White matter changes in microstructure associated with a maladaptive response to stress in rats

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INTRODUCTION

Stress is a major risk factor to the development of severe mental illnesses, including major depression, anxiety, bipolar disorders and schizophrenia (for review, see Walker et al.20) and overall one of the more common factors in eliciting dynamic changes in brain states.21 Stress is known to trigger the activation of the hypothalamus–pituitary–adrenal axis, culminating in the production of glucocorticoids by the adrenals22,5 that will in turn generate, depending on the individual and the stress stimulus characteristics, adaptive or maladaptive psychoneuroendocrine responses to the stressful stimulus.7 In patients with major depressive disorder, dysregulation of the hypothalamus–pituitary–adrenal axis elicits specific and long-lasting functional and structural changes on a network of regions encompassing the hippocampus,8–10 the medial prefrontal cortex11,12 and amygdala.13,14 Subjects with ultrahigh risk for psychosis are particularly sensitive to social stress, life events and daily hassles, which have the potential to trigger psychiatric symptoms; they have an increased basal cortisol level15,16 and a smaller hippocampal volume.17,18 Moreover, stressful life events in non-psychiatric subjects are associated with a gray matter volume decrease in a network encompassing the anterior cingulate cortex, the hippocampus and the parahippocampal gyrus that was observed within a 3-month period.19

Animal models have confirmed the drastic effects that stress can have on the brain, including changes in dendritic trees, synaptic plasticity inhibition in the hippocampus and the hippocampal-to-prefrontal pathway,12,20,21 decreased neurogenesis in the hippocampus22 and apoptosis, involving corticosteroids and glutamate receptors.23 Taken together, these findings support the effect of stress on structural changes within networks of spatially distributed gray matter regions.

In addition to these regional changes, increasing evidence suggests that stress may also disrupt the structural and functional connectivity within neural networks.24–28 Diffusion magnetic resonance imaging (dMRI) is an advanced technique for examining white matter (WM) anatomy providing insights on the pathway microstructure within neural networks.29 A commonly used feature in dMRI studies is fractional anisotropy (FA), which estimates the degree to which tissue organization limits diffusion of water molecules in brain WM.30 In animals, different recent dMRI studies investigated changes in diffusion signal associated to...
chronic stress exposure. Delgado y Palacios et al.\textsuperscript{31} were the first to report the effects of stress using in vivo dMRI in rats: using diffusion kurtosis imaging (DKI), hippocampus microstructure was revealed to be altered in chronically stressed rats, independently of the hedonic state. More recently, the same team evaluated the mean kurtosis in the PFC, caudate–putamen (CPu) and amygdala in anhedonic-like and resilient rats and found a decrease in the CPu in the anhedonic-like.\textsuperscript{32} In addition, using a similar chronic mild stress (CMS) model, Kumar et al.\textsuperscript{33,34} showed increases in axial diffusion (AD) and radial diffusion (RD) specifically in the CPu and the amygdala of stressed rats. Another study using in vivo dMRI showed an increase in the mean diffusion (MD) in the lateral ventricles of chronically stressed rats, although no other changes were found.\textsuperscript{35} Finally, using a mice and a social defeat stress paradigm, Anacker et al.\textsuperscript{36} have shown correlations between diffusion metrics and social avoidance correlating positively with FA in the hypothalamus and hippocampus.

Here, we used dMRI and the tract-based spatial statistics (TBSS) approach\textsuperscript{37} adapted to brain rat to investigate the WM microstructure on the entire brain. We selected two strains of rats, Fischer 344 (F344) and Sprague–Dawley (SD), known to have differential response to stress,\textsuperscript{38,39} and compared their WM microstructure, assessed by four complementary dMRI measures (FA, MD, AD and RD), after exposure to repeated inescapable stress. Repeated exposure to the same stressor very often results in habituation, which leads to a decrease in the hypothalamic–pituitary–adrenal axis response.\textsuperscript{37} In contrast to SD rats, F344 rats show virtually no habituation or adaptation of the corticosterone stress response during repeated stress but an exaggerated acute stress-induced corticosterone secretion\textsuperscript{35,36} and increased anxiety-related behaviors\textsuperscript{37,38} with increased amygdala volume.\textsuperscript{39} Such a design allowed studying the effect, but also the responsiveness, to stress.

**MATERIALS AND METHODS**

**Animals**

Experiments were performed with male adult SD (n = 14) and Fisher 344 (F344; n = 14) rats (Charles River, Saint-Germain-sur-l’Arbresle, France) at 8 weeks’ age (average of 200 g for SD and 180 g for F344). Rats were housed in groups of two animals with ad libitum access to food and water and maintained in a temperature-controlled room, with a light/dark cycle of 12/12 h (lights on at 0600 hours). For each strain, rats were randomly assigned to stressed (N = 14) and non-stressed (N = 14) groups. Two animals of the SD strain of the control group were killed before the end of the 2 weeks. The protocols have been approved by the Comité d’Éthique de l’Expérimentation Animale du Commissariat à l’Énergie Atomique et aux Énergies Alternatives—Direction des Sciences du Vivant Ile de France (CETEA/CEA/DSV IdF) under protocol ID 12-058. All procedures were conducted in conformity with National (JO 887–848) and European (86/609/EEC) rules for animal experimentation.

**Stress protocol**

The behavioral stress protocol has been previously described elsewhere.\textsuperscript{20} Briefly, rats were placed on an elevated and unsteady platform for 30 min. The platform was positioned 1 m above the ground and illuminated with a high-intensity light source (1500 Lux). While on the platform, animals showed urination, defecation, grooming and freezing. This inescapable stress exposure (called a session) was repeated daily during 15 days between MD. AD, RD and parallel to axonal fibers and AD decreases are thought to reflect pathology of the axon itself, such as from trauma or ischemic changes.\textsuperscript{47} RD measures diffusivity perpendicular to axonal fibers and appears to be more strongly correlated with myelin abnormalities, like demyelination, as observed in multiple sclerosis.\textsuperscript{51}

**TBSS**

Whole-brain voxel-based statistical analysis was performed using the TBSS approach\textsuperscript{39} distributed as part of FSL adapted to the rat brain. The FA maps...
of all subjects obtained in the tensor-fitting step were aligned into a common space using a study-dedicated template and the nonlinear registration tool FNIRT. The template was defined as the most representative animal, calculated during the TBSS pipeline as the one that minimizes transformations. Next, all the FA images were averaged and thinned in order to create the mean FA skeleton. A threshold of 0.3 was applied to this skeleton in order to restrict the analysis to the WM tracts, and thus defining the final voxels for analysis. The AD, MD and RD maps of all animals were then warped into this skeleton map using the nonlinear transformations previously calculated for the FA maps.

Figure 1. Representative image of the diffusion MRI data (b0 map) and diffusion metrics (FA, MD, RD, AD) in a rat brain. AD, axial diffusivity; FA, fractional anisotropy; MD, mean diffusivity; MRI, magnetic resonance imaging; RD, radial diffusivity.

Figure 2. Three-dimensional reconstructions of the white matter skeleton used for TBSS analyses (lateral and top views). White matter tracts were color-coded based on the Paxinos and Watson atlas. TBSS, tract-based spatial statistics.
Figure 3. Plasma levels of corticosterone (mean ± s.d.) obtained in control and after stress in F344 and SD rats. (a) Longitudinal data at baseline (D0), after acute stress (D1) and after chronic stress (D15). (b) Comparison between strains before stress (D0) and after chronic stress (D15). Significance levels: *P < 0.05, **P < 0.001. F344, Fischer 344; SD, Sprague–Dawley.

Figure 4. White matter tracts with microstructural differences between control rats and stressed rats. Top panel represents an axial brain slice with voxels with significant main effect of stress (red–yellow scale) superimposed on the white matter skeleton used for TBSS analyses. Bottom panel provides histogram of white matter tracts microstructure (FA, MD or RD) in control (black) and stressed (light gray) rats. Only tracts with significant main effect of stress on FA (a), MD (b) or RD (c) are represented. For illustration purpose, the tracts were slightly dilated. FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity; TBSS, tract-based spatial statistics.
Statistical analysis for the skeletonized maps of FA, AD, MD and RD was performed using the 'randomise' fsl command-line tool, yielding a non-parametric test based on randomization methods. A total of 10,000 random permutations were used with threshold-free cluster enhancement, and multiple comparison corrections for family-wise error results were considered significant at $P < 0.05$. Six different contrasts were calculated, testing for the effect of stress ('Stress > No stress'); stress + no stress), strain (SD > F344', SD < F344') and stress-by-strain interaction.

Labeling of significant clusters in the FA skeleton was done with the standard 58,59 and cross-validated by visual inspection (Figure 2). Descriptive statistics were then calculated separately for each WM bundles.

RESULTS
Corticosterone plasma level
As expected, we found a significant main effect of stress on corticosterone plasma levels ($F(2,5) = 54.87$, $P = 2.7 \times 10^{-7}$) after chronic stress exposure (increase) in both strains (SD rats: $n = 6$, 197 ± 63.34 ng ml$^{-1}$ and F344 rats: $n = 8$, 273.75 ± 59.50 ng ml$^{-1}$) when compared with non-stressed rats (SD rats: $n = 6$, 63.66 ± 41.86 ng ml$^{-1}$ and F344 rats: $n = 6$, 92.50 ± 44.01 ng ml$^{-1}$).

There was also a significant difference between the two strains after 15 days of stress exposure with a higher plasma corticosterone level in F344 rats compared with SD rats ($T(11) = 2.18$, $P = 0.02$; Figure 3). There was no variance difference in corticosterone plasma levels between strains (SD versus F344) in control and stressed animals nor difference between conditions (stress versus no stress) in SD and F344 (Fligner–Killeen non-parametric test of homogeneity of variances, all $P$-values > 0.2).

White matter microstructure
The final number of animals involved in the analysis was as follows: 11 SD rats (four control and seven stressed) and 13 F344 (six control and seven stressed).

The WM skeleton in which statistical tests were conducted was constituted by a total of 6254 voxels. All the statistical tests were done with 23 degrees of freedom.

TBSS analyses revealed no significant main effect of strain ('F344' versus 'SD') in FA, AD, MD or RD maps ($P_{\text{corrected}} > 0.05$). In contrast, we found a main effect of stress ('Control' versus 'stress') in several WM bundles, with increased FA (peak $T$-value = 5.720, peak-corrected $P$-value = 0.012, cluster size of 3126 voxels) and decreased RD (peak $T$-value = 4.621, peak-corrected $P$-value = 0.001, cluster size of 3480 voxels) and MD (peak $T$-value = 4.598, peak-corrected $P$-value = 0.037, cluster size of 1515 voxels) in stressed animals compared with controls (Figure 4). These stress-related differences were distributed over the entire brain and involved WM bundles in posterior and anterior areas, on both hemispheres (Table 1).

Finally, significant strain-by-stress interactions were found in MD, RD and AD maps (Figure 5), with a stress-related decrease in SD rats and an absence of change in F344 rats. Significant interactions involved WM bundles in the left hemisphere and included the following: the corpus callosum (cc), external capsule (ec) and deep cerebral WM (dcw) for MD, RD and AD measures; the anterior commissure (ac), dorsal and intermediate endopiriform nucleus (DeNi/En) and amygdala for MD and RD measures; and dorsal hippocampus commissure (dhh) on MD. All statistics related to these results can be found in Table 2.

DISCUSSION
This diffusion MRI study reveals that 15 days of repeated exposure to the same inescapable stressor in rats leads to microstructural WM changes—increased FA and decreased MD and RD—of several WM bundles distributed in the entire brain. Furthermore, differential stress effects were observed in SD and F344 rat strains, which are known to have a different behavioral and physiological habituation to repeated stress.

Several WM bundles reported in this study (including amygdala fibers, dcw, DeNi/En fibers, dorsal hippocampus, fimbria of the hippocampus, external capsule and corpus callosum) connect brain areas associated with emotion formation and processing, attention, and learning and memory. Changes found in ac, proximal to the olfactory bulb and in Deni, may indicate a stress-related alteration in sensory circuits, possibly because of a readjustment of the perception of their surroundings.

To our knowledge, this is the first study to show the effects of repeated acute stress exposure in two strains with different stress sensitivity and habituation. Indeed, we show decreased MD, RD and AD in several brain bundles in SD rats, whereas no such differences were observed in F344 rats. This is particularly relevant, as SD rats were able to adjust their stress response to the repeated exposure to acute stress (resilience), therefore, showing an adaptive response that may be triggered by the acquisition of coping mechanisms that are paralleled by the decreases in MD, RD and AD, despite the overall increase in FA. In contrast, F344 (nonresilient) rats, which display a maladaptive response, do not reveal significant changes in these parameters. These findings suggest that differential response to repeated acute stressors may be revealed by or are associated with the ability to trigger structural plastic events in WM.

A few preclinical dMRI studies previously reported measurable effects of stress on several brain regions, and in all cases addressing the impact of chronic stress. Indeed, a significant decrease in the mean and radial kurtosis in the hippocampus was detected following CMS in rats.21 More recently, the same team reported significant stress-related increases in AD and RD in the CPu and in the amygdala, respectively, along with a mean kurtosis decrease in the CPu in anhedonic-like animals compared with resilient animals.22 Such effects were interpreted as the result of

| Table 1. Abbreviations of the white matter tracts investigated in the study |
|-----------------|-----------------|
| Abbreviation    | White matter tract |
| ac              | Anterior commissure |
| amygdala        | Amygdala fibers |
| cc              | Corpus callosum |
| dcw             | Deep cerebral white matter |
| DeNi/En         | Dorsal and intermediate endopiriform nucleus fibers |
| dhh             | Dorsal hippocampus commissure |
| ec              | External capsule |
| fi              | Fimbria of the hippocampus |
| ic              | Internal capsule |
| inwh            | Intermediate white layer |
| lobo            | Lateral orbital cortex/ventral orbital cortex |
| mfb             | Medial forebrain bundle |
| nsplh           | Nigrostriatal bundle/peduncular part of the lateral hypothalamus |
| nv              | Navicular nu basal forebrain |
| opt             | Optic tract |
| optot           | Optic tract |
| prlcg           | Prelimbic cortex/cingulate cortex |
| strbers         | Striatum fibers |
| strmf            | Superior thalamic radiation/medial lemniscus/ fasciculus retroflexus |

White matter tracts were labeled based on the Paxinos and Watson atlas.
Figure 5. White matter tract changes in microstructure associated with maladaptative response to stress. Interaction graphs provide the mean values of white matter tract microstructure - FA (a), MD (b) or RD (c) - in control (black) and stressed (light gray) animals, in F344 (circles with solid lines) and SD (squares with dotted lines) rats. ac, anterior commissure; amygFib, amygdala fiber; cc, corpus callosum; dcw, deep cerebral white matter; denien, dorsal and intermediate endopiriform nucleus fiber; dhc, dorsal hippocampus commisure; ec, external capsule; FA, fractional anisotropy; F344, Fischer 344; MD, mean diffusivity; RD, radial diffusivity; SD, Sprague–Dawley.
axonal degeneration and demyelination within WM bundles with disrupted microstructural spatial coherence. A FA decrease interpreted as a potential loss of myelin sheath was also found in the corpus callosum, bilateral frontal cortex and bilateral hypothalamus in rats after a similar CMS protocol.\(^3\)

Such contrasting results are likely to reflect the temporal dynamics of the stress response (and its successful, or not, adaptation). Yet, we cannot exclude that other methodological differences may also explain the difference in FA change direction, including the stress paradigm (repeated acute stress versus CMS), image acquisition (in vivo dMRI versus ex vivo data with higher spatial resolution and higher signal-to-noise ratio) or image analysis (measure in \textit{a priori} preselected regions of interest, mostly within gray matter structures versus voxel-wise analysis on the whole WM tracts).

Increased WM FA has been repeatedly associated to learning\(^{59-61}\) via neuronal plasticity processes (for example, synaptogenesis and dendritic branching) and glial remodeling (for example, modification of astrocyte processes).\(^62\) An increased FA was found in the corpus callosum after a spatial learning task and such increase was supported by significant increases in immune reactivity for a myelin marker, suggesting an increase in the cellular organization and packing of axons or myelin.\(^{59,63}\) More recently, TBSS analysis also revealed higher FA in skilled learning rats in comparison with control\(^64\) that could be explained by increases in myelination. On the other hand, a reduction in MD was found in both rat hippocampi before and after learning a hippocampal-dependent spatial navigation task.\(^61\) Data from both human and animal studies indicate the potential for rapid changes in dMRI indices,\(^{61,65}\) suggesting changes in structural plasticity in specific brain regions. The patterns of FA increase/RD decrease are likely related to a tissue density increase due to reshaping of neuronal or glial processes, and/or enhancement of tissue organization, including strengthening of axonal or dendritic backbones and surrounding tissue.\(^66\) Myelination, known to be modified by experience and maturation,\(^{57,64}\) may also partly explain the RD decrease observed in the stressed rats as RD increases have previously been associated with demyelination processes.\(^{69,70}\) Of note, an activity-dependent myelination has been recently proposed in a human study of motor training, where the FA change in WM was accompanied by adjacent gray matter density alterations.\(^71\)

This study presents some limitations that should be considered. Here the susceptible/resilient differences are achieved by using different strains. We cannot discard the possibility that the mechanisms that lead to different responses to stress within a single strain are different, or if the results found are specific to the SD strain, making its generalization harder. In addition, corticosterone was the only measure used to access the stress response, and although it is known to be one of the more representative markers of stress, the use of complementary behavioral assessment could be beneficial. Other limitations include the lack of direct histological correlations between DTI indices and morphological markers due to the exclusive \textit{ex vivo} approach. \textit{In vivo} longitudinal measurements would have allowed comparisons before and after stress, however, at the expense of signal-to-noise ratio and diffusion MRI spatial and angular resolution.

To conclude, we identified microstructural changes in the key WM tracts like the corpus callosum and the amygdala fibers linked to the frontolimbic circuitry with a functional relevance for cognitive performance and emotional response. Our data demonstrate that SD rats able to adjust to repeated exposure to an acute stress leads to significant changes in dMRI indices. These changes are not well understood, but we demonstrate that dMRI may offer a novel measure of microstructural remodeling occurring in response to stress to further explore the neural basis of adaptive and maladaptive response to stress in rodents and provide quantitative biomarkers to evaluate novel treatments to the protection of stress effects.

**CONFLICT OF INTEREST**
The authors declare no conflict of interest.

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