

Neuroendocrinology

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Online Supplemental Material

Hypothalamic structural and functional imbalances in anorexia nervosa

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Supplementary Tables

Metabolites	Comparisons	Mean diff.	95,00% CI of diff.	Significant?	Adjusted P Value	N1	N2	q	DF
Glx/Cr $F_{(2, 23)} = 5.807$ $P = 0.0091$	CTL vs. CL	0.299	-1.23 to 1.828	No	0.877	8	10	0.692	23
	CTL vs. AN	2.011	0.399 to 3.623	Yes	0.013	8	8	4.418	23
	CL vs. AN	1.712	0.183 to 3.241	Yes	0.026	10	8	3.965	23
NAA/Cr $F_{(2, 25)} = 2.251$ $P = 0.1262$	CTL vs. CL	0.619	-0.163 to 1.401	No	0.140	8	10	2.790	25
	CTL vs. AN	0.548	-0.234 to 1.329	No	0.209	8	10	2.468	25
	CL vs. AN	-0.071	-0.808 to 0.665	No	0.968	10	10	0.341	25
Ins/Cr $F_{(2, 20)} = 0.3412$ $P = 0.7150$	CTL vs. CL	0.164	-0.454 to 0.782	No	0.783	7	10	0.949	20
	CTL vs. AN	-0.017	-0.715 to 0.681	No	0.998	7	6	0.088	20
	CL vs. AN	-0.181	-0.828 to 0.467	No	0.762	10	6	0.999	20
GPC/Cr $F_{(2, 24)} = 2.571$ $P = 0.0973$	CTL vs. CL	0.077	-0.100 to 0.254	No	0.533	8	10	1.533	24
	CTL vs. AN	0.164	-0.017 to 0.345	No	0.081	8	9	3.198	24
	CL vs. AN	0.0875	-0.084 to 0.259	No	0.424	10	9	1.800	24

Table S1. Feeding-evoked response of the metabolite-to-Creatine (Cr) ratio in the hypothalamus. One-way ANOVA followed by a Tukey's multiple comparisons test. CTL, control; CL, constitutional leanness; AN, anorexia nervosa. Glx, glutamine + glutamate; NAA, *N*-acetyl-aspartate; Ins, myoinositol; Cho, choline.

Comparisons	Mean diff.	95,00% CI of diff.	Significant?	Adjusted <i>P</i> Value	n1	n2	t	DF
CTL fasting vs. CTL fed	-0.662	-1.833 to 0.510	No	0.406	8	8	1.454	23
CTL fasting vs. CL fasting	-0.357	-1.635 to 0.922	No	0.870	8	10	0.691	46
CTL fasting vs. AN fasting	-1.505	-2.853 to -0.157	Yes	0.020	8	8	2.767	46
CTL fed vs. CL fed	-0.058	-1.337 to 1.221	No	0.999	8	10	0.1118	46
CTL fed vs. AN fed	0.506	-0.842 to 1.854	No	0.735	8	8	0.9294	46
CL fasting vs. CL fed	-0.363	-1.411 to 0.685	No	0.764	10	10	0.8912	23
CL fasting vs. AN fasting	-1.148	-2.427 to 0.130	No	0.090	10	8	2.225	46
CL fed vs. AN fed	0.563	-0.716 to 1.842	No	0.628	10	8	1.091	46
AN fasting vs. AN fed	1.349	0.177 to 2.521	Yes	0.021	8	8	2.964	23

Table S2. Evolution of the Glx/Cr ratio in the hypothalamus. Two-way repeated measures ANOVA followed by a Sidak's multiple comparisons test. CTL, control; CL, constitutional leanness; AN, anorexia nervosa.

Metabolites	Comp.	Mean diff.	95,00% CI of diff.	Significant?	Adjusted P Value	n1	n2	q	DF
Glx/Cr $F_{(2, 24)} = 0.913$ $P = 0.415$	CTL vs. CL	0.218	-1.048 to 1.485	No	0.903	9	10	0.608	24
	CTL vs. AN	0.711	-0.629 to 2.050	No	0.396	9	8	1.874	24
	CL vs. AN	0.493	-0.815 to 1.800	No	0.620	10	8	1.330	24
NAA/Cr $F_{(2, 26)} = 1.593$ $P = 0.223$	CTL vs. CL	0.475	-0.429 to 1.379	No	0.405	9	10	1.846	26
	CTL vs. AN	0.627	-0.278to 1.531	No	0.216	9	10	2.435	26
	CL vs. AN	0.152	-0.728 to 1.031	No	0.904	10	10	0.605	26
Ins/Cr $F_{(2, 20)} = 0.841$ $P = 0.446$	CTL vs. CL	0.496	-0.496 to 1.488	No	0.431	8	10	1.789	20
	CTL vs. AN	0.155	-1.037 to 1.347	No	0.942	8	5	0.465	20
	CL vs. AN	-0.341	-1.486 to 0.805	No	0.735	10	5	1.065	20
Cho/Cr $F_{(2, 26)} = 2.163$ $P = 0.135$	CTL vs. CL	0.135	-0.052 to 0.323	No	0.192	9	10	2.536	26
	CTL vs. AN	0.004	-0.184 to 0.191	No	0.999	9	10	0.0679	26
	CL vs. AN	-0.132	-0.314 to 0.051	No	0.192	10	10	2.536	26

Table S3. Feeding-evoked response of the metabolite-to-Creatine (Cr) ratio in the thalamus. One-way ANOVA followed by a Tukey's multiple comparisons test. CTL, control; CL, constitutional leanness; AN, anorexia nervosa. Glx, glutamine + glutamate; NAA, *N-acetyl*-aspartate; Ins, myoinositol; Cho, choline.

Comparisons	Mean diff.	95,00% CI of diff.	Significant?	Adjusted <i>P</i> Value	n1	n2	t	DF
CTL fasting vs. CTL fed	0.056	-0.888 to 1.000	No	0.998	9	9	0.152	24
CTL fasting vs. CL fasting	-0.011	-0.887 to 0.864	No	>0.999	9	10	0.032	48
CTL fasting vs. AN fasting	-0.206	-1.132 to 0.720	No	0.928	9	8	0.551	48
CTL fed vs. CL fed	0.207	-0.669 to 1.082	No	0.916	9	10	0.585	48
CTL fed vs. AN fed	0.504	-0.422 to 1.430	No	0.457	9	8	1.347	48
CL fasting vs. CL fed	0.274	-0.622 to 1.170	No	0.825	10	10	0.785	24
CL fasting vs. AN fasting	-0.195	-1.099 to 0.709	No	0.934	10	8	0.534	48
CL fed vs. AN fed	0.298	-0.606 to 1.201	No	0.804	10	8	0.814	48
AN fasting vs. AN fed	0.766	-0.235 to 1.768	No	0.173	8	8	1.964	24

Table S4. Evolution of the Glx/Cr ratio in the thalamus. Two-way repeated measures ANOVA (Interaction: $F_{(2, 24)} = 0.913$, $P = 0.415$; Subjects: $F_{(2, 24)} = 0.099$; $P = 0.844$; Time: $F_{(1, 24)} = 2.934$; $P = 0.100$) followed by a Sidak's multiple comparisons test. CTL, control; CL, constitutional leanness; AN, anorexia nervosa.

ROI	Comparisons	Mean diff.	95.00% CI of diff.	Significant?	Adjusted P Value	n1	n2	q	DF
ARH $F_{(2, 22)} = 5.059$ $P = 0.0156$	CTL vs. CL	299	38 to 560	Yes	0.023	8	9	4.073	22
	CTL vs. AN	282	14 to 550	Yes	0.038	8	8	3.733	22
	CL vs. AN	-16.99	-278 to 244	No	0.985	9	8	0.231	22
LHA $F_{(2, 27)} = 4.345$ $P = 0.0231$	CTL vs. CL	-267.9	-2166 to 1630	No	0.935	10	10	0.495	27
	CTL vs. AN	-2075	-3973 to -176	Yes	0.030	10	10	3.832	27
	CL vs. AN	-1807	-3705 to 91	No	0.064	10	10	3.337	27
MPO $F_{(2, 26)} = 2.425$ $P = 0.1082$	CTL vs. CL	-435.5	-1184 to 313	No	0.3330	10	9	2.045	26
	CTL vs. AN	-631.9	-1361 to 97	No	0.0983	10	10	3.048	26
	CL vs. AN	-196.4	-945 to 552	No	0.7930	9	10	0.922	26
DMH $F_{(2, 26)} = 1.6$ $P = 0.2212$	CTL vs. CL	452.6	-247.6 to 1153	No	0.2610	9	10	2.272	26
	CTL vs. AN	49.59	-650.6 to 750	No	0.9831	9	10	0.249	26
	CL vs. AN	-403	-1085 to 279	No	0.3217	10	10	2.078	26
VMH $F_{(2, 25)} = 0.1238$ $P = 0.8841$	CTL vs. CL	-104.6	-648 to 439	No	0.8816	8	10	0.678	25
	CTL vs. AN	-82.18	-625.4 to 461	No	0.9250	8	10	0.533	25
	CL vs. AN	22.4	-490 to 535	No	0.9935	10	10	0.154	25
PaPe $F_{(2, 22)} = 1.326$ $P = 0.2858$	CTL vs. CL	-708.9	-2186 to 768	No	0.4625	8	8	1.705	22
	CTL vs. AN	182.3	-1253 to 1618	No	0.9456	8	9	0.451	22
	CL vs. AN	891.2	-544 to 2327	No	0.2838	8	9	2.205	22
PH $F_{(2, 27)} = 0.3141$ $P = 0.7331$	CTL vs. CL	47	-982 to 1076	No	0.9930	10	10	0.160	27
	CTL vs. AN	305.5	-724 to 1335	No	0.7445	10	10	1.041	27
	CL vs. AN	258.5	-771 to 1288	No	0.8090	10	10	0.881	27
MR $F_{(2, 26)} = 0.4215$ $P = 0.6604$	CTL vs. CL	-258.4	-965 to 448	No	0.6397	10	10	1.285	26
	CTL vs. AN	-95.77	-822 to 630	No	0.9426	10	9	0.464	26
	CL vs. AN	162.6	-563 to 889	No	0.8440	10	9	0.787	26

Table S5. Diffusion weighted imaging showing tractography analysis with the number of fibers present in different regions of interest (ROI). One-way ANOVA followed by a Tukey's multiple comparisons test. CTL, control; CL, constitutional leanness; AN, anorexia nervosa; ARH, arcuate nucleus of hypothalamus; LHA, lateral hypothalamic area; MPO, medial preoptic area; DMH, dorsomedial hypothalamic nucleus; VMH, ventromedial hypothalamic nucleus; PaPe, paraventricular hypothalamic nucleus; PH, posterior hypothalamic nucleus; MR, mammillary region.

Supplementary Figures

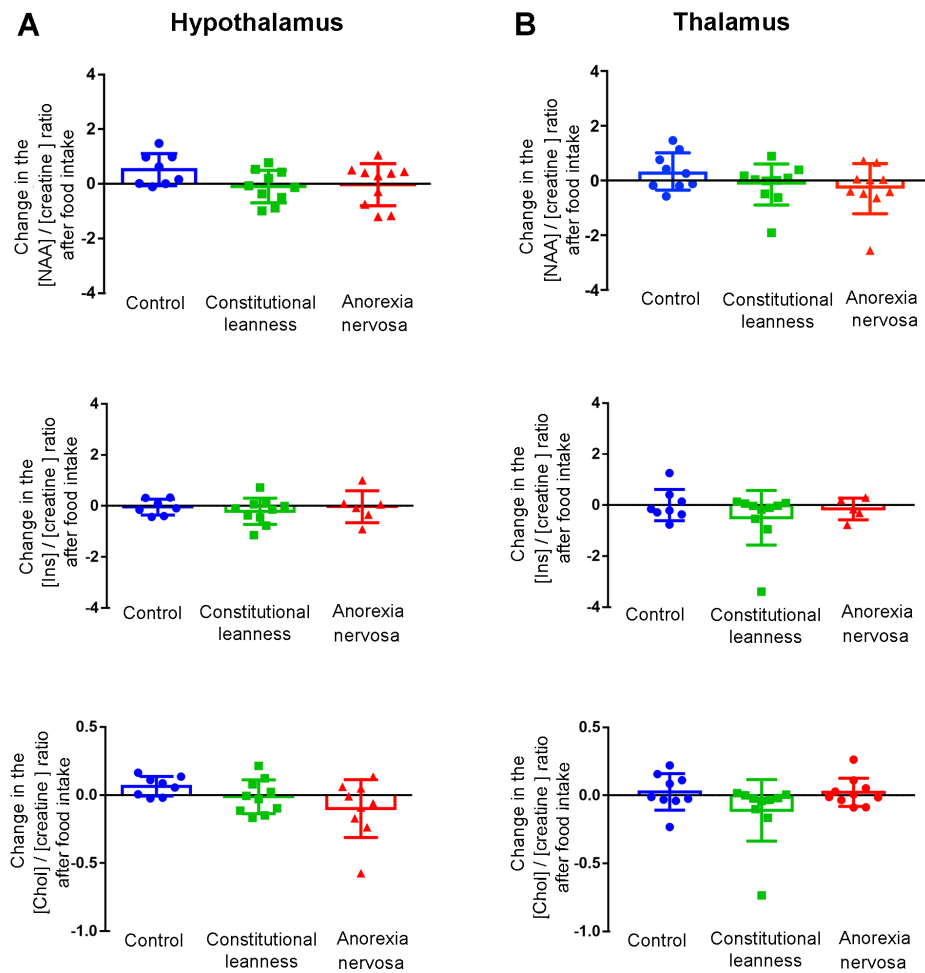


Figure S1. Task-based MR spectroscopic analysis of *N*-Acetyl-aspartate (NAA), myoinositol (Ins) and choline (Cho) within the hypothalamus (A) and the thalamus (B).

Coronal

Axial

Sagittal

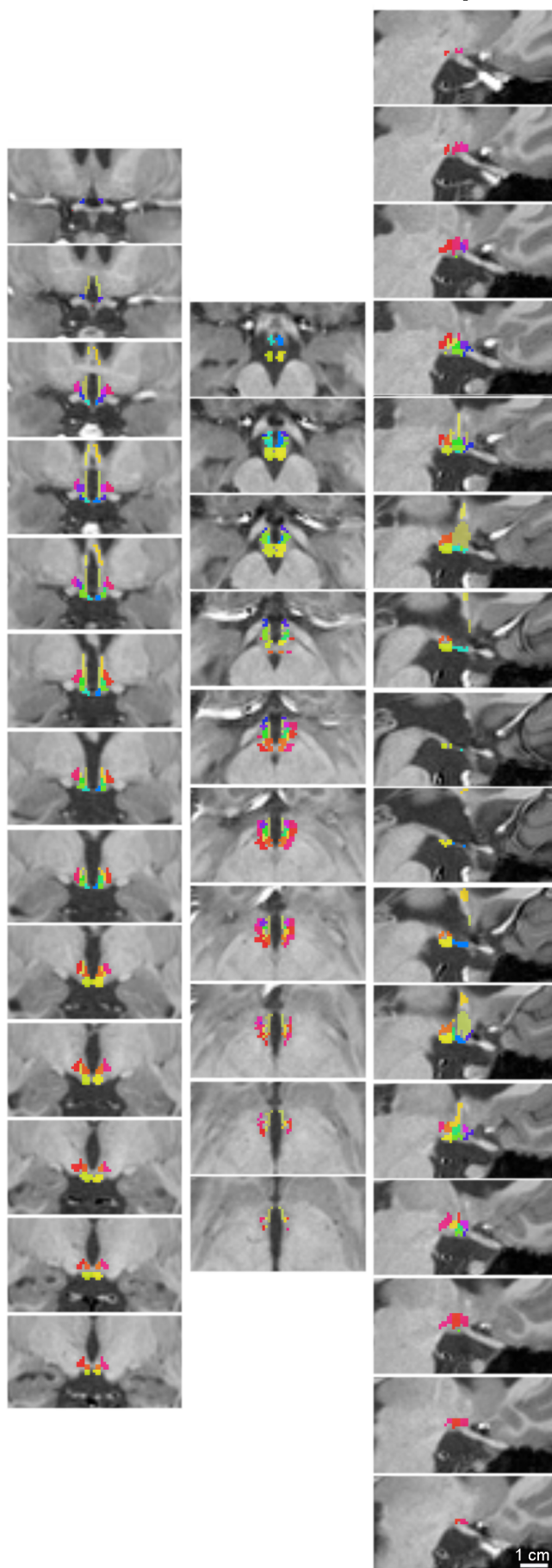


Figure S2. Segmentation of the hypothalamus in a single subject. The different regions of the hypothalamus were identified manually from thirteen 1-mm thick T1-weighted anatomical coronal sections using the MRI atlas of the hypothalamus (see reference ¹³) for each participant to the study. The color-codes are as follows (LH, left hypothalamus; RH, right hypothalamus; MR, mammillary region; PH, posterior hypothalamic nucleus; LHAp, posterior lateral hypothalamic area; VMH, ventromedial hypothalamic nucleus; DMH, dorsomedial hypothalamic nucleus; LHAt, tuberal LHA; ARH, arcuate nucleus of the hypothalamus; LHAA, anterior LHA; PaPe, paraventricular nucleus; MPO, medial preoptic nucleus; SO, supraoptic nucleus; fx, fornix):

 MR_LH: Color Hex color code DDFB00

 MR_RH: FFFF00

 PH_LH: FF6A00

 PH_RH: FF4700

 LHAp_RH: FF0000

 LHAp_LH: FF007A

 VMH_RH: 82FF00

 VMH_LH: 69FF00

 DMH_RH: 06FF00

 DMH_LH: 00FF65

 LHAt_RH: FF0060

 LHAt_LH: FF1900

 ARH_RH: 03FFDA

 ARH_LH: 007EFF

 LHAA_RH: FF00A7

 LHAA_LH: FF0065

 PaPe_RH: C4C44B

 PaPe_LH: CCE253

 MPO_RH: 7900FF

 MPO_LH: EE00FF

 SO_RH: 0011FF

 SO_LH: 3F00FF

 fx_RH: FFFF00

 fx_LH: FFD800

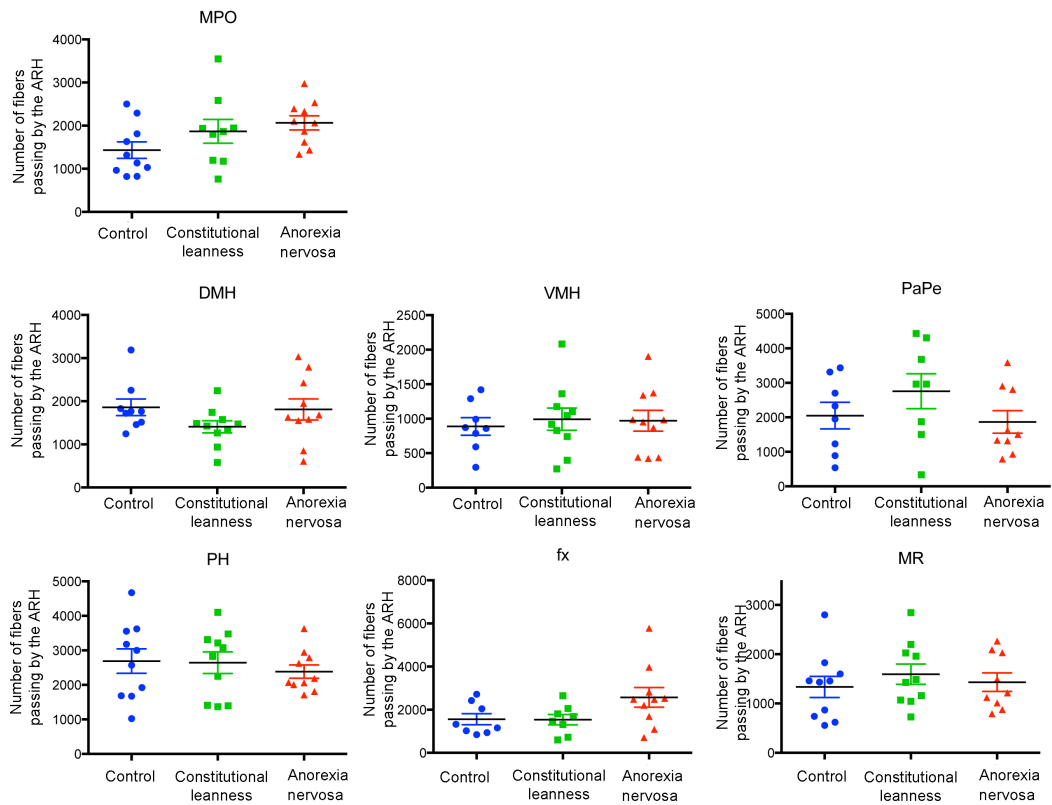


Figure S3. Tractography analysis from distinct regions of the hypothalamus and the fornix (fx). MPO, medial preoptic area; DMH, dorsomedial hypothalamic nucleus; VMH, ventromedial hypothalamic nucleus; PaPe, paraventricular hypothalamic nucleus; PH, posterior hypothalamic nucleus; MR, mammillary region.

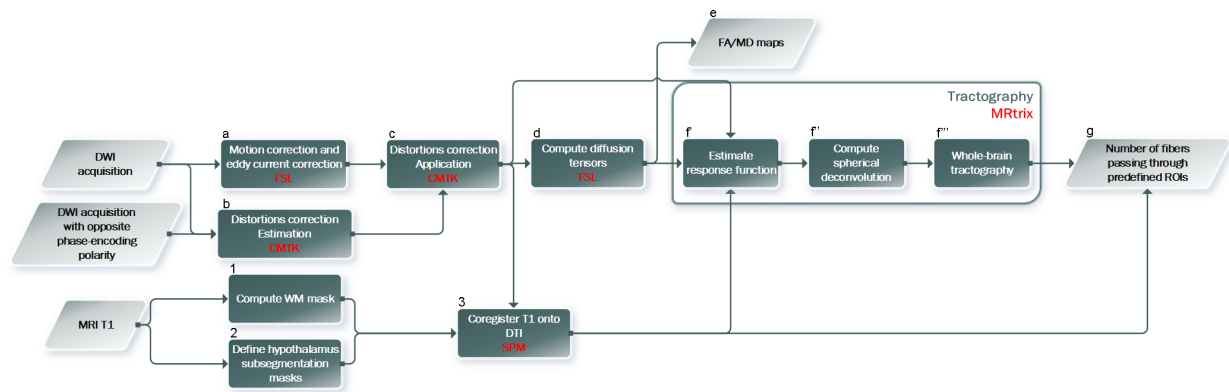


Figure S4. Flowchart of tractography data processing. Numbers and letters differentiate the different steps of the process detailed in the text of the materials and methods. The red labels correspond to the name of the software's that have been used at each one of these steps.