BRAIN INJURY

Local changes in network structure contribute to late communication recovery after severe brain injury

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Spontaneous recovery of brain function after severe brain injury may evolve over a long time period and is likely to involve both structural and functional reorganization of brain networks. We longitudinally tracked the recovery of communication in a patient with severe brain injury using multimodal brain imaging techniques and quantitative behavioral assessments measured at the bedside over a period of 2 years and 9 months (21 months after initial injury). Structural diffusion tensor imaging revealed changes in brain structure across interhemispheric connections and in local brain regions that support language and visuomotor function. These findings correlated with functional brain imaging using functional magnetic resonance imaging and positron emission tomography, which demonstrated increased language network recruitment in response to natural speech stimuli, graded increases in interhemispheric interactions of language-related frontal cortices, and increased cerebral metabolic activity in the language-dominant hemisphere. In addition, electrophysiological studies showed recovery of synchronization of sleep spindling activity. The observed changes suggest a specific mechanism for late recovery of communication after severe brain injury and provide support for the potential of activity-dependent structural and functional remodeling over long time periods.

INTRODUCTION

Recovery of brain function after severe brain injury can evolve over a range of time scales (*1*, *2*). Very late recovery of communication has been reported in patients with severe brain injury (*2*–*4*), but the mechanisms that underpin such recovery are unclear. Here, we longitudinally studied a single subject, who had suffered severe multifocal structural ischemic brain injury. This patient remained behaviorally unresponsive for 21 months until the identification of an intermittent communication channel using vertical movement of the left eye. After this discovery, we tracked the subject for 2 years and 9 months using both quantitative behavioral assessments and multiple independent imaging measurements of brain structure and function to gain insight into the underlying biological mechanisms of late functional recovery of communication after brain injury.

RESULTS

Quantitative behavioral evaluation of a patient with severe brain injury

A 22-year-old right-handed female had sustained extensive brainstem stroke and focal thalamic stroke (bilateral within the anterior intralaminar nuclei) (movie S1). The patient presented with a previous diagnosis of vegetative state, originally determined after the onset of brain injury. At our first clinical evaluation (21 months after the diagnosis of vegetative state), during the first 24 hours of observation by our investigative team, neither standard neurological examination nor quantitative behavioral testing at the bedside using the Coma Recovery Scale—Revised (CRS-R) revealed evidence of behavioral responsiveness. These findings showed consistency with the previous evaluation that led to the original diagnosis of vegetative state. However, the continuing evaluations over the next 48 hours revealed evidence of volitional yet inconsistent movement to commands using the downward movement of the left eye (Fig. 1). This clear demonstration of following commands immediately shifted the patient's diagnosis to minimally conscious state (MCS) (*5*). Follow-up evaluations done as part of this study at ~1-year intervals revealed behavioral and cognitive improvements with reliable but inconsistent left downward eye movements (Fig. 1). Notably, the patient demonstrated use of the eye movement to accurately respond to questions of varying complexity and increasing cognitive demands including evaluation of descriptors and conditional statements. For example, the patient correctly identified objects from a field of two with respect to name or function independently using eye gaze alone. The patient then harnessed these abilities further using assistive technologies to communicate yes/no responses to verbal commands (Fig. 1 and movie S4; see the Supplementary Materials for clinical history and detailed behavioral assessments).

Longitudinal analyses using diffusion tensor imaging

To identify changes in brain structure that accompanied the patient's spontaneous recovery of communication, we prospectively tracked changes in brain structure using diffusion tensor imaging (DTI). We used an omnibus method (*6*), unbiased in terms of brain regions, using standard FMRIB (functional magnetic resonance imaging of the brain) Software Library (FSL) parcellations of high-resolution T1-weighted imaging (for detailed DTI analysis, see "Clinical history and behavioral assessments" in the Supplementary Materials). Figure 2 (A and B) shows the mean fractional anisotropy (FA) across white matter fibers between any two cortical regions in a graphical form, with the colors corresponding to FA values. Quantification of FA values within and across most cortical regions revealed relative preservation of structures at the first evaluation (21 months after injury) and the final evaluation (33 months since the first evaluation; 54 months after injury), but with marked increases in interhemispheric connectivity (Fig. 2, A and B, and movies S2 and S3). Further regional analyses revealed marked increases in connectivity and FA values in the pars triangularis and pars opercularis in the left inferior gyrus, which are two key brain regions involved in the expressive language network (Fig. 2C). Finally, statistical analyses also revealed significant increases in FA ($P < 0.05$, corrected for multiple

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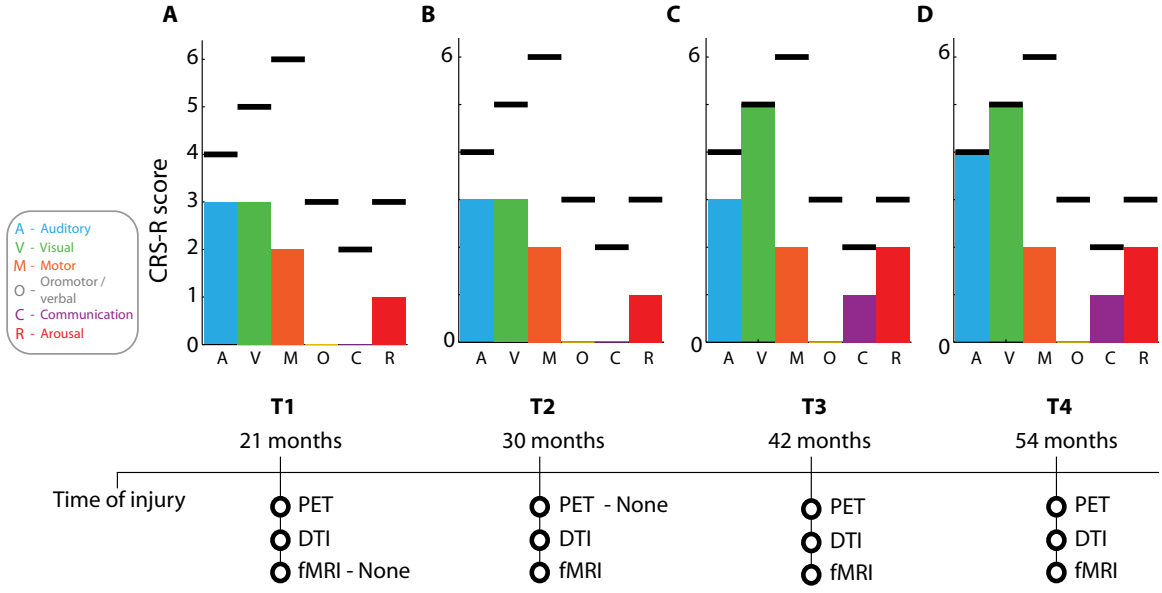

Fig. 1. Quantitative behavioral assessments over time. (**A** to **D**) Maximum scores the subject attained at each time point of evaluation (T1 to T4) using the CRS-R subscales. A, auditory function scale; V, visual function scale; M, motor function scale; O, oromotor/verbal function scale; C, communication scale; R, arousal scale. The maximum possible score on each subscale is denoted by the thick black lines. T1 to T4 indicate the time points starting at 21 months after brain injury at which the three imaging measurements—DTI, fMRI, and ^{18}F-FDG PET—were acquired over the 2-year and 9-month testing period.

comparisons) within the white matter of the right thalamus, left cingulate cortex, right lateral occipital area, and precentral and postcentral regions along both motor cortices from the first to the last evaluation (Fig. 2C and fig. S1).

Blood oxygen level–dependent fMRI of Broca's area during language stimuli

To test for functional activation of Broca's area in response to language, we measured the subject's BOLD (blood oxygen level–dependent) response to language stimuli using fMRI over 3 years of testing beginning at 30 months after the injury. Whole-brain imaging analysis showed statistically significant clusters of activity in Broca's area during the presentation of language stimuli at three consecutive time points (30, 42, and 54 months after injury), with a significantly larger response at the final time point ($P < 0.05$, FDR-corrected) (Fig. 3). We quantified the amount of interhemispheric cross-talk and functional connectivity by analyzing the left and right hemispheric regions activated during the presentation of language stimuli. Clusters of activation identified in Broca's area in the left hemisphere showed no correlation (slightly negative correlation), with language-induced activation identified in the homologous region of the right hemisphere at the first fMRI evaluation, 30 months after injury ($r^2 = 0.083$) (Fig. 3). At the next evaluation (12 months later; 42 months after injury), language-induced clusters of activation appeared to be more positively correlated between the right and left hemispheres ($r^2 = 0.143$). At the final evaluation (12 months after the second evaluation; 54 months after injury), the interhemispheric activation was highly correlated ($r^2 = 0.652$), demonstrating emergence of synchronized interhemispheric activity in response to language stimuli (Fig. 3).

Resting-state brain metabolism measured by ^{18}F-FDG PET

To track possible changes in underlying cerebral metabolic activity, we measured the subject's resting-state brain metabolism using ^{18}F-labeled fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET). At the initial time of evaluation (21 months after injury), ^{18}F-FDG PET imaging demonstrated metabolic down-regulation of the left frontotemporal cortical regions and bilateral superior medial frontal cortices (fig. S2). Repeat ^{18}F-FDG PET imaging at the final time point of evaluation (54 months after injury) revealed a shift in overall pattern of cerebral metabolism. The left frontotemporal cortical regions demonstrated greater metabolic activity compared to the right frontal lateral cortices; increased superior medial frontal cortical metabolic activity appeared to be bilateral but was more prominent in the left hemisphere (fig. S2).

Quantification of sleep spindles using electroencephalography

To track possible changes in sleep architecture over the 2-year and 9-month period, we analyzed serial long-term electroencephalography (EEG) recordings over multiple nights obtained at the first evaluation (21 months after injury) and the last evaluation (33 months after first evaluation; 54 months after injury). We focused on spectral analysis of stage 2 sleep spindling activity, a feature prevalent in normal sleep attributed to an intact corticothalamic circuit. Coherence analysis of the EEG revealed a dip in coherence at the spindling frequency across multiple intra- and interhemispheric electrode pairs at the first time point of evaluation (21 months after injury) (fig. S3). Although the power spectra showed a sharp increase in power at 14.3 Hz across most channels at both time points, a three- to fourfold decrease in coherence was present at ~14 to 14.4 Hz across several Laplacian channel pairs at the first time point. This coherence dip appeared in channel pairs both within and across hemispheres and demonstrated asynchronous interhemispheric spindling activity at the first time point. At the second evaluation (54 months after injury), coherence spectra revealed a marked increase in coherence in the same frequency range, indicating the recovery of inter- and intrahemispheric synchronization of the spindle frequency oscillation (fig. S3, blue). Figure 4 summarizes the longitudinally tracked changes in local function and global connectivity as identified by multimodal brain imaging and electrophysiological studies carried

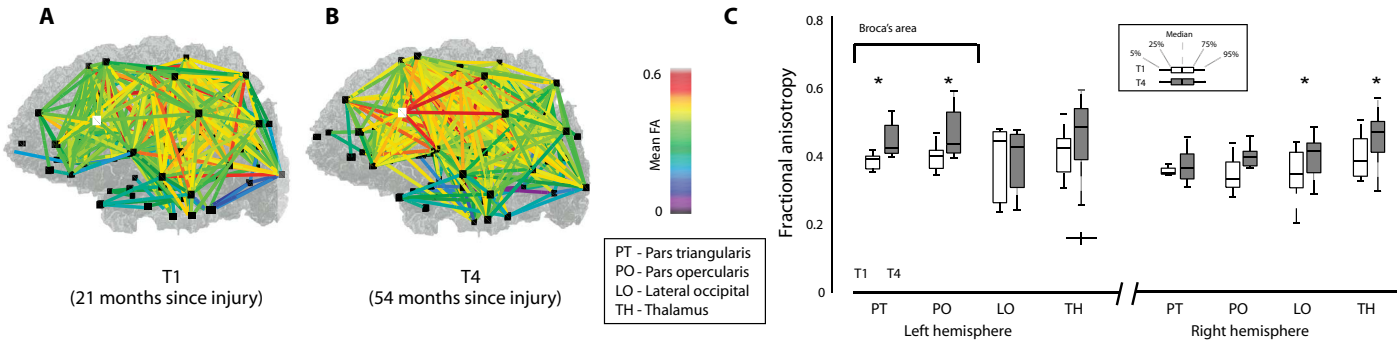

Fig. 2. Longitudinal structural remodeling identified by DTI. FA values measured at the first (**A**) and last (**B**) time point of evaluation are shown as colored lines between the connected brain regions (black squares). The inferior temporal gyrus is denoted with a white square. (**C**) Boxplot showing the distribution of FA values measured within the pars opercularis and pars triangularis, lateral occipital region, and thalamic regions in the left and right hemisphere. Distribution of FA values compared nonparametrically using Kolmogorov-Smirnov test ($P < 0.05$) and corrected for multiple comparisons with the false discovery rate (FDR).

out over 2 years and 9 months as the patient recovered language communication after severe brain injury.

DISCUSSION

Our findings isolate local structural changes in brain structure and function of Broca's area, accompanied by global changes in interhemispheric connectivity, and changes in local structure within the visual cortex in association with this subject's late recovery of communication after severe brain injury. The multiple independent brain functional measurements, taken concurrently over the 2.75-year period after brain injury, all concordantly identified evidence for functional changes within Broca's area supporting the patient's late recovery of a communication system involving the left eye. The local increases in FA in Broca's area and the occipital cortex, and increased metabolism within the left frontotemporal cortices were associated with the patient's observed ability to communicate yes/no responses, albeit inconsistently, using eye movements alone as quantified by the increase in CRS-R score for communication over the course of 2.75 years (Fig. 1 and the Supplementary Materials). Moreover, these findings were functionally linked to the selectively increased activation of BOLD signal within Broca's area in response to passive language stimuli and the increasing correlation of interhemispheric functional activation across language-responsive brain regions. Collectively, these data revealed an underlying late recovery of executive language network function over time.

The convergence of the findings across multiple imaging modalities and behavioral examinations suggested key mechanistic insights into the evolution of the recovery of communication after severe neurological insult. The measured changes in the BOLD response to language stimuli (Fig. 3) showed a precise correspondence to the regionally increased structural connectivity of Broca's area identified over a similar time course (Fig. 2). Furthermore, the increase in interhemispheric connectivity identified in the structural studies was accompanied by the positively correlated interhemispheric activation clusters revealed in the fMRI responses by the final time point of the evaluation. Notably, electrophysiological studies of this patient's sleep state identified recovery of synchronization of sleep spindle frequency oscillations across interhemispheric frontal-parietal cortices by the final time point of evaluation (fig. S3) (*7*). Sleep spindling has been previously proposed to provide a process linking structural reorganization to the normalizing of brain functions disrupted by structural injury (*7*) and stroke (*8*). Synchronization across the hemispheres may both reflect the structural changes measured and reveal an element of the recovery processes specific to the use of Broca's area and the wider frontal lobe components of the language system.

Regional increases in FA values outside of Broca's area further support the role of increased activity and connectivity across the expressive language network in the observed behavioral changes. The specific connections demonstrating the greatest increases in FA values within Broca's area (left caudal medial frontal, precentral region) are consistent with studies of expressive language networks, which indicate that these components of neuronal circuits are bilaterally recruited in expressive speech and may play a role in the broader engagement of the body in speech acts (*9*). Notably, the posterior aspect of Broca's area, in particular the pars opercularis, is known to have precise projections to the supplementary motor area (SMA) and pre-SMA, a network that has been implicated in the initiation and coordination of complex oculomotor movements (*10*). This is consistent with the earlier finding that stimulation of the SMA and pre-SMA can also produce both vocalization and arrest of speech, as well as coordinated movements of the eyes and head (*11*). Moreover, the FA changes in visuomotor regions of the right hemisphere (the right occipital visual areas and right precentral region along the motor cortex) were linked to the volitional movements of the left eye to communicate. This association suggests that the observed increases in FA values likely reflected the recruitment of resources across both hemispheres by the expressive language networks involved in higher executive function in conjunction with control of vertical eye movements. Our subject suffered brainstem and thalamic strokes with no injuries to cortical structures within the language system; thus, the observed local increases in cortical anisotropy over a total of 4 years cannot be attributed to reactive astrocytes and gliosis (*12*). This suggests the possibility of structural modifications directly supporting recovery of language function (*2*).

The marked shift in lateralization of hemispheric metabolic activity observed over the same time course of recovery of communication suggested that reorganization of metabolic activity might be a marker of the process of recovery of communication. This lateralization in metabolic activity from the first to the final time point of evaluation revealed by ^{18}F-FDG PET imaging was consistent with the pattern of structural injury in this patient. Specifically, the metabolic down-regulation of bilateral medial frontal cortices and left frontotemporal cortices at the first time point was consistent with the impact of withdrawal of excitatory projection neurons within the anterior intralaminar thalamic nuclei, primarily the central lateral nuclei (*13*), as a result of the patient's

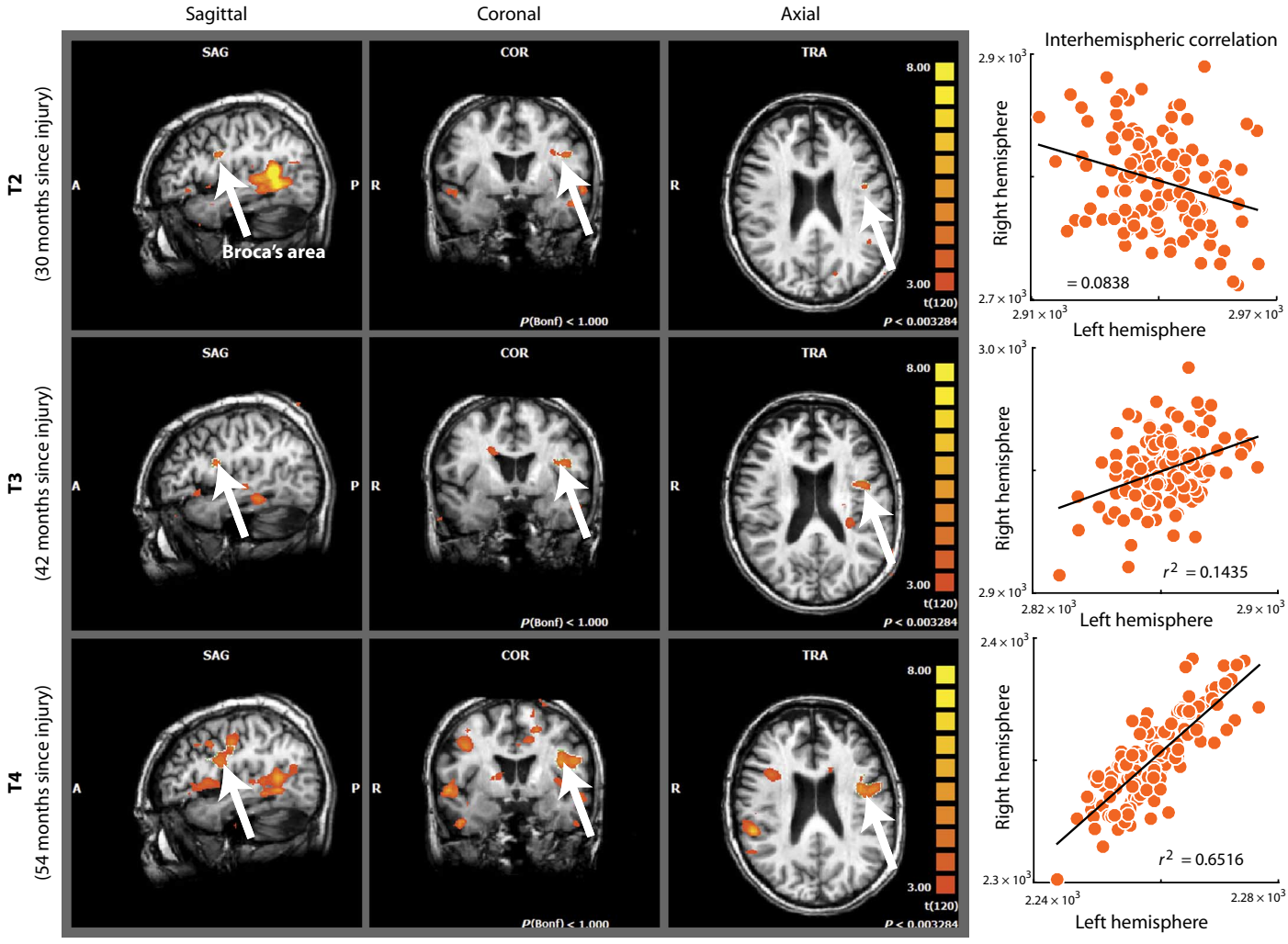

Fig. 3. Changes in fMRI BOLD signals in response to passive language stimuli. From the first time point of evaluation to the last time point (T2 to T4), whole-brain analyses showed graded increases in activation of Broca's area when comparing language stimulation to rest. Scatter plots show correlation of right and left hemisphere cluster activation across the brain during the presentation of passive language stimuli (voxels, 128; maximum cluster radius, 50). The BOLD responses measured by fMRI (modeled by Γ functions) were thresholded using FDR ($P < 0.05$).

discrete structural lesions (fig. S2 and movie S1) (*14*, *15*). Notably, the shift in metabolic lateralization seen in this subject was similar to changes seen in normal brain development. Chiron *et al.* showed that in typically developing children, at the age of 3.5 months, blood flow is dominant in the posterior sensory regions of the right hemisphere and subsequently shifts to the frontal cortex of the left hemisphere after the age of 3 years (*16*). Similar asymmetry in structural changes in the left hemisphere accompanies acquisition of language function in normal development (*17*). Amunts *et al.* found that the dominance of left hemisphere homologous regions of the right hemisphere within the language area (Brodmann's area 45) does not appear until 5 years of age and is delayed in Broca's area 44 until 11 years of age in normal development (*17*). In our subject, late emergence of the structural brain changes after injury revealed by ^{18}F-FDG PET and DTI may reflect a similar use-dependent reorganization in the pattern of resting cerebral metabolism and cortical structure associated with the active use of a communication channel. Notably, at the final time point of evaluation, the marked increase in left-lateralized frontal cortical metabolism correlated with the increase in FA values in pars opercularis and pars triangularis seen in the DTI studies, as well as increased functional activation clusters induced by passive language stimuli as revealed by fMRI.

Having identified these concordant changes in language-related networks, we considered the underlying mechanisms driving late recovery of communication in our subject and the generalizability of these findings for other patients with severe brain injury. The discovery of this subject's communication channel via eye movements on command opened an avenue for iterative engagement by family and caretakers nearly 2 years after the injury. Once this ability was identified, its use resulted in an increase in the patient's responsiveness (Fig. 1). The subject was tested on a range of augmentative communication devices and found to be able to respond to simple yes/no questions via communication cards. It is important to consider that, in this subject, impaired arousal regulation arose directly from bilateral injuries within the central thalamus. These lesions represented the end arterial distribution of the P1 segment of the basilar communicating artery (arising from distal occlusion of the basilar artery) and are correlated in pathological studies with a dominant involvement of the central lateral nuclei of the intralaminar thalamus (*14*). Recovery from isolated and paired injuries

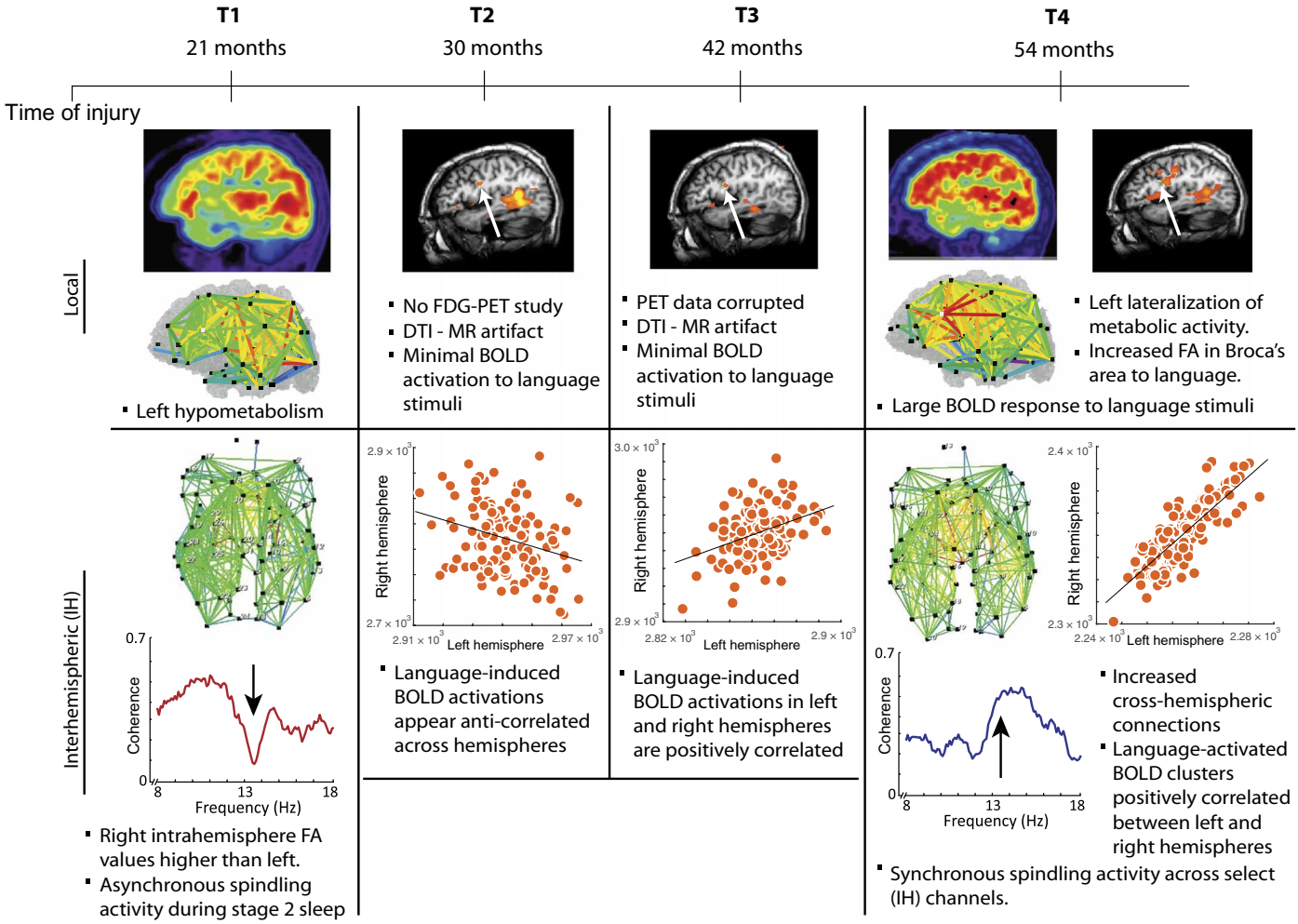

Fig. 4. Summary of the local and global changes in brain structure and function captured longitudinally using multimodal imaging analyses. The time line shows the four time points of evaluation (T1 to T4) since the patient suffered a traumatic brain injury. From the first (at 21 months after injury) to the last (at 54 months after injury) time points of evaluation, structural and functional brain imaging and electrophysiological studies revealed concordant findings in global remodeling and local changes supporting the recovery of language abilities over the 2-year and 9-month period.

restricted to the central thalamus in this arterial territory is variable. In some instances, full recovery is possible over years (*18*, *19*); however, with larger lesions extending caudally into the tegmental brainstem, persistent disorders of consciousness may result without any interruption of the motor pathways (*14*). Fluctuations in arousal regulation are the sine qua non of injuries within the central thalamus (*20*, *21*) even when isolated to one hemisphere (*15*). The critical role of these central thalamic neuronal populations in broadly driving activity across the frontal cortex and striatum has been experimentally verified (*13*). Loss of driving input and denervation of the central thalamus have been proposed to underlie a general down-regulation of the anterior forebrain mesocircuit in all types of severe brain injuries producing disorders of consciousness (*22*); several studies support predictions of this model (*23*–*25*). In our subject, both her behavioral fluctuations in arousal level and the bimesial frontal resting hypometabolism present in both ^{18}F-FDG PET studies directly correlated with her bilateral thalamic injury.

The active use of communication by the subject required the engagement of expressive language regions within the left frontal cortex. The broad recruitment of additional areas participating in language-related networks across each hemisphere is supported by the specificity of the other changes measured including increasing correlation of Broca's area with its homologous region within the right hemisphere during presentation of language, and the changes in interhemispheric synchronization of sleep spindles. Thus, reengagement of expressive language may be able to drive bihemispheric frontal networks and partially compensate for the persistent metabolic down-regulation resulting from loss of central thalamic and brainstem afferents. The specificity of changes in both structural connections and the language-related functional activity measured are consistent with this hypothesis. Graded recovery of anterior mesocircuit function as a result of reengagement of expressive language producing increased arousal regulation within the frontal language–related networks may be a potentially generalizable mechanism for recovery of communication after severe brain injury. The initial novelty of restored contact with the environment combined with iterative engagement could explain the gradual yet consistent process of recovery captured in our study over 2.75 years.

A resulting increase in arousal may support restoration of interhemispheric connectivity and local changes within language networks across the frontal lobes, which are most vulnerable to down-regulation in the setting of multifocal severe brain injury and are selectively reengaged

by expressive language function. In addition to the changes observed in the left language areas, marked increases in FA values were noted in the right visuomotor region (lateral occipital) in the subject, consistent with the behavioral improvements in visual communication, suggesting that such use-dependent recovery could be generalizable across sensory modalities as well. Similar neurophysiological reorganization of cortical areas (motor and somatosensory areas) has been extensively reported in stroke recovery patients (*8*, *26*). Notably, the FA changes within right visual cortex arose in a region deafferented by both direct structural injury and loss of the optic radiation at the level of the right geniculate body (movie S1). Moreover, in our previous report (*2*) of one subject with diffuse axonal injury who had recovered communication using spoken language and motor function, DTI analyses revealed marked increases in FA values in the posterior white matter, corpus callosum, and the midline cerebellar region, further supporting a role for increased structural interhemispheric connectivity in the late recovery of communication.

The quantified changes in the multiple imaging modalities invite further consideration of the biological mechanisms underlying the observed use-dependent alterations in brain structure and function in the injured brain. Use-dependent changes in corticocortical fiber connectivity and white matter plasticity, as demonstrated by intensive piano practice (*27*, *28*), have been linked to increased transcription of genes such as that encoding brain-derived neurotrophic factor (*28*) and recruitment of neuronal progenitors, particularly oligodendrocytes (*29*). Similar experience-induced remodeling has also been proposed to underlie DTI-measured changes in patients after traumatic brain injury (*4*), diffuse axonal injury, and stroke (*8*). Thus, our observations offer support that identifying any window for interaction with the environment may initiate use-dependent ongoing remodeling of structure and function in cells and networks in the severely injured adult brain that are recruitable and may harbor latent capacity.

Finally, it is important to emphasize the diagnostic challenges presented by patients such as the subject of this study. We could identify volitional eye movements to commands identified via CRS-R examination in our patient. However, similar patients may not show behavioral signs but, like our subject, may demonstrate command-following using standard fMRI or electrophysiological (EEG) methods, as previously reported (*30*, *31*). Such patients point to a key challenge in establishing their cognitive level when a wide dissociation of motor and cognitive function is present. Like our subject, such persons are typically diagnostically mislabeled as in a vegetative state, hindering any further attempts to establish a communication channel or to further harness their latent capacities via assistive devices. Adding to the difficulty of establishing such latent capacity is the presence of dysregulated arousal from multifocal brain injuries not present in locked-in state patients who may be the closest group to our patient. The term cognitive motor dissociation has been proposed (*32*) to reflect the combination of relatively well-preserved corticothalamic function measured in cerebral metabolic and electrophysiological studies (*15*) in patients with very limited or no behavioral responsiveness.

Our study had a number of limitations. Patients with cognitive motor dissociation who retain relatively broad preservation of integrative brain function and structural connectivity are more likely to harbor a capacity for both inter- and intrahemispheric plasticity as observed here than most of the patients with disorders of consciousness who do not show this level of cerebral preservation (*24*, *32*). Thus, our findings cannot be generalized to suggest a potential for all patients who demonstrate a vegetative state or MCS behavioral profile to recover a communication system. Although the previous report by Voss *et al.* (*2*) demonstrated similar changes in interhemispheric connectivity after diffuse axonal injury and late recovery of communication to those seen in our patient who sustained ischemic injury, it will be necessary to verify that similar changes in brain structure and function can occur in patients with severe brain injuries due to other causes.

In our subject, the identification of a communication ability initiated her active engagement with the environment and allowed the continued reorganization of brain structure and function. After the final time point of our evaluation, the subject was subsequently fitted with a custom device that enabled her to repeatedly and consistently use her eye movements to respond accurately to computer commands (movie S4). Our findings provide an incentive for therapeutic engagement of this patient population (*33*) and further development of therapeutic and rehabilitation measures (*34*, *35*), including consideration of the role of advanced augmentative devices for patients with severe brain injury (*36*).

MATERIALS AND METHODS

Study design

We performed a prospective longitudinal study of a single patient, who suffered severe brain injury 21 months before the first evaluation. The Institutional Review Board of Weill Cornell Medical College approved all experiments, and informed consent was obtained from the legally authorized representative of the patient. MRI studies that revealed excessive motion artifact were excluded in the longitudinal analyses. Behavioral analysis using the CRS-R examination was performed at each of the 3- to 4-day clinical visits at multiple times throughout the day, including the days of the fMRI, ^{18}F-FDG PET, DTI, and EEG studies. In addition to the CRS-R examination, additional behavioral assessments were performed (see the Supplementary Materials).

Quantitative behavioral assessment using CRS-R

We used the CRS-R for quantitative assessment of behavior at each of the four time points of the study. The CRS-R is a graded assessment scale with large functional differences represented for each single point change of a subscale; in clinical studies, even a one-point change can be statistically significant because of the wide separation of functional requirements of scoring (*5*). See the Supplementary Materials for detailed clinical history and quantification of behavior using CRS-R, and additional behavioral assessments.

DTI analyses

The Connectome Mapping Toolkit (CMTK) was used to perform whole-brain structural DTI analyses. The details of the methods used are described by Gerhard *et al.* (*6*) and will be described briefly here. CMTK is a python-based open-source software that implements a full diffusion MRI processing pipeline, from raw diffusion data to connection maps (www.cmtk.org) (*6*). The magnetization-prepared rapid gradient echo volumes were registered to the b_0 volumes by using a rigid body alignment with the FLIRT tool (FMRIB Linear Image Registration Tool) from the FSL Toolbox (University of Oxford, Oxford, England). With the FreeSurfer package (http://surfer.nmr.mgh.harvard.edu), implemented within the CMTK (*6*), the T1-weighted images were used to parcellate cortical surface representations, segment the gray and white matter regions, and define 32 cortical regions of interest (ROIs) per hemisphere and 7 subcortical ROIs. Because of the nature of the injury and the intact preservation of brain structures in this patient

subject, we could use standard Talairach atlas to identify and segment cortical regions accurately. After the non–brain tissue was removed, and subcortical gray matter structures were segmented as described earlier, the gyral and sulcal structures were subdivided into 16 frontal regions, 5 parietal regions, 4 occipital regions, 7 temporal regions, and 7 subcortical regions (amygdala, hippocampus, pallidum, putamen, caudate, thalamus). The cortical surfaces were further divided into 1000 ROIs with about a 1.5-cm^2 surface area that spanned the entire cortex by using Connectome Mapper Lausanne 2008 parcellation (www.connectomics.org). The diffusion data were motion-corrected, and only data without visible motion (after correction) were included for further longitudinal analysis, thus excluding the data from one time point. Diffusion Toolkit (www.trackvis.org/dtk) was used to reconstruct the tensor data, and the Connectome Mapper was used to perform deterministic streamline fiber-tracking algorithm on the entire white matter by using 32 random seed points per voxel. Fibers shorter than 2 cm and longer than 50 cm were removed, and all remaining fibers were spline-filtered for smoothing. This graphical representation helped to understand the large-scale structural topology of brain and the FA values measured along the reconstructed fibers, and the locations of the cortical and subcortical regions (center of mass) were extracted from the output for further analyses. The FA values measured across cortical and subcortical regions were compared (using nonparametric approaches) locally using a Kolmogorov-Smirnov test, and the resulting *P* values were corrected for multiple comparisons (5% significance level) (FDR-corrected). All statistical analyses were performed with MATLAB R2014b (MathWorks).

BOLD analyses

Data were acquired on a 3.0T General Electric Signa Excite HDx MRI scanner. An axial three-dimensional (3D) inversion recovery fast spoiled gradient recalled (IRFSPGR) sequence (BRAVO) was acquired (acquisition and reconstruction matrix, 256 × 256; slices, 120; thickness, 1.2 mm; field of view, 24 cm; repetition time, 8.864 ms; echo time, 3.524 ms; inversion time, 400 ms; and flip angle, 13°). For detailed acquisition and analysis methodology, see methods by Bardin *et al.* (*30*).

For fMRI analysis, statistical parametric maps were computed using BrainVoyager QX version 1.10.4.1250. Analyses were performed comparing BOLD activation regions during the presentation of language stimuli and rest condition. Time series were corrected for slice scan time differences and for motion, spatially filtered with a 3D Gaussian smoothing kernel, corrected for linear trends, and then co-registered to the 3D anatomical volumes, and these were normalized to Talairach coordinates. Volumes of time series were motion-corrected and co-registered to 3D anatomical volumes normalized to Talairach coordinates. General linear models were used to compute statistical parametric maps, and the motion variables were regressed out. The hemodynamic response functions (modeled by Γ functions) were thresholded using FDR ($P < 0.05$).

^{18}F-labeled FDG PET

Resting brain metabolism was assessed at different time points using ~370 MBq of ^{18}F-FDG during PET acquired on a General Electric Medical Systems combined PET–computed tomography (CT) (Biograph mCT scanner). Standard uptake values were computed in the selected ROIs using PMOD v.3.309 (PMOD Technologies Ltd.) and then scaled to the global mean normalized uptake values according to Fridman *et al.* (*25*). To obtain cortical ROIs, cortical regions were extracted using a standard nonrigid normalization to the Montreal Neurological Institute space and then applied, using an inverse transformation, to the brain-injured subject natural space. The analyses performed here are described in further detail in a previous report from our laboratory by Fridman *et al.* (*25*).

Statistical analyses

For the DTI analyses, FA values measured across the FSL parcellated cortical and subcortical areas were extracted from the graphical output, and the distributions of FA values across time points were compared nonparametrically using the Kolmogorov-Smirnov and Wilcoxon rank sum test, both at 5% significance (boxplot histograms are shown in Fig. 2 and fig. S1). All *P* values from the DTI analyses and the fMRI analyses were corrected for multiple comparisons using FDR. All statistical analyses were performed using custom software written in MATLAB R2014b (MathWorks).

SUPPLEMENTARY MATERIALS

www.sciencetranslationalmedicine.org/cgi/content/full/8/368/368re5/DC1
Clinical history and behavioral assessments
Fig. S1. Whole-brain DTI analyses.
Fig. S2. ^{18}F-FDG PET resting-state metabolic activity.
Fig. S3. Coherence analysis of stage 2 sleep EEG.
Movie S1. FSPGR MRI structural imaging of whole brain.
Movie S2. 3D rendered video of DTI data in graphical form at first evaluation (21 months after injury).
Movie S3. 3D rendered video of DTI data in graphical form at first evaluation (54 months after injury).
Movie S4. Eye movement triggered binary signaling using high-resolution charge-coupled device camera and eye tracking.

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Funding: This work was supported by NIH HD051912 (to N.D.S.), Charles A. Dana Foundation (to N.D.S.), James S. McDonnell Foundation (to N.D.S.), Jerold B. Katz Foundation (to N.D.S.), Weill Cornell Center for Translational Sciences UL1TR000457 (to N.D.S.), and Fred Plum Fellowship in Systems Neurology and Neuroscience (to D.J.T.). **Author contributions:** D.J.T., H.U.V., and N.D.S. conceived the project. H.U.V. and N.D.S. collected the fMRI, DTI, ^{18}F-FDG PET, and EEG data. N.D.S. collected the quantitative behavioral data. D.J.T. and H.U.V. performed the DTI analyses. H.U.V. performed the fMRI analyses. D.J.T. performed the EEG analyses. E.A.F. performed the ^{18}F-FDG PET analyses. D.J.T. and N.D.S. wrote the paper with discussions and comments from H.U.V. and E.A.F. **Competing interests:** N.D.S. is a scientific advisor to Enspire DBS. The other authors declare that they have no competing interests. **Data and materials availability:** Enquiries or requests for the data should be directed to N.D.S.

Submitted 2 March 2016
Resubmitted 12 August 2016
Accepted 17 November 2016
Published 7 December 2016
10.1126/scitranslmed.aaf6113

Citation: D. J. Thengone, H. U. Voss, E. A. Fridman, N. D. Schiff, Local changes in network structure contribute to late communication recovery after severe brain injury. *Sci. Transl. Med.* **8**, 368re5 (2016).

Science Translational Medicine AAAS

Local changes in network structure contribute to late communication recovery after severe brain injury

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Science Translational Medicine **8** (368), 368re5. [doi: 10.1126/scitranslmed.aaf6113]

Editor's Summary

Establishing communication after severe brain injury

Recovery of communication after severe brain injury can occur over long time scales, yet the biological underpinnings of this uncertain process remain unknown. Thengone *et al*. longitudinally captured the 2.75-year evolution of the recovery of communication in a severely brain-injured patient by repeatedly measuring brain structure and function using multimodal imaging. Concordant findings of structural and functional reorganization of expressive language networks in both hemispheres suggested that recovery of communication involved both local changes within the language-dominant hemisphere and increased connections between hemispheres. These findings support a specific mechanism underlying the recovery of communication after severe brain injury.

Science Translational Medicine (print ISSN 1946-6234; online ISSN 1946-6242) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005.