METHODS: MAGNETIC RESONANCE IMAGING AND ANALYSIS

MRI Scan Acquisition

Ten normal right-handed, neurologically normal female control subjects age 20-29 and one female, right-handed, traumatic brain injury patient, age 21 were imaged identically. All participants were scanned with a 3T Allegra MRI scanner (Siemens, Erlangen, Germany). Participants were instructed to stay awake with their eyes open and to not perform any specific cognitive task (e.g., mental arithmetic, meditation). Conventional structural imaging included a high-resolution (1x1x1.25 mm) T1 sagittal magnetization-prepared rapid gradient-echo (MP-RAGE), a T2-weighted sagittal fast spin echo, and an axial fluid attenuated inversion recovery (FLAIR) scan. Resting state fMRI fluctuation data were acquired using a T2* weighted echo-planar sequence (3 runs × 5 minutes) sensitive to blood oxygenation level dependent (BOLD) contrast. Diffusion tensor imaging (DTI) is detailed below. Total scanning time was approximately 1 hour/subject.

Diffusion Tensor Imaging

DTI involves measuring the diffusion properties of water, which are strongly dependent on local tissue micro-structure (1). Thus, in normal myelinated white matter, diffusion is highly anisotropic (directionally dependent) because water molecules tend to move parallel as opposed to perpendicular to fiber tracts.-Prior studies in humans (2-10) as well as in animal models (11-15) of traumatic brain injury have shown that DTI parameters appear to be sensitive to changes following trauma.

The present DTI methodology is similar to that previously described by Shimony et al 2006 (16). Diffusion weighted images were sensitized in 48 directions using a locally modified echo planar imaging (EPI) sequence (TR=6400ms, TE=87ms, 1.5 x 1.5 x 1.5 mm voxels). 12 directions of diffusion were acquired at b=800, 1000 and 1200 s/mm^2, and 6 directions of diffusion were acquired at b=600 and 1400 s/mm^2. Diffusion tensor parameters were measured in regions of interest (ROI) traced on each subject's MP-RAGE image. Accordingly, the first image processing step was to define the spatial relationships between all images in terms of affine transforms computed by image registration. Multimodal (e.g., T2W/T1W) image registration was performed using vector gradient measure (VGM) maximization (17). The first acquired, unsensitized (b = ~0 s/mm^2; I0) DTI volume was registered to the T2W image; stretch and shear were enabled (12-parameter affine transform) to partially compensate for EPI distortion. Atlas transformation was computed via the T1 weighted image, which itself was registered to an atlas representative target produced by mutual coregistration of MP-RAGE images from a separate group of 12 normal young adults. The atlas target conformed to the Talairach system (18). Algebraic composition of transforms (matrix multiplication) enabled resampling any data type in register with any other (19). Thus, ROI generated on anatomical images were resampled in register with the DTI data for purposes of white matter tract selection and DTI parameter measurement.

Diffusion tensor tracking (DTT) was performed using a streamline algorithm (21, 22). The procedure for defining a volume using DTT was previously described (16). Start and end ROIs were selected visually in the cingulum bundle on coronal DTI images at the anterior and posterior margins of the corpus callosum. Cingulum bundle tracks were required to pass through both ROIs. A visitation count was calculated for every voxel based on how many of the filtered tracks pass through it. The cingulum bundle volume was defined as the collection of voxels with a visitation count above 25% of the

total number of tracks. These volumes were inspected visually in each subject for location and size. The final DTI parameter values were determined by averaging over the final volume in each subject.

Relative anisotropy was measured bilaterally in the fornix. To this end, ROIs were manually traced using Analyze 6.1 (Mayo Foundation, Rochester, Minn) on individual subject RA images while simultaneously viewing the coregistered MP-RAGE. Structure boundaries were determined according to Woolsey et al. (20).

Resting-State fMRI correlation analysis

Intrinsic fluctuations of the blood oxygen level dependent (BOLD) signal normally are correlated within anatomically connected and functionally related cortical regions (23-25). Thus, we hypothesized that traumatic axonal injury severe enough to cause clinical deficits should be accompanied by abnormal *functional connectivity* between the affected regions. To test this hypothesis, we studied the correlation structure of low frequency (<0.1 Hz) fluctuations of the blood oxygen level dependent (BOLD) signal acquired in the resting state. This technique has also been referred to as functional correlation MRI (fcMRI). A major advantage of this approach is that it does not rely on the subject's task performance. Subjects who are cognitively impaired or uncooperative can be studied without the confound of variable task performance. Moreover, as the correlation structure of spontaneous BOLD fluctuations is minimally affected by light sleep (26) and persists (albeit in somewhat altered form) even under anesthesia (27). The BOLD data required for fcMRI can be acquired in about 15 minutes using standard fMRI sequences, although the post-processing algorithms used in this work are not currently available commercially.

The following BOLD sequence parameters were employed: 25 ms echo time (TE), 90° flip angle, 4 x 4 x 4 mm voxel size, acquisition time 2.012 seconds per whole brain volume. Whole brain coverage was obtained with 32 contiguous slices. The BOLD data were analyzed as previously described (28). Preprocessing included compensation for systematic, slice-dependent time shifts, correction of odd-even slice intensity differences and correction for rigid body head motion. Registration of the resting-state functional data was achieved by composition of affine transforms as follows: BOLD$\rightarrow$T2W$\rightarrow$MP-RAGE$\rightarrow$atlas. Prior to correlation analysis, variance unlikely to represent local neuronal activity was removed by regression. These nuisance regressors included the BOLD signal averaged over white matter, CSF, and the whole brain as well the six rigid body parameters derived by head motion correction. Inter-regional correlations were computed using the Pearson product moment formula applied to regional time courses averaged over each ROI. Correlation maps were similarly computed between seed ROI and all other voxels in the brain. Each seed region was a 12-mm diameter sphere centered on previously reported Talairach coordinates (29, 30). These seeds included: left hippocampus (-21 -25 -14), and right hippocampus (23 -23 -14). For quantitative analysis, a 12 mm diameter sphere centered on area 24 of the ventral anterior cingulate cortex (-5 35 -4) was used. As there was some variability between subjects, analysis of correlations with the anterior thalami used two alternative 2 mm diameter spheres, centered on (7, -5, 3) or (8, -7, 7) for the right anterior thalamus and (-7, -5, 3) or (-8, -7, 7) for the left anterior thalami.

Hippocampal Volumetry

Hippocampal volumetry was performed on each individual subject's high resolution MP-RAGE images transformed to atlas space and resampled to 1 mm^3 voxels. Each hippocampus was manually traced using Analyze 6.1. The rostral border was the most anterior slice showing the emergence of the hippocampus from the inferior side of the amygdala; the caudal border was the slice displaying the fornices leaving the fimbria of the hippocampus. Volumes were calculated using the Cavalieri principle as previously described (31). The presently reported volumes include the hippocampal formation, dentate gyrus, alveus, and fimbria.

DTI DATA RESULTS

Relative Anisotropy	Left Cingulum	Right Cingulum	Left Fornix	Right Fornix
Controls, (n=10 Mean ± SD)	0.35 ± 0.02	0.37 ± 0.03	0.36 ± 0.03	0.34 ± 0.02
Subject	0.33	0.34	0.32	0.33

NEUROPSYCHOLOGICAL TEST RESULTS

TEST	RAW SCORE	PERCENTILE	INTERPRETATION
Wechsler Abbreviated Scale of Intelligence			
Vocabulary	53	39	Average
Block Design	48	58	Average
Similarities	37	66	Average
Matrix Reasoning	30	82	High Average
Verbal IQ	100 (IQ score)	50	Average
Performance IQ	108 (IQ score)	70	Average
Full Scale IQ	105 (IQ score)	63	Average
Wechsler Memory Scale-III			
Logical Memory I Recall Score	31	16	Low Average
Logical Memory II Recall Score	13	10	Low Average
Logical Memory II Percent Retention	59	5	Mildly Impaired
Logical Memory Recognition	19/30	…	
Letter Number Sequencing	12	64	Average
California Verbal Learning Test-II			
Trial I Recall Correct	5	7	Borderline
Trial II Recall Correct	8	16	Low Average
Trial III Recall Correct	9	7	Borderline
Trial IV Recall Correct	8	<1	Severely Impaired
Trial V Recall Correct	11	7	Borderline
Trials 1-5 Total	41	7	Borderline
Short Delay Free Recall	8	7	Borderline
Short Delay Cued Recall	8	<1	Severely Impaired
Total Intrusions	1	50	Average
Total Repetitions	3	31	Average
Long Delay Free Recall	9	7	Borderline
Long Delay Cued Recall	8	<1	Severely Impaired
Long Delay Recognition Hits	12	<1	Severely Impaired
Long Delay Recognition False Positives	5	7	Borderline
Recognition Discriminablility	1.7	<1	Severely Impaired
Brief Visual Memory Test-Revised			
Trial 1	2	<1	Severely Impaired
Trial 2	7	4	Mild-Moderately Impaired
Trial 3	10	21	Low Average
Total Recall	19	2	Severely Impaired
Learning	5	79	High Average
Delayed Recall	8	3	Moderately Impaired
Percent Retained	80	6-10%	Low Average
Recognition Hits	6	>16	Average

Recognition False Alarms		0	>16	Average
Recognition Discrimination Index		6	>16	Average

NOTE: Based on the patient's normal learning, percent retention and recognition scores, the consulting neuropsychologist did not feel that her visual memory deficit was clinically significant.

Benton Judgment of Line Orientation Test		26	56	Average
Symbol Digit Modalities Test				
Written		49	28	Average
Oral		58	39	Average
Auditory Naming Test		46	10	Low Average
Wisconsin Card Sorting Test				
Trials Administered		70	…	
Categories Completed		6	>16	Average
Perseverative Responses		4	>99	Very Superior
Perseverative Errors		4	>99	Very Superior
Failures to Maintain Set		0	>16	Average
Delis-Kaplan Executive Function System				
Trail Making Test	13 seconds		85	High Average
Visual Scanning	30 seconds		50	Average
Number Sequencing	21 seconds		74	High Average
Letter Sequencing	82 seconds		37	Average
Number-Letter Sequencing	20 seconds		74	High Average
Motor Speed				
Verbal Fluency Test				
Letter Fluency Total Correct		35	37	Average
Category Fluency Total Correct		34	25	Average
Category Switching: Total Correct		10	10	Low Average
Category Switching: Total Accuracy		8	10	Low Average
Wide Range Achievement Test-4th Edition				
Word Reading		61	53	Average
Sentence Comprehension		44	42	Average
Spelling		42	42	Average
Math Computation		42	39	Average
Conners' Continuous Performance Test-II				All scores within Average Range
Barkley Scales of Attention Deficit Hyperactivity Disorder				

Childhood Symptoms-Self Report			
Inattention	0		Not Significant
Hyperactivity	0		Not Significant
Childhood Symptoms-Other Report (father)			
Inattention	0		Not Significant
Hyperactivity	0		Not Significant
Current Symptoms-Self Report			
Inattention	0		Not Significant
Hyperactivity	0		Not Significant
Current Symptoms-Other Report (father)			
Inattention	0		Not Significant
Hyperactivity	0		Not Significant

Frontal Systems Behavior Scale

Self Rating-Before Injury			
Apathy	25	58	Within Normal Limits
Disinhibition	15	3	Within Normal Limits
Executive Dysfunction	22	14	Within Normal Limits
Total	62	16	Within Normal Limits
Self Rating-After Injury			
Apathy	23	42	Within Normal Limits
Disinhibition	15	3	Within Normal Limits
Executive Dysfunction	22	14	Within Normal Limits
Total	60	14	Within Normal Limits
Family Rating-Before Injury (father)			
Apathy	22	50	Within Normal Limits
Disinhibition	17	7	Within Normal Limits
Executive Dysfunction	30	46	Within Normal Limits
Total	69	27	Within Normal Limits
Family Rating-After Injury (father)			
Apathy	20	34	Within Normal Limits
Disinhibition	17	7	Within Normal Limits
Executive Dysfunction	23	16	Within Normal Limits
Total	60	12	Within Normal Limits

Supplementary References

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