Appendix 1 to McFadden KL, Tregellas JR, ME Shott, et al. Reduced salience and default mode network activity in women with anorexia nervosa. *J Psychiatry Neurosci* 2013

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Supplementary methods

Conditioned stimulus task

Taste stimuli consisted of 1M sucrose solution (100 trials), no solution (100 trials), or artificial saliva (80 trials). Participants learned to associate each taste stimulus with a unique paired visual conditioned stimulus (a geometric shape), presented for 2 s. Following the visual cue, the taste stimulus was delivered when a black fixation cross appeared on a white background. Taste fluid delivery occurred over 1 s, with intertrial interval fixed at 6 s. Participants were instructed to swish their tongue once, look at the fixation cross, and await the next trial. Taste stimuli were applied using a customized programmable syringe pump (J-Kem Scientific) controlled by E-Prime Software (Psychological Software Tools), and individual taste applications were triggered by the magnetic resonance imaging (MRI) scanner's radiofrequency pulse.[1] Task duration was 28 minutes.

Functional MRI acquisition

Functional images were acquired with T_2*-weighted echo-planar imaging for blood oxygen level–dependent (BOLD) functional activity, with voxel size 3.4 x 3.4 x 2.6 mm, repetition time (TR) 2100 ms, echo time (TE) 30 ms, angle 70°, 30 slices, interleaved acquisition, and 2.6 mm slice thickness with 1.4 mm gap. Structural images were also acquired for analysis of brain anatomy (T_1, SPGR field of view 22 cm, flip angle 10°, slice thickness 1.2 mm, scan matrix 256 x 256, TR 10, TE 3, voxel size 1.2 mm^3).

Functional MRI data preprocessing methods

Functional data from each participant were realigned to the first volume and normalized to Montreal Neurological Institute space. Normalized data were smoothed with an 8 mm full-width at half-maximum Gaussian kernel. Each image sequence was manually inspected, and images with artifacts or movement greater than 1 voxel size were removed.

GIFT analysis methods

The dimensionality of the data from each participant was reduced using principle component analysis and concatenated into an aggregate dataset. Twenty independent components were estimated using the infomax algorithm.[2] Individual participant independent component analysis (ICA) data sets were then back-reconstructed. The default mode network (DMN) and salience network (SN) components were identified using spatial sorting in GIFT to assess the spatial correlation to DMN and SN masks, following removal of artifact components (i.e., white matter and cerebrospinal fluid). Both masks were defined anatomically from the Wake Forest University Pickatlas (http://www.fmri/wfubmc.edu). The SN mask consisted of the anterior cingulate cortex and bilateral insula.[3,4] The DMN mask consisted of the lateral posterior parietal cortex, precuneus, posterior cingulate cortex, frontal pole and occipitotemporal junction.[4,5] For the SN, the component with the highest correlation to the mask was selected for further analyses. For the DMN, the 2 most highly correlated components were averaged together, as both components were part of the DMN. The term "activity" reflects the amplitude of the SN and DMN signals identified by ICA and spatial template matching; as such, this reflects the degree of engagement within each functionally connected network. Components for all participants, as z score maps, were evaluated across the entire brain on a voxel-wise basis with directional contrasts (SPM t contrasts) in a 1-way analysis of variance (ANOVA) in SPM8. Based on a priori hypotheses, the specific contrasts of interest were control > anorexia nervosa, control > recovered, and recovered > anorexia nervosa. To ensure differences reflected activity of only the network of interest, results were masked with the main effect of network-related activity ($p < 0.05$, uncorrected). Results were considered significant at a whole-brain level if they exceeded a voxel-wise threshold of $p < 0.01$ and cluster-level correction for multiple comparisons of $p < 0.05$, using a false discovery rate (FDR) approach.[6]

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Structural data analyses

Structural data were analyzed to test if group volumetric differences in grey matter, white matter or total intracranial volume might have influenced functional differences. These data have been presented elsewhere,[7] but in short, voxel-based morphometry (VBM) analysis was conducted on the T_1-weighted data sets for all participants, with the exception of 1 woman in the anorexia nervosa group for whom structural data were not available.

Network modulation by task condition

Within the GIFT software, a regression analysis was performed on the ICA time courses to identify how each task condition was associated with each network of interest. This regression utilized the SPM design matrix for each individual participant, with regressors entered for 3 task conditions: expected reward (expected sucrose administration/received sucrose administration), positive prediction error (expected no sucrose/received sucrose), and negative prediction error (expected sucrose/did not receive sucrose). This analysis utilized the temporal sorting function in GIFT, resulting in association estimates (β weights) representing how the time course of each network was modulated by task condition.[8] The temporal sorting procedure also identified which networks were most highly correlated with the task (across task conditions), revealing that the sensorimotor network (SMN) was most highly correlated, followed by the SN, basal ganglia network (BGN), and DMN. Given this, we also conducted analyses on the SMN and BGN, in addition to the a priori networks of interest (SN and DMN). The SMN and BGN networks were identified as per templates described by Shirer and colleagues.[9] The SMN included the bilateral precentral gyrus, postcentral gyrus, and supplementary motor area (SMA). The BGN included the bilateral caudate, putamen, and inferior frontal gyri. Differences in β weights among task conditions (expected reward, positive prediction error, negative prediction error) for each network were analyzed using 1-way ANOVA in SPSS 21.0 (IBM Corp). Group differences (anorexia nervosa, recovered, control) were also analyzed with 1-way ANOVA. Post hoc *t* tests determined directionality of significant ANOVA results, for an $\alpha < 0.05$, Bonferroni-corrected.

Behavioural analyses

Group comparisons of participant characteristics and behavioural measures were assessed using 1-way ANOVA in SPSS. For correlations between fMRI and anorexia nervosa–related behavioural data, *z* scores reflecting network activity for each participant were extracted from SPM at the local maxima for the region of interest in each network. Relationships between *z* scores and behavioural measures were determined using partial correlation analyses in SPSS, covarying for age, BMI, BDI score, STAI score (State), and psychiatric medication use (yes/no).

Supplementary results

Network modulation by task condition

An exploratory analysis of the β weights derived from temporal sorting in GIFT was performed to identify how each network was modulated by task condition (expected reward [ER], positive prediction error [PPE], negative prediction error [NPE]). Within each group (anorexia nervosa, recovered, control), there were significant differences among the conditions in each network of interest (SN, DMN, BGN, and SMN; Fig. S1). Post hoc *t* tests (Bonferroni-corrected) demonstrated that in SN, BGN and SMN, positive β values (across the entire network) seen for PPE and ER were significantly different from negative β values seen for NPE trials. As would be expected, the opposite was seen in the DMN, in which PPE and ER were associated with negative β values, which differed from the positive β values seen during NPE. There were no significant group differences (anorexia nervosa, recovered, control) among β weights in any condition in the networks of interest.

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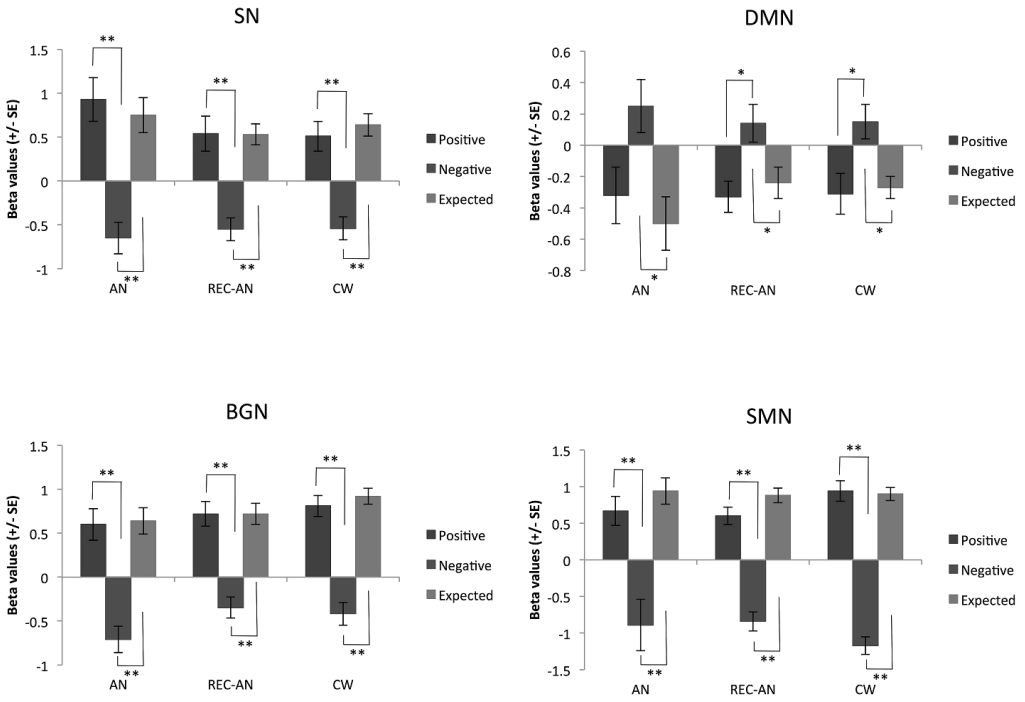

Fig. S1: Network modulation by task condition. Association estimates (β weights) indicate the degree to which each network time course was associated with task conditions. This is shown for the salience network (SN), default mode network (DMN), basal ganglia network (BGN) and sensorimotor network (SMN) for the following conditions: positive prediction error (positive), negative prediction error (negative), and expected reward (expected). These values represent each network as a whole. AN: anorexia nervosa; CW = control women; REC-AN: recovered anorexia nervosa. *$p < 0.05$, **$p < 0.001$.

References

1. Frank GK, Reynolds JR, Shott ME, et al. Altered temporal difference learning in bulimia nervosa. *Biol Psychiatry* 2011;70:728-35.
2. Bell AJ, Sejnowski TJ. An information-maximization approach to blind separation and blind deconvolution. *Neural Comput* 1995;7:1129-59.
3. Seeley WW, Menon V, Schatzberg AF, et al. Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci* 2007;27:2349-56.
4. Kullmann S, Heni M, Veit R, et al. The obese brain: association of body mass index and insulin sensitivity with resting state network functional connectivity. *Hum Brain Mapp* 2012;33:1052-61.
5. Tregellas JR, Wylie KP, Rojas DC, et al. Altered default network activity in obesity. *Obesity (Silver Spring)* 2011;19:2316-21.
6. Storey JD, Tibshirani R. Statistical significance for genomewide studies. *Proc Natl Acad Sci U S A* 2003;100:9440-5.
7. Frank GK, Shott ME, Hagman JO, et al. Alterations in Brain Structures Related to Taste Reward Circuitry in Ill and Recovered Anorexia Nervosa and in Bulimia Nervosa. *Am J Psychiatry* 2013 [Epub ahead of print].
8. Meda SA, Stevens MC, Folley BS, et al. Evidence for anomalous network connectivity during working memory encoding in schizophrenia: an ICA based analysis. *PLoS One* 2009;4:e7911.
9. Shirer WR, Ryali S, Rykhlevskaia E, et al. Decoding subject-driven cognitive states with whole-brain connectivity patterns. *Cereb Cortex* 2012;22:158-65.