

Appendix 1 to Boehm I, Geisler D, Tam F, et al. Partially restored resting-state functional connectivity in women recovered from anorexia nervosa. *J Psychiatry Neurosci* 2016.

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Supplemental material

SM 1 Methods

SM 1.1 Participant

RecAN participants were recruited from specialized eating disorder programs of a university child and adolescent psychiatry and psychosomatic medicine department and diagnosed according to DSM-IV criteria using semi-structured clinical interviews. The expert version of the Structured Interview for Anorexia and Bulimia Nervosa for DSM-IV (SIAB-EX) was used to confirm the diagnosis and to ensure the absence of eating disorder symptoms, i.e. the recovery status before scanning. Within the recAN group all participants were of the restrictive subtype and 28.8% (n=8) of the participants had associated psychiatric comorbidity at the time of treatment (7 depressive disorders including dysthymia and 1 obsessive compulsive disorder). Comorbid psychiatric diagnoses were made by an expert clinician and included examination of the participant and careful archival chart review (including medical and psychiatric history, physical examination and several psychiatric screening instruments).

HCs were recruited through advertisement among middle school, high school and university students.

Exclusion criteria and possible confounding variables, e.g. the use of psychotropic medications and medical comorbidities, were obtained using the SIAB-EX and our own semistructured interview.

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HC participants were excluded if they had any history of psychiatric illness, a lifetime BMI below the 10th age percentile (if younger than 18 years) or BMI below 18.5kg/m² (if older than 18 years), or were currently obese (BMI not over 97th age percentile if younger than 18 years; BMI not over 30kg/m² if older than 18 years). Participants of all study groups were excluded if they had a lifetime history of any of the following clinical diagnoses: organic brain syndrome, schizophrenia, substance dependence, psychosis NOS, bipolar disorder, bulimia nervosa or binge-eating disorder (or “regular” binge eating - defined as bingeing at least once weekly for three or more consecutive months). Further exclusion criteria for all participants were IQ lower than 85; psychotropic medication within six weeks prior to the study; current substance abuse; current inflammatory, neurologic or metabolic illness; chronic medical or neurological illness that could affect appetite, eating behavior, or body weight (e.g., diabetes); clinical relevant anemia; pregnancy; breast feeding.

Pairwise case-control age-matching was carried out using the Munkres algorithm¹ resulting in a maximum difference of 1.6 years between the individuals within one pair.

Study data were collected between September 2011 and February 2014 and managed using secure, web-based electronic data capture tools REDCap (Research Electronic Data Capture).²

SM 1.2 Clinical measures

For all participants, current and/or past diagnoses of eating disorders were evaluated by the expert form of the SIAB,³ a well-validated 87-item semi-standardized interview that assesses the prevalence and severity of specific eating-related psychopathology over the past three months. The interview provides diagnoses according to the ICD-10 and DSM-IV.

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Interviews were conducted by clinically experienced and trained research assistants under the supervision of the attending child and adolescent psychiatrist.

Intelligence quotient (IQ) was assessed with a short version of the German adaption of the Wechsler Adult Intelligence Scale⁴ for participants aged 16 years and older or a short version of the German adaption of the Wechsler Intelligence Scale for Children⁵ for participants aged 15 years or younger.

SM 1.3 Instrumental target reaction task

The task comprised 48 trials. Each trial included an (A) preparatory phase, (B) motor response phase and (C) feedback phase. During (A) a visual cue (3s) represented the reward level (reward levels: 0 [no reward], 1, 10, 100) of this trial. The motor response phase started after a 2s fixation period. Acoustic feedback of each button press was provided through headphones. After another fixation period of 4s, feedback was provided for 3s by displaying the amount of money gained in this trial and the cumulative total amount. The monetary reward per trial was determined by multiplying the number of button presses (#bp) during (B) in this trial x the reward level x an individual adjustment factor (which was calculated based on the individual maximum #bp in the test run before the main experiment). Between trials, the subjects fixated on crosshairs for 3s on average. Since we focused on general psychomotor processing, the behavioural performance was calculated irrespectively of the reward levels by computing average reaction time (RT) and #bp.

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SM 1.4 Data Acquisition

The parameters of the rapid acquisition gradient echo (MP-RAGE) sequence were the following: number of slices=176; repetition time=1900ms; echo time=2.26 ms; flip angle=9°; slice thickness=1 mm; voxel size=1 x 1 x 1 mm³; field-of-view=256 x 224 mm²; bandwidth=2004Hz/pixel.

The parameters of the gradient-echo T2*-weighted echo planar imaging (EPI) were the following: tilted 30° towards AC–PC line (to reduce signal dropout in orbitofrontal regions); number of volumes = 190; number of slices = 40; repetition time = 2200ms; echo time = 30ms; flip angle (FA) of 75°; 3,4 mm in-plane resolution; slice thickness of 2,4 mm (1 mm gap resulting in a voxel size of 3,4 x 3,4 x 2,4 mm³); FoV=220 x 220mm²; bandwidth of 200 Hz/pixel.

SM 1.5 Image Data Preprocessing

The applied standard image data preprocessing procedure included slice time correction of the functional data, realignment and registration to the mean. The realigned files were coregistered to the subject's structural brain image. A DARTEL template was created using structural images from all subjects.⁶ The EPI volumes were then normalized to MNI space using the DARTEL template and corresponding flow field. The resulting data were smoothed with an isotropic 8mm FWHM Gaussian kernel. The quality of the fMRI data was evaluated by manual inspection and by using artifact detection tools (ART⁷). Volumes that exceed an intensity threshold of three standard deviations or a threshold of 2mm normalized movement in any direction were classified as outliers (motion-outlier: recAN : 0.13 ± 0.71, HC: 0.926 ± 1.26; intensity-outlier: recAN: 1.65 ± 2.44, HC: 1.48 ± 1.71); the two groups did

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not differ regarding numbers of motion- and intensity-outliers (motion-outlier: $t(60)=0.49$, $p=0.62$; intensity-outlier: $t(60)=-0.30$, $p=0.76$). Participants with more than 25% of frames classified as motion outlier were not included in the sample.

SM 1.6 Cortical thickness measurement

Surface reconstruction was conducted for each hemisphere including tessellation of the gray matter-white matter boundary, automated topology correction, and surface deformation following intensity gradients to optimally place the gray-white and gray-cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class. Based on the resulting surfaces cortical thickness were calculate at each vertex as the closest distance from the gray-white boundary to the pial surface. Segmentation and surface reconstruction quality were assured by manual inspection of all raw MRI volumes, segmented volumes in three planes and pial as well as inflated volumes. Possible misclassification of brain tissue was resolved manually by providing points of the tissue boundaries via the graphical user interface tkmedit. After rerunning the reconstruction procedure, which uses the information given in tkmedit, the quality of the result was reassessed.

SM 1.7 Component selection

Components correlating significantly with white matter and/or cerebral spinal fluid (MNI template provided in SPM 8) were identified as artifacts and subsequently removed from the analysis. Remaining components were then spatially correlated with RSN templates obtained in over 1000 healthy subjects by Yeo et al.⁸ to identify components covering the

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frontal-parietal network (FPN), the DMN and the salience network ($p < 0.05$, two-tailed). In case of significant overlap with two templates, the RSN with the highest correlation was assigned. The evaluation of “Dynamic Range” - the difference in power between the maximum and the minimum of the distribution and “Low to High Power Ratio” – the ratio of the integral of spectral power below 0.1Hz to the integral of the power between 0.15 and 0.25Hz confirmed that the components investigated in this study are clearly separated in the distribution from components reflecting physiological noise (see Figure SM 1.7).⁹

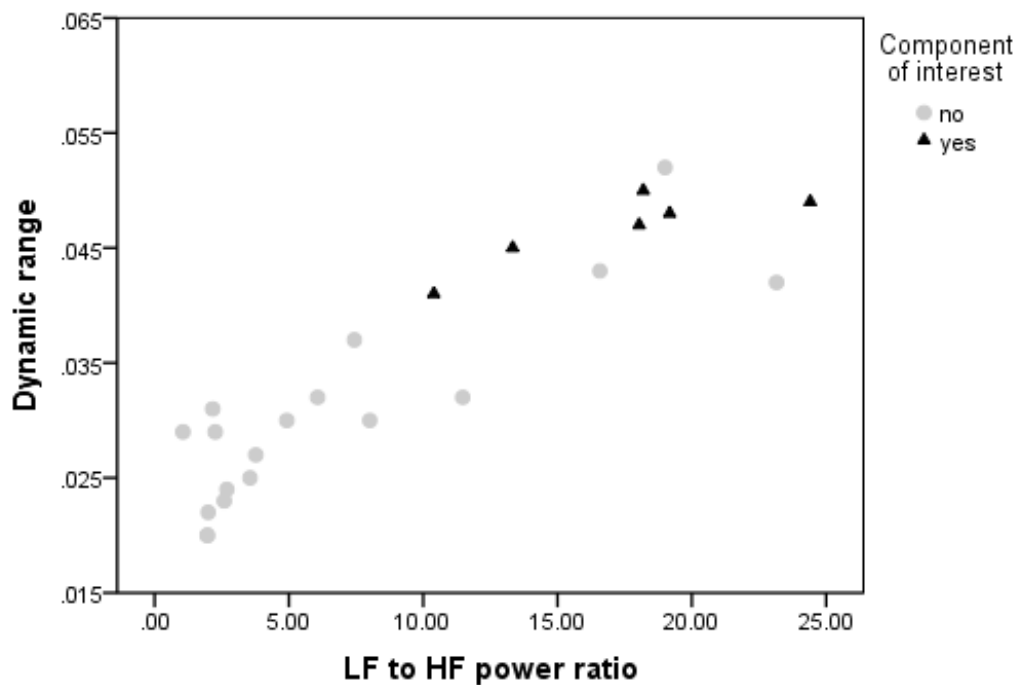


Figure SM 1.7: Scatterplot of low frequency (LF) to high frequency (HF) power ratio versus dynamic range for all components. Spectral characteristics were used to confirm component selection

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SM 1.8 Statistical analyses of independent components

The mask for our targeted follow-up approach was based on the cluster of increased FPN rsFC in acAN>HC at a threshold of $p=0.01$ (uncorrected, to allow for some anatomical variability). This approach was chosen to specifically probe the effect of weight recovery on aberrant network connectivity in acAN.

SM 1.9 Functional network connectivity

$$p_{\Delta i} = \frac{(X_{i_0}^T)(Y_{i_0+\Delta i}^T)}{\sqrt{(X_{i_0}^T X_{i_0})} * \sqrt{(Y_{i_0+\Delta i}^T Y_{i_0+\Delta i}^T)}}$$

We assume p to be the correlation between two time courses X and Y of dimension T x 1, where T is the number of time points within a time course. The starting reference point is i_0 and Δi is the noninteger change in time in seconds. When X is at an initial time point (X_{i_0}) and Y is circularly shifted Δi units from -5 to +5 ($Y_{i_0+\Delta i}$), the correlation of the overlapping time points $p_{\Delta i}$ is calculated as described in the formula. The maximal correlation value $p_{\Delta i_{max}}$ and the corresponding lag value $\Delta i_{p_{\Delta i_{max}}}$ is saved for each participant and afterwards averaged for control and patient group separately.

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SM 2 Results

2.1 Group comparison of independent components

Since our HC sample includes nine HC participants that were also included in our previous study¹⁰ we reran the analyses excluding those participant and could show that the finding remained significant ($t_{\text{peak}}=3.18$; $p=0.05$ (FWE)).

SM 2.2

	recAN	HC	<i>t</i>	<i>p</i>
Cortical thickness right dlPFC	2.97 ± 0.14	2.94 ± 0.15	-0.62	0.538
Cortical thickness left AG	2.364 ± 0.15	2.61 ± 0.18	-0.65	0.519
Psychomotor processing: #bp	12.70 ± 3.36	12.15 ± 3.53	-0.62	0.536
Psychomotor processing: RT	423.21 ± 83.44	424.89 ± 113.39	0.07	0.948
Persistence	53.90 ± 6.53	50.03 ± 8.65	-1.99	0.051

Table SM 2.2: Mean and standard deviation of parameters used in the paragraph of additional statistical analyses; Cortical thickness in mm; RT in ms; Group differences were tested using Student's t-tests

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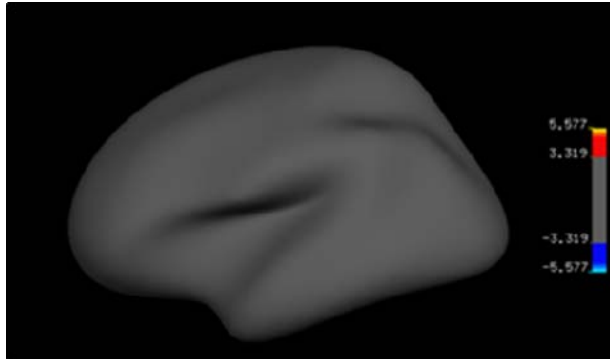


Figure SM 2.2: Statistical map displaying no group difference ($p < 0.05$ FDR) of vertexwise cortical thickness (as analyzed using FreeSurfer) in recAN patients relative to age-matched HCs plotted on the inflated surface of the standard average subject (for more details regarding the statistical modelling approach please refer to King et. al (2014)¹¹).

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