Persistent and reversible consequences of combat stress on the mesofrontal circuit and cognition

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Prolonged stress can have long-lasting effects on cognition. Animal models suggest that deficits in executive functioning could result from alterations within the mesofrontal circuit. We investigated this hypothesis in soldiers before and after deployment to Afghanistan and a control group using functional and diffusion tensor imaging. Combat stress reduced midbrain activity and integrity, which was associated to compromised sustained attention. Long-term follow-up showed that the functional and structural changes had normalized within 1.5 y. In contrast, combat stress induced a persistent reduction in functional connectivity between the midbrain and prefrontal cortex. These results demonstrate that combat stress has adverse effects on the human mesofrontal circuit and suggests that these alterations are partially reversible.

functional MRI | working memory | prospective | posttraumatic stress disorder | dopamine

It is well known that prolonged stress increases the risk for the development of psychiatric symptoms (1). At the same time, stress also compromises neurocognitive functioning (2). People often experience cognitive failures during stressful periods that usually disappear when the stress decreases. However, severe stress, such as experienced during military deployment can lead to long-lasting cognitive impairments that contribute substantially to its negative influence on social and occupational functioning (3, 4). Different animal models have been developed to explain the long-term consequences of stress on cognition. Its adverse effect on memory is thought to result from damage to hippocampal neurons (5), whereas its influence on executive functions such as sustained attention and working memory may result from alterations in the catecholaminergic system (6). This system includes the mesofrontal dopaminergic neurons that originate from the midbrain and project to the prefrontal cortex (7), which are crucial for several higher-order executive functions such as sustained attention and working memory (8, 9). Chronic stress reduces the activity of these neurons in the substantia nigra and ventral tegmental area and reduces dopamine turnover in terminal regions in the prefrontal cortex, leading to impaired neurocognitive functioning (10–12). In humans, acute stress impairs working memory and attention by affecting the prefrontal cortex (13, 14), but the long-term consequences of severe stress are unknown. Patients that have developed posttraumatic stress disorder (PTSD) after severe stress exposure also show working memory abnormalities in the prefrontal cortex (15, 16), but it is unclear whether this is caused by severe stress exposure, the experience of stress symptoms, preexisting abnormalities, or a combination of those factors.

Here, we used a prospective longitudinal study design in combination with functional MRI (fMRI), diffusion tensor imaging (DTI), and neuropsychological testing to study the neural mechanisms underlying the long-term effects of severe stress on cognition in humans. We investigated 33 healthy soldiers before (baseline) and 1.5 mo (SD = 0.8) after (short-term follow-up) their first military deployment to a combat zone, which is typically associated with severe stress exposure. The soldiers were deployed for 4 mo to Afghanistan as part of the North Atlantic Treaty Organization International Security Assistance Force peacekeeping operation. Because recent studies suggest that the effects of stress on the brain may reverse but can also be persistent, we investigated them again 1.6 y (SD = 0.1) after deployment (long-term follow-up) (17–19). In addition, we investigated 26 healthy soldiers, who were never deployed, at similar time intervals to control for repeated testing and nonspecific time effects. Nine participants of the combat group and nine participants of the control group did not complete the long-term follow-up.

Results

Combat Exposure and Stress Symptoms. We measured combat exposure during deployment to quantify the level of stress exposure using a previously validated questionnaire. The average score for combat exposure (mean ± SD; 4.7 ± 2.3) was similar to that of a previously reported reference population of Gulf War veterans (4.0 ± 3.2) (20), confirming that the combat group was exposed to typical combat zone stressors, such as armed combat, combat patrols, and exposure to enemy fire, as well as asymmetric warfare with a risk of exposure to improvised explosive devices. However, this exposure did not lead to an increase in stress symptoms, as no significant differences in PTSD, state anxiety, positive and negative affect scores between groups were observed (Ps > 0.4) (Table S1). This finding suggests that any neural changes are unlikely to have been caused by increases in psychiatric symptoms.

Executive Functioning. To assess neuropsychological functioning under demanding conditions, we used a standard neuropsychological test of sustained attention that was completed under time pressure (Bourdon–Wiersma dot cancellation test). There were no significant differences in sustained attention scores at baseline (Ps > 0.1). However, although the number of errors decreased at each assessment across groups [i.e., a practice effect; time: $F(1.8, 60.3) = 17.4$, $P < 0.001$], the decline from baseline to short-term follow-up was reduced in the combat group [$F(1, 33) = 4.2$, $P = 0.048$; overall group×time: $F(1.8, 60.3) = 3.4$, $P = 0.044$] (Fig. S1

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and Table S2). No significant differences in completion times were observed ($P > 0.6$), indicating that there was no change in speed-accuracy trade-off. Thus, the combat group benefitted less from repeated testing after deployment, suggesting that combat stress interfered with sustained attention under high cognitive demand.

Midbrain Activity. To investigate the influence of combat stress on neural functioning, we used a working memory task during fMRI scanning that is known to recruit midbrain dopamine and prefrontal cortex activity, and is sensitive to stress and stress hormones (13, 21, 22). The *n*-back task consisted of a high and a low working memory load condition. In the high-load condition, participants had to memorize a string of single digits and to press a button when a particular digit was the same as two items before (2-back). In the low-load condition, participants were requested to merely indicate when the digit "1" was presented (0-back). These conditions were chosen such that task performance was expected to be high even in the high-load condition, and participants rehearsed the tasks outside the scanner to ensure near optimal task performance. As expected, working memory performance was high (average accuracy >90%) and did not differ significantly between the combat and control group because of ceiling performance (accuracy: $P > 0.2$; reaction times: $P > 0.2$) (Table S2). Across groups and assessments, the task activated the frontal cortex and midbrain, as well as the parietal cortex, cerebellum, thalamus, caudate nucleus, and temporal cortex (Fig. S2 and Table S3) [all statistical comparisons were family-wise error rate-corrected at $P < 0.05$; see the specific tables for coordinates]. No significant differences were observed between groups at baseline.

Comparison of the baseline and short-term follow-up data showed that combat stress reduced activity in the midbrain (Fig. 1*A* and Table S4). The group×time interaction confirmed that this reduction was significantly different from the control group, and showed no significant differences in the prefrontal cortex or other brain regions. The midbrain activity reduction included the substantia nigra from which the dopaminergic projections to the lateral prefrontal cortex originate (7), but the imaging resolution that was used was insufficient to localize this reduction specifically to the substantia nigra.

Midbrain Integrity. To investigate whether the change in midbrain activity was accompanied by alterations in midbrain structure, we analyzed the DTIs that were collected at each assessment. We generated mean diffusivity and fractional anisotropy images from each of the scans, which are different measures for the diffusion of water in brain tissue. Mean diffusivity indexes the overall presence of tissue, and fractional anisotropy indexes the presence and coherence of oriented tissue. Thus, these measures are complementary indices for the density and integrity of brain tissue (23). No significant differences in these measures were observed at baseline in the midbrain, but baseline fractional anisotropy in the left superior parietal lobule was higher in the

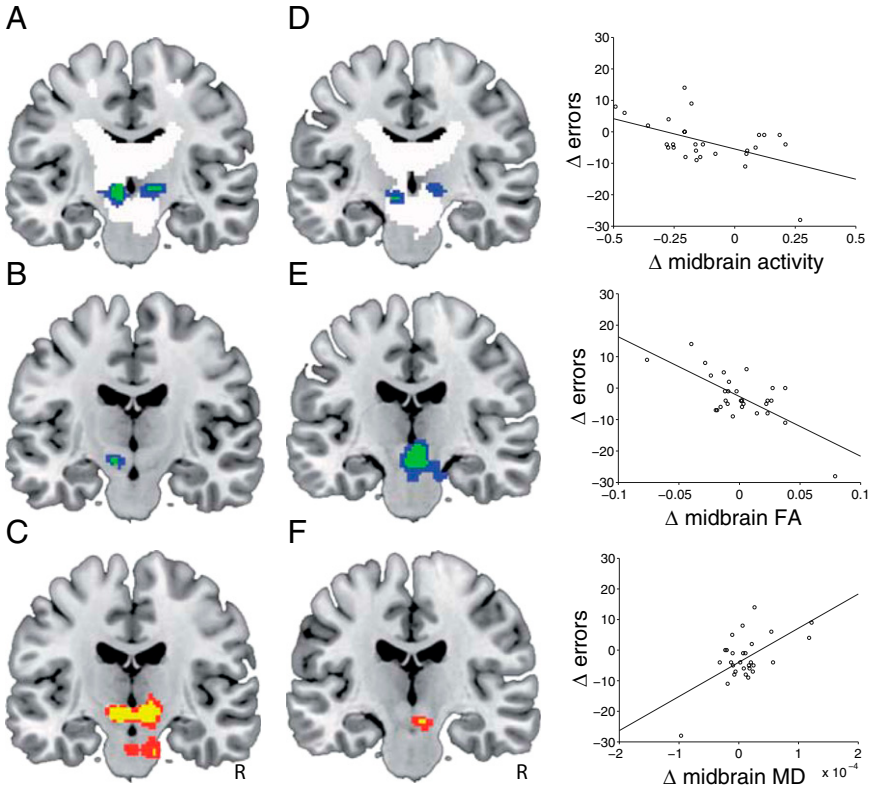

Fig. 1. Combat stress reduces midbrain activity and integrity, which is associated to reduced executive functioning. (*A*) The reduction in midbrain activity from baseline to short-term follow-up in the combat group (blue), projected on the main effect of working memory load (white). The corresponding reduction in midbrain integrity as measured with DTI is presented (*B*) for reduced fractional anisotropy, and (*C*) for increased mean diffusivity. (*D*) The reduction in midbrain activity is associated to attenuated improvement in sustained attention from baseline to short-term follow-up (blue), projected on the main effect of working memory load (white). This correlation shows that a larger reduction in midbrain activity led to less improvement on the sustained attention test. (*E*) The corresponding negative association for fractional anisotropy. (*F*) The positive association for mean diffusivity. All statistical tests were corrected for multiple comparisons ($P < 0.05$, SVC). The panels are presented at $P < 0.005$ uncorrected to illustrate the spatial extent of the results ($y = -16$). The clusters that are significant after correction for multiple comparisons ($P < 0.05$, SVC) are presented in green or yellow. The scatter plots illustrate the voxel-wise correlations at the peak voxel.

combat group. More importantly, combat stress reduced fractional anisotropy (Fig. 1*B* and Table S4) and increased mean diffusivity (Fig. 1*C*) in the midbrain. The increase in mean diffusivity was confirmed by the group×time interaction, but this analysis did not reach significance for fractional anisotropy. The interactions further showed a reduction in fractional anisotropy in the right corticospinal tract. Taken together, these results show that combat stress reduces the structural integrity of the midbrain and its activity during cognitive processing.

Brain–Behavior Relationship. Next, we aimed at determining whether the stress-related changes in midbrain function and structure could explain the reduction in cognitive performance. Because the influence of combat stress on executive functioning was observed in the sustained attention test, we selected this measure to perform correlation analyses between performance and changes in brain function and structure. Although sustained attention and working memory are separate constructs, they recruit overlapping neural resources and working memory can be conceptualized as sustained attention focused on internal representations (24, 25), suggesting that there might also be meaningful overlap in the influence of stress on these measures. Indeed, a voxel-wise correlation analysis showed a negative association between the change in midbrain activity during the working-memory task and the change in number of errors on the sustained attention test, indicating that combat soldiers with the largest decrease in midbrain activity benefited least from repeated testing (Fig. 1*D* and Table S5). Similarly, the decrease in fractional anisotropy (Fig. 1*E*) and increase in mean diffusivity (Fig. 1*F*) were also associated to a lower reduction in number of errors. These correlations remained significant after exclusion of one outlier. Furthermore, these correlations were not observed in the control group or even in the opposite direction for fractional anisotropy and were significantly different from the control group, indicating that these associations were specific to the combat group. Thus, these results suggest that the influence of combat stress on midbrain function and structure leads to reduced performance on sustained attention.

Midbrain-Prefrontal Cortex Connectivity. To assess whether the influence of combat stress on the midbrain affected its functional integration with the prefrontal cortex, we performed a functional connectivity analysis. We extracted the time-course from the midbrain and correlated that to the time-course of the rest of the brain. Across groups and assessments, the midbrain was positively coupled to a widespread network, including regions in the lateral and medial prefrontal cortex, parietal cortex, temporal cortex, occipital cortex, thalamus, basal ganglia, and cerebellum (Table S6). No significant differences were observed between groups at baseline.

Comparison of the baseline and short-term follow-up images showed that combat stress reduced functional coupling between the midbrain and lateral prefrontal cortex (Fig. 2 and Table S7). The reduction was confirmed by the group×time interaction that showed no significant differences in other brain regions. Thus, the impact of combat stress on the midbrain influences the functioning of the larger cognitive neurocircuitry.

Long-Term Follow-Up. These changes in midbrain structure, activity, and connectivity persisted for at least 1.5 mo after return from combat. To determine whether these alterations persisted even over 1.5 y or whether they normalized, we analyzed the long-term follow-up data. Quadratic polynomial contrasts were used to test whether changes from baseline to short-term follow-up reversed from short-term to long-term follow-up. These voxel-wise analyses showed that the changes in midbrain activity (Fig. 3*A* and Table S8), fractional anisotropy (Fig. 3*B*), and mean diffusivity (Fig. 3*C*) had normalized within 1.5 y after deployment. Group×quadratic polynomial contrast interactions confirmed

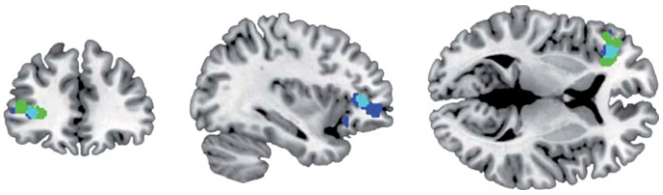

Fig. 2. Combat stress reduces functional connectivity of the midbrain with the lateral prefrontal cortex. The reduction from baseline to short-term follow-up is presented in blue. The persistent reduction from baseline to long-term follow-up at 1.5 y after military deployment is presented in green. The overlap between the short-term and long-term effects is presented in cyan. All statistical tests were corrected for multiple comparisons ($P < 0.05$, SVC). The figures are presented at $P < 0.005$ uncorrected to illustrate the spatial extent of the results (−36, 36, 6).

that these patterns were significantly different from the control group. Unlike the normalization of midbrain structure and activity, the quadratic polynomial contrast showed no significant normalization of midbrain-prefrontal coupling. Instead, the difference between coupling at baseline and long-term follow-up remained significant (Fig. 2 and Table S8). The group×linear polynomial contrast interaction confirmed that combat stress led to a persistent reduction in functional coupling of the midbrain to the lateral prefrontal cortex.

Discussion

These results show that the adverse effects of combat stress on sustained attention are related to functional and structural changes in the midbrain. These alterations normalize within 1.5 y in soldiers without psychiatric complaints, which may explain why long-term cognitive deficits following combat are mainly observed in soldiers with posttraumatic stress symptoms (26). In contrast to the reversible effects on the midbrain itself, its reduced interaction with the prefrontal cortex persists for at least 1.5 y. Taken together, these results suggest that the human brain can largely recover from the adverse effects of stress, supporting the view that neural plasticity in response to prolonged stress is adaptive (27). However, the results also reveal long-term changes within the mesofrontal network that may increase the vulnerability to subsequent stressors and lead to long-lasting cognitive deficits.

The normalization of midbrain structure and function, but not midbrain-prefrontal cortex coupling, is notable. Interestingly, we also observed a normalization of amygdala reactivity to emotional stimuli 1.5 y after combat exposure in combination with persistent changes in amygdala connectivity with the dorsal anterior cingulate cortex (18, 19). This finding could imply that local subcortical networks are highly plastic and readily adapt to ongoing environmental changes, whereas alterations in long-ranging cortical-subcortical networks are resistant to recovery. This finding concurs with the relative persistence of distal dendrite retraction of layer V medial prefrontal cortex pyramidal neurons after chronic stress in rodents, which mainly project to and regulate subcortical output systems (28). We speculate that the local networks may regain their normal functioning under normal circumstances, as evidenced by normalized midbrain activity and sustained attention performance, but that they may be more susceptible to environmental stressors because of suboptimal cortical regulation. Although prefrontal cortex projections to the midbrain are sparse in primates (29), the prefrontal cortex can regulate midbrain activity via the striatum and subthalamic nucleus (30). Optimal dopamine signaling is crucial for cognitive functioning and too much dopamine release under acute stress impairs performance (6). Because prior chronic stress exposure increases stress-evoked dopamine release in the prefrontal cortex in rodents (31), the remaining alterations in mesofrontal

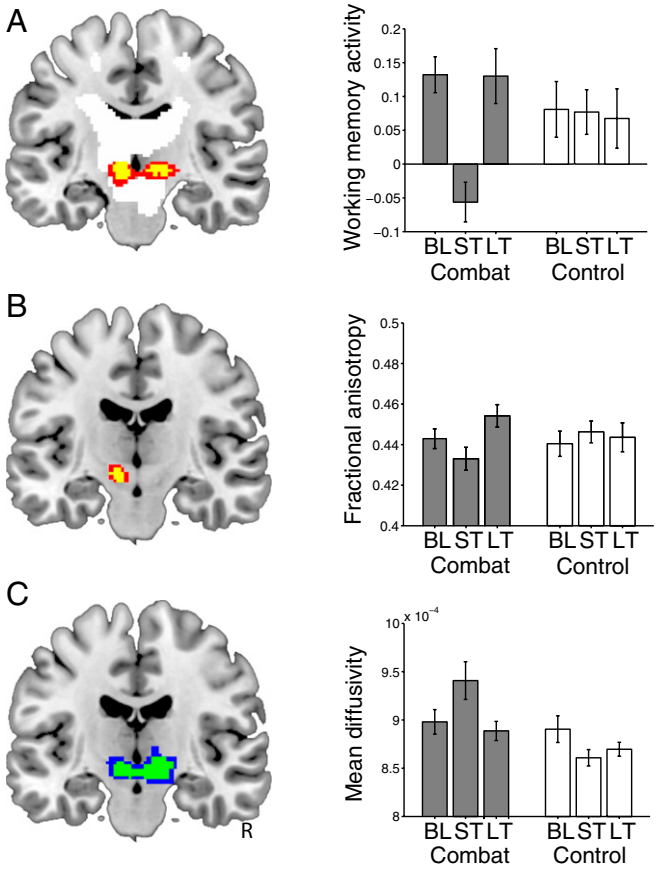

Fig. 3. Reversible effects of combat stress on midbrain function and structure. (*A*, *Left*) The normalization of midbrain activity at 1.5 y after military deployment (red; quadratic polynomial contrast), projected on the main effect of working memory load (white). (*Right*) Bar graph illustrating the normalization of midbrain activity. Data for the combat group (Combat) and control group (Control) are presented as mean (± SEM) at baseline (BL), short-term follow-up (ST), and long-term follow-up (LT). The corresponding normalization of midbrain integrity as measured with DTI is presented for fractional anisotropy (*B*) and mean diffusivity (*C*). All statistical tests were corrected for multiple comparisons ($P < 0.05$, SVC). The figures are presented at $P < 0.005$ uncorrected to illustrate the spatial extent of the results ($y = -16$). The clusters that are significant after correction for multiple comparisons ($P < 0.05$, SVC) are presented in yellow or green. The bar graphs illustrate the voxel-wise comparisons at the peak voxel.

coupling may sensitize the midbrain response to stress. However, our functional connectivity analysis does not assess the direction of connectivity; thus, the influence of combat stress on mesofrontal coupling may reflect changes in top-down as well as bottom-up processes. Further studies are required to test this hypothesis.

The results showed that combat stress reduced the practice effect on the sustained attention test, but no behavioral effects were observed on the working-memory task. The lack of behavioral results on the working-memory task was anticipated, because we designed the experiment to achieve ceiling performance during fMRI scanning. However, as combat stress reduced midbrain activity, it can be expected that working memory performance will also be compromised when the experiment becomes more difficult.

Whereas the adverse effects of combat stress point to changes in the midbrain, the effects of acute stress on cognition appear to be mediated by the frontoparietal network and medial temporal lobe (13, 14, 32, 33). The influence of prolonged and acute stress on different brain regions may be explained by the increased release of stress hormones during acute stress that also exert direct effects on the brain (6, 34). However, as discussed above, prolonged stress exposure sensitizes the mesofrontal response to acute stress in animals (31), suggesting that the remaining mesofrontal alterations may also influence the response to acute stressors in humans.

Many stress-related neuropsychiatric disorders are characterized by impaired cognitive functioning (35). Rather than being a mere consequence of psychiatric symptomatology, reduced neurocognitive functioning also increases the vulnerability for its development (36). As such, the persistent changes in the mesofrontal circuit may also play a role in the development of psychiatric symptoms, and contribute to the neural abnormalities observed in patients with PTSD (15, 16).

The combat soldiers were recruited from battle groups. This selection was chosen to maximize the probability of significant stress exposure. A resulting limitation is that part of the effects may be explained by blast exposure to improvised explosive devices. However, blast exposure mainly appears to influence brain structure when it leads to traumatic brain injury (37), and none of the participants in our study were physically injured during deployment. This result indicates that the influence of blast exposure is presumably limited, and suggests that the changes in brain structure are primarily mediated by psychological factors. Another consequence of the recruitment of a specific sample is that it limits the possibility to generalize the findings to the general population. Our participants were relatively resilient to the consequences of combat, because only 1 of the 33 soldiers (3%) scored above the clinical threshold for PTSD symptoms on a self-report questionnaire. This finding suggests that in more vulnerable individuals with less capacity to compensate for the adverse effects of stress (36), the mesofrontal alterations may be more pronounced, more persistent, or have a larger impact on cognitive functioning.

In conclusion, our results demonstrate that combat stress affects the midbrain and thereby compromises sustained attention. These consequences of combat stress were reversible. However, the persistent changes in mesofrontal connectivity may increase the vulnerability to subsequent stressors and promote later development of difficulties with cognitive, social, and occupational functioning (4).

Materials and Methods

Participants. The participants for the combat-stress group were recruited from a larger prospective study on the development of stress-related disorders following military deployment in the Dutch armed forces. Their duties included combat patrols, clearing or searching homes and buildings, participation in demining operations, and transportation across enemy territory. The combat-stress group was exposed to typical war-zone stressors, such as exposure to enemy fire, armed combat, and seeing seriously injured and dead fellow soldiers and civilians (including women and children). Participants for the control group had never been deployed and were recruited from training bases and army divisions currently not involved in combat missions. One deployed and one never-deployed soldier scored above the threshold for possible PTSD on a self-report questionnaire, and both were therefore excluded from the analyses. The groups did not differ significantly in sex ratio, age, and intelligence quotient. Furthermore, we observed no significant differences in PTSD, state anxiety, and positive and negative affect scores at baseline (Table S1). The study was in accordance with the declaration of Helsinki and institutional guidelines of the local ethics committee (Commissie Mensgebonden Onderzoek Regio Arnhem-Nijmegen, The Netherlands), and all participants provided written informed consent after written and oral description of the study.

Questionnaires. To assess PTSD symptoms, we used the short version of the Self-Rating Inventory for PTSD (38). We excluded individuals with possible PTSD from all analyses, as defined by the cutoff score of 52. The Positive and Negative Affect Schedule was used to assess positive and negative mood (39), and the State Trait Anxiety Inventory was administered to assess state anxiety (40). To quantify the level of combat exposure, we used the Combat

Experiences Scale of the Deployment Risk and Resilience Inventory (20), which included questions such as "I or members of my unit were attacked by terrorists or civilians" and "I personally witnessed someone from my unit or an ally unit being seriously wounded or killed."

Behavioral Tasks. The experimental paradigm during fMRI scanning consisted of a blocked design, including a high and a low working-memory load condition. In the high-load condition, participants had to memorize a pseudorandom string of single digits (numbers 1–9) and to press a button when a particular digit was the same as two items before (2-back). In the low-load condition, participants were requested to indicate when the digit "1" was presented (0-back). Five blocks of each condition were presented in alternating order. Each block started with a cue signaling the 2-back or 0-back condition for 3,000 ms followed by an interstimulus interval of 2,000 ms, and consisted of 15 digits that were presented for 400 ms with an interstimulus interval of 1,500 ms. Each block contained one, two, or three targets, and each condition contained 15% targets on average. Participants rehearsed the tasks outside the scanner to ensure understanding of task demands. Nevertheless, three deployed and two never-deployed soldiers performed at chance level during scanning. Data from those participants were excluded from the analyses, because this indicates inattentiveness during scanning.

As neuropsychological test of sustained attention the Bourdon–Wiersma dot cancellation test was used (41). This test consists of groups of three, four, or five dots, and participants are requested to mark the groups of four dots as fast and accurately as possible. All except one of the participants scored within the normative range for the number of errors (first decile $\geq$ 50) or completion time per line (first decile $\geq$ 17.35 s) on each session. The participant that scored among the 10% worst was also an outlier on the change score, and was excluded from the correlation analyses.

MRI Data Acquisition. MR data were acquired with a 1.5T Siemens Avanto MR scanner, equipped with a standard head coil. T2*-weighted blood-oxygen level-dependent (BOLD) images were acquired using echo-planar imaging (EPI), and each volume consisted of 32 axial slices (3.5 mm, 0.35 mm slice-gap, TR = 2.340 s, TE = 35 ms, 64 × 64 matrix, FOV = 212 mm, FA = 90°). Diffusion tensor images were acquired using EPI with 30 diffusion directions and four unweighted images (voxel size 2.5 × 2.5 × 2.5 mm^3, b-value 900 s/mm^2, TR = 9.100 s, TE = 88 ms, FOV = 240 mm). In addition, a high-resolution T1-weighted structural MR image was acquired for normalization purposes (3D MP-RAGE, voxel size 1 × 1 × 1 mm^3, TR = 2.730 s, TE = 2.95 ms, TI = 1000 ms, FOV = 256 mm, FA = 7°).

fMRI Data Analysis. Image analysis was performed with SPM5 (http://www.fil.ion.ucl.ac.uk/spm). The first five EPI-volumes were discarded and the remaining images were realigned to the first volume. Images were then coregistered to the anatomical scan, corrected for differences in slice acquisition time, spatially normalized to the Montreal Neurological Institute (MNI) T1 template, resampled into 2 × 2 × 2 mm^3 voxels, and spatially smoothed (8 mm FWHM). Statistical analysis was performed within the framework of the general linear model (42). The two experimental conditions were modeled as box-car regressors convolved with the canonical hemodynamic response function of SPM5. In addition, the realignment parameters were included to model potential movement artifacts, as well as a constant. Furthermore, a high-pass filter (cut-off 1/128 Hz) was included, temporal autocorrelation was modeled with an AR (1) process, and proportional scaling was used to minimize effects related to global signal variations between scan sessions. Contrast images comparing the high and low working-memory load conditions were obtained, and analyzed in random-effects models. To assess midbrain connectivity, the time-course of midbrain activity was obtained for each scanning session. The first eigenvariate of a sphere with 5-mm radius around the peak group×time interaction in the midbrain was extracted and entered as additional regressor to the original fMRI model to account for task-related signal fluctuations. The midbrain connectivity images were obtained, and analyzed in random effects models.

Diffusion Tensor Imaging. The preprocessing of the DTI data were performed using in-house developed software, written in Matlab (MathWorks). Preprocessing was executed on the Dutch Grid (www.biggrid.nl) using a Web interface to the e-Bioinfra gateway (43). Head motion and deformations induced by eddy currents were corrected for by using an affine registration of the diffusion-weighted images (DWIs) to the non-DWIs. The gradient directions were corrected by the rotation component of the transformation, and Rician noise in the DWIs was reduced by an adaptive-noise filtering method (44). Diffusion tensors were estimated in a nonlinear least squares sense. From the tensors, fractional anisotropy and mean diffusivity maps were computed. Using SPM5, these images were coregistered to the anatomical images based on a rigid-body mutual information registration between the fractional anisotropy and anatomical images. The coregistered images were subsequently spatially normalized to the MNI T1 template, resampled into 2 × 2 × 2 mm^3 voxels, and spatially smoothed (8 mm FWHM). The resulting images were analyzed in random effects models and were masked to exclude voxels with a mean fractional anisotropy value below 0.2.

Statistical Testing. Mixed-model ANOVAs with the factors group and time were used to test whether changes in brain structure and function over time were different between the combat stress and control groups. In addition, voxel-wise correlation analyses with the change in number of errors on the sustained attention test were performed to investigate associations between brain changes and performance changes. Statistical tests were family-wise error rate-corrected ($P < 0.05$) for multiple comparisons across the entire brain, or for the search volume for regions of interest using a small volume correction (SVC) (45). The search volume for the midbrain was anatomically defined using the WFU Pickatlas toolbox implemented in SPM5 (46), and the search volume for the prefrontal cortex was defined as sphere with 10-mm radius around the geometric center of the left and right prefrontal working memory clusters as identified in a meta-analysis of working-memory studies (47). Peak coordinates are reported in MNI space.

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