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RESEARCH ARTICLE

Uncal apex position varies with normal aging

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Abstract

The uncal apex is an anatomical landmark frequently used for segmenting the hippocampus into its anterior and posterior segments, a necessary step for computing many measurements of its long axis. It functions well, as it is both local to the hippocampus and easy to identify. However, in spite of widespread use and definition in the EADC-ADNI Harmonized Hippocampal Protocol (HarP), how the uncal apex is influenced by gross hippocampal changes during normal aging has not been established, nor has the possible impact on measures of anterior hippocampus (aHPC) and posterior hippocampus (pHPC) volume. Here I drew upon three large data sets to describe and confirm these relationships, investigating them in one large data set and replicating my findings in the two others, evaluating a total of 4,434 hippocampi. I found the uncal apex fell in an increasingly more anterior position with increasing age. This age-related retraction of the uncus began after age 36, with the sharpest effects arising after age 60. This phenomenon exaggerates age-related aHPC volume decreases while simultaneously underestimating age-related pHPC volume decreases, a pattern I confirmed by juxtaposing uncal apex and MNI space-based landmarking. A hippocampally based reference frame was also rendered unstable by age-related shifts in the posterior extent of the hippocampus. Both the uncal apex and hippocampal reference frame should therefore be used with caution in aging research, or in research involving other demographic or disease factors known to evoke gross changes in the hippocampus. Instead, MNI coordinate-based heuristics may be appropriate for segmenting the hippocampus in study designs involving such factors. Apex-based segmentation is still attractive, however, in study designs where advanced age and atrophy are not used as regressors, including investigations into long-axis effects in healthy young adults. Progress toward localizing functional divisions within the hippocampus is needed to identify best practices for the field.

KEYWORDS

aging, anterior hippocampus, long axis, posterior hippocampus, uncal apex

1 | INTRODUCTION

The hippocampus long axis has emerged as a topic of great interest over the past decade, with numerous proposals emerging regarding its functional organization (Brunec et al., 2018; Fanselow & Dong, 2010; Poppenk, Evensmoen, Moscovitch, & Nadel, 2013; Strange, Witter, Lein, & Moser,

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu), Cambridge Centre for Ageing and Neuroscience (CamCAN) repository (mrc-cbu.cam.ac.uk/datasets/camcan), and Human Connectome Project (HCP) database (humanconnectome.org). As such, investigators from these groups contributed to the design and implementation and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

2014). Issues regarding how to best distinguish anterior hippocampus (aHPC) from posterior hippocampus (pHPC) have therefore also risen in importance. Many researchers take the approach of segmenting the hippocampus at the coronal slice containing the uncal apex (Poppenk et al., 2013; Yushkevich et al., 2010), a landmark that is both local to the hippocampus and clearly visible even in lower-quality neuroimaging scans. This approach was also recently described in the EADC-ADNI Harmonized Hippocampal Protocol (HarP) for manual hippocampal segmentation (Boccardi et al., 2015), an influential guideline aimed at consolidating the measures used for investigation of the hippocampus, such that research results may be more readily compared across studies. The use of this landmark is further supported by evidence that there are numerous transitions that occur proximal to the uncal apex and that distinguish aHPC from pHPC, including structural changes (Duvernoy, 2005), intrinsic connectivity (Strange et al., 2014), and structural and functional extrinsic connectivity (Blessing, Beissner, Schumann, Brünner, & Bär, 2016; Ge et al., 2019; Plachti et al., 2019; Poppenk, *in press*; Vogel et al., 2019).

The above offer many excellent reasons to use the uncal apex as a landmark for investigation of the hippocampus long axis, yet the properties of the uncal apex have not been evaluated at the level of individual differences. Yet, effects arising from major structural changes in the hippocampus could be present: for example, the hippocampus is known to undergo major changes during the lifespan, decreasing in volume as part of normal aging (Cespedes et al., 2017). Because the uncal apex is part of the hippocampus, it is possible, if not likely, that such developmental changes have an impact on the positioning of uncal apex.

Where the uncal apex is to be used as a spatial reference, repositioning could be cause for concern. To illustrate, should the uncus decrease in volume by retracting as a result of atrophy, measures of the volume of the aHPC would, correctly, decrease due to the loss of uncal tissue, which falls in the aHPC (Figure 1). However, the portion of the hippocampus inferior to the uncus, previously falling below a larger uncus and defined as aHPC, would simultaneously become redefined as pHPC. The result could be nominal growth of the pHPC and volume loss in aHPC, which could lead to the incorrect conclusion that aHPC and pHPC segments each grow at the expense of the other. Even if the effects were not so extreme so as to suggest the pHPC was increasing in size, concurrent pHPC atrophy could be obscured.

To better understand variation of the uncal apex in the population and its effects on segmentation of aHPC from pHPC, here I document the uncal apex position of several thousand healthy adult participants, evaluate its stability as a function of basic demographic factors including age, gender, and handedness, and consider its place within the system of neuroanatomical measures that together undergo changes over the lifespan.

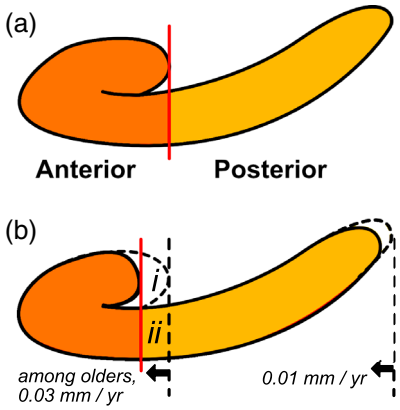

FIGURE 1 Mechanism for possible hippocampal volume misclassification as a function of landmark migration. (a) aHPC volume (orange) and pHPC volume (yellow) are often defined as hippocampal tissue falling anterior to and posterior to the coronal slice containing the uncal apex (red line), respectively. (b) When the hippocampus is undergoing atrophy, the uncus may retract, again yielding not only appropriate indexing of volume loss in the uncus (i) as well as and other aspects of the hippocampus, but also reclassification of hippocampal tissue from aHPC to pHPC (ii). As a result, even where these hippocampal segments undergo atrophy at an even rate, the pHPC could erroneously appear to increase in volume, whereas the aHPC could appear to decrease in size more rapidly than is actually taking place. Changes in posterior extent also impact reference systems local to the hippocampus. Values reflect age-related displacements reported in Section 3. aHPC, anterior hippocampus; pHPC, posterior hippocampus [Color figure can be viewed at wileyonlinelibrary.com]

2 | METHODS

2.1 | Data sets

To conduct this analysis, I used several public data sets featuring distinctive demographics as well as T1-weighted anatomical images. I began with a data set that encompassed the full adult lifespan, which I accessed from the Cambridge Centre for Ageing and Neuroscience (CamCAN) repository (available at http://www.mrc-cbu.cam.ac.uk/datasets/camcan/). Briefly, the CamCAN project uses epidemiological, cognitive, and neuroimaging data to understand how individuals can best retain cognitive abilities into old age (for an overview of this project, see Shafto et al., 2014). I accessed data from Stage II of the project (Taylor et al., 2017), in which 700 adults were scanned at a single site using a 3T Siemens Tim Trio scanner. The scans originally acquired in the project included structural Magnetic Resonance Imaging (MRI), functional MRI (both resting and task-based), and magnetoencephalography (MEG; resting and task-based). Participants included in this data set were drawn from a large community sample with uniform distribution of age to maximize statistical power for age-based analysis. Some participants were withheld due to data quality issues, and I was provided with access to 637 participants with brain data (age range = 18–87 years). I made use of structural T1- and T2-weighted data provided in participant native space at $1 \times 1 \times 1$ mm^3. Linear transforms to MNI space, as well as hippocampal segmentations, were also provided by the data set authors based on FreeSurfer analysis of these same images (v.6.0.0, Fischl et al., 2002; see Taylor et al., 2017, for details). Output from the subcortical processing stream was used to obtain hippocampal segmentations.

Upon inspecting this data set, I observed apparently discrepant trends in younger and older adults. To more closely examine these, I investigated additional data obtained from the Human Connectome

Project (HCP), a large sample of healthy young adults aged 22–37 years (n = 1,113) who were scanned at a single site with a customized 3T Siemens Skyra scanner. This age range was selected by the data set authors to best sample healthy adults beyond the age of major brain development who were not yet experiencing age-related neurodegenerative effects (for an overview of the project, see Glasser et al., 2013). This sample was valuable for inclusion into the current analysis for similar reasons. Briefly, the HCP data set involved diffusion imaging (dMRI), resting-state fMRI (R-fMRI), task-evoked fMRI (T-fMRI), T1- and T2-weighted MRI for structural and myelin mapping, plus combined MEG and electroencephalography imaging. T1- and T2-weighted data were provided in participant native space at $0.7 \times 0.7 \times 0.7$ mm^3. I also obtained curated FreeSurfer output (v.5.3.0) from the HCP consortium, again using its linear transforms to MNI space as well as segmentations from the subcortical processing stream.

To separately investigate the discrepant trends in older adults, I obtained brain images and demographic data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public–private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment and early Alzheimer's disease (AD). In the current case, however, I inspected only data from healthy older adults (aged 60–90 years) included as control participants in ADNI1 (n = 190) and ADNI2 (n = 277), for a combined sample of n = 467. Although the current analyses relate to the HarP, which was conducted using an ADNI-based reference data set (n = 135), I did not limit investigation to that subset, which was curated with different goals in mind (e.g., generalization of protocol to demented patients) and represented a group that was rather small for evaluation of the relationship between hippocampal features and age. For each participant, I obtained from the ADNI database T1-weighted anatomical scans in native space, as well as linear transforms to MNI space and hippocampal segmentations from the subcortical stream of FreeSurfer (v.5.3.0).

2.2 | Landmark identification

The position of the uncal apex in AC–PC space was recorded for the left and right hippocampus of each participant. This was achieved by identifying the last slice of the anterior, defined as the last slice moving posteriorly in which the uncus was visible (or as described in HarP: "where the hippocampus can be seen as a folded structure on the sagittal view, or as a double-level structure in the coronal view," Boccardi et al., 2015; see also Poppenk et al., 2013). Due to the large scope of the task (with thousands of hippocampi to be evaluated), several raters contributed, with a different rater for each data set.

After preliminary training about how to recognize the landmark, raters completed a procedure to establish reliable ratings. In particular, raters recorded landmarks for a set of 10 initial participants from the HCP data set, which featured the highest resolution, and which we found required a strong understanding of distracting features appearing in the vicinity of the apex (e.g., blood vessels) in order to obtain an exact slice match. After raters completed 10 participants (20 hippocampi), I computed the DICE coefficient evaluating the rate at which the rater selected the exact same slice as another rater's evaluations of the same participants. If this coefficient fell below a threshold of 0.80, then we discussed cases with discrepancies, the rater discarded their scores, and they undertook landmarking for a new set of 10 participants. Final inter-rater agreement was high for the CamCAN rater, DICE = 0.80, ICC = 0.97, mean error = 0.27 mm (first iteration); for the HCP rater, DICE = 0.80, ICC = 0.99, mean error = 0.07 mm (third iteration); and for the ADNI rater, DICE = 0.85, ICC = 0.98, mean error = 0.16 mm (third iteration).

Where required, the obtained landmark coordinates were transformed into MNI space using the linear transforms provided with each data set. In a small minority (1.5%) of cases, these transforms yielded coordinates falling far outside of normal apex y-coordinate values, suggesting a registration failure. In these cases, the transformation was re-evaluated using FSL's *flirt* tool (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012), and applied to obtain a corrected MNI coordinate value.

As part of a further evaluation of hippocampal spatial reference points, I identified the anterior and posterior extent of each participants' hippocampus by identifying the most anterior or posterior slice of their FreeSurfer hippocampus segmentation, repeating this step for each hemisphere. I calculated hippocampus length by taking the difference between these two values.

2.3 | Evaluation of landmark position

For each data set, I computed descriptive statistics for left and right uncal apex position in the y-plane. I then further computed the relationship between the position of the uncal apex in MNI space, other measures of the hippocampus, and participants' basic demographic characteristics. Correlations were performed using bootstrap resampling statistics (see Sunavsky & Poppenk, in press). Briefly, correlations were computed using 1,000 random subsets of participants, and the correlation obtained using all participants was then evaluated relative to the bootstrap standard error to obtain a bootstrap ratio (BSR), analogous to a z-score, which was used to evaluate statistical significance alongside the 95% bootstrap confidence interval. In addition, to quantify the impact of various relationships with the uncal apex, a series of regressions were run and the relevant slopes recorded.

3 | RESULTS

3.1 | CamCAN

Because it was the only data set featuring the full lifespan, I began analysis with the CamCAN data set. Collapsing across adults of all ages, there was no significant difference between average left uncal apex y-position (M = −20.61, SD = 2.05) and right uncal apex

y-position (M = −20.49, SD = 1.95), M_{diff} = 0.13, SD_{diff} = 1.50, BSR_{diff} = −1.17, p_{diff} = 0.24. The two were also highly correlated, r = .72, 95% CI = [0.65 0.78], BSR = 18.50, p < .001. I therefore took the average uncal apex position across hemispheres (M = −20.58, SD = 1.71) to streamline subsequent analyses of this data set.

The critical test for purposes of the current investigation concerned the relationship between age and apex position over the lifespan. There was a significant correlation between age and apex position, r = .31, 95% CI = [0.24 0.38], BSR = 9.01, p < .001. To quantify the age-related change in apex position, I regressed age against uncal apex position, and observed an average rate of change of 0.028 mm per year toward the aHPC extent.

Visual inspection of the data on a scatterplot (Figure 2) suggested this rate of change was not static across the lifespan. I therefore conducted post-hoc analyses on three separate age groups: young adults (18–36 years old; n = 139), middle-aged adults (37–59 years old; n = 228), and older adults (60+ years old; n = 270). There was no relationship between age and apex position in the younger group (M = −21.21, SD = 1.67), r = .07, 95% CI = [−0.09 0.23], BSR = 0.85, p = 0.395. However, the relationship was significant in middle-aged adults (M = −20.81, SD = 1.72), r = .13, 95% CI = [0.01 0.26], BSR = 2.11, p = .035 with an average difference of 0.035 mm per year, and older adults (M = −20.06, SD = 1.57), r = .24, 95% CI = [0.13 0.35], BSR = 4.49, p < .001, with an average difference of 0.050 mm per year.

For descriptive purposes, I further investigated demographic factors of sex and handedness. The mean MNI apex position of men (M = −20.44, SD = 1.68) and women (M = −21.71, SD = 1.74), was slightly different, M_{diff} = 0.28, BSR_{diff} = 2.01, p < .001. However, Edinburgh handedness score was not correlated with mean uncal apex position, r = .02, 95% CI = [0.02 0.09], BSR = 0.87, p = .383, nor the difference between left and right uncal apex position, r = −.05, 95% CI = [−0.12 0.01], BSR = −1.58, p = .114.

3.2 | Hippocampal indexing

As an alternative to MNI-based spatial localization, the uncal apex can be indexed in terms of its position along the hippocampal axis (i.e., as a percentage progression, where 0% represents the anterior extent and 100% the posterior extent). This positioning system is local to the hippocampus, and potentially more resistant to noise arising from whole-brain registration issues associated with aging. However, it is also more exposed to displacement of the hippocampus' anterior and posterior extent. I and others have previously proposed an alternative heuristic in this space: rather than using an MNI *y*-coordinate of y = −21 mm as the division between head and tail, a value of 35% anterior–posterior progression can be used, which is both an existing convention and the approximate location of the uncal apex on the MNI template (Poppenk et al., 2013).

I conducted further analyses using this coordinate frame to test its stability. Uncal apex position in the left hemisphere (M = 38.70%, SD = 4.11%) relative to the right hemisphere (M = 40.31%, SD = 4.11%) was more posterior (M_{diff} = 1.60%, SD_{diff} = 5.53%), BSR_{diff} = 7.04, p_{diff} < 0.001. Uncal apex position was nonetheless correlated across hemispheres, r = .52, 95% CI = [0.48 0.58], BSR = 19.53, p < .001, so I merged them for subsequent analyses. Like in the MNI coordinate

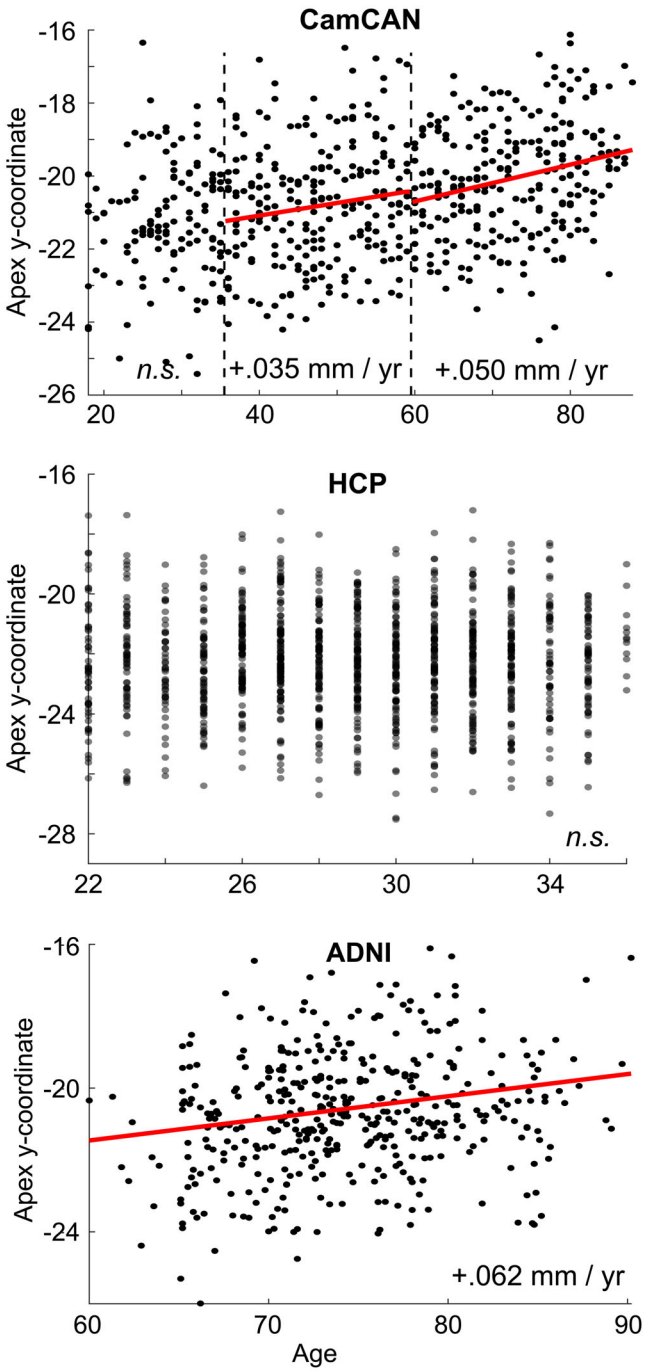

FIGURE 2 Uncal apex *y*-coordinate as a function of age in three data sets. In the CamCAN data set (top), post-hoc testing revealed age-related uncal apex displacement toward the anterior (more positive values) in middle-aged and older adults (black dashed lines distinguish age groupings; red lines designate significant linear fits). This was confirmed upon inspection of HCP healthy young adult data (middle), where no significant relationship was observed between these variables, and ADNI healthy older adult data (bottom), where a robust trend was observed. HPC, hippocampus [Color figure can be viewed at wileyonlinelibrary.com]

system, I found the position of the uncal apex was correlated with age, $r = -.32$, 95% CI = [−0.39 −0.25], BSR = −8.80, $p < .001$, with a displacement of 0.06% per year toward the anterior extent (Figure 3; young adult $M = 40.67\%$, $SD = 3.51\%$; middle-aged adult $M = 40.11\%$, $SD = 3.43\%$; older adult $M = 38.39\%$, $SD = 3.47\%$). Focusing on specific age groups, I observed a higher displacement of 0.08% per year for middle-aged adults, and 0.12% per year for older adults.

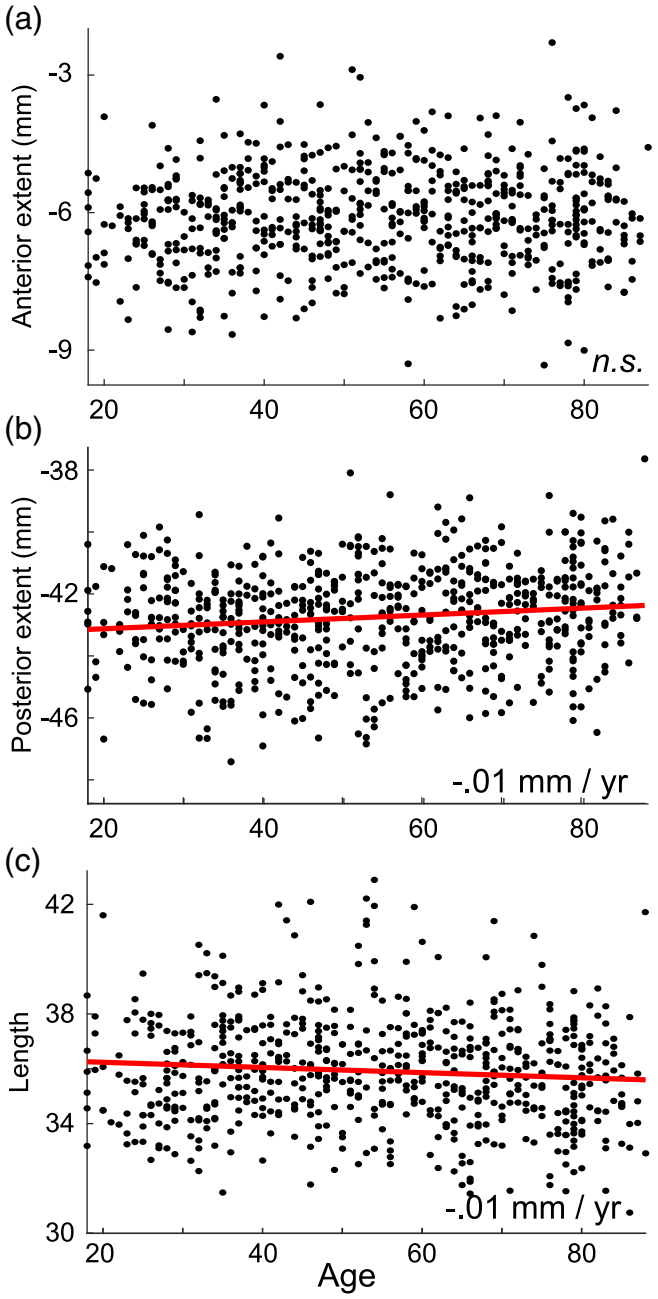

FIGURE 3 Hippocampal reference frame as a function of age. Across the lifespan (CamCAN data set), there was no change in the anterior extent of the hippocampus (a), but there was displacement of the posterior extent toward the anterior (b) and a corresponding age-related shortening of the hippocampus overall (c). These patterns, which were apparent only when evaluating across the entire lifespan rather than a particular demographic group, reveal that the whole-hippocampal reference frame also fluctuates with age [Color figure can be viewed at wileyonlinelibrary.com]

As the hippocampal coordinate frame is referenced by the anterior and posterior extent of the hippocampus, I also evaluated the stability in MNI position of these elements as a function of age. I observed no age-related displacement of the anterior extent of the hippocampus, $r = .03$, 95% CI = [−0.05 0.11], BSR = 0.72, $p = .474$, but I did observe forward age-related displacement of the posterior extent, $r = .12$, 95% CI = [0.05 0.20], BSR = −3.30, $p < .001$, indicating that not only was uncal apex position fluid within this reference frame, but the reference frame itself was unstable with respect to aging.

It follows from observation of a more forward posterior extent with increasing age that the hippocampus should also be shorter among participants with greater age, a pattern I confirmed, $r = -.09$, CI = [−0.17–0.01], BSR = −2.26, $p = .024$. However, age-related length was unrelated to apex position, with no relationship between length and apex y-coordinate, $r = -.00$, CI = [−0.09 0.07], BSR = −0.09, $p = .925$.

3.3 | Effect on volumes

To quantify the effects of age-related uncal apex migration on aHPC and pHPC volumes, again using the CamCAN data set, I correlated uncal apex position with aHPC and pHPC volume. Its position was both negatively correlated with aHPC volume (as defined using this same landmark), $r = -.55$, 95% CI = [−0.60–0.49], BSR = −20.53, $p < .001$ (approximately 30.3% of variance) and positively correlated with pHPC volume, $r = .18$, 95% CI = [0.10 0.27], BSR = 4.37, $p < .001$. On average across hemispheres, for each 1 mm of uncal apex displacement toward the anterior (in MNI space), ICV-corrected aHPC volume was 95.2 mm^3 lower. With an average difference of 1.15 mm in the uncal apex position of younger and older adults, this means that apex-based mislabeling could be expected to account for about 110 mm^3 of aHPC tissue that is potentially misclassified when comparing these groups and not accounting for changes in uncal apex position (roughly 5% of the average CamCAN aHPC volume of 2,229 mm^3).

To verify the idea that, based on the above, using the uncal apex would mask age-related atrophy of the pHPC while exaggerating age-related atrophy of the aHPC, I tested the relationship between age and both aHPC and pHPC volumes where their labels were defined using the uncal apex, and then repeated this test after segmenting aHPC and pHPC using a fixed landmark instead (MNI $y = -21$ mm). I found that aHPC volume was negatively correlated with age when using the uncal apex landmark, $r = -.37$, 95% CI = [−0.33–0.40], BSR = −10.41, $p < .001$, as well as when using the fixed landmark, $r = -.26$, 95% CI = [−0.19–0.33], BSR = −7.25, $p < .001$, but that the relationship was weaker using the fixed landmark, $r_{diff} = -.11$, 95% CI_{diff} = [−0.21–0.02]. Conversely, I found that the pHPC volume was negatively correlated with age when using the uncal apex landmark, $r = -.32$, 95% CI = [−0.38–0.25], BSR = −9.09, $p < .001$, as well as

when using the fixed landmark, $r = -.43$, 95% CI = [−0.49–0.37], BSR = −14.05, $p < .001$, but that this relationship was stronger using the fixed landmark, $r_{diff} = .11$, 95% CI_{diff} = [0.02 0.20].

As a further exploration of the relationship between age-related atrophy and uncal apex position, I attempted to predict apex position using overall volume of the hippocampus. I first confirmed that age-related atrophy was reflected in the correlation between age and hippocampus volume, $r = -.45$, CI = [−0.52–0.40], BSR = −15.57, $p < .001$. I then evaluated the relationship between apex position and hippocampal volume, and observed a negative relationship, $r = -.35$, CI = [−0.43–0.28], BSR = −9.04, $p < .001$. Regression revealed that with every 76 mm^3 decrease in hippocampus volume, the uncus was located 1 mm more anteriorly.

3.4 | Younger adults

Young adults comprised only on a small portion of the CanCAN data set, making confirmation of the null hypothesis in that group somewhat less convincing (particularly in light of trend-level significance). Accordingly, I supplemented the current analysis with inspection of the HCP data set. This data set includes healthy young adults of an age range (22–35 years) similar to the post-hoc grouping analyzed in CamCAN (18–35 years). With more than 1,100 participants, it afforded a more authoritative test of the potential null effect.

In the HCP data set, there was a significant but small difference between left uncal apex *y*-position ($M = -22.42$, $SD = 1.85$) and right uncal apex *y*-position ($M = -22.10$, $SD = 1.86$), $BSR_{diff} = -7.17$, $p_{diff} < .001$, although left and right uncal apex were still highly correlated, $r = .70$, 95% CI = [0.67 0.73], BSR = 47.34, $p < .001$. As with young adults in the CamCAN data set, apex position and age were not significantly related, $r = .01$, 95% CI = [−0.06 0.07], BSR = 0.16, $p = .867$.

The uncal apex *y*-position of men ($M = -22.22$, $SD = 1.80$) and women ($M = -22.30$, $SD = 1.63$), was not different, $M_{diff} = 0.08$, $BSR_{diff} = 0.76$, $p_{diff} = .448$. Similar to the CamCAN analysis, Edinburgh handedness score was neither correlated to mean apex position, $r = -.00$, 95% CI = [−0.06 0.05], BSR = −0.11, $p = .914$, nor the difference between left and right apex position, $r = .03$, 95% CI = [−0.02 0.10], BSR = 1.18, $p = .234$.

3.5 | Older adults

To confirm the effects found among older adults in the CamCAN data set, I inspected older adult data from the ADNI data set, which featured 467 healthy older adult controls. There was no difference between left uncal apex *y*-position ($M = -20.62$, $SD = 3.02$) and right uncal apex *y*-position ($M = -20.53$, $SD = 2.91$), $M_{diff} = 0.08$, $BSR_{diff} = 0.94$, $p_{diff} = .345$, with left and right uncal apex position again being highly correlated, $r = .83$, 95% CI = [0.56 0.92], BSR = 7.68, $p < .001$. I observed a displacement in apex position of 0.062 mm per year toward the anterior, and as in the CamCAN data set, apex position and age were correlated, $r = .21$, 95% CI = [0.12 0.30], BSR = 4.48, $p < .001$.

The average uncal apex *y*-position of men ($M = -20.52$, $SD = 1.68$) and women ($M = -20.62$, $SD = 3.53$), were not different, $M_{diff} = 0.10$, $BSR_{diff} = 0.38$, $p_{diff} = .702$. As most participants were right-handed; as only left/right handedness values, rather than graded Edinburgh handedness scores, were available; and as analyses in the first two data sets were negative, I did not conduct a handedness analysis on the ADNI data set.

I also attempted to replicate the volumetric findings of the CamCAN data set. Uncal apex again showed a negative correlation with aHPC volume (as defined using this same landmark), $r = -.54$, 95% CI = [−0.60 −0.47], BSR = −16.6, $p < .001$ (or approximately 29.2% of aHPC variance) and positive correlation with pHPC volume, $r = .25$, 95% CI = [0.17 0.33], BSR = 5.70, $p < .001$. For each 1 mm of uncal apex displacement toward the anterior, ICV-corrected aHPC volume was 72.7 mm^3 lower. I found apex-referenced aHPC to be negatively correlated with age, $r = -.32$, 95% CI = [−0.40 −0.23], BSR = −7.43, $p < .001$, but also when using the fixed landmark, $r = -.27$, 95% CI = [−0.36 −0.18], BSR = −5.74, $p < .001$, with no significant difference, $r_{diff} = -.02$, 95% CI_{diff} = [−0.10 0.14]. I also found that the pHPC was negatively correlated with age when using the uncal apex landmark, $r = -.22$, 95% CI = [−0.31–0.12], BSR = −4.38, $p < .001$, as well as when using the fixed landmark, $r = -.32$, 95% CI = [−0.39 −0.24], BSR = −7.18, $p < .001$, with the fixed landmark relationship again being stronger for the posterior, as assessed by a one-way confirmatory analysis, $r_{diff} = 0.10$, 95% CI_{diff} = [0.00 0.20].

4 | DISCUSSION

Through inspection of three large data sets of healthy younger and older adults, I have demonstrated that the position of the uncal apex in MNI space varies with age, with the uncus beginning a slow age-related retraction toward the anterior extent of the hippocampus after age 36. Where the uncal apex is used as a landmark to segment aHPC from pHPC, this age-related displacement can be expected to cause hippocampal tissue beneath the uncus to be relabeled, exaggerating age-related volumetric decline in the aHPC, while understating decline in pHPC. Age-related hippocampal change was also shown to impact the use of a hippocampal reference frame. These phenomena were demonstrated with regard to age-related hippocampal atrophy, but similar phenomena are likely to occur in any population where differences in the gross structure of the hippocampus can be found. Accordingly, although the uncal apex does feature a number of advantages in distinguishing anterior from pHPC, it should be used with careful consideration of the population under investigation and the goals of research.

Although the current work focuses on use of the uncal apex in research on aging, the scope of this study does not include validation of a specific alternative. Nonetheless, several factors should be considered. The problem arises not from a lack of reliable means for

demarcating the uncus from the rest of the hippocampus, which can in principle already be solved by either manual or computer segmentation. Instead, the problem is finding a static local transition point from aHPC to pHPC in the subuncular hippocampus that is easy to observe, has a meaningful relationship with the structure, and remains relatively fixed as the hippocampus changes. Although it is possible to look to proximal brain structures instead—for example, radiologists and researchers sometimes reference the tectal plate, located at the midbrain (Beattie et al., 2017; although this is traditionally a segmentation point of body from tail; Gan, Di Muzio, & Gaillard, 2015)—none are known to align with the "high water mark" of uncal development.

In place of a static local landmark of this kind, the main alternative would appear to be a fixed, global one. Currently, most studies on the aging hippocampus use either the uncal apex or an MNI coordinate as a reference point. Speaking to the latter approach, my colleagues and I have suggested that $y = -21$ mm in MNI space could be used as a heuristic for demarcating the posterior extent of the aHPC (Poppenk et al., 2013). We established this value based on of segmentation of the MNI atlas. This strategy does introduce noise into measures of aHPC and pHPC, as it is likely to lead the tip of the uncus to fall into either aHPC or pHPC about half the time (because $y = -21$ mm represents the population mean of the young adults demographic used to construct the MNI atlas; Cocosco, Kollokian, Kwan, & Evans, 1997). Use of this heuristic also suffers from imprecise localization of the hippocampus (which features some variability in its global positioning), and atlas registration challenges in groups with more variable morphology (including older adults). But in spite of these drawbacks, the overall brain affords a relatively fixed coordinate system that is less prone to systematic distortion in a particular direction. Based on the factors above, measurement error should be random with respect to whether tissue is mislabeled as aHPC versus pHPC.

The current analyses afford an opportunity to better describe this population mean. As the CamCAN data set featured the most complete coverage of the lifespan, I will draw values from that data set. Investigating young adults, the average position of the uncal apex ($y = -21.2$ mm) was very close to the original heuristic, consistent with the young adult composition of the MNI template. In this way, the current study contributes converging evidence toward $y = -21$ mm as the average uncal apex position in the young adult population. The hippocampal reference frame was less consistent with the 35% demarcation point heuristic used by some researchers (see Poppenk et al., 2013), with an observed value of 40.67% among young adults; it therefore appears appropriate to update this heuristic. However, the current results highlight that this reference frame may only be appropriate for younger adults (see Section 3). Use of a hippocampal reference frame for older adults is not recommended, since it is subject to repositioning due to age-related displacement of the pHPC extent, as well as concomitant age-related shortening of the hippocampus.

Where the uncal apex is used, further steps can be taken to reduce measurement error. One interesting approach, applied by Nordin, Herlitz, Larsson, and Söderlund (2017), was to exclude a 4 mm buffer between aHPC and pHPC measurements (dropping slices from $y = -19$ through $y = -23$). This can be expected to reduce uncus-related noise, but omitting data is generally a last resort, and as has been illustrated here, the uncal apex still sometimes falls outside these boundaries. Along these lines, however, a coordinate value falling more posteriorly than the normal range of the uncus could address this noise. For example, adding a buffer of two standard deviations to the young adult group, $y = -21.21$ mm $- (1.67$ mm $\times 2)$ would result in a reference point of 24.55 mm, for which none of the uncus would be misclassified as pHPC in 95% of adults, albeit at the cost of a consistently overstated aHPC. Finally, a hybrid approach may be possible, in which the uncus is segmented separately from the subuncal portion of the hippocampus. Under this strategy, all of the uncus would be assigned to aHPC, while the subuncular hippocampus would be partitioned at $y = 21$ mm (regardless of uncal apex position), thereby circumventing any reclassification of tissue due to uncal apex migration. Separate segmentation of the uncus would be laborious, however, unless a computer algorithm were designed to perform this step.

These possible alternatives diverge from the uncal apex-based head definition used in HarP, an influential protocol and product of a large collaborative effort aimed at standardizing procedures for hippocampal measurement (Boccardi et al., 2015). This may generate confusion about what methods are best to apply. Notably, it is written into the protocol that partitioning of the head, body, and tail was "not a matter of decision of the Delphi panel" (which generated the protocol). Abstaining from use of the uncal apex in defining the head should therefore not be regarded as deviating from the protocol. The current report also does not deliver decisive guidance: although problems arise with use of the uncal apex, it has the discussed advantages; no confounds were revealed in young adults; and age-related bias in aHPC and pHPC volume assessments need not amount to a confound in careful designs. It will be valuable for ongoing harmonization efforts to consider these issues carefully in pursuit of their goal of a convergent hippocampal science. In the meantime, researchers deciding which approach to implement will need to reflect carefully on the issues raised here in relation to their own research aims.

Several limitations should be noted. First, the current work is based on cross-sectional analysis, and longitudinal research will be required to confirm that the effects represent change over time. Second, I did not attempt to characterize development of the uncus among children and adolescents, a developmental stage that is also associated with structural change in the hippocampus (Tamnes, Bos, van de Kamp, Peters, & Crone, 2018). Uncal apex should therefore be used as a landmark with caution in child and adolescent data until its properties can be investigated in greater detail in those populations. Also, the segmentations obtained from the different databases were originally generated using different versions of FreeSurfer: CamCAN was processed with version 6.0, and the other two data sets with version 5.3. The latter version has been found to slightly overestimate hippocampal size due to inclusion of boundary voxels; however, this overestimation does not obscure differences between groups (Tae, Kim, Lee, Nam, & Kim, 2008), does not affect correlations between hippocampal volume and cognitive variables (Cherbuin, Anstey, Reglade-Meslin, & Sachdev, 2009), and the variance structure is

sustained in both anterior and pHPC (Poppenk & Moscovitch, 2011). Finally, in the HCP data set, the uncal apex was observed about 1 mm further posterior than expected based on observations in the similarly aged CamCAN group, as well as from the MNI template, even though apex position in older adults was in close agreement across CamCAN and ADNI data sets. It is possible that drift in rater evaluations led to the discrepancy, since these data sets were evaluated by different raters. However, as all analyses were performed within data set, and as these data sets were used principally to confirm generalization of the patterns in the CamCAN data set, these small deviations do not undermine the study's conclusions.

It is interesting to note that aHPC and pHPC volumes have sometimes expressed opposite relationships with cognitive variables (Poppenk & Moscovitch, 2011). As the current results show that the position of the uncal apex contributes in opposing ways to the volume of both structures, it is therefore interesting to speculate that in such cases, the variance contributing to these mirror-like relationships might be related specifically to the uncus, since its development, as has been argued here, can contribute to opposing effects on aHPC and pHPC volumes.

In summary, the current study documents age-related migration of the uncal apex toward the anterior extent of the hippocampus during normal aging, with this effect arising across large data sets in middle-aged and older, but not younger adults. However, as the age effect is shown to have corresponding influences on aHPC and pHPC measurements, care should be taken when using the uncal apex for their segmentation when also making inferences about the relationship of these structures to age (or other factors involving gross hippocampal changes). An MNI-based heuristic may provide a preferable static reference for partitioning the hippocampus that sidesteps apex position and a shifting hippocampal reference frame as potential confounds.

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DATA AVAILABILITY STATEMENT

All data (brain images and transformations) were obtained from public datasets and are available from the respective repositories described above. A list of uncal apex positions derived from the CamCAN and ADNI datasets are available at https://data.mendeley.com/datasets/c4gyz845yx.

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