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REGULAR ARTICLE

Differential longitudinal changes of hippocampal subfields in patients with anorexia nervosa

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Background: Anorexia nervosa (AN) is a mental disorder characterized by dietary restriction, fear of gaining weight, and distorted body image. Recent studies indicate that the hippocampus, crucial for learning and memory, may be affected in AN, yet subfield-specific effects remain unclear. We investigated hippocampal subfield alterations in acute AN, changes following weight restoration, and their associations with leptin levels.

Methods: T1-weighted magnetic resonance imaging scans were processed using FreeSurfer. We compared 22 left and right hemispheric hippocampal subfield volumes cross-sectionally and longitudinally in females with acute AN ($n = 165$ at baseline, $n = 110$ after partial weight restoration), healthy female controls (HCs; $n = 271$), and females after long-term recovery from AN ($n = 79$) using linear models.

Results: We found that most hippocampal subfield volumes were significantly reduced in patients with AN compared with HCs (∼−3.9%). Certain areas such as the subiculum exhibited no significant reduction in the acute state of AN, while other areas, such as the hippocampal tail, showed strong decreases (∼−9%). Following short-term weight recovery, most subfields increased in volume. Comparisons between participants after long-term weight-recovery and HC yielded no differences. The hippocampal tail volume was positively associated with leptin levels in AN independent of body mass index.

Conclusions: Our study provides evidence of differential volumetric differences in hippocampal subfields between individuals with AN and HC and almost complete normalization after weight rehabilitation. These alterations are spatially inhomogeneous and more pronounced compared with other major mental disorders (e.g. major depressive disorder and schizophrenia). We provide novel insights linking hypoleptinemia to hippocampal subfield alterations hinting towards clinical relevance of leptin normalization in AN recovery.

Keywords: *anorexia nervosa, FreeSurfer, hippocampus, leptin, longitudinal study.*

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Anorexia nervosa (AN) is a severe and persistent mental disorder, characterized by fears of food and weight gain, distorted body image, and dietary restriction, leading to a significantly low body weight (American Psychiatric Association, 2013). This loss of body mass has consequences for the brain, such as cortical thickness reductions of ∼6.4% and subcortical volume reductions of ∼4%.[1–3] One subcortical structure that might be particularly relevant to unraveling the underlying neurobiology of AN is the hippocampus. This small seahorse-shaped brain region, part of the limbic system and located in the medial temporal lobes, has several important functions including long-term memory, learning, spatial processing, and emotional regulation[4,5]; all of which have gained recent interest in research on AN.[6–9] Although prior structural neuroimaging research in AN has largely scrutinized the hippocampus as a unitary structure, it comprises 12 structurally and functionally distinct subfields that can be subsegmented with modern analysis techniques.[10] Here we investigate volumetric differences of the hippocampus and its subfields in young females with AN compared with healthy control (HC) female participants, their longitudinal development across short-term weight recovery, as well as hippocampal volumes in individuals with former AN after long-term weight recovery in the largest sample to date.

Previous research in animal models has explored hippocampal subfields and their task-specific functions. For example, the dentate gyrus is involved in pattern separation, while pattern completion

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occurs in CA3.[11] In terms of autobiographical memory retrieval, the anterior hippocampus stores recent and remote memories, whereas the dentate gyrus and CA3 store remote memories.[12] There is evidence for difficulties in some of these hippocampus-related cognitive functions (e.g. autobiographical memory and pattern completion) in adults with AN.[8,13]

Whole hippocampal volumes have been examined in numerous previous investigations of individuals with AN.[2,14,15] The majority of these studies found global decreases that were restored with weight gain.[13] To date, only three studies have examined the hippocampal subfield volumes in AN samples.[16–18] These studies have yielded conflicting results regarding total hippocampal gray matter (GM) volume and specific subfield volumes. Compared with control participants, studies have found volumetric reductions in only one of seven subfields,[16] in 11 of 12 hippocampal subfields,[18] and in six of 18 subfields.[17] Furthermore, it remains unclear whether the observed reductions in subfield volumes are lateralized or can be attributed to particular subfield components, as in these previous studies the subfield volumes were mostly averaged across hemispheres and were defined based on a coarser atlas. Data on Alzheimer disease indicates an increased accuracy of hippocampal estimates when using the newer atlas.[19]

Longitudinal structural magnetic resonance imaging (MRI) studies in AN suggest rapid normalization of global reductions in GM volumes after weight restoration.[1,3,20] As the hippocampal subfields are structurally and functionally distinct,[10] a detailed investigation across stages of recovery promises new insight into temporal dynamics of hippocampal substructure alterations in AN.

Several symptoms of acute AN are strongly associated with low levels of the hormone leptin, a key biomarker of AN (e.g. physical activity, other hormone axes, and potentially depressive symptom severity).[21] Increases in leptin levels are observed over the course of short-term weight restoration, indicative of nutritional/metabolic recovery.[21,22] Studies in rodents have shown links between leptin and hippocampal growth,[23,24] but there is a lack of corresponding data in humans.[25] Investigating longitudinal associations could elucidate how changes in leptin levels affect changes in hippocampal subfields during weight restoration, which may have implications for new pharmacological treatment options for AN, e.g. recombinant human leptin.[26]

In addition to investigating hippocampal subfield volumetric differences in individuals with (a history of) AN relative to HCs and longitudinal trajectories across short-term weight recovery, we were interested in potential associations with changes in weight and clinical markers, particularly leptin. Longitudinal studies on AN investigating global cortical and subcortical alterations[1,3,27] and recent findings from the detailed investigation of the amygdala nuclei[28] led us to hypothesize that the hippocampus and its subfields would show: (i) decreased volumes in the acute state of AN and (ii) an increase/normalization following weight restoration (except for the fissure, which represents a sulcus between hippocampal tissue). In recovered individuals with a history of AN, we expected that: (iii) hippocampal subfield volumes would be fully normalized and would not differ from HCs. Importantly, we anticipated that: (iv) the magnitude of these alterations might be more pronounced in some subfields, but less in others, mirroring results from a similarly complex structure, the amygdala.[28] In addition, we investigated associations between hippocampal subfield volume alterations and leptin as well as eating disorder (ED)–specific, depressive, and general psychiatric symptoms.

Methods and Materials

Participants

The cross-sectional sample comprised 515 female participants (Table 1): 165 underweight patients with acute AN at time point 1 (AcAN-TP1, aged 12–29 years), 271 HC participants (aged 12–29 years) and 79 individuals who were long-term recovered from AN (RecAN, aged 14–29 years). Of the AcAN patients, 110 were reexamined longitudinally at time point 2 (TP2) after partial weight restoration (>14% increase in body mass index [BMI]; AcAN-TP2). This combined sample included 625 individual MRI scans from 515 female participants (Table 2) who were recruited using the same inclusion/exclusion criteria and assessment methods as in our previous studies.[1,20] All AcAN patients were admitted to intensive treatment with specialized ED programs at a child and adolescent psychiatry and psychosomatic medicine department of a university hospital, where they completed all TP1 assessments within 96 h of commencing nutritional rehabilitation.

Current and past diagnoses of EDs and other pertinent information including potential confounding variables (e.g. medication, comorbidities, and menstrual cycle) were obtained from all participants using the expert form of the Structured Interview for Anorexia and Bulimia Nervosa (SIAB-EX),[29] supplemented with our own semistructured interview. A diagnosis of AN required a BMI <10th age percentile (if younger than 18 years) or < 17.5 kg/m^2 (if older than 18 years). To be included in the RecAN group, participants previously had to have met diagnostic criteria for AcAN. RecAN had to maintain a BMI >18.5 kg/m^2 (or the 10th age percentile if younger than 18 years), have regular menstruation, and not engage in significant restrictive eating behaviors (or binging/purging) for at least 6 months before study participation (average recovery was 5 years). HC participants were required to have a normal BMI (18.5–29 kg/m^2 or >10th, <95th age percentile if <18 years), regular menstruation, no history of any mental illness, and normal eating behavior as assessed with SIAB-EX. Further details on participant recruitment, diagnostic procedures, and inclusion/exclusion criteria are included in the online supplement (SM 1.1). This study was approved by the institutional review board of the TU Dresden and conforms to the provisions of the Declaration of Helsinki. All study participants (and their legal guardians if underage) gave written informed consent.

Clinical measures

We assessed ED-related symptoms with the Eating Disorder Inventory-2 (EDI-2)[30] and depressive symptoms with the Beck Depression Inventory-II (BDI-II).[31] "Core" ED-related symptoms were assessed with a summary score averaging EDI-2 subscales "drive for thinness," "body dissatisfaction," and "bulimia."[32,33] Trait anxiety symptoms were measured with the State-Trait Anxiety Inventory (STAI[K])[34] and general psychiatric symptoms with the Symptom Checklist-90-Revised (SCL-90-R Global Severity Index/GSI).[35] General intelligence (intelligence quotient) was estimated with the Wechsler Adult Intelligence Scale (WIE; if age older than 16 years)[36] or the Wechsler Intelligence Scale for Children (HAWIK-IV; if age 16 years or younger).[37] BMI SD scores (BMI-SDS)[38] were computed to provide an age-corrected index of weight-to-height ratio. Plasma leptin level was measured in fasting venous blood samples collected into EDTA vacutainer tubes between 7 and 9 a.m. (for AcAN-TP1 within 96 h after treatment initiation, see SM 1.2 for processing and storage of blood samples). Measured leptin levels were logarithmically transformed ($\log_{10}$ leptin) to reduce deviations from normal distribution.[39] Left-censored leptin levels below the lower limit of detection of the leptin assay (LOD = 0.20 µg/L, in $n = 23$ AcAN-TP1) were imputed using censored likelihood multiple imputation (SM 1.2).[40]

Structural MRI acquisition and image data processing

All participants underwent MRI between 8 and 9 a.m. following an overnight fast to standardize their metabolic state prior to the scan.[41] For AcAN-TP1, the first scan was performed within 96 h after treatment initiation. High-resolution three-dimensional T1-weighted structural scans were acquired on the same Siemens Magnetom Trio 3T Scanner. The cerebral cortex was automatically reconstructed using FreeSurfer 7.1.1.[42,43] This was followed by standardized quality control of (sub-)cortical regions by trained raters, resulting in the exclusion of 8% of the scans. For more details and specific exclusion rates, refer to SM 1.3 (Table S1). Quality controlled images were processed with FreeSurfer's automated longitudinal processing stream.[44] Subsequently, images underwent combined amygdala and hippocampus

Table 1. Demographic and Clinical Data of the Cross-Sectional Sample

Variable	*n* AcAN-TP1/HCs	Sample (mean ± SD) AcAN-TP1	Analyses HCs	|t|	df	*P*-value	Cohen d
Demographics and BMI							
Age (years)	165 / 271	16.44 ± 3.06	19.06 ± 4.35	7.36	434	<0.001	0.669
IQ	151 / 269	111.97 ± 12.07	112.22 ± 10.11	0.22	418	0.829	0.023
BMI (kg/m^2)	165 / 271	14.69 ± 1.39	21.12 ± 2.14	37.96	434	<0.001	3.397
BMI-SDS	165 / 271	−3.25 ± 1.25	−0.11 ± 0.65	29.92	434	<0.001	3.399
BMI_{min} (kg/m^2)	163 / 237	14.33 ± 1.37	19.90 ± 1.80	35.02	398	<0.001	3.392
Hormone parameter							
Leptin (μg/L)	142 / 256	1.57 ± 2.27	12.60 ± 8.70	NA	NA	NA	NA
Log_{10} leptin	142 / 256	−0.33 ± 0.82	1.00 ± 0.30	18.33	679.29	<0.001	2.334
Brain segmentation volumes							
eTIV (mm^3)	165 / 271	1,495,628 ± 121,184	1,512,205 ± 105,310	1.45	434	0.147	0.149
Subcortical GM volume (mm^3)	165 / 271	57,981 ± 4611	60,664 ± 4179	8.93	434	<0.001	0.610
Total GM volume (mm^3)	165 / 271	645,881 ± 52,692	686,662 ± 52,622	15.51	434	<0.001	0.774
Total brain volume (mm^3)	165 / 271	1,104,736 ± 90,109	1,166,463 ± 85,392	13.66	434	<0.001	0.703
Psychiatric symptom measures							
EDI-2 core	159 / 267	26.09 ± 7.07	14.73 ± 4.35	18.28	424	<0.001	2.055
BDI-II total	162 / 268	23.32 ± 11.07	4.10 ± 4.48	21.08	428	<0.001	2.511
SCL-90-R GSI	140 / 237	49.06 ± 12.20	33.48 ± 7.66	13.61	375	<0.001	1.624

Note: Number of participants and mean ± SD for each variable and study group (acute anorexia nervosa [AN] and healthy controls [HCs]) are shown. Group differences were tested using two-sample *t* tests, except for estimated total intracranial volume (eTIV) and subcortical gray matter (GM) volume, where general linear models were used (age-, age^2-, and eTIV-adjusted). As test statistics, *t* value (absolute), degrees of freedom (df), *P*-value, and effect size estimate Cohen d[72] are stated. Censored likelihood multiple imputation[40] was applied for left-censored leptin values in patients with AN below the lower limit of detection (LOD = 0.20 μg/L) of the leptin assay (39 of 142 available leptin values in AN (27.46%) were imputed, leptin below the LOD did not occur in HCs). In patients with AN, the mean (SD) age at first onset of AN was 14.2 ± 2.8 years (assessed in 162 patients) and the mean duration of the current AN episode (duration of illness) was 14.1 ± 18.2 months (n = 162). AN subtype was determined *via* SIAB-EX: 139 AN (84.24%) were restrictive and 22 (13.33%) were binge/purge (subtype not assessed in n = 4 [2.42%]). All study participants were female and identified as European.
Abbreviations: AcAN-TP1, acute anorexia nervosa at time point 1; BDI-II, Beck Depression Inventory-II; BMI, body mass index; BMI_{min}, minimum lifetime body mass index; BMI-SDS, body mass index SD score; EDI-2 core, averaged score comprising the core subscales "drive for thinness," "body dissatisfaction," and "bulimia" of Eating Disorder Inventory-2; IQ, intelligence quotient; log_{10} leptin, logarithmically transformed (base 10) leptin concentration; NA, not available; SCL-90-R GSI, Global Severity Index of the Symptom Checklist-90-Revised.

subsegmentation,[45] using both cross-sectional and longitudinal processing streams.[46] The following subfields were examined bilaterally (left hemisphere = lh; right hemisphere = rh): the whole hippocampus, whole hippocampal body, whole hippocampal head, hippocampal tail, cornu ammonis 1 body (CA1_body), cornu ammonis 1 head (CA1_head), cornu ammonis 3 body (CA3_body), cornu ammonis 3 head (CA3_head), cornu ammonis 4 body (CA4_body), cornu ammonis 4 head (CA4_head), fimbria, granule cell (GC) and molecular layer (ML) of the dentate gyrus (DG) body (GC_ML_DG_body), GC and ML of the DG head (GC_ML_DG_head), hippocampus amygdala transition area (HATA), hippocampal fissure (a cerebral spinal fluid cleft, rather than hippocampal tissue), molecular layer of the hippocampus body (molecular_layer_HP_body), molecular layer of the hippocampus head (molecular_layer_HP_head), parasubiculum, presubiculum body, presubiculum head, subiculum body, and subiculum head. The whole hippocampus encompasses all volumes, the whole hippocampal head encompasses all "head" volumes as well as the HATA and the parasubiculum, and the whole hippocampal body encompasses all "body" volumes as well as the fimbria. We included the hippocampal fissure in the main analysis model but excluded it from further examination since it does not measure GM mass, but rather cerebrospinal fluid and has high numeric and spatial measurement uncertainty.[47]

Statistical analyses of the cross-sectional data

For the cross-sectional analyses, hippocampal subfield volumes were modeled using general linear models (GLMs) with the study group (AcAN-TP1, HC) as the predictor of interest, and, as covariates, age up to quadratic terms based on previous literature[10,48,49] in line with ENIGMA studies,[50,51] and estimated total intracranial volume (eTIV) as an established correction method of brain volumes for head size variation.[52] Multiple testing adjustment of *P*-values using false discovery rate (FDR)[53] was applied across all hippocampal subfields.

We considered supplementary GLMs (Fig. S1) to account for the potential effects of: (i) psychiatric comorbidities (coexisting depressive, anxiety, obsessive-compulsive, or posttraumatic stress disorder[s]) and/or psychoactive medication (i.e. selective serotonin reuptake inhibitor or mirtazapine intake in the past 6 months before study participation); (ii) AN subtype; and (iii) hydration status (urine-specific gravity) and (iv) oncotic pressure (serum albumin concentration).

For regions in which a significant volume reduction in AN was observed in our main model (Fig. 1), we considered follow-up analyses within the AN group to assess the effect of: (i) nutritional and endocrine markers (BMI-SDS; log_{10} leptin); and (ii) psychiatric symptom severity markers (duration of illness [DOI]; ED-specific [EDI-2 core], depressive [BDI-II], and general psychiatric [SCL-90-R-GSI] symptoms). Specifically, we used robust linear regression models (RLMs) to examine the effect of the clinical marker of

Table 2. Demographic and Clinical Data of the Longitudinal Sample

	Acute AN sample at two study time points				Change	Control samples				Test statistics			Post hoc contrasts
	AcAN-TP1		AcAN-TP2		AcAN-TP2–AcAN-TP1	RecAN		HCs					
Variable	*n*	Mean ± SD	*n*	Mean ± SD	88 ± 27 days	*n*	Mean ± SD	*n*	Mean ± SD	*F* value (between) |*F* (within)	df (between) |df (within)	*P*-value (between) |*P* (within)	Significant at *P* (mvt) < 0.05
Demographics													
Age (years)	110	16.07 ± 2.32	110	16.31 ± 2.31	0.24 ± 0.09	79	23.08 ± 3.77	271	19.06 ± 4.35	75.83|872.39	2;457|1;109	<0.001|<0.001	AcAN-TP1 < HCs ($P < 0.001$)|AcAN-TP2 > AcAN-TP1 ($P < 0.001$)|AcAN-TP2 < RecAN ($P < 0.001$)|AcAN-TP2 < HCs ($P < 0.001$)|RecAN>HCs ($P < 0.001$)
IQ	107	112.92 ± 12.43	NA	NA	NA	79	111.53 ± 10.00	269	112.22 ± 10.11	0.39	2;452	0.677	NA
BMI (kg/m^2)	110	14.77 ± 1.22	110	18.96 ± 1.13	4.19 ± 1.11	79	20.95 ± 1.87	271	21.12 ± 2.14	472.82|1450.25	2;464|1;94	<0.001|<0.001	AcAN-TP1 < HCs ($P < 0.001$)|AcAN-TP2 > AcAN-TP1 ($P < 0.001$)|AcAN-TP2 < RecAN ($P < 0.001$)|AcAN-TP2 < HCs ($P < 0.001$)
BMI-SDS	110	−3.12 ± 1.06	110	−0.69 ± 0.59	2.43 ± 0.83	79	−0.48 ± 0.62	271	−0.11 ± 0.65	687.84|1122.64	2;540|1;159	<0.001|<0.001	AcAN-TP1 < HCs ($P < 0.001$)|AcAN-TP2 > AcAN-TP1 ($P < 0.001$)|AcAN-TP2 < HCs ($P < 0.001$)|RecAN<HCs ($P < 0.001$)
Minimal lifetime BMI (kg/m^2)	108	14.60 ± 1.22	NA	NA	NA	69	14.29 ± 1.65	237	19.90 ± 1.80	550.36	2;411	<0.001	AcAN-TP1 < HCs ($P < 0.001$)|RecAN<HCs ($P < 0.001$)
Hormone parameter													
Leptin (µg/L)	63	1.51 ± 2.29	60	11.20 ± 7.46	9.61 ± 6.25	43	9.28 ± 6.50	159	12.60 ± 8.70	NA	NA	NA	NA
Log_{10} leptin	63	−0.32 ± 0.76	60	0.94 ± 0.36	1.29 ± 0.69	43	0.88 ± 0.26	159	1.00 ± 0.30	218.02|341.95	2;319|1;144	<0.001|<0.001	AcAN-TP1 < HCs ($P < 0.001$)|AcAN-TP2 > AcAN-TP1 ($P < 0.001$)
Brain segmentation volumes													
eTIV (mm^3)	110	1,447,137 ± 121,813	NA	NA	NA	79	1432,794 ± 110,792	271	1,455,562 ± 104,583	1.35	2;457	0.261	NA
Subcortical GM volume (mm^3)	110	61,605 ± 4723	110	63,846 ± 4551	2241 ± 1432	79	61,579 ± 4392	271	63,941 ± 4449	26.34|281.85	2;469|1;119	<0.001|<0.001	AcAN-TP1 < HCs ($P < 0.001$)|AcAN-TP2 > AcAN-TP1 ($P < 0.001$)
Total GM volume (mm^3)	110	662,594 ± 54,780	110	704,112 ± 54,947	41,518 ± 26,592	79	669,263 ± 45,324	271	701,490 ± 53,330	105.38|332.01	2;513|1;151	<0.001|<0.001	AcAN-TP1 < HCs ($P < 0.001$)|AcAN-TP2 > AcAN-TP1 ($P < 0.001$)|AcAN-TP2 < HCs ($P = 0.009$)
Total brain volume (mm^3)	110	1,101,201 ± 90,566	110	1,150,478 ± 89,662	49,277 ± 31,010	79	1,128,358 ± 84,087	271	1,167,805 ± 86,183	88.91|324.91	2;497|1;142	<0.001|<0.001	AcAN-TP1 < HCs ($P < 0.001$)|AcAN-TP2 > AcAN-TP1 ($P < 0.001$)|AcAN-TP2 < HCs ($P < 0.001$)
Symptoms													
EDI-2 core symptoms	107	26.08 ± 7.01	106	24.49 ± 7.19	−1.70 ± 6.35	78	18.59 ± 6.37	267	14.73 ± 4.35	151.57|8.67	2;530|1;161	<0.001|0.004	AcAN-TP1 > HCs ($P < 0.001$)|AcAN-TP2 < AcAN-TP1 ($P = 0.016$)|AcAN-TP2 > RecAN ($P < 0.001$)|AcAN-TP2 > HCs ($P < 0.001$)|RecAN>HC ($P < 0.001$)
BDI-II total	108	23.52 ± 10.49	105	14.27 ± 9.95	−9.48 ± 9.63	78	7.95 ± 8.45	268	4.10 ± 4.48	259.97|141.48	2;541|1;199	<0.001|<0.001	AcAN-TP1 > HCs ($P < 0.001$)|AcAN-TP2 < AcAN-TP1 ($P < 0.001$)|AcAN-TP2 > RecAN ($P < 0.001$)|AcAN-TP2 > HCs ($P < 0.001$)|RecAN>HCs ($P < 0.001$)

Table 2. (*Continued*)

	Acute AN sample at two study time points				Change	Control samples				Test statistics			Post hoc contrasts
	AcAN-TP1		AcAN-TP2		AcAN-TP2–AcAN-TP1	RecAN		HCs					
Variable	*n*	Mean ± SD	*n*	Mean ± SD	88 ± 27 days	*n*	Mean ± SD	*n*	Mean ± SD	*F* value (between) |*F* (within)	df (between) |df (within)	*P*-value (between) |*P* (within)	Significant at *P* (mvt) < 0.05
SCL-90-R GSI	109	1.00 ± 0.58	104	0.67 ± 0.53	−0.35 ± 0.46	78	0.40 ± 0.38	267	0.25 ± 0.28	134.82|77.40	2;531|1;179	<0.001|<0.001	AcAN-TP1 > HCs ($P < 0.001$)|AcAN-TP2 < AcAN-TP1 ($P < 0.001$)| AcAN-TP2 > RecAN ($P < 0.001$)| AcAN-TP2 > HCs ($P < 0.001$)| RecAN>HCs ($P = 0.014$)

Note: Number of participants and mean ± SD for each variable and study group (acute anorexia nervosa [AN] at time point 1 [AcAN-TP1], acute AN at time point 2 [AcAN-TP2], long-term recovered from AN [RecAN], and healthy controls [HCs]) as well as longitudinal change scores (AcAN-TP2–AcAN-TP1) are shown. Between- (AcAN, RecAN, HCs) and within- (AcAN-TP1/TP2) group differences were tested using linear mixed effects (LME) models computed as *F* tests/mixed ANOVA type III (variance and covariance parameters estimated *via* restricted maximum likelihood, Satterthwaite method applied for degrees of freedom (df),[72] The LME model for total subcortical gray matter (GM) volume included additional covariates age, age$^{2,\perp}$, and estimated total intracranial volume [eTIV]). As test statistics, *F* value (between-subjects|within-subject), *P*-value (between-subjects|within-subject), and effect size estimate partial η^2 (between-subjects|within-subject)[53] are given. Significant ($q < 0.05$) post hoc pairwise contrasts are stated after multiple testing adjustment of *P*-values using false discovery rate[53] across all assessed contrasts per variable. Intelligence quotient (IQ) was estimated with short versions of the German adaptation of the Wechsler Adult Intelligence Scale (WIE)[36] or the Wechsler Intelligence Scale for Children (HAWIK-IV)[37] for participants aged 15 years or younger. Censored likelihood multiple imputation (CLMI)[40] was applied for left-censored leptin values in AcAN-TP1 below the lower limit of detection (LOD = 0.20 µg/L) of the leptin assay (25 of 89 available leptin values in AcAN-TP1 [28.09%] were imputed, leptin lower than the LOD did not occur in AcAN-TP2, RecAN, or HCs). The mean age of AN onset in the AcAN sample was 14.5 ± 2.2 years and the mean duration of illness was 10.1 ± 10.5 months. The RecAN participants had a mean age at onset of former AN of 14.6 ± 1.9 years and were recovered for 69.4 ± 41.0 months on average. A total of 93 AcAN were of the restrictive subtype (84.55%) and 17 (15.45%) were of the binge/purge subtype. A total of 51 (64.56%) RecAN were of the restrictive subtype during illness and 18 (22.78%) were binge/purge. Ten RecAN (12.66%) had an unknown subtype. All study participants were female and identified as European.

Abbreviations: BDI-II, Beck Depression Inventory-II; BMI, body mass index; BMI-SDS, body mass index SD score; EDI-2 core, averaged score comprising the core subscales "drive for thinness", "body dissatisfaction", and "bulimia" of Eating Disorder Inventory-2; $\log_{10}$ leptin, logarithmically transformed (base 10) leptin level; NA, not available; SCL-90-R GSI, Global Severity Index of the Symptom Checklist-90-Revised.

PCN Psychiatry and Clinical Neurosciences

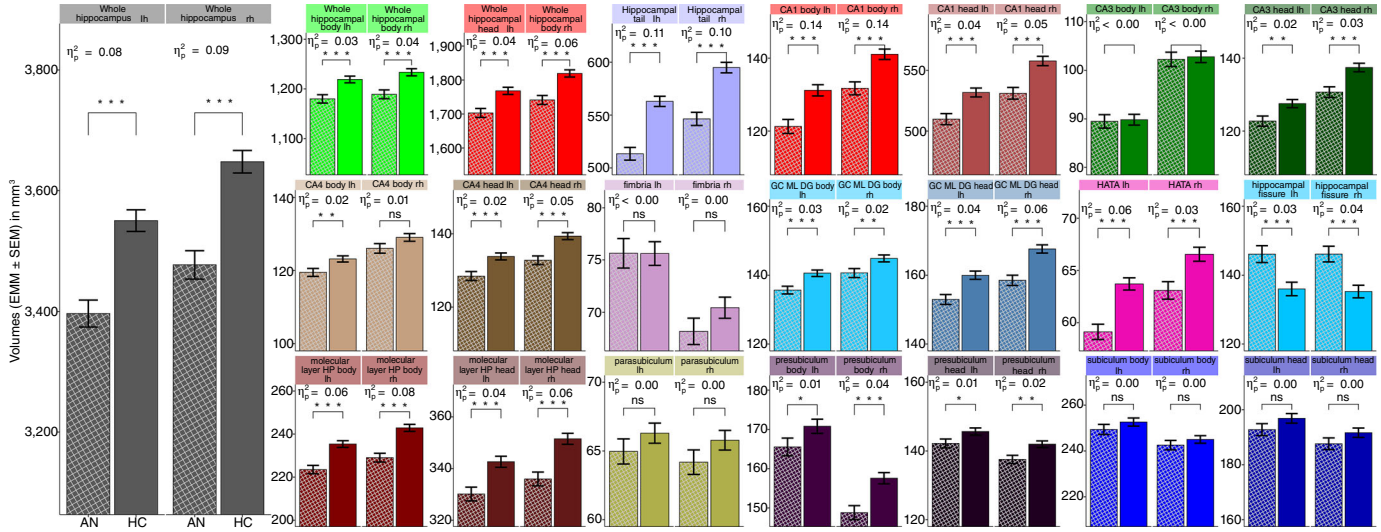

Fig. 1 Hippocampal (subfield) volumes in patients with acute anorexia nervosa (AN) vs healthy controls (HCs). Bar graphs with error bars for study groups AN ($n = 165$) and HCs ($n = 271$) displaying adjusted means (estimated marginal means [EMMs], mm^3) ± SEM of individual whole hippocampus and hippocampal subfield volumes in separate brain hemispheres, covarying for age at the date of research (linear and quadratic effects) and estimated total intracranial volume. FDR-q: *P*-values were multiple testing–adjusted using false discovery rate (FDR)[53] across all hippocampal subfields (whole hippocampus adjusted separately using FDR). Significance levels for volume differences between study groups are stated as: ***q < 0.001; **q < 0.01; *q < 0.05; ns, nonsignificant. Effect size statistics are provided as partial η^2.[72] lh, left brain hemisphere; rh, right brain hemisphere; GLM, general linear model.

interest on hippocampal volume alterations after accounting for the potential confounding effects of age, age^2, and eTIV (SM 2.3). FDR adjustment was applied across all RLMs per group of clinical markers.

Statistical analyses at different stages of weight recovery in AN

The volumes of the hippocampal subfields in the longitudinal arm of this study were modeled in all study groups (AcAN-TP1, AcAN-TP2, RecAN, and HCs) using a random-intercept linear mixed-effects (LME) model, established in our previous studies,[1,20,54] which assumes that the longitudinal differences in hippocampal subfield volumes are a linear function of BMI-SDS changes across short-term weight rehabilitation. Again, age (linear and quadratic effects) and eTIV were added as covariates. By also including participants with a single time point in the LME model (i.e. some participants from the AcAN group and all participants from the RecAN and HC groups), a more precise estimation of fixed and random (i.e. individual variability) effects is achieved. In particular, this leads to a more precise subtraction of the confounding effects of age and eTIV. In sum, we modeled the hippocampal subfields as follows:

$$\mathrm{HP}_{(\mathrm{Sub-})\mathrm{Volumes}} = \mathrm{A} + \Delta_{\mathrm{recAN}} + \Delta_{\mathrm{acAN}} + \mathrm{B}_{\mathrm{acAN}}(\mathrm{b_t} - \mathrm{b_{TP2}}) + \mathrm{C_1\,age} + \mathrm{C_2\,age}^{2,\perp} + \mathrm{D\,eTIV}.$$

For each subfield volume measure, we computed the contrasts: (i) AcAN-TP2 vs RecAN ($-\Delta_{\mathrm{recAN}} + \Delta_{\mathrm{acAN}} + \mathrm{B}_{\mathrm{AcAN}} \times$ mean $[\mathrm{b_{T2}} - \mathrm{b_{T1}}]$); (ii) the speed of longitudinal changes as a function of linear changes in BMI-SDS ($\mathrm{B}_{\mathrm{AcAN}}$, AcAN-TP1 vs AcAN-TP2); (iii) RecAN vs HC (Δ_{RecAN}); and (iv) AcAN-TP2 vs HC ($\Delta_{\mathrm{AcAN}} + \mathrm{B}_{\mathrm{AcAN}} \times$ mean$[\mathrm{b_{T2}}\text{-}\mathrm{b_{T1}}]$). We corrected for multiple comparisons using FDR across all contrasts and all subfields. Further analysis details are provided in the SM Methods.

Supplementary LME models aimed to: (i) confirm main LME model findings after excluding AcAN and RecAN with current/former binge-purge AN subtype; (ii) confirm main LME model findings after excluding AcAN and RecAN with comorbidities and/or psychoactive medicine intake; and (iii) investigate the effect of change in $\log_{10}$ leptin levels (independent of ΔBMI-SDS) on significantly altered and longitudinally changing hippocampal subfield volumes.

The effect of demographic and clinical characteristics on hippocampal subfield volumes was examined by considering extensions of the main LME model in which interaction effects of ΔBMI-SDS with: (iv) age, (v) DOI, and (vi) AN subtype were examined as well as the effect of changes in: (vii) ED symptoms, (viii) depressive symptoms, and (ix) general psychiatric symptoms independent of ΔBMI-SDS. Further, associations between hippocampal subfield volumes that were persistently altered in AcAN-TP2 compared with HC and demographic and clinical variables (BMI-SDS, $\log_{10}$ leptin, and psychiatric symptom levels) were analyzed using supplementary age-, age^2-, and eTIV-adjusted GLMs. For all analyses, we performed two-tailed significance testing at $\alpha = 0.05$. Statistical analyses were performed using R version 4.1.1.[55]

Results

Demographic and clinical characteristics

The demographic and clinical data of the cross-sectional sample are presented in Table 1 and the data of the longitudinal sample are presented in Table 2. As expected, AcAN-TP1 patients had lower BMI-SDS, total/subcortical GM volume and leptin concentrations, and higher symptom levels (EDI-2, BDI-II, SCL-90-R-GSI) than HCs, which improved after short-term weight restoration. BMI-SDS increased by a mean of 2.43 (SD, 0.83) in AcAN during weight-restoration treatment (duration mean, 2.77 months [SD, 0.84 months]) but was still under the age-appropriate average at follow-up (AcAN-TP2), mirrored in the leptin levels with an increase by a mean of 9.61 μg/L (SD, 6.25 μg/L). RecAN patients were significantly older and still had residual symptoms (EDI-2, BDI-II, SCL-90-R-GSI) despite long-term weight recovery, but displayed no differences in total/subcortical GM volume compared with HCs.

Hippocampal subfields show differential alterations in the acute state of AN

When correcting for age and head size, estimates of the whole hippocampus (lh = −4.33%; rh = −4.69%) and most of the hippocampal subfield volumes were significantly smaller in AcAN-TP1 patients than HCs (Fig. 1). Fourteen of the regions were smaller in both

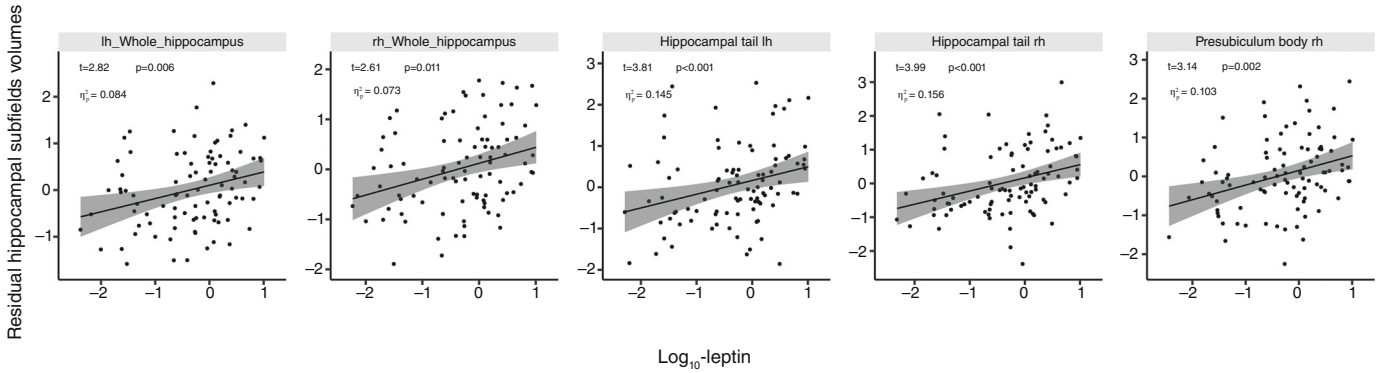

Fig. 2 Associations between significantly altered hippocampal subfield volumes and leptin concentrations in anorexia nervosa (AN). Scatter plots with individual data points, linear regression lines, and 95% confidence intervals around the regression line (gray band) in the AN study group (plasma leptin measurement available in 142 of 165 patients) displaying associations between individual hippocampal subfield volumes that were significantly altered in patients with AN vs healthy controls (HCs) and logarithmically transformed (base 10) ($\log_{10}$) leptin concentration (leptin values lower than the limit of detection were multiply imputed using censored likelihood multiple imputation,[40] associations were examined *via* robust linear models [RLMs] applying M estimation and Huber weighting for fitting *via* iterated reweighted least squares). Standardized residuals of hippocampal subfield volumes are plotted after adjustment of raw volume measures for age at the date of research (linear and quadratic effects) and estimated total intracranial volume using robust multiple linear regression. RLM statistics are provided as *t* values (unstandardized β divided by its standard error), unadjusted *P*-value (computed *via* robust Wald *F* test), and effect size estimate partial η^2.[72] lh, left brain hemisphere; rh, right brain hemisphere.

hemispheres in patients with AN. The most substantial volumetric reductions were observed in hippocampal tail (lh = −8.80%; rh = −8.15%), presubiculum body (lh = −3.08%; rh = −5.56%), and HATA (lh = −7.22%; rh = −5.17%), while CA3 body (lh = −0.37%; rh = −0.51%), parasubiculum (lh = −1.91%; rh = −2.23%), and subiculum head (lh = −1.28%; rh = −1.01%) were only nominally smaller in patients with AN. The hippocampal fissure (lh = 7.38%; rh = 7.99%) was significantly larger in volume in patients with AN than HCs. These group differences were not affected by excluding AN with coexisting depressive, anxiety, obsessive-compulsive and/or posttraumatic stress disorder diagnoses, and/or antidepressant pharmacotherapy (SM Results 2.1, Fig. S2). Hippocampal subfield volumes were also not related to AN subtype (Table S2) and hydration status in AN (mostly within normal range, dehydration/hyperhydration in two of six patients with AN, Tables S3 and S4). Hypoalbuminemia did not occur in patients with AN (Table S3).

Associations with clinical measures in acute AN

RLMs within AN showed no effect of BMI-SDS on any hippocampal subfield volumes (Table S5). Hippocampal tail lh and rh and presubiculum body rh volumes were significantly and positively associated with leptin, the hippocampal tail with a large effect size ($\eta_{p,lh}^2 = 0.145$, $\eta_{p,rh}^2 = 0.156$, Fig. 2, Table S5). Leptin effects on the hippocampal tail persisted when accounting for BMI-SDS effects in the same model ($\eta_{p,lh}^2 = 0.076$, $\eta_{p,rh}^2 = 0.112$, Table S5). There were no relevant effects of DOI or psychiatric symptom severity markers (ED-specific, depressive, anxiety, and general psychiatric

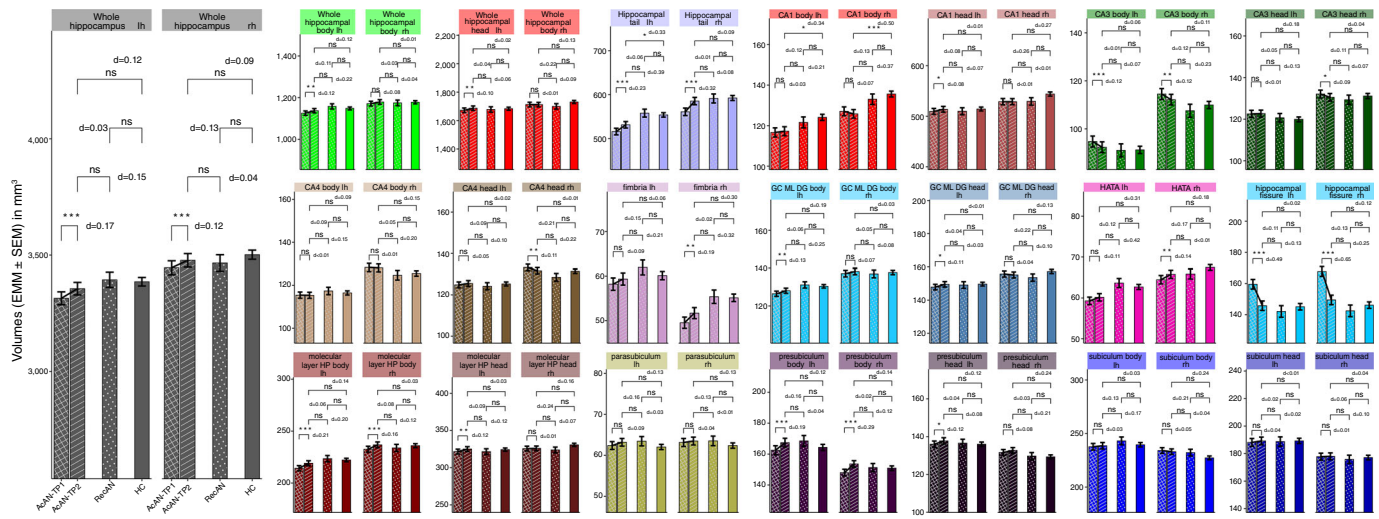

Fig. 3 Hippocampal subfield comparison between patients with acute, short- and long-term treated anorexia nervosa (AN) and healthy controls (HCs). Bar graph with error bars for study groups with acute AN at time point 1 (AcAN-TP1) and acute AN at time point 2 (AcAN-TP2) ($n = 110$), long-term recovered from AN (RecAN) ($n = 79$), and HCs ($n = 271$) displaying estimated marginal mean (EMM, mm^3) ± SEM of individual whole hippocampus and hippocampal subfield volumes in separate brain hemispheres, covarying for age at the date of research (linear and quadratic effects) and estimated total intracranial volume. Variance and covariance parameters estimated *via* restricted maximum likelihood, and Satterthwaite method applied for degrees of freedom.[73,74] FDR-q: *P*-values were multiple testing–adjusted using false discovery rate (FDR)[53] across all assessed pairwise contrasts (AcAN-TP2–AcAN-TP1, AcAN-TP2–HCs, RecAN–HCs, and AcAN-TP2–RecAN) per hippocampal subfield and across all hippocampal subfields. Significance levels are stated as: ***q < 0.001; **q < 0.01; *q < 0.05; ns, nonsignificant. Effect size statistics for linear mixed effects (LME) model contrasts are provided as Cohen d.[72] CA1, cornu ammonis 1; CA3, cornu ammonis 3; CA4, cornu ammonis 4; DG, dentate gyru; GC, granule cell; HATA, hippocampus amygdala transition area; lh, left brain hemisphere; ML, molecular layer; rh, right brain hemisphere.

symptoms; Table S5) on any of the examined hippocampal subfield volumes.

Differential alterations in hippocampal subfield volumes at different stages of weight recovery in AN

LME modeling in all study groups (AcAN at TP1 and TP2, RecAN, and HCs) showed that volumetric recovery was most prominent for subfields that were substantially reduced in the acute phase of AN (Figs. 3 and S3, Table S6). Accordingly, the highest effect sizes for longitudinal change were observed in the hippocampal tail ($d_{lh} = 0.23$; $d_{rh} = 0.32$) and the presubiculum body ($d_{lh} = 0.19$; $d_{rh} = 0.29$), while the parasubiculum ($d_{lh} = 0.09$; $d_{rh} = 0.04$) and the subiculum head ($d_{lh} = 0.04$; $d_{rh} = 0.01$) showed effects sizes close to zero. The hippocampal fissure ($d_{lh} = -0.49$; $d_{rh} = -0.65$) mirrors the cross-sectional results with a sizeable decrease following partial weight restoration. In short-term weight-restored AcAN-TP2, the hippocampal tail lh (−4%) and CA1 body lh and rh (lh = −6%, rh = −7%) were still reduced with small to medium effect size compared with HCs (Fig. 3). After long-term weight recovery, there were no group differences in hippocampal subfield volumes compared with HCs. When comparing short-term (AcAN-TP2) with long-term (RecAN) weight recovery, no significant differences were detected (Fig. 3).

The aforementioned group differences obtained with the main LME model remained significant when including only the restrictive subtype of AcAN and RecAN (with a few minor exceptions, Fig. S4). Likewise, these differences largely persisted when excluding AcAN and RecAN with a history of coexisting psychiatric diagnoses and/or current antidepressant medication (with a few minor exceptions, Fig. S5).

Associations between hippocampal subfields volumes, leptin levels, and clinical measures over the course of short-term weight restoration

Among hippocampal subfields significantly increased in AcAN as a function of BMI-SDS increase, there was no association with increases in leptin levels above and beyond ΔBMI-SDS. Neither participant age and DOI nor AN subtype (restrictive/binge-purge) significantly affected the speed of ΔBMI-SDS–related change or contributed to volumetric change in hippocampal subfields above and beyond ΔBMI-SDS. Changes in ED, depressive, and general psychiatric symptoms were unrelated to changes in hippocampal subfield volumes in AcAN during weight restoration.

Supplementary GLMs including hippocampal subfield volumes that were still reduced at AcAN-TP2 (Fig. 3) and demographic/clinical variables (Table S7) did not yield any notable associations.

Discussion

This study provides the first insight, to our knowledge, into the dynamic alterations of hippocampal subfield volumes during the underweight, partially weight-restored, and long-term weight-recovered states of AN. We confirm previous findings of sizeable reductions in hippocampal subfield volumes in young, acutely underweight patients with AN[18] and present new evidence of rapid reversibility of these changes after short-term weight gain. Hippocampal subfields were fully normalized following long-term weight recovery. Notably, the effects of hippocampal volume reductions were not equally distributed across the hippocampus. In acutely underweight patients with AN, regions including, most notably, the subiculum were less severely affected and a trend towards smaller hippocampal subfield volumes in the left hemisphere was observed. Going beyond previous findings, we found that the extent of volumetric increase during weight gain was different across the hippocampal subfields, with subfields increasing the most representing regions that were the most significantly reduced in the acute state (e.g. presubiculum body). These findings indicate a "rebound" of the reductions observed in the acute state of AN, but are not fully congruent with the finding of some volumetric reductions persisting after short-term weight restoration (in the hippocampal tail and the CA1 body). When evaluating further possible clinical markers, we found that hypoleptinemia might be linked to reductions of hippocampal subfields in the acute state. Specifically, in individuals with AN, lower leptin levels predicted a larger volumetric reduction of the hippocampal tail independently of BMI-SDS.

The mean volume of the hippocampal complex in patients with acute AN (lh = −3.68%; rh = −4.08%) was significantly lower than in HCs, confirming previous reports of hippocampal volumetric reduction.[17,18,20] Reported rates of cortical thinning in the acute stage of AN (6.4% on average)[1,3] exceed the whole hippocampus volume reduction observed in this study, whereas the volumetric reduction of the amygdala is comparable in magnitude (lh = −4.34%; rh = −4.22%).[28] However, while the direction of the effects in all of the larger subfields aligned with those observed for the entire hippocampus, the spatial distribution of the volumetric reduction within the hippocampus appears more heterogeneous than the pattern of cortical thickness reduction. For example, reductions range from almost 9% in the left hippocampal tail to <0.5% in the left CA3 body. Of note, similar to recent studies of cortical thickness in AN,[1,3,20] the mean reductions in the hippocampal complex are greater than in other mental disorders (2%–3% for schizophrenia and 0.7% for major depression)[56,57] but smaller compared with Alzheimer disease (38%).[58]

As predicted, we found that the observed heterogeneity in hippocampal subfield volumes between individuals with acute AN and HC participants was largely resolved by short-term weight restoration, with subfields showing greater reductions during acute AN also exhibiting the largest increases during weight restoration. This aligns with our recent finding that cortical areas with greater thickness reductions during acute AN demonstrated larger gains in cortical thickness following weight restoration.[1] The rapid increase in volume of severely affected hippocampal subfields during the underweight state (rebound effect) suggests that these subfields have a high recovery potential (resilience) indicative of neuroplasticity during weight restoration in AN. However, the effect sizes of these increases were smaller than those reported for weight restoration–related changes in cortical thickness and other subcortical segmentations.[1,3] Furthermore, while most GM aberrations return to normal after short-term weight restoration,[41,59] the still reduced hippocampal tail and CA1 body volumes after short-term weight restoration in our study suggest a delayed but ongoing recovery of these regions after discharge from inpatient treatment. This emphasizes the necessity of treatment support strategies after discharge, even if patients have regained weight in the short term.

Importantly, all of our main cross-sectional findings were robust when controlling for potentially confounding factors including age, duration of illness, AN subtype, coexisting psychiatric diagnoses, antidepressant use (selective serotonin reuptake inhibitor and mirtazapine exclusively), and hydration status.[27,41] Additionally, we adjusted our longitudinal models to incorporate possible confounders and longitudinal changes in psychological symptoms and found no evidence of additional explained variance in hippocampal subfield volumes beyond our investigated predictors (i.e. mainly increasing BMI).

We also evaluated whether BMI-SDS predicted lower volumes of hippocampal subfields in AcAN based on the longitudinal findings described above and previous similar findings.[1,3,27] Interestingly, while for some regions, such as the molecular layer of the hippocampal head and the presubiculum head, a significant relationship was found prior to FDR correction, this was not the case after FDR adjustment. This hints towards effects beyond BMI, which influence hippocampal volumes in the acute underweight state. For example, in AcAN, the volumes of the hippocampal tail and presubiculum body of the right hemisphere were significantly related to leptin levels. While not significant after FDR adjustment, the whole hippocampus, in both hemispheres, showed a similar correlation with leptin levels allowing speculation towards partial autocorrelation effect within the hippocampal structure. Importantly, only the hippocampal tail was linked with leptin levels above and beyond the degree of underweight as measured by BMI-SDS, indicating a unique effect of leptin.

Therefore, we hypothesize that hypoleptinemia, in conjunction with extreme underweight, might be a plausible (patho-)mechanism underpinning or influencing the degree of hippocampal volume alterations in AN. Prior research lends some support to our speculation. For example, a greater leptin increase during weight restoration has been shown to predict less thinking about food after discharge independently of BMI in AN.[60] Moreover, the influence of hypoleptinemia on many neuroendocrine systems and AN-related symptoms is well-established.[21,22,61–63] First results also link hypoleptinemia to alterations of the amygdala in the acute stage of AN.[28] As evidence mounts suggesting that leptin regulates hippocampal synapses, which subsequently affect hippocampal-dependent memory,[64] we hypothesize that through its interactions with the cellular processes governing hippocampal learning and sleep-dependent memory consolidation, leptin may influence food-related cognition in AN.[65–67] Recombinant human leptin administration in patients with AN is currently planned for the evaluation in clinical trials[21,68] due to its tolerability and potential beneficial effects on cognitive, emotional, and behavioral functions, as reported in case studies.[26,69,70]

The following limitations of the study should be noted. Our findings may be limited to young patients with nonchronic AN. In particular, while we did not detect differences between HC and RecAN, we remark that our RecAN group might constitute a selected group of patients with a particularly favorable outcome due to our strict inclusion criteria (e.g. BMI >18.5 kg/m^2, regular menstruation, and no significant restrictive eating behaviors or binging/purging for at least 6 months before study participation). Many individuals with AN achieve these objectives only after many years, and up to 30% develop a chronic form of AN.[71] Furthermore, due to the small number of patients with binge/purge subtype AN in our study, we were unable to evaluate the influence of AN subtype. When we removed participants with binge/purge subtype AN, we obtained results qualitatively identical to our main model, suggesting that subtype had no strong influence. Finally, our 3T-based 1-mm resolution structural MRI methods captured macrostructural alterations of very fine subfields within the hippocampus. Therefore, it is possible that such MRI studies miss persistent microstructural changes within the hippocampus in AcAN-TP2 and RecAN and therefore may overestimate or underestimate the volumes of the very small structures (e.g. granule cell and molecular layer of the dentate gyrus).[47] Including additional scans of the hippocampus in higher resolutions or incorporating T2 imaging sequences could mitigate such underestimations.

In summary, this study in a large sample of young women with AN receiving weight-restoration treatment provides novel evidence of varied dynamic and lateralized hippocampal subfield reductions in the acute state of illness and almost complete normalization of hippocampal volumes following partial weight restoration. The effects are more pronounced than those seen in other major psychiatric disorders (e.g. major depressive disorder and schizophrenia) and are congruent with volumetric alterations shown in other subcortical substructures in AN, such as the amygdala. Furthermore, we demonstrate that extreme underweight and, in particular, the accompanying hypoleptinemia in AN may be relevant for hippocampal volume loss. This observation highlights the importance of renutrition for brain health and might support the therapeutic relevance of leptin supplementation to support hippocampal recovery during renutrition.

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Disclosure statement

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Author contributions

Klaas Bahnsen: conceptualization, methodology, investigation, data curation, formal analysis, software, visualization, writing—original draft. Marie-Louis Wronski: methodology, investigation, software, data curation, writing—review and editing. Johanna Louise Keeler: methodology, formal analysis, writing—review and editing. Joseph A. King: conceptualization, methodology, data curation, writing—review and editing. Quirina Preusker: data curation, writing—review and editing. Theresa Kolb: investigation, data curation, writing—review and editing. Kerstin Weidner: resources, funding acquisition, writing—review and editing. Veit Roessner: resources, funding acquisition, writing—review and editing. Fabio Bernardoni: conceptualization, methodology, software, data curation, writing—review and editing, supervision. Stefan Ehrlich: conceptualization, methodology, validation, resources, funding acquisition, writing—review and editing, supervision, project administration.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section at the end of this article.