Supplementary Information

Association of plasma tryptophan concentration with periaqueductal gray matter functional connectivity in migraine patients

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Supplementary Table S1. Descriptive characteristics of participants in the sex matched case control design

		Migraine	Control	Test-statistic	p-value
Sex		F=21, M=6	F=21, M=6	$\chi^2 = 0$	1
Age (years)		25.9 ± 4.6	25.6 ± 4.0	U = 362	p=0.965
TRP (µg/ml)					
	1.Blood Sample	9.32 ± 2.88	7.12 ± 3.52	U = 136	0.002
	2.Blood Sample	9.07 ± 2.66	7.52 ± 2.27	t(2.21)=4.78	0.032
	Mean	9.16 ± 2.35	7.33 ± 2.51	F(1,53)=9.467	0.003*
LNAA (µg/ml)					
	1.Blood Sample	108.79 ± 37.00	116.80 ± 33.62	U=228	0.219
	2.Blood Sample	101.67 ± 30.85	115.10 ± 28.80	t(44)=-1.50	0.140
	Mean	104.15 ± 28.09	117.45 ± 26.56	F(1,53)=3.886	0.054**
Zung		35.27 ± 5.34	33.44 ± 7.05	t(52)=1.065	0.292
STAI-T		39.34 ± 8.63	37.63 ± 10.85	t(52)=0.639	0.526
Age of migraine onset (years)		14.65 ± 1.2	NA		
Migraine attack frequency (month)		3.51 ± 0.57	NA		

Note. Data are expressed as mean value ± SD. The *p* values are based on Pearson chi-square tests for sex, Mann-Whitney U tests for Age, 1. blood sample of TRP and LNAA, and Independent samples t-test for 2. blood sample of TRP and LNAA, STAI-T and Zung. *The difference of mean tryptophan concentration between migraine and control groups was corrected for age, sex and plasma LNAA level using univariate ANOVA.

**The difference of mean LNAA level between migraine and control groups was corrected for age and sex using univariate ANOVA.

LNAA: Large neutral amino-acids, N: Number of participants, F: Female, M: Male, STAI-T: Trait-anxiety level scores, TRP: L-tryptophan plasma concentration, Zung: Depressive symptoms scores, Significance threshold: p<0.05,

Supplementary Table S2. Intrinsic functional connectivity of left and right side of PAG in the sex matched case control design

Seed region	Region	Cluster size (voxel)	Peak T- value	MNI coordinates (x y z)		
Left PAG	R Thalamus	324	4.37	10	-28	-2
	R Precuneus	139	4.85	4	-70	54
Right PAG	R Thalamus	514	19.87	12	-22	10
	L Cerebelum Crus 1	1576	5.42	-40	-52	-34
	L Cerebelum_VI		4.62	-12	-68	-26
	L Cerebelum Crus 2		3.86	-38	-70	-44
	L Vermis VI		3.73	-4	-58	-22
	R Cerebelum VI	428	4.41	26	-62	-30
	R Cerebelum Crus 1		3.60	32	-56	-36
	R Cerebelum VIII		3.49	18	-64	-38

Note. Results are corrected for age and sex. All the listed clusters showed significant positive correlations with PAG, while no negative correlation survived the cluster-level $p_{FWE}< 0.05$ significance threshold.

R: right, L: left, MNI: Montreal Neurological Institute

Supplementary Table S3. Alterations of PAG connectivity in association with migraine frequency, migraine onset, trait-anxiety level and depressive symptoms in migraine patients with and without correction for plasma tryptophan concentration

Without correction for tryptophan concentration						After correction for tryptophan concentration					
Region	Cluster-size	Peak T-value	MNI-coordinates (x y z)			Region	Cluster-size	Peak T-value	MNI-coordinates (x y z)		
Attack frequency						**Attack frequency**					
Left PAG: *Positive correlation*						Left PAG: *Positive correlation*					
R Triangular part of inferior frontal gyrus	181	5.68	50	40	10	R Triangular part of inferior frontal gyrus	232	7.12	50	40	10
R Middle frontal gyrus	141	5.04	24	48	4	R Middle frontal gyrus		3.98	54	34	22
Right PAG: *Positive correlation*						Right PAG: *Positive correlation*					
R Triangular part of inferior frontal gyrus	272	5.4	50	28	26	R Triangular part of inferior frontal gyrus	271	5.47	50	28	26
R Middle frontal gyrus	161	5.21	24	50	4	R Superior frontal gyrus	126	4.07	32	58	14
Age of onset						**Age of onset**					
Left PAG: *Positive correlation*						Left PAG: *Positive correlation*					
R Precuneus	197	5.23	14	-44	4	R Lingual gyrus	289	5.45	14	46	4
R Lingual gyrus		4.31	6	-56	4	R Cuneus		4.94	6	66	20
R Cuneus		4.18	6	-64	20						
Trait-anxiety						**Trait-anxiety**					
Left PAG: *Positive correlation*											
L Middle frontal gyrus	166	5.33	-30	56	28						
Right PAG: *Positive correlation*						No significant result					
L Middle frontal gyrus	190	5.73	-26	54	30						
L Superior medial frontal gyrus	123	5.09	-4	36	32						
Depressive symptoms						**Depressive symptoms**					
Left PAG: *Positive correlation*						Left PAG: *Negative correlation*					
L Middle frontal gyrus	283	6.65	-26	54	28	R Middle cingulate gyrus	206	-4.08	4	-40	42
Left PAG: *Negative correlation*						L Precuneus		-4.44	-10	56	26
R Fusiform gyrus	158	-4.89	26	-40	-18						
R Parahippocampal gyrus		-4.14	30	-30	-16						
Right PAG: *Positive correlation*											
L Middle frontal gyrus	309	6.70	-26	54	30						

*Note.*Significance threshold was cluster-level $p_{FWE} < 0.05$ including at least 20 contiguous voxels. Results are corrected for age, sex and LNAA concentration.
R: right, L: left, MNI: Montreal Neurological Institute

Supplementary Table S4. Association between PAG intrinsic functional resting-state connectivity and plasma tryptophan concentration in migraine patients after correction for trait-anxiety or depressive symptoms.

Region	Cluster-size	Peak T-value	MNI-coordinates (x y z)		
After correction for trait-anxiety level					
Left PAG					
Negative correlation					
L Fusiform gyrus	143	- 6.86	-32	-40	-22
Right PAG					
Positive correlation					
L Superior frontal gyrus	155	6.12	20	44	32
L Middle frontal gyrus		4.43	26	38	30
Negative correlation					
L Fusiform gyrus	162	-4.15	-40	-46	-24
After correction for depressive symptoms					
Left PAG					
Negative correlation					
L Fusiform gyrus	133	-6.65	-32-	-38	-22
Right PAG					
Positive correlation					
R Superior frontal gyrus	175	6.74	20	44	32
R Superior medial part of Frontal gyrus		4.37	8	56	38
Negative correlation					
L Fusiform gyrus	135	-3.89	-40	-46	-24

Note. Significance threshold was cluster-level $p_{FWE} < 0.05$ including at least 20 contiguous voxels.

Results are corrected for age, sex and LNAA concentration. R: right, L: left, MNI: Montreal Neurological Institute

Supplementary Table S5 Descriptive characteristics of participants in the extended unbalanced sample

	1. Blood Sample		Test statistic	p -value
Total	74			
Diagnosis	Migraine	Control		
N	31	43		
Sex	F=25, M=6	F=19, M=24	χ^2=4.965	0.026
Age (years)	26.71 ± 4.84	25.91 ± 4.06	U=614	0.564
TRP (µg/ml)	9.48 ± 2.92	7.85 ± 3.14	U=423	0.008
LNAA (µg/ml)	111.49±35.20	116.86±32.40	U=573	0.306
Age of migraine onset (years)	14.52 ± 1.14	NA		
Migraine attack frequency (month)	3.33 ± 0.50	NA		
	2. Blood Sample			
Total	68			
Diagnosis	Migraine	Control		
N	27	41		
Sex	F=21, M=6	F=17, M=24	χ^2 =2.693	0.101
Age (years)	26.19 ± 4.73	25.44 ± 3.96	U=515	0.628
TRP (µg/ml)	9.07 ± 2.66	7.42 ± 2.1	U=333	0.006
LNAA (µg/ml)	101.67±30.85	110.80±27.46	U=410	0.114
Age of migraine onset (years)	14.85 ± 1.15	NA		
Migraine attack frequency (month)	3.40 ± 0.56	NA		
	Resting-state fMRI			
Total	64			
Diagnosis	Migraine	Control		
N	27	37		
Sex	F=21, M=6	F=21, M=16	χ^2=3.058	0.080
Age (years)	25.9 ± 4.6	25.7 ± 4.3	U=493	0.929
TRP (µg/ml)	9.16 ± 2.35	7.51 ± 2.34	$F(1,63)$=10.005	0.002*
LNAA (µg/ml)	103.14±26.69	117.11±23.44	U=360	0.058
Age of migraine onset (years)	14.65 ± 1.2	NA		
Migraine attack frequency (month)	3.51 ± 0.57	NA		
STAI-T	39.34 ±8.63	37.66 ± 10.47 (N = 35)	$t(60)$=0.980	0.503
ZUNG	35.27 ± 5.34	33.73 ± 6.72 (N = 35)	$t(60)$=0.674	0.331

Note. Data are expressed as mean value ± SD. The *p* values are based on Pearson chi-square tests for sex, Mann-Whitney U tests for Age, TRP, LNAA, and Independent samples t-test for STAI-T and Zung. *The difference of mean tryptophan concentration between migraine and control groups was corrected for age, sex and plasma LNAA level in the resting state fMRI group using univariate ANOVA. LNAA: Large neutral amino-acids, N: Number of participants, F: Female, M: Male, Significance threshold: p<0.05, STAI-T: Trait-anxiety level scores, TRP: L-tryptophan plasma concentration, Zung: Depressive symptoms scores

Supplementary Table S6. Intrinsic functional connectivity of left and right side of PAG in the extended sample (27 migraineurs and 37 controls)

Seed region	Region	Cluster size (voxel)	Peak T- value	MNI coordinates (x y z)		
Left PAG	L Thalamus	1435	4.44	-12	-26	-8
	R Thalamus		4.35	12	-18	12
	L Caudate		4.15	-10	4	12
	L Superior frontal gyrus		4.90	-26	62	10
	L Middle frontal gyrus		3.68	-24	60	16
	L Superior frontal gyrus, medial part		3.64	-12	66	10
	L Cerebelum Crus2	426	4.54	-40	-68	-42
	L Cerebelum_VI		4.24	-28	-58	-36
	L Cerebelum Crus1		4.16	-42	68	-32
	L Cuneus	316	4.17	-2	-68	24
	L Precuneus		3.21	-12	-66	38
	R Middle frontal gyrus	277	4.10	42	6	56
			3.88	36	12	46
	L Vermis_VI	268	4.22	-2	-58	-24
	L Cerebelum_VI		4.15	-8	-66	-22
	R Vermis_VII		3.74	4	-74	-24
Right PAG	R Thalamus	1567	4.78	12	-18	12
	L Thalamus		3.73	-14	-26	8
	L Cerebelum Crus2	2654	4.99	-36	-68	-40
	L Cerebelum_VI		4.68	-28	-60	-34
	L Cerebelum_IV_V		4.25	-6	-60	-22
	L Superior frontal gyrus	167	4.42	-26	62	10
	L Middle frontal gyrus		3.47	-26	48	16
	R Caudate	215	4.09	10	6	6
	R Pallidum		4.02	22	0	6
	L Precuneus	323	4.21	-4	-70	30
	L Cuneus		4.12	-2	-68	22
	R Middle frontal gyrus	237	4.18	44	26	32

Note. Results are corrected for age and sex. All the listed clusters showed significant positive correlations with PAG, while no negative correlation survived the cluster-level $p_{FWE} < 0.05$ significance threshold.

R: right, L: left, MNI: Montreal Neurological Institute

Supplementary Table S7 Significantly different association between PAG intrinsic resting-state connectivity and plasma tryptophan concentration in the extended unbalanced sample (27 migraine patients compared to 37 controls)

Region	Cluster size (voxel)	Peak F-value	MNI coordinates (x y z)		
Left PAG					
R Middle Occipital gyrus	1020	21.64	28	-92	20
L Middle Occipital gyrus	944	19.07	-20	-88	20
L Superior Occipital gyrus		17.39	-16	-86	30
L Fusiform gyrus	916	20.98	-36	-52	-18
L Cerebellum IV-V		20.83	-6	-64	-6
R Fusiform gyrus	334	14.67	34	-38	-24
Right PAG					
L Fusiform gyrus	146	25.94	-34	-66	-12
L Cerebellum VI		14.5	-22	-62	-14
L Middle Occipital gyrus	123	20.61	-36	-76	8

Note. Significance threshold was cluster-level $p_{FWE}< 0.05$ including at least 20 contiguous voxels. Results are corrected for age, sex and LNAA concentration. R: right, L: left, MNI: Montreal Neurological Institute

Supplementary Table S8 Significantly different association between PAG intrinsic functional resting-state connectivity and plasma tryptophan concentration in migraine patients compared to controls using PAG as a single seed

Region	Cluster size (voxel)	Peak F-value	MNI coordinates (x y z)		
L Fusiform gyrus	1060	31.18	-44	-70	-18
L Cerebellum IV-V		24.06	-6	-64	-6
R Fusiform gyrus	1176	23.14	30	-46	-16
R Middle Occipital gyrus		23.79	28	92	20
L Middle Occipital gyrus	696	20.54	-28	-84	15
L Superior Occipital gyrus		17.44	-14	-96	24

Note. Significance threshold was cluster-level pFWE< 0.05 including at least 20 contiguous voxels. Results are corrected for age, sex and LNAA concentration. MNI: Montreal Neurological Institute

Supplementary Table S9 Significant association between PAG intrinsic functional resting-state connectivity and plasma tryptophan concentration in migraine patients using a single seed for PAG

Region	Cluster size (voxel)	Peak T-value	MNI coordinates (x y z)		
Negative correlation					
L Fusiform gyrus	718	-6.76	-32	-48	-18
R Fusiform gyrus	191	-6.10	28	-44	-18
R Cerebellum VI		-4.65	34	-44	-26
L Middle Occipital gyrus	210	-5.38	-24	-84	14
Positive correlation					
R Superior medial part of Frontal gyrus	224	5.05	10	54	36
L Superior medial part of Frontal gyrus	199	4.93	-2	34	34
L Superior Frontal gyrus	183	6.11	-18	48	36

Note. Significance threshold was cluster-level pFWE< 0.05 including at least 20 contiguous voxels. Results are corrected for age, sex and LNAA concentration. MNI: Montreal Neurological Institute

Supplementary Table S10 Alterations of PAG connectivity in association with migraine frequency, migraine onset, trait-anxiety level and depressive symptoms in migraine patients with and without correction for plasma tryptophan concentration, using PAG as a single seed

Without correction for tryptophan concentration						After correction for tryptophan concentration					
Region	Cluster-size	Peak T-value	MNI-coordinates (x y z)			Region	Cluster-size	Peak T-value	MNI-coordinates (x y z)		
Attack frequency						**Attack frequency**					
Positive correlation						Left PAG: *Positive correlation*					
R Middle frontal gyrus	162	5.03	24	50	4	R Triangular part of inferior frontal gyrus	286	6.29	50	40	10
R Triangular part of inferior frontal gyrus	273	5.17	50	40	10	R Middle frontal gyrus		4.41	54	34	22
Age of onset						**Age of onset**					
Positive correlation						Left PAG: *Positive correlation*					
R Lingual gyrus R	179	4.93	14	-46	4	R Lingual gyrus	225	5.04	14	-46	4
R Precuneus		4.14	12	-64	24						
Trait-anxiety						**Trait-anxiety**					
Positive correlation											
L Middle frontal gyrus	213	5.58	-26	54	30	*No significant results*					
L Superior medial frontal gyrus	129	5.13	-4	36	32						
Depressive symptoms						**Depressive symptoms**					
Positive correlation											
L Middle frontal gyrus	314	6.73	-26	54	28						
Negative correlation						*No significant results*					
R Fusiform gyrus	146	-5.49	26	-40	-18						
R Hippocampus		-4.28	34	-26	-10						

Note. Significance threshold was cluster-level $p_{FWE} < 0.05$ including at least 20 contiguous voxels. Results are corrected for age, sex and LNAA concentration.
MNI: Montreal Neurological Institute

Preprocessing pipeline

Freesurfer (version 5.3) (http://surfer.nmr.mgh.harvard.edu/) was used to perform intensity non-uniformity correction and intensity normalization on the T1-weighted images. The Freesurfer workflow also segmented the structural images to extract the brain volume and to separate white matter and gray matter tissues from cerebrospinal fluid (CSF). Spatial normalization was performed with the ANTs registration tool [1], which transformed the extracted brain volumes to match the 2 mm isovoxel brain template in MNI152 space provided by the Montreal Neurological Institute (MNI). The first four frames of the fMRI time-series were deleted from further processing to remove variance introduced while magnetization stabilized. Primary motion correction was performed using the MCFLIRT [2] utility of the FMRIB's Software Library (FSL version 6.0), by moving every image with rigid-body transformation to the first volume of the time-series. The six movement parameters were completed with the movement at the previous time point and with the squared value of each obtained parameter [3]. These 24 time-invariant variables were used at later steps to reduce motion artefacts in the data. For spatial standardization the functional images were coregistered with the corresponding subject's structural scan with the FSL linear registration tool, then transformed them into MNI152 space using the same transformation we normalized the T1 images with. CompCor technique [4] was investigated to compute the first five principal components of fMRI time-series within the white matter and CSF, which are considered to describe uninteresting variance regarding cortical activation. Then spatial filtering was applied with the FSL application SUSAN [5], using a Gaussian-kernel of 6 mm full-width at half maximum. Further signal correction was performed with the independent component analysis based ICA-AROMA [6], and we linearly regressed the 24 parameters derived from primary motion correction and the 5 CompCor components from the data. Four subjects were excluded due to excessive movements defined as frame-wise relative RMS displacement > 0.25 mm in more than 20 volumes [7]. Finally, temporal band-pass filtering was applied between 0.009 Hz and 0.08 Hz to remove noise and eliminate signal drifting effects.

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