Stress-induced brain activity, brain atrophy, and clinical disability in multiple sclerosis

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Prospective clinical studies support a link between psychological stress and multiple sclerosis (MS) disease severity, and peripheral stress systems are frequently dysregulated in MS patients. However, the exact link between neurobiological stress systems and MS symptoms is unknown. To evaluate the link between neural stress responses and disease parameters, we used an arterial-spin–labeling functional MRI stress paradigm in 36 MS patients and 21 healthy controls. Specifically, we measured brain activity during a mental arithmetic paradigm with performance-adaptive task frequency and performance feedback and related this activity to disease parameters. Across all participants, stress increased heart rate, perceived stress, and neural activity in the visual, cerebellar and insular cortex areas compared with a resting condition. None of these responses was related to cognitive load (task frequency). Consistently, although performance and cognitive load were lower in patients than in controls, stress responses did not differ between groups. Insula activity elevated during stress compared with rest was negatively linked to impairment of pyramidal and cerebral functions in patients. Cerebellar activation was related negatively to gray matter (GM) atrophy (i.e., positively to GM volume) in patients. Interestingly, this link was also observed in overlapping areas in controls. Cognitive load did not contribute to these associations. The results show that our task induced psychological stress independent of cognitive load. Moreover, stress-induced brain activity reflects clinical disability in MS. Finally, the link between stress-induced activity and GM volume in patients and controls in overlapping areas suggests that this link cannot be caused by the disease alone.

multiple sclerosis | psychological stress | functional magnetic resonance imaging | clinical disability | brain atrophy

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system leading to demyelination, axonal damage. and neuronal degeneration (1). In addition to sensorimotor symptoms, stress-related syndromes such as depression and anxiety disorders are among the most frequent comorbidities in MS (2).

A role for psychological stress in the pathobiology of MS was hypothesized as early as the 19th century when Charcot first described the disease, and a link between stress and the risk of MS relapse is now supported by numerous prospective clinical studies (e.g., 3). Moreover, MS patients frequently exhibit dysregulated psychobiological stress systems, and these systems interact with the key neurologic characteristics. Neuroendocrine studies revealed a link between MS and altered regulation of both stress systems, the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS) (4). Specifically, pharmacological challenge tests have shown that glucocorticoid responsivity is elevated in MS patients (5) and that impaired HPA axis feedback control is linked to brain atrophy (6) and subsequent deterioration of clinical disability in MS (7). Furthermore, the application of corticotropin-releasing factor has been shown to reduce the severity of experimental autoimmune encephalomyelitis (EAE), the animal model of MS (8). For hormones of the SNS it has been shown that the density of β-adrenoreceptors on peripheral blood mononuclear cells correlates positively with lesion load in MS (9) and that norepinephrine (NE)-related antidepressants reduce the severity of EAE (10). Finally, a stress-reduction intervention based on cognitive behavioral therapy reduced the number of new contrast-enhancing lesions in a randomized controlled trial (11), providing the best evidence to date that stress and MS disease severity indeed might be linked directly.

Given the relatively small number of functional MRI (fMRI) stress studies that experimentally manipulated the degree of psychological stress and measured neural responses in healthy subjects (e.g., refs. 12–14), it is not surprising that stress-related brain activity has not yet been investigated in MS. This investigation is important, however, because psychosomatic studies suggest that the impact of stressors on health depends on the cognitive processing of stressors or affective stimuli (e.g., refs. 15, 16), which is closely reflected by immediate brain responses (17).

Consequently, we investigated in voxelwise fMRI analyses the neural responses to psychological stress in 36 patients with MS and 21 healthy control subjects with an arterial-spin–labeling (ASL) fMRI stress paradigm and the relation of these responses to disease

Significance

Psychological stress is linked to multiple sclerosis (MS) severity (e.g., to a heightened risk of brain lesion development). The exact mechanisms underlying this association are unknown. To investigate the link between brain activity induced by mild psychological stress and MS disease parameters, we conducted a mental arithmetic neuroimaging task involving performance feedback in MS patients and healthy controls and related the brain activity signals to clinical disability and brain volume. In patients, motor and cognitive impairment were related to activity in the insular cortex. Brain volume was related to activity in overlapping cerebellar areas in patients and controls. This overlap suggests that the link between activity and volume cannot reflect a passive response to clinical disability alone.

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parameters [i.e., clinical disability (18), clinicoradiographic disease severity measures, gray (GM) and white matter (WM) volume]. GM and WM volume were assessed across the whole brain. The fMRI paradigm comprised seven experimental stages (Fig. 1). Brain activity and heart rate were measured during three of the seven stages: stage II, baseline 1; stage IV, stress; and stage VI, baseline 2. Salivary cortisol and perceived stress were measured at four stages: stage I, prebaseline 1; stage III, prestress; stage V, poststress; and stage VII, postbaseline 2. In stage IV, psychological stress was induced by a mental arithmetic task. This task was subdivided in an adaptation stage (IVa) during which the participant's performance level was determined, and a subsequent performance stage (IVb) comprising performance-dependent adjustments in task frequency and performance feedback. Finally, measures of fast and slow (14) neural stress responses were derived from the task and were related separately to MS disease parameters.

Results

Demographic and Clinical Participant Characteristics. Twenty-two of thirty-six patients and 13 of 21 controls were female ($\chi^2 = 0.00$, $P > 0.999$). Twenty-one of thirty-six patients and 16 of 21 controls had at least a high school diploma ($\chi^2 = 1.86$, $P = 0.250$). The mean age (±SD) of patients was 47.4 (±9.1) y and of controls was 49.1 (±11.7) y ($t = -0.59$; $P = 0.547$). Eight of 36 patients were treated with fumarate, 7 with β-interferons, 7 with glatiramer acetate, 6 with fingolimod, and finally 2 with teriflunomide. For a subgroup of 22 patients, a T2-weighted (T2w) brain MRI scan acquired within a time period of roughly 1 y before participation in our study was available [median = 293 d before participation; range, 132–435 d]. Comparing these images with T2w images acquired during study participation revealed that only 7 of 22 patients had developed new lesions in this period (median = 0 new lesions; range, 0–4 new lesions). Consistently, the median number of days since the end of the last relapse across all 36 patients was 654 d (range, 22–3,550 d). Together, these findings suggest that disease activity at or around the time of our study was fairly small. See Table 1 and Fig. S1 for further patient characteristics.

Psychophysiological Stress Responses, Mental Arithmetic Performance, and Cognitive Load. As expected because of the performance-dependent adjustments in task frequency in the stress paradigm, the link between performance (the number of correct trials during the last 8 min of stage IVb) and cognitive load (the mean duration of intertrial intervals during that period) across participants was strong ($t = -18.89$; $P < 10^{-4}$). Please note that only trials in the last 8 min of IVb were evaluated to control for measurement duration across conditions and equal feedback settings (*Materials and Methods*, *Experimental Design*). Patients performed worse than controls ($t = -2.16$; $P = 0.019$) and had a lower cognitive task load ($t = 2.10$; $P = 0.021$). The paradigm induced a fast psychological stress response (i.e., a significant positive difference in stress ratings for stage V vs. stage III: $t = 6.20$; $P < 10^{-6}$) and a fast response of SNS-related measures (i.e., the difference between the average heart rate in the last 8 min of stage IVb and the average rate across stage II; $t = 7.20$; $P < 10^{-7}$). For salivary cortisol, no fast stress response was observed. The paradigm did not induce a slow or lasting stress response in any of the three response measures (heart rate: differences between stages VI and II; perceived stress and cortisol: differences between stages VII and III). None of the stress responses (i.e., fast increases in perceived stress and heart rate) was (linearly) related to cognitive task load. Please also see Table 2, the supplementary analysis of psychophysiological stress response measures investigating nonlinear associations in *SI Materials and Methods*, *Psychophysiological Stress Responses*, *Mental Arithmetic Performance, and Cognitive Load*, and Figs. S2 and S3 for further details.

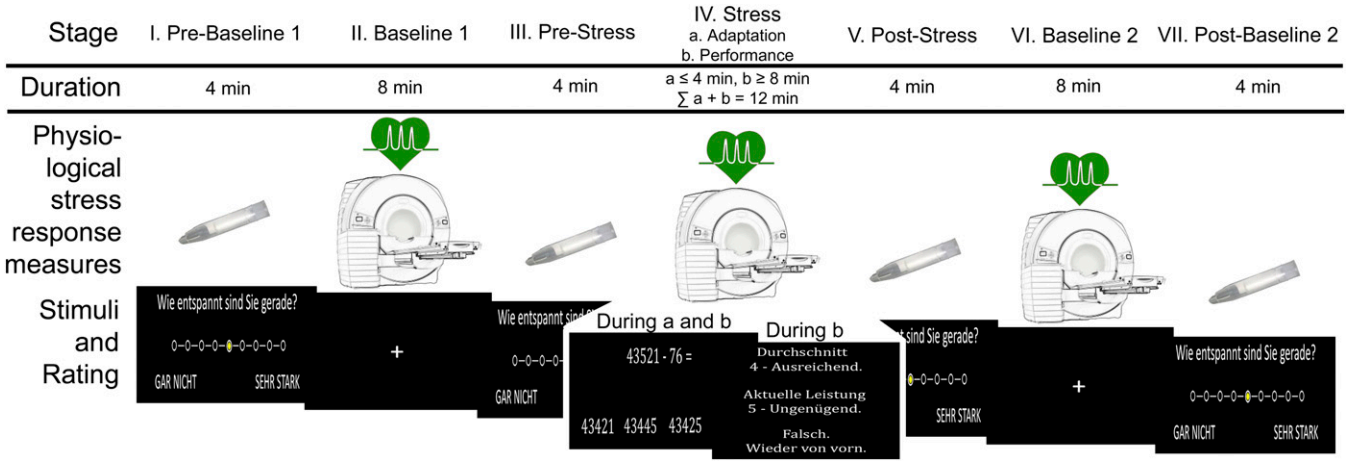

Fig. 1. The stress task. In the first experimental stage (stage I, pre-baseline 1), an initial salivary cortisol sample was taken, and participants were asked questions related to their currently perceived stress level (referred to as 'rating' in the following). These questions were presented on a projection screen and answered with MRI-compatible button boxes. In the next stage (stage II, baseline 1), the first ASL fMRI measurement was conducted; patients were asked to fixate on a crosshair. In stage III, pre-stress, a second rating and cortisol measurement were performed. In stage IV (stress), the fMRI stress measurement was conducted. The participant was asked to perform repeated subtraction tasks having the form operand X minus operand Y. The participant had to select the correct answer from a set of four answers shown on the screen below the operands. The task started with a constant value of X, 43,521, for all subjects. Operand Y ranged from 1 to 99 and was determined randomly in each trial. If a patient gave the correct answer, the difference between the two operands was used as operand X in the next trial. The course of the stress task was divided into two substages, an adaptation stage (stage IVa, duration ≤4 min) and a performance stage (stage IVb, lasting for the remainder of the total 12-min duration of stage IV). In each trial in stage IVa, participants had 8 s to select the response, and response times were recorded. Stage IVa ended either when the participant made 10 correct answers or when a 4-min period had elapsed. Stage IVb began without a break. Stage IVb differed from stage IVa in three aspects. First, the calculation times for correct trials determined during the adaptation stage were used to provide performance feedback in terms of school grades ranging from 1 [*Sehr gut* (very good)] to 5 [*Ungenügend* (insufficient)]. Second, if the answers were incorrect or too slow, the subject had to begin again with the initial starting value for X. Third, the time provided for subtraction was adjusted (only) in IVb based on the subject's performance (i.e., starting with 8 s after the transition from IVa to IVb, this time was decreased or increased by ten percent in a given trial based on the correctness or incorrectness in the preceding trial). Finally, a third rating and cortisol measurement (stage V, post-stress), a second resting fMRI measurement (stage VI, baseline 2), and a fourth rating and cortisol measurement (stage VII, post-baseline 2) were performed.

Table 1. Patient characteristics

Parameter	Median (range)
Disease duration since diagnosis, y	6.4 (0.3–21.2)
T2w lesion volume, 10^2 mm^3	44.6 (0.9–720.9)
Relapses since diagnosis	5 (1–21)
Days since last relapse	654 (22–3550)
EDSS	3.5 (1.5–6)
FSS, BB	1 (0–4)
FSS, BS	1 (0–3)
FSS, CB	2 (0–3)
FSS, CE	1 (0–2)
FSS, PY	2 (0–4)
FSS, SE	2 (0–4)
FSS, VI	1 (0–3)

BB, bowel and bladder; BS, brainstem; CB, cerebellar; CE, cerebral; PY, pyramidal; SE, sensory; VI, visual.

Stress-Induced Brain Activity and MS Disease Parameters. Four fMRI analyses were conducted to investigate mechanisms of fast and slow neural stress responses and their link to MS disease parameters. Specifically, subject-specific voxel contrast maps denoting the difference in local cerebral blood flow (CBF, measured in milliliters per 100 g per minute) averaged across the last 8 min of stage IVb (the mental arithmetic task with feedback) minus the CBF averaged across stage II (baseline 1) were used as parameters of fast neural stress effects. Maps assessing this difference between stages VI and II were used as indicators of slow stress effects. We report coordinates significant on a familywise error (FWE)-corrected level ($\alpha_{FWE} = 0.05$).

fMRI analysis 1: Neural stress response. In analysis 1a, one-sample *t* tests were conducted on the voxel level across all participants to identify brain areas showing fast or slow neural stress responses (i.e., stress-induced increases in activity). In analysis 1b, we tested for differences between MS patients and controls in these responses using two-sample *t* tests. Both analyses were restricted to coordinates located in a GM group mask (see *Materials and Methods*, *MRI Preprocessing* and *SI Materials and Methods*, *MRI Preprocessing*), and in both analyses gender and age were modeled as covariates of no interest. Analysis 1a identified a variety of brain areas showing a fast generic stress response across MS patients and controls, primarily in visual, insular, and cerebellar cortex areas. See Table S1 for further details. No slow neural stress responses were found. Analysis 1b showed that fast and slow neural stress responses do not differ in patients and controls.

fMRI analysis 2: Clinical disability. In fMRI analysis 2 (and in fMRI analyses 3, and 4) we searched for brain activity that is linked to MS disease parameters using voxelwise regression analyses modeling gender, age, and a constant term as covariates of no interest. Because we were particularly interested in the link between neural stress responses and disease parameters, we restricted these analyses to regions identified in analysis 1a. Because no slow neural stress effects were found in analysis 1a, we analyzed the link between alterations in the slow neural signal and disease parameters across all areas located in GM (*SI Materials and Methods*, *fMRI analysis S4*). We report effect size measures (*r*) for significant associations (weak effect: $0.1 \leq r < 0.3$; moderate effect: $0.3 \leq r < 0.5$; strong effect: $r \geq 0.5$) (19).

To investigate the link between fast neural stress responses and clinical disability, we tested the association between the respective activity changes and Expanded Disability Status Scale (EDSS) scores in analysis 2a and the Functional System Scores (FSS) subscales in analyses 2b–2h (2b, bowel and bladder; 2c, brainstem; 2d, cerebellar; 2e, cerebral; 2f, pyramidal; 2g, sensory; and 2h, visual). These analyses consistently revealed negative associations for coordinates in the left anterior insula. In particular, activity in a cluster of voxels surrounding coordinates −30, 23, 2 [$t = -3.97$; $p_{FWE} = 0.026$; cluster size (CS) = 54 mm^3; $r = 0.57$] in the anatomical standard space defined by the Montreal Neurological Institute (MNI) (20) was negatively linked to the EDSS score (analysis 2a). Activity in this peak voxel coordinate also showed a significant negative association ($t = -2.38$; $P = 0.021$; $r = 0.39$) with fatigue [i.e., measured with the Modified Fatigue Impact Scale (MFIS)] (21) (see *Discussion*). When controlling for fatigue in addition to gender and age in a voxelwise analysis, the link between the EDSS score and activity in this coordinate was no longer significant.

For the FSS cerebral scale (analysis 2e), activity in a cluster of left insula coordinates surrounding MNI −30, 20, 5 ($t = -4.39$; $p_{FWE} = 0.007$; CS = 459 mm^3; $r = 0.61$) was found [in addition to activity in a cerebellar area surrounding MNI coordinates −33, −64, −25 ($t = -4.01$; $p_{FWE} = 0.019$; CS = 432 mm^3; $r = 0.58$)]. Activity in this single insula peak coordinate was not linked significantly with fatigue ($t = -1.88$; $P = 0.065$; $r = 0.32$). The link between activity in the peak insula coordinate and the FSS cerebral scale remained significant in a voxelwise analysis when fatigue was additionally controlled for ($t = -3.96$; $p_{FWE} = 0.023$; CS = 27 mm^3; $r = 0.58$).

Finally, activity in a cluster of voxels surrounding MNI coordinates −30, 23, −1 in the insula ($t = -5.24$; $p_{FWE} = 0.003$; CS = 270 mm^3; $r = 0.68$) was negatively associated with the score on the FSS pyramidal scale (analysis 2f). The association remained significant when additionally controlling for fatigue ($t = -4.59$; $p_{FWE} = 0.009$; CS = 108 mm^3; $r = 0.64$), and activity was significantly linked to the MFIS score on a single-voxel level ($t = -2.50$; $P = 0.017$; $r = 0.4$) (see Fig. S4).

fMRI analysis 3: Clinico-radiographic measures of disease severity. In fMRI analyses 3a (total lesion volume), 3b (total number of relapses since diagnosis), and 3c (disease duration), no significant relations were found between stress-induced increases in fast brain activity and clinico-radiographic measures of disease severity.

fMRI analysis 4: Brain volume. In analysis 4a, a significant positive association between fast neural stress responses and the GM fraction (GMF) in patients was found in the left cerebellum (MNI coordinates −42, −61, −22; $t = 4.71$; $p_{FWE} = 0.004$; CS = 108 mm^3; $r = 0.64$) and in the supplementary motor area (SMA; MNI coordinates 0, 20, 47; $t = 4.10$; $p_{FWE} = 0.030$; CS = 81 mm^3; $r = 0.59$). Interestingly, when we repeated this voxelwise analysis for controls in the peak voxel cluster identified in patients, a significant positive association between stress-induced brain activity and GMF was found for cerebellar MNI coordinates −45, −61, −22 ($t = 2.10$; $p_{FWE} = 0.047$; CS = 27 mm^3; $r = 0.45$). Analysis 4b revealed a negative link between fast neural stress responses and WM

Table 2. Psychophysiological stress response variables and cognitive task load

	Fast response		Slow response	
Response variable	*t*	*P*	*t*	*P*
Heart rate	Stage IVb–II		Stage VI–II	
Main effect	7.2	$<10^{-7}$	−0.6	0.442
Group effect	−1.5	0.133	−0.1	0.900
Task load	0.4	0.724	0.0	0.983
Cortisol	Stage V–III		Stage VII–III	
Main effect	−3.0	>0.99	−2.7	>0.99
Group effect	−0.6	0.557	−1.1	0.306
Task load	0.8	0.408	0.7	0.528
Perceived stress	Stage V–III		Stage VII–III	
Main effect	6.2	$<10^{-6}$	1.3	0.145
Group effect	−1.1	0.271	−1.5	0.150
Task load	0.1	0.937	1.1	0.262

Main effects of stress on response variables (response differences between pairs of experimental stages) were tested using sign tests across variables of all participants (corrected for mean-centered gender, age, and cognitive task load but not for the overall mean). Group effects on response variables were tested with regression and permutation testing (covariates of no interest: gender, age, task load, constant) as well as the association of task load and response variables (covariates of no interest: gender, age, constant).

fraction (WMF) in the left middle occipital gyrus (MNI coordinates −30, −64, 38; $t = -4.64$; $p_{FWE} = 0.011$; CS = 189 mm^3; $r = 0.63$) in patients. No significant associations were found between stress-induced brain activity and WMF for the corresponding analysis based on data for controls (Fig. 2).

Discussion

In this study, we investigated fast and slow neural and psychophysiological stress responses and related these signals to MS disease parameters in a cohort of clinically stable MS patients. We demonstrate that fast neural stress responses are associated with clinical disability and brain atrophy in MS.

First we investigated a basic stress response across patients and controls. On the level of neural activity, this response was investigated in fMRI analysis 1a by evaluating fast increases of activity (from stage II to stage IVb) or slow increases (from stage II to stage VI) across all participants. We searched only for increases in stress-related activity because the great majority of stress-response parameters described in the literature are parameters of increased (not decreased) activity. For example, psychological stress induces increased cerebral perfusion (22), heart rate, growth hormone, prolactin, and cortisol secretion (23), NE secretion (24), and cognitive coping (25). fMRI analysis 1a revealed a distributed set of regions showing a fast stress response that largely overlapped with those areas found in healthy subjects (14), especially in dorsomedial prefrontal cortex (the SMA), inferior parietal areas, and insular cortex. Analysis 1a also identified visual regions and coordinates in the cerebellum, a brain area receiving strong norepinephrinergic inputs (26; also see below). On the contrary, no brain areas showing slow stress responses were found.

The analysis of psychophysiological stress parameters showed that the mental arithmetic task triggered fast increases in perceived stress and heart rate. Importantly, none of these task effects was (linearly) related to individual variations in cognitive task load (see *SI Materials and Methods*, *fMRI Analysis S1* for neural data). Furthermore, a supplementary analysis of psychophysiological stress response measures (*SI Materials and Methods*, *Psychophysiological Stress Responses, Mental Arithmetic Performance, and Cognitive Load*, *Nonlinear Associations Between Psychophysiological Stress Responses and Cognitive Load*) showed that heart rate accelerations and cognitive load are not related in a nonlinear fashion. Together, these findings strongly suggest that we are not falsely interpreting as psychological stress (27) the functioning of a cardiac mechanism that simply serves to adjust brain glucose delivery to increased cognitive and thus increased metabolic demands during the mental arithmetic task (stage IVb) relative to baseline (stage II). Finally, when additionally considering that cognitively demanding and socially evaluative tasks such as our task trigger NE release (24), one can conclude that our task successfully induced psychological stress.

Group differences in fast or slow neuronal stress responses were investigated in analysis 1b, which failed to identify such differences. This result might appear counterintuitive, given that pharmacological challenge studies have found differences in glucocorticoid responsivity (e.g., ref. 5). However, our result is consistent with the absence of group differences in markers of fast or slow psychological (perceived) stress or SNS-related stress (heart rate accelerations) in our study.

fMRI analyses 2–4 investigated the link between brain activity and MS disease parameters. Because we were particularly interested in the link between stress-related brain activity and MS disease parameters, in analyses 2–4 we searched only in those brain areas that showed a significant generic stress response in analysis 1a. Because no slow neural stress responses were found, we first discuss findings made for fast responses in analyses 2–4 and then briefly discuss the findings made in the corresponding supplementary fMRI analysis S4 for slow signal variations.

Specifically, the association between stress-responsive areas and clinical disability was evaluated in fMRI analysis 2. This analysis revealed a consistent negative link between fast neural stress responses and three disability markers (i.e., the EDSS and the cerebral and the pyramidal FSS subscales) in overlapping left anterior insular areas and thus might suggest an important association between insula functioning and MS disability, in general. At this point, it must be noted that the interrelatedness among EDSS scores and FSS subscales might have contributed to these consistent associations (cf. ref. 18). However, because the functions underlying these scales are realized by distributed networks, and MS neuropathology typically evolves in distributed regions across the disease course (28, 29), activity in a single region very well might be coupled to different but interrelated disability markers via a single functional mechanism. Thus, the interrelatedness among clinical scales does not severely affect the validity of this finding.

Findings made in an fMRI study investigating neural foundations of so-called "sickness behavior" in healthy subjects (30) may help explain the functional link between insular activity and disability in MS. Specifically, sickness behavior denotes a group of symptoms observed during the course of systemic infections including fatigue, depression, reduced exploratory behaviors, fever, and impaired cognitive performance (e.g., ref. 31). Sickness behavior is understood as an adaptive mechanism aiming to suppress nonimmunologically relevant behaviors during infections to reserve metabolic resources for immunological processes (30; see ref. 32 for further details on the complex interplay between immune processes and biologically relevant behaviors such as eating). In ref. 30, either a typhoid vaccination or placebo was applied on one of two measurement days. Subsequently, inflammation, self-report variables (including fatigue), and cognitive performance were measured in an fMRI Stroop task. As expected, vaccination induced a marked inflammatory response and simultaneously induced fatigue. Neural activity in the insular cortex was of outstanding importance because it increased in response to vaccination, and inflammation-induced activity in the insula correlated positively with fatigue. Consequently, the insular cortex may be understood as a region monitoring inflammation and (directly or as part of a larger network) inducing sickness behavior (e.g., fatigue) in response to inflammation.

To investigate whether similar mechanisms might contribute to findings made in analysis 2, we tested whether the insular activity related to clinical disability in the peak voxel coordinates identified in analyses 2a, 2e, and 2f was also related to patients'

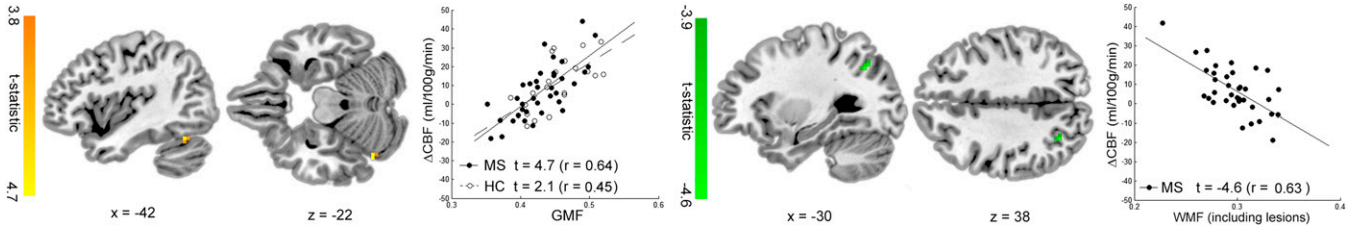

Fig. 2. The association between fast neural stress responses and brain volume. (*Left*) The brain slices depict coordinates with a positive link of stress-induced brain activity and GMF in patients surrounding the peak coordinate identified in fMRI analysis 4a (MNI coordinates −42, −61, −22). The scatterplot illustrates the association of GMF and stress-induced brain activity (corrected for gender and age) at MNI coordinates −42, −61, −22 for patients and the association between activity and GMF at MNI coordinates −45, −61, −22 in controls. (*Right*) The corresponding results for WMF identified in fMRI analysis 4b.

fatigue (measured with the MFIS). These analyses revealed a significant link between fatigue and EDSS-related activity ($t = -2.38$; $P = 0.021$) and activity related to the FSS pyramidal scale ($t = -2.50$; $P = 0.017$) and thus supported the relevance of the findings in ref. 30. However, contrary to the findings in ref. 30, the link was negative. Interestingly, such a negative association is consistent with inverse associations of other stress-related parameters and inflammatory processes in MS [e.g., elevated glucocorticoid activity (5) and reduced glucocorticoid suppression of inflammatory cytokine production (33)] and thus might indicate the existence of a depleted neuroimmunological process. Speculatively, this depletion might result from the immune system being confronted with a type of inflammation that it cannot cope with adequately (i.e., inflammation driven by autoimmune processes rather than by external pathogens) and that leads to continuous but ineffective neuroimmunological activity. As a consequence, the immune system triggers sustained fatigue in a misguided attempt to save metabolic resources for a process falsely assumed to be of a short, temporary nature. Please note, however, that other MS studies found a link between fatigue and HPA-axis hyperactivity (34), regional GM and WM atrophy, and lower fractional anisotropy in several WM areas (35). Consequently, dysregulated and inflammation-triggered sickness behavior might be considered as only one among several complex factors contributing to fatigue in MS.

To determine the relevance of the covariation between activity and fatigue for the covariation of activity and disability, we conducted voxelwise analyses modeling the MFIS as an additional covariate of no interest in analysis 2. Importantly, the link between both the FSS subscales and fast insular stress responses remained significant under these circumstances. These findings clearly argue that the link between disability and activity must be driven by further processes in addition to fatigue, an assumption that is consistent with other immunity-related processes regulated by the insula such as autonomic nervous system activity (36).

In analysis 3, we investigated the link between stress-induced activity and clinico-radiographic measures of disease severity, i.e., T2w lesion volume, the number of relapses, and disease duration. However these analyses failed to identify such associations. Speculatively, this lack of association can explained in part be by the rather weak link between brain lesions and clinical disability in MS (e.g., ref. 37).

In analysis 4 we investigated the associations between fast neural stress responses and GM (analysis 4a) and WM volume (analysis 4b). In 4a, a positive association between stress-induced activity and GM volume (i.e., a negative association between activity and atrophy) was found in the SMA and the left cerebellum in MS patients. The latter finding is compatible with the observations that the cerebellum receives major NE projections from the locus coeruleus (26) and that the stress hormone NE is closely linked to inflammatory processes (9, 10). Thus, given that inflammation is a source of GM atrophy (38), which is pronounced in the cerebellum (29, 39), one might conclude that the negative link of stress-induced brain activity and GM atrophy might be mediated by the NE system. Importantly, analysis 4a also showed that stress-induced activity is positively related to GM volume in overlapping cerebellar areas of controls. Thus, contrary to the conclusions that might be derived using only patient data (and supported by the absence of a link between stress-induced activity and disease duration), the association between stress-induced activity and GM volume cannot reflect only heightened stress sensitivity in response to MS. Instead, it might be at least partly indicative of a generic mechanism of neurodegeneration.

We conducted several supplementary analyses in addition to those described above. In these analyses, we found GMF and WMF were lower in patients than in controls, as is compatible with neurodegeneration being a key feature of MS (1). Furthermore, in fMRI analysis S2 we evaluated whether the associations between brain activity and MS disease parameters tested in analyses 2–4 depend on task load and found that they did not. Thus, together with the findings on fast generic stress responses, analysis S2 strongly suggests that the associations between neural signals and MS severity are driven by psychological stress. fMRI analysis S3 evaluated the specificity of the link between areas showing a fast neural stress response and MS disease parameters by repeating fMRI analyses 2–4 across all GM areas and revealed results very similar to those of analyses 2–4. Thus, fMRI analysis S3 nicely confirmed the functional relatedness of neural stress signals and MS disease parameters. Finally, in analysis S4 we tested the association between slow neural signal alterations and MS disease parameters across all GM areas in the brain and found links to the FSS cerebellar scale, the number of relapses, and WMF. However, given the absence of a slow generic neural stress response, the nature of these slow signal alterations and their link to MS disease parameters must be clarified in future studies.

Further explanations are required for the lack of stress-induced increases in cortisol in our study even though we used an experimental protocol almost identical to that described in ref. 14. A possible explanation is that, unlike the Trier Social Stress Test (TSST) (23), our protocol did not include a free speech task and that the induced stress level is lower under these conditions. Alternatively, the delay between the stress task and the last cortisol measurement might have been too short to account for the slow temporal characteristics of the salivary cortisol response (22), and thus a longer interval should be chosen in future studies. However, given the findings we made and that our task is less burdensome than the TSST (an important consideration in a study involving patients), we believe our task is well suited for addressing the research objectives we investigated.

Materials and Methods

Participants. Forty-three MS patients and 27 controls were recruited for participation in this study. Suitable participants were referred by the Charité MS outpatient clinic. Controls were recruited through advertisements. The inclusion criteria for patients were (*i*) a diagnosis of relapsing-remitting MS (RRMS) or secondary progressive MS (SPMS) according to McDonald Criteria 2010 (28); (*ii*) stable disease-modifying treatment for at least 6 mo or no disease-modifying treatment; (*iii*) age ≥18 y; and (*iv*) the physical and mental capability to use the test devices without restrictions. Patients were examined and diagnosed by experienced neurologists. Clinical disability was assessed using the EDSS and FSS (18). Potential participants presenting with mental or addictive disorders, neurologic diseases other than MS, acute MS relapses, acute infections, MRI contraindications, or pregnancy were excluded. Except for MS diagnosis, relapses, and treatment, the inclusion and exclusion criteria were the same for patients and controls. From the 64 participants remaining after the application of these criteria, seven subjects were additionally excluded from the analysis because of incomplete imaging datasets. Consequently, the fMRI data from 57 participants [35 females and 22 males; 36 MS patients (27 RRMS; 9 SPMS) and 21 controls] were available for analyses of fast neural stress effects, and data from 52 participants (34 patients) were available for analyses of alterations in slow neural signals. Data from 57 participants were available for analyses of fast and slow variations in perceived stress. Heart rate data for analyses of fast and slow signal variations were available from 46 participants (30 MS patients), and salivary cortisol data were available from 20 participants (13 patients). The two groups (36 MS patients and 21 controls) were comparable in terms of gender and age. Written consent was obtained from participants according to the Declaration of Helsinki. The study was approved by the research ethics committee of the Charité–Universitätsmedizin Berlin (EA1/182/10, amendment V).

Experimental Design. We used a version of an established ASL fMRI stress protocol (14) derived from the TSST (23) to induce and measure the impact of mild psychological stress (a mental arithmetic task with social performance feedback) on regional brain activity, SNS (heart rate), HPA axis (salivary cortisol), and perceived stress (Fig. 1). Before the experiment started, participants were told that they would participate in a mental arithmetic task and would receive feedback relating their performance to performance parameters in the overall population. After the protocol, participants were informed that the performance evaluation (expressed as a school grade) was computed based on their arithmetic performance in the adaptation stage.

MRI Sequences. Brain images were measured with a 3-T tomograph (Magnetom Trio; Siemens) and a 12-channel head coil. Stress-related brain activity was measured with a pseudocontinuous ASL echo-planar imaging (EPI) sequence (40) covering the whole brain. Using this sequence, 120 images were acquired during stages II (baseline 1) and VI (baseline 2) (8 min each), and 180 images were acquired during stage IV (stress) (12 min). To determine

brain morphological parameters (see *MRI Preprocessing* below), anatomical T1-weighted (T1w; 1 min 43 s) and T2w (5 min 52 s) sequences were measured. See *SI Materials and Methods*, *MRI Sequences* for further details.

MRI Preprocessing. Preprocessing of anatomical T1w and T2w images comprised manual lesion mapping and determination of lesion volume, generation of a group mask used to constrain several fMRI analyses to areas of GM, and determination of GM and WM volume (i.e., GMF and WMF). Preprocessing of fMRI images included realignment, distortion correction, coregistration to T1w anatomical images, spatial smoothing, determination of local CBF, spatial normalization, and computation of voxelwise contrast maps denoting the difference in average local CBF for fast stress responses (stage IVb minus stage II) or slow responses (stage VI minus stage II). These maps were computed in the anatomical standard space defined by the MNI (20) and served as source data for fMRI group analyses. Please note that only ASL images acquired during the last 8 min of stage IVb were preprocessed and analyzed to control for measurement duration across conditions and equal feedback settings (see *Experimental Design*, above).

Psychophysiological Stress Responses, Mental Arithmetic Performance, and Cognitive Load. For details on psychophysiological stress responses, mental arithmetic performance, and cognitive load please see *Results* and Table 2.

Stress-Induced Brain Activity and MS Disease Parameters. fMRI analyses were conducted with the SnPM13 (www2.warwick.ac.uk/fac/sci/statistics/staff/academic-research/nichols/snpm), and the SPM12 toolboxes (www.fil.ion.ucl.ac.uk/spm) (Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College London, London) using permutation testing for inference. Effect size measures (i.e., correlations, *r*) (19) for significant associations between brain activity and MS disease parameters were computed based on the *t*-statistic of a corresponding regression coefficient and the given degrees of freedom (df) following the equation $r = (t^2/[t^2 + df])^{0.5}$. In addition to the fMRI analyses described in the main text, several supplementary fMRI analyses were conducted. In particular, in *SI Materials and Methods*, *fMRI Analysis S1* we investigated the link between neural stress responses and cognitive load. In *SI Materials and Methods*, *fMRI Analysis S2* we tested whether the findings made in voxelwise fMRI analyses 1–4 for patients and controls depend on the (linear) covariation between fast neural stress responses and cognitive task load by repeating the corresponding analyses but modeling cognitive load as additional covariate of no interest (Table S2). Furthermore, we investigated the link between alterations in fast (*SI Materials and Methods*, *fMRI Analysis S3*; Table S3) and slow (*SI Materials and Methods*, *fMRI Analysis S4*; Table S4) brain activity variations and MS disease parameters in patients across all coordinates contained in the GM group mask (not constrained to the areas showing a stress response as identified in analysis 1a). These analyses were performed to evaluate the functional specificity of areas showing a fast neural stress response for MS severity parameters and to evaluate whether alterations in slow brain activity are at all linked to these parameters.

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