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Supplemental Materials

SM 1 Methods

SM 1.1 Participants

AN participants were recruited from specialized eating disorder programs of a university child and adolescent psychiatry and psychosomatic medicine department and diagnosed according to DSM-5 criteria using semi-structured clinical interviews. Comorbid psychiatric diagnoses were made by an expert clinician and included examination of the participant and careful chart review (including medical and psychiatric history, physical examination, laboratory values and several psychiatric screening instruments). The AN participants were amenorrheic with two exceptions: One patient took oral contraceptives; thus the natural menstrual cycle could not be evaluated and the other continued to maintain a menstrual cycle.

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 semi-structured interview.

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/m^2 (if older than 18 years), or were currently obese (BMI not over 94th age percentile if younger than 18 years; BMI not over 28kg/m^2 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 other than SSRI within four 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.

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Pairwise case-control age-matching was carried out using the Munkres algorithm[1] resulting in a maximum difference of 1.6 years between the individuals within one pair. 24 AN participants and 20 HC were already included within the sample of Boehm et al.[2]

Study data were managed using secure, web-based electronic data capture tools REDCap (Research Electronic Data Capture)[1].

SM 1.2 Bisulfite Pyrosequencing Protocol

Genomic DNA was bisulfite treated using the EZ DNA Methylation Gold Kit (Zymo Research, Range, CA, USA). One amplicon (fragment 5HTT_P3 as described in Wankerl et al. [4]) was generated from bisulfite-treated DNA. PCR protocol was run as follow: HotStarTaq polymerase (Qiagen, Hilden, Germany) 95°C 15', 49x (95°C 35", 52°C 35", 72°C 35"); 72°C 5'. Sample preparation was carried out using Vacuum Prep Tool according to standard procedures. 12-15µl PCR product was immobilized to 2µl Streptavidin Sepharose™ HP beads (GE Healthcare) followed by annealing to 0.8-1.0µl sequencing primer (5µM) for 2' at 80°C. Amplicon and sequencing primers are depicted in Table SM 1.2a.

TYPE OF PRIMER	NAME OF PRIMER	PRIMER SEQUENCE (5'-3')
FORWARD	5HTT-F	ggg gaa gta tta agt tta t
REVERSE	5HTT-R	Biotin-ccc cta caa caa taa aca
SEQUENCING	5HTT-S1new	att tag aga tta gat tat gtg

Table SM 1.2a: Primers used for bisulfite pyrosequencing of parts the *SLC6A4* promoter-associated CpG island; All primers refer to bisulfite treated DNA.

A sequence within the *SLC6A4* promoter-associated CpG island as previously described by Philibert et al. [5] (GenBank accession number: NG_011747) is shown in Table SM 1.2b. We focused on 15 CpG sites within the amplicon 3 of a 799-bp promoter region (originally CpG 43-57, referred to in the current study as CpG 1-15) [6]. CpG sites analyzed by means of bisulfite pyrosequencing are numbered and base pair positions are depicted according to the NCBI genome browser on the left hand side.

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5161 Tctttggcggcggctatctagagatcagaccatgtgagggcccg^{1}cg^{2}ggtacaaatacg^{3}c

5221 Cg^{4}cg^{5}ccg^{6}gcg^{7}cccctccg^{8}cacagccagcg^{9}ccg^{10}ccg^{11}ggtgcctcg^{12}agggcg^{13}cg^{14}aggccagc

5281 Ccg^{15}cctgcccagccggaccagcctcccgcgcagcctggcaggtgggtccgcttttcc

Table SM 1.2b: Sequence of the SLC6A4 promoter–associated CpG island

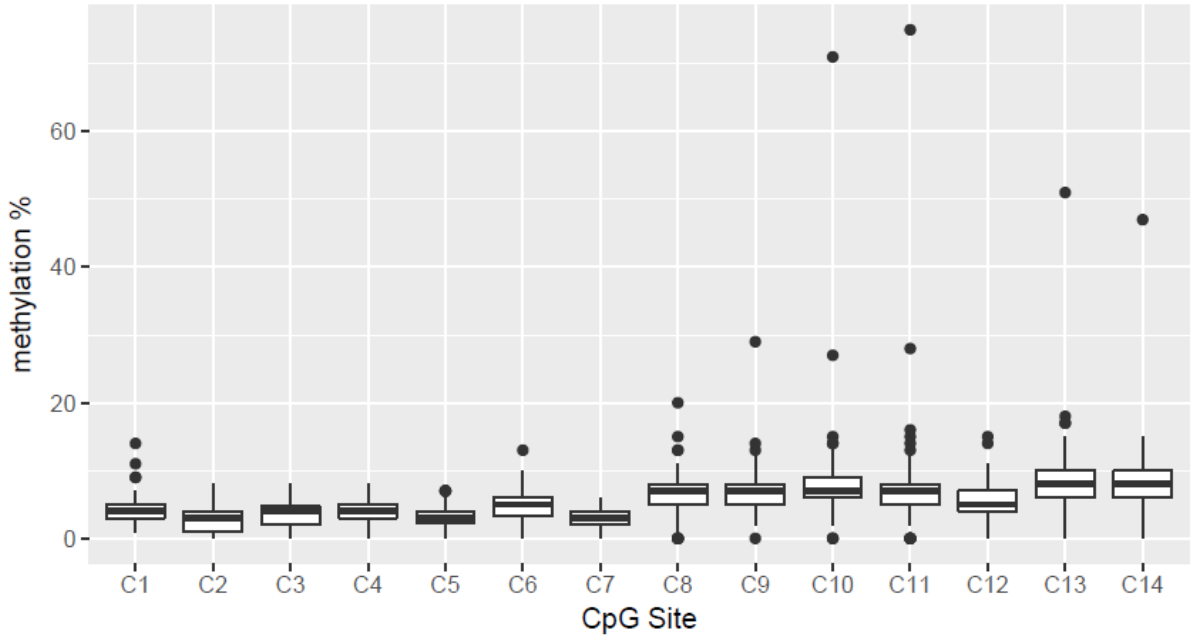

Figure SM 1.2a: Boxplots showing DNA methylation levels for each of the 14 investigated CpG sites across groups; The box includes methylation values for each CpG site between 25th - 75th quantile (median ± 1 interquartile range), the whiskers represent the range of estimates within 1.5-fold of the interquartile range.

Correlation between methylation sites were varied (mean pairwise correlations between CpGs=0.31 ; Figure SM 1.2b). Inspection of transcription factor binding site information, based on ENCODE data, indicated that most binding sites covered the whole region from CpG1-14 (Figure SM 1.2c) justifying the approach of averaging across CpG sites which has been used here and in previous reports on the SLC6A4 promoter region[6,7].

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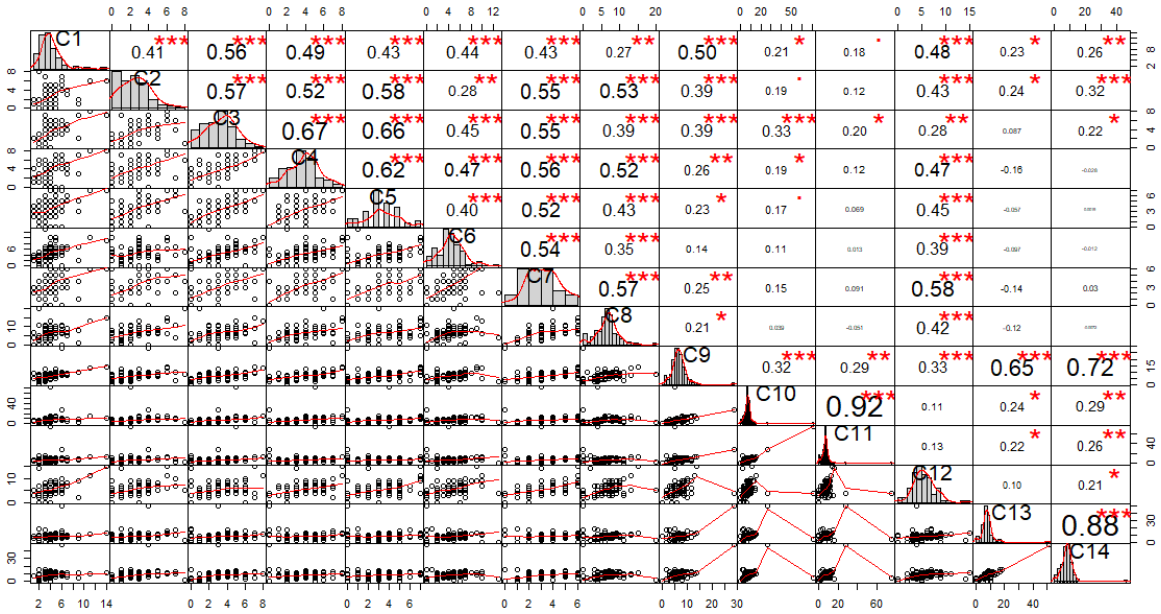

Figure SM 1.2b. Covariance plot between all 14 CpG sites.

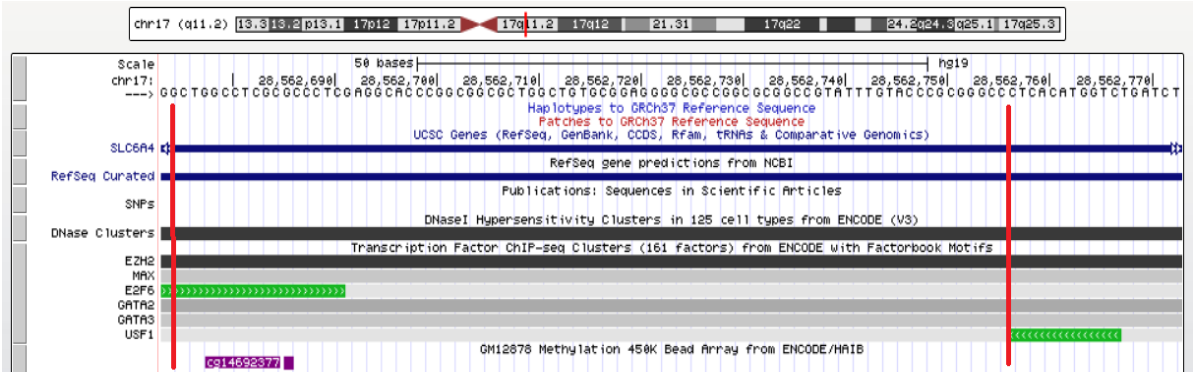

Figure SM 1.2c.: Transcription factor binding sites (horizontal bars in lower panel) covered the whole genomic region investigated (within vertical red bars), based on ENCODE data. Figure produced via the UCSC Genome Browser (access date: May 24th 2019).

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1.3 fMRI 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=2.26ms; flip angle=9°; slice thickness=1mm; voxel size=1x1x1mm^3; field-of-view=256x224mm^2; 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°; 3,4mm in-plane resolution; slice thickness of 2,4mm (1mm gap resulting in a voxel size of 3,4x3,4x2,4mm^3); FoV=220x220mm^2; bandwidth of 200Hz/pixel.

1.4 fMRI 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. The EPI volumes were then normalized to MNI space using the DARTEL template and corresponding flow field [8]. 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 (ART).

1.5 Independent component analysis

Spatial group independent component analysis was conducted to extract 24 temporally coherent networks.

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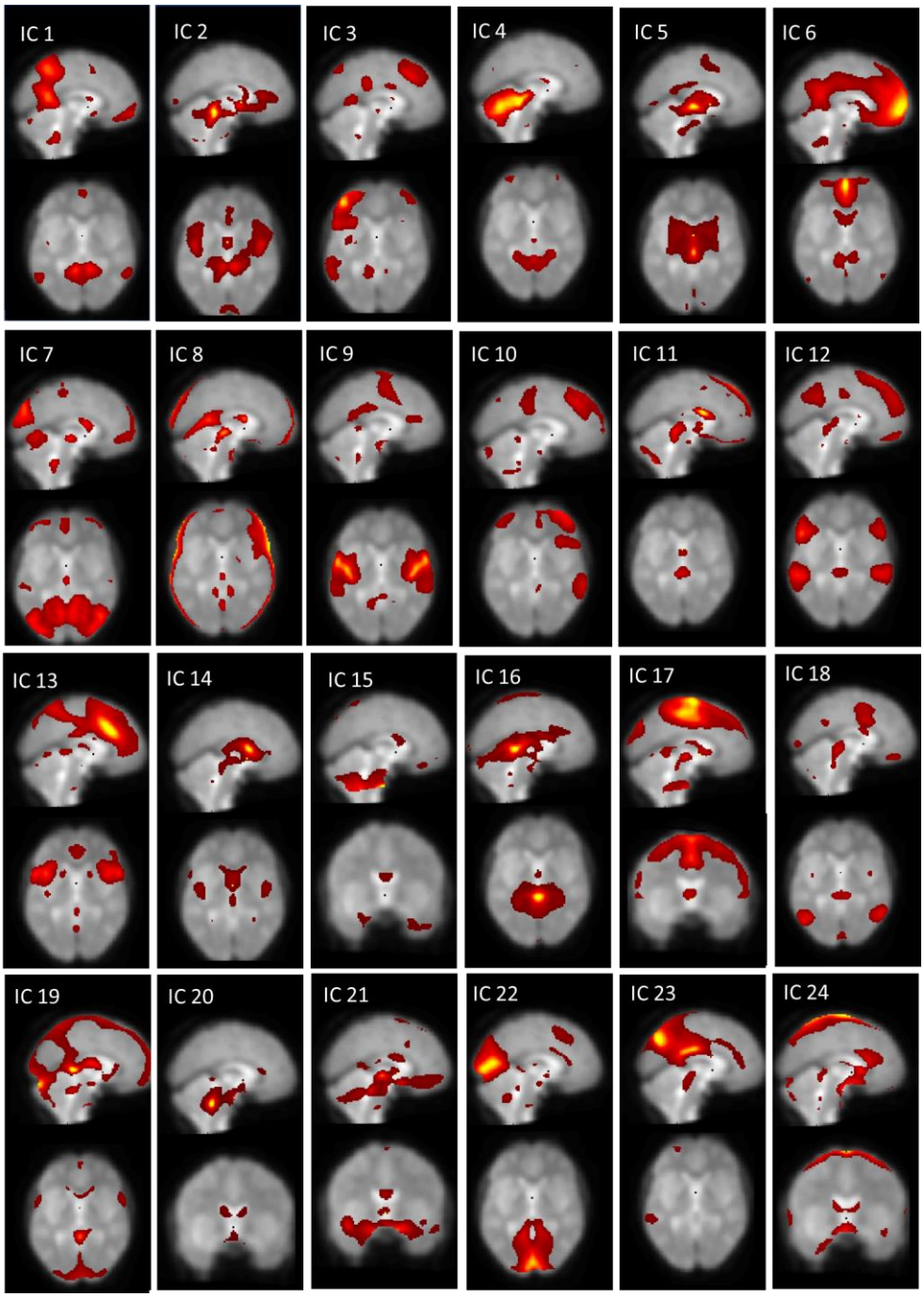

Figure SM 1.5: Spatial maps of the 24 extracted independent components; Selected slices of all 24 independent components; IC=independent component.

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1.6 Identification of components of interest

Components of interest were identified by spatial correlation with the relevant templates by Yeo et al. [9]. Two components (IC9 and IC13) were identified as SN, while the visual network (IC7) was employed as negative control.

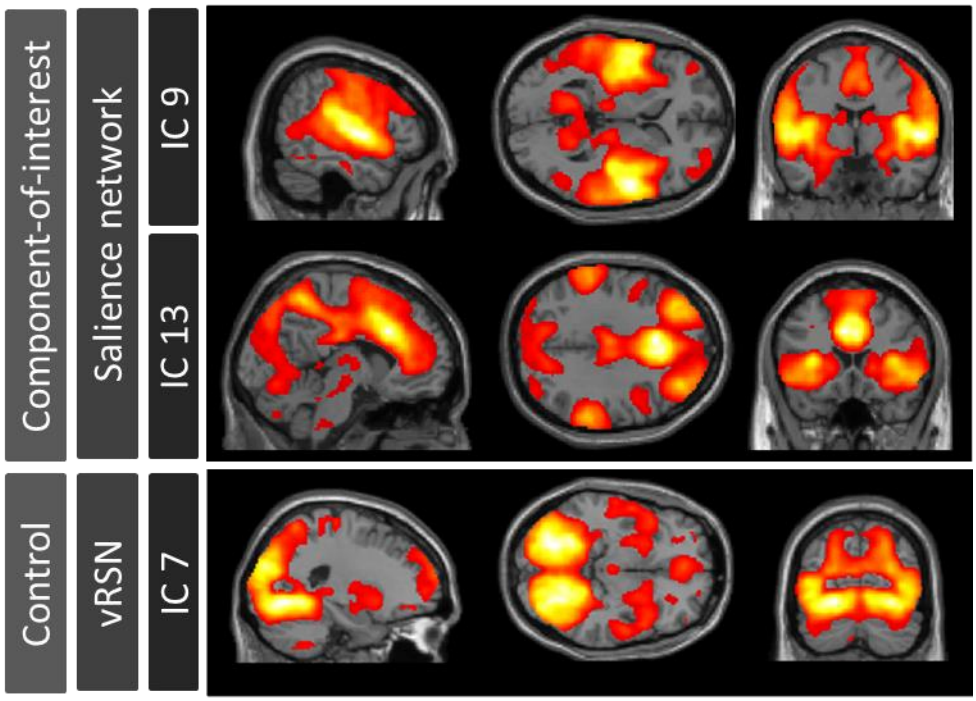

Figure SM 1.6: Spatial maps of the independent components; Selected slices of spatial maps of the two identified independent components that showed significant spatial correlation with the SN template [9] and the visual network as negative control network; IC=independent component, vRSN=visual resting state network; spatial maps are plotted as t-statistics thresholded at p=0.05 (FWE).

SM 2 Results

2.1 Group x methylation$_{CpG13}$ interaction

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Investigating the group x methylation$_{CpG13}$ interaction with age as a covariate revealed a significant finding at the right dlPFC (t=4.73; p=0.014 (FWE)).

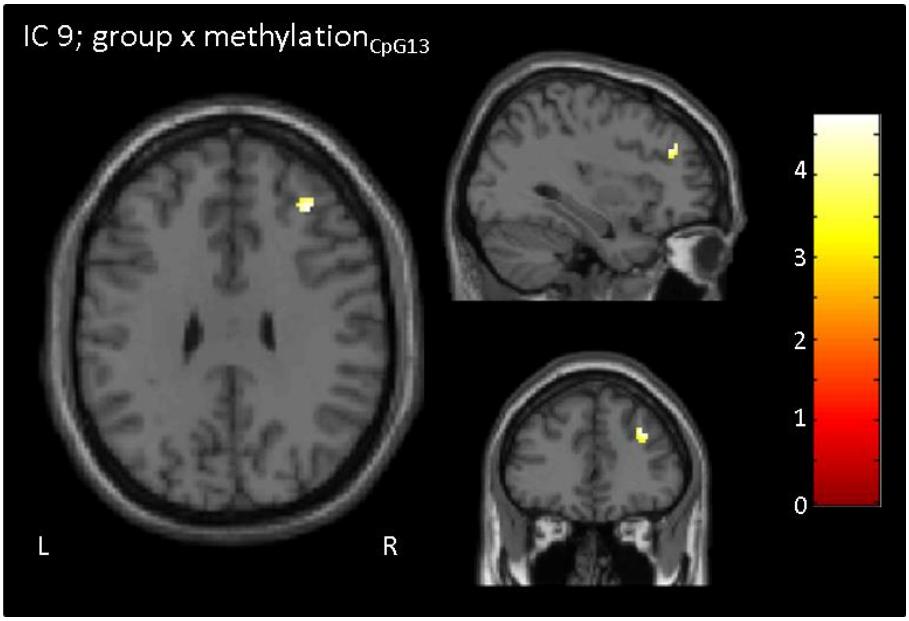

Figure SM 2.1: Group x SLC6A4 methylation$_{CpG13}$ interaction; Significant *group x SLC6A4 methylation$_{CpG13}$* interaction at the right dlPFC, for visualization purpose displayed at a threshold of p=0.001 (uncorrected); color bar represents t-values.

2.2 *Group x methylation$_{mean}$ interaction at a lower threshold*

When lowering the threshold to p=0.05 (uncorrected) the finding appears in both hemispheres.

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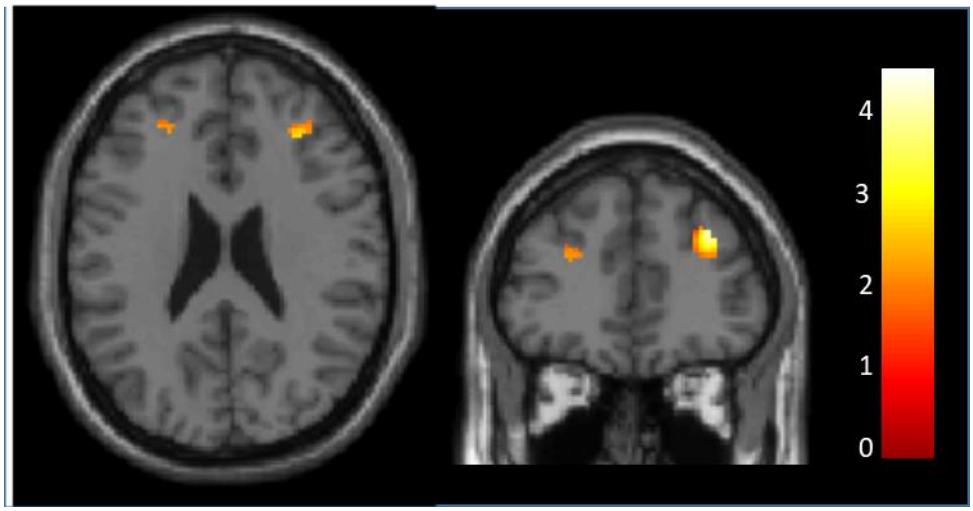

Figure SM 2.2: Group x SLC6A4 methylation$_{mean}$ interaction at p=0.05 (uncorrected); *Group x SLC6A4 methylation$_{mean}$* interaction at the left and right dlPFC, for visualization purpose displayed at a threshold of *p*=0.05 (uncorrected); color bar represents t-values.

2.3 Group x SLC6A4 methylation$_{mean}$ and the fronto-parietal network

In order to specify whether our finding of a significant group x SLC6A4 methylation$_{mean}$ interaction at the dlPFC exclusively constitutes a methylation-dependency of the frontal-limbic circuit (reflected by the SN), we also conducted post-hoc tests of the group x SLC6A4 methylation$_{mean}$ interaction in the fronto-parietal network. The fronto-parietal network is also anchored by the dlPFC, but in contrast to the SN is characterized by synchronized activity with parietal brain regions instead of subcortical limbic regions. Results showed no group x SLC6A4 methylation$_{mean}$ interaction.

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2.4 Mediation analysis

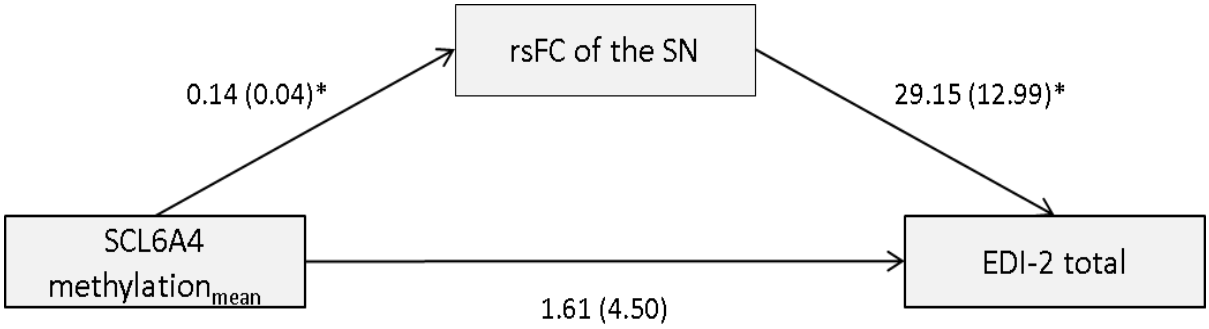

Figure SM 2.3: Mediation analysis between SCL6A4 methylation$_{mean}$ and EDI-2 total with rsFC of the SN as mediator; Unstandardized coefficients and standard error are displayed; *significant with p<0.05; rsFC=resting state functional connectivity; SN=salience network; EDI-2 total=eating disorder inventory; SCL6A4 methylation$_{mean}$=mean *SLC6A4* methylation score

2.6 Analysis of genetic influences

To investigate whether our findings were driven by underlying methylation quantitative trait loci, we queried two different databases. mQTL.org is a catalogue of the genetic influences on DNA methylation in human blood, based on samples of 1018 mother-child pairs at five different life stages[10]. Brain-based mQTLs are described in a data catalogue hosted on epigenetics.essex.ac.uk.mQTL, based on a collection (n=166) of human fetal brain samples spanning 56-166 days post-conception [11].

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1 *SM 3 List of selected task-based fMRI studies reporting insula dysfunction in AN*

2

Author	Year	Journal	Title	fMRI-task targeting
Bär et al.[12]	2013	Acta Psychiatr Scand	Insular dysfunction and descending pain inhibition in anorexia nervosa	Pain processing
Bischoff-Grethe et al.[13]	2018	Transl Psychiatry.	Neural hypersensitivity to pleasant touch in women remitted from anorexia nervosa	Interoceptive experience
DeGuzman et al.[14]	2017	Am J Psychiatry	Association of Elevated Reward Prediction Error Response With Weight Gain in Adolescent Anorexia Nervosa	Reward learning processing
Frank et al.[15]	2013	Front Hum Neurosci	Food related processes in the insular cortex	Food processing
Frank et al.	2016	J Psychiatry Neurosci.	Prediction error and somatosensory insula activation in women recovered from anorexia nervosa	Reward learning processing
Holsen et al.[16]	2012	J Psychiatry Neurosci.	Food motivation circuitry hypoactivation related to hedonic and nonhedonic aspects of hunger and satiety in women with active anorexia nervosa and weight-restored women with anorexia nervosa	Food processing
Kerr et al.[17]	2016	Neuropsychopharmacology	Altered Insula Activity during Visceral Interoception in Weight-Restored Patients with Anorexia Nervosa	Visceral interoceptive processing
Kerr et al.[18]	2017	Psychosom Med	Influence of Visceral Interoceptive Experience on the Brain's Response to Food Images in Anorexia Nervosa.	Visceral interoceptive processing
Leppanen et	2017	Biol Psychol	Blunted neural response to implicit negative facial affect in	Social-emotional

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al.[19]			anorexia nervosa	processing
Oberndorfer et al.[20]	2013	Am. J. Psychiatry	Altered insula response to sweet taste processing after recovery from anorexia and bulimia nervosa.	Food processing
Oberndorfer et al.[21]	2013	Psychiatry Res	Greater anterior insula activation during anticipation of food images in women recovered from anorexia nervosa versus controls	Food processing
Strigo et al.[22]	2013	Int J Eat Disord	Altered insula activation during pain anticipation in individuals recovered from anorexia nervosa: evidence of interoceptive dysregulation.	Pain processing
Vocks et al.[23]	2011	J. Psychiatr. Res.	Effects of gustatory stimulation on brain activity during hunger and satiety in females with restricting-type anorexia nervosa: an fMRI study.	Food processing
Wagner et al.[24]	2007	Neuropsychopharmacology	Altered insula response to taste stimuli in individuals recovered from restricting-type anorexia nervosa.	Food processing
Wierenga et al.[25]	2017	Front Nutr	Aberrant Cerebral Blood Flow in Response to Hunger and Satiety in Women Remitted from Anorexia Nervosa.	Food processing

Table 3: Selected task-based fMRI studies reporting insula dysfunctions in AN

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