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Supplemental information

Similar neural pathways link psychological stress and brain-age in health and multiple sclerosis

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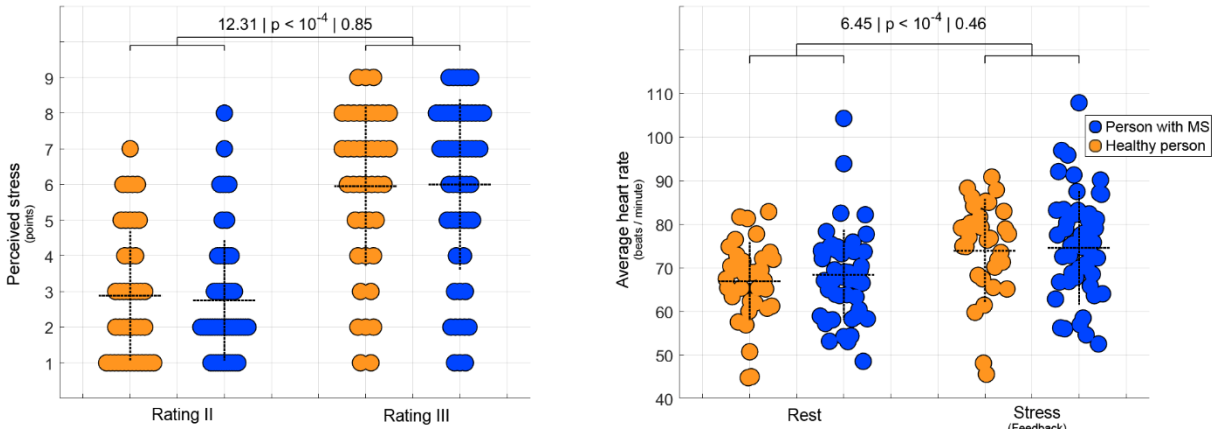

Figure S1. Psychological and physiological stress responses (related to Figure 3). The figure illustrates the results of supplementary analysis 1 which tested main effects of condition (i.e., stress) on perceived stress and heart rate as well as interaction effects condition × group on these factors. This revealed a main effect of condition for both markers but no interaction effects. S1A illustrates the results for perceived stress, S1B those for heart rate. In the analysis, perceived stress and heart rate variations were evaluated based on the cross-sectional data with a factorial repeated measures analyses using linear mixed models. For perceived stress (ratings during stages 3 & 5), data of 98 participants were available, for heart rate (average pulse across stage 2 & 4b) data of 84 participants. Each model included three fixed effects regressors (i.e., covariates of interest): One main effect regressor for experimental stage, one for group, and a regressor reflecting their interaction. Regressors for age, sex, project, cognitive task load, time to feedback, severity of depressive symptoms, plus constant modelled fixed effects of no interest. Clinical severity, disease duration, and progressive MS (y/n) were not modelled here as this might artificially reduce potential group differences as only patients have non-zero scores in these parameters. A constant modelling each participant's average perceived stress or pulse across experimental stages was included as random nuisance parameter. The type-I-error rate for the undirected tests performed were determined with permutation testing (10,000 within-subject permutations of each covariate of interest). An uncorrected significance threshold of $\alpha = 0.05$ was applied. The dotted horizontal lines depict the mean, the dotted vertical line the standard deviation for the respective parameter. The statistical parameters in S1A and S1B correspond to (from left to right) the t-statistic, p-value, and effect size f^2.

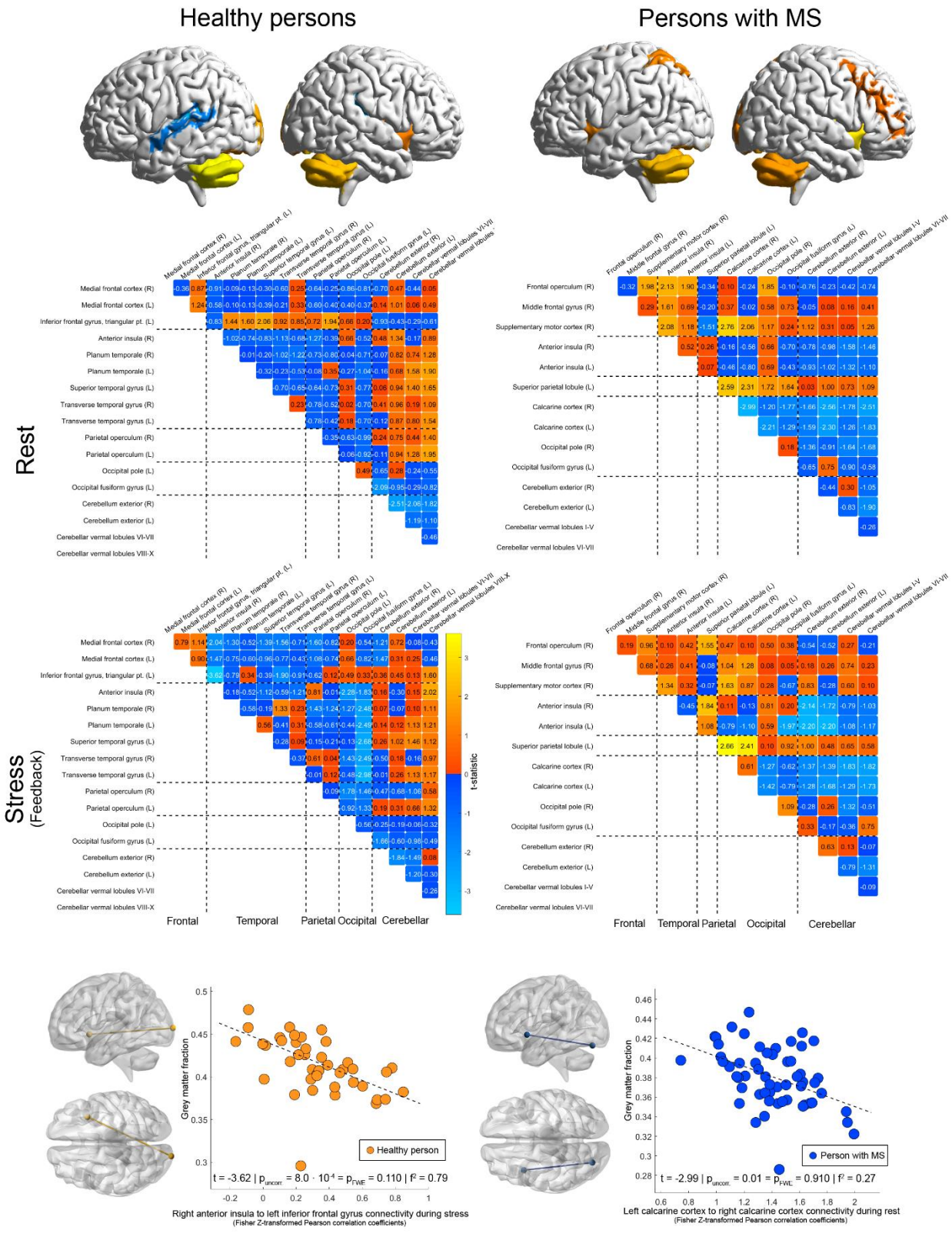
Healthy persons
Persons with MS
Rest
Stress
(Feedback)
t-statistic
Frontal
Temporal
Parietal
Occipital
Cerebellar
Grey matter fraction
Healthy person
t = -3.62 | p_uncorr. = 8.0 · 10^-4 = p_FWE = 0.110 | f² = 0.79
Right anterior insula to left inferior frontal gyrus connectivity during stress
(Fisher Z-transformed Pearson correlation coefficients)
Person with MS
t = -2.99 | p_uncorr. = 0.01 = p_FWE = 0.910 | f² = 0.27
Left calcarine cortex to right calcarine cortex connectivity during rest
(Fisher Z-transformed Pearson correlation coefficients)

Figure S2. Functional connectivity and grey matter fraction (related to Figure 4). Figure S2 illustrates the results of supplementary analysis 2, which tested associations between FC of stress-responsive brain regions and whole-brain GM fraction separately for each group and condition. Supplementary analysis 2 was identical to main analysis 2 except for the fact that we used participants' whole-brain GM fraction as dependent variable instead of brain-PAD. Although again, stress-related FC between right anterior insula and another region (here: inferior frontal gyrus), showed the strongest association between FC and GM fraction for HPs across all pairs of stress-responsive regions in terms of the obtained t-statistic, supplementary analysis 2 did not reveal significant results on an FWE-corrected level.

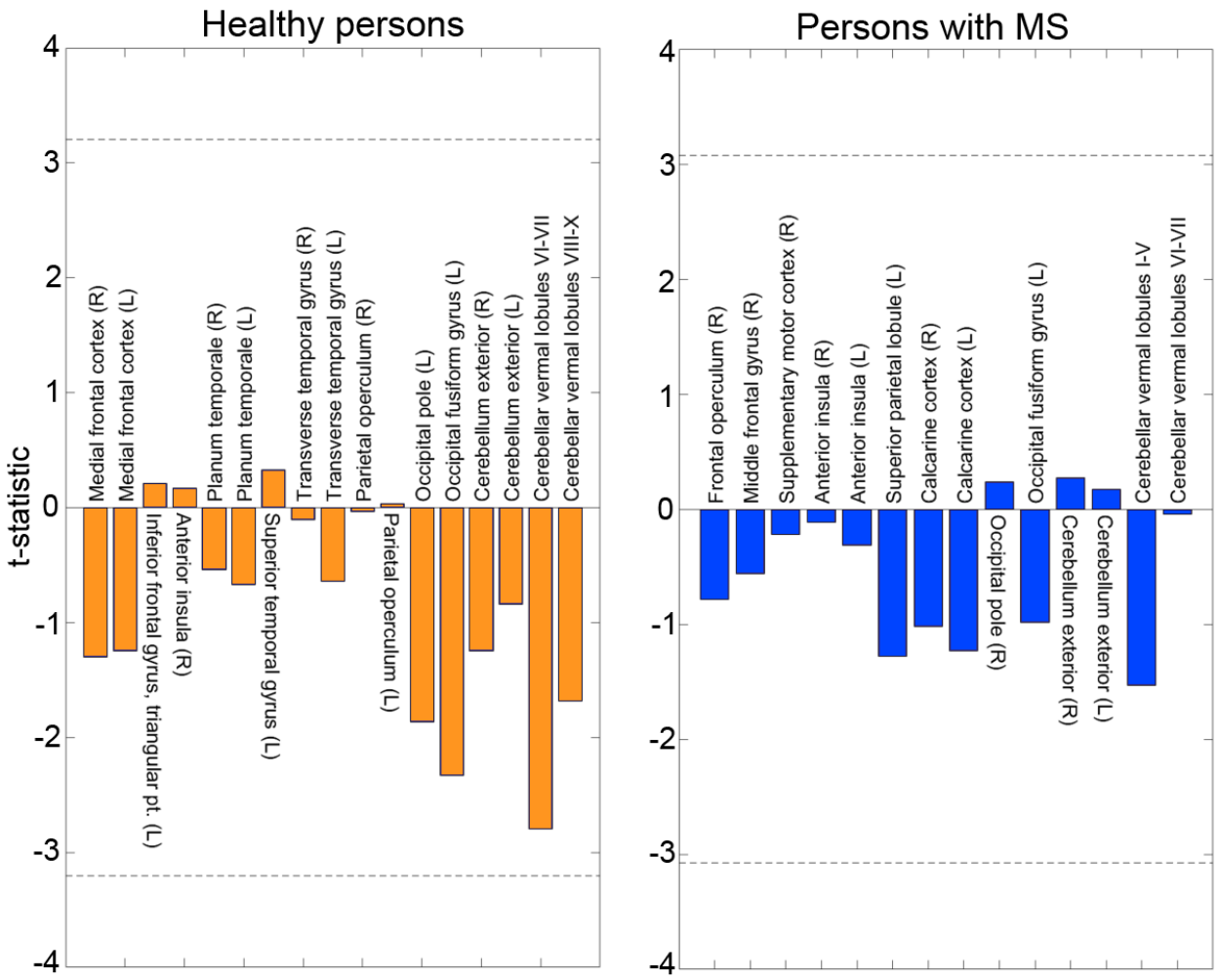

Figure S3. Regional neural stress response activity and brain-PAD (related to Figure 4). Figure S3 illustrates the results of supplementary analysis 3, which tested associations between stress-responsivity of stress-responsive brain regions and brain-PAD separately for each group. Supplementary analysis 3 was identical to main analysis 2 except for the fact that we used the regression coefficients computed for assessing regional neural stress responsivity in main analysis 1 to test these parameters' relation to brain-PAD. Supplementary analysis 3 did not reveal significant results on an FWE-corrected level. In the analysis, we applied an FWE-corrected significance threshold that corrected for the number of regions with significant stress responses (instead of the number of pairs of regions with significant stress responses as in main analysis 2) per group. Consequently, the uncorrected equivalent of an FWE-corrected threshold of $\alpha_{FWE} = 0.05$ corresponds to 0.05 / 17 = 0.003 in HPs and to 0.05 / 14 = 0.004 in PwMS. The dashed line indicates the t-statistic that would have been necessary to achieve a significant undirected association on an FWE-corrected level.

Table S1. Sex and age of individual participants (related to Table 1).

Cross-sectional main analyses 1 - 3						Longitudinal main analysis 4		
PwMS			HP			PwMS		
Study project	Sex	Age	Study project	Sex	Age	Study project	Sex	Age
I	M	33	I	F	31	I	M	33
I	F	22	I	M	25	I	F	27
I	M	48	I	F	31	I	M	52
I	M	46	I	M	41	I	F	52
I	M	40	I	M	40	I	M	48
I	F	53	I	F	60	I	M	46
I	F	53	I	F	47	I	M	40
I	F	52	I	F	42	I	M	52
I	F	62	I	M	64	I	F	50
I	F	51	I	M	58	I	M	42
I	F	33	I	M	48	I	F	53
I	F	49	I	F	51	I	F	53
I	F	55	I	M	60	I	F	62
I	F	47	I	F	59	I	F	45
I	M	54	I	F	60	I	F	51
I	F	48	I	F	43	I	M	46
I	M	53	I	F	60	I	F	53
I	M	46	I	F	57	I	F	55
I	F	46	I	F	63	I	F	47
I	F	44	II	M	21	I	M	54
II	F	62	II	F	47	I	F	48
II	F	27	II	F	45	I	M	46
II	F	33	II	F	57	I	M	59
II	F	43	II	F	32	I	F	46
II	M	35	II	F	46			
II	M	55	II	M	53			
II	F	56	II	M	30			
II	F	52	II	F	27			
II	M	49	II	M	74			
II	F	40	II	F	46			
II	F	52	II	F	45			
II	F	64	II	F	23			
II	F	40	II	M	31			
II	M	36	II	F	69			
II	F	36	II	M	75			
II	M	45	II	F	54			
II	F	53	II	F	24			
II	M	56	II	F	44			
II	F	55	II	M	26			

II	M	61	II	F	24			
II	F	62	II	F	23			
II	F	34	II	F	21			
II	F	41						
II	M	31						
II	M	41						
II	F	49						
II	M	37						
II	F	48						
II	F	31						
II	F	56						
II	M	43						
II	M	56						
II	F	66						
II	F	27						
II	F	29						
II	F	60						

Abbreviations: F, Female; M, Male;

Table S2. Regional brain aging (related to Figure 5). The table lists coordinates significantly related to overall grey matter volume and overall T2-weighted lesion load as well as coordinates separating significantly between groups.

Variable / Group / Region	**Dist. (mm)**	**CS (mm^3)**	**x**	**y**	**z**	**t**	**p_{FWE}**
Grey matter fraction							
HP							
Supramarginal gyrus	0	51	-54	-40	38	-6.25	0.012
Supramarginal gyrus	2	20	56	-22	32	-6.25	0.013
Supramarginal gyrus	2.2	7	-50	-51	32	-5.84	0.034
Angular gyrus	1	7	-47	-51	44	-5.75	0.041
Supramarginal gyrus	0	7	-39	-48	44	-5.73	0.043
Supramarginal gyrus	3.6	10	-54	-25	29	-5.73	0.043
Posterior cingulate gyrus	0	3	-9	-46	2	-5.67	0.048
PwMS							
Angular gyrus	0	2791	-48	-57	41	-8.85	10^{-4}
Superior occipital gyrus	0	128	-26	-90	19	-6.33	0.003
Middle temporal gyrus	0	71	-59	-37	-6	-6.31	0.003
Supramarginal gyrus	0	128	54	-34	55	-6.24	0.004
Supramarginal gyrus	1.4	27	-56	-22	31	-5.89	0.010
Angular gyrus	3	24	-32	-63	38	-5.77	0.014
Angular gyrus	0	34	53	-57	28	-5.77	0.014
Supramarginal gyrus	2.2	17	-42	-49	41	-5.68	0.018
Supramarginal gyrus	0	47	-53	-37	50	-5.65	0.019

Supramarginal_gyrus	0	10	59	-46	37	-5.65	0.019
Angular gyrus	0	61	47	-61	41	-5.64	0.019
Angular gyrus	0	20	-42	-75	38	-5.60	0.021
Supramarginal gyrus	1.4	7	-45	-31	35	-5.36	0.042
Angular gyrus	0	3	44	-70	37	-5.34	0.043
Middle frontal gyrus	1.4	3	-35	14	47	-5.30	0.048
Supramarginal gyrus	0	3	63	-36	25	-5.28	0.049
T2-weighted lesion load							
PwMS							
Cerebral white matter	0	2028	33	-49	10	8.45	0.0002
Cerebral white matter	0	135	-23	-25	26	7.00	0.0008
Lateral ventricle	0	361	-26	-51	8	6.64	0.0018
Cerebral white matter	0	41	-27	-51	22	6.60	0.0018
Cerebral white matter	0	159	21	-22	28	6.51	0.0026
Cerebral white matter	0	138	20	35	8	6.19	0.0066
Caudate	0	78	17	23	2	6.18	0.0066
Cerebral white matter	0	54	14	29	-7	6.15	0.0072
Cerebral white matter	0	44	42	-54	11	6.14	0.0078
Cerebellum exterior	0	30	-24	-49	-18	5.95	0.0146
Cerebral white matter	0	44	-35	-21	22	5.92	0.0160
Cerebral white matter	0	24	-23	-6	28	5.80	0.0228
Cerebral white matter	0	17	14	31	4	5.71	0.0294
Cerebral white matter	0	7	-26	31	13	5.69	0.0324

Cerebral white matter	0	7	-29	-45	23	5.58	0.0406
Group							
HPs & PwMS							
Thalamus proper	0	213	18	-27	11	5.51	0.0038

Abbreviations: CS, cluster size; Dist., distance.