HSG:

Harmonized Hippocampal Subfield Segmentation Working Group

Report of a JPND Working Group on Harmonisation and Alignment in Brain Imaging Methods

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Background

The hippocampus and surrounding medial temporal lobe (MTL) cortices are a primary focus in neurodegeneration research of normal aging and dementia. For example, the *in vivo* measurement of structural integrity of the hippocampus and MTL cortical regions is a useful and sensitive measure in detecting early neurodegeneration in prodromal Alzheimer's disease (Frisoni et al., *Nature Reviews Neurology*, 2010). However, the hippocampus is a complex structure composed of several subfields that are distinct in cytoarchitecture and function, and their *in vivo* measurement has been demonstrated to be differentially sensitive to mechanisms of aging and disease (Kerchner et al., *NeuroImage*, 2010; La Joie et al., *NeuroImage*, 2010; Mueller et al., *Human Brain Mapping*, 2010; Kerchner et al., *NeuroImage*, 2012; La Joie et al., *NeuroImage*: Clinical, 2013; Bender et al. 2013 *J Cog Neuro;* Raz et al., *Brain Struct Funct*, 2014; Wisse et al., *Neurobio Aging*, 2014). Due to the non-uniform progression of Alzheimer's disease pathology within the MTL, measurement of particular subfields (e.g. CA1) has been proposed to more accurately discriminate between early stages of disease and typical atrophy in cognitively-normal aging as compared to measures of whole hippocampal volume and global brain atrophy (Kerchner et al., *NeuroImage*, 2010; La Joie et al., *NeuroImage*, 2010; Mueller et al., *Human Brain Mappin*g, 2010; La Joie et al., *NeuroImage*: Clinical, 2013).

Despite this positive progress, the exact structures vulnerable to specific types of neurodegenerative disease is still uncertain, in large part due to discrepancies in extant evidence (de Flores et al., *Neuroscience*, 2015). Existing protocols are numerous (>20), have employed different terminology and definitions of the subfield boundaries (using 3-11 different labels), were developed from a variety of histology references, and also use significantly different subfield boundary locations (Yuskevich et al., *NeuroImage*, 2015). The multitude of protocols has produced widely discrepant results, exemplified by up to 6-fold differences in subfield volumes (Wisse et al., *Front Aging Neurosci*, 2014), even when studying similar populations and phenomena. The solution is to develop a reliable, validated harmonized protocol for segmentation of the hippocampal subfields that can be applied to all populations of variable age and health; an initiative that was launched in 2013 by the Harmonized Hippocampal Subfield Segmentation Working Group (HSG; http://hippocampalsubfields.com/).

The current harmonization effort follows a successful model of a previous harmonization effort (led by European Alzheimer's Disease Consortium [EADC] - Alzheimer's Disease Neuroimaging Initiative [ADNI]), which developed a segmentation protocol to measure volumes of the whole hippocampus as a single structure (known as "HarP" for harmonized protocol; http://www.hippocampal-protocol.net). Critically, HarP does not divide the hippocampus into subfields—which is the goal of the current effort and an instrumental methodological step in the study of neurodegenerative processes. Three members of the HarP working group are also involved in the HSG and served on our Advisory Reference Group. These members provide first-hand experience in the development, evaluation, and dissemination of a harmonized protocol for the hippocampus.

Workflow and Consensus Process

A common means of segmenting the hippocampus is to divide the region into head, body and tail sections. In the current proposal, we aim to develop guidelines to further segment each of these regions into individual subfields, beginning first within the hippocampal body (currently segmented by the largest number of research groups), followed by subfields within the head and then tail. The harmonized protocol is being developed with experts in neuroanatomy and *in vivo* neuroimaging, and will undergo evaluation by the larger community prior to finalization of the protocol. The process for each of these steps is described below.

The overall HSG community currently consists of over 200 members; however, the main harmonization efforts are conducted by smaller groups of members. Specifically, the boundary working group (which is composed of a small team of HSG members) have contributed to protocol development during the funded period (see Figure 1 and Wisse et al., *Hippocampus* 2017 for a thorough description of our workflow and group structure). A separate small team of HSG members comprise the questionnaire working group, which serves as the communication arm between the boundary working group and the larger HSG community. A team of neuroanatomists: Augstinack, Ding, Insausti, and Kedo have provided their expertise throughout and have helped the boundary working group in the development and initial vetting of the harmonized protocol.

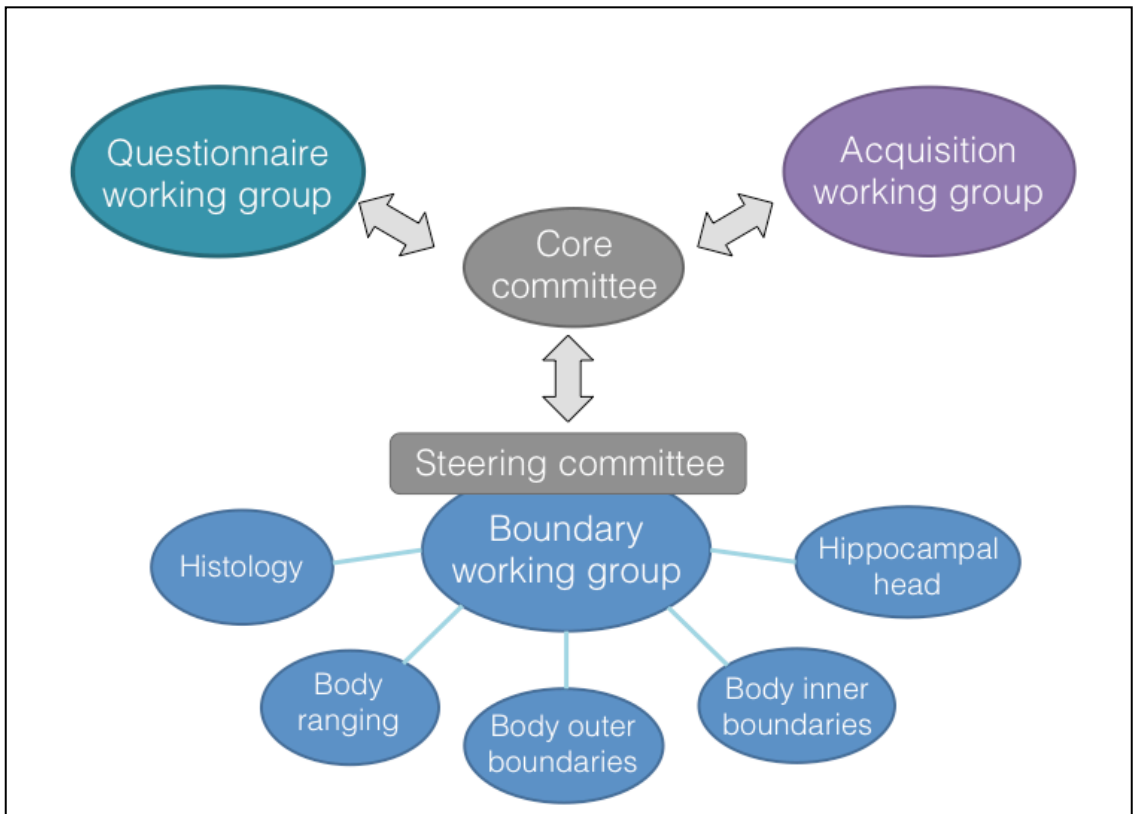

Figure 1. Organization of HSG. JPND funding covered the costs of three boundary working group meetings, in which we developed a draft of the hippocampal subfield protocol within the hippocampal head. During the funded period the questionnaire working group also prepared an online survey which will serve to collect feedback and obtain consensus from the larger HSG community. For more information about these working groups, please see our website: http://www.hippocampalsubfields.com/working-groups/

To obtain consensus within the larger HSG, the questionnaire working group has adopted the Delphi method, with some adaptations (see Figure 2). Namely, the following process is used: 1) obtain anonymous feedback on the protocol from a group of experienced segmenters via a questionnaire, 2) iterate through this feedback process such that responses from previous rounds are used to revise questionnaire content, and 3) cease the iterative process once majority consensus is reached. While the obtainment of consensus will add time to the total length of the harmonization process, the HSG is committed to this process as it is anticipated that consensus building will greatly facilitate widespread adoption of the harmonized protocol.

Responses from the consensus questionnaire will be analyzed both qualitatively and quantitatively. Qualitative responses will be assessed to determine whether updates to rule descriptions or to the rules themselves are needed. Qualitative responses will be made available to the HSG community in the next iteration of the questionnaire and also will become publicly available with the publication of the harmonized protocol. Quantitative responses will be analyzed to assess the level of agreement among respondents. Agreement will be measured via previously established methods used by HarP, and is achieved by counting the number of respondents agreeing (1-3 on the likert scale) and disagreeing (5-7) with each rule. A binomial test will be used to assess the significance of agreement versus disagreement. Additionally, the median and measures of dispersion will be calculated as secondary measures to demonstrate level of agreement. If the binomial test indicates significant agreement and no further updates to the rules or language are requested,

consensus is reached. If consensus is not reached, updates to the rule's description or content, potentially together with proposed alternative rules, will be included in the next iteration of the questionnaire, together with statistical results. This iterative process will continue until consensus is reached for all subfield boundaries. If consensus on a given rule is not reached after four rounds, the details of the rule agreed upon by the majority of respondents will be taken as the final rule.

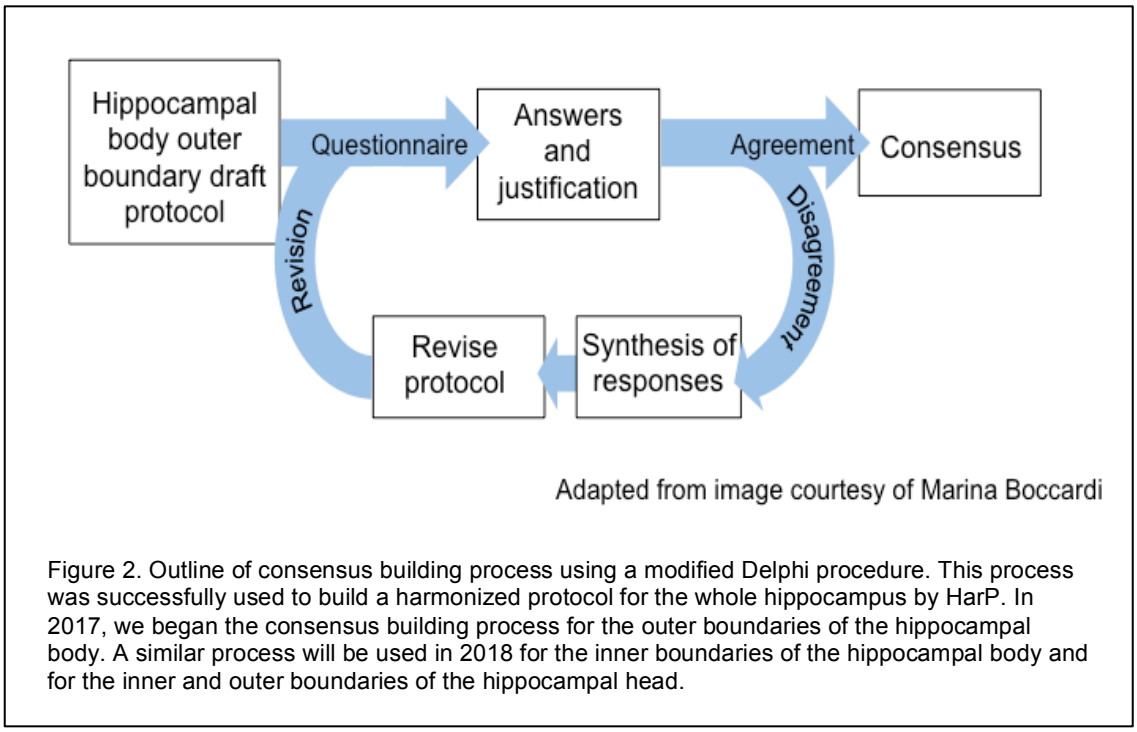

Figure 2. Outline of consensus building process using a modified Delphi procedure. This process was successfully used to build a harmonized protocol for the whole hippocampus by HarP. In 2017, we began the consensus building process for the outer boundaries of the hippocampal body. A similar process will be used in 2018 for the inner boundaries of the hippocampal body and for the inner and outer boundaries of the hippocampal head.

Previous work (Steps 1-3)

Prior to obtaining funding from the Joint Programme on Neuodegenerative disease (JPND), the HSG completed Steps 1-3 of the harmonization effort (see Figure 3). The first step culminated in a published paper entitled, "Quantitative comparison of 21 protocols for labelling hippocampal subfields and parahippocampal subregions in *in vivo* MRI: Towards a harmonized segmentation protocol" (Yushkevich et al., *NeuroImage*, 2015). This manuscript provided detailed examples of the 20 protocols used to segment the hippocampal subfields and MTL cortex (plus the HarP protocol). This was a critical first step in identifying commonalities, as well as discrepancies, among the current and commonly published segmentation protocols for the hippocampal subfields.

In Step 2, three HSG neuroanatomists (Augustinack, Amunts, Insausti) labeled six slices of the *hippocampal body* (the mid section of the hippocampus), in three different histological datasets. These tracings served several purposes: 1) providing a more comprehensive set of reference images currently lacking in canonical anatomy atlases, which can now be used to evaluate the location of subfield boundaries in consideration of natural variability between individual brains, as well as measurement variability due to different histological procedures and raters, and 2) visualization of subfields in the same orientation as is customary for *in vivo* MRI (i.e. perpendicular to the long axis of the hippocampus). Thus, these new samples were used by the HSG as the "ground truth" when evaluating the anatomical validity of the draft harmonization protocol rules developed in Steps 3 & 5.

In Step 3, boundary working group members met in-person in a working group held just prior to the 2015 Society for Neuroscience conference in Chicago, Illinois. This working group meeting generated a working document for the inner and outer boundaries of the hippocampal body. Subsequently, the outer and inner boundary rules have been formalized into a draft protocol through a series of phone and video conferences. This draft protocol uses neuroanatomical landmarks, image contrast features, and geometric rules to delineate the following subregions of the hippocampal body: subiculum, CA1, CA2, CA3, and a region which combines the dentate gyrus and CA4/hilus. A draft

protocol of the hippocampal subfields within the hippocampal body was shared at a satellite meeting in San Diego in November of 2016 (prior to the annual Society for Neuroscience meeting) and informal, initial feedback was obtained.

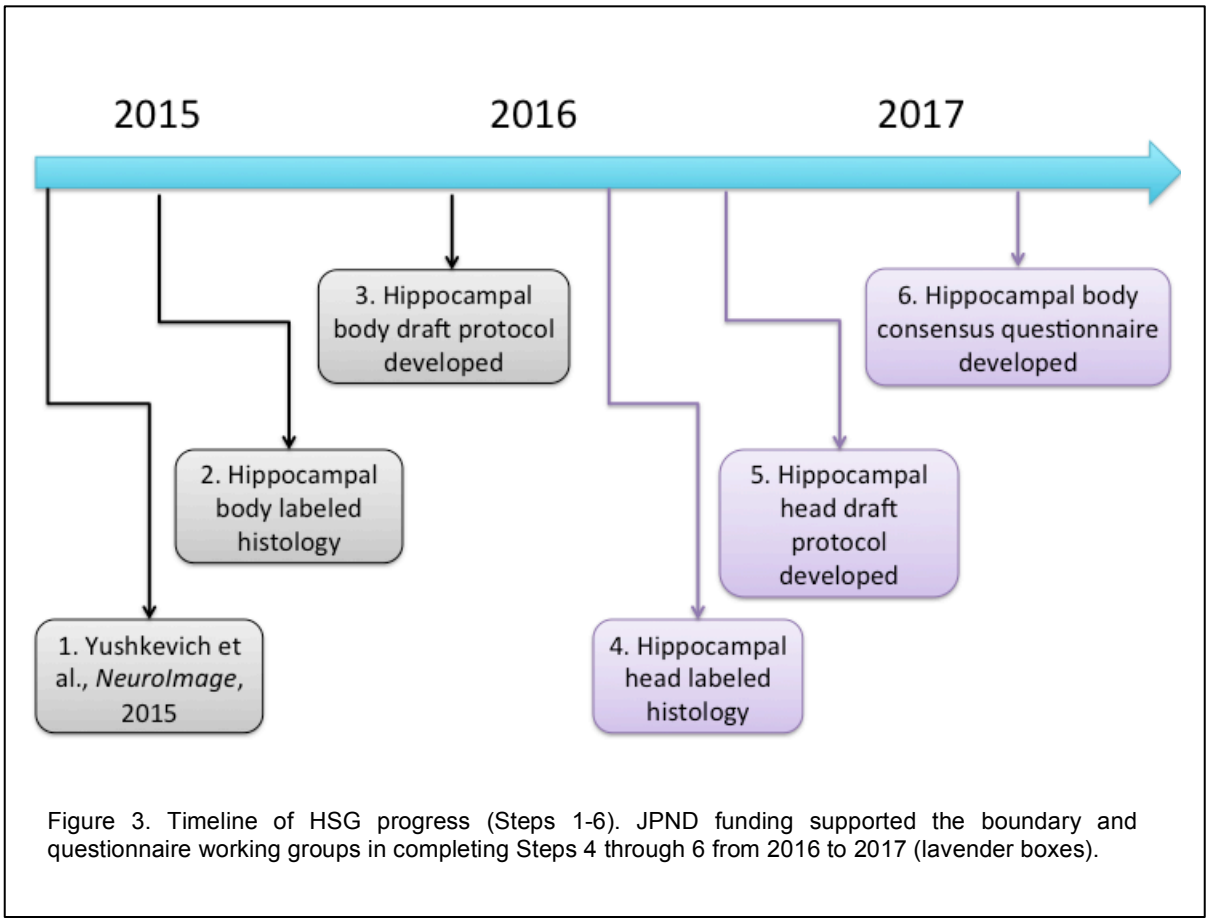

Figure 3. Timeline of HSG progress (Steps 1-6). JPND funding supported the boundary and questionnaire working groups in completing Steps 4 through 6 from 2016 to 2017 (lavender boxes).

Progress made with JPND funding (Steps 4-6)

Three steps of the harmonization effort were completed with support from JPND funding (Figure 3). JPND funding was used to organize three in-person working groups, which focused on developing a draft protocol of the hippocampal subfields for the hippocampal head (most anterior part of the hippocampus). Due to lack of established guidelines, this part of the hippocampus is less commonly segmented by researchers using *in vivo* 3T MRI. For the hippocampal head, we obtained four histological samples and accompanying annotations (Insausti, Kedo, Amunts, and Ding were involved in this step of the effort). For two of the samples we obtained two sets of labels from our team of neuroanatomists to characterize differences between neuroanatomy laboratories. The collection of these samples (Step 4) has provided a most comprehensive reference set of the hippocampal head, which extends well beyond the existing published atlases.

These in-person JPND-funded working group meetings took place in San Diego, California, U.S.A. (November 2016, nine participants), Montreal, Quebec, Canada (April 2017, 13 participants) and London, UK (July 2017, 19 participants). JPND funding allowed the working group participants to travel from their home institutions in Canada, France, Spain, Germany, UK, and USA to attend the working group sessions. The ability to meet in person to discuss the segmentation rules allowed our group to make much more progress than would have been possible otherwise, and also allowed participants from more laboratories to participate in the harmonization effort. Neuroanatomist Ding attended the meeting in Montreal and neuroanatomist Insausti attended the meeting in London, which was greatly beneficial for the subfield protocol development of the hippocampal head. As mentioned

above, this part of the hippocampus is not commonly segmented by human *in vivo* neuroimagers; thus JPND funding was integral in supporting a meeting of members from around the world that have expertise regarding the neuronanatomy of this region. A draft protocol was completed in London (Step 5), and is currently being edited by the participants that attended the London working group. Boundary working group members will finalize the hippocampal head draft protocol through phone and video conferences. The draft protocol delineates the same subregions as in the hippocampal body (subiculum, CA1, CA2, CA3, and a region that combines the dentate gyrus and CA4/hilus). A draft protocol of the hippocampal subfield protocol for the hippocampal head was presented at a small meeting in November of 2017 in Washington DC (prior to the annual Society for Neuroscience meeting).

In addition to the protocol development achieved during the working groups and the feedback obtained during satellite meetings in San Diego and Washington DC in 2016 and 2017, during the funded period the questionnaire working group also finalized and disseminated the survey on the hippocampal body outer boundaries, which will be used to obtain feedback and establish consensus with the entire HSG community (Step 6). The results from the consensus questionnaire will be collected and analysed in early 2018.

Summary, future work and knowledge dissemination

To review, in 2015-2016, the HSG established a new histological reference set and a working protocol for the delineation of the hippocampal subfields within the *hippocampal body*. In 2016-2017, the HSG utilized JPND funding to establish a second histological reference set and a working protocol for segmentation of the *hippocampal head* (see Figure 3 for timeline). In 2017 the questionnaire group began the process of obtaining consensus from the larger HSG community regarding the proposed hippocampal body protocol. We expect that the hippocampal body protocol will be finalized by June 2018 and that the hippocampal head protocol will be finalized in January 2019. In subsequent steps of this project, we will establish a new histological reference set and develop a harmonized protocol for the hippocampal tail and parahippocampal subregions (entorhinal, perirhinal and parahippocampal cortices).

We have presented our work in several venues, including a poster at the 2016 Alzheimer's Association International Conference in Toronto, a mini-symposium at the 2016 Society for Neuroscience in San Diego, the 2017 BigBrain Workshop in Montreal, an invited presentation to the fMRI users at the National Institutes of Mental Health in 2017, and during a one-day workshop at the 2017 Society for Neuroscience meeting in Washington DC. Dr. Olsen will present the draft protocol for the hippocampal body as well as the results from the consensus process in a poster at the International Conference on Learning and Memory in April 2018. We aim to present the final protocol for the hippocampal head and body at several conferences in 2019 (Organization of Human Brain Mapping, and the Alzheimer's Association International Conference).