Dynamic changes in cerebral and peripheral markers of glutamatergic signaling across the human sleep-wake cycle

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Supplementary material

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Supplementary Table S1: Behavioral Effects of Sleep Deprivation

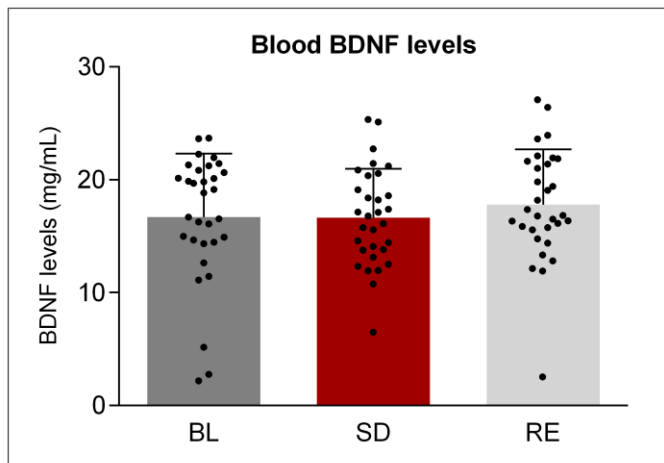|	Baseline (BL)	Sleep deprivation (TSD)	Recovery (RE)	TSD *vs.* BL *p* value	RE *vs.* TSD *p* value	RE *vs.* BL *p* value
Karolinska Sleepiness Scale	3.23 ± 0.19	4.92 ± 0.32	2.81 ± 0.19	**< 0.001**	**< 0.001**	**< 0.013**
Tiredness Symptoms Scale	0.52 ± 0.12	1.59 ± 0.29	0.43 ± 0.15	**< 0.001**	**< 0.001**	0.609
Visual Analogue Scales of States						
Mood	66.55 ± 1.90	61.69 ± 2.20	70.98 ± 2.05	**< 0.001**	**< 0.001**	**< 0.001**
Energy	63.48 ± 1.64	55.07 ± 2.11	65.59 ± 2.22	**< 0.001**	**< 0.001**	< 0.240
Motivation	32.81 ± 2.00	38.14 ± 2.36	27.45 ± 2.13	0.066	**< 0.001**	**< 0.048**
Agitation	41.43 ± 3.38	35.09 ± 2.56	33.88 ± 2.98	0.073	0.561	**0.006**

Values represent means ± SEM (n = 31). *P* values refer to two-tailed, paired *t* tests. Significant differences between conditions are highlighted in bold.

Supplementary Figure S1
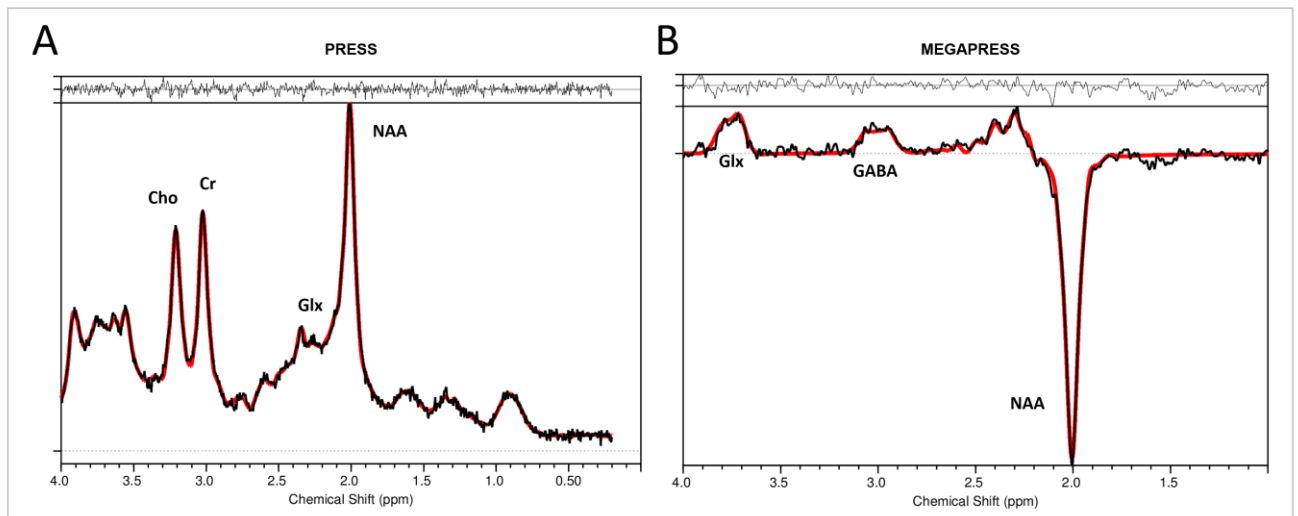
Example spectra depicting the signal detected for each resonance frequency (represented by the chemical shift in ppm). Representative spectra from the PRESS and MEGAPRESS sequences are shown in the left and right panels, respectively. Each spectrum is plotted in black with the LCModel fit overlaid in red. The residuals of the fit are also plotted above each spectrum.

Supplementary Figure S2

Effects of sleep deprivation and recovery sleep on circulating blood brain derived neurotrophic factor (BDNF)protein levels. Columns display blood BDNF levels in baseline (BL, dark grey), sleep deprivation (TSD, red) and recovery (RE, light grey) conditions. Data represent means + standard error of the mean (SEM) in n = 31. Black dots represent individual subjects. Mixed model ANOVA main effect of 'condition': $F_{2,90} = 0.23$, p > 0.7.

Supplementary Table S2: Mixed-model repeated-measures ANOVA with factor *'condition'*.

Variable	Region-of-interest	Metabolite	NumDF	DenDF	F-value	p-value
FMRP			2	44	3.37	**0.043**
BDNF			2	90	0.23	0.731
PET	Global mean		2	36	4.52	**0.017**
	Caudate nucleus		2	36	6.25	**0.005**
	Putamen		2	36	4.61	**0.017**
	Ventral striatum		2	36	0.86	0.431
	Amygdala		2	36	5.54	**0.008**
	dlPFC		2	36	4.15	**0.024**
	Orbitofrontal cortex		2	36	0.10	0.902
	Medial superior frontal cortex		2	36	4.57	**0.017**
	Anterior cingulate cortex		2	36	4.89	**0.013**
	Parietal cortex		2	36	6.85	**0.003**
	Inferior parietal cortex		2	36	7.18	**0.002**
	Precuneus		2	36	6.96	**0.003**
	Medial temporal lobe		2	36	3.18	0.054
	Parahippocampal gyrus		2	36	0.82	0.448
	Hippocampus		2	36	5.47	**0.008**
	Insula		2	36	7.42	**0.002**
MRS	Basal ganglia	Glutamate	2	37	4.83	**0.014**
		GLX	2	37	6.32	**0.004**
		GABA	2	37	0.63	0.754
		NAA	2	37	0.68	0.515
		Choline	2	37	0.83	0.444
		Glutathione	2	37	1.41	0.258
	dlPFC	Glutamate	2	37	0.08	0.924
		GLX	2	37	1.61	0.209
		GABA	2	37	0.48	0.624
		NAA	2	37	0.65	0.526
		Choline	2	37	0.74	0.483
		Glutathione	2	37	0.04	0.957

Results of mixed-model repeated-measures ANOVA with within-subject factor *'condition'* (BL, TSD, RE). Significant p-values are highlighted in bold. FMRP: fragile-x mental retardation protein; BDNF: brain-derived neurotrophic factor; PET: positron emission

tomography; MRS: magnetic resonance spectroscopy. dlPFC: dorsolateral prefrontal cortex;

GLX: glutamate-to-glutamine ratio; GABA: γ-aminobutyric acid; NAA: N-acetyl-aspartate;

Supplementary Table S3: Post-hoc comparisons of blood protein levels, PET and MRS variables to localize differences between conditions.

Variable	Region-of-interest	Metabolite	DF	Corrected p-value					
				TSD *vs.* BL		RE *vs.* TSD		RE *vs.* BL	
				Tukey-Kramer	Benjamini-Hochberg	Tukey-Kramer	Benjamini-Hochberg	Tukey-Kramer	Benjamini-Hochberg
FMRP			44	**0.035**		0.620		0.259	
BDNF			90	0.975		0.999		0.984	
PET	Global Mean		36	**0.044**		**0.004**		0.465	
	Caudate nucleus		36		**0.027**		**0.027**		0.657
	Putamen		36		0.120		**0.027**		0.384
	Ventral striatum		36		0.383		0.406		0.709
	Amygdala		36		0.057		**0.027**		0.523
	dlPFC		36		**0.031**		0.231		0.406
	Orbitofrontal cortex		36		0.657		0.657		0.724
	Medial superior frontal cortex		36		**0.031**		0.092		0.657
	Anterior cingulate cortex		36		0.357		**0.027**		0.131
	Parietal cortex		36		**0.027**		**0.024**		0.614
	Inferior parietal cortex		36		0.053		**0.020**		0.350
	Precuneus		36		0.071		**0.020**		0.283
	Medial temporal lobe		36		0.100		0.100		0.687
	Parahippocampal gyrus		36		0.657		0.366		0.485
	Hippocampus		36		0.092		**0.027**		0.384
	Insula		36		0.101		**0.020**		0.142
MRS	Basal ganglia	Glutamate	37		0.057		0.298		0.503

		DF			
	GLX	37	**0.031**	0.139	0.464
	GABA	37	0.862	0.969	0.865
	NAA	37	0.621	0.947	0.538
	Choline	37	0.712	0.865	0.865
	Glutathione	37	0.902	0.496	0.448
dlPFC	Glutamate	37	0.862	0.865	0.902
	GLX	37	0.487	0.290	0.865
	GABA	37	0.816	0.503	0.865
	NAA	37	0.865	0.902	0.869
	Choline	37	0.865	0.902	0.862
	Glutathione	37	0.902	0.865	0.862

Results of post-hoc analyses of blood protein levels, PET and MRS data. Based upon *a priory* hypotheses, p-values to localize condition-dependent difference in FMRP and BDNF levels and global mGluR5 availability were Tukey-Kramer corrected (conditions: BL, TSD, RE). The secondary analyses including 15 pre-defined regions-of-interest (see: Hefti et al., Biol Psychiatry 73, 161-168, 2013) across BL, TSD and RE conditions and 6 MRS metabolites in two brain regions (basal ganglia and dlPFC) across BL, TSD and RE conditions, were corrected by the Benjamini-Hochberg procedure to reduce the false discovery rate.

FMRP: fragile-x mental retardation protein; BDNF: brain-derived neurotrophic factor; PET: positron emission tomography; MRS: magnetic resonance spectroscopy; DF: degrees of freedom; BL: baseline; TSD: total sleep deprivation; RE: recovery; dlPFC: dorsolateral prefrontal cortex; GLX: glutamate-to-glutamine ratio; GABA: γ-aminobutyric acid; NAA: N-acetyl-aspartate. Significant p-values are highlighted in bold.