1 **Supplementary material**

2 **1 Method**

3 **1.1 Sample**

4 General exclusion criteria were current SSRI intake, psychotropic drug intake within the last 4 weeks,

5 being pregnant or breast feeding, anemia, being younger than 12 or 30 and older, having an IQ below

6 85, suffering from organic brain syndrome, dementia, schizophrenia, bipolar disorder, drug abuse,

7 obesity (BMI>97th percentile for age <18, BMI>30 for age 18 and older) as well as chronic medical or

8 neurological illnesses that could affect appetite, eating behavior or body weight (e.g. diabetes).

9 As for the healthy controls, they were excluded if they had a lifetime diagnosis of any psychiatric

10 disorder, the lowest lifetime BMI below the 10th percentile (below age 18) or <17.5 (for age 18 and

11 older) or if they were currently underweight, showed abnormal eating behavior (diet, binge eating) or

12 a binge eating disorder.

13 Patients recovered from AN were excluded if they got diagnosed with atypical AN, bulimia nervosa or

14 binge eating disorder.

15 Due to feasibility, this study included only female participants. AN has a 10:1 female-to-male

16 prevalence ratio [1], which is one of the most striking gender differences in psychiatry. However, this

17 large gap between the prevalence rates presents a major challenge for research. At the same time, the

18 comparatively low lifetime prevalence of AN (0.1% to 3.6%) and the strict criteria for defining recovery

19 make recruitment of participants difficult. Therefore, most studies, including this one, involve only

20 female participants.

21 **1.2 ATD mixture**

22 The dose of amino acids in the experimental mixtures were adjusted to the weight of each participant

23 [2–5]. Experimental and sham mixtures contained the same amount of large neutral amino acids

24 (LNAA), but differed in their tryptophan content. Accordingly, tryptophan was completely absent in

25 the mixture of the ATD condition, while the mixture of the sham condition contained 7mg/kg body

26 weight [recommended daily dose for adults; ,6]. The dose of large neutral amino acids (LNAA) was

27 constant for every participant across sessions: L-phenylalanine (132 mg/kg), L-leucine (132 mg/kg), L-

28 isoleucine (84 mg/kg), L-methionine (50 mg/kg), L-valine (96 mg/kg), L-threonine (60 mg/kg), and L-

29 lysine (96 mg/kg).

30 Participants were informed about the bad taste of the mixtures and it was recommended to drink it all

31 at once. If desired, participants were allowed to drink a provided tryptophan free beverage afterwards.

1 **1.3 Biochemical measures**

2 Immediately after the blood sampling, the samples were centrifuged at 4,000g and 4°C for 10 minutes.

3 Afterwards, the plasma was stored in a -81°C fridge. Finally, blood analyses were conducted at the

4 Institute for Clinical Chemistry and Laboratory Medicine of the Technische Universität Dresden,

5 Medizinische Fakultät [for a detailed description, see 7]. The analyses of physiological amino acids were

6 performed by cation exchange chromatography with post-column derivatization, using the Biochrome

7 amino acid analyzer B30 (http:\\www.biochrom.co.uk). To avoid influences of calibration, all samples

8 from each patient were run within one and the same series, using additional internal standard in each

9 sample.

10 Missing values (n=3 during the first session, n=6 during the second) due to complications during blood

11 sampling.

12 **1.4 fMRI data acquisition**

13 The parameters of the rapid acquisition gradient echo (MP-RAGE) sequence were the following:

14 number of slices=176; repetition time=1900ms; echo time=2.26ms; flip angle=9°; slice thickness=1mm;

15 voxel size=1x1x1mm³; field-of-view=256x224mm²; bandwidth=2004Hz/pixel.

16 The parameters of the gradient-echo T2*-weighted echo planar imaging (EPI) were the following: tilted

17 30° towards AC–PC line (to reduce signal dropout in orbitofrontal regions); number of volumes=190;

18 number of slices=40; repetition time=2200ms; echo time=30ms; flip angle (FA) of 75°; 3,4mm in-plane

19 resolution; slice thickness of 2,4mm (1mm gap resulting in a voxel size of 3,4x3,4x2,4mm³);

20 FoV=220x220mm²; bandwidth of 200Hz/pixel.

21 **1.5 fMRI data preprocessing**

22 The applied standard image data preprocessing procedure included slice time correction of the

23 functional data, realignment and registration to the mean. The realigned files were coregistered to the

24 subject's structural brain image. A DARTEL template was created using structural images from all

25 subjects. The EPI volumes were then normalized to MNI space using the DARTEL template and

26 corresponding flow field [8]. The resulting data were smoothed with an isotropic 8mm FWHM Gaussian

27 kernel. The quality of the fMRI data was evaluated by manual inspection and by using artifact detection

28 tools (ART).

29 **2 Results**

30 **2.1 Main effect of group and condition**

Main effect	Seed-region	Brain-region	Peak-cluster	k	p-FWE
group	Left anterior insula	left MFG and IFG	-34, 8, 32	184	0.02

ACC	Right MFG/ frontal pole	28, 40, 40	168	0.045
	Left rostral PFC	Left ITG and MTG	-54, -56, -14	221	0.011
		Left lateral occipital gyrus	-22, -72, 54	168	0.037
		Occipital pole	-12, -104, 14	157	0.049
condition	Left rostral PFC	Left and right thalamus	04, -10, 06	200	0.001

1 *Note. MFG=medial frontal gyrus; IFG=inferior frontal gyrus; PFC=prefrontal cortex; ITG=inferior*

2 *temporal gyrus; ACC=anterior cingulate cortex; k=number of voxels; FWE=Family-wise error.*

3 **2.2 Contrasts of the extracted connectivity values of the significant cluster of the group x condition**
4 **interaction**

Contrast	t	p-value
HC sham versus recAN sham	-3.096	0.004
HC depletion versus recAN depletion	3.855	<0.001
HC depletion versus HC sham	4.449	<0.001
recAN depletion versus recAN sham	-3.607	0.002
HC sham versus recAN depletion	-0.662	0.512

5 *Note. HC=healthy control; recAN=patients recovered from Anorexia nervosa*

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