Hippocampal maturity promotes memory distinctiveness in childhood and adolescence

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Adaptive learning systems need to meet two complementary and partially conflicting goals: detecting regularities in the world versus remembering specific events. The hippocampus (HC) keeps a fine balance between computations that extract commonalities of incoming information (i.e., pattern completion) and computations that enable encoding of highly similar events into unique representations (i.e., pattern separation). Histological evidence from young rhesus monkeys suggests that HC development is characterized by the differential development of intrahippocampal subfields and associated networks. However, due to challenges in the in vivo investigation of such developmental organization, the ontogenetic timing of HC subfield maturation remains controversial. Delineating its course is important, as it directly influences the fine balance between pattern separation and pattern completion operations and, thus, developmental changes in learning and memory. Here, we relate in vivo, high-resolution structural magnetic resonance imaging data of HC subfields to behavioral memory performance in children aged 6–14 y and in young adults. We identify a multivariate profile of age-related differences in intrahippocampal structures and show that HC maturity as captured by this pattern is associated with age differences in the differential encoding of unique memory representations.

hippocampal subfields | episodic memory | specificity | pattern separation | child development

Many years ago, the Swiss developmentalist Jean Piaget noted an imbalance between assimilation and accommodation during early and middle childhood in the sense that children tend to extract schematic knowledge at the expense of learning and recollecting specific events (1, 2). This imbalance has resurfaced in computational models of memory (3), and later as the imbalance between pattern completion and pattern separation, processes linked to computational properties of subfields within the hippocampus (HC) (4–6). Understanding the developmental organization of HC subfields is therefore crucial to understand how associated changes in HC-subfield computations drive concomitant changes in learning and memory.

An important step toward unraveling controversies about human hippocampal maturation (7, 8) is to acknowledge that the HC is not a homogeneous structure, but rather is composed of cytoarchitectonically and functionally distinct subfields (9). The availability of high-resolution, in vivo magnetic resonance imaging (MRI) of the HC permits the study of specific contributions of different HC subfields in humans (10–12). Computational and rodent models of HC function and high-resolution MRI studies in humans have sought to establish the contributions of individual HC subfields to specific mnemonic functions. For example, the dentate gyrus (DG) has been closely linked to pattern separation (6). Developmental findings from animal models (13) and initial evidence from human studies (14) suggest that the DG matures later than other HC subfields. Likewise, memory functions associated with pattern separation, such as recollection (6), show a protracted course of development that extends well into middle childhood (15). Thus, the DG is a candidate region of interest (ROI) for investigating developmental associations between HC and pattern separation.

However, a sole focus on DG is not warranted. Hippocampal subfields are intricately interconnected (13, 16), and their independent demarcation on MRI images remains imperfect (17). Moreover, extant high-resolution MRI studies in human samples are inconsistent in assigning specific HC computations to specific subfields. For instance, pattern separation has been linked not only to DG, but also to the adjacent area 3 of Cornu Ammonis (CA3; e.g., refs. 10 and 18), entorhinal cortex (EC; ref. 19) and in some cases, data have suggested links to the subiculum (Sub; ref. 14). Thus, it appears oversimplified to assign computations, such as pattern separation and completion, to specific parts of the HC in a one-to-one manner (6). Rather, the HC network may be relatively biased toward more pattern separation or more pattern completion, reflecting differential contributions of its constituent parts (6, 10). In a similar vein, based on domain-specific pattern separation signals within the human EC, Reagh and Yassa (19) suggested a conceptual model of interference resolution in the medial temporal lobe (MTL), whereby incremental decrease in representational overlap is reached by pattern separation in domain-specific, parallel pathways already upstream of DG, including EC.

In sum, there is a clear tension between the desire to link structure and function at the level of specific subfields to behavior and the presence of massive connections and interactions within the HC network. Moreover, existing animal and human data suggest that all HC subfields undergo maturational changes during early development (13, 14, 20), albeit along different trajectories. Both of these observations call for a multivariate approach to investigate HC

Significance

Children tend to extract schematic knowledge at the expense of learning and recollecting specific events. Our findings allow us to speculate that the heterogeneous development of subregions within the hippocampus—a brain region crucial for laying down novel memories—contributes to this developmental lag in memory. Specifically, we used in vivo high-resolution structural MRI and memory tests in a large sample of children aged 6–14 years and young adults to characterize hippocampal development. We show that hippocampal maturity as expressed in the multivariate pattern of age-related differences in hippocampal subregions is specifically related to the ability to lay down highly specific memories.

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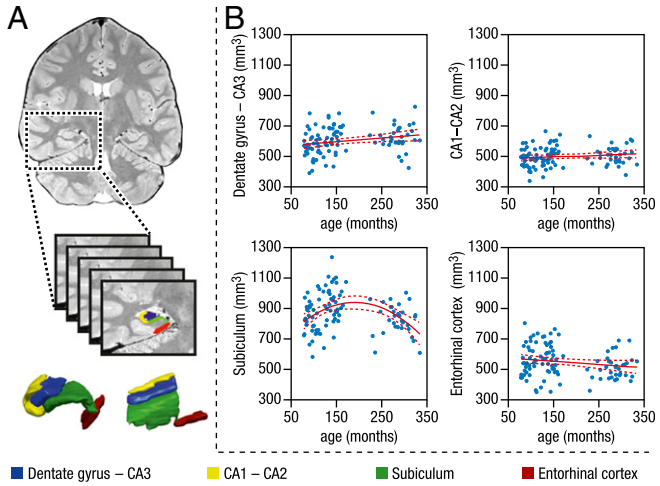

Fig. 1. Age-related differences in hippocampal structures suggest differential maturational trends that extend well into middle childhood and beyond. (*A*) Three ROIs, limited to HC body, comprising the CA regions 1 and 2, CA3 and DG, and Sub, and one ROI comprising EC were manually demarcated on MR slices to obtain volumetric measures for HC subfields (*Methods* and *SI Methods*). (*B*) Linear regression models fitted on the relationship of ROI volumes with age reveal a complex pattern with differential and often nonlinear maturational tracks in the different ROIs. Including a quadratic term improved fit for DG-CA3 and Sub. Confidence bounds capture 95% confidence intervals.

subfield–behavior associations during development (see ref. 21 for a similar approach on studying brain maturation).

Here, we investigated the pattern of HC maturation and its relation to pattern separation and completion in children and young adults. We acquired high-resolution MRI scans of the MTL in 70 children (aged 6–14 y) and 33 young adults (aged 18–27 y) and determined volumes of HC subfields from manually delineated ROI. Following common use (16), we use the term hippocampus as a shorthand notation for the hippocampal formation including the EC, a crucial input-output hub of the HC (16). In addition to our goal of exploring potential associations between EC and memory development (22), the inclusion of EC in our analyses was also justified by human fMRI data supporting its role in pattern separation (19) and animal data, suggesting that lateral EC development may follow that of DG (20).

We used multivariate statistical techniques to estimate individual maturation profiles of HC anatomy and examined the association between HC maturity and different behavioral measures previously associated with HC. The behavioral measures included a bias score of pattern separation versus pattern completion as the primary target of investigation. In addition, we also included indicators of age-sensitive mnemonic processes such as source memory, associative memory, and item memory that also rely on extra-hippocampal areas (21, 23–26). The inclusion of the latter measures was exploratory and served the purpose of probing the specificity of a potential association between pattern separation/completion and HC subfield maturation. For instance, previous studies reported age-related differences in functional and structural HC contributions to source memory (27, 28) and associative inference (29) along the longitudinal HC axis. However, the relationship between age differences in source memory and HC subfield development remains elusive.

We expected to replicate initial evidence for the relatively late maturation of the DG (14). In addition, we reasoned that individual differences in a multivariate index of HC maturity would predict individual differences in processes that support the specific encoding of unique events such as pattern separation. Furthermore, we expected that this index of HC maturity would be only weakly associated with, or unrelated to, memory processes that rely on early maturing aspects of the HC, such as familiarity, or late-maturing memory processes that are less exclusively HC-dependent and also heavily dependent on extra-hippocampal areas, such as source memory (23).

Results and Discussion

Age-Related Differences in Hippocampal Structures Suggest Differential Development That Extends Well into Middle Childhood and Beyond. In an initial set of analyses, we examined whether individual HC subregions (*Methods*) show evidence for maturation across childhood. We identified age-related neuroanatomical differences between 6 and 27 y of age by regressing ROI volumes on age (Fig. 1*B*). In the total sample, only the ROI including the DG and the CA3 (DG-CA3) subfields showed a significant linear age trend ($R^2_{\text{adjusted}} = 0.05$, $p_\beta = 0.023$). Adding a quadratic term revealed a significant age trend in Sub ($R^2_{\text{adjusted}} = 0.14$, $p_{\beta\ \text{linear}} < 0.001$, $p_{\beta\ \text{quadratic}} < 0.001$). When the same analyses were restricted to children, we found significant linear age trends for DG-CA3 ($R^2_{\text{adjusted}} = 0.11$, $p_\beta = 0.005$), Sub ($R^2_{\text{adjusted}} = 0.17$, $p_\beta < 0.001$), as well as CA1-2 ($R^2_{\text{adjusted}} = 0.08$, $p_\beta = 0.02$). F tests on ΔR^2 values showed that adding quadratic terms did not result in significant increments in explained variance.

These univariate results suggest a protracted development of HC subfields, including the CA1-2, DG-CA3, and the Sub, until late middle childhood and early adolescence. In addition, they also show age-related changes in DG-CA3 volume until young adulthood and an onset of volume decrements in Sub around adolescence. The results are in partial agreement with one earlier study by Lee et al. (14) who investigated age differences in HC subfields in four arbitrarily defined age groups in a sample of 8- to 14-y-old children and observed significant quadratic age trends in DG-CA3 and CA1-2. Differences in results may reflect subtle variations in tracing protocols between the two studies [see *SI Methods* in relation to the delineation of CA1-2 and Sub (cf. ref. 17)], the grouping of the individuals into age groups by Lee et al., or both. Despite these differences, the two studies provide converging evidence for a protracted maturation of HC subfields through middle childhood. This pattern of maturation is followed by later volumetric reductions that may extend into young adulthood, at least for the DG-CA3.

A Specific Multivariate Profile of HC Substructures Is Associated with Age. The HC subfields form a highly interconnected hard-wired processing circuitry (16). Therefore, if maturation would only affect a single substructure (with no effect on others), it would

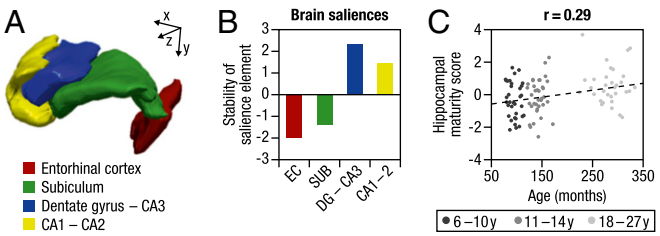

Fig. 2. A specific multivariate profile of HC subfields is associated with age. (*A*) Example ROIs defined by manual tracings. (*B*) Latent variable weights (brain saliences) for each ROI used to transform original volumetric data of each participant into one latent variable expressing the largest amount of information common to the multivariate pattern of ROI volumes and age. *Z* score-like values of stability suggest a positive relationship between DG-CA3 and CA1-2 and age, and a negative relationship between EC and Sub and age, and also show that DG-CA3 and EC are the most stable elements of the weight vector. (*C*) The resulting latent variable, termed HC maturity score, plotted against age with least-squares line (dashed). The large overlap of HC-maturity scores between age groups (defined arbitrarily for illustrative purposes) underscores that chronological age only partly relates to differences in HC maturity.

most likely harm the fine balance between pattern separation and completion operations required for a flexible and adaptive memory system (6). Age-graded changes in the balance between pattern separation and completion are likely to result from a multivariate pattern of subfield changes. Therefore, we examined the age-graded link of HC-subfields maturity to different memory processes from a multivariate perspective. Using partial least squares correlation analysis (PLSC), we extracted a single composite score that captures individual differences in the structural maturity of HC subfields. For simplicity, we refer to this score as "HC-maturity score" (*Methods*). Our PLSC analysis identified a single reliable latent variable (LV; $P = 0.038$) that optimally represents the association between participants' age and ROI volumes ($r = 0.29$). Bootstrap ratios (BSR) indicated an age-associated increase of DG-CA3 (BSR = 2.3) volume, and a decrease of EC volume (BSR = –1.98) as the two stable components of the LV expressing the largest amount of information common to both age and the multivariate pattern of ROI volumes (Fig. 2).

Several previous reports (8, 30, 31) have suggested that the HC and associated memory functions reach maturity by middle childhood. In contrast, other studies have provided evidence for prolonged maturation of HC-dependent memory process until adolescence (7). In our view, these apparent contradictions can be overcome by acknowledging the heterogeneous course of HC maturation (e.g., refs. 13, 14, and 32). In particular, in light of the subfields' different maturational trajectories, HC subfield data can yield a more fine-grained picture of age-graded volumetric differences than total HC volume can. With a whole HC analysis run on standard resolution MRI data, subtle maturational effects detectable by high-resolution MRI derived subfield data may go unnoticed. To check this claim empirically, we ran two additional analyses. First, to mimic whole HC analyses, we aggregated DG-CA3, CA1-2, Sub volumes, and computed an analogous PLSC with age, whole HC, and EC. In contrast to the original analysis based on subfield volumes, the analysis with age, whole HC, and EC failed to extract a significant and generalizable latent variable ($P = 0.17$). Second, a voxel-based morphometry (VBM) analysis revealed widespread age-related differences in gray matter volume (GM) over the cortex, but not in the MTL (see Fig. S2 for results and description of the VBM methods). In sum, previous research may have failed to find age-related differences in HC during middle childhood because their total HC target measure collapsed regions with heterogeneous maturational trajectories and/or did not include EC.

Multidimensional Structural Maturity of the Hippocampal Formation Is Associated with Memory Processes Enabling the Unique Encoding of Similar Representations. Next, we assessed the association between individuals' HC-maturity scores and memory performance. Memory development across childhood is characterized by an overall improvement of mnemonic functions (21, 24). Nevertheless, the developmental timing and interdependence of different mnemonic operations remains controversial (33–36), especially in relation to their dependence on HC maturity (7 vs. 8). Therefore, in the present study, we comprehensively assessed memory processes potentially associated with HC maturity: pattern separation/completion, source memory, item, and associative memory.

A mnemonic similarity task adapted from (10, 37) was used to behaviorally assess pattern separation versus pattern completion bias (Fig. 3*A* and *SI Methods*). From this task, we computed a pattern separation/completion bias index that expresses the degree to which mnemonic similarity judgments are biased toward pattern separation (or against pattern completion) (10, 37). Several studies have corroborated the suggestion that this index is a reasonable estimate of the relative strength of HC pattern separation (37–39). Using two alternating contexts during learning blocks of the same task, we also assessed source memory. A second task adapted from

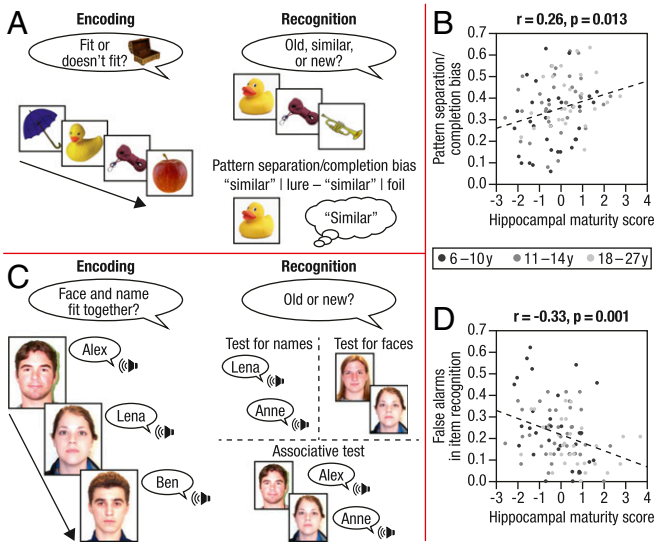

Fig. 3. Multidimensional structural maturity of the hippocampus is associated with memory processes enabling the unique encoding of similar representations. (*A*) Mnemonic similarity task used to assess pattern separation/completion bias. After incidentally encoding pictures of everyday objects, in a recognition task, participants saw the same target pictures intermixed with highly similar lures and novel foils. Their task was to identify image types by responding "old," "similar," or "new." Pattern separation/completion bias was calculated by subtracting the proportion of similar responses to foils from the proportion of similar responses to lures. The resulting score weighs the tendency to encode two highly similar inputs into separate mnemonic representations against the tendency to assimilate the incoming information to already existing mnemonic representations. Trials that were responded either similar or old were followed by a source memory decision trial (not depicted; see *SI Methods* for details of material, design, and procedure). (*B*) Increasing HC maturity is associated with a shift in bias toward pattern separation. (*C*) The faces-and-names task used to assess item and associative memory. After incidentally encoding face-name pairs, participants performed an old/new recognition task composed of three tests administered in a counterbalanced order. Performance on the two item tests was merged to provide one item memory score. Hits and false alarms were calculated for both item and associative memory (see *SI Methods* for details). (*D*) Increasing HC maturity is related to a decrease in false item recognition. (*B* and *D*) Dashed lines represent least-square lines. Different shades of gray represent different age segments to illustrate that the HC maturity–behavior associations hold across age.

Naveh-Benjamin et al. (40) was used to assess item and associative memory (Fig. 3*C* and *SI Methods*).

To assess the dependence of each memory process on hippocampal maturity, we ran bivariate correlation analyses between the PLSC-derived HC maturity scores and the behavioral indicators. As predicted, the pattern separation/completion bias score correlated positively with the HC maturity score ($r = 0.26$, $P = 0.013$), revealing a moderate shift toward pattern separation with increasing HC maturity (Fig. 3*B*). In addition, false recognition of item memories (*Methods* and Fig. 3*C*) showed a significant negative association (Fig. 3*D*), $r = –0.33$, $P < 0.001$, with HC maturity. No other memory measure revealed significant associations with HC-maturity scores (Table S1). Importantly, the strength of correlations between HC maturity and pattern separation/completion and between HC maturity and false recognition of item memories were significantly stronger than the nonsignificant correlations between HC maturity and the other memory measures [indicated by a significant contrast among correlated correlation coefficients (41), $z = 2.67$, $P = 0.004$].

Both mnemonic similarity judgments and the rejection of foils in a recognition memory task involving highly similar items crucially depend on the orthogonalization of overlapping feature sets in representational space. Therefore, our results suggest that the multidimensional maturity of structures in the HC is specifically related to processes that enable the construction of unique

mnemonic representations of highly overlapping feature sets during memory encoding.

Conversely, the present results also suggest that age-associated differences in item memory, source memory, and associative memory performance (Fig. S1) depend less on HC maturity than the age-associated changes in the disambiguation of highly similar events. Clearly, performance on item memory, source memory, and associative memory relates to hippocampal functioning (42). However, the demand characteristics of these tasks, under most conditions at least, presumably depend less on pattern separation than the demand characteristics of making mnemonic similarity judgments and rejecting highly similar foils. In addition, source memory and associative memory are likely to require prefrontally mediated control processes such as monitoring during source memory decisions (23) and inhibition of combinations of familiar items (25) during old/new decisions in an associative memory task (26). This enhanced prefrontal dependence may inject additional age-related variance into task performance, which may weaken or mask potential associations with HC maturity. To test these assumptions, we applied the PLSC approach used to construct the HC-maturity score to also establish a maturity score for frontal ROIs based on GM measures obtained from our VBM analyses (see Fig. 4 for results, and see *Methods* and Fig. S2 for methods). In support of our considerations, the frontal maturity score correlated significantly with source memory ($r = 0.26$, $P = 0.009$) but not with any other memory measure except for hits in item memory ($r = 0.40$, $P < 0.001$; Table S1). The strength of the two significant correlations significantly differed from the strength of the nonsignificant ones ($z = 2.95$, $P = 0.002$).

General Discussion

Using multivariate correlational techniques on high-resolution structural MRI data of the MTL in a sample of 6- to 27-y-old individuals, we identified a multivariate profile of developmental differences in HC substructures that expresses the structural maturity of the HC. We then showed that HC maturity is specifically related to the development of memory processes promoting the unique encoding of overlapping memory representations. Our results suggest that key contributors of this specific connection between HC maturity and memory are age-associated changes in the DG-CA3 and the EC. HC maturity scores did not reveal a robust association with any of the other memory measures, although these measures also showed age-associated improvements (Fig. S1). Compared with the mnemonic similarity task, our additional recognition measures (item and associative recognition memory and source memory) apparently were less sensitive to shifts in pattern separation/completion bias. The mnemonic similarity task has been specifically designed to assess this bias on a continuous scale between separation and completion (37), whereas performance on the other memory measures may more heavily depend on extrahippocampal areas not incorporated in our HC-maturity score (23, 25, 26).

Our observation that the association between HC maturity and memory is restricted to age-related increases in specificity may reflect one or both of the following underlying processes. First, the development of memory processes that require less specificity with regard to unique feature combinations may depend more strongly on age-related changes in extrahippocampal areas. As discussed above, maturation of prefrontal cortex can, in part, drive improvements in both associative recognition memory and source memory (23, 25, 26), possibly moderated by increases in demands on strategic processes rather than associative memory operations (34). This conjecture is in part supported by our analyses showing that maturation of frontal areas was significantly correlated with source memory, but not with pattern separation/completion bias. However, we should note that, based on standard-resolution MRI, some studies have found age differences in the functional division along the longitudinal axis of the HC (27, 28) that may also contribute to age differences in source memory ability (43). Second, pattern completion may be relatively mature by middle childhood despite ongoing structural changes in HC, whereas computations underlying specificity are still developing, thus promoting the observed age-graded shift in bias from pattern completion to pattern separation.

Our results complement earlier findings (14) demonstrating age-associated differences in HC subfields in middle childhood and extend those observations to a large sample of children aged 6 to 14 y. In addition, we provide an initial picture of HC subfield development in middle childhood. This picture highlights the presence of subfield-specific, heterogeneous maturational tracks. By demonstrating that estimates of whole HC volumes failed to detect HC-age associations in our sample, the results of the present study also help to resolve conflicting observations, with some studies suggesting that HC maturation levels off early in middle childhood (8, 13, 31, 34) and others suggesting that HC maturation extends well into, and possibly beyond, this period (7). Previously available standard resolution MRI techniques may not be sensitive enough to reveal extended HC maturation.

Our study revealed effects that complement earlier studies linking the DG and CA3 to pattern separation (6, 10, 18). Beyond the crucial role of the DG-CA3 region for providing separable inputs to downstream HC subfields, the development of memory specificity appears associated with a common maturational process that potentially affects all HC subfields to varying degrees. Our finding that EC development is a key component of HC maturity associated to pattern separation fits nicely with observations in animals that layer 2 and 5 of the lateral EC follows DG development (20), and with human data suggesting that lateral EC may perform pattern separation on overlapping object representations before passing its input onto the DG (19). It is worth noting that EC by itself did not show significant age-related differences in the present sample. The contribution of EC to HC maturity was revealed only when applying a multivariate approach that expresses the common variance between individual differences in HC subfield volumes and age. Methodologically, our approach follows the longstanding claim to conceptualize and analyze developmental change from a multivariate perspective (44). Earlier work has shown that multivariate composites of individual differences in

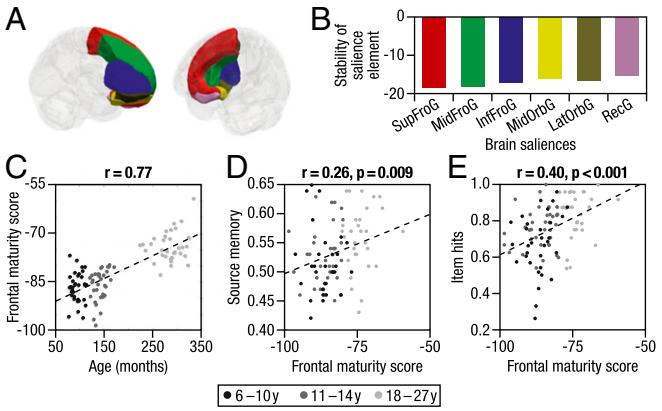

Fig. 4. (*A*) Six frontal ROIs defined by the lpba40 atlas (54). SupFroG, MidFroG, InfFroG: Superior, Middle, and Inferior frontal gyrus; MidOrbG, LatOrbG: Middle and Lateral orbitofrontal gyrus, RecG: gyrus rectus. (*B*) Latent variable weights (brain saliences) for each ROI used to transform individual GM volumetric estimates, extracted using VBM, into one latent variable expressing the largest amount of information common to the multivariate pattern of GM and age. *Z* score-like values of stability suggest a negative relationship between all ROIs and age. (*C*) The resulting latent variable, termed frontal maturity score, plotted against age. Increasing frontal maturity related to an increase in source memory accuracy (*D*) and increase in correct item recognition (*E*). (*C–E*) Dashed lines represent least-square lines.

brain anatomy can serve as a summary description of biological maturity (45). The dimensionality reduction associated with these methods helps to test and refine theories of age-graded changes in brain–behavior relations.

The present study has several limitations, which can guide future research in the field. Given that development is a process unfolding in ontogenetic time, repeated within-subjects assessments are needed to directly capture longitudinal relationships between neural and behavioral variables of interest (46). For this reason, we refrained from using hierarchical linear regression models with age as independent variable, memory processes as dependent variables, and HC subfields as mediator variables. It has been shown analytically that these methods may fail to detect longitudinal mediation when it is present (false negatives) and detect mediation when it is absent (false positives) (47; see also ref. 48). A second limitation is related to the bias score used in this study, which pits pattern separation against pattern completion. Future studies need to obtain measures that separately index age differences in the efficiency of pattern separation and pattern completion mechanisms. The restriction of our analyses to HC body is a third limitation. Previous studies found both structural and functional age-related differences in source memory contributions of the HC head and tail, but not the body (27, 28). Investigating subfield contributions along the full anatomical extent of HC could therefore refine our understanding of how HC subfield and memory development are related (see ref. 29; however, this study also highlights the controversies regarding methods for identifying HC subfield in the head and tail, see ref. 49). Fourth, recent fMRI findings suggest that pattern separation may not be restricted to the HC (50, 51). In the present study, we selected a task that aims at studying age differences in pattern separation performed by the HC, but one that is not well-suited for examining pattern separation, and age differences therein, in other brain areas, such as visual cortex. Future studies need to address the maturational course of pattern separation in other brain areas and their contributions to behavioral development. Last, we devised this study to test the suggestion that HC and related mnemonic functions may develop beyond the onset of middle childhood, but had no a priori reason to postulate that this development may continue beyond middle childhood. Therefore, we did not include individuals aged 15–18 y in the present sample. Also, we did not include children below 6 y of age, reflecting practical limitations when conducting MRI studies with young children. Our results should encourage future research to explore HC subfields and related mnemonic development in a more extended age range.

We found that age-related shifts from pattern completion toward pattern separation are associated with maturational changes in HC subfields. If corroborated by longitudinal evidence from tasks directly measuring some form of knowledge extraction from invariances (i.e., category learning), this result has fundamental implications for theories of episodic memory development: It leads us to speculate that the extraction of invariance across a range of different experiences may precede the encoding, consolidation, and retrieval of detail for reasons that are rooted, at least in part, in the uneven maturational course of substructures within the HC. Returning to Piaget (1, 2), we conclude that this décalage, or developmental lag may be developmentally advantageous, as it helps children to recognize regularities, form stable representations of recurring episodes, predict the structure of future events, and build semantic knowledge.

Methods

Participants. Seventy children (35 girls; age range: 6–14 y; M = 9.80, SD = 2.39 y), and 33 young adults (18 women; age range: 18–27 y; M = 23.21, SD = 2.5 y) participated in the study. Participants provided written informed consent, also signed by the primary caregiver for all children. Participants were right-handed and had no history of neurological or psychiatric disorders. They completed the study in two sessions lasting 2 h each and were paid 40 €. Behavioral data were not available for one child and one young adult because of technical issues. The Ethics Committee of the German Psychological Society (Deutsche Gesellschaft für Psychologie) approved the study.

Delineating ROIs in the MTL. Four ROIs were manually demarcated bilaterally (Fig. 1*A*) by two expert tracers on coronal slices of the high-resolution structural MR volume (voxel size: 0.4 mm × 0.4 mm × 2 mm). The segmentation protocol included three ROIs (Sub, CA1-2, and DG-CA3) segmented along the full range of the HC body, excluding head and tail, and the EC segmented in six slices anterior to the HC body. Bilateral ROIs were collapsed across hemisphere and adjusted for intracranial volume (ICV) for all following analyses (*SI Methods*).

Assessing the Multivariate Relationship Between ROI Volumes and Age Using PLSC. We chose to use PLSC (52, 53) to assess HC maturation on conceptual and methodological grounds. First, deciphering the role of various subregions in memory has proven difficult because the HC forms a hard-wired interconnected processing circuit of interdependent nodes. Second, from a developmental perspective, chronological age is only a proximate index of any assumed "latent maturational process." Therefore, capturing a maturational process by sampling from participants of different ages is an oversimplified process. By extracting a latent HC subfield profile that maximally shares common variance with age, we aimed at increasing precision for sampling from a latent maturational process. Third, while univariate analyses can capture age-related differences in subregions separately, they ignore intercorrelated patterns of developmental processes affecting the different subparts in a concerted fashion.

Here, PLSC starts by calculating a between-subject correlation matrix (CORR) between (*i*) an *n*-element vector containing AGE (in month) and (*ii*) a *n*×4-matrix of volumetric measures for each HC ROI. CORR is then decomposed by using singular value decomposition (SVD). SVD $_{CORR}$ (AGE, ROI) = USV′. This decomposition produces a left singular vector of AGE weights (U), a right singular vector of ROI weights (V), and a diagonal matrix of singular values (S). A single estimable latent variable (LV) results that optimally (in a least-squares sense) represents the associations between AGE and ROI volumes. This LV contains a profile depicting the ROIs that show the strongest relation to AGE. Significance of the detected association was assessed by using 5,000 permutation tests of the singular value corresponding to the LV. A subsequent bootstrapping procedure revealed the robustness of within-LV ROI weights across 5,000 bootstrapped resamples of the data. By dividing each ROI's weight (from V) by its bootstrapped SE, we obtained BSRs as normalized estimates of robustness (Fig. 2*B*). BSRs are comparable to conventional *z* values, where a value larger/smaller than ±1.96 is treated as reliably robust. We also obtained a summary measure of each participant's robust expression of the estimated LV's profile, a within-person HC-maturity score, by multiplying the model-based vector of ROI weights (V) by each subject's vector of ROI volume estimates (Q), producing a single within-subject value, the HC-maturity score = VQ (Figs. 2*C* and 3 *B* and *D*).

A comparable procedure was used to derive "frontal maturity score" for control analyses. Instead of volumetric measures of HC subfields, we used VBM derived gray matter volume (GM) estimates in six frontal ROIs of the lpba40 Atlas (54). Results of this analysis are presented in Fig. 4. Whole-brain PLSC analyses, probing the associations between GM and age are reported in Fig. S1.

Voxel-Based Morphometry. We used the standard preprocessing pipeline of the CAT12 toolbox (dbm.neuro.uni-jena.de/cat) run in SPM12 (Wellcome Trust Centre for Neuroimaging, www.fil.ion.ucl.ac.uk/spm) (version 6906) to obtain voxelwise and ROI specific GM estimates (see Fig. S2 for details). For ROI analyses (Fig. 4), GM estimates were collapsed across hemispheres, and ICV corrected using the same approach as for our HC analysis (*SI Methods*).

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1. Chapman M (1988) *Constructive Evolution: Origins and Development of Piaget's thought* (Cambridge Univ Press, New York).
2. Piaget J, Grize JB, Szeminska A, Bang V (1977) *Epistemology and Psychology of Functions* (Reidel, Dordrecht, The Netherlands).
3. McClelland JL, McNaughton BL, O'Reilly RC (1995) Why there are complementary learning systems in the hippocampus and neocortex: Insights from the successes and failures of connectionist models of learning and memory. *Psychol Rev* 102:419–457.
4. Kumaran D, McClelland JL (2012) Generalization through the recurrent interaction of episodic memories: A model of the hippocampal system. *Psychol Rev* 119:573–616.
5. Oehrn CR, et al. (2015) Human hippocampal dynamics during response conflict. *Curr Biol* 25:2307–2313.
6. Yassa MA, Stark CE (2011) Pattern separation in the hippocampus. *Trends Neurosci* 34: 515–525.
7. Ghetti S, DeMaster DM, Yonelinas AP, Bunge SA (2010) Developmental differences in medial temporal lobe function during memory encoding. *J Neurosci* 30:9548–9556.
8. Ofen N, Chai XJ, Schuil KD, Whitfield-Gabrieli S, Gabrieli JD (2012) The development of brain systems associated with successful memory retrieval of scenes. *J Neurosci* 32: 10012–10020.
9. Duvernoy H, Cattin F, Risold P-Y (2013) *The Human Hippocampus* (Springer, Berlin).
10. Bakker A, Kirwan CB, Miller M, Stark CEL (2008) Pattern separation in the human hippocampal CA3 and dentate gyrus. *Science* 319:1640–1642.
11. Carr VA, Viskontas IV, Engel SA, Knowlton BJ (2010) Neural activity in the hippocampus and perirhinal cortex during encoding is associated with the durability of episodic memory. *J Cogn Neurosci* 22:2652–2662.
12. Eldridge LL, Engel SA, Zeineh MM, Bookheimer SY, Knowlton BJ (2005) A dissociation of encoding and retrieval processes in the human hippocampus. *J Neurosci* 25:3280–3286.
13. Lavenex P, Banta Lavenex P (2013) Building hippocampal circuits to learn and remember: Insights into the development of human memory. *Behav Brain Res* 254:8–21.
14. Lee JK, Ekstrom AD, Ghetti S (2014) Volume of hippocampal subfields and episodic memory in childhood and adolescence. *Neuroimage* 94:162–171.
15. Friedman D, de Chastelaine M, Nessler D, Malcolm B (2010) Changes in familiarity and recollection across the lifespan: An ERP perspective. *Brain Res* 1310:124–141.
16. Amaral D, Lavenex P (2007) Hippocampal neuroanatomy. *The Hippocampus Book*, eds Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J (Oxford Univ Press, New York), pp 37–114.
17. Yushkevich PA, et al.; Hippocampal Subfields Group (HSG) (2015) Quantitative comparison of 21 protocols for labeling hippocampal subfields and parahippocampal subregions in in vivo MRI: Towards a harmonized segmentation protocol. *Neuroimage* 111: 526–541.
18. Lacy JW, Yassa MA, Stark SM, Muftuler LT, Stark CEL (2010) Distinct pattern separation related transfer functions in human CA3/dentate and CA1 revealed using high-resolution fMRI and variable mnemonic similarity. *Learn Mem* 18:15–18.
19. Reagh ZM, Yassa MA (2014) Object and spatial mnemonic interference differentially engage lateral and medial entorhinal cortex in humans. *Proc Natl Acad Sci USA* 111: E4264–E4273.
20. Donato F, Jacobsen RI, Moser M-B, Moser EI (2017) Stellate cells drive maturation of the entorhinal-hippocampal circuit. *Science* 355:eaai8178.
21. Riggins T (2012) Building blocks of recollection. *Origins and Development of Recollection: Perspectives from Psychology and Neuroscience*, eds Ghetti S, Bauer PJ (Oxford Univ Press, New York), pp 42–72.
22. Daugherty AM, Bender AR, Yuan P, Raz N (2016) Changes in search path complexity and length during learning of a virtual water maze: Age differences and differential associations with hippocampal subfield volumes. *Cereb Cortex* 26:2391–2401.
23. Mitchell KJ, Johnson MK (2009) Source monitoring 15 years later: What have we learned from fMRI about the neural mechanisms of source memory? *Psychol Bull* 135: 638–677.
24. Ghetti S, Lyons KE, DeMaster DM (2012) The development of episodic memory: Binding processes, controlled processes, and introspection on memory states. *Origins and Development of Recollection: Perspectives from Psychology and Neuroscience*, eds Ghetti S, Bauer PJ (Oxford University Press, New York), pp 144–167.
25. Achim AM, Lepage M (2005) Dorsolateral prefrontal cortex involvement in memory post-retrieval monitoring revealed in both item and associative recognition tests. *Neuroimage* 24:1113–1121.
26. Spaniol J, et al. (2009) Event-related fMRI studies of episodic encoding and retrieval: Meta-analyses using activation likelihood estimation. *Neuropsychologia* 47:1765–1779.
27. Sastre M, 3rd, Wendelken C, Lee JK, Bunge SA, Ghetti S (2016) Age- and performance-related differences in hippocampal contributions to episodic retrieval. *Dev Cogn Neurosci* 19:42–50.
28. DeMaster D, Pathman T, Lee JK, Ghetti S (2014) Structural development of the hippocampus and episodic memory: Developmental differences along the anterior/posterior axis. *Cereb Cortex* 24:3036–3045.
29. Schlichting ML, Guarino KF, Schapiro AC, Turk-Browne NB, Preston AR (2017) Hippocampal structure predicts statistical learning and associative inference abilities during development. *J Cogn Neurosci* 29:37–51.
30. Ofen N, et al. (2007) Development of the declarative memory system in the human brain. *Nat Neurosci* 10:1198–1205.
31. Shing YL, Brehmer Y, Heekeren HR, Bäckman L, Lindenberger U (2016) Neural activation patterns of successful episodic encoding: Reorganization during childhood, maintenance in old age. *Dev Cogn Neurosci* 20:59–69.
32. Daugherty AM, Bender AR, Raz N, Ofen N (2016) Age differences in hippocampal subfield volumes from childhood to late adulthood. *Hippocampus* 26:220–228.
33. Reyna VF, Brainerd CJ (1995) Fuzzy-trace theory: An interim synthesis. *Learn Individ Differ* 7:1–75.
34. Shing YL, et al. (2010) Episodic memory across the lifespan: The contributions of associative and strategic components. *Neurosci Biobehav Rev* 34:1080–1091.
35. Squire LR, Zola SM (1998) Episodic memory, semantic memory, and amnesia. *Hippocampus* 8:205–211.
36. Tulving E, Markowitsch HJ (1998) Episodic and declarative memory: Role of the hippocampus. *Hippocampus* 8:198–204.
37. Stark SM, Yassa MA, Lacy JW, Stark CEL (2013) A task to assess behavioral pattern separation (BPS) in humans: Data from healthy aging and mild cognitive impairment. *Neuropsychologia* 51:2442–2449.
38. Yassa MA, et al. (2011) Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults. *Hippocampus* 21:968–979.
39. Das T, Ivleva EI, Wagner AD, Stark CEL, Tamminga CA (2014) Loss of pattern separation performance in schizophrenia suggests dentate gyrus dysfunction. *Schizophr Res* 159:193–197.
40. Naveh-Benjamin M, et al. (2009) Adult age differences in memory for name-face associations: The effects of intentional and incidental learning. *Memory* 17:220–232.
41. Meng X-L, Rosenthal R, Rubin DB (1992) Comparing correlated correlation coefficients. *Psychol Bull* 111:172–175.
42. Squire LR, Wixted JT (2011) The cognitive neuroscience of human memory since H.M. *Annu Rev Neurosci* 34:259–288.
43. Lee JK, Wendelken C, Bunge SA, Ghetti S (2016) A time and place for everything: Developmental differences in the building blocks of episodic memory. *Child Dev* 87: 194–210.
44. Baltes PB, Nesselroade JR (1973) The developmental analysis of individual differences on multiple measures. *Life-Span Developmental Psychology: Methodological Issues*, eds Nesselroade JR, Reese HW (Academic, Oxford), pp 219–251.
45. Brown TT, et al. (2012) Neuroanatomical assessment of biological maturity. *Curr Biol* 22:1693–1698.
46. Raz N, Lindenberger U (2011) Only time will tell: Cross-sectional studies offer no solution to the age-brain-cognition triangle: Comment on Salthouse (2011). *Psychol Bull* 137:790–795.
47. Lindenberger U, von Oertzen T, Ghisletta P, Hertzog C (2011) Cross-sectional age variance extraction: What's change got to do with it? *Psychol Aging* 26:34–47.
48. Maxwell SE, Cole DA (2007) Bias in cross-sectional analyses of longitudinal mediation. *Psychol Methods* 12:23–44.
49. Wisse LE, et al.; Hippocampal Subfields Group (2017) A harmonized segmentation protocol for hippocampal and parahippocampal subregions: Why do we need one and what are the key goals? *Hippocampus* 27:3–11.
50. Schlichting ML, Mumford JA, Preston AR (2015) Learning-related representational changes reveal dissociable integration and separation signatures in the hippocampus and prefrontal cortex. *Nat Commun* 6:8151.
51. Pidgeon LM, Morcom AM (2016) Cortical pattern separation and item-specific memory encoding. *Neuropsychologia* 85:256–271.
52. Krishnan A, Williams LJ, McIntosh AR, Abdi H (2011) Partial Least Squares (PLS) methods for neuroimaging: A tutorial and review. *Neuroimage* 56:455–475.
53. McIntosh AR, Bookstein FL, Haxby JV, Grady CL (1996) Spatial pattern analysis of functional brain images using partial least squares. *Neuroimage* 3:143–157.
54. Shattuck DW, et al. (2008) Construction of a 3D probabilistic atlas of human cortical structures. *Neuroimage* 39:1064–1080.
55. Jenkinson M, Bannister P, Brady M, Smith S (2002) Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* 17: 825–841.
56. Mueller SG, Schuff N, Raptentsetsang S, Elman J, Weiner MW (2008) Selective effect of Apo e4 on CA3 and dentate in normal aging and Alzheimer's disease using high resolution MRI at 4 T. *Neuroimage* 42:42–48.
57. Mueller SG, et al. (2007) Measurement of hippocampal subfields and age-related changes with high resolution MRI at 4T. *Neurobiol Aging* 28:719–726.
58. Shing YL, et al. (2011) Hippocampal subfield volumes: Age, vascular risk, and correlation with associative memory. *Front Aging Neurosci* 3:2.
59. Jack CR, Jr, et al. (1989) Anterior temporal lobes and hippocampal formations: Normative volumetric measurements from MR images in young adults. *Radiology* 172: 549–554.
60. Raz N, et al. (2005) Regional brain changes in aging healthy adults: General trends, individual differences and modifiers. *Cereb Cortex* 15:1676–1689.
61. Smith SM (2002) Fast robust automated brain extraction. *Hum Brain Mapp* 17:143–155.
62. Bender AR, Daugherty AM, Raz N (2013) Vascular risk moderates associations between hippocampal subfield volumes and memory. *J Cogn Neurosci* 25:1851–1862.
63. Konkle T, Brady TF, Alvarez GA, Oliva A (2010) Conceptual distinctiveness supports detailed visual long-term memory for real-world objects. *J Exp Psychol Gen* 139:558–578.