Evidence for Thalamocortical Circuit Abnormalities and Associated Cognitive Dysfunctions in Underweight Individuals with Anorexia Nervosa

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Anorexia nervosa (AN) is characterized by extremely low body weight resulting from pathological food restriction, and carries a mortality rate among the highest of any psychiatric illness. AN, particularly during the acute, underweight state of the illness, has been associated with abnormalities across a range of brain regions, including the frontal cortex and basal ganglia. Few studies of AN have investigated the thalamus, a key mediator of information flow through frontal-basal ganglia circuit loops. We examined both thalamic surface morphology using anatomical MRI and thalamo-frontal functional connectivity using resting-state functional MRI. Individuals with AN ($n = 28$) showed localized inward deformations of the thalamus relative to healthy controls (HC, $n = 22$), and abnormal functional connectivity between the thalamus and the dorsolateral and anterior prefrontal cortices. Alterations in thalamo-frontal connectivity were associated with deficits in performance on tasks probing cognitive control (Stroop task) and working memory (Letter-Number Sequencing (LNS) task). Our findings suggest that abnormalities in thalamo-frontal circuits may have a role in mediating aspects of cognitive dysfunction in underweight individuals with AN.

Neuropsychopharmacology (2016) **41**, 1560–1568; doi:10.1038/npp.2015.314; published online 4 November 2015

INTRODUCTION

Anorexia nervosa (AN) is a serious disorder characterized by aberrant eating behavior and inappropriately low body weight, and is associated with a mortality rate among the highest of any psychiatric illness (Swanson *et al*, 2011). Neuroimaging research has demonstrated metabolic, morphological, and functional connectivity abnormalities among patients with AN in frontal, temporal, and visual cortical regions, as well as within subcortical structures including the basal ganglia (Amianto *et al*, 2013; Cowdrey *et al*, 2014; Delvenne *et al*, 1995; Husain *et al*, 1992; Matsumoto *et al*, 2006; Takano *et al*, 2001; Yau *et al*, 2013). Relatively few studies, however, have investigated the thalamus, a subcortical region that, in part, relays output from the basal ganglia to the cortex through parallel cortico-striato-thalamo-cortical (CSTC) loops (Alexander and Crutcher, 1990; Haber and Calzavara, 2009; Parnaudau *et al*, 2013).

Studies of individuals with AN have previously documented signs of thalamic dysfunction, particularly during the acute, underweight phase of the illness. Amianto *et al* (2013)

used anatomical MRI and voxel-based morphometry and reported reduced gray matter in a cluster overlapping the right thalamus. Moreover, single-photon emission computed tomography has shown hyperperfusion in the thalamus in AN, indicative of hypermetabolism (Takano *et al*, 2001). One cross-sectional diffusion tensor imaging study of low weight and weight-restored individuals with AN reported altered white-matter integrity in the left and right posterior thalamic radii (Frieling *et al*, 2012). These data suggest that patients with AN may exhibit thalamic abnormalities at the level of morphology as well as thalamocortical connectivity.

Emerging evidence suggests that neural connections between the thalamus and the frontal cortex (ie, thalamo-frontal circuits) may subserve executive functions including set-shifting, working memory, and cognitive control (Hughes *et al*, 2012; Marenco *et al*, 2012; Mitelman *et al*, 2006; Parnaudau *et al*, 2013). These executive functions may be disrupted in patients with AN (Bodell *et al*, 2014; Park *et al*, 2014; Steinglass *et al*, 2006; Talbot *et al*, 2014). However, the relationship between alterations in thalamo-frontal circuits and cognitive dysfunction in AN has not been fully explored.

The primary aim of this study was to investigate whether the thalamus shows abnormalities in morphology or functional connectivity among individuals with AN relative to healthy controls (HC). Using anatomical MRI scans, we performed surface analysis of the thalamic structure. Surface analysis examines discrete areas of deformation computed from group differences in the shape of a defined brain region, in this case the thalamus. Since different regions of

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Received 24 June 2015; revised 29 September 2015; accepted 29 September 2015; accepted article preview online 14 October 2015

Table 1 Participant Demographics and Clinical Characteristics

	Thalamic morphology (main group)				Thalamic connectivity (subgroup)			
	AN (n = 28)	HC (n = 22)	Test statistic (d.f.)	P-value ^a	AN (n = 15)	HC (n = 16)	Test statistic (d.f.)	P-value ^a
Age (years)	19.36 ± 0.44	20.50 ± 0.63	t(48) = 1.54	0.13	19.00 ± 0.50	20.31 ± 0.77	t(29) = 1.41	0.17
IQ (estimated)	106.10 ± 1.45	108.30 ± 2.68	t(44) = 0.77	0.44	103.30 ± 2.13	106.60 ± 3.28	t(26) = 0.86	0.40
BMI (kg/m ²)	17.16 ± 0.26	21.26 ± 0.38	t(48) = 9.25	< 0.000	17.37 ± 0.29	21.57 ± 0.45	t(29) = 7.75	< 0.000
Lowest lifetime BMI (kg/m ²)	15.17 ± 0.26				15.23 ± 0.39			
Age of onset (years)	14.87 ± 0.38				14.26 ± 0.53			
Illness duration (months)	53.82 ± 7.16				56.87 ± 9.96			
EDE (score)	3.08 ± 0.27	0.08 ± 0.23	t(48) = -9.80	< 0.000	3.23 ± 0.38	0.07 ± 0.03	t(28) = -8.24	< 0.000
Education (years)	13.18 ± 0.38	13.91 ± 0.49	t(48) = 1.26	0.21	12.93 ± 0.36	13.63 ± 0.59	t(29) = 0.99	0.33
SES	4.3 ± 0.4	4.1 ± 0.4	t(45) = 0.4	0.68	3.2 ± 0.5	4.5 ± 0.6	t(27) = 1.7	0.11
Race	89% Caucasian	68% Caucasian	χ ² (1) = 3.40	0.06	80% Caucasian	69% Caucasian	χ ² (1) = 0.50	0.47
Subtype	11 Binge-Purge, 17 Restricting				8 Binge-Purge, 7 Restricting			

Abbreviations: AN, anorexia nervosa; BMI, body mass index; EDE, Eating Disorder Examination Interview; HC, healthy control; IQ, full-scale IQ estimated by the Wechsler Test of Adult Reading (WTAR); SES, socioeconomic status. Data are presented as mean ± SEM (see Supplementary Table S1 for scoring details).

^aTwo-tailed in case of t-tests.

P-values in bold denote statistical significance ≤ 0.05.

the thalamus project to discrete regions of the cortex (Johansen-Berg *et al.*, 2005), this analysis can reveal potential abnormalities in specific thalamocortical circuits and their related cognitive functions. Resting-state functional MRI scans were used to assess group differences in functional connectivity, a measure of the coherence of fMRI signal across brain regions, between the thalamus and the frontal cortex. We hypothesized that relative to HC, individuals with AN would show localized alterations in thalamic morphology, as well as abnormal functional connectivity within thalamo-frontal circuitry. We also explored whether thalamic morphology and functional connectivity correlated with performance on cognitive tasks that probe executive functions.

MATERIALS AND METHODS

The Institutional Review Board of the New York State Psychiatric Institute approved the study procedures. Adult participants provided written informed consent. Adolescent participants provided informed assent, and a legal guardian provided written informed consent.

Participants

Study subjects were included if they had participated in a neuroimaging study of AN described elsewhere (Decker *et al.*, 2014). Participants were females with AN, aged 16–25 years, receiving in-patient treatment through the Eating Disorders Research Clinic at the New York State Psychiatric Institute (NYSPI)/Columbia University Medical Center, and group-matched HC (Table 1). Individuals with AN participated in this study within their first week of hospital admission, and were medically stable as determined by their clinical team. Eligible patients met DSM-5 (APA, 2013) criteria for AN, both restricting (AN-R) and binge-purging (AN-BP) subtypes. Individuals were excluded if they had an estimated

IQ of < 80; a history of a neurological, bipolar, or psychotic disorder; substance abuse in the last 6 months; were currently taking psychotropic medication; had contraindications to MRI; or if they were pregnant. Anxiety or depressive disorders, which commonly co-occur with AN (Godart *et al.*, 2007), were not exclusionary when AN was the primary diagnosis. HC were group matched to the participants with AN by age, sex, IQ, and ethnicity, and had a body mass index (BMI) in the normal range (18–25 kg/m²). Additional exclusion criteria for HC were any current or past psychiatric illness, significant medical illness, or history of psychotropic medication.

Neuroimaging and Cognitive Testing: Overview

Participants (AN, *n* = 28; HC, *n* = 22) were scanned with anatomical MRI and a subgroup of participants (AN, *n* = 15; HC, *n* = 16) also underwent resting-state functional MRI. All participants underwent cognitive testing as part of a neuropsychological battery within 1 week of MRI scanning.

Anatomical MRI Pulse Sequences

Images were acquired on a 1.5-T Philips Intera scanner with an 8-channel head coil. Acquisition of T1-weighted sagittal localizing images was followed by a 3D spoiled gradient recall (SPGR) image using the following parameters: TR = 25 s, TE = 3.7 ms, flip angle = 30°, FOV = 256 mm, 256 × 204 matrix, 128 slices, voxel size 1 × 1 × 1 mm.

Resting-State Functional MRI Pulse Sequences

Two 5-min resting-state scans were obtained using the following parameters: TR = 2000 ms, TE = 40 ms, flip angle = 77°, 33 slices, voxel size = 3 × 3 × 4 mm, 150 volumes. During scan acquisition, participants were instructed to stay still with their eyes open, and to let their minds wander freely.

Processing of Surface Morphology using Vertex Analysis

To perform semi-automated segmentation and surface vertex analysis of subcortical structures in each participant, we used the FIRST pipeline through FSL (v5.0, fsl.fmrib.ox.ac.uk/fsl/fslwiki) (Patenaude *et al*, 2011). This method uses the 'active appearance model' within a Bayesian framework to segment 15 subcortical structures. The structures are defined *a priori* by 336 manually traced 'training' images, after which vertex analysis is used to compute local differences in structural morphology across groups. Briefly, high-resolution T1-weighted anatomical images for each subject, as well as the training images, were subjected to linear affine registration followed by non-linear registration to the Montreal Neurological Institute (MNI) 152 standard template using FSL FLIRT and FNIRT (Jenkinson *et al*, 2012; Patenaude *et al*, 2011). Manually delineated training image structures were then modeled by fitting a deformable mesh consisting of triangle vertices connected by edges with known three-dimensional coordinates. Subject-specific subcortical regions were then modeled by fitting a triangle/vertex mesh to the overlapping locations of the corresponding vertices derived from the training data. The subcortical regions of interest (ie, left and right thalamus) modeled for every participant were then concatenated in a single four-dimensional file, and vertex analysis was performed using rigid alignment in MNI152 standard space to estimate local variation in thalamic vertex position (and thus localized thalamic deformations) between groups. Brain size differences were controlled for by normalizing and registering each participant's brain scan to MNI152 standard space using the FLIRT pipeline within FSL (Jenkinson *et al*, 2002; Jenkinson and Smith, 2001). For subsequent correlation analyses, we used SPM8 (Statistical and Parametric Mapping, v8, www.fil.ion.ucl.ac.uk/spm/software/spm8) to extract the average thalamic deformation value for each participant.

Processing of Resting-State Functional Connectivity

Computation of resting-state functional MRI-based connectivity maps was performed using the functional connectivity toolbox (Conn, v14, www.nitrc.org/projects/conn) as detailed in Whitfield-Gabrieli and Nieto-Castanon (2012). Briefly, anatomical and fMRI images for each participant were spatially pre-processed using Artifact Detection Tools (Art, www.nitrc.org/projects/artifact_detect) through SPM8 to perform slice-timing correction, realignment, co-registration, spatial smoothing, and CompCor-based noise removal. Estimated temporal confounds including head motion and background blood oxygen level-dependent (BOLD) signal intrinsic to white matter and cerebral spinal fluid were used as covariates in the BOLD time series to regress out non-neural sources of BOLD signal. Finally, head motion parameters FD_{PEAK} , FD_{MEAN} , $DVAR_{PEAK}$, and $DVAR_{MEAN}$ (see Supplementary Methods for description, and Supplementary Table S2 for group comparisons) were estimated and extracted using SPM 8 for use as covariates in all subsequent analyses involving functional connectivity results.

Seven bilateral thalamic seed masks, each corresponding to a thalamic region with preferential connections to a specific cortical region or lobe (Johansen-Berg *et al*, 2005), were

thresholded at 25% connection probability, binarized, and extracted from the Oxford thalamic connectivity atlas (OTCA) included with FSL (Supplementary Figure S1). We used bilateral masks as (a) the thalamus has been shown to be bilaterally engaged during the performance of tasks probing the same cognitive domains as were examined in our study (Forsyth *et al*, 2014; Hendrick *et al*, 2010; Moore *et al*, 2013; Wagner *et al*, 2013) and (b) the thalamus in the AN group showed evidence of bilateral morphometric deformation (see Results). For each subject, functional connectivity maps were estimated using voxel-wise seed-to-voxel bivariate correlations between the BOLD time series of each thalamic seed and the whole brain. To improve data normality for second-level analysis using the General Linear Model, a Fisher-z transform was applied to the resulting connectivity data. We limited our analyses to thalamic connectivity with the frontal lobes (ie, Brodmann areas (BA) 8–10, 45–47) because of our goal of examining the contribution of altered thalamo-frontal circuitry to executive dysfunction in AN (Hughes *et al*, 2012; Marenco *et al*, 2012; Mitelman *et al*, 2006; Parnaudau *et al*, 2013).

We used SPM8 to extract the average connection strength for each thalamo-frontal cluster in which significant differences in connectivity were detected between patients with AN and HC. Brain coordinates for each cortical cluster were used to find the nearest local maxima within each cluster. The average connectivity values within a 6-mm radius of this peak coordinate were then extracted as eigenvalues for each cluster and compared with outcomes of cognitive performance, as well as various control measures, for each subject as described in statistical analyses.

Cognitive Measures

The neuropsychological battery included the Stroop task to assess cognitive control (Golden, 1978), the LNS task to measure working memory (WAIS-III; Wechsler, 1997), and the Trail-Making Test (TMT) to assess processing speed and visual attention (Bowie and Harvey, 2006). The administration and scoring of these tasks are included in Supplementary Methods.

Hypothesis Testing

Vertex analysis in FSL FIRST was used to compute morphological thalamic differences between participants with AN and HC using the General Linear Model through the randomize function in FSL. Thalamic morphology was used as a dependent variable, while group designation was used as the independent variable. We used the HC–AN contrast to measure the group difference in inward thalamic deformation, and the AN–HC contrast to measure the group difference in outward deformation, both at a family-wise error (FWE)-corrected $P \leq 0.05$ using threshold-free cluster enhancement. This analysis was then repeated while controlling for age and IQ.

For each thalamic seed, group differences in whole brain seed-to-voxel resting-state functional connectivity were independently estimated using the General Linear Model through SPM8. Resting-state connectivity maps for each of the seven thalamic seeds (Supplementary Figure S1) were used as the dependent variable, while group designation was

Figure 1 Thalamic morphology. Compared with healthy controls, patients with anorexia nervosa showed inward surface deformations (orange) spanning the mid dorsal-ventral aspects of the left (a) and right (b) thalamus (blue) ($P_{FWE} < 0.05$). Arrows indicate anterior–posterior ('A', 'P') orientation of the thalamic structure.

used as the independent variable. The false-positive rate was controlled by both a voxel-level threshold of $P = 0.005$ and a cluster extent threshold using false discovery rate (FDR) correction. $P_{FDR} \leq 0.05$ was considered as statistically significant. Bonferroni correction was used to control for multiple comparisons stemming from independently examining each of the seven thalamic seeds. These analyses were then repeated while controlling for age, IQ, and head motion parameters.

Exploratory Analyses

Two sample *t*-tests were used to assess group differences in performance on the Stroop, TMT, and LNS between the individuals with AN and HC, who were included in the resting-state functional connectivity analysis. This analysis was then repeated using the general linear model to control for age and IQ.

Partial correlations were used to compute the association between thalamo-frontal functional connectivity strength, and performance on all three cognitive tasks across all subjects while controlling for group (AN or HC), age, IQ, and head motion parameters. Partial correlations were also used to compute the association between (a) thalamic morphology and thalamo-frontal functional connectivity strength and (b) between thalamic morphology and performance on cognitive tasks. For these exploratory analyses, a $P \leq 0.05$ was considered as statistically significant. Potential effects of outliers were controlled for using the bias-corrected and accelerated method on bootstrapped samples ($n = 1000$).

Sensitivity Analyses

We performed several sensitivity analyses to examine the potential impact of AN-associated malnutrition, illness severity, and comorbid diagnoses on our findings, detailed in Supplementary Information. More specifically, we examined the extent to which AN subtype, current BMI, lowest

lifetime BMI, illness duration, comorbid diagnoses, and average low-frequency fluctuations (ALFF) in BOLD signal may have influenced our study outcomes.

RESULTS

Study Participants

Clinical characteristics are described in Table 1. Groups differed significantly in BMI ($P < 0.001$) and EDE scores ($P < 0.001$), but did not differ in age, IQ, years of education, socioeconomic status (SES), or race/ethnicity. The subgroup of participants who were included in resting-state functional MRI did not differ significantly from the full sample (see Supplementary Table S1). Among the individuals with AN, eight patients met criteria for a comorbid psychiatric illness (three with specific phobia, four with major depressive disorder (MDD), and one with post-traumatic stress disorder). The same AN participants with comorbid diagnoses were also part of the subgroup, excluding one individual with MDD.

Hypothesis Testing

Thalamic morphology. Compared with HC, patients with AN exhibited significant inward deformations in several regions of the left and right thalamus, spanning the central dorsal-ventral aspects of the thalamus both medially and laterally ($P_{FWE} < 0.05$, Figure 1). The group difference remained significant after controlling for the effects of age and IQ ($P_{FWE} < 0.05$).

Thalamo-frontal functional connectivity. Relative to HC, individuals with AN showed greater connectivity between the central-medial thalamus (OTCA thalamic seed 5, 'Thal5', Supplementary Figure S1) and the bilateral dorsolateral prefrontal cortex (DLPFC, BA9/BA46; Thal5-DLPFC(L) and Thal5-DLPFC(R), Figure 2a and b), and lower functional

Figure 2 Thalamo-frontal functional connectivity. Relative to healthy controls (HC), individuals with anorexia nervosa (AN) exhibited abnormally high functional connectivity between the thalamus and the left dorsolateral prefrontal cortex (Thal-DLPFC(L)) (a) as well as right DLPFC (Thal-DLPFC(R)) (b) ($P_{FDR} < 0.05$). The yellow–red bar scale indicates increasingly higher group connectivity differences in the positive direction (ie, AN > HC). (c) Conversely, relative to HC, individuals with AN exhibited abnormally low functional connectivity between the thalamus and the left anterior prefrontal cortex (Thal-AntPFC(L)) ($P_{FDR} < 0.05$). The white–blue bar scale indicates increasingly higher group connectivity differences the negative direction (ie, AN < HC).

connectivity between the anterior thalamus (OTCA thalamic seed 4, ‘Thal4’) and the left anterior prefrontal cortex (AntPFC, BA10; Thal4-AntPFC(L), Figure 2c). The group differences remained significant after controlling for the effects of age, IQ, and head motion parameters ($P_{FDR} < 0.05$), as well as Bonferroni correction for multiple seed comparisons (threshold $P = 0.0071$; cluster $P_{s_{unc}} < 0.001$).

Exploratory Analyses

Cognitive performance. Compared with HC, patients with AN showed significant impairment in performance on the Stroop task ($t(28) = 1.78$, $P = 0.04$) and the LNS task ($t(28) = 1.71$, $P = 0.05$), but not the TMT ($P = 0.28$) (Table 2); these results remained significant after controlling for age and IQ (P 's ≤ 0.05).

MRI measures and cognitive performance. Partial correlations controlling for age, IQ, group (AN or HC), and head motion revealed a significant negative correlation between performance on the Stroop and both the Thal5-DLPFC(L) and Thal5-DLPFC(R) connectivity strength (Stroop vs Thal5-DLPFC(L): $r(21) = -0.45$, $P = 0.02$; Stroop vs Thal5-DLPFC(R): $r(21) = -0.38$, $P = 0.04$; Figure 3a and b). Lower

scores on the Stroop reflect higher interference and greater impairment. Group-wise bivariate correlations revealed a strong trend toward negative correlation between Stroop performance and Thal5-DLPFC(L) as well as Thal5-DLPFC(R) connectivity strength in AN (Stroop vs Thal5-DLPFC(L): $r(14) = -0.44$, $P = 0.06$; Stroop vs Thal5-DLPFC(R): $r(14) = -0.43$, $P = 0.06$) but not in HC (Stroop vs Thal5-DLPFC(L): $P = 0.10$; Stroop vs Thal5-DLPFC(R): $P = 0.26$).

Partial correlations controlling for age, IQ, group (AN or HC), and head motion revealed a significant positive correlation between performance on the LNS task and Thal4-AntPFC(L) connectivity ($r(21) = 0.58$, $P = 0.002$; Figure 3c), where higher LNS scores reflect better performance. Group-wise bivariate correlations revealed a significant positive correlation between LNS performance and Thal4-AntPFC(L) connectivity strength in AN ($r(15) = 0.45$, $P = 0.05$) but not in HC ($P = 0.14$).

No significant association was found between performance on the TMT and thalamo-frontal connectivity (P 's > 0.10). Thalamic morphology did not correlate with measures of thalamo-frontal connectivity nor performance on any of the cognitive measures (P 's > 0.14).

Table 2 Group Comparisons on Cognitive Task Performance

Test	Group	N	Mean $\pm$ SEM	Test statistic (d.f.)	P-value ^a	95% CI	
						Lower	Upper
Stroop	AN	14	3.02 $\pm$ 1.88	$t(28) = 1.78$	0.04	-0.72	10.23
	HC	16	7.77 $\pm$ 1.88				
LNS	AN	15	10.60 $\pm$ 0.42	$t(28) = 1.71$	0.05	-0.23	2.49
	HC	15	11.73 $\pm$ 0.51				
TMT	AN	14	0.45 $\pm$ 0.03	$t(28) = 0.58$	0.28	-0.03	0.18
	HC	15	0.48 $\pm$ 0.05				

Abbreviations: AN, anorexia nervosa; CI, confidence interval; HC, healthy control; LNS, Letter-Number Sequencing task; TMT, Trail-Making Test.

^aOne-tailed *t*-test.

Figure 3 Thalamo-frontal functional connectivity and cognitive performance. Partial correlations controlling for age, IQ, group (AN or HC), and head motion revealed a significant negative correlation between performance on the Stroop task and connection strength between the thalamus and both the left dorsolateral prefrontal cortex (Thal-DLPFC(L)) (a), $r(21) = -0.45$, $P = 0.02$ and right DLPFC (Thal-DLPFC(R)) (b), $r(21) = -0.38$, $P = 0.04$, where lower scores on the Stroop reflect higher interference and greater impairment. These analyses also revealed a significant positive correlation between performance on the Letter-Number Sequencing (LNS) task and thalamic–anterior prefrontal cortex (Thal-AntPFC(L)) connectivity (c), $r(21) = 0.56$, $P = 0.002$, where higher LNS scores reflect better performance. Colored perforated lines are fitted to group-wise data, while the solid black line is fitted to data across both groups.

Sensitivity Analyses

Results from our sensitivity analyses are detailed in Supplementary Information.

DISCUSSION

Using anatomical and resting-state functional MRI, we examined surface morphology and functional connectivity of the thalamus in patients with AN in comparison with HC. Individuals with AN in the acute, underweight phase of the illness exhibited altered thalamic surface morphology, as well as abnormal functional connectivity between the thalamus, the DLPFC, and the AntPFC. The alterations in thalamo-frontal connectivity were associated with impairments in performance on tasks probing working memory and cognitive control, but not visuospatial processing speed and attention.

Compared with HC, individuals with AN showed localized inward deformations in bilateral thalamus. These deformations may result from a number of factors, including cellular loss, atrophy of neuronal dendritic arbors, demyelination, reduction in somatic size, or loss of afferent input (Sowell *et al*, 2004). In rodents, it has been shown that increased neuronal excitability is associated with a decrease in dendritic complexity (Cazorla *et al*, 2012). One previous study has shown hypermetabolism in the thalamus in AN (Takano *et al*, 2001), which may reflect higher intrinsic neural activity, and potentially explain the thalamic deformation seen in our study as resulting from dendritic atrophy. If thalamic metabolism normalizes following weight restoration, as suggested by two prior studies of AN (Delvenne *et al*, 1996; Frank *et al*, 2007), then thalamic deformations in AN may also normalize with successful treatment. Alternatively, these deformations may represent loss in cellular number, myelination, or synaptic input into the thalamus, the analysis of which would require post-mortem tissue.

Evidence from both animal and human studies suggests that the DLPFC, AntPFC, and anterior cingulate gyrus act in concert during various executive functions including working memory, set-shifting and behavioral flexibility, attention, and cognitive control (Botvinick *et al*, 2001; MacDonald *et al*, 2000; Milham *et al*, 2003; Niendam *et al*, 2012; Parnaudeau *et al*, 2013; Van Snellenberg *et al*, 2014). Modulation of these cognitive domains is achieved by parallel processing through CSTC loops, the final output of which involves thalamic interactions with frontal regions (Ferguson and Gao, 2014; Giguere and Goldman-Rakic, 1988; Graybiel, 2005; Haber and Calzavara, 2009; Parnaudeau *et al*, 2013). Our study found that when compared with HC, individuals with AN exhibited altered thalamo-frontal functional connectivity that was associated with deficits in performance on tasks probing cognitive control and working memory. This suggests that altered thalamo-frontal connectivity may have a role in mediating aspects of cognitive dysfunction in this disorder. In this regard, it is noteworthy that in a recent study in rodents, pharmacogenetic disruption of thalamo-frontal interactions yielded deficits in similar cognitive domains (Parnaudeau *et al*, 2013).

We did not find associations between localized thalamic deformations and either thalamo-frontal functional

connectivity or executive function performance. This suggests that either thalamic morphometry may be independent of alterations in thalamo-frontal functional connectivity, or the included measures lack the sensitivity needed to detect such an association. Little is known about the relationship between structural changes in the thalamus and their effect on thalamic function, and future investigations are needed to clarify how thalamic morphology and function interact to ultimately influence cognition and behavior.

In this study, we examined the acute, underweight state of AN, when it is difficult to discern the impact of malnutrition alone from disease-specific processes. To address this issue, we performed two sensitivity analyses in which (a) we examined the potential impact of malnutrition on our resting-state connectivity findings by assessing group differences in the ALFF in blood-oxygen-level dependent (BOLD) signal, a proxy measure of glucose metabolism in the brain (Nugent *et al*, 2015; Tomasi *et al*, 2013), within the thalamus, AntPFC, and DLPFC and (b) we assessed the extent to which current BMI, one index of malnutrition, correlated with our main study findings in the AN group. We found no group differences in ALFF within any of the examined thalamo-frontal regions, suggesting that the AN group did not exhibit alterations in BOLD signal that may have confounded the results of our resting-state connectivity analyses. In addition, current BMI in the AN group did not correlate with the extent of deformation in the left or right thalamus, or Thal4-AntPFC connectivity. However, this measure did significantly correlate with Thal5-DLPFC functional connectivity, bilaterally. Given this finding, it is conceivable that connectivity dynamics within certain thalamo-frontal circuits are more sensitive to the effects of malnutrition than others; by implication, these circuits may also be more amenable to normalization in response to weight restoration irrespective of AN diagnosis. Nonetheless, future work will be needed to fully establish the role of thalamic dysfunction in the cognitive and behavioral disturbances that are central to AN.

The present study examined morphological and functional connectivity differences between individuals with AN and HC within thalamo-frontal circuits. An important limitation that future studies could address is the specificity of our findings by performing the same imaging analyses in other subcortical regions. In addition, it would be of interest to determine whether the frontal regions identified as having differential connectivity with the thalamus in this study also show abnormalities in morphology or functional connectivity with other brain regions, including the thalamus. A limitation inherent in resting-state functional MRI is the correlational nature of the measurement. To assess for differences in thalamo-frontal structural connectivity between individuals with AN and HC, additional imaging techniques such as diffusion tensor imaging with fiber tracking are needed. The cognitive measures in this study were selected because of their known relationship to the thalamo-frontal circuits under study; however, they have limitations. Although we found that working memory, as measured by the LNS, was significantly impaired in patients with AN in our study, other studies have not found impairment in this cognitive domain (Lao-Kaim *et al*, 2014; Nikendei *et al*, 2011). Cognitive flexibility is often impaired in AN (Steinglass *et al*, 2006; Wu *et al*, 2014), but

findings with the Stroop and TMT have been inconsistent, perhaps because these tasks are less cognitively demanding. Furthermore, the relationship between these cognitive disturbances and the core behavioral disturbances in AN has not been established. Finally, although we did not detect effects of AN subtype on our outcome measures, it should be noted that these subtype analyses were underpowered due to small sample sizes comprising either subtype.

In conclusion, the current study begins to investigate thalamic dysfunction and the relationship with cognitive functioning in AN. Implicating thalamic dysfunction in AN adds to the growing literature suggesting CSTC loop dysfunction as an endophenotype of several disease states, including schizophrenia, ADHD, obsessive-compulsive disorder, and Tourette syndrome (Debes *et al*, 2014; Mitelman *et al*, 2005; Nigg and Casey, 2005; Posner *et al*, 2014a,b). Since alterations in thalamo-frontal connectivity might indicate general CSTC loop dysfunction, future studies could investigate whether other components of CTSC circuitry show alterations in AN. In addition, animal research may be a useful approach to test the causal relationship between altered functional connectivity and cognitive deficits with manipulations of thalamo-frontal interactions (eg, optogenetics) during behavioral performance of tasks probing these cognitive domains. Unveiling how CSTC loops contribute to cognitive function at a molecular, cellular, and system level using translational approaches may ultimately allow the generation of therapeutic interventions beneficial for both AN and other psychiatric conditions.

FUNDING AND DISCLOSURE

Dr Posner is a principal investigator on an investigator-initiated grant from Shire Pharmaceuticals.

ACKNOWLEDGMENTS

This study was supported by NIMH grants R21-MH099388 (JS and JP), K23 MH76195 (JS), K23-MH091249 (JP), the Klarman Family Foundation (JS), and the Paul Janssen Fellowship in Translational Neuroscience Research (DB).

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (<http://www.nature.com/npp>)