Towards A Harmonized Protocol for Segmentation of Hippocampal Subfields and Parahippocampal Cortical Subregions in *In Vivo* MRI

A White Paper

The Hippocampal Subfields Group (HSG)

November 24, 2014

1 Introduction

An increasing number of human *in vivo* magnetic resonance imaging (MRI) studies have focused on examining the structure and function of the subfields of the hippocampal formation (the dentate gyrus, CA fields 1-3, and the subiculum) and the cortical subregions of the parahippocampal gyrus (entorhinal, perirhinal, and parahippocampal cortices). These small substructures within the medial temporal lobes of the brain are understood to subserve different functions in the memory system [30, 21, 3, 36], and different psychiatric and neurological disorders are believed to affect hippocampal subfields and parahippocampal cortical subregions differently, selectively, and in a complex progression [10, 2, 28, 12, 33, 19, 29].

Currently, different research groups that use MRI to study hippocampal and parahippocampal substructures rely on largely independently developed protocols for labeling the anatomical extents of these substructures. Already, close to twenty subfield segmentation protocols have been described in the literature [15, 24, 4, 14, 18, 16, 22, 20, 11, 25, 37, 40, 39, 27, 17, 13, 34, 35, 32, 9]. Different protocols label different subsets of structures and use different rules, landmarks, and cues to define their anatomical extents. Such variability among protocols complicates comparison of results obtained by different groups, and slows down scientific progress. The ability to interpret and interrelate the results of studies that examine medial temporal lobe substructures would be vastly improved if a common standard existed for labeling hippocampal subfields and parahippocampal cortical subregions.

The Hippocampal Subfield Group (HSG, `hippocampalsubfields.com`) was formed in 2013 with the mission to quantitatively characterize the differences between existing hippocampal/parahippocampal subfield segmentation protocols, and where those differences are reconcilable, to move towards reducing them. The end product envisioned by the HSG is a harmonized subfield segmentation protocol that achieves high reliability in *in vivo* MRI while being as faithful as possible relative to the true anatomical subfield boundaries. The HSG has held meetings in June 2013, November 2013 and August 2014, during which progressive steps have been taken towards assessing the current state of the art in subfield segmentation, quantifying differences between existing segmentation protocols, and developing a roadmap towards protocol harmonization. The participants in these meetings include MRI researchers and neuroanatomists, as a strong collaboration between these two communities is crucial for developing a segmentation protocol that is both reliable and consistent with anatomy.

2 Towards a Harmonized Protocol

The formation of the HSG and its efforts have been influenced strongly by the pioneering work on the EADC-ADNI harmonized protocol for whole hippocampus volumetry [7, 6, 5]. The EADC-ANDI effort began by quantitatively comparing existing protocols [7], then defined a set of three-dimensional regions that would serve as building blocks for a harmonized protocol [6], and employed a Delphi procedure to collect and integrate feedback from the developers of different existing segmentation protocols and other experts [5]. The resulting harmonized protocol is highly reliable and is becoming a standard in the field [8]. However, there are a number of important factors that make the strategy followed by Boccardi et al. difficult to apply in the context of subfield protocol harmonization:

- **Label Set Complexity.** In the Boccardi et al. effort, all protocols produced just one anatomical label (hippocampus), while in the subfield context, each protocol uses several labels, and labels used by different protocols frequently overlap. This key difference essentially precludes the use of "building blocks" employed by Boccardi et al.
- **Imaging Protocol and Application Heterogeneity.** The Boccardi et al. effort was focused on hippocampal volumetry in Alzheimer's disease. The harmonized protocol and all input protocols targeted the

same MRI modality: T1-weighted MRI with roughly isotropic resolution. By contrast, subfield segmentation protocols represented in the HSG target diverse applications and imaging modalities. It is unlikely, for example, that a single protocol can satisfy those interested in integrating functional MRI signal over subfields using 3T scans, and those interested in performing morphometry on hippocampal layers using high-resolution 7T scans.

- **Organizational Structure.** The EADC-ADNI effort was driven by the central coordinating site, and researchers at that site did the bulk of the preparatory and protocol development work, such as learning and applying a dozen existing protocols to a common set of subjects [7]. By contrast, subfield harmonization has so far been a community effort with broader division of labor.

Taking these factors into account, the HSG adopted a preliminary vision and roadmap for hippocampal subfield harmonization at its August 2014 working group meeting. The harmonized protocol will consist of a set of rules for defining and tracing boundaries between adjacent anatomical subfields. Boundaries will be defined by visual cues when these cues can be seen consistently across different MRI acquisitions, different age groups, and different diseases. An example of such cue is the hypointense band seen in T2-weighted MRI at the boundary between DG and a large portion of the CA and SUB subfields. However, for many boundaries (CA/SUB, CA1/CA2, etc.), heuristic rules combining known anatomical landmarks and geometric constructions will be preferred because such rules can be more consistently and reliably applied than rules based on visual cues such as subtle changes in MRI intensity or thickness.[1]

It is inevitable that geometric and heuristic rules will result in subfield boundaries being placed in locations that differ on an individual participant's brain from the true underlying anatomical boundaries (as would be defined by a neuroanatomist in a histological image). Recognizing that such discrepancy is inevitable, the HSG will use histological imaging data from multiple specimens as part of its protocol harmonization effort, and will focus its effort on minimizing the systematic differences between true anatomical

[1] As MRI technology improves, it may become possible for more and more subfield boundaries to be reliably drawn based purely on visual cues. For other boundaries however, there is variability in the boundary definition itself, i.e. even the researchers looking at histological slices do not always agree where the exact boundary for a specific subfield is. As a consequence, some boundaries will have to be agreed on arbitrarily no matter how clear (or high quality) the resolution and signal of MRI will become.

boundaries and the boundaries derived from applying the heuristic and geometric rules in the harmonized protocol. It will do so by applying the proposed heuristic landmark-based and geometric rules directly to histological data and adjusting the rules to minimize the systematic error between rule-based and histologically derived boundaries across all specimens.

The envisioned harmonized protocol will not consist of a single fixed set of labels, as different applications require anatomical labels of different complexity. Users of the protocol will have a choice of the set of boundaries which to draw and which not to draw. They will also have a choice of the extent to which the hippocampus is segmented along the main axis. For instance, in the context of fMRI analysis, a user may choose to draw the CA1/CA2 boundary and the CA1/SUB boundary, but to combine CA2, CA3 and DG into a single subfield. A user focused on imaging biomarker extraction may instead choose to label all CA subfields, but only in the body of the hippocampus. It would be unreasonable and impractical to require both of these users to adopt a single set of labels or to label the whole extent of the hippocampus. However, given a common set of rules for drawing boundaries, the two users would still be able to generate segmentations that are consistent and can be compared to each other.

The HSG August 2014 working group meeting put forth a vision for the development of the harmonized subfield protocol, to proceed in three stages outlined below.

- **Stage 1: Collaborative Definition of Subfield Boundaries.** In the first stage, representatives from multiple existing subfield segmentation protocols, together with neuroanatomists, will form a Boundary Working Group (BWG). This group will hold regular online collaborative meetings during which successive subfield boundaries will be discussed and defined. In these meetings, boundaries will be drawn, discussed and adjusted on a single common set of images, which will consist of several stacks of Kluver-Barrera stained histology slices taken at 1 mm intervals through the length of the hippocampal formation, similar to [1]; several clinical quality *in vivo* 3T-T2 scans of patients with mild cognitive impairment and older controls from the ADNI2 subfield imaging effort [23]; and several *in vivo* scans of younger adults. As rules are developed, feedback from neuroanatomists will be obtained. In between meetings, reliability testing of each boundary will be performed by multiple BWG participants, as a way to inform rule development. As rules are agreed upon, they will be formally documented. The complete set of written rules spanning the entire hippocampal for-

mation and the parahippocampal gyrus will constitute Version 1 of the protocol.

- **Stage 2: Feedback from the Community and Application-Specific Refinement.** Once the initial protocol has been defined, it will be shared with the larger community, using a Delphi procedure similar to the EADC-ADNI approach [5]. The Delphi procedure will be used to solicit feedback on the boundaries in the initial protocol, collect proposed changes, and accept or reject such changes based on community feedback. During this stage, successive versions of the protocol will be developed. In addition, this stage will be used to define protocol addenda for specific applications, including a compatible derived protocol suitable for fMRI analysis; a compatible but more complex protocol for 7T MRI, which will include separate labels for the SRLM layers of the CA and DG; and a compatible fast body-only protocol suitable for volumetry in clinical and imaging biomarker applications.

- **Stage 3: Formal Reliability Analysis.** Groups will submit representative datasets for reliability analysis. Reliability of the main protocol and derived protocols will be measured in terms of intra-rater and inter-rater reliability for both expert and novice raters. Each dataset will have on the order of 10 hippocampi, and will be segmented by at least three different participating groups.

If successful, this three-stage effort will produce a written document consisting of rule definitions for subfield boundaries that can be applied reliably across sites, scanners, and applications, and which is consistent with underlying anatomy to the extent possible. Once the protocol is completed, groups participating in the harmonization effort are expected to commit to adopt it in their future work, unless there is a good scientific reason not to do so. Furthermore, we expect the harmonized protocol to be integrated into some of the automatic subfield segmentation algorithms that are available [31, 38, 26], as the developers of these techniques are already part of the harmonization effort. Overall, a successful protocol harmonization effort will allow different groups working in the subfield imaging domain to produce measurements that are consistent and comparable, which will in turn make published studies easier to relate to each other and easier to replicate, leading to better science.

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