Archival Report

The Hippocampus in Depression: More Than the Sum of Its Parts? Advanced Hippocampal Substructure Segmentation in Depression

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ABSTRACT

BACKGROUND: Hippocampal volume reduction is the most replicated finding in neuroimaging studies of major depressive disorder (MDD). Varying hippocampal volume definition is a well-established problem in this field. Given that hippocampal function can be mapped onto anatomically defined substructures and that detailed examination of substructure volumes is now possible, we examined different hippocampal composite measures in MDD to look for hippocampal markers of MDD.

METHODS: Magnetic resonance imaging brain scans were compared between 80 patients with a range of MDD duration and 83 healthy control subjects. High-resolution T1-weighted and T2-weighted–fluid-attenuated inversion recovery magnetic resonance images were examined using the automated hippocampal substructure module in FreeSurfer 6.0. Between-group volumetric assessments were performed at substructure and composite substructures levels.

RESULTS: Patients with MDD showed a bilateral pattern of volume reduction in principal hippocampal substructures: the cornu ammonis (CA1–CA4), dentate gyrus, and subiculum. Changes were more pronounced on the left of these structures and in recurrent depression. CA2 to CA4 were the only substructures reduced in first-presentation depression. Overall changes were most marked in the left CA1, and CA1 volume was a predictor of illness duration.

CONCLUSIONS: Hippocampal involvement in MDD is confined to principal substructures only. Differences between patients with MDD and healthy control subjects increased with progressively restricted hippocampal definitions, with the left CA1 emerging as a potential marker of MDD. Changes were more extensive in patients with recurrent, as opposed to first-presentation, MDD, suggesting a hippocampal disease process. These findings identify core hippocampal regions in the pathology of MDD, suggesting a potential marker of disease progression in MDD.

Keywords: Depression, First-presentation, FreeSurfer, Hippocampus, Recurrent, Substructure

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Dysfunction in the hippocampus, a key hub within the limbic system, is proposed in the neuropathology of major depressive disorder (MDD) (1). Various meta-analyses examining magnetic resonance imaging (MRI) of the hippocampus, solely (2–4) or as part of a greater limbic system analysis (5–7), have found volume reductions of 4% to 10% in depression. Voxel-based morphometry also demonstrates hippocampal gray matter volume loss in depression (8–10). Although changes have been found in first-episode patients, most evidence indicates that volume reduction is associated with chronicity of depressive illness and multiple episodes (4,11,12). A minority of studies show no hippocampal volume differences in depression (6,13–16). However, global hippocampal volumetric change needs to be interpreted with caution because of the multifaceted nature of the relationship between volumetric changes and MDD (17,18). Hippocampal neuroimaging studies are conducted on heterogenous groups of persons with MDD with different grades of severity and subtypes of depression, with inconsistent states of relapse and/or remission, and with different treatments—all of which may be reflected in different changes in the hippocampus.

Further variability may occur because the hippocampus consists of related deep substructures that combine organizationally to form the traditional functional unit (19) (Figure 1 and Supplemental Figure S1). Definitions of what constitutes the hippocampus often lack consensus among researchers (20) and may compromise interstudy comparison. In a database of 423 hippocampal MRI studies, approximately 60 different anatomical guidelines were used (21). Classically, the hippocampus proper consists of the allocortical cornu ammonis subfields 1 to 4 (CA1–CA4) (22). This core hippocampal circuit operates within the hippocampal formation that also includes the subiculum inferiorly and the dentate gyrus medially (23). Broader definitions again may include anatomically adjacent areas such as the presubiculum and parasubiculum, functionally connected areas such as the entorhinal

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and parahippocampal cortices, and accessory white matter areas such as the fimbriae and fornix (22). Substructure changes may influence the total hippocampal volume, and subfield variations may be obscured when the hippocampus is examined as a whole. Some specific substructural changes have been reported. Dentate gyrus and CA1 to CA3 volume reductions have been found in nonmedicated, but not in medicated, patients with depression (24). CA3 volume reduction has been associated with depression in persons with Parkinson's disease (25). In one study, the number of episodes of depression was found to affect dentate gyrus (26) and subiculum volumes (27). Reductions in the CA3, dentate gyrus, and subiculum on the left side have been found in a study focusing on female persons with depression (28). A recent study, however, failed to find significant substructure changes in persons with MDD compared with substructures in persons with bipolar disorder or in healthy control subjects (HCs) (29).

Varying hippocampal definitions is a known problem in neuroimaging research (21) with up to 60 different definitions used in previous research. Variable hippocampal volumes may also pose difficulties in generalizing preclinical hippocampal research to clinical populations. Basic hippocampal research often investigates hippocampal function at individual substructural and circuit-based and/or combined substructural level (30). An approach examining individual substructures could assemble individual substructures into anatomicofunctional or composite regions and may help bridge the elusive gap between clinical and preclinical hippocampal research (18,31). This approach would help to identify specific hippocampal pathology associated with, and functional consequences of, hippocampal neuroimaging changes in MDD.

Increasing MRI field strengths and advances in automated tissue-segmentation protocols have enabled examination of hippocampal substructures with ever-increasing precision (32,33). Software advances such as FreeSurfer 6.0 (http://surfer.nmr.mgh.harvard.edu/) can exploit multimodal T1- and T2-weighted MRI data using in vivo and ex vivo atlas probabilistic segmentation to identify more subregions with improved accuracy (34).

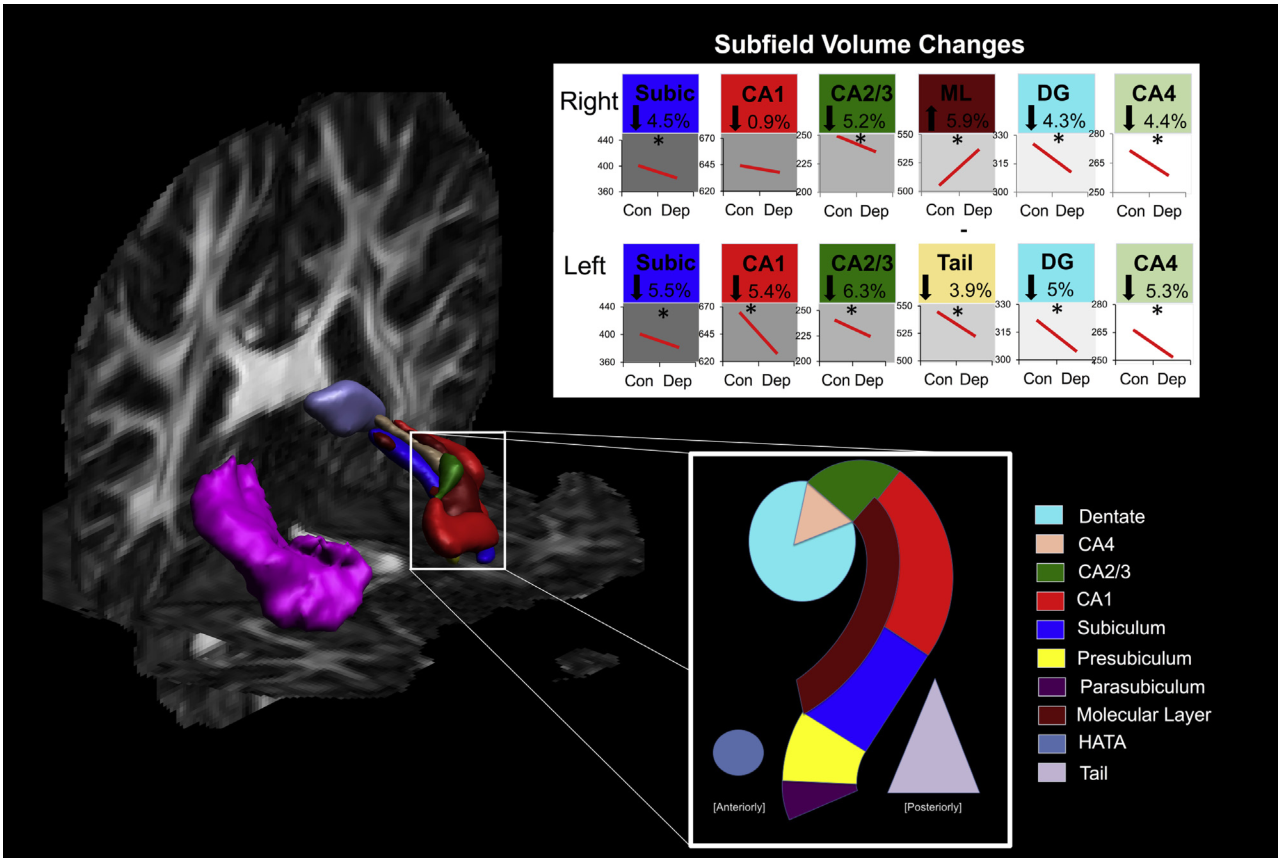

Figure 1. FreeSurfer output and percentage substructure changes. FreeSurfer output showing left and right hippocampi. (Left panel) The right hippocampus is depicted in solid magenta, and the image represents the total of all FreeSurfer substructure output. The left hippocampus shows segmented FreeSurfer substructures (not all are visible). Significant substructure volume changes in depression on the left and right are presented graphically (left panel) and as percentage change (top right panel). Values marked with an asterisk in the top right panel are significant after false discovery rate correction for multiple comparisons. (Lower right panel) A schematic cross section of the left hippocampus is shown. This schematic diagram shows the relative sizes of substructures. The hippocampal amygdalar transition area and tail are not cross-sectional in this schematic diagram; they are represented as the anterior or posterior poles of the hippocampus. See Supplemental Figure S1 for more information. CA, cornu ammonis; Con, control subject group; Dep, total persons with depression group; DG, dentate gyrus; HATA, hippocampal amygdalar transition area; ML, molecular layer; Subic, subiculum.

Automated approaches have advantages over gold-standard manual segmentation because of improved reliability and, if adapted into routine neuroimaging research, should increase interstudy comparability (35).

In this study, hippocampal MRI findings in groups of persons with and without depression were evaluated using this latest technology. Patients with first-presentation depression (FPD) and recurrent depression (RD) were studied using automated hippocampal substructure segmentation of high-definition T1-weighted and T2-weighted–fluid-attenuated inversion recovery (FLAIR) magnetic resonance images. Substructure volumes were combined according to defined anatomical criteria (known as composite measures) to examine whether predicted reductions in overall hippocampal volume depended on specific anatomical hippocampal definitions. Exploratory composites were also created to further investigate the importance of particular subregions. Our hypotheses were that the impact of hippocampal change in depression depends on the definition of hippocampus used (i.e., proper, formation, total) and that the more specific the hippocampal definition (i.e., the core CA regions), the greater the degree of change.

METHODS AND MATERIALS

Participants and Clinical Data

Eighty people with MDD were compared with 83 HCs without MDD. Full inclusion and exclusion criteria are listed in the Supplement. All participants completed the Structured Clinical Interview for DSM-IV (36) and Hamilton Depression Rating Scale (37). All patients required a current Structured Clinical Interview for DSM-IV diagnosis of MDD with a Hamilton Depression Rating Scale score $>$20. Control subjects were required to have no active or previous Structured Clinical Interview for DSM-IV diagnosis and a Hamilton Depression Rating Scale score $<$8. Patients with depression were further subdivided into FPD and RD groups based on the number of previous episodes of depression (FPD = 0; RD $\geq$ 2).

Ethical approval was obtained from the Tallaght Hospital/St James Hospital Joint Research Ethics Committee, Dublin, and fully written informed consent was obtained from subjects prior to participation.

MRI Acquisition

All data were acquired on a Philips (Best, Netherlands) Intera Achieva 3.0T magnetic resonance system (32-channel head coil) at Trinity College Institute of Neuroscience, Dublin.

The following T1-weighted images were acquired: 180 axial high-resolution T1-weighted anatomical images (T1W-IR1150 sequence, echo time = 3.8 ms, repetition time = 8.4 ms, field of view 230 mm, 0.898 $\times$ 0.898-mm^2 in-plane resolution, slice thickness 0.9 mm, flip angle alpha = 8°). For T2-weighted–FLAIR images, we acquired 60 axial T2-weighted–FLAIR images (echo time = 120 ms, repetition time = 2800 ms, 0.49 $\times$ 0.49-mm^2 in-plane resolution, slice thickness = 3 mm, flip angle alpha = 8°, field of view = 230 mm). Slices were taken in the axial plane, with the resulting smaller in-plane resolution corresponding to the longitudinal axis of the hippocampus.

Image Analyses

Cortical reconstruction and segmentation was performed using the FreeSurfer 6.0 image analysis suite (38,39). The technical details of these procedures are described elsewhere (32,40–44). FreeSurfer interrogates contrast differences between substructures using previously defined in vivo and ex vivo hippocampal atlases to determine substructure characteristics. By combining T1-weighted and T2-weighted–FLAIR inputs and selecting the 3T MRI flag and multispectral segmentation in FreeSurfer, the procedure was optimized. Eleven substructures were computed (Figure 1, Supplemental Figure S1, and Supplemental Table S1).

Composite Measures

Computed substructure volumes were summed to generate composite measures. We initially generated a restrictive hippocampal definition: the hippocampus proper (HP), consisting of the CA regions only. Secondly, a functional hippocampal definition was calculated: the hippocampal formation (HF), comprising, in addition to the HP, the dentate gyrus, the subiculum, and the tail. This definition incorporated all the principal substructures involved in classic hippocampal circuitry. Thirdly, an expansive definition, hippocampal extended (HE), was defined, incorporating all computed hippocampal substructures. Four exploratory anatomical regions (complete dentate gyrus, CA only, combined dentate gyrus and CA, and CA2 to CA4–only regions) were created (Supplemental Table S2).

Statistical Analysis

All extracted subfield volumes were systematically inspected visually, and measures were exported to SPSS 24 (https://www.ibm.com/analytics/us/en/technology/spss/).

Mixed-method analyses of variance (ANOVAs) were used to investigate groupwise differences in substructure and/or composite volumes and across all substructures and/or composites and hemisphere (left/right) of the hippocampi. To clarify any driving effects identified, additional post hoc analyses of covariance were used to compare between-group differences (control vs. all with depression, and control vs. FPD vs. RD) for each substructure and/or composite and hemisphere independently. Age, sex, and estimated total intracranial volume were entered as covariates throughout. Multiple-comparison correction was performed using false discovery rate (FDR) correction (45). Finally, the predictive ability of substructures on disease duration was examined using two-step hierarchical multiple regression analysis (see the Supplement).

RESULTS

Demographics

Eighty patients with depression (43 FPD, 37 RD) and 83 HCs participated (Table 1). No significant differences between HC and total patient groups or between HC and FPD groups were found for age, sex, or handedness. The RD group was older (10–12 years older) and had a higher proportion of female subjects compared with either the FPD or HC group. The average duration of depression was 10 months in the FPD group and 60 months in the RD group. All patients with RD and

Table 1. Demographic Information

	Study Group				Con vs. Dep	Con vs. FPD	Con vs. RD	FPD vs. RD
	Con, n = 83	Dep, n = 80	FPD, n = 43	RD, n = 37	p Value			
Age, Years, Mean (SEM)	31.5 (1.4)	34.5 (1.4)	29 (1.7)	41 (1.8)	.13	.22	.0001	3.90×10^{-6}
Range, Years	16–64	17–64	17–61	22–64	—	—	—	—
Sex, n_{Male}/n_{Female}, % Male	34/49, 41	23/57, 29	19/24, 44	4/33, 11	χ^2 = .14	χ^2 = .85	χ^2 = .001	χ^2 = .001
Handedness, n_R/n_L	75/8	73/7	—	—	NS	—	—	—
HDRS Score, Mean (SEM)[a]	1.3 (0.4)	22.2 (0.4)	21.4 (0.4)	23 (0.6)	1.10×10^{-75}	1.98×10^{-65}	2.00×10^{-53}	.04
MDD Duration, Months, Mean (SEM)[b]	—	29 (4)	10 (4)	60 (5)	—	—	—	—

The table shows demographic data and between-group differences for control subjects, total persons with depression, FPD, and RD groups.

Con, control subjects; Dep, total individuals with depression; FPD, first-presentation depression; HDRS, Hamilton Depression Rating Scale; L, left-handed; MDD, major depressive disorder; NS, not significant; R, right-handed; RD, recurrent depression.

[a]Data corrected for age, sex, and estimated total intracranial volume.

[b]Transformed (normalized) data.

73% of patients with FPD (27 of 37 patients) were taking antidepressants.

Hippocampal Volumetrics

Mixed-method ANOVA revealed a strong trend toward significance for the main effect for group (F_1 = 3.69, p = .057, η_p^2 = .03, power = 0.48). No main effect for hemisphere was found, but a significant group × hemisphere interaction (F_1 = 6.55, p = .012, η_p^2 = .05, power = 0.72) was found with reduced left substructure volumes in depression. A main effect for substructure ($F_{3.2}$ = 6.91, p = .0001, η_p^2 = .05, power = 0.98) and group × hemisphere × substructure interaction ($F_{2.68}$ = 6.89, p = .0004, η_p^2 = .05, power = 0.96) was found.

Post hoc analyses of covariance revealed that the interaction was driven by smaller volumes bilaterally for CA2/CA3, CA4, subiculum, and dentate gyrus and lateralized differences for left tail, CA1 (decreased), and right molecular layer (increased) volumes in depression (Figure 1). Left CA2/CA3 and CA4 were reduced only in FPD. No correlation was found between duration of depression and substructure volumes (Table 2).

Composite Measures

HC Versus All Patients With Depression. Mixed-method ANOVA revealed a significant main effect for group (F_1 = 6.97, p = .009, η_p^2 = .045, observed power = 0.75) but not hemisphere. A significant group × hemisphere interaction (F_1 = 7.66, p = .006, η_p^2 = .05, power = 0.79) was found with reduced left composite volume observed in patients with depression. A main effect for composite volume ($F_{1,1}$ = 20.94, p = .000005, η_p^2 = .13, power = 0.99) was found. Hemisphere × composite interaction was not significant, but group × composite × hemisphere interaction was significant ($F_{1.18}$ = 4.99, p = .022, η_p^2 = .033, observed power = 0.65).

HC Versus FPD and RD. Mixed-method ANOVA revealed a pattern of smaller volumes in RD compared with FPD, lateralized to the left, with a main effect for group (F_1 = 6.97, p = .024, η_p^2 = .05, observed power = 0.69), significant group × hemisphere interaction (F_2 = 5.09, p = .007, η_p^2 = .065, power = 0.81), and significant main effect for composite volume ($F_{1.1}$ = 20.96, p = .000005, η_p^2 = .13, power = 0.99).

Post hoc analyses of covariance showed reduced composite volumes on the left in depression. Only the HP showed volume change on the right. As the three composites focused more toward core hippocampal substructures (HE to HF to HP), the significance of the change in depression increased on both the left (p = .005 to p = .003 to p = .0002) and right (p = .232 to p = .121 to p = .0129).

Hierarchical multiple regression revealed the left CA1 as the only substructure with a predictive relationship between disease duration and substructure volume, with 6% of the variance in CA1 volume explained by disease duration (R^2 change = .057, $F_{1,63}$ change = 4.6, p = .036).

Exploratory anatomical composite analyses showed volume reductions across all composites on the left in patients with depression (Table 3, Supplemental Table S4, Supplemental Figure S3).

DISCUSSION

This study investigated hippocampal substructural volumes in 80 individuals with depression and 83 HCs using advanced automated segmentation. Extensive bilateral volume reductions across multiple principal substructures were found in persons with MDD, with left core hippocampal volume reduction being the most salient finding. Differences were generally more pronounced on the left hemisphere and with increasing duration of depression. Analyzing the hippocampus using composite anatomical definitions highlighted the importance of core CA regions over peripheral areas in the pathogenesis of depression (Table 3, Figure 2, Supplemental Figure S2). Persons with FPD showed CA2 to CA4 changes only while the recurrent MDD group had a extending pattern of substructure involvement (Figure 3). It is likely that this finding reflects evolving hippocampal deterioration with increasing duration of depression.

The extent of hippocampal change in depression depends on which hippocampal region is defined; definition of the hippocampus is important for interpretation of past and future research.

There are recent moves toward developing a harmonized protocol for whole hippocampus segmentation (46,47). This is, to the best of our knowledge, the first study in which multiple composite definitions of the hippocampus have been used to

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Table 2. Volumetric Between-Group Differences for Individual Substructures

	p Value, Con vs. Dep	Effect Size, Con vs. Dep, η_p^2 [a]	Pairwise Comparison, Con vs. FPD, p Value	Pairwise Comparison, Con vs. RD, p Value	Effect Size, CON vs. FPD and CON vs. RD, η_p^2 [a]
Left Substructure					
CA1	.0004[b]↓	.08[c]	.021↓	.006[b]↓	.08[c]
CA2/CA3	.0001[b]↓	.09[c]	.002[b]↓	.018[b]↓	.09[c]
CA4	.001[b]↓	.07[c]	.0063[b]↓	.005[b]↓	.08[c]
Dentate gyrus	.002[b]↓	.06[c]	.120↓	.008[b]↓	.07[c]
Subiculum	.001[b]↓	.06[c]	.094↓	.004[b]↓	.08[c]
Tail	.016[b]↓	.04	.999↓	.003[b]↓	.07[c]
Molecular layer	.181↑	.01	.615↑	.999↑	.01
Presubiculum	.501↑	.003	.999↑	.999↑	.003
Parasubiculum	.632↑	.002	.999↑	.999↑	.002
Fimbria	.837↑	.0005	.999↑	.999↑	.001
Fissure	.175↑	.012	.391↑	.999↑	.016
HATA	.905↑	.0005	.999↑	.999↑	.0005
FDR Across All Left Measures	.021		.006	.025	
Right Substructure					
CA1	.12↓	.016	.456↓	.951↓	.02
CA2/CA3	.003[b]↓	.06[c]	.014↓	.179↓	.06[c]
CA4	.003[b]↓	.06[c]	.149↓	.014↓	.06[c]
Dentate gyrus	.004[b]↓	.05	.166↓	.018↓	.06[c]
Subiculum	.006[b]↓	.05	.265↓	.014↓	.06[c]
Tail	.267↓	.008	.883↓	.883↓	.008
Molecular layer	.006[b]↑	.05	.120↑	.056↑	.05
Presubiculum	.066↑	.02	.272↑	.706↑	.02
Parasubiculum	.064↑	.03	.734↑	.184↑	.03
Fimbria	.344↓	.006	.659↓	.999↓	.01
Fissure	.139↑	.014	.999↑	.366↑	.02
HATA	.384↑	.005	.999↑	.730↑	.009
FDR Across All Right Measures	.013		0	.009	

The table shows between-group volumetric differences for individual substructures following analysis of covariance correcting for age, sex, and estimated total intracranial volume and after correcting for multiple comparisons using false discovery rate (FDR). Arrows indicate the direction of volume change between controls and depressed. Up means an increase in depression. Down means a decrease in depression. For details of substructures, see Supplemental Table S1. See Supplemental Table S3 for an expanded version that includes actual representative volumes.

CA, cornu ammonis; Con, control subjects; Dep, total persons with depression; FPD, first-presentation depression; HATA, hippocampal amygdalar transition area; MDD, major depressive disorder; RD, recurrent depression.

[a] η_p^2 describes effect size (.01 = low, .06 = moderate, .14 = large).

[b] p values that survived FDR correction.

[c] Moderate or large effect size.

investigate hippocampal volumes in depression. Three total hippocampal composite volumes were calculated through the summation of appropriate substructures (Figure 2, Supplemental Table S2). Using the broader definitions of hippocampal volume—HF and HE—we found a reduced hippocampal volume in depression exclusively on the left side, which was attributable to changes in the RD group. The most restrictive definition of hippocampus (HP) yielded volume differences bilaterally in MDD, and, unlike the reductions we found in the HE and HF, these volume reductions were maintained across FPD and RD groups. Of note, the left HP volume reduction in depression was highly significant (p = .0002).

As we progressively constricted our hippocampal definition from the larger, nonspecific HE through the more midsized, functional HF and finally to the smaller, highly specific HP, the volume reductions seen in depression became progressively more pronounced (Table 3, Figure 2). The implications of this finding are twofold. First, it highlights the importance of particular hippocampal definitions in studies of MDD, where conflicts may arise when interpreting research because of differing hippocampal definitions. Previous nonsignificant findings examining a larger hippocampal corpus may obscure deeper hippocampal substructural changes in depression (13–16). Ours is the first study to build multiple "ground up" composite definitions of total hippocampal volumes by assembling substructural components. Such an approach may resolve some interstudy inconsistencies moving forward. Second, the conservative HP measure supports our hypothesis implicating the classic core CA subfields in the MDD disease process. In essence, the more precise the hippocampal definition, the greater the degree of change in persons with depression. Disruption of the

Table 3. Volumetric Between-Group Differences for Composite Measures

	p Value, Con vs. Dep	Effect Size, Con vs. Dep, η_p^2[a]	Pairwise Comparison, Con vs. FPD, *p* Value	Pairwise Comparison, Con vs. RD, *p* Value	Effect Size, CON vs. FPD and CON vs. RD, η_p^2[a]
Left Hippocampal Composite					
Hippocampal extended	.005[b]↓	.05	.261↓	.009[b]↓	.07[c]
Hippocampal formation	.003[b]↓	.05	.118↓	.021[b]↓	.06[c]
Hippocampal proper	.0002[b]↓	.09[c]	.0041[b]↓	.0049[b]↓	.09[c]
Left Anatomical Composite					
Complete dentate gyrus	.0004[b]↓	.06[c]	.0087[b]↓	.0028[b]↓	.06[c]
CA only	.0003[b]↓	.09[c]	.0058[b]↓	.0051[b]↓	.09[c]
Combined dentate gyrus/CA	.0001[b]↓	.08[c]	.0021[b]↓	.0042[b]↓	.08[c]
CA2–CA4	.0002[b]↓	.07[c]	.0013[b]↓	.0080[b]↓	.07[c]
FDR Across All Left Measures	.021		.006	.025	
Right Hippocampal Composite					
Hippocampal extended	.232↓	.01	.3498↓	.4208↓	.01
Hippocampal formation	.121↓	.02	.2493↓	.2757↓	.02
Hippocampal proper	.0129[b]↓	.04	.3742↓	.8751↓	.05
Right Anatomical Composite					
Complete dentate gyrus	.003[b]↓	.06[c]	.080↓	.030↓	.06[c]
CA only	.034↓	.03	.078↓	.1816↓	.03
Combined dentate gyrus/CA	.010[b]↓	.04	.061↓	.0572↓	.04
CA2–CA4	.002[b]↓	.06[c]	.022↓	.108↓	.06[c]
FDR Across All Right Measures	.013		0	.009	

The table shows between-group differences for all composite measures following analysis of covariance correcting for age, sex, and estimated total intracranial volume and after correcting for multiple comparisons using false discovery rate (FDR). Total hippocampal volume and anatomical composite volume differences are shown. Arrows show direction of volume change between controls and depressed. Up means an increase in depression. Down means a decrease in depression. For details of composite components, see Supplemental Table S2. See Supplemental Table S4 for an expanded version that includes actual representative volumes.

CA, cornu ammonis; Con, control subjects; Dep, total persons with depression; FPD, first-presentation depression; HATA, hippocampal amygdalar transition area; MDD, major depressive disorder; RD, recurrent depression.

[a] η_p^2 describes effect size (.01 = low, .06 = moderate, .14 = large).

[b] *p* Value that survived FDR correction.

[c] Moderate to large effect size.

CA regions could interfere with all aspects of the trisynaptic circuit and alter fundamental sensory and emotional information processing. The trisynaptic circuit allows entorhinal cortical inputs to be processed through the key hippocampal subfields (entorhinal cortex to dentate gyrus/CA4→ CA2/3→CA1) before outputting through the subiculum (22,30). Disruption of any of these principal hippocampal substructures could result in symptoms of depression (31).

Only Principal Hippocampal Substructures Show Differences in Depression

Substructure changes were found only in those considered part of the classic hippocampal formation—that is, CA1–CA4, dentate gyrus, subiculum, tail (composed largely of CA and dentate gyrus), and the molecular layer (the upper layer of the subiculum and CA) (Figure 1). No differences were found for the nonclassic hippocampal substructures: the presubiculum, parasubiculum, fimbria, hippocampus-amygdala transition area, and hippocampal fissure. Replicating findings of a recent substructural study, we found no correlation between MDD duration and any individual substructure volume (29). Differences in persons with MDD were generally observed bilaterally in principal substructures, with differences consistently more pronounced on the left and frequently more prominent in the RD subgroup. Once again, our findings were broadly consistent with those of recent studies looking at hippocampal substructures in patients with depression (24–26,28). A novel finding in the present study is that the CA1 and hippocampal tail demonstrated lateralization of pathology exclusively to the left side. The tail comprises elements of all principal substructures (CA, dentate gyrus, and subiculum). However, the relative dominance of CA1 within the hippocampal tail compared with other substructures may account for some of the lateralization found comparably in the CA1 region and the tail.

The CA1 was the only principal substructure that showed no change on the right. Volumetric reductions in CA1 were lateralized exclusively to the left side. The left CA1 exhibited reduced volumes across both first presentation and recurrent groups, but only the recurrent group survived the strict FDR correction. The CA1 region is particularly vulnerable to various insults (48,49) and has been found in postmortem studies of MDD patients to contain greater evidence of neuronal apoptosis relative to that in control subjects without depression (50). CA1 is involved in autobiographical memory (51), contextual memory retrieval (52), and self-awareness (53). MDD is associated with autobiographical memory difficulties

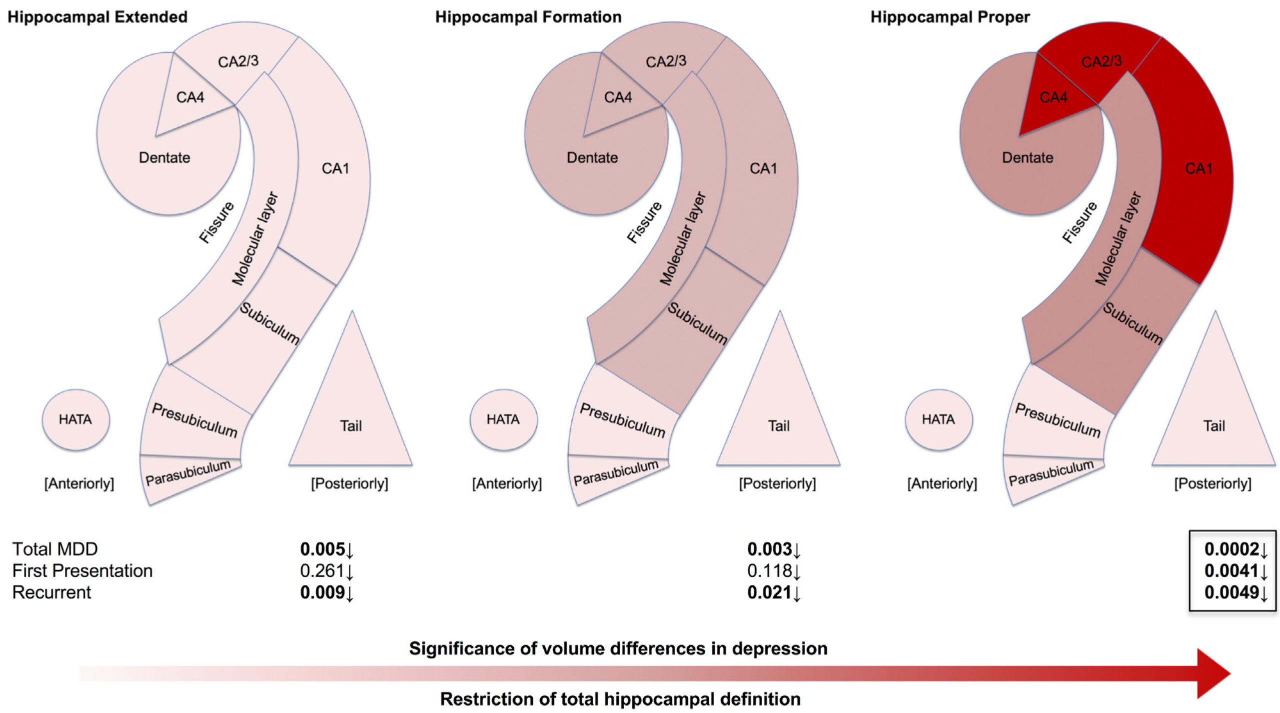

Figure 2. Total hippocampus volumes in depression. Schematic cross sections of different left total hippocampal definitions. (Left panel) Hippocampal extended includes all computed substructures. (Middle panel) Hippocampal formation includes only dentate gyrus, subiculum, and cornu ammonis (CA) substructures. (Right panel) Hippocampal proper includes CA substructures only. See Supplemental Table S2 for further details on composite assembly. See Supplemental Figure S2 for a graphical representation of differences in volume between control subject, first-presentation depression, and recurrent depression groups. Note that as the hippocampal definition becomes more restrictive and exclusive, the greater the significance of volumetric change in depression. HATA, hippocampal amygdalar transition area; MDD, major depressive disorder.

and overgeneralization deficits (54). Left CA1 volume was the only individual component to survive regression analysis, suggesting that left CA1 volume represents a predictor of illness duration in depression. CA1 tissue also has a particularly high expression of many 5-hydroxytryptamine receptor subtypes (55–57). As such, the CA1 region may be especially sensitive to disturbances of the serotonergic system or, alternatively, limbic 5-hydroxytryptamine neurotransmission could be disrupted secondary to CA1 disease. Long-term corticosteroid exposure, a preclinical animal model of MDD, has been shown to attenuate serotonin responses in the CA1 region (56). Consequently, long-term overexposure to stress and cortisol, known occurrences in depression (58), may result in CA1 changes and limbic serotonergic system disturbance.

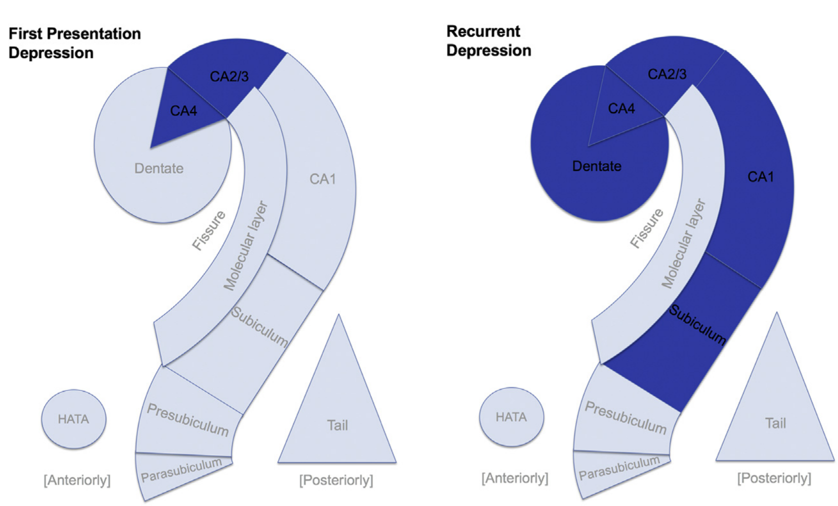

Figure 3. (Left panel) First-presentation and (right panel) recurrent depression substructures. Schematic cross sections of the left hippocampus showing substructures that are reduced in volume in first-presentation depression and in recurrent depression. Note the greater number of substructures involved in the image for recurrent depression, suggesting a possible extension of disease process in depression. CA, cornu ammonis; HATA, hippocampal amygdalar transition area.

CA2 to CA4 Involvement in FPD and Further Extension With Chronicity

Our findings for total hippocampal volumes in depression are consistent with many studies showing hippocampal volume reductions in persons with FPD (2,12) and RD (3,4). Although we did not find a statistical correlation between duration of depression and total hippocampal volumes, the differences between FPD and RD groups are broadly consistent with the premise that the chronicity of depression has an inverse relationship with hippocampal volumes (4,11,59,60).

The CA2/3 and CA4 regions individually demonstrated significant volume reduction in patients with FPD (Figure 3). Combining them to form an exploratory CA2 to CA4 composite measure further augmented the significance of this region (Table 3), especially in the FPD group (p = .0013). This finding suggests a potential locus across these two substructures for initial hippocampal pathology in MDD. CA1 also displayed a distinct trend in this direction (p = .021), but the trend did not survive strict FDR correction. These results imply that the classic CA hippocampal subfields (CA2–CA4 in particular) may be affected early in the disease process, as is also suggested by our restrictive HP total hippocampal volume measure. Conversely, the left dentate gyrus, subiculum, and tail showed volume reductions only in the RD group. This finding suggests that these substructures may be recruited later, as depression progresses from first presentation into chronicity (Figure 3). This finding, importantly, also suggests that MDD displays the characteristics of a disease process, and hints how early intervention may help prevent further extension of pathology throughout the hippocampus with chronicity.

Molecular Layer

Only one substructure showed a volume increase in depression. The right molecular layer measure was larger in MDD and driven by FPD only. This measure incorporates the upper layer of the subiculum and CA1 to CA3 regions (34). Lining the fissure just below the cerebrospinal fluid, this layer is populated sparsely with neuronal bodies and contains connecting axons, interneurons, and glial cells (61). It is noteworthy that this was the only substructure whose volume increased in depression. Potential explanations include an initial inflammatory response, glial scarring along the fissure edge, or extensive dendritic branching in this layer in the early stages of the disease.

Can Exploratory Anatomical Composites Aid Deeper Substructural Analysis?

We calculated additional exploratory composite measures to further investigate the volumes of anatomical subregions within the hippocampal complex (Table 3, Supplemental Table S2).

The CA4 lying within its hilum can be considered part of the dentate gyrus (62). As such, a more anatomically and functionally inclusive complete dentate gyrus measure, combining the dentate gyrus with CA4, showed significant volume reduction (p = .0004) in MDD on the left side, and this finding was maintained across both FPD and RD groups. This measure also showed volume reduction on the right side in persons with depression (p = .003). Conversely, a measure removing CA4 from the other CA regions (i.e., a genuine CA-only region) was calculated by adding the volumes of CA1 through CA3. This composite showed increased significant differences between depressed and control subjects (p = .0003) on the left side, with reductions being found across both depression groups. Finally, by adding the dentate gyrus and all CA areas together to form a combined dentate gyrus/CA measure, we found our most significant volume reduction overall in depression groups (p = .0001), with the differences driven by both FPD and RD groups.

The exceptional significance of the volume changes found in these exploratory composites and in the restrictive hippocampus proper (CA1–CA4 regions) reinforces the importance of the left CA and dentate regions as key substructures in depression. Together, these regions correspond to the path of the classic trisynaptic circuit, the fundamental hippocampal computational unit (63). This circuit is important for the memory functions of pattern separation and completion (64) as well as adult neurogenesis (65), which is postulated to be disrupted in MDD. It is also a central region in the cortisol and serotonin responses, which experience key disturbances in depression (58,66,67). This finding supports our hypothesis that the principal hippocampal pathophysiology in depression is confined within classic principal hippocampal loci only, confirming extensive preclinical research showing the CA and dentate gyrus involvement in stress and emotional regulation (31,64,68–70).

Strengths and Limitations

This is the first time that hippocampal substructures in both FPD and RD have been examined. There are recent moves toward developing a harmonized protocol for whole hippocampus segmentation (46,47), and these findings suggest this novel method of assembling substructures according to anatomical subregion definitions. Having distinct hippocampal definitions is necessary for the rational interpretation of future findings in MDD. Other groups using FreeSurfer 6.0 have used the FreeSurfer-generated total hippocampal volume (equivalent to our HE measure). The combination of two MRI modalities (T1-weighted and T2-weighted–FLAIR) allows increased accuracy and resolution of the automated technique by providing an additional contrast. This approach allows superior substructure demarcation compared with T1 weighting alone. This outcome has previously been shown in an Alzheimer's disease cohort (34). We are the first to demonstrate this improved substructure differentiation in MDD. Differences between persons with depression and HCs observed only in T1-weighted images can be found in Supplemental Table S5.

Some limitations need to be considered. The average duration of depression in the FPD group was 10 months, with 73% of persons being treated with antidepressants. Ideally, an FPD cohort would consist of treatment-naïve patients, but most of our patients had treatment from their family doctor prior to referral. We were thus unable to assess the effect of medication in our sample. Sex-specific changes have been found in previous hippocampal research (3,12,71,72). We corrected for sex and age in our study by removing these variables as covariates and were thus unable to directly assess the effects of sex and age. Automated segmentation has been argued to have reduced interpretation and validity precision compared with those of expert manual segmentation (73). Current automated protocols can differ considerably from one another in terms of substructure terminology, regional boundaries, and segmentation protocols (74). Such discrepancies are particularly evident for smaller substructures, and this may be a limitation, as FreeSurfer 6.0 creates a number of smaller divisions such as the parasubiculum and hippocampus-amygdala transition area. The previous iteration of FreeSurfer (5.3) has been criticized because of defined substructures not being in line with anatomical studies (75). In particular, it has been suggested that portions of the large CA1 region are redistributed to the neighboring CA2/CA3 with the resultant CA1 region rendered smaller than in anatomical studies (76). This situation has been addressed in FreeSurfer 6.0 (34), with CA1 volumes now in line with histological studies (77). Considering the data generated in neuroimaging studies—in our study, 163 subjects yielded almost 2000 individual substructures—automated segmentation is a more reliable and more viable alternative than manual segmentation. Finally, even though we had FPD and RD groups, disease progression cannot be definitively inferred based on a cross-sectional sample. We are currently following up participants as part of a longitudinal study.

Conclusions

In conclusion, using advanced high-quality automated hippocampal segmentation, this study found patterns of differences across principal hippocampal substructures in cases of depression. The left hippocampus was found to be smaller in MDD, similar to findings of previously published studies. This volume difference was found irrespective of hippocampal definition and was driven by chronicity. The more restricted the hippocampal definition, the greater the volume change in cases of depression. No changes were found among the more peripheral hippocampal substructures. This confirms preclinical research identifying the importance of core hippocampal substructures in the pathophysiology of depression and stress. In particular, CA1 volume findings were compelling because of lateralization of volume differences to the left side only and because the CA1 volume measure was predictive of MDD duration. The uniqueness of this finding suggests that left CA1 volumes may represent a marker of depressive illness. The initial first-presentation involvement of the CA2 to CA4 regions and additional extension of pathology into adjacent substructures with chronicity suggest a potential MDD disease process. This suggestion reinforces the need for effective early therapeutic intervention in depression to try and halt possible progressive hippocampal damage.

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