Smaller volumes of the cornu ammonis (CA1 and CA3) may be a biological
endophenotype for suicide risk in mood disorders

Authors: Spiro P. Pantazatos, Ph.D.^{1,2,*}, Todd Ogden, Ph.D.^{1,3}, Nadine M. Melhem
Ph.D.⁴, David A. Brent M.D.⁴, Mohammad Lisanpezeski M.D.,^{1,2} Ainsley Burke
Ph.D.^{1,2}, Jeffrey M. Miller, M.D.^{1,2}, J. John Mann, M.D.^{1,2,*}

¹Molecular Imaging and Neuropathology Area, New York State Psychiatric Institute,
New York, NY; ²Department of Psychiatry, Columbia University Irving Medical Center,
New York, NY; ³Mailman School of Public Health, Columbia University, New York, U. S. A,
⁴Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA,
United States;

* To whom correspondence should be addressed:

E-mail: spp2101@columbia.edu

Correspondence can also be addressed to:

E-mail: jjm@columbia.edu

This manuscript contains:

Abstract

Main text

4 Tables

1 Figures

1 Figure Legends

References

Running title: Hippocampus substructure volumes and familial suicide risk

Keywords: hippocampus subfields, risk factors, suicidal behavior, familial transmission,
early life adversity, heritable

Abstract

Mood disorders and suicidal behavior have moderate heritability and are associated with smaller hippocampal volumes. However, it is unclear whether these alterations reflect heritable risk or epigenetic effects of childhood, compensatory mechanisms, or illness-related changes. We sought to separate effects on hippocampal substructure volumes due to mood disorder, suicidal behavior, and risk and resilience to both by examining high familial risk individuals (HR) who have passed through the age of greatest risk for psychopathology onset. Structural brain imaging and advanced hippocampal substructure segmentation quantified cornu ammonis (CA1-4), dentate gyrus, and subiculum volumes in healthy volunteers (HV, N=25) and three groups with one or more relatives with early-onset mood disorder and suicide attempt: 1. unaffected HR (N=20); 2. HR with lifetime mood disorder and no suicide attempt (HR-MOOD, N=25); and 3. HR-MOOD+SA with mood disorder and a previous suicide attempt (N=18). Findings were tested in two independent cohorts not selected for family history (HV, MOOD, and MOOD+SA, total N=199). Lower CA1 and CA3 volumes were found in both HR-MOOD+SA and in HR as compared to HV, suggesting a biological endophenotype for suicide risk and mood disorder. No group differences were observed in MOOD+SA or MOOD vs. HV, suggesting the effects are associated with familial risk for, but not previous, suicide attempt and mood disorder. Our findings suggest familial suicide risk may be mediated in part by smaller CA1 and CA3 volumes. The structures may serve as therapeutic targets for suicide prevention strategies in high risk families.

Introduction

Smaller hippocampal volume is the most replicated and well documented finding in brain imaging studies of mood disorder (MOOD)(1,2). Although most studies have found smaller hippocampal volumes in MOOD, findings have been mixed and include several negative studies (3–6). The finding has been linked to markers of MOOD illness progression, including longer illness duration (7,8), non-remission/treatment resistance (6,9), and greater number of major depressive episodes (10). Preclinical and human research suggests that chronic stress and stress-mediated neurotoxic processes, including inflammation, hypothalamic-pituitary-adrenal axis dysfunction, and neurotransmitter disturbances, contribute to hippocampal volume loss as illness progresses (11). In addition, studies have found more pronounced hippocampal reductions in depressed suicide attempters relative to depressed non-attempters (12) .

With the advent of automated hippocampal subfield segmentation routines, more recent studies have examined alterations in hippocampal subfields in mood disorders. One study found smaller volumes in the bilateral cornu ammonis 1 (CA1), CA4, the granule cell layer, molecular layer, and whole hippocampus in major depressive disorder (13). Another study found pronounced volume deficits in principal hippocampal substructures in major depression that were most marked in left CA1 (1). The study used a cross-sectional design and found patients with recurrent, as opposed to first-episode depression, had the most pronounced volume deficits in principal hippocampal substructures: the cornu ammonis (CA1-CA4), dentate gyrus, and subiculum, and that CA1 volume correlated with illness duration. Longitudinal studies

are required to definitively demonstrate an effect of ongoing depression on CA1 volume (1).

There is debate about whether hippocampal volume reductions observed in mood disorders are a consequence of stress and MOOD, or whether they predate symptom onset and a contributor to pathophysiology (14). Some suggest that although hippocampal volume loss may result from stress, the development of mood disorder or suicidal behavior may be an independent hippocampal based process (11). While previous studies have reported smaller hippocampal volume in individuals at high familial risk for MOOD (15,16), it is unknown whether hippocampus and/or hippocampal subfields deficits are associated with familial risk and pathogenesis for suicidal behavior and mood disorder, or whether they are downstream stress responses or damage due to allostatic load.

To test whether hippocampal subfield alterations are associated with risk for developing psychiatric disorder or whether they are related to compensatory adaptation possibly marking resilience or illness related changes, the current study examined regional differences in hippocampal subfield volumes in low risk healthy volunteers (HV) and in medication-free high-risk individuals, having a first or second degree relative with both an early onset mood disorder and a history of suicidal behavior. High risk individuals were either unaffected (i.e. HR, recruited when largely beyond the age of risk for mood and suicidal behavior), or affected with lifetime history of mood disorder alone (HR-MOOD) or mood disorder plus one or more lifetime suicide attempts (HR-MOOD+SA). We first sought to identify subfields with lower volumes in HR-MOOD+SA relative to HV. We hypothesized the strongest differences would occur

in CA1-4 based on previous research (1). We then tested for similar alterations in these regions in HR relative to HV individuals.

Methods

Inclusion/exclusion criteria

The FAMPATH sample consisted of high-risk individuals and healthy volunteers (HV) that were 25 years of age or older, an age by which mood disorder and suicidal behavior in our familial transmission studies are generally phenotypically clinically expressed (17,18). All high-risk subjects were categorized as being at-risk for a mood disorder and suicide because they had a first or second degree relative with early-onset (<40 years) mood disorder and a lifetime history of suicide attempt. The subjects in the high-risk group were separated into vulnerable and resilient groups based on their phenotype: individuals with a lifetime history (current or past) of mood disorders and a history of suicide attempt (HR-MOOD+SA, n=18); individuals with a lifetime history (current or past) of mood disorders but no suicide attempt (HR-MOOD, n=25); and resilient (HR, n=20) individuals with no personal history of mood disorder or suicide attempt. Qualifying mood disorders included major depressive disorder, bipolar disorder, dysthymia and depressive disorder not otherwise specified. The HR-MOOD sample consisted of 20 participants with major depressive disorder, 1 with bipolar disorder, and 4 participants with other diagnoses. The HR-MOOD+SA sample consisted of 14 participants with major depressive disorder, 3 with bipolar disorder, and 1 with other diagnosis. Healthy volunteers (HVs, n=35) had no personal or family history of psychiatric disorders or suicidal behavior. The sample was recruited at two sites, University of Pittsburgh Medical Center and New York State Psychiatric Institute, in

protocols approved by the respective Institutional Review Boards (IRB) at the University of Pittsburgh and The New York State Psychiatric Institute. Participants were recruited through outpatient clinics, clinician referrals and IRB-approved advertisements and all participants provided written, informed consent. See Supplementary Methods for assessments and additional inclusion/exclusion criteria for FAMPATH and for the two replication (CONTE and NODA) datasets.

Final sample sizes across the three included studies

The primary discovery dataset (FAMPATH) consisted of 25 HV, 20 HV, 25 HR-MOOD), and 18 HR-MOOD+SA). The primary replication (CONTE) dataset included 35 HV, 26 individuals with current mood disorder (MOOD), and 20 participants with MOOD and one or more lifetime suicide attempts (MOOD+SA). A secondary replication dataset (NODA) included 12 HV and 18 MOOD participants. The total replication sample size (CONTE and NODA) is N=111, while the total in all sets is N=199. Note that the replication cohorts (CONTE and NODA) were acquired and analyzed the same way as was the FAMPATH cohort in terms of brain imaging acquisition and segmentation analysis. Our group has not previously published on volumetric MRI findings in the CONTE and NODA cohorts.

T1 structural brain image acquisition and preprocessing

The Pittsburgh and NY sites have standardized MRI protocols. MRI images were acquired on a GE 3 Tesla MR750 scanner utilizing 18 high order superconducting shim coils and 5 resistive shim coils, and acquiring images using a 32-channel head-coil at the New York site and on a Siemens 3T Trios scanner in Pittsburgh. A sagittal

T1-weighted structural scan was acquired over the whole brain with the following parameters: repetition time (TR), 6 ms; echo time (TE), 2.4 ms; flip angle, 8°; 1-mm slice thickness (zero gap); 178 slices; and field of view, 256 x 256mm. All images were reconstructed to a size of 256 x 256, with an isotropic resolution of 1mm³. This T1-weighted structural image was used for identification of regions of interest, gray-white matter segmentation, skull-stripping, and for PET co-registration. The structural images were first processed using recon-all with FreeSurfer 7.1 (19). Details of the recon-all and FreeSurfer segmentation process are described elsewhere (20,21). Next the hippocampus subfields were segmented using the segmentHA_T1.sh bash script (22). Residual plots and histograms of resulting volumes were inspected for outliers.

Linear Mixed Effects modeling

Matlab table structures were used to store hippocampus and eTIV volumes and clinical (group) labels. Linear mixed effects (LME) by Restricted Maximum Likelihood (REML) with t-tests using Satterthwaite's method (lmerModLmerTest) models were applied using R Version 3.6.3. Analysis with log volume as outcome focused on 3 main models/comparisons: 1) HV vs. HR-MOOD+SA; 2) HV vs. HR-MOOD; and 3) HV vs. HR-R. Since we anticipated that group difference would vary across subfields (1), the primary models included the fixed effects region, age, sex, log of total intracranial volume, site, and group x region interaction, and subject was treated as a random effect. The first two models (HV vs. HR-MOOD+SA and HV vs. HR-MOOD) were used to identify subfields of interest altered in mood disorder (HR-MOOD) and suicide attempt in the context of mood disorder (HR-MOOD+SA) in high familial risk individuals.

The subfields found to differ in these comparisons were considered as ROIs in the third analysis comparing HV vs. HR-R. Note that this analysis (and all preceding analyses) still include all 8 hippocampus subfields in order to “borrow strength” across regions within each subject. A secondary set of models included the same terms as the primary models with the exception that the group x region interaction term was replaced with the main effect of group.

Results

Demographic and clinical results

Within the primary discovery and replication datasets (FAMPATH and CONTE), the groups did not differ in sex ratio or age (all p-values > 0.2, Table 1). The HR-MOOD and HR-MOOD+SA groups in the FAMPATH dataset had relatively lower current Beck Depression Inventory (BDI) and Hamilton Depression Rating Scale (HAM17) scores since the study included individuals with lifetime diagnosis of mood disorder but they were not required to be currently ill (Table 1). The primary replication CONTE dataset MOOD and MOOD+SA subgroups had higher BDI and HAM17 scores that were comparable to the scores in the exploratory replication NODA dataset because both studies examined currently depressed subjects (Table 1).

The FAMPATH HR, HR-MOOD, and HR-MOOD+SA groups were selected to have at least one or more 1st or 2nd degree relative with early onset MOOD+SA. Although the CONTE and NODA samples were not selected for family history of MOOD+SA, it is possible the sample included some high familial risk individuals. We therefore compared the number of 1st degree relatives who had completed suicide (a

variable in common across all cohorts) in the affected MOOD and MOOD+SA subsamples of each cohorts (Table 1). Thirty-five of 43 participants (42%) in the FAMPATH HR-MOOD and HR-MOOD+SA subsamples had a 1st degree relative with completed suicide, compared to 2 of 46 (4%) in the CONTE MOOD and MOOD+SA subsamples (Fisher's exact test $p < 0.00001$) and 1 (6%) in the NODA MOOD sample (Fisher's exact test $p < 0.00001$). The results indicate significantly lower proportions of high familial risk individuals in the CONTE and NODA samples.

Smaller CA1 and CA3 in both depressed suicide attemptors and unaffected individuals with high familial suicide risk

A model with HV and HR-MOOD+SA found no evidence for an overall region x group interactions ($F_{7,134}=1.32$, $p=0.24$). However, post hoc t-tests suggested lower volumes in CA1 body ($p=0.02$ uncorrected) and CA3 body ($p=0.01$ uncorrected) in HR-MOOD+SA relative to HV (gray shaded rows in Table 2). A similar model with HV and HR-MOOD found no evidence for region x group interaction (Table 3). A similar model with HV and HR found no evidence for overall region x group interaction ($F_{1,141}=1.44$, $p=0.19$). Post-hoc t-tests showed trend-level evidence for lower volumes in CA1 body ($p=0.067$ uncorrected) and CA3 body ($p=0.0057$ uncorrected) in HR relative to HV (grey shaded rows in Table 4). Thus, the same regions that were smaller in HR-MOOD+SA vs. HV (CA1 body and CA3 body) were also smaller in HR relative to HV.

An additional set of models similar to the above were estimated which included a main effect of group term instead of a group x region interaction term. In these models,

the main effects of group were not detected at $p < 0.05$ in equivalent models in which the group x region interaction term was replaced with the main effect of group. This revealed trend-level evidence for reduced volumes across regions in HR vs. HV ($F_{1,39} = 3.54$, $p = 0.07$) and HR-MOOD+SA vs. HV $F_{1,47} = 4.0$, $p = 0.05$), with no main effect detected in the HR-MOOD vs. HV comparison ($F_{1,44} = 0.79$, $p = 0.38$).

Depressed suicide attemptors and non-attemptors not selected for familial risk do not show reduced CA1 body and CA3 body relative to HV

Similar models in the CONTE dataset found no region x group interactions in MOOD+SA vs. HV, while there was trend-level evidence for *higher* CA1 body volumes in MOOD vs. HV ($t(169) = 1.9$, $p = 0.06$, Supplementary Table S1). A similar model in the secondary replication dataset (NODA) also found evidence for *higher* CA3 body volume in MOOD vs. HV ($t(71) = 2.8$, $p = 0.006$, Supplementary Table S1).

Post hoc analysis of hippocampus subvolumes in samples not selected for familial risk

The CONTE MOOD+SA and NODA MOOD samples contained 2 and 1 individuals, respectively with one or more 1st degree relatives with MOOD+SA (Table 1). We hypothesized these individuals tend to have lower CA1 and CA3 volumes relative to other participants in their group that had no 1st degree relatives with MOOD+SA. To explore this, the CA1 volumes for CONTE MOOD+SA and NODA MOOD samples were adjusted for TIV, and the residuals were z-score normalized. Consistent with our hypothesis, the 2 high familial risk individuals in CONTE MOOD+SA had z-scores of -2.24 and -0.4 (CA1) and -1.47 and -0.54 (CA3). The single high

familial risk individual in the NODA MOOD sample had z-score -1.15 (CA1) and -0.96 (CA3). We converted the z-scores to 1-sided p-values and submitted them to Fisher's combined probability test (23) across the 3 participants for CA1 and CA3 separately. This yielded Fisher's combined $p=0.02$ for CA1 and $p=0.08$ for CA3, suggesting that our observation of lower CA1 and CA3 volumes in the 3 high familial risk individuals in the CONTE and NODA datasets was not due to chance.

Discussion

In this study we find evidence for smaller hippocampal CA1 and CA3 volumes in individuals with high familial risk for MOOD and suicide, but with no lifetime MOOD diagnosis or suicide attempt and who have passed through the age of greatest risk for psychopathology onset. Low hippocampal volumes have been consistently implicated in mood disorders (1,2) and other groups have also found smaller hippocampal volumes in MOOD+SA vs. MOOD (12,24). In addition, studies have found similar effects in healthy individuals at-risk for depression (15,16). Taken together, the above findings suggest low hippocampal CA1 and CA3 may be a biological endophenotype for suicidal behavior. That the HR individuals did not develop depression and suicidal behavior (despite having smaller low CA1/3 volumes) suggest the absence of other risk factors (i.e. environment, chronic stress, etc.) (11) and/or the presence of compensatory mechanisms explains the absence of psychopathology in this HR group.

Our results are consistent with a recent study that found hippocampal proper subfields (CA1-4, DG) have high heritability ($h^2 \sim 0.75-0.85$) (25). The study analyzed young, healthy twins from the Queensland Twins Imaging Study (N=728) and found that

of 12 hippocampal subfields examined, the CA1 had the second highest heritability ($h^2 = 0.84$) while CA3 was near the median ($h^2 = 0.75$). By comparison the hippocampal fissure had relatively low (but still significant) heritability ($h^2 = 0.56$). These results are also consistent with those from a large mega-analysis ($N > 20K$) that found CA1 and CA3 had high heritability relative to other subfields using SNP-based heritability analysis (26).

In addition to heritability (i.e. genetic factors), early environmental factors such as early life trauma or stress may comprise a familial risk factor for mood disorder and suicide that results in low hippocampal volumes (27–30). Consistent with our findings, previous studies using advanced hippocampal segmentation found smaller volumes specifically in CA1/3 or DG in children with early life adversity (30–32). It is possible the genetics or early adversity, or both, contribute to lower CA1/3 volumes in individuals with high familial risk for mood disorder and suicidal behavior.

Contrary to our expectations, we did not find low hippocampal subfield volumes in MOOD or MOOD+SA groups (individuals with lifetime diagnosis of mood disorder with or without previous suicide attempt **not** selected for high familial risk). This is consistent with previous mixed results and negative finding studies that found no differences in hippocampal volumes in mood disorder (3–6). These null findings are consistent with the interpretation that low hippocampal volumes are associated with risk for psychopathology, but not illness severity and sequelae. This interpretation may also explain findings of lower CA1-4 and other principal hippocampus substructure volumes in patients with recurrent vs. first presentation depression and with longer

symptom durations (1), since these outcomes may reflect a higher risk for depression rather than effects of the illness progressions.

Our study is limited by a relatively small sample size. Additional research in independent cohorts is required to confirm and extend the findings. Future research should also examine the role of CA1/CA3 in emotional and cognitive functioning associated with psychopathology, which may reveal more sensitive neural markers of risk or resilience for mood disorders and suicidal behavior.

Acknowledgments

This study was supported by National Institute of Mental Health MH108039 (NMM) MH056390, 5P50MH090964 and 5R01MH040695 (JJM) and American Foundation for Suicide Prevention (AFSP) SRG-0-102-16 (SPP). The funding sources did not participate in the design and conduct of the study; in the collection, management, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

Conflicts of interest

Authors SPP NMM ML JMM have no conflicts of interest to report. JJM and AB receive royalties for commercial use of the C-SSRS from the Research Foundation for Mental Hygiene. DAB receives royalties from the electronic self-rated version of the C-SSRS from eRT, Inc.

Data availability statement

The derived data that support the findings of this study are available upon

request from the corresponding authors. The raw data are not publicly available as participants of this study did not agree for their data to be shared publicly.

Table 1. Demographics and behavioral data. Group differences were assessed using Kruskal–Wallis one-way ANOVA (continuous variables) or chi² test (categorical variables). Risk is indicated by FamDep and FamSui where No/Yes=0/(1 or more) 1st degree relatives with MOOD or completed suicide, respectively. iqr=interquartile range.

FAMPATH	HV (N=25)	HR (N=20)	HR-MOOD (N=25)	HR-MOOD+SA (N=18)	pval
Age: mean (iqr)	40.24 (15.25)	38.85 (20.50)	36.24 (15.50)	38.17 (18.00)	0.56
HAM17: mean (iqr)	0.88 (1.25)	1.30 (2.00)	3.56 (4.75)	9.61 (12.00)	0
BDI: mean (iqr)	1.24 (1.25)	2.05 (4.00)	6.72 (8.50)	16.39 (8.00)	0
eTIV: mean (iqr)	1415283.80 (255923.50)	1334939.75 (157165.97)	1415271.33 (222531.34)	1317348.85 (192865.95)	0.06
Sex: count (%)	8/17 (0.32/0.68)	5/15 (0.25/0.75)	9/16 (0.36/0.64)	6/12 (0.33/0.67)	0.89
FamDep: count (%)	25/0 (1.00/0.00)	5/15 (0.25/0.75)	4/21 (0.16/0.84)	4/14 (0.22/0.78)	0
FamSui: count (%)	25/0 (1.00/0.00)	7/13 (0.35/0.65)	13/12 (0.52/0.48)	12/6 (0.67/0.33)	0
site: count (%)	15/10 (0.60/0.40)	7/13 (0.35/0.65)	11/14 (0.44/0.56)	8/10 (0.44/0.56)	0.39
CONTE	HV (N=35)	NA	MOOD (N=26)	MOOD+SA (N=20)	pval
Age: mean (iqr)	32.43 (10.75)	NA	32.15 (11.00)	29.15 (13.00)	0.36
HAM17: mean (iqr)	0.77 (1.00)	NA	16.50 (8.00)	17.85 (7.50)	0
BDI: mean (iqr)	0.80 (1.00)	NA	23.23 (12.00)	23.53 (5.75)	0
eTIV: mean (iqr)	1380106.18 (239227.71)	NA	1353303.14 (260311.46)	1314553.76 (155533.03)	0.54
Sex: count (%)	14/21/0 (0.40/0.60/0.00)	NA	9/16/1 (0.35/0.62/0.04)	8/12/0 (0.40/0.60/0.00)	0.69
FamDep: count (%)	35/0 (1.00/0.00)	NA	12/14 (0.46/0.54)	11/9 (0.55/0.45)	0
FamSui: count (%)	35/0 (1.00/0.00)	NA	26/0 (1.00/0.00)	18/2 (0.90/0.10)	0.04
site: count (%)	35 (1.00)	NA	26 (1.00)	20 (1.00)	NA
NODA	HV (N=12)	NA	MOOD (N=18)	NA	pval
Age: mean (iqr)	32.25 (19.50)	NA	37.83 (16.00)	NA	0.02
HAM17: mean (iqr)	1.08 (1.50)	NA	18.44 (5.00)	NA	0
BDI: mean (iqr)	1.08 (0.00)	NA	25.28 (7.00)	NA	0
eTIV: mean (iqr)	1437456.07 (200838.75)	NA	1451848.70 (171051.70)	NA	0.83
Sex: count (%)	3/9 (0.25/0.75)	NA	6/12 (0.33/0.67)	NA	0.63
FamDep: count (%)	12/0 (1.00/0.00)	NA	9/9 (0.50/0.50)	NA	0
FamSui: count (%)	12/0 (1.00/0.00)	NA	17/1 (0.94/0.06)	NA	0.41
site: count (%)	6/6 (0.50/0.50)	NA	9/9 (0.50/0.50)	NA	1

Table 2. Results from LME comparing 8 hippocampal subfields in HV vs. HR-MOOD+SA.
Grey rows indicate CA1 body and CA3 body are smaller in HR-MOOD+SA relative to HV (p<0.05 uncorrected).

Estimate	Std.	Error	df	t-value	Pr(> t)
regionCA1_body	-2.361	2.416	37.003	-0.977	0.3348
regionCA1_head	-0.913	2.416	37.003	-0.378	0.7076
regionCA3_body	-2.775	2.416	37.003	-1.148	0.2581
regionCA3_head	-2.398	2.416	37.003	-0.993	0.3274
regionCA4_body	-2.387	2.416	37.003	-0.988	0.3296
regionCA4_head	-2.356	2.416	37.003	-0.975	0.3359
regionGC_ML_DG_body	-2.255	2.416	37.003	-0.933	0.3568
regionGC_ML_DG_head	-2.155	2.416	37.003	-0.892	0.3782
age	-0.001	0.002	37.000	-0.462	0.6467
sex2	-0.023	0.038	37.000	-0.615	0.5421
log(e_tiv)	0.506	0.170	37.000	2.971	0.0052
site2	-0.031	0.033	37.000	-0.933	0.3567
regionCA1_body:groupHV	0.098	0.042	70.218	2.348	0.0217
regionCA1_head:groupHV	0.042	0.042	70.218	1.008	0.3170
regionCA3_body:groupHV	0.110	0.042	70.218	2.65	0.0100
regionCA3_head:groupHV	0.060	0.042	70.218	1.449	0.1517
regionCA4_body:groupHV	0.061	0.042	70.218	1.468	0.1466
regionCA4_head:groupHV	0.065	0.042	70.218	1.562	0.1228
regionGC_ML_DG_body:groupHV	0.057	0.042	70.218	1.376	0.1733
regionGC_ML_DG_head:groupHV	0.058	0.042	70.218	1.407	0.1637

Table 3. Results from LME comparing 8 hippocampal subfields in HV vs. HR-MOOD. No regions differed between groups at $p < 0.05$ uncorrected.

Estimate	Std.	Error	df	t-value	Pr(> t)
regionCA1_body	-1.304	1.849	44.010	-0.705	0.4845
regionCA1_head	0.118	1.849	44.010	0.064	0.9496
regionCA3_body	-1.707	1.849	44.010	-0.923	0.3611
regionCA3_head	-1.355	1.849	44.010	-0.733	0.4675
regionCA4_body	-1.335	1.849	44.010	-0.722	0.4741
regionCA4_head	-1.310	1.849	44.010	-0.709	0.4823
regionGC_ML_DG_body	-1.202	1.849	44.010	-0.65	0.5191
regionGC_ML_DG_head	-1.116	1.849	44.010	-0.603	0.5494
age	-0.001	0.001	44.000	-0.396	0.6942
sex2	-0.018	0.030	44.000	-0.608	0.5466
log(e_tiv)	0.433	0.130	44.000	3.342	0.0017
site2	-0.001	0.025	44.000	-0.056	0.9560
regionCA1_body:groupHV	0.040	0.033	131.400	1.195	0.2342
regionCA1_head:groupHV	0.011	0.033	131.400	0.322	0.7480
regionCA3_body:groupHV	0.042	0.033	131.400	1.247	0.2148
regionCA3_head:groupHV	0.017	0.033	131.400	0.511	0.6104
regionCA4_body:groupHV	0.009	0.033	131.400	0.262	0.7936
regionCA4_head:groupHV	0.019	0.033	131.400	0.576	0.5655
regionGC_ML_DG_body:groupHV	0.004	0.033	131.400	0.126	0.9001
regionGC_ML_DG_head:groupHV	0.019	0.033	131.400	0.568	0.5711

Table 4. Results from LME comparing 8 hippocampal subfields in HV vs. HR-R Grey rows indicate CA1 body (trend level) and CA3 body are smaller in HR-MOOD+SA relative to HV (p=0.066 and p=0.006 uncorrected, respectively).

Estimate	Std.	Error	df	t-value	Pr(> t)
regionCA1_body	-1.336	1.789	39.006	-0.747	0.4596
regionCA1_head	0.092	1.789	39.006	0.052	0.9591
regionCA3_body	-1.770	1.789	39.006	-0.989	0.3286
regionCA3_head	-1.385	1.789	39.006	-0.774	0.4434
regionCA4_body	-1.373	1.789	39.006	-0.768	0.4473
regionCA4_head	-1.334	1.789	39.006	-0.746	0.4601
regionGC_ML_DG_body	-1.238	1.789	39.006	-0.692	0.4930
regionGC_ML_DG_head	-1.142	1.789	39.006	-0.638	0.5271
age	-0.002	0.001	39.000	-2.14	0.0387
sex2	-0.078	0.029	39.000	-2.751	0.0090
log(e_tiv)	0.442	0.126	39.000	3.502	0.0012
site2	0.013	0.025	39.000	0.524	0.6035
regionCA1_body:groupHV	0.062	0.033	100.444	1.857	0.0662
regionCA1_head:groupHV	0.025	0.033	100.444	0.764	0.4468
regionCA3_body:groupHV	0.094	0.033	100.444	2.826	0.0057
regionCA3_head:groupHV	0.037	0.033	100.444	1.102	0.2733
regionCA4_body:groupHV	0.037	0.033	100.444	1.1	0.2738
regionCA4_head:groupHV	0.033	0.033	100.444	0.991	0.3243
regionGC_ML_DG_body:groupHV	0.030	0.033	100.444	0.895	0.3727
regionGC_ML_DG_head:groupHV	0.034	0.033	100.444	1.033	0.3042

Figure 1

Figure Legends

Figure 1. Mean volume differences of hippocampus proper (HP) in the Fampath dataset.

Bar plots represent mean volume differences relative to the HV group for the HR-R group (blue), HR-MOOD (grey), HR-MOOD+SA (yellow). Results show larger CA1-body in HV vs. MOOD+SA (yellow bar, $p=0.02$) and trend level evidence for larger CA1-body in HV vs HR-R vs. HV (blue bar, $p=0.066$). Results also show larger CA3-body in HV vs. MOOD+SA (yellow bar, $p=0.01$) and larger CA3-body in HV vs. HR-R (blue bar, $p=0.0057$). Error bars represented 2*standard error. Reported p-values are uncorrected.

References

1. Roddy DW, Farrell C, Doolin K, Roman E, Tozzi L, Frodl T, et al. The Hippocampus in Depression: More Than the Sum of Its Parts? Advanced Hippocampal Substructure Segmentation in Depression. *Biol Psychiatry*. 2019 Mar 15;85(6):487–97.
2. Haukvik UK, Gurholt TP, Nerland S, Elvsåshagen T, Akudjedu TN, Alda M, et al. In vivo hippocampal subfield volumes in bipolar disorder-A mega-analysis from The Enhancing Neuro Imaging Genetics through Meta-Analysis Bipolar Disorder Working Group. *Hum Brain Mapp*. 2022 Jan;43(1):385–98.
3. Greenberg DL, Payne ME, MacFall JR, Steffens DC, Krishnan RR. Hippocampal volumes and depression subtypes. *Psychiatry Res*. 2008 Jul 15;163(2):126–32.
4. Rusch BD, Abercrombie HC, Oakes TR, Schaefer SM, Davidson RJ. Hippocampal morphometry in depressed patients and control subjects: relations to anxiety symptoms. *Biol Psychiatry*. 2001 Dec 15;50(12):960–4.
5. Videbech P, Ravnkilde B. Hippocampal volume and depression: a meta-analysis of MRI studies. *Am J Psychiatry*. 2004 Nov;161(11):1957–66.
6. Phillips JL, Batten LA, Tremblay P, Aldosary F, Blier P. A Prospective, Longitudinal Study of the Effect of Remission on Cortical Thickness and Hippocampal Volume in Patients with Treatment-Resistant Depression. *Int J Neuropsychopharmacol*. 2015 Mar 30;18(8):pyv037.
7. Bludau S, Bzdok D, Gruber O, Kohn N, Riedl V, Sorg C, et al. Medial Prefrontal Aberrations in Major Depressive Disorder Revealed by Cytoarchitectonically Informed Voxel-Based Morphometry. *Am J Psychiatry*. 2016 Mar 1;173(3):291–8.
8. Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. *Proc Natl Acad Sci U S A*. 1996 Apr 30;93(9):3908–13.
9. MacQueen GM, Yucel K, Taylor VH, Macdonald K, Joffe R. Posterior hippocampal volumes are associated with remission rates in patients with major depressive disorder. *Biol Psychiatry*. 2008 Nov 15;64(10):880–3.
10. MacQueen GM, Campbell S, McEwen BS, Macdonald K, Amano S, Joffe RT, et al. Course of illness, hippocampal function, and hippocampal volume in major depression. *Proc Natl Acad Sci U S A*. 2003 Feb 4;100(3):1387–92.
11. Belleau EL, Treadway MT, Pizzagalli DA. The Impact of Stress and Major Depressive Disorder on Hippocampal and Medial Prefrontal Cortex Morphology. *Biol Psychiatry*. 2019 Mar 15;85(6):443–53.
12. Colle R, Chupin M, Cury C, Vandendrie C, Gressier F, Hardy P, et al. Depressed suicide attempters have smaller hippocampus than depressed patients without suicide attempts. *J Psychiatr Res*. 2015 Feb;61:13–8.
13. Han K-M, Kim A, Kang W, Kang Y, Kang J, Won E, et al. Hippocampal subfield volumes in major depressive disorder and bipolar disorder. *Eur Psychiatry*. 2019;57:70–7.
14. Sheline YI, Liston C, McEwen BS. Parsing the Hippocampus in Depression: Chronic Stress, Hippocampal Volume, and Major Depressive Disorder. *Biol Psychiatry*. 2019 Mar 15;85(6):436–8.
15. Chen MC, Hamilton JP, Gotlib IH. Decreased hippocampal volume in healthy girls at risk of depression. *Arch Gen Psychiatry*. 2010 Mar;67(3):270–6.
16. Amico F, Meisenzahl E, Koutsouleris N, Reiser M, Möller H-J, Frodl T. Structural MRI correlates for vulnerability and resilience to major depressive disorder. *J Psychiatry Neurosci*. 2011 Jan;36(1):15–22.
17. Brent DA, Melhem NM, Oquendo M, Burke A, Birmaher B, Stanley B, et al. Familial pathways to early-onset suicide attempt: a 5.6-year prospective study. *JAMA Psychiatry*. 2015 Feb;72(2):160–8.
18. Melhem NM, Porta G, Oquendo MA, Zelazny J, Keilp JG, Iyengar S, et al. Severity and

Variability of Depression Symptoms Predicting Suicide Attempt in High-Risk Individuals. *JAMA Psychiatry*. 2019 Jun 1;76(6):603–13.

19. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proceedings of the National Academy of Sciences [Internet]*. 2000; Available from: <http://www.pnas.org/content/97/20/11050.short>
20. Fischl B, Salat DH, van der Kouwe AJW, Makris N, Ségonne F, Quinn BT, et al. Sequence-independent segmentation of magnetic resonance images. *Neuroimage*. 2004;23 Suppl 1:S69-84.
21. Salat DH, Buckner RL, Snyder AZ, Greve DN, Desikan RSR, Busa E, et al. Thinning of the cerebral cortex in aging. *Cereb Cortex*. 2004 Jul;14(7):721–30.
22. Iglesias JE, Augustinack JC, Nguyen K, Player CM, Player A, Wright M, et al. A computational atlas of the hippocampal formation using ex vivo, ultra-high resolution MRI: Application to adaptive segmentation of in vivo MRI. *Neuroimage*. 2015 Jul 15;115:117–37.
23. Mosteller F, Fisher RA. Questions and Answers. *The American Statistician*. 1948;2(5):30–1.
24. Sarkinaite M, Gleizniene R, Adomaitiene V, Dambrauskiene K, Raskauskiene N, Steibliene V. Volumetric MRI Analysis of Brain Structures in Patients with History of First and Repeated Suicide Attempts: A Cross Sectional Study. *Diagnostics (Basel)*. 2021 Mar 10;11(3):488.
25. Whelan CD, Hibar DP, van Velzen LS, Zannas AS, Carrillo-Roa T, McMahon K, et al. Heritability and reliability of automatically segmented human hippocampal formation subregions. *Neuroimage*. 2016 Mar;128:125–37.
26. van der Meer D, Rokicki J, Kaufmann T, Córdova-Palomera A, Moberget T, Alnæs D, et al. Brain scans from 21,297 individuals reveal the genetic architecture of hippocampal subfield volumes. *Mol Psychiatry*. 2020 Nov;25(11):3053–65.
27. Woon FL, Hedges DW. Hippocampal and amygdala volumes in children and adults with childhood maltreatment-related posttraumatic stress disorder: a meta-analysis. *Hippocampus*. 2008;18(8):729–36.
28. Weissman DG, Lambert HK, Rodman AM, Peverill M, Sheridan MA, McLaughlin KA. Reduced hippocampal and amygdala volume as a mechanism underlying stress sensitization to depression following childhood trauma. *Depress Anxiety*. 2020 Sep;37(9):916–25.
29. Vythilingam M, Heim C, Newport J, Miller AH, Anderson E, Bronen R, et al. Childhood trauma associated with smaller hippocampal volume in women with major depression. *Am J Psychiatry*. 2002 Dec;159(12):2072–80.
30. Teicher MH, Anderson CM, Ohashi K, Khan A, McGreenery CE, Bolger EA, et al. Differential effects of childhood neglect and abuse during sensitive exposure periods on male and female hippocampus. *Neuroimage*. 2018 Apr 1;169:443–52.
31. Dahmen B, Puetz VB, Scharke W, von Polier GG, Herpertz-Dahlmann B, Konrad K. Effects of Early-Life Adversity on Hippocampal Structures and Associated HPA Axis Functions. *Dev Neurosci*. 2018;40(1):13–22.
32. Teicher MH, Anderson CM, Polcari A. Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum. *Proc Natl Acad Sci U S A*. 2012 Feb 28;109(9):E563-572.

Supplemental Material for “Smaller volumes of the cornu ammonis (CA1 and CA3) may
be a biological endophenotype for suicide risk in mood disorders”

Authors: Spiro P. Pantazatos, Ph.D.^{1,2,*}, Todd Ogden, Ph.D.¹, Nadine M. Melhem Ph.D.³,
David A. Brent M.D.³, Mohammad Lisanpezheshki M.D.,^{1,2} Ainsley Burke Ph.D.^{1,2} ,
Jeffrey M. Miller, M.D.^{1,2} , J. John Mann, M.D.^{1,2,*}

¹Molecular Imaging and Neuropathology Area, New York State Psychiatric Institute,
New York, NY; ²Department of Psychiatry, Columbia University Irving Medical Center,
New York, NY; ³Department of Psychiatry, University of Pittsburgh School of Medicine,
Pittsburgh, PA, United States;

* To whom correspondence should be addressed:

E-mail: spp2101@columbia.edu

Correspondence can also be addressed to:

E-mail: jjm@columbia.edu

Contents:

Supplementary Methods

Supplementary Results

Supplementary Figure 1

Supplementary References

Running title: Hippocampal substructure volumes and familial suicide risk

Keywords: hippocampus subfields, risk factors, suicidal behavior, familial transmission,
early life adversity, heritable

Supplementary Methods

Inclusion/exclusion criteria and assessments

We required all subjects to be off psychotropic medication and other serotonergic medications for at least 3 weeks, and off any anticoagulant medication for a minimum of 10 days. The NYC site allowed individuals to enroll if they were taking medication that was ineffective after a trial of adequate dose and duration. Those individuals underwent medication taper followed by 3-week medication washout prior to scanning. We permitted use of benzodiazepines up to 3 days prior to scanning. Exclusion criteria consisted of: other Axis I psychiatric diagnosis, current alcohol or drug use disorder, history of IV drug use or ecstasy use more than twice, family history of schizophrenia, significant active physical illness, lacking capacity to consent, pregnancy, presence of metal implants or a medicinal patch, medical or occupational radiation exposure within the past 12 months, or a head injury causing loss of consciousness for more than three minutes or measureable memory impairment.

Psychiatric diagnoses were assessed in all groups including HVs using the Structured Clinical Interview for DSM-5 disorders (SCID-5) ¹ by an experienced clinician with at least a Master's degree and on the basis of other data such as past medical records, and were reviewed by senior clinicians at a consensus conference. Familial history of psychiatric disorders was assessed using the FH-RDC ². Suicidal behavior was assessed using the Columbia Suicide History Form and the Columbia Suicide Severity Rating Scale (C-SSRS) ³ and depressive symptoms were assessed using the Hamilton Depression Rating Scale (HAMD) ⁴. Self-reported symptoms were assessed using the Beck Depression Inventory (BDI) ⁵ and the Beck Hopelessness Inventory

(BHI)⁶. We also assessed impulsivity and aggression using the Brown-Goodwin Lifetime History of Aggression Scale⁷ and the Barratt Impulsivity Scale (BIS-II)⁸, respectively. Childhood abuse and neglect was assessed using the Childhood Trauma Questionnaire (CTQ)⁹.

Inclusion/exclusion criteria: CONTE and NODA

The CONTE study enrolled individuals with current major depressive disorder (MDD), with and without a history of past suicide attempt (MOOD and MOOD+SA), as well as healthy volunteers (HV). MOOD and MOOD+SA groups had a 17-item Hamilton Depression Rating Scale of ≥ 16 at the time of enrollment. Other eligibility criteria and assessments were consistent with the FAMPATH sample except for the family history requirements and psychiatric illness assessment used SCID-1 for DSM-4 disorders¹⁰.

The (Neurobiology of Depression and Antidepressant Action) NODA study participants were individuals with current MDD with a current 17-item HDRS score ≥ 16 ; remitted MDD with 17-item HDRS score < 10 , in remission for ≥ 1 year and off antidepressants and mood stabilizers for ≥ 6 months; and HVs. For the current analysis, current and remitted MDD participants were combined, and categorized by suicide attempt history into MOOD and MOOD+SA groups. Other eligibility criteria and assessments were consistent with the FAMPATH sample except for the family history requirements and psychiatric illness assessment used SCID-1 for DSM-4 disorders¹⁰.

Supplementary Table S1. Results from LME comparing 8 hippocampal subfields in HV vs. MOOD+SA in the CONTE dataset. Grey rows indicate CA1 body and CA3 body are smaller in HR-MOOD+SA relative to HV ($p < 0.05$ uncorrected).

Fixed effects of interest	Estimate	Std. Error	df	t-value	p-value
<u>CONTE MOOD+SA vs. HV</u>					
regionCA1_body:groupMOOD_SA	0.001526	0.04289	129.222368	0.036	0.971671
regionCA1_head:groupMOOD_SA	-0.018477	0.04289	129.222368	-0.431	0.667326
regionCA3_body:groupMOOD_SA	-0.007051	0.04289	129.222368	-0.164	0.869674
regionCA3_head:groupMOOD_SA	-0.005595	0.04289	129.222368	-0.13	0.896407
regionCA4_body:groupMOOD_SA	0.009604	0.04289	129.222368	0.224	0.82317
regionCA4_head:groupMOOD_SA	-0.024797	0.04289	129.222368	-0.578	0.56417
regionGC_ML_DG_body:groupMOOD_SA	0.00703	0.04289	129.222368	0.164	0.870051
regionGC_ML_DG_head:groupMOOD_SA	-0.018723	0.04289	129.222368	-0.437	0.663169
<u>CONTE MOOD vs. HV</u>					
regionCA1_body:groupMOOD	5.80E-02	3.05E-02	1.69E+02	1.899	0.0593
regionCA1_head:groupMOOD	6.32E-03	3.05E-02	1.69E+02	0.207	0.8362
regionCA3_body:groupMOOD	5.36E-02	3.05E-02	1.69E+02	1.753	0.0814
regionCA3_head:groupMOOD	2.55E-02	3.05E-02	1.69E+02	0.835	0.405
regionCA4_body:groupMOOD	7.35E-03	3.05E-02	1.69E+02	0.241	0.8102
regionCA4_head:groupMOOD	1.01E-02	3.05E-02	1.69E+02	0.332	0.7402
regionGC_ML_DG_body:groupMOOD	5.79E-03	3.05E-02	1.69E+02	0.189	0.8499
regionGC_ML_DG_head:groupMOOD	8.14E-03	3.05E-02	1.69E+02	0.267	0.7901
<u>NODA MOOD vs. HV</u>					
regionCA1_body:groupMOOD	0.039004	0.043899	71.215642	0.888	0.37727
regionCA1_head:groupMOOD	0.057493	0.043899	71.215642	1.31	0.19452
regionCA3_body:groupMOOD	0.124328	0.043899	71.215642	2.832	0.00601
regionCA3_head:groupMOOD	0.100414	0.043899	71.215642	2.287	0.02515
regionCA4_body:groupMOOD	0.047358	0.043899	71.215642	1.079	0.28432
regionCA4_head:groupMOOD	0.069428	0.043899	71.215642	1.582	0.11819
regionGC_ML_DG_body:groupMOOD	0.029857	0.043899	71.215642	0.68	0.49863
regionGC_ML_DG_head:groupMOOD	0.073691	0.043899	71.215642	1.679	0.09761

Supplementary Figure Legends

Figure 1. Slices showing hippocampus proper (HP) from a sample subject. The 8 HP regions are shown overlaid on a subject's MRI scan.

Supplementary Figure 1

References

1. First, M., Williams, J., Karg, R. & Spitzer, R. Structured clinical interview for DSM-5—Research version (SCID-5 for DSM-5, research version; SCID-5-RV). *Arlington, VA: American Psychiatric Association* 1–94 (2015).
2. Andreasen, N. C., Endicott, J., Spitzer, R. L. & Winokur, G. The family history method using diagnostic criteria. Reliability and validity. *Arch Gen Psychiatry* **34**, 1229–1235 (1977).
3. Posner, K. *et al.* The Columbia-Suicide Severity Rating Scale: initial validity and internal consistency findings from three multisite studies with adolescents and adults. *The American journal of psychiatry* **168**, 1266–1277 (2011).
4. Hamilton, M. A rating scale for depression. *Journal of Neurology, Neurosurgery & Psychiatry* (1960).
5. Beck, A. T., Steer, R. A. & Brown, G. K. Manual for the beck depression inventory-II. *San Antonio, TX: Psychological Corporation* (1996).
6. Beck, A. T. & Steer, R. A. Clinical predictors of eventual suicide: a 5- to 10-year prospective study of suicide attempters. *J Affect Disord* **17**, 203–209 (1989).
7. Brown, G. L. *et al.* Aggression, suicide, and serotonin: relationships to CSF amine metabolites. *Am J Psychiatry* **139**, 741–746 (1982).
8. Patton, J. H., Stanford, M. S. & Barratt, E. S. Factor structure of the Barratt impulsiveness scale. *J Clin Psychol* **51**, 768–774 (1995).
9. Bernstein, D. P., Ahluvalia, T., Pogge, D. & Handelsman, L. Validity of the Childhood Trauma Questionnaire in an Adolescent Psychiatric Population. *Journal of the American Academy of Child & Adolescent Psychiatry* **36**, 340–348 (1997).
10. First, M. B., Spitzer, R. L., Gibbon, M. & Williams, J. B. W. Structured clinical interview for DSM-IV axis I disorders. *New York: New York State* (1995).