appropriate comprehension and execution of the tracing. This is an important point because the complexity of the anatomical structure of the hippocampal formation requires clarification of protocol details from the expert protocol designers with first-hand tracing experience.

Study limitations

We adopted the criterion of selecting protocols widely used in the literature of AD although different selection criteria could have been used. Nevertheless, our results were entirely in line with the previous larger review.

Another possible limitation is the inclusion of both recent and older protocols. These may differ in landmark definition since the older protocols are based on the view of coronal slices. Moreover, our categorization of landmarks for these protocols includes subsequent modifications that the authors carried out in more recent years to benefit of more recent tools like the 3D visualization, and that we ascertained through the individual teleconferences. Other subsequent changes to protocols were dictated by focusing on different study targets than those expressed in the paper reporting the original protocols. One example is the desire (or lack thereof) to accurately segment adjacent regions, like the parahippocampal gyrus, that overlaps in part with hippocampal tissue from the point of view of its anatomical definition. Moreover, alternative criteria may be used by the same author, depending on image quality. For example, the medial border may be segmented following visible morphology or through arbitrary linear demarcations depending on visibility of any boundary on the MR slices [J], and the inclusion of alveus and fimbria could be an acceptable criterion if their exclusion was made difficult due to poor visibility or contrast [B, K, Pa]. In cases like these, we gave priority to the criterion that the author would adopt in cases where anatomical structures are fully visible, even though more than one criterion may be appropriately reported for the same author.

The next steps towards the development of a harmonized protocol

The global project, described and updated on www.hippocampal-protocol.net, joins experts from the ADNI and EADC consortia, and other centres with advisory role. It is aimed to achieve and validate a homogeneous protocol, and implement its standard learning and use across laboratories. Standard hip-pocampal segmentation is indeed required in clinical trials and as a gold standard in the development and validation of more automated approaches to define hippocampal volumes, as they demonstrate utility. The development of a standard approach that relies on now commonly used T1-weighted high resolution images will allow a broader comparison across studies. The immediate next step of the project will be the completion of the operationalization of differences among protocols in order to model and quantify them. Information about differences in reliability, across different tracers, and in test re-test assessments will be provided for each of the relevant differences among protocols detected by this survey. Moreover, their value to inform on AD pathology will be computed on an adequate sample of representative patients with a diagnosis of AD. A panel of experts will then be provided with quantitative information that would support decisions on which features should be included in a harmonized protocol.

This effort is particularly relevant in light of the new diagnostic clinical and pre-clinical criteria for AD, which can be found at http://www.alz.org/research/diagnostic criteria. In line with the 2007 research criteria by Dubois *et al.* [1], these criteria place emphasis on hippocampal volumetry, stating that, although not specific for this kind of disorder, this biomarker better correlates with disease progression than the more specific molecular biomarkers, such as the Abeta and Tau CSF concentration levels [1, 82]. A uniform method

for the computation of hippocampal volume would improve AD diagnosis across laboratories, and hopefully provide a comparable hippocampal outcome measure in clinical trials for disease modifying drugs.

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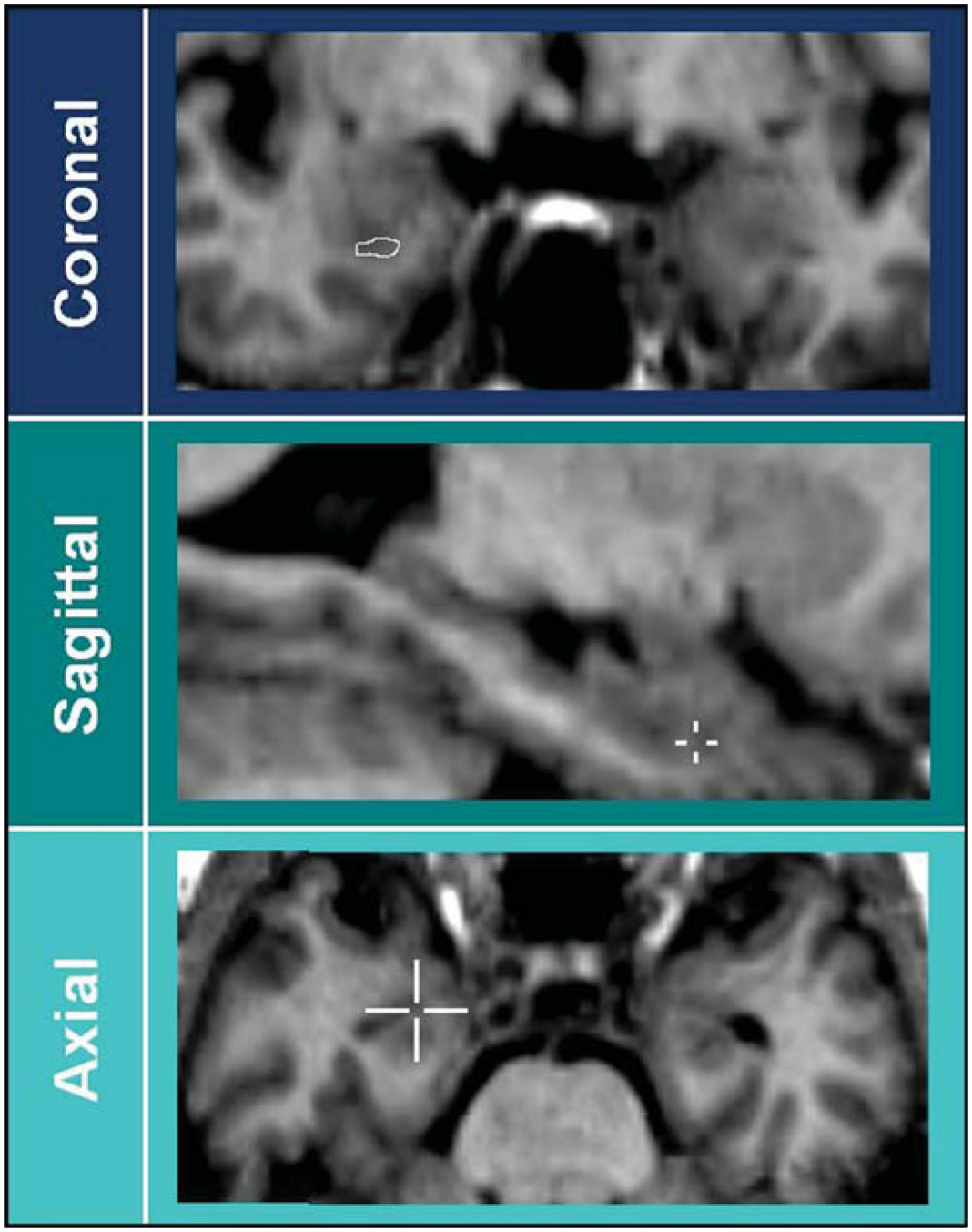

Fig. 1.
Most anterior slice. Differences in the definition of the most anterior slice are overcome due to currently used software for 3D brain navigation. The visualization of the cursor position in 3D provides unequivocal information about its location in the amygdale or in the hippocampus, even if a single coronal section may not provide sufficiently clear information.

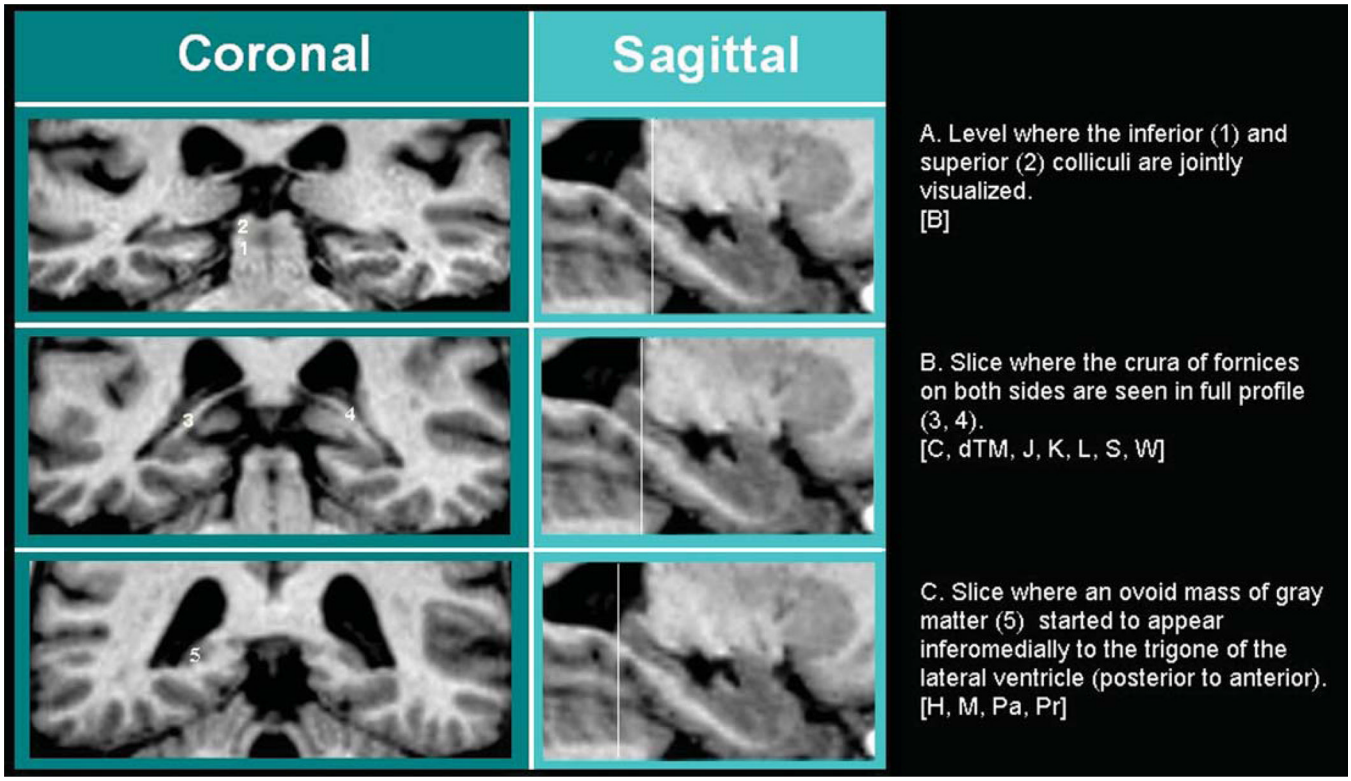

Fig. 2.
Most posterior slice. The criterion in A) is followed by protocol [B], B) by protocols [C, dTM, J, K, L, S, W], C) by [H, M, Pa, Pr].

Fig. 3.

Superior border. The superior border concerns the hippocampal white matter, i.e. the alveus and fimbria, that can be excluded [B, H, K, Pa, S] or included in different tracing protocols [C, dTM, J, L, M, Pr, W]. Additional criteria for separation of vestigial gray matter tissue in posterior-most slices are reported in [Pr] (last column). In the first line, histological pictures corresponding to the MRI slice of the same column are presented. Line II: MRI images without tracings. Lines III-IV: same MRI images with tracing example of exclusion (III) and inclusion (IV) of the hippocampal white matter.

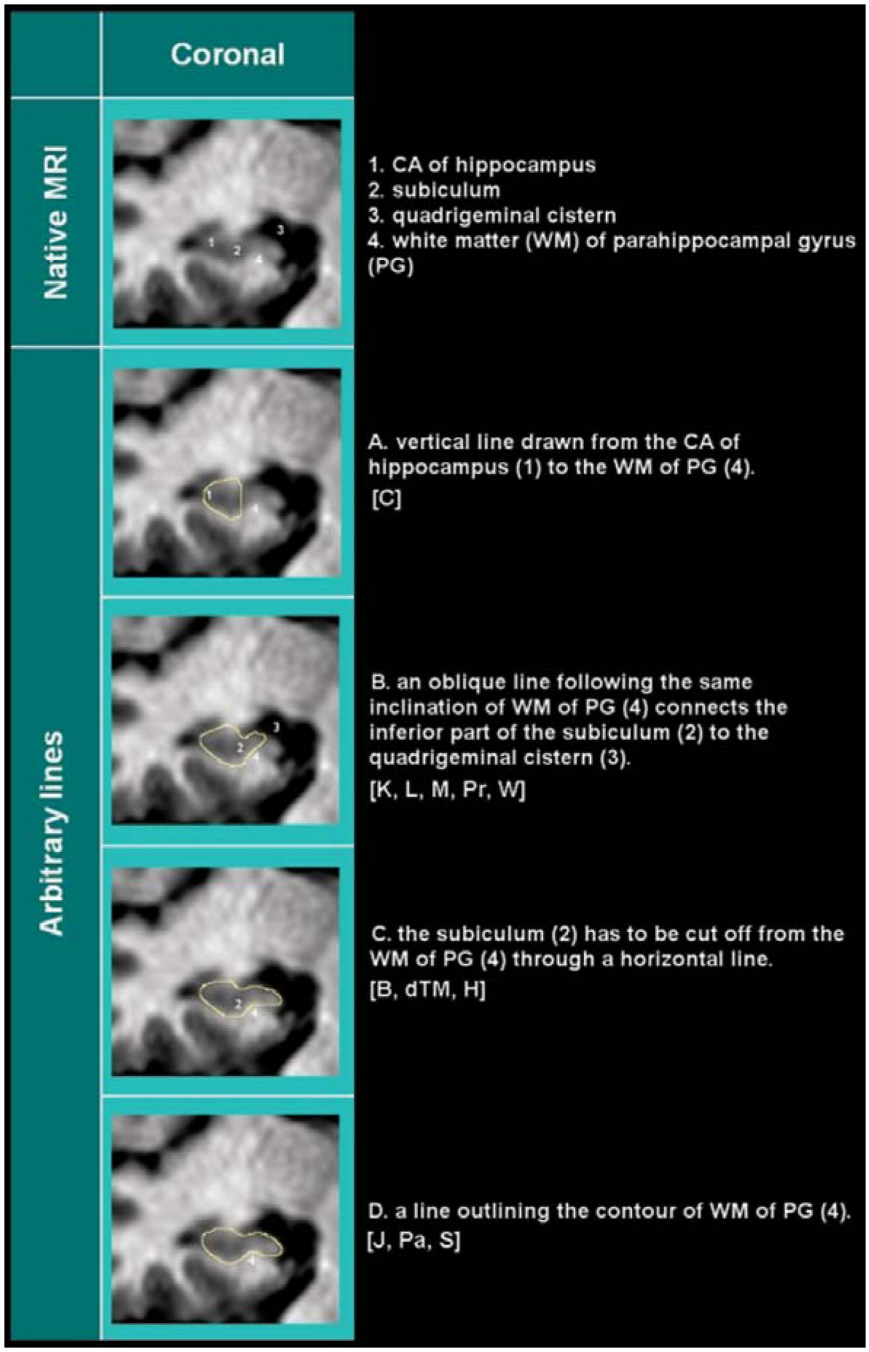

Fig. 4.

Medial border. Five different methods are described across protocols to separate the subiculum, medially, from the rest of the parahippocampal gyrus. CA=cornu Ammonis; WM=white matter; PG=parahippocampal gyrus. The method in A is adopted by [C], that in B by [K, L, M, Pr, W], C by [B, dTM, H], D by [J, Pa, S].

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Table 1

Surveyed protocols for hippocampal segmentation.

Protocol	Citations in the AD literature	Cited by Konrad and colleagues	Cited by Geuze and colleagues	Compliance with selection criteria	Inclusion stage	Selected for harmonization	Ref
Killiany, 1993	173	No	No	Satisfying criteria	Experimental	Yes	[24]
Convit, 1997	143	Yes	Yes	Satisfying criteria	Experimental	Yes	[26]
Watson, 1992	122	Yes	Yes	Satisfying criteria	Experimental	Yes	[30]
Soininen, 1994	118	Yes	Yes	Satisfying criteria	Experimental	Yes	[28]
Sheline, 1996	79	Yes	No	Author not available	Experimental	No	[31]
Lehericy, 1994	78	No	No	Satisfying criteria	Experimental	Yes	[25]
Pruessner, 2000	78	Yes	No	Satisfying criteria	Pilot	Yes	[18]
Bremner, 1995	53	Yes	Yes	Only hippocampal body included	Experimental	No	[32]
deToledo-Morrell, 2004	50	No	No	Satisfying criteria	Experimental	Yes	[23]
Haller, 1997	44	Yes	No	Satisfying criteria	Experimental	Yes	[27]
Cook, 1992	44	Yes	Yes	Plexus choroideus included	Experimental	No	[33]
Bigler, 1997	38	Yes	Yes	Less than 40 citations	Experimental	No	[34]
Shenton, 1992	33	Yes	Yes	Less than 40 citations	Experimental	No	[35]
MacQueen, 2003	30	Yes	No	Less than 40 citations	Experimental	No	[36]
Bogerts, 1993	30	No	Yes	Less than 40 citations	Experimental	No	[22]
Narr, 2004	25	Yes	No	Less than 40 citations	Experimental	No	[37]
Mervaala, 2000	21	Yes	No	Less than 40 citations	Experimental	No	[38]
Steffens, 2002	20	Yes	No	Less than 40 citations	Experimental	No	[39]
Jack, 1994	18	Yes	Yes	Satisfying criteria except citations number	Pilot	Yes	[17]
O'Brien, 2004	18	Yes	No	Less than 40 citations	Experimental	No	[40]
Ashtari, 1999	17	Yes	No	Less than 40 citations	Experimental	No	[41]
Bartzokis, 1993	17	Yes	Yes	Satisfying criteria except citations number (<40 in AD literature)	Pilot	Yes	[21]
Pantel, 2000	17	No	No	Satisfying criteria except citations number (<40 in AD	Pilot	Yes	[19]

Protocol	Citations in the AD literature	Cited by Konrad and colleagues	Cited by Geuze and colleagues	Compliance with selection criteria	Inclusion stage	Selected for harmonization	Ref
				literature)			
Zipursky, 1994	16	Yes	Yes	Less than 40 citations	Experimental	No	[42]
Giedd, 1996	16	Yes	Yes	Less than 40 citations	Experimental	No	[43]
Kates, 1997	16	Yes	No	Less than 40 citations	Experimental	No	[44]
Honeycutt, 1998	15	Yes	Yes	Less than 40 citations	Experimental	No	[45]
von Gunten, 2000	13	Yes	No	Less than 40 citations	Experimental	No	[46]
Vythilingam, 2004	10	Yes	No	Less than 40 citations	Experimental	No	[47]
Hastings, 2004	9	Yes	No	Less than 40 citations	Experimental	No	[48]
Niemann, 2000	9	Yes	No	Less than 40 citations	Experimental	No	[49]
Lobnig, 2006	7	Yes	No	Less than 40 citations	Experimental	No	[50]
Lloyd, 2004	6	Yes	No	Less than 40 citations	Experimental	No	[51]
Driessen, 2000	6	Yes	No	Less than 40 citations	Experimental	No	[52]
Barr, 1997	5	Yes	No	Less than 40 citations	Experimental	No	[53]
Neumeister, 2005	5	Yes	No	Less than 40 citations	Experimental	No	[54]
Caetano, 2004	5	Yes	No	Less than 40 citations	Experimental	No	[55]
Frodl, 2004	5	Yes	No	Less than 40 citations	Experimental	No	[56]
Rusch, 2001	4	Yes	No	Less than 40 citations	Experimental	No	[57]
Nakano, 2002	4	Yes	No	Less than 40 citations	Experimental	No	[58]
MacMillan, 2003	3	Yes	No	Less than 40 citations	Experimental	No	[59]
Yucel, 2007	3	Yes	No	Less than 40 citations	Experimental	No	[60]
Brambilla, 2003	3	Yes	No	Less than 40 citations	Experimental	No	[61]
Saylam, 2006	3	Yes	No	Less than 40 citations	Experimental	No	[62]
MacMaster & Kusumakar, 2004	2	Yes	No	Less than 40 citations	Experimental	No	[63]
Malykhin, 2007	2	No	No	Satisfying criteria except citations number (<40 in AD literature)	Pilot	Yes	[20]
Scott, 2004	2	Yes	No	Less than 40 citations	Experimental	No	[64]
Chen, 2004	2	Yes	No	Less than 40 citations	Experimental	No	[65]
Vermetten, 2006	1	Yes	No	Less than 40 citations	Experimental	No	[66]
Arango, 2003	1	Yes	No	Less than 40 citations	Experimental	No	[67]
Chang, 2005	1	Yes	No	Less than 40 citations	Experimental	No	[68]

Protocol	Citations in the AD literature	Cited by Konrad and colleagues	Cited by Geuze and colleagues	Compliance with selection criteria	Inclusion stage	Selected for harmonization	Ref
Rosso, 2005	1	Yes	No	Less than 40 citations	Experimental	No	[69]
Xia, 2004	1	Yes	No	Less than 40 citations	Experimental	No	[70]
Frazier, 2005	1	Yes	No	Less than 40 citations	Experimental	No	[71]
Bossini, 2008	1	Yes	No	Less than 40 citations	Experimental	No	[72]
Starkman, 2007	0	Yes	No	Less than 40 citations	Experimental	No	[73]

Protocols are sorted in order of citation in the AD literature (first the most cited)

Table 2

General features of the 12 selected protocols

Protocol	Slice	Direction of tracing	MR Sequence	Validation Sample	Included Hippocampal tissue	Reproducibility measures
Bartzokis et al., 1998 [21]	coronal	from rostral to caudal	T1	healthy controls	Head, body, and tail until visualization of colliculi	5% error
Convit et al., 1997 [26]	coronal	from rostral to caudal	T1	AD	Head, body, and tail until visualization of crus/crura of fornix/ces in full profile	0.85 inter-rater reliability (ICC)
deToledo-Morrell et al., 2004 [23]	coronal	from rostral to caudal	T1	MCI	Head, body, and tail until visualization of crus/crura of fornix/ces in full profile	0.97 intra-rater and inter-rater reliability (ICC)
Haller et al., 1997 [27]	coronal, sagittal and axial	from caudal to rostral	T1	schizophrenia	Head, body, and whole tail	77.9% overlap of voxels
Jack et al., 1994 [17]	coronal	from caudal to rostral	T1	epilepsy	Head, body, and tail until visualization of crus/crura of fornix/ces in full profile	1.2% and 3.4% intra- and inter-observer variability (5% error with slices below a thickness of 3mm)
Killiany et al., 1993 [24]	coronal	from rostral to caudal	T1	AD	Head, body, and tail until visualization of crus/crura of fornix/ces in full profile	0.91 and 0.92 intra- and inter-rater reliability (ICC)
Lehericy et al., 1994 [25]	coronal	from caudal to rostral	T1	AD	Head, body, and tail ntil visualization of crus/crura of fornix/ces in full profile	7% inter-rater relative error on volume [81]
Malykhin et al., 2007 [20]	coronal; sagittal at the level of the head	from the hippocampal body to the tail, then from the posterior part of head to its rostral part	T1	Parkinson's disease; depression	Head, body, and whole tail	0.86 and 0.96 intra- and inter-rater reliability (ICC)
Pantel et al., 2000 [19]	coronal	from rostral to caudal	T1+ T2	healthy controls	Head, body, and whole tail	intraclass $R = 0.78$
Pruessner et al., 2000 [18]	coronal	from caudal to rostral	T1	healthy controls	Head, body, and whole tail	0.92 and 0.99 intra- and inter-rater reliability (ICC)
Soininen et al., 1994 [28]	coronal	from rostral to caudal	T1	MCI	Head, body, and tail until visualization of crus/crura of fornix/ces in full profile	0.95 intra-rater reliability (ICC)
Watson et al., 1992 [29]	coronal	from rostral to caudal	T1	healthy controls	Head, body, and tail until visualization of crus/crura of fornix/ces in full profile	intra-rater score range between 0.88 and 0.99

Table 3

Differences of anatomical landmarks among the 12 selected protocols, after semantic harmonization

a) Plane of tracing			
Axis of hippocampus [B, C, dTM, J, L, S, W]	AC-PC line [H, K, M, Pa, Pr]		
b) Most posterior slice			
Where inferior and superior colliculi are jointly visualized [B]	Where crus/crura of fornix/ces is/are visible in full profile [C, dTM, J, K, L, S, W]	Where gray matter is visible inferomedially to the trigone of the lateral ventricle [H, M, Pa, Pr]	
c) Superior border			
Lower border of alveus/fimbria [B, H, K, Pa, S]	Upper border of alveus/fimbria [C, dTM, J, L, M, Pr, W]		
d) Medial border at subiculum level			
vertical line from the CA to the WM of the parahippocampal gyrus [C]	Oblique line with same inclination of parahippocampal WM, connecting the inferior part of the subiculum to the quadrigeminal cistern [K, L, M, Pr, W]	Horizontal line from the highest medial point of the parahippocampal WM to the cistern [B, dTM, H]	Line outlining the contour of white matter of parahippocampal gyrus [J, Pa, S]

AC = anterior commissure; PC=posterior commissure; CA =cornu Ammonis; WM = white matter; [B] Bartzokis et al., 1998, [C] Convit et al., 1997, [dTM] deToledo-Morrell et al., 2004; [H] Haller et al., 1997, [J] Jack et al., 1994, [K] Killiany et al., 1993, [L] Lehericy et al., 1994, [M] Malykhin et al., 2007, [Pa] Pantel et al., 2000, [Pr] Pruessner et al., 2000, [S] Soininen et al., 1994, [W] Watson et al., 1992.