

Progress update from the hippocampal subfields group

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Working Group Summaries for European Joint Programming For Neurodegenerative Research (JPND)

Progress update from the hippocampal subfields group

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Abstract

Introduction: Heterogeneity of segmentation protocols for medial temporal lobe regions and hippocampal subfields on *in vivo* magnetic resonance imaging hinders the ability to integrate findings across studies. We aim to develop a harmonized protocol based on expert consensus and histological evidence.

Methods: Our international working group, funded by the EU Joint Programme–Neurodegenerative Disease Research (JPND), is working toward the production of a reliable, validated, harmonized protocol for segmentation of medial temporal lobe regions. The working group uses a novel *postmortem* data set and online consensus procedures to ensure validity and facilitate adoption.

Results: This progress report describes the initial results and milestones that we have achieved to date, including the development of a draft protocol and results from the initial reliability tests and consensus procedures.

Discussion: A harmonized protocol will enable the standardization of segmentation methods across laboratories interested in medial temporal lobe research worldwide.

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1. Introduction

Current neuroimaging technology enables detailed structural and functional investigations of distinct medial temporal lobe (MTL) components *in vivo*, including the hippocampal allocortical subfields (usually denominated cornu ammonis or CA regions, dentate gyrus, and subiculum) and parahippocampal gyrus regions (entorhinal, perirhinal, and parahippocampal cortices). Here, we review the fundamental need for standardization [1] and describe our own progress toward this goal of harmonizing MTL segmentation methods using high-resolution structural magnetic resonance imaging (MRI).

MRI-based MTL volumetry is used in basic and clinical research and is of particular importance in the study of Alzheimer's disease (AD) to relate *in vivo* changes to underlying histopathology [2,3]. Because AD pathology, especially tau-containing neurofibrillary tangles and neuronal loss, first emerges in particular MTL regions [4,5], regional MTL volumetry may be used as an imaging biomarker that can identify the earliest disease stages and distinguish AD from typical aging [6–9]. Regional MTL atrophy tracks disease progression and may improve diagnosis, evaluation of novel therapeutics, and case selection for clinical trials [10]. In addition, accurate and reliable identification will also facilitate research into the basic cognitive functions of different subfields [11]. high-resolution *in vivo* volumetry provided critical insights into distinct hippocampal subfield functions supporting human memory processes [12–14]. Researchers worldwide have since used submillimeter

resolution MRI to investigate lifespan changes in MTL regional volumes and functions, and the relation to neurodegenerative pathologies and genetic risk factors [11,15]. Yet, protocols vary considerably across research groups with respect to histological and neuroimaging references, imaging resolution, and MTL nomenclature [16–20]. Thus, boundary locations and, in turn, measured volumes, vary across protocols. The lack of a gold-standard consensus protocol for MTL regional segmentation contributed to discrepant findings across studies [17,21] when analyzing similar populations and diseases, or even identical data sets [15,22]. Protocol differences can partly explain inconsistent evidence in mild cognitive impairment, AD, and aging (see [12] for a review). Thus, protocol discrepancies hinder the integration of findings across research studies. Clearly, developing a harmonized, reliable, and validated protocol for segmentation of MTL regions—for populations that vary in age and health—is a critical necessity. To accomplish this harmonization initiative, the Hippocampal Subfield Group (HSG; http://hippocampalsubfields.com/), was launched in 2013 [16,23].

1.1. What is the HSG and what is our goal?

The HSG, the only group of its kind, currently consists of over 200 researchers from 18 countries (Fig. 1A). This organization began as an informal group of hippocampal subfield researchers concerned about differences among existing segmentation protocols. Efforts to quantify these differences resulted in our first article, which highlighted regions of agreement and disagreement across protocols, underscoring the need for harmonization [14]. As a result, the group was formalized in 2015, at which time members developed a workflow [23] and the harmonization process began in earnest.

The Boundary Working Group (BWG, http://www.hippocampalsubfields.com/people/boundary-working-group/), a subset of the larger membership, is now developing a highly reliable and valid harmonized segmentation protocol for defining and measuring the hippocampal subfields and adjacent MTL cortical regions on *in vivo* MRI. The membership comprises representatives of many of the laboratories conducting manual segmentation of MTL regions *ex vivo* and *in vivo*. Our aim is to develop, validate, and disseminate a segmentation protocol for MTL regions on T2-weighted structural MRI images with submillimeter in-plane resolution collected in high field ($\geq$3 tesla) scanners, following the current recommended practice for manual segmentation (see http://www.hippocampalsubfields.com/people/acquisition-working-group/ for details). The HSG aims to enable broad adoption of the harmonized protocol across laboratories and thus foster direct comparison of research findings, similar in methods to the previous harmonized whole hippocampus protocol (referred to as the "Harmonized Protocol" and abbreviated as "HarP") [24,25]. In contrast to extant procedures used for hippocampal subfield research, the harmonized protocol will be based on the most comprehensive histology data set to date, specifically designed with an eye toward usage by neuroimaging researchers, and will be based on a consensus of an international group of experts. The ultimate goal will be to create a finished protocol that will be applicable to MRI data obtained from individuals of all ages and populations and to facilitate robust and reproducible scientific discovery.

2. Methods

2.1. HSG work plan

The harmonization workflow was outlined previously [23], and here we report on milestones achieved within each workflow component. This article thus serves as both a "progress report" and a more detailed resource for the neuroimaging community regarding our methods and procedures. Our aim in this endeavor is to communicate our methods as widely as possible throughout the process; we expect that this transparency will facilitate and promote adoption of the harmonized protocol once complete.

Owing to the regional complexity of the MTL, the HSG has divided the standardization procedure and protocol development into stages. The HSG first developed a draft

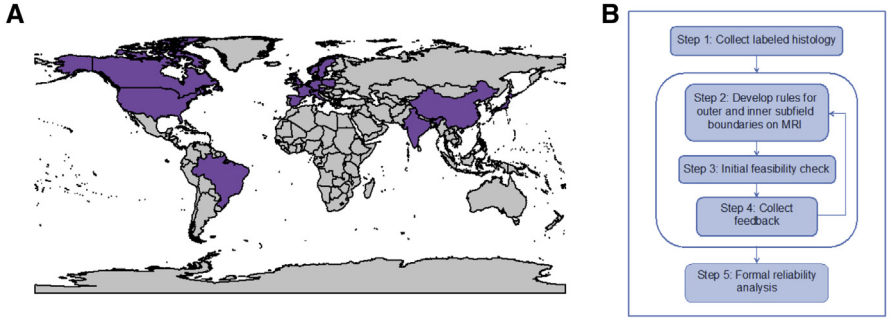


Fig. 1. (A) Map highlighting the global membership of the HSG. Over 200 members from 18 countries are currently in the HSG. (B) Workflow procedure for the HSG. Abbreviations: HSG, Hippocampal Subfield Group.

Table 1
Case descriptions and methods used for the anatomical labeling of the hippocampal body and head samples contributed to the HSG postmortem data set

Case number (brain hemisphere)	Age/Sex	Neurological disease	Type of stain	Slice spacing	Origin	Annotations
Hippocampal body						
1 (left)	65/Male	None	Silver Stain	2mm, 6 slices	Juelich	JA, OK, RI
2 (right)	60/Male	None	Nissl	2mm, 6 slices	Massachusetts General Hospital	JA, OK, RI
3 (left)	75/Male	None	Kluver-Barrera	2mm, 6 slices	University of Pennsylvania	JA, OK, RI
Hippocampal head*						
1 (right)	75/Male	None	Kluver-Barrera	1mm, 14 slices	University of Pennsylvania	OK S-LD and RI
2 (right)	60/Male	AD	Kluver-Barrera	1mm, 16 slices	University of Pennsylvania	OK S-LD and RI
3 (left)	80/Male	None	NeuN, PV, NPNFP, CB	0.8 mm, 16 slices	Allen Institute	S-LD

Abbreviations: AD, Alzheimer's disease; CB, antibody to calbindin-d28k; HSG, Hippocampal Subfield Group; JA, Jean Augustinack; NeuN, antibody to neuron-specific nuclear binding proteins; NPNFP, antibody to a nonphosphorylated site of the neurofilament triplet proteins; OK, Olga Kedo; PV, antibody to parvalbumin; RI, Ricardo Insausti; S-LD, Song-Lin Ding.
*In progress.

protocol for the hippocampal subfields in the body (hippocampal mid-section) and is now developing the draft protocol for the remainder (i.e., head and tail) of the hippocampus. After this step, the working group will focus on the entorhinal, perirhinal, and parahippocampal cortices (i.e. MTL cortical regions).

The workflow comprises five iterative steps (see Fig. 1B). First, several neuroanatomists (K.A., O.K., J.C.A., S-L.D., and R.I.) label *ex vivo* data sets, the results of which are serving as an additional reference set for boundary definition. This step has been completed for the hippocampal body and is in progress in the head (details in Table 1). This new HSG postmortem data set allows us to better account for interindividual and across-laboratory variability than previously possible. Second, the HSG working groups, through meetings and conference calls, regularly discuss and establish guidelines for subfield boundaries based on reference atlases, neuroanatomical landmarks visible on MRI, and geometric heuristics. This step has been completed for the hippocampal body and is currently in progress for the hippocampal head. Third, boundary rules and definitions are subjected to an initial feasibility test. This step has been completed for the inner boundaries of the hippocampal body. Fourth, feedback and consensus on the protocol from the wider HSG community is collected via questionnaire (see the section "*How will the HSG achieve consensus?*" in the following). This step has been completed for the outer boundaries of the hippocampal body and will be completed for the inner boundaries of the hippocampal body in the coming months. Boundary descriptions are revised based on feedback, and following protocol consensus, reliability is evaluated (step 5).

2.2. Resources used for in vivo segmentation of the MTL regions

In guiding the delineation of MTL regions [16], MRI researchers typically rely on published human MRI atlases, including Duvernoy [26,27], Ding and Van Hoesen [28], and Mai et al. [29], and book chapters from R.I. and R.S.C.A. [30–32]. Additional reference materials include early high-resolution imaging studies of MTL [14,33,34] or published protocols developed specifically for *in vivo* MRI [35,36]. We hypothesized that the discrepancies in the extant segmentation protocols (Fig. 2A) are primarily due to differences in the reference materials used [16,23].

The published atlases described previously provide detailed information about the organization, structure, and relative position of the hippocampal subfields that has guided the development of MRI protocols. However, for several reasons, these resources are insufficient for protocol harmonization. First, the nomenclature and boundary definitions vary across atlases—e.g., CA4 is a separate hippocampal subfield according to Duvernoy [26,27] and Ding et al [37,38], whereas R.I. and R.S.C.A. [31] consider this area as the "hilus" region of the dentate gyrus. Second, these references include few cases and do not address individual differences in anatomy. Third, the neuroanatomical cutting plane in these atlases does not match that of typical high-resolution T2-weighted MRI slices used in hippocampal subfield research. The slices and diagrams in published atlases typically show coronal cuts in a plane perpendicular to the anterior-posterior commissure line, whereas submillimetric T2-weighted MRI slices are typically acquired and viewed in a plane perpendicular to the long axis of the hippocampus [11]. This difference is particularly problematic in anatomically complex regions such as the hippocampal head.

To overcome the outlined limitations inherent to the extant references, the HSG developed a novel data set corresponding to the oblique-coronal plane used in MRI research (i.e. perpendicular to the long axis of the hippocampus; Table 1). The data set includes delineations of subfields in histological sections obtained from multiple specimens, each labeled by multiple neuroanatomists). This HSG histology data set thus enables us to develop a harmonized protocol that better addresses discrepancies across neuroanatomy laboratories and individual differences in regional

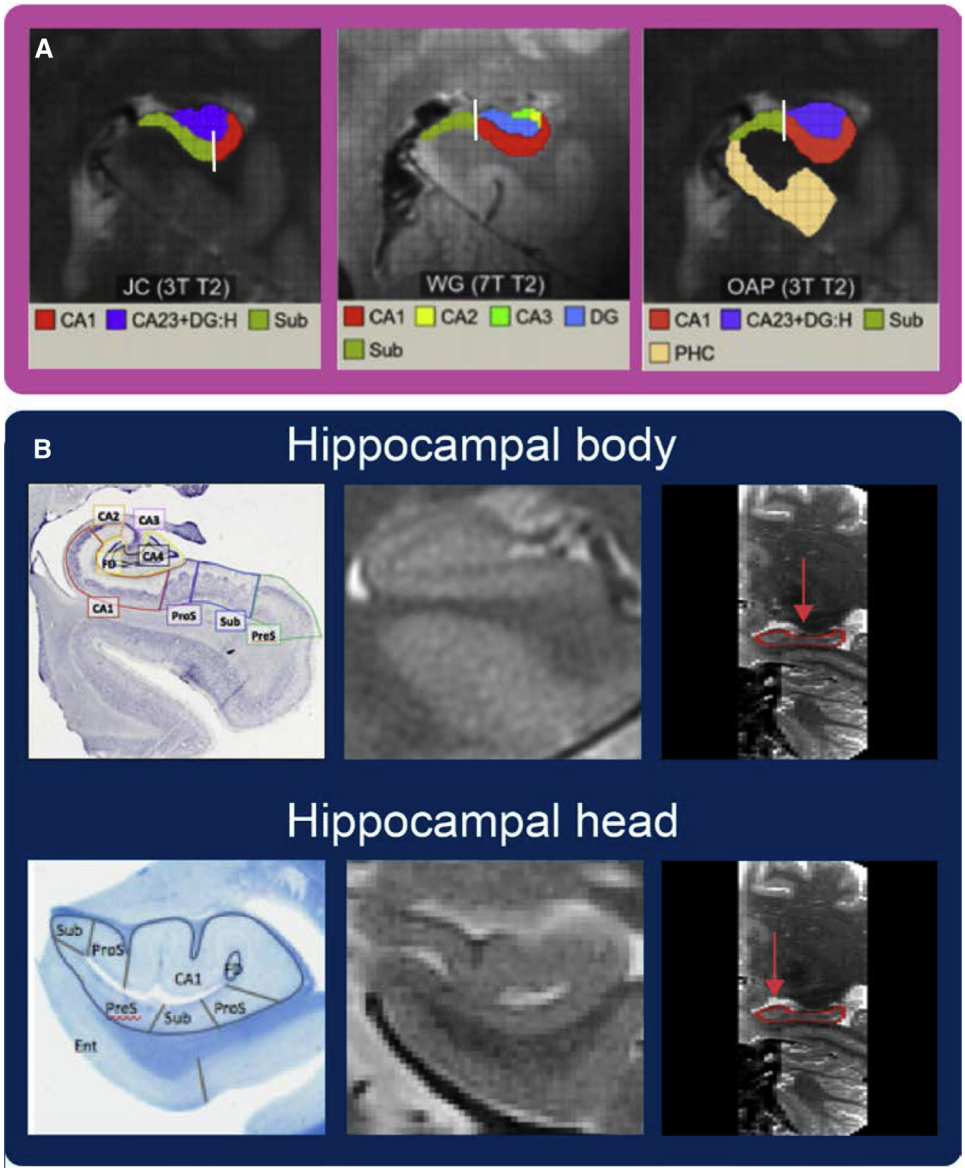


Fig. 2. (A) Examples of the variability in the position of the CA1, subiculum/prosubiculum boundary (white line) across three different segmentation protocols overlaid on the same brain (adapted from Yushkevich et al., 2015, Fig. 3). This variability is likely due to differences in reference atlases. (B) Histological and MRI slices of the human hippocampal formation depicting the body (upper) and head (lower) regions. Note that the subfield anatomy in the body and head differ in composition and organization relative to macroscopic features. Abbreviations: FD, fascia dentata (dentate gyrus); Ent, entorhinal cortex; JC, La Joie, Chetalat protocol; OAP, Olsen, Amaral, Palombo protocol; PreS, presubiculum; ProS, prosubiculum; Sub, subiculum; WG, Wisse, Geerlings protocol.

delineation than previously possible. In creating this data set, as noted previously, the HSG includes MTL neuroanatomy experts who actively participate in this harmonization effort (see Table 1). Moreover, to address limitations of our HSG histology data set regarding sample size, gender distribution, and inclusion of subjects with neurodegenerative disease, we also have access to a newly developed postmortem data set from the University of Pennsylvania containing $0.2 \times 0.2 \times 0.2\,mm^3$ resolution MRI scans of 31 hippocampal specimens (25 individuals; 12 male; 16 with and 9 without dementia), nine of which have densely sampled serial histology sections, and two of which also have *antemortem* imaging [39]. The University of Pennsylvania data set will continue to grow with additional cases over time.

2.3. How are the histological data used in protocol development?

Following a workflow established in 2015 (Fig. 1B), the BWG has used the newly acquired histological data labeled by the HSG neuroanatomists, the University of Pennsylvania specimens, and previously published reference atlases to prepare a draft protocol for the inner and outer subfield boundaries in the hippocampal body and

head (hippocampal head protocol still in progress). The protocol is designed to maximize validity and reliability and is developed by members who have substantial expertise in manual MTL segmentation. The histology data are analyzed for the location of anatomical boundaries corresponding to macroscopic features visible on MRI, anterior-to-posterior variability in boundaries, individual variability, and for variability in boundary definitions between neuroanatomists (Fig. 3). Many cytoarchitectonically defined subfield boundaries detectable on postmortem histology are not always represented by detectable differences in image intensity on 3T MRI; thus, the location of anatomical boundaries in relation to macroscopic features were used to develop candidate geometric segmentation rules in reference to multiple examples of MRI. Validity is continuously considered throughout this process by comparing candidate rules and geometric heuristics to the labeled histology data. Via this iterative process, rules and descriptions are reviewed and updated in collaboration with neuroanatomists.

2.4. How will the HSG achieve consensus?

We are using the same Delphi convergence procedure used by the whole hippocampus harmonized protocol group [24], a project jointly funded by the European Alzheimer's Disease Consortium and Alzheimer's Disease Neuroimaging Initiative and focused on AD [1]. Similarly, the Delphi procedure, which is a formal method for achieving consensus [40], will enable the HSG to achieve consensus regarding hippocampal subfield and MTL cortex segmentation.

Working in stages, the Questionnaire Working Group (http://www.hippocampalsubfields.com/people/questionnaire-working-group/) uses the Qualtrics platform to transform the draft protocol into a web-friendly questionnaire. To evaluate each subsection of the protocol in a digestible format, each subfield boundary rule or major anatomical landmark is explained using text and images (see Fig. 4D for an example of the questionnaire used to obtain feedback on the proposed outer boundaries in the hippocampal body). Each boundary rule is presented side-by-side with both histological evidence (step 1 of workflow; Figs. 3, and 2B) and results from an initial feasibility test (step 3 of workflow; Fig. 1B). Feedback regarding each rule's clarity and appropriateness is solicited from all HSG laboratories. Importantly, HSG laboratories (one response per laboratory) indicate in their questionnaire response number of years of experience with manual hippocampal subfield segmentation to ensure that sufficient numbers of respondents have expertise in the field. By design, laboratories with limited segmentation experience are not excluded, as this ensures that the protocol can also be interpreted and implemented by relative novices.

For each boundary definition or rule, respondents assess the clarity of the written description and figures accompanying the rule, and level of agreement with the rule on a 9-point Likert-scale. Respondents explain their responses, including motivation for suggested changes. The proportions of responses agreeing with a given rule are analyzed via a binomial test, and significant proportion favoring a rule is counted as a consensus endorsement. If consensus is not reached, updates to the rule description or content are made based on respondents' qualitative responses and are included in the next iteration with the binomial test results. This process continues until consensus is reached for all boundaries. If statistically significant consensus on a given rule is not reached after four rounds, the details of the rule agreed on by most respondents are taken as the final rule [24].

3. Results

3.1. What milestones have we achieved so far?

Over the past three years, the HSG has held six international working group meetings to develop the harmonized protocol. These working groups have taken place in Chicago, the USA (15 attendees, October 2015), San Diego, the USA (9 attendees, November 2016), Montreal, Canada (13 attendees, April 2017), London, the UK (18 attendees, July 2017), Irvine, the USA (10 attendees, April 2018), and Magdeburg, Germany (13 attendees, October

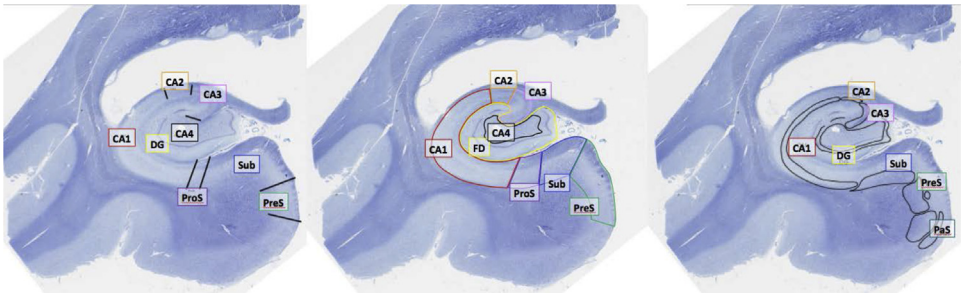


Fig. 3. The same single histological slice of the hippocampal body stained with Kluver-Barrera stain, segmented by three different neuroanatomists. Abbreviations: DG/FD, dentate gyrus/fascia dentata; ProS, prosubiculum; PreS, presubiculum; PaS/ParaS, parasubiculum; S/Sub, subiculum.

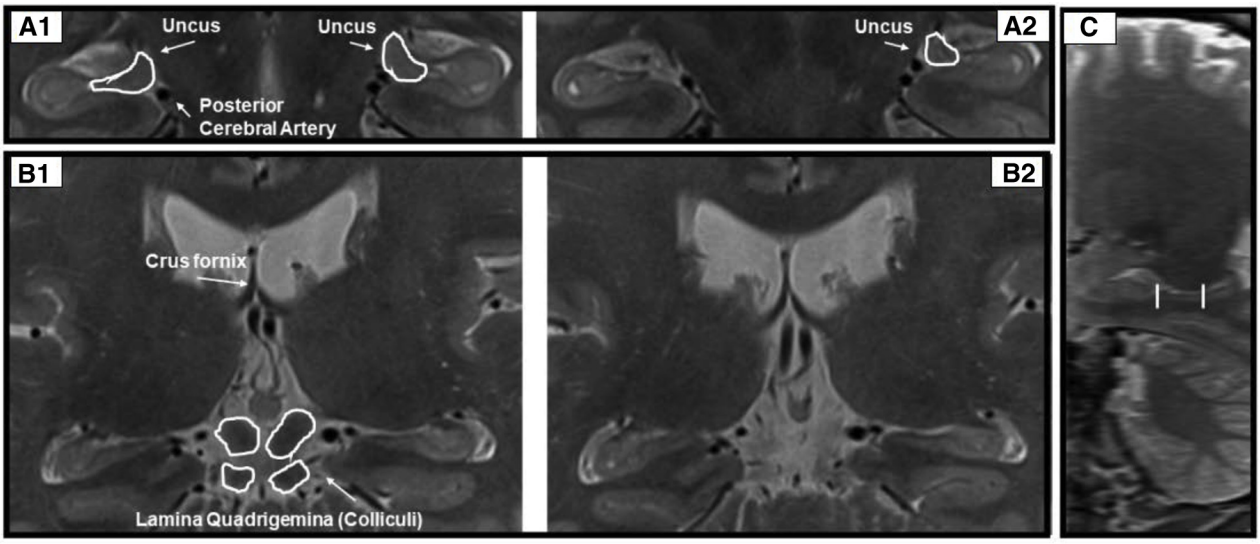


Fig. 4. Hippocampal head displaying the uncus in both hemispheres. (A2) Slice showing the disappearance of the uncus in the left hemisphere, and as such, the anterior-most slice of the hippocampal body in the left hemisphere. (B1) Final posterior slice of the hippocampal body displaying the colliculi, the crus fornix, and the "tear drop" shape of the hippocampal body. (B2) Colliculi are no longer visualized, and as such this slice is considered the first slice of the hippocampal tail. (C) Sagittal view, illustrating the anterior and posterior limits of the inclusive hippocampal body range. All images are T2-weighted, resolution 0.39 × 0.39 × 2 mm. (D) Screenshot from the online questionnaire used to reach consensus on the outer boundaries of the hippocampal body.

2018). The JPND funded two of these working groups (Montreal and London), and the HSG members funded the remaining working groups internally. These small meetings have enabled close collaboration among attendees from around the world and enabled valuable discussions of protocol rules between the BWG neuroimaging experts and attending neuroanatomists. In the following, we describe four milestones achieved to date, including the creation of the specialized histological reference data set (milestone #1), initial reliability results (milestone #2), body protocol development (milestone #2-5), and consensus procedures (milestone #3).

3.1.1. ***Milestone #1:*** *Histological data sets of the hippocampal body and head created for the HSG*

Histologically stained slices of the hippocampal body originated from three research sites: Research Centre Jülich, Massachusetts General Hospital, and the University of Pennsylvania, and were prepared using the methods customary for each laboratory (sample characteristics in Table 1). Three neuroanatomy laboratories labeled the hippocampal subfields, on all samples, enabling the evaluation of across-laboratory differences in boundary definitions (see Fig. 3). The HSG is developing a similar novel histological data set to guide harmonization using samples from the hippocampal head (Table 1). Three samples were obtained from two research sites (the University of Pennsylvania and the Allen Institute) and labeled by the HSG neuroanatomists.

3.1.2. ***Milestone #2:*** *Anterior and posterior hippocampal body limits have been developed*

The subfield anatomy in the anterior portion (hippocampal head) differs from the middle (body) and posterior (tail) sections (Fig. 2B). Thus, our first rule development was establishing reliable landmarks of the anterior and posterior body limits accommodating different subfield segmentation rules along the anterior-posterior axis. A review of extant subfield segmentation protocols, hippocampal macrostructure, and histological atlases identified the uncal apex (*gyrus intralimbicus* in the neuroanatomical nomenclature) as a canonical landmark of transition from the hippocampal head to the body. No consensus in the current literature emerged on the body's posterior landmark or to denote transition to the hippocampal tail [41]. However, many commonly used landmarks in histology (e.g., lateral geniculate nucleus) and *in vivo* MRI (e.g., visualization of the crus fornix in the coronal plane)

Table 2
Example questions from the HSG questionnaire along with agreement responses obtained from the respondents

Boundary	Question	Range	Mean (SD)	% Agree (>5)	binomial test (*P* value)
Anterior	How clear is the boundary description?	7-9	8.31 (0.76)	100	<.001
Anterior	Do you agree with the boundary rule?	7-9	8.89 (0.41)	100	<.001
Posterior	How clear is the boundary description?	6-9	7.93 (0.88)	100	<.001
Posterior	Do you agree with the boundary rule?	5-9	8.48 (0.99)	96.55	<.001
Dorsal	How clear is the boundary description?	5-9	8.10 (1.21)	96.55	<.001
Dorsal	Do you agree with part 1 of the boundary rule?	5-9	8.31 (1.31)	93.10	<.001
Dorsal	Do you agree with part 2 of the boundary rule?	3-9	8.35 (1.26)	96.55	<.001
Ventral	How clear is the boundary description?	7-9	8.76 (0.51)	100	<.001
Ventral	Do you agree with the boundary rule?	5-9	8.79 (0.78)	96.55	<.001
Medial	How clear is the boundary description?	4-9	7.03 (1.70)	72.41	.004
Medial	Do you agree with part 1 of the boundary rule?	5-9	7.62 (1.50)	82.76	<.001
Medial	Do you agree with part 2 of the boundary rule?	4-9	8.10 (1.45)	89.66	<.001
Lateral	How clear is the boundary description?	5-9	8.66 (0.90)	96.55	<.001
Lateral	Do you agree with the boundary rule?	6-9	8.62 (0.82)	100	<.001
Vessels	How clear is the boundary description?	5-9	8.38 (0.98)	96.55	<.001
Vessels	Do you agree with the boundary rule?	7-9	8.79 (0.56)	100	<.001
CSF/cysts	How clear is the boundary description?	3-9	7.52 (1.66)	89.66	<.001
CSF/cysts	Do you agree with part 1 of the boundary rule?	4-9	8.24 (1.41)	89.66	<.001
CSF/cysts	Do you agree with part 2 of the boundary rule?	5-9	8.00 (1.31)	89.66	<.001
CSF/cysts	Do you agree with part 3 of the boundary rule?	3-9	7.90 (1.65)	86.21	<.001

Abbreviation: HSG, Hippocampal Subfield Group.

appear in approximately the same plane as the most posterior aspect of the lamina quadrigemina (LQ) comprised superior and inferior colliculi (Fig. 4B). LQ is easily visualized on MRI and is robust to variability in head positioning in the scanner. Therefore, LQ visualization is a suitable landmark of the posterior end of the hippocampal body.

The intent of this ranging protocol was to demarcate the portions of the hippocampus with distinct subfield anatomy and to accommodate different parcellation rules and definitions for the same subfield labels in the hippocampal head, body, and tail. In a reliability test, expert raters with at least 3 years experience segmenting the hippocampus achieved excellent agreement with training rater [42,43] [left anterior, kappa (κ) = 0.87 (89% agreement); right anterior, κ = 0.75 (82% agreement); left posterior, κ = 0.79 (84% agreement); right posterior, κ = 0.76 (84% agreement)]. Changes in brain volume and macrostructure are expected due to typical development, aging, or neurodegenerative disease, and these changes may affect visualization of these landmarks. However, such variability is not expected to introduce substantial bias in hippocampal subfield volumes, especially when the full length of the hippocampus is segmented. Nonetheless, these factors should be considered in applying the protocol to between-subjects or cohort study designs.

3.1.3. ***Milestone #3:*** *Body "outer boundary" rules have been drafted*

The outer boundary BWG defined rules delineating the medial, lateral, ventral, and dorsal borders of the hippocampal body. In creating these rules, the group referred to the definitions used by harmonized protocol [24], but some rules were modified to accommodate application to the type of MRI scans typically acquired for subfield segmentation [11] (e.g., 0.4 × 0.4 × 2 mm T2-weighted images acquired perpendicular to the hippocampal long axis).

3.1.4. ***Milestone #4:*** *Consensus achieved on initial set of boundary rules*

The Questionnaire Working Group (Fig. 4D) obtained feedback on the first set of rules via online survey. These rules defined the anterior and posterior extent of the hippocampal body as well as its outer boundaries. Of the 29 laboratories participating in the questionnaire process, 72.4% had 5+ years of experience manually segmenting hippocampal subfields. Binomial tests revealed that the description of all 8 rules was rated as overwhelmingly more clear than unclear (mean clarity level on a 9-point Likert scale: 8.1, all *P's* < .004; see Table 2 for details). There was significantly more agreement than disagreement with all rules: mean level of agreement: 8.3, all *P*'s < .001. The high level of endorsement of the rules merited acceptance of that part of the protocol as final after only one Delphi round.

3.1.5. ***Milestone #5:*** *Inner boundary rules in the body defined; initial feasibility test performed*

A draft protocol of the inner boundaries within the hippocampal body has been completed, and we are in the process of developing a questionnaire to obtain feedback from the community on this section of the protocol. This protocol contains guidelines and definitions for boundaries of the dentate gyrus, subiculum (inclusive of pre- and parasubiculum), CA1, CA2, and CA3. An initial assessment of each rule's feasibility is currently being conducted by three expert raters with >5 years of experience, two of whom are naïve to the protocol. The raters will manually segment three

MRI data sets representative of data collected in children, healthy older adults and AD cases, and two combined *in vivo* and *ex vivo* data sets that have accompanying histological study and labeling [44]. The inter-rater agreement and correspondence to histological labeling will be evaluated with Dice similarity and intraclass correlation coefficients, and systematic bias will be evaluated with Bland-Altman plots and statistics. This study will evaluate feasibility of protocol execution by multiple raters, and gauge external validity *vis-a-vis* histological samples.

After the initial feasibility assessment, the Questionnaire Working Group will proceed to the online questionnaire process to obtain feedback and consensus with the outlined Delphi procedure. On achieving consensus using the methods described previously, the inner and outer boundaries will be combined and submitted to reliability assessment. As this draft protocol is still under development, we do not provide here details regarding the subfield boundary rules. On completion, the full protocol will be available on the HSG website.

4. Discussion

Segmentation of the MTL is important for the study of development, neurodegeneration, and the neural basis of memory and other cognitive functions. The integration of knowledge and replication of results across laboratories has been hindered by variable methodology. Although a harmonization process that requires time and resources from scientists worldwide is challenging, the HSG is committed to the effort.

We are encouraged by the success of the Delphi procedure in obtaining consensus on the outer boundaries of the hippocampal body (Milestone 4), and we anticipate that this procedure, although time-intensive, will result in a protocol that is more readily adopted by the community. This project addresses the "interoperability" and "reusability" terms in the "FAIR" guiding principles for reproducible science (FAIR is an abbreviation for: findability, accessibility, interoperability, and reusability) [45], and is part of a larger movement in the field to provide greater access to neuroimaging data sets and standardized research procedures to pool data and increase reliability of individual studies [46,47].

We aim to provide a valid and reliable protocol that can be easily adopted by laboratories worldwide that are conducting research using submillimetric, high-field MRI. We anticipate that the harmonized protocol will be adopted widely, in part thanks to the consensus procedures we have used. The HSG plans to provide online tools, videos, and other resources and promote the dissemination and adoption of the protocol. These resources, which will also include the integration with automated segmentation methods, will benefit experienced and novice MTL researchers alike. We anticipate that our efforts will facilitate great progress in the study of AD and other neurodegenerative, neuropsychiatric, and neurodevelopmental diseases involving MTL degeneration and dysfunction. Moreover, harmonization can lead to improved preclinical and differential diagnostic tools, and will facilitate the ability to reliably assess the efficacy of AD therapeutic interventions using noninvasive and readily available MRI technology.

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RESEARCH IN CONTEXT

1. Systematic review: Imaging of medial temporal lobe subregions, including the hippocampal subfields, has become increasingly popular due to the improvement of neuroimaging techniques. The Hippocampal Subfield Group (HSG), which includes more than 200 international scientists with various fields of expertise, was formed in 2013 to develop a harmonized, valid, and reliable protocol for in vivo medial temporal lobe segmentation.
2. Interpretation: Our group has gathered a unique comprehensive histology dataset to support the anatomical validity of the proposed harmonized segmentation protocol and achieved community consensus on an initial set of boundary definitions using online questionnaires. Adoption of the finalized harmonized protocol will facilitate aggregation of data across research sites and across-lab replications.
3. Future directions: Following final approval and reliability testing by the HSG, the segmentation protocol for the hippocampal body will be disseminated to the broader scientific community. The HSG will next develop segmentation protocols for the hippocampal head and tail and the parahippocampal cortices.

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