Severity of Traumatic Brain Injury

In order to be able to compare the control, military and civilian traumatic brain injury groups along the severity dimension, we used an ordinal scale based in the duration of post-traumatic amnesia. This information was available for the 3 groups. According to this scale, non-traumatic brain injury exposure (i.e., control group) was given a value of 0, absence of post-traumatic amnesia in the presence of traumatic brain injury exposure was categorized as 1, post-traumatic amnesia duration < 30 minutes was categorized as 2, and post-traumatic amnesia duration between 30 minutes and 24 hours was categorized as 3. Glasgow Coma Scale (*1*) scores were only available for the Civilian traumatic brain injury group.

Neuropsychological Assessment

Given that traumatic brain injury is selectively associated with alterations in executive and memory functions (*2*) and to limit the number of statistical tests examined, we collapsed the results of the neuropsychological tests into two domains respectively assessing executive and memory functions. The executive function domain score was the average of the following standardized (i.e., mean=0, SD=1) scores: the Groove Pegboard dominant hand time, the Ruff 2&7 time accuracy standardized score, the negative value of the Trails Making Test B time and the Digit Span total standardized score. The memory domain score was the average of the following standardized (i.e., mean=0, SD=1) scores: the Brief Visuospatial Memory Test-Revised total recall standardized score and the California Verbal Learning Test total number of correct standardized score.

Neuroimaging Methods

MR imaging was conducted on a Siemens TIM Trio scanner and included a multi-modal imaging study including T1, T2, fluid-attenuated inversion recovery, SWI, and Diffusion Tensor Imaging sequences. The T1-weighted images were acquired using a 3D MP-RAGE sequence with the following parameters: TE=2.8 ms, TR=2530 ms, TI=900ms, flip angle = 10°, FOV=256x256x220 mm, Matrix=256x256x220, NEX=1, Bandwidth=180Hz/pixel, iPAT=2. The T2-weighted images were collected using a 3D SPACE sequence in the sagittal plane with the following parameters: TE=452ms, TR=4800ms, NEX=1, FOV=256x230x192mm, Matrix=256x230x192, Bandwidth=592 Hz/pixel. The SWI data were acquired using the following parameters: TE=20 ms, TR=28 ms, flip angle = 15°, FOV=230x211x256 mm, Matrix=320x294x128, NEX=1, Bandwidth=120Hz/pixel. The diffusion tensor imaging data were acquired using a single shot twice-refocused spin-echo echo-planar sequence in the axial plane with the following parameters TE=82 ms, TR=8700 ms, FOV=256x256 mm, matrix = 128x128, slice thickness/gap = 2.0/0.0 mm, NEX = 1, b-value=1750 s/mm2, Bandwidth=1396 Hz/pixel. Diffusion gradients were applied along 64 directions.

Image Analysis

Quality assurance of the diffusion tensor images was performed using DTIPrep (*3*). This contains the following checks: 1) verification of protocol, 2) slice-wise checking, and 3) identification of venetian blind artifacts. Diffusion gradients with artifacts were removed and the remaining high quality diffusion weighted images retained.

As a first step, the data were converted from DICOM to NIFTI format using MRIConvert (http://lcni.uoregon.edu/~jolinda/MRIConvert/). All further preprocessing was carried out using FDT, part of the FMRIB's Software Library FSL (*4*). Here, all diffusion-weight volumes were aligned to the first b0 volume using affine registration and corrected for Eddy current distortions. Then, non-brain tissue and background noise were removed from the b0 image using the Brain Extraction Tool and the quality of image acquisition was inspected prior to further processing. Fractional anisotropy and mean diffusivity images, as well as, tensor eigenvalues that describe $\lambda_{||}$ and $\lambda_{\perp}$, were created by fitting a tensor model to the raw diffusion data using FDT.

Voxel-based analysis of fractional anisotropy data across subjects was performed using Tract-Based Spatial Statistics, part of FSL (*5*) First, all fractional anisotropy images were registered to the standard FMRIB58 fractional anisotropy template, which is in MNI152 standard space using a non-linear registration algorithm, Then, aligned fractional anisotropy maps were visually inspected after registration and to confirm that the result of the previous step was correct. Next, a mean fractional anisotropy image was created from the data coming from all the subjects in this common space and narrowed to generate a mean fractional anisotropy skeleton that represents the center of all tracts common to the entire group. A threshold fractional anisotropy value of 0.2 was then applied to exclude voxels that are primarily GM or CSF. The aligned fractional anisotropy image for each subject was then projected onto the skeleton by filling it with fractional anisotropy values from the nearest relevant tract centre. The resulting skeletonized data were then fed into the voxel-wise statistics analysis.

To investigate changes in the distribution of fractional anisotropy and mean diffusivity between all groups we used the FSL randomize program that is based on a non-parametric approach utilizing permutation test theory (5000 permutations) (*4*). The Threshold-Free Cluster Enhancement option was used (*6*) and age and handedness were introduced as nuisance variables in the matrix. The statistical threshold was set at $p < 0.05$ Family Wise Error corrected, which is a conservative procedure that allows a high control of Type I error, being the probability of one or more fractional false positives the same as the significance level.

The fractional anisotropy images produced by Tract-Based Spatial Statistics processing were used as input for analysis of "potholes." Potholes are small regions of the brain which had abnormally low fractional anisotropy compared to what was expected based on the control population. The methodology for identifying these potholes has been extensively described in a previous publication (*7*). In summary, the analysis of the fractional anisotropy images of all subjects in the control population was used to create average and standard deviation images. Using these statistical images, the fractional anisotropy images were used to create an image of z-scores for each subject, where

the fractional anisotropy at each voxel was converted to a z-score based on the mean and standard deviation from the control group. To find "potholes" this z-score image was then thresholded at a z value of -3.00. Pothole size was estimated according to the number of voxels that exceeded the threshold and the sum of potholes of all sizes constituted the measure of white matter disruption. In addition to the number of potholes in the total white matter, the number of potholes was also determined for every subject in each of the 48 regions of the John Hopkins University white matter atlas (*8*).

Statistical Analysis

We assessed the association between traumatic brain injury and the number of potholes in the brain after controlling for possible confounders. The confounders investigated were age, time since trauma, number of blast episodes, HAM-D, and CAPS total scores. The possible confounders were selected among the characteristics that resulted different among the groups. We built multiple linear regression models including the aforementioned covariates. The model with the smallest corrected Akaike's information criterion was preferred(*9*). Models' assumptions were assessed with residual and influence analyses. Since the residuals were not normally distributed the dependent variable (i.e., total number of potholes) was log-transformed.

All analyses were performed using SAS 9.2 (SAS Institute Inc, Cary, NC). All p-values reported are two-tailed. The significance level was set at 0.05.

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