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**Supplementary Methods**

*Patients with anorexia nervosa*

Characteristics of the anorexia nervosa group: mean age at assessment 25.0 (standard deviation [SD] 6.9) years; mean age at onset 17.8 (SD 4.1) years; mean lowest lifetime body mass index 15.2 (SD 1.6, range 10.5–17.3); mean level of education 14.0 (SD 2.8) years. Of these patients, 93 belonged to the restricting subtype (no binging or purging behaviour in their lifetime); 73 patients had bulimic symptoms either during or before/after anorexia nervosa episode.

Criteria for participation in the study were a lifetime diagnosis of anorexia nervosa according to the DSM-IV criteria and age older than 14 years. Exclusion criteria were traumatic brain injury, any neurologic or systemic illness independent of the eating disorder, Axis I comorbidity (except for depressive and anxious disorders), alcohol or substance abuse, and psychoactive medication (except for antidepressants, which were used by 23% of patients).

Comparing underweight and weight-restored patients, we found no significant differences in age at onset of illness. Weight-restored patients were significantly older at the time of assessment (26.1 [SD 5.9] yr v. 23.7 [SD 7.9]; t = 2.18, p = 0.030), reported on average a less severe lowest lifetime BMI (15.7 [SD 1.4] v. 14.5 [SD 1.7]; t = 4.83, p < 0.001) and had more years of educational (14.7 [SD 2.6] v. 13.0 [SD 2.9] yr; t = 3.81, p < 0.001).

*Healthy women*

Characteristics of the control group: mean age at assessment 27.2 (SD 4.8) years; mean level of education 16.0 (SD 2.4) years.

Exclusion criteria were BMI below 18; a first-degree relative with a lifetime eating disorder; traumatic brain injury; any neurologic, psychiatric or systemic illness; alcohol or substance abuse; and use of psychoactive medication.

Some of the participants in this study (113 patients, 84 controls) participated in our previous neuropsychological study.1 Of all patients with anorexia nervosa who participated in the neuroimaging study (n = 50), 17 who were scanned after full recovery (at least 6 months of normal weight and regular menses) were excluded because the few participants in genotype groups (2 Met-Met, 11 Val-Met, 4 Val-Val) did not allow powerful analysis.

Handedness was assessed with the Edinburgh Handedness Inventory;2 which yields scores ranging from –100, denoting consistent left-handedness, to +100, denoting consistent right-handedness. As in our previous study,1 more patients than controls were more left-handed (13% v. 5%; odds ratio [OR] 2.9, 95% confidence interval [CI] 1.2–6.9; p = 0.020). In the subsample that underwent resting-state functional magnetic resonance imaging (fMRI), no difference in handedness emerged.

Given the high frequency of fluid balance disturbances in patients with anorexia nervosa, we performed a bioimpedence analysis (Akern Corporation) in a subgroup of patients (n = 16) to be able to verify the role of dehydration on our findings in the anorexia nervosa group. A hydration index was obtained by bioimpedence output.3 As also brief-term dehydration can confound the assessment of brain atrophy,4 and effects of dehydration are unknown as regards intrinsic brain activity and assessment, we instructed all participants to normally eat and drink in the hours preceding the scanning.

*Participants in the MRI study*

Patients with anorexia nervosa who participated in the MRI study did not differ from those who did not in terms of age, age at onset and education. To obtain comparable samples, healthy women who underwent scanning were slightly younger than those who did not (t = 2.25, p = 0.030), whereas no differences emerged for education (t = 0.87, p = 0.38) and hand lateralization (t = 1.53, p = 0.13). Patients with anorexia nervosa and healthy women who participated in the MRI study had similar age (26.9 [SD 7.3] v. 25.8 [SD 6.7] yr; t = 0.63, p = 0.53), educational level (14.4 [SD 2.3] v. 15.5 [SD 2.4] yr; t = 1.88, p = 0.08) and hand lateralization scores (56.9 [SD 38.3] v. 52.5 [DF 46.8]; t = 0.41, p = 0.68).

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Genotype analysis

Polymerase chain reaction (PCR) primers were designed with open-source Primer3 software (http://frodo.wi.mit.edu/primer3/): FOR 5’-CATCACCATCGAGATCAACC-3’ and REV 5’-CCTTTTTCCAGGTCTGACAC-3’. The PCR and High Resolution Melt (HRM) analysis were carried out using Takara Ex Taq R-PCR custom (Takara Bio Europe S.A.S.). The PCR was performed in a 25 µL total volume containing 1X PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 300 nM of each primer, 1.5 µM of Eva Green, 1.25 IU Taq DNA polymerase and 3 × 10⁹ copies/µL DNA; amplification conditions were 95°C 3 min, 40 cycles of 95°C 10 s, 56°C 20 s, and final step of 72°C 5 min. The HRM analysis was performed with the temperature ramping (81°C to 91°C, rising by 0.1°C).

Three different melting profiles were obtained: 1 for heterozygotes Val158Met, 1 for homozygotes Val158Val and 1 for homozygotes Met158Met. In the HRM analyses, homozygote and heterozygote curves were confirmed by sequencing as reference for genotype analysis of unknown samples. The PCR products were sequenced by Big Dye Terminator kit (Applied Biosystems Inc.) and resolved on an ABI-PRISM 3100 Genetic Analyzer (Applied Biosystems Inc.).

Melting curves were normalized throughout the calculation of “line of best fit” of 2 normalization regions, before and after major fluorescence drop, corresponding to melting of the PCR product, with software provided with Rotor-Gene 6000 (Corbett Research).

Functional MRI image analysis

The first 5 volumes of every scan were discarded, to remove any stabilization effects. Preprocessing consisted of motion correction using Fourier interpolation (volume registration using least squares alignment of 3 translational and 3 rotational parameters), spatial smoothing with a 6 mm full-width at half maximum Gaussian kernel, mean-based intensity normalization of all volumes by the same factor, linear and quadratic detrending, and spatial normalization via estimation of a linear transformation from the individual functional spaces to MNI152 standard brain space using each individual’s high-resolution anatomic image. A high-pass filter setting of 200 seconds (< 0.005 Hz) was used to reduce very low-frequency artifacts, such as scanner drift, and a low-pass filter was used to remove any components in the high-frequency spectrum (> 0.1 Hz).

A seed voxel correlation approach was used to explore functional connectivity in the prefrontal cortex. Nuisance signals were removed by multiple regression before functional connectivity analyses. Each individual’s 4-dimensional (4-D) time series was regressed on 9 predictors, consisting of white matter, cerebrospinal fluid, the global signal and 6 motion parameters (3 cardinal directions and rotational movement around 3 axes). The time series of the nuisance signals were extracted by:

- averaging all voxels in the brain (global signal) across the time series;
- segmenting each individual’s high-resolution structural image, applying a threshold at 80% tissue type probability, and averaging all voxels within the thresholded mask (white matter and cerebrospinal fluid) across each time series; and
- using the residuals obtained from motion correction by FLIRT (FMRIB).

Each participant’s residual 4-D time series was transformed into Montreal Neurological Institute space by means of a linear affine transformation implemented in FSL, and the time series was extracted for each seed. Time series were averaged across all voxels in the seed ROI and then, for each participant, the correlations between the time series of the seed ROI and of each voxel in the brain were determined. Lastly, correlation maps were converted to Z value maps. The connectivity maps derived from the left and right seed were averaged to create a single map. The resulting standardized maps were then used to perform group comparisons and correlations using age and hand lateralization as nuisance variables.

Supplementary Results

Genotype distribution

Among patients with anorexia nervosa, there were 80 homozygotes (45 Val-Val and 35 Met-Met) and 86 heterozygotes; healthy controls numbered 72 homozygotes (34 Val-Val and 38 Met-Met) and 68 heterozygotes. In both samples, the distribution was consistent with expectations based on the Hardy-Weinberg equilibrium (patients $\chi^2_1 = 0.26, p = 0.60$; controls $\chi^2_1 = 0.11, p = 0.74$). No differences emerged in genotype distribution between the restricting or bingeing/purging patients ($\chi^2_2 = 2.59, p = 0.27$) or between underweight and weight-restored patients ($\chi^2_2 = 2.93, p = 0.23$).

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Set-shifting impairment

Patients with anorexia nervosa performed significantly worse than healthy controls on the WCST (Table S1). In the control group, WCST scores showed significant correlations with education and BMI at the time of assessment (Table S2). In the anorexia nervosa sample, there were no significant correlations between WCST scores and age or BMI at the time of assessment, whereas education correlated significantly with WCST perseverative responses (Table S2). In both samples, no significant correlations were found between hand lateralization and WCST.

<table>
<thead>
<tr>
<th>WCST</th>
<th>Group; mean (SD) raw score†</th>
<th>Group; mean (SD) raw score†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Underweight AN, n = 73</td>
<td>Weight-restored, n = 93</td>
</tr>
<tr>
<td>Global score</td>
<td>50.7 (36.6)</td>
<td>50.3 (34.2)</td>
</tr>
<tr>
<td>Perseverative responses</td>
<td>17.3 (14.8)</td>
<td>16.9 (13.6)</td>
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<tr>
<td>Nonperseverative errors</td>
<td>16.2 (14.2)</td>
<td>15.1 (10.9)</td>
</tr>
<tr>
<td></td>
<td>t&lt;sub&gt;1&lt;/sub&gt;,306 = 0.31</td>
<td>t&lt;sub&gt;1&lt;/sub&gt;,306 = 0.51</td>
</tr>
<tr>
<td>All patients, n = 166</td>
<td>50.5 (35.2)</td>
<td>37.4 (30.4)</td>
</tr>
<tr>
<td>Controls, n = 140</td>
<td>17.1 (14.1)</td>
<td>12.9 (11.1)</td>
</tr>
<tr>
<td></td>
<td>15.6 (12.4)</td>
<td>11.5 (10.1)</td>
</tr>
<tr>
<td></td>
<td>t&lt;sub&gt;1&lt;/sub&gt;,306 = 4.34‡</td>
<td>t&lt;sub&gt;1&lt;/sub&gt;,306 = 3.97‡</td>
</tr>
</tbody>
</table>

SD = standard deviation; WCST = Wisconsin Card Sorting Task.
*No differences emerged between underweight and weight-restored patients.
†WCST scores adjusted for age and education were used for statistical analysis after logarithmic transformation.
‡p < 0.001.

<table>
<thead>
<tr>
<th>Group; WCST</th>
<th>Age</th>
<th>BMI</th>
<th>Education</th>
<th>Onset</th>
<th>Illness duration</th>
<th>Edinburgh</th>
<th>HSCL depression</th>
<th>HSCL anxiety</th>
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<tbody>
<tr>
<td>Anorexia nervosa</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Global score</td>
<td>−0.03</td>
<td>0.10</td>
<td>−0.25</td>
<td>−0.04</td>
<td>−0.06</td>
<td>−0.01</td>
<td>−0.03</td>
<td>−0.07</td>
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<tr>
<td>Perseverative responses</td>
<td>0.01</td>
<td>0.06</td>
<td>−0.31*</td>
<td>−0.03</td>
<td>−0.01</td>
<td>0.05</td>
<td>−0.06</td>
<td>0.05</td>
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<tr>
<td>Nonperseverative errors</td>
<td>0.01</td>
<td>0.08</td>
<td>−0.17</td>
<td>−0.03</td>
<td>0.02</td>
<td>−0.01</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td></td>
</tr>
<tr>
<td>Global score</td>
<td>0.15</td>
<td>0.28*</td>
<td>−0.48*</td>
<td>−</td>
<td>−</td>
<td>0.10</td>
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<td>0.10</td>
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<td>Perseverative responses</td>
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<td>−</td>
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<td>−</td>
<td>0.10</td>
<td>0.11</td>
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</table>

BMI = body mass index; HSCL = Hopkins Symptom Checklist; WCST = Wisconsin Card Sorting Task.
* p < 0.001.

Set-shifting impairment and oral contraceptives

In the control sample, 40 participants (10 Val-Val, 15 Val-Met, 15 Met-Met) were taking oral contraceptives at the time of assessment. In the anorexia nervosa sample, 17 patients were taking oral contraceptives (4 Val-Val, 9 Val-Met, 4 Met-Met) and 26 had amenorrhea (25 Val-Val, 34 Val-Met, 17 Met-Met) at the time of assessment. In controls, no significant differences were found in the WCST performance of those who were taking oral contraceptives in any of the genotype groups. In the anorexia nervosa group, there were significant differences between those who were or were not taking oral contraceptives in the Met-Met group (t = 3.80, p = 0.001), and there was a trend toward significance in the Val-Val group (t = 2.22, p = 0.06; Fig. S1).

References


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Fig. S1: Perseverative responses according to menstrual status and oral contraceptive use in patients with anorexia nervosa and healthy controls. In the 2 samples, 17 patients (4 Val-Val, 9 Val-Met, 4 Met-Met) and 40 controls (10 Val-Val, 15 Val-Met, 15 Met-Met) were taking oral contraceptives.

Fig. S2: Seed-based negative correlations of seeds located in the dorsolateral prefrontal cortex. Figure shows area of significant difference ($p < 0.05$, TFCE-corrected) between Met-Met genotype and Val carriers in the anorexia nervosa (AN) group (Met-Met > Val); peak: –30, –93, –9 (Brodmann area 18). Graph shows average coactivation ($z$ score) according to the catechol-O-methyltransferase enzyme (COMT) genotype in patients with anorexia nervosa. No significant effect was found in healthy controls. Analyses by nonparametric permutation test, with age, education and hand lateralization as covariates. TFCE = threshold-free cluster enhancement.

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Fig. S3: Seed-based negative correlations of seeds located in the ventrolateral prefrontal cortex. Figure shows area of significant difference ($p < 0.05$, TFCE-corrected) between Met-Met genotype and Val carriers in the anorexia nervosa (AN) group (Met-Met > Val); peaks: –3, –90, –6 (Brodmann area 17) and –9, –69, –48 (cerebellum). Graph shows average coactivation ($z$ score) according to the catechol-O-methyltransferase enzyme (COMT) genotype in patients with anorexia nervosa. No significant effect was found in healthy controls. Analyses by nonparametric permutation test, with age, education and hand lateralization as covariates. TFCE = threshold-free cluster enhancement.

Fig. S4: Seed-based negative correlations of seeds located in the ventromedial prefrontal cortex. (A) Area of significant genotype × group interaction ($p < 0.05$, TFCE-corrected) between Met-Met genotype and Val carriers in the anorexia nervosa (AN) group (Met-Met > Val); peaks 18, –84, –36, cerebellum; –39, 24, 51, Brodmann area [BA] 9; and 33, –54, 30, BA 39. (B) Area of significant difference ($p < 0.05$, TFCE-corrected) between Met-Met genotype and Val carriers in the anorexia nervosa (AN) group (Met-Met > Val); peaks 18, –84, –36, cerebellum), –51, 9, 51 (BA 6) and 33, –57, 30 (BA 39). Graphs show average coactivation ($z$ score) according to the catechol-O-methyltransferase enzyme (COMT) genotype in patients with anorexia nervosa and healthy controls for the brain area shown in panel B. No significant effect was found in healthy controls. Analyses by nonparametric permutation test, with age, education and hand lateralization as covariates. TFCE = threshold-free cluster enhancement.