Functional Magnetic Resonance Imaging Technology and Traumatic Brain Injury Rehabilitation: Guidelines for Methodological and Conceptual Pitfalls

Objectives: To illuminate the current methodological and conceptual pitfalls inherent in conducting functional magnetic resonance imaging (fMRI) research with individuals who have sustained traumatic brain injury (TBI) and to discuss appropriate remedies. The aim is describe fMRI research, its limitations, and how to best use this technology to examine TBI. **Discussion:** The topics discussed in this article include issues regarding signal detection, brain activation measurement, head movement, and sources of signal artifact. Issues surrounding data interpretation and the importance of analyzing the brain as a connected neural network is also discussed. Finally, problems with spatial normalization when examining individuals with TBI are reviewed. **Conclusions:** To date, there is a scarcity of research applying fMRI technology to the study of TBI. However, because it is a noninvasive procedure with high availability in hospital settings across the country, the next decade of TBI research will likely include a proliferation of this form of investigation. At this time, much work is needed to better understand how to optimally use this technology to examine the effects of TBI on behavior. For fMRI to enhance TBI research it will be imperative to establish valid research protocols and reliable methods of data interpretation. Key words: *fMRI*, *methodology*, *rehabilitation*, *TBI*

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TRAUMATIC BRAIN INJURY

In the United States traumatic brain injury (TBI) accounts for more than 70,000 new cases of disability each year,[1] and the financial costs of injury have been estimated at $21 billion per year.[1] By definition, individuals sustaining significant TBI commonly experience impairments in the areas of physical, cognitive, and psychosocial functioning.[2] As a result, a large number of individuals with TBI endure life-long impairment and disability.

Functional neuroimaging techniques continue to provide researchers with important opportunities to study the anatomy and pathophysiology of brain dysfunction after TBI. In the field of TBI rehabilitation, for example, functional magnetic resonance imaging (fMRI) provides researchers with an

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alternative method to examine brain changes, evaluate patient outcomes, and validate treatment interventions.[3] Both clinical researchers[4] and the National Institutes of Health[2] have emphasized that advanced neuroimaging techniques, such as fMRI, will be important for assessing outcomes and the success of novel brain injury rehabilitation treatments and interventions. Importantly, however, the application of fMRI to TBI remains in its infancy, and much work is to be done before these future possibilities can be realized. For fMRI to be useful clinically, it will be important to devise established protocols and to guarantee accurate and consistent interpretation of the resultant data.

Advanced neuroimaging techniques such as fMRI hold the promise of greater integrating what is known about behavioral and brain changes after TBI. It is anticipated that fMRI will receive wider application in clinical studies primarily because of the accessibility of MR technology, its noninvasiveness, and its low cost compared with positron emission tomography.[3] Therefore, the aim of this article is to provide an overview of the practical issues facing researchers conducting fMRI research and to offer solutions to potential problems that may arise when applying fMRI technology to the study of TBI.

BLOOD OXYGEN LEVEL–DEPENDENT MR

The mechanisms of MRI rely on the nuclei of certain atoms (normally 1 H) absorbing and then re-emitting radio waves when in a magnetic field. The frequency of the absorbed and emitted waves depends on the strength of the magnetic field, so by varying the strength of the field over the head it, is possible to record waves of different frequencies from different brain regions.

The MR signal is largely determined by "observing" the behavior of the body's water protons when in the presence of a strong magnetic field (typically referred to as the B_0 field). The strength of the magnetic field is measured in Tesla (T) (a unit of magnetic field strength, 1T is equal to 10,000 Gauss), and, at 1.5 T, roughly .0005% of the body's water protons align with the B_0 field. Through the application of a radio frequency (RF) pulse at an angle perpendicular to the B_0 field, the influence of the B_0 field is temporarily disrupted, resulting in a misalignment of the protons with the large magnetic field and a period of "excitation" of the protons. After the application of this RF pulse, the protons will eventually return to alignment with the B_0 field, by precessing about the axis of the B_0 field, moving closer with each precession. During this precession, the protons emanate a signal that is detectable by the MR scanner; Fourier transform methods are used to reconstruct images from these emanating signals. Depending on the properties of the surrounding tissue, the protons will process at different rates, so not only does MRI produce a map of the density of 1 H atoms, but it also provides information about the environment in which the atoms are found. For example, the signal will vary depending on whether it emanates from protons in white matter, gray matter, or cerebrospinal fluid.

Currently, the index of neuronal activity most commonly used for fMRI is the blood oxygenation level dependent (BOLD) contrast.[5] The signal in BOLD fMRI is based on the same principles as traditional fMRI (i.e., B_0 field, proton precession), but the very fast imaging sequence used in BOLD fMRI (echo planar images or EPI) is sensitive to blood-based properties. The assumption in BOLD fMRI is that an increase in neuronal activity within a brain region results in an increase in local blood flow, leading to reduced concentrations of deoxyhemoglobin (a product of oxygen consumption) in nearby vessels. Compared with oxyhemoglobin, deoxyhemoglobin has a differential magnetic susceptibility (or magnetic property) in relation to

the surrounding tissue. Therefore, relative decreases in deoxyhemoglobin concentration lead to a reduction in local inhomogeneity in the B_0 field and a slower decay of the MR signal, resulting in higher intensities in the images. For a more detailed review of MR physics see Ref. 6; for a review of fMRI (as well as other functional imaging techniques) see Ref. 7.

In sum, BOLD fMRI detects secondary effects of neuronal firing, or changes in blood flow and oxygen consumption, or the *hemodynamic response*, allowing researchers to indirectly measure neuronal responses to task demands. Figure 1 illustrates the nature of the hemodynamic response from the point of stimulus onset and subsequent neuronal firing to the inflow of oxygenated blood, resulting in an increase in the BOLD signal and, finally, the decline in the BOLD response. This illustration shows the lag time between the initiation of neuronal firing and the maximum hemodynamic response. Also illustrated in Figure 1 are the "pre-undershoot" and "post-undershoot" responses, which remain only inconsistently elicited in fMRI studies. There has been some indication that the "undershoot" is related to oxygen consumption resulting in increased signal heterogeneity and signal reduction,[7a] although this notion has not been universally accepted.[7b] Thus, it is important for investigators to keep in mind that the BOLD signal is based on a consistent, but secondary, physiological response that is mediated by multiple factors, including blood volume, blood flow, orientation of vessels within the slice, and the metabolic demand of the tissue oxygenated.

The fast acquisition time of fMRI allows whole brain images to be collected in about

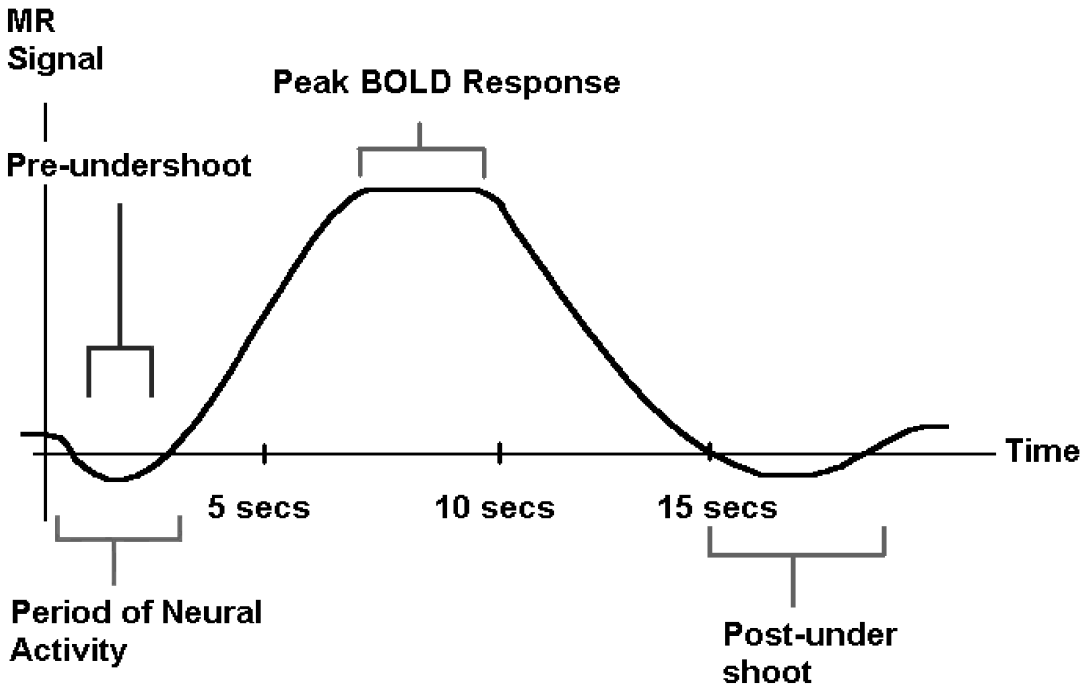

Fig 1. The nature of the hemodynamic response. The y-axis represents the BOLD signal, and the x-axis represents time. Note the significant lag time between the onset of neuronal firing and the peak BOLD response.

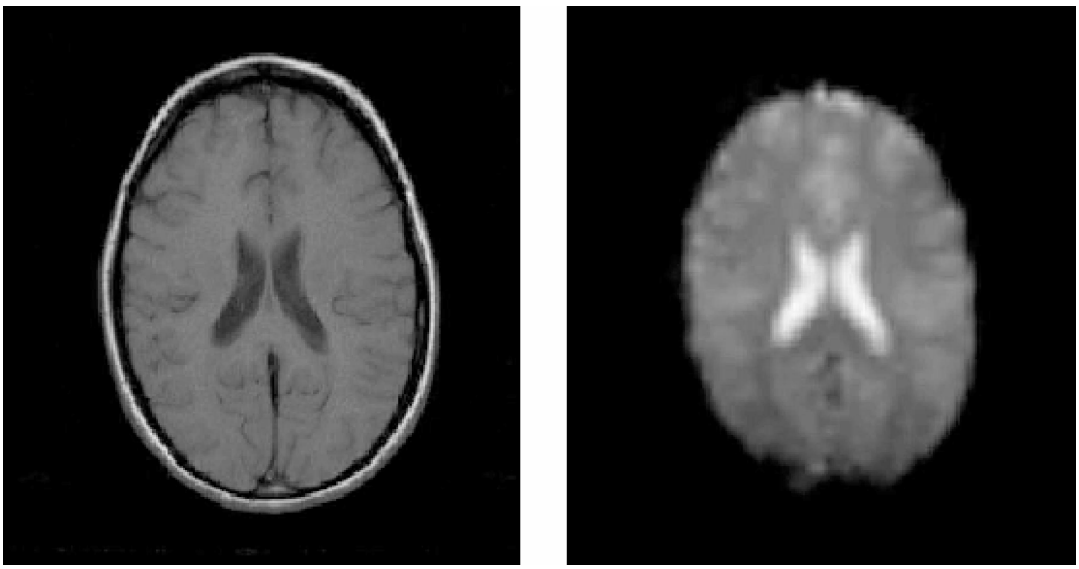

Fig 2. Left is high-resolution T1-weighted image with cervical spinal fluid appearing black. Right is a low-resolution T2-weighted image with cerebral spinal fluid appearing white.

6 seconds, meaning that hundreds of fMRI volumes are often collected for each subject. However, because of this increased acquisition speed, the examiner forfeits the fine degree of spatial resolution evident in structural MR. Figure 2 illustrates the qualitative differences between a traditional structural T1 and an EPI taken from the same healthy adult.

Determining brain activation

Most brain tissue receives greater oxygen than is metabolically necessary for functioning, resulting in an oversupply of oxyhemoglobin to the active area. In large part, the BOLD signal is based on the relationship between this "oversupply" of oxyhemoglobin and the deoxyhemoglobin residing in the area of synaptic activity.[5] It should be noted that both the magnitude of blood flow and oxygen consumption are different across brain regions. For example, the hippocampus has the largest oxygen extraction fraction compared with any other brain substrate and, therefore, uses the most oxyhemoglobin.[8] Therefore, because of differences related to flow and metabolism between brain structures, signal intensity may vary across brain regions irrespective of the degree of neuronal activity.

If the "signal" from the BOLD response is to be detected, it must be greater than the background "noise." That is, the signal of interest must be isolated from the remainder of the cerebral activity and blood flow (certainly the entire brain will be consuming oxygen and not just the area driven by the investigator's paradigm). In fMRI data sets, this noise comes from such sources as cardiac rate, which increases blood flow and volume; the respiration rate, which increases oxygenation levels; and the imperfections of the system's instrumentation.[9] Thus, the BOLD signal associated with the paradigm can only be detected if the signal-to-noise ratio (SNR) is sufficient, and this ratio may vary between studies.[10]

The basis for detecting brain activation from visual, motor, or cognitive stimuli with fMRI lies in making thousands of comparisons between each brain voxel (a single cubic volume). The basic premise of fMRI research is that blood flow, volume, and oxygenation levels change in response to the neuronal firing associated with some cognitive, motor, or sensory event.[5] To determine which brain areas are active in relationship to the presented stimulus, multiple *t* tests or *F* tests, based on the assumptions of the general linear model, are generally used requiring voxel-by-voxel comparisons throughout the brain.[11] Because of the very high number of statistical comparisons made (each activation map consists of roughly 15,000 voxels and therefore, comparisons[12]), a conservative threshold for statistical significance that has accounted for multiple comparisons must be adhered to reduce the potential for false-positive findings.[11]

An alternate method for determining brain activation is through the use of cross-correlation analysis.[13] An activation map based on cross-correlation analysis is achieved by measuring activation in all of the voxels of the brain and by comparing a time course of each voxel's signal intensity to an idealized function of both the task (or time period of stimulus presentation) and the rest periods or baseline (period of no stimulation, but active recording of BOLD signal). Those voxels with a correlation coefficient above a predetermined level (e.g., $r = .40$) are considered "active." Thus, the resulting activation map contains voxels that are consistently "active" and "inactive" with the rise and fall of the reference waveform, and, for a block design, the investigator would choose a function that approximates the paradigms "on" and "off" periods, such as a cosine waveform.[13] An additional advantage to cross-correlation analysis is that the functional connectivity between adjacent (or remote) brain regions may be inferred by observing the synchrony in the fluctuations across regions.[14]

APPLICATION OF fMRI TO TBI

To date, there are only a handful of studies using fMRI to examine individuals with TBI. For example, McAllister et al[15,16] conducted separate investigations examining working memory in individuals sustaining mild TBI, whereas Christodoulou et al[17] examined individuals diagnosed with moderate and severe TBI. Taken together, these investigations document significant alterations at the level of the cerebral substrate in individuals with TBI and represent an important first step in applying fMRI technology to examine brain changes after TBI. There are currently several impediments for investigators attempting to reliably examine the subtypes of TBI and the various stages of recovery. First, in acute cases of TBI, there have been no systematic examination of the effects of collecting or loose blood, such as subarachnoid hemorrhage or subdural hematoma, or increased intracranial pressures among factors that may alter hemodynamic response measured by fMRI. Second, there has been no systematic investigation of the effects of brain lesions after TBI on the preprocessing of fMRI data. Finally, the relationship between the location of the injury and the nature of the pathophysiology, such as diffuse axonal injury, and observable changes the brain activation measured by fMRI remain unknown.

GUIDELINES FOR CONDUCTING fMRI RESEARCH

The following discusses specific problems associated with conducting fMRI research with specific attention given to methodological and conceptual problems born from conducting fMRI research with individuals with TBI. Among the issues we aim to address

include measurement and interpretation of brain activation, how to handle "lesioned brains," and issues related to image acquisition, such as head movement and study design. Therefore, the following will focus on the general pitfalls and potential solutions associated with fMRI research and how they affect the examination of TBI.

Head movement

Head movement is the prototypical confounding factor to be addressed by fMRI researchers. This is primarily because the fMRI signal changes caused by the hemodynamic response are small compared with apparent signal differences that result from head movement. Some head movement will not be obvious (such as when a participant sneezes in the scanner) and cannot be completely eliminated, so retrospective motion correction is imperative during preprocessing of imaging data. Typically, brain activation related to gross motion artifact is most evident on the brain surface and at the interface between parenchyma and the cerebrospinal fluid.[18] Movements in fMRI data are assumed to be "rigid-body," that is, the shape and position of internal structures remain preserved (unlike a nonrigid or gelatinous body). Because of this, the alignment algorithm works by estimating a set of translations and rotations for each scan and then spatially transforming the images according to these parameters. Even after motion correction, however, there are many sources of residual motion-related artifact. Because of this, it is possible to use models based on previous and current motion estimations to detect false brain activations, and one example of this is autoregressive moving average (ARMA).[19]

Motion correction is especially important for experiments in which subjects may move in the scanner in a way that is correlated with the different experiment conditions.[20] That is, even tiny systematic differences can result in significant signal accumulation over numerous scans. For example, if a study participant moved consistently with each stimulus presentation or at the time of each response, artifacts arising from subject movement correlated with the experimental paradigm may appear as brain activation.

Motion correction is important not only for eliminating spurious activation but also to increase sensitivity, because *t* tests are often conducted to determine the areas of brain activation, and these analyses are based on the signal change in each voxel relative to the residual signal variance.[21] Movement artifacts add to the residual variance, which, in turn, reduces the sensitivity of the statistical test to detect true activations.

During the fMRI experiment, it may be helpful to track patient movement and to examine the data to determine whether any positional changes occurred during the session. Individuals who have sustained brain injury may have poor attention or they may be disinhibited, which may increase the propensity to alter their positioning while in the scanner. In particular, providing head cushioning around the head and/or taping the head are safe and effective methods to diminish inadvertent head movement. Some examiners use a "bite bar" to guarantee stabilization of the participant's head, although this procedure may not be well tolerated in certain clinical samples. Also, shorter experimental times may reduce the propensity for participant movement,[22] and, in our laboratory, more frequent "check-in" periods have been useful when examining someone with a brain injury. Because head positioning is so critical, and participants with TBI may be unable to cooperate for extended periods of time, it may also be useful to reposition the head between functional imaging runs and conduct a new localizer scan (structural imaging scan that provides the

investigator with information about head positioning) at the time of each adjustment.

The problem of measuring "brain" and not "vein"

Measurement of the hemodynamic response is based on several factors, including the ratio of oxyhemoglobin to deoxyhemoglobin and blood volume.[5,23] During any given cognitive, motor, or sensory protocol, the investigator should expect a 2% to 3% signal change (at 1.5 T) associated with the behavioral task.[24] Signal change, however, is dependent on the type of vasculature of the region from which the signal emanates. The gradient echo (GE) sequence (one method for measuring the precession of protons) detects large vessel effects in addition to changes in the hemodynamic response in the capillary beds that irrigate the gray matter tissue, yet only the latter is likely to be directly related to the paradigm. Investigators have determined that, when using a GE sequence, the signal change associated with the macrovasculature lining the cortical sulci was at 4.3%, and the signal change associated with even larger bridging veins jumped to 7.3%.[24] This finding is important to consider, because the venous system and, in particular, collecting veins at the surface of the cortex may allow for "pooling" of venous blood several millimeters from the primary site of cortical activity.[25] There are two potential problems with measuring the activation in the macrovasculature and bridging veins. First, very large signal changes secondary to collections of venous blood will mislead the examiner regarding the magnitude of the brain activation directly driven by the experimental protocol.[26] Second, determining the neuroanatomical structure(s) associated with the observed "activation" may be very difficult, if not impossible, because the signal may emanate from a vein and, therefore, cannot be linked to any particular brain structure (or worse, the observed "activation" is erroneously related to the brain structure[s] proximal to the large vessel responsible for the signal). This problem can result in dubious patterns of activation once data are analyzed.

This issue of measuring "vein" and not "brain" is less problematic when a spin echo (SE) sequence is used because of its primary sensitivity to the microvasculature (i.e., capillaries). The tradeoff for using the SE sequence in place of the GE sequence is significant, however, because the SNR is decreased dramatically (the average signal change with SE is about one half that of the GE sequence).[24] This brings about a fundamental problem facing behavioral researchers in fMRI. If the GE sequence is used, the resultant maps could potentially include false areas of activation and poor localization of results. However, if the SE sequence is used, then the power is reduced, and failure to detect potentially relevant and important changes in brain activation may result.

There are several solutions to this problem. First, in cases in which a large SNR is necessary to detect paradigm-driven changes, the examiner may need to use the GE and track the "percent signal change." Because brain activation related to capillary functioning should result in a signal change from 2% to 4%, investigators can eliminate signal change that is much higher than 4%.[24] Also, activation from the brain parenchyma has been observed at 4.4 seconds after stimulus presentation, whereas activation secondary to the macrovasculature was not apparent until 6.6 seconds after the stimulus presentation.[24] Once again, Figure 1 illustrates that the peak BOLD response is not apparent until 6 to 10 seconds after neuronal firing. Thus, by monitoring the timing and magnitude of the signal change, investigators can make determinations whether or not activation was secondary to capillary response and, therefore, more

likely to be related to the experimental protocol. This procedure allows for a conservative measurement of signal changes based on the GE sequence.

Second, if the examiner is less concerned about localizing the specific anatomical structure associated with brain activation, conservative estimates of the signal produced by the GE sequence may be adequate. For example, if the investigator is studying working memory performance after TBI and plans to analyze the total activation in the "left frontal lobe" or "the right hemisphere," the need to determine the exact location of brain activation is less critical. However, if the examiner is analyzing the role of very small anatomical structures in the paradigm (e.g., comparing activation in anterior and posterior portions of the parahippocampal gyrus), the GE sequence may not be interpretable for this level of specificity.

A more obvious solution is to use the SE sequence; however, as noted, the SE sequence may not provide the investigator with the optimal SNR. An alternative to both the SE and GE sequences is to use multiple GE sequences. When using multiple GE sequences, each provides its own signal, so that the contributions of small, medium, and large vessels may be determined.[27]

An alternative to traditional BOLD fMRI that may address issues surrounding the measurement of large unrelated veins is perfusion fMRI. Investigators using this technique use "arterial spin labeling," which is the application of an RF pulse at the level of the internal carotid, so that a portion of the blood flow can be "labeled" and measured during cerebral perfusion (for a comparison of BOLD and perfusion fMRI see Ref. 28). Perfusion fMRI and can be used with an SE sequence, and because it measures the arterial system, the signal is based on early hemodynamic changes (i.e., at the time of the pre-undershoot). Moreover, examiners using perfusion fMRI can effectively eliminate problems associated with artificial inflation of the BOLD signal because of the size or orientation of the venous system.[28]

In sum, to reliably determine the magnitude and location of brain activations, investigators of TBI should consider a method that accounts for the "brain versus vein" problem. It has been emphasized that, for the potential of fMRI to be maximized in clinical populations, the various contributions to the fMRI signal change must be discerned.[29]

SUSCEPTIBILITY ARTIFACT

The most common site for brain damage after TBI is in the frontal lobes and anterior temporal lobes.[30] The frontal lobes, of course, are critical for a variety of functions (including integration of sensory input, memory, planning, and taking action)[31] and are therefore of much interest when using fMRI to examine behavior after TBI. Several of the studies to date applying fMRI to TBI have primarily examined the frontal systems.[15–17] Moreover, there is a particular emphasis in brain injury rehabilitation to help individuals compensate for frontal lobe dysfunction,[32] making examination of the frontal systems with fMRI important for the evaluation of clinical interventions.

In fMRI, there is significant signal dropout or distortion (i.e., voxel shifting) in areas where there is an interface between brain tissue and air, such as the anterior portions of the frontal lobes. This signal loss is commonly referred to as susceptibility artifact.[33–35] As noted, "susceptibility" refers to the magnetic properties of a tissue, and there are a range of susceptibilities across brain compositions. Air, from sinus cavities, has no susceptibility, or no magnetic properties, and therefore, emits no signal within the scanner. Susceptibility artifact may cause signal loss and/or distortion when attempting to image the temporal lobes or frontal lobes, and more precisely, the anterior most portions of the frontal

lobes adjacent to the sinuses.[34] In some cases, the EPI sequence may lack imaging data for these regions altogether, making it impossible to study the relationship between brain areas typically of interest in TBI research (e.g., orbitofrontal cortex) and the cognitive challenge presented. Moreover, the influence of intracranial air after TBI, such as that introduced with skull fracture, on the BOLD signal is not known.

For TBI investigators interested in examining the effects of orbitofrontal or inferior temporal lobe damage on cognitive functioning, it will be important to make determinations about the potential effect of susceptibility artifact on the resultant data. Although adjustments are typically study specific, there are some general rules that reduce susceptibility artifact. First, susceptibility artifact may be reduced when scanning is performed axially. Second, some examiners maintain that fine-tuned shimming (refinement of B_0 field homogeneity with correction coils) near the source of interference eliminates some of this artifact by maximizing the homogeneity of the MR signal.[35] Other investigators have minimized susceptibility artifact by segmenting, or dividing up and capturing individually, signals from the SE sequence[36] and by using multiple excitations[37] or a "refocusing echo."[38] However, multiple excitations have been shown to reduce temporal resolution.[39] More recently, investigators have examined a form of single-shot EPI as a method to recover signal loss associated with susceptibility artifact.[39] To examine the frontal lobe during recovery from TBI, the issue of susceptibility artifact must be addressed. For a more elaborate review of this issue and potential design solutions, see Song.[39]

EXAMINING THE BRAIN AS A NETWORK

Some investigators have expressed concern that, in many ways, fMRI is the latest scientific phrenology[40] or, in some ways, has reoriented cognitive neuroscience toward theories of localization of function.[41] This issue is particularly relevant when investigators are interested in linking the responsibility of a brain function to a specific neuroanatomical structure. As fMRI garners greater application in the study of TBI, it will be important for researchers to continue to conceptualize the brain as a parallel processor with distributed networks (this is especially critical when examining secondary and tertiary cortices). The observation that a particular brain structure is associated with a specified function likely indicates that the neuroanatomical structure is necessary for a function, but its activation alone is likely not sufficient. Although there is a lure to conclude that the "blobs" on the activation map are responsible for the observed behavioral output, each area of activation should be conceptualized as having only a relationship to or role within a specific cerebral function.[42,43]

The interpretation of fMRI images has also been characterized as a phrenological science because of the preponderance of region of interest (ROI) analyses. ROI analyses allow investigators to test a priori hypotheses regarding the relationship between a specified brain area and a behavior. However, the importance, or role, of any anatomical structure in a particular brain function is difficult to determine in ROI analysis, because the timing of this activation and its relationship to the remainder of the neural network (which is potentially wide reaching) cannot be assessed. One brain region may be subservient to multiple brain regions. In TBI rehabilitation, the benefits of fMRI research are likely to be related to the information it provides about adaptation to injury through the expression of preexisting or development of new alternate neural networks.[3] Although ROIs will continue to be included in analyses, they should not be performed at the expense of

studying the interrelationship between neural systems.

Structural equation modeling, or path analysis, is a regression-based analysis that allows for the relationship between areas of activation to be assessed.[43] Path analysis is based on the premise that the brain is composed of multiple, integrated, and inter-reliant networks acting in concert for motor, sensory, and/or cognitive output. Based on this assumption, brain activations cannot be entirely independent of one another, and a measure of their covariance through models of multiple linear regression allows the examiner to observe the relationship between areas of activation.[42,43] This relationship is not only determined in magnitude but the direction of activation is examined, allowing for determinations about the sequence of activations in a neural network.

In the study of TBI, statistical approaches measuring covariance (e.g., path analysis, partial least squares) allows examiners to test hypotheses about the effect of TBI on the functioning of neural networks. Furthermore, using path analysis to examine the brain as an integrated system will provide researchers with information about how the patterns and the timing of brain activation are altered after injury.

STUDY DESIGN

There are two primary methods for analyzing brain activations: block designs and event-related designs. In block designs, the stimulus paradigm alternates between periods of experimental and control tasks and areas of activation represent the average brain activation over some predetermined period of time. Block paradigms have predominated clinical studies, because they are typically shorter in duration than event-related paradigms and, therefore, more easily tolerated by patients. Block designs are relatively simple to implement and provide a good SNR for behaviors that can be averaged over long blocks of time (e.g., 32 seconds); however, there are shortcomings to the traditional block design. If, for example, a brain region is active for only a short period of time to initiate, or terminate, some process, it may not seem active in a block paradigm that averages the fMRI signal over several minutes.[12] An additional drawback is that block design paradigms were originally developed for positron emission tomography and do not maximize the superior temporal resolution offered by fMRI.[44]

Event-related paradigms allow for brain activation associated with short periods of time (e.g., single responses) to be extracted from the hemodynamic response.[45,46] Event-related paradigms also allow for the timing of activations within a neural network model to be assessed, and through the use of "overlapping responses," investigators can pull out specific activations associated with stimulus presentations separated by only a few seconds.[47] Because of this, event-related paradigms are particularly useful when measuring brain activations associated with cognition and for activation periods that may be very short in nature. Moreover, event-related designs allow investigators to compare the activations associated with the quality of response (e.g., correct, incorrect).[48] In the study of the memory and attentional systems after TBI, this comparatively fine level of analysis of brain function may prove very useful.

MAKING SENSE OUT OF BRAIN ACTIVATION

To accurately interpret activation patterns, several factors that influence brain activation should be considered. Specific methodological manipulations may be necessary to account for such factors when studying brain changes over time in individuals with TBI.

Task load

There is growing evidence that task load, or the degree of task demand placed on the study participant, may affect the magnitude and extent of brain activation.[49] For example, in healthy adults, investigators have noted increased right hemisphere activation during working memory tasks (that typically demand left frontal lobe resources) as the task load was increased.[49,50]

The role of task load on brain activation in healthy adults is important to consider when examining individuals with TBI. For example, investigators have noted increased activation on a working memory task in individuals with TBI compared with healthy adults.[15,17] However, if the investigator has not controlled for the effect of task load on brain activation, explaining the differences between groups may be difficult. In other words, the pattern of activation observed in individuals with TBI may be a departure from that of healthy adults because of brain reorganization or quite simply, it could be because the task is more difficult for individuals with a brain injury. In the case of the latter, these alterations in brain activation may be explained by increased demand, and presumably task difficulty, for individuals with TBI, resulting in a "normal" recruitment of resources. This interaction between task load and brain activation is important to ascertain when interpreting the differences in fMRI results between individuals with TBI and healthy adults.

In sum, investigators interested in determining the cause for differences in activation between healthy adults and individuals with TBI may first have to control for the differential effects of task load between groups. One method for doing this is by titrating the task, so that all participants (both healthy adults and individuals with TBI) maintain the same behavioral output (e.g., all participants achieve a 75% accuracy rate). By doing so, the examiner can be certain that the residual activation differences between an individual with TBI and a healthy adult are not related to difference in task load (and difficulty) between groups.

Stimulus novelty

There is also evidence in the functional imaging literature that the novelty of the stimuli presented in the paradigm can influence the degree and location of brain activation.[51,52] For example, during investigations of working memory, repeated exposure to task demands and stimuli reduces the activation as the participants become more efficient with the task.[53] This issue will be important to consider when investigators of TBI are using serial fMRI scans over the course of the recovery period to track changes in the brain substrate associated with therapies. Investigators using this method must account for the natural changes in brain activation associated with increased task efficiency and acclimation to the paradigm that occurs with repeated trials.

Emotional status

Dysphoric mood is common after TBI,[54] and patterns of brain activation in functional neuroimaging studies have been shown to be affected by emotional states, such as sadness,[55,56] anxiety,[57] or pain.[58] As such, changes in the magnitude of the BOLD signal thought to have been elicited by cognitive, sensory, or motor challenges may be, at least in part, attributable to the emotional state of the individual. In fact, investigators have determined that brain regions commonly implicated in cognitive processes, including the amygdala,[59] anterior cingulate,[60] and frontal lobe regions,[61] are also involved in emotional processing. To increase the probability that the magnitude of the BOLD signal can be attributed to cognitive, sensory, or motor challenges, emotional states need to be assessed behaviorally before image acquisition.

Although the presence of elevated scores on test instruments (e.g., Beck Depression Inventory) alone is not proof that brain activations have been affected by mood or emotional processing, behavioral findings should be taken into account during the statistical analysis of the imaging data. In our laboratory, the relationship between symptoms of depression or anxiety are accounted for through the use of post-hoc correlational analyses between scores on behavioral measures and the observed pattern of brain activation.

GROUP DATA

There has been some debate in the fMRI literature regarding the use and interpretation of "group data" or the generation of a single activation map to represent the data generated by a group of participants. Concern about the reliability of group data originated in studies of healthy adults, in which researchers noted significant anatomical and functional variability across individuals.[62] Although it has not been established, this problem could be even more pronounced in cases of TBI because of the wide variability in anatomical and functional organization after injury.

For imaging analysis software packages such as SPM99,[63] group data are generally achieved through fixed effects analysis or random effects analysis. Fixed effects analysis provides the examiner with a group image based on the activations that are present "on the average" across subjects. The problem with this form of analysis with small groups (such as those typically observed in fMRI studies) is obvious: one very large activation in one subject can artificially drive the group activation map. Random effects analysis, however, is based on determining the areas of activation that are present in much the same way across all participants in the group. Each of these methods may provide different activation maps for the same group data. In clinical fMRI studies, random effects analysis is typically preferred because, although conservative, the group activation maps are generally a good representation of the commonalties in activation between subjects.

For a balanced statistical design, a random effects analysis can be achieved by applying simple statistical tests to summary images derived from each subject in the study. The tests are therefore based on intersubject error variance estimates, allowing the results to be generalized to the population from which the subjects were drawn. Sample size is also important to consider, and it has been recommended when using random effects analysis that each group include at least 10 participants.[11]

Because there is such great variation in the brain activations associated with many cognitive tasks, it will be important to guard against drawing conclusions about "pathological" activation in persons with TBI. For example, in any "group brain" representative of a number of healthy adults, activation related to the experimental paradigm that is evident in only one or two individuals may be eliminated. Thus, the "group brain" rendered during analysis does not include the activation occurring in all individuals, and because of this, even a healthy control participant may exhibit brain activation that is not present in the group representative. Therefore, when considering the activation patterns of individuals with TBI, it is necessary to determine if the location and magnitude of the activation represents some departure from what was elicited from your paradigm across healthy adults and not just the group representative.

fMRI AND LESIONED BRAINS

Much of the current software available for image analysis was developed to handle image processing in healthy adults (e.g., SPM99, Human Brain Atlas). Because of this, it is

important to consider the effect of applying these standardized procedures to lesioned brains.[64] This issue is particularly important in studies with persons with TBI. Specifically, caution is recommended during the "preprocessing" steps of image analysis, with specific attention given to coregistration and normalization.

Coregistration

For studies of a single subject, sites of activation can be accurately localized by superimposing the results of the low-resolution functional imaging data (EPI) on a high-resolution structural image of the subject (typically a T1-weighted MRI). This requires coregistration (or overlaying) of the functional images on the structural image. Although there is some difficulty in coregistering the low-resolution EPI with the high-resolution T1, the advanced algorithms (e.g., nonlinear transforms, information theoretic objective functions) available in imaging software are able to accommodate this registration.[65] Even so, because of the differences in resolution between the EPI and the T1, the results of this match should serve as a guide and may not indicate the exact locations of activation. In cases of individuals with TBI, an advantage to coregistration is that the lesions present on the structural scan will remain intact during this coregistration, so that the relationship between brain activation and lesion location may be observed.

Normalization and lesioned brains

To make comparisons between participants and between groups of participants, it is necessary to "warp" or translate images from several subjects into roughly the same standardized brain space (a template image). This procedure is known as spatial normalization and typically occurs after coregistration of the functional and structural images. An advantage of using spatially normalized images is that activation sites can be reported according to their Euclidean coordinates within a standard space.[66] The most commonly adopted coordinate system within the brain imaging community is that described by Talairach and Tournoux,[67] although new standards are now emerging that are based on digital atlases.[68–70]

Matching an image of a normal healthy brain to a template derived from normal healthy subjects is a relatively straightforward procedure. For proper normalization, most imaging software uses "linear" and "nonlinear" transformations. Simple linear transformation methods that estimate translations, rotations, zooms, and shears have fewer problems with brain lesions than methods that use multiple parameters to describe the relative shapes of brains. As seen in Figure 3, separate linear transformations operate to make adjustments in three-dimensional space (the x, y, and z planes), and these transforms may be less affected by brain lesions.

However, most fMRI data processing software, such as SPM99, uses nonlinear transformations in conjunction with linear transformations to "warp" the source brain into standardized space.[71] Unlike linear transformations, nonlinear transformations are very sensitive to differences in image intensity between the source and template. Many of these methods work by optimizing a cost function that is related to the mean squared difference in signal intensity between a source image and a template image that defines the standard space.[72] In cases in which the source brain has areas of abnormal signal intensity (i.e., brain lesion), the algorithm will work to match the source and template brains at the lesion site at the expense of the remainder of the brain. Therefore, the presence of a lesion in an image (such as that observed after TBI) makes the matching more difficult for nonlinear transformations, because there is no longer a simple relationship between the image intensities. Is the surrounding healthy

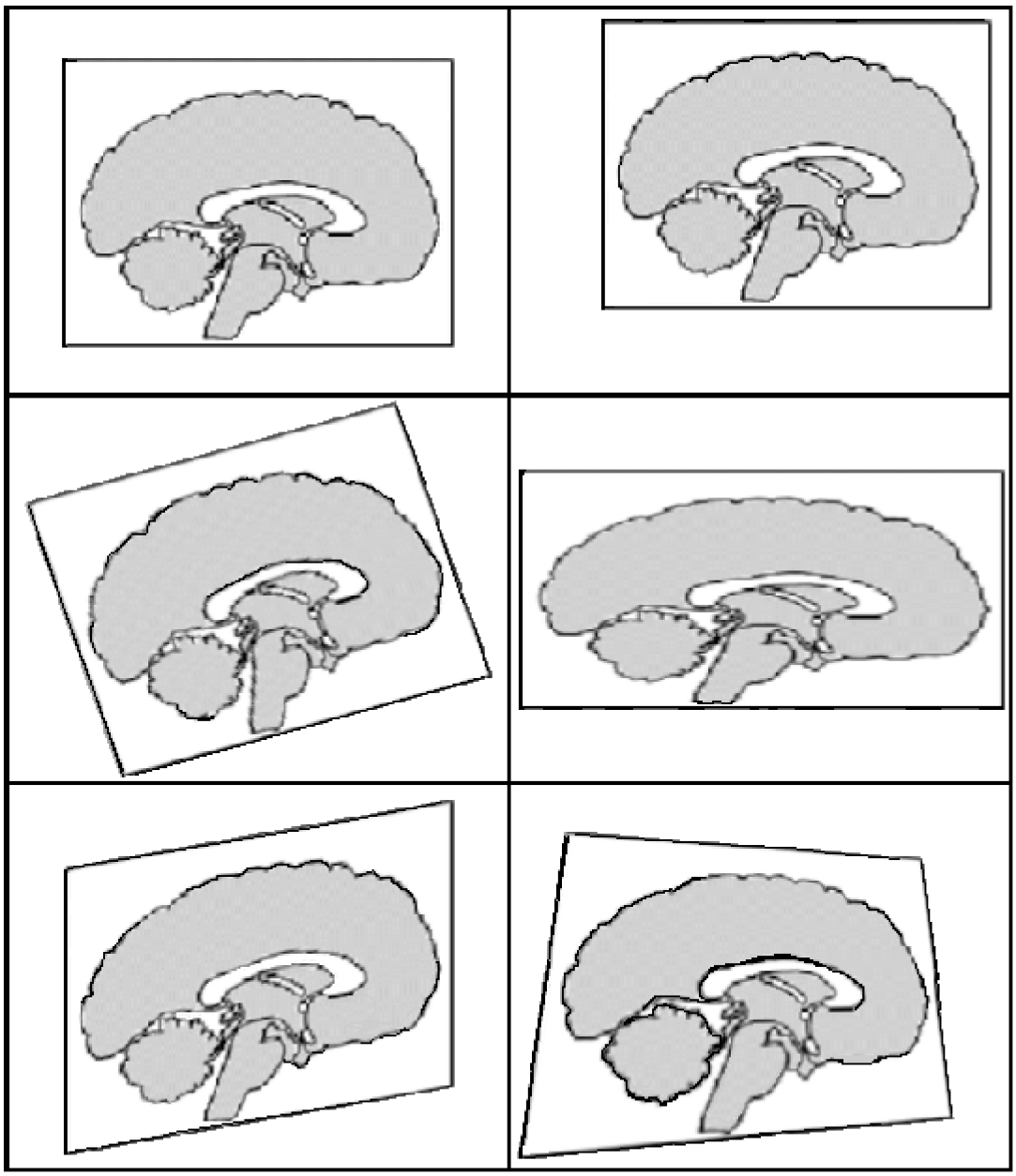

Fig 3. The methods of linear transformation for spatial normalization, depicting five linear transforms: first, the original image (upper left), the second picture (upper right) is translated, the third (second row left) is rotated, the fourth picture (second row right) is zoomed, the fifth picture (bottom left) is sheared, and the sixth picture (bottom right) is perspective projection. Rotations and translations are rigid-body transformations, and zooms and shears are affine transformations (because parallel lines remain parallel). A perspective projection is not affine, but it is occasionally used for intersubject registration (e.g., in the software AIR). *Source*: Reprinted with permission from Christopher Rorden, School of Psychology, University of Nottingham, UK.

Table 1. Changes in magnitude and location of brain activation based on normalization process

Cluster size	X	Y	Z	T value	Brain area
Result using SPM99 default normalization settings					
1118	28	−24	50	14.64	Right frontal lobe—precentral gyrus white matter
	26	−26	60	12.89	Right frontal lobe—precentral gyrus
2131	−20	−56	−30	10.40	Left cerebellum—anterior lobe
	−36	−50	−34	6.72	Left cerebellum—posterior lobe, tonsil
	−26	−78	−10	6.69	Left cerebrum—occipital lobe lingual gyrus
81	36	30	26	5.58	Right frontal lobe—middle frontal white matter
Result using affine (linear) transforms only					
1108	0	−24	52	15.06	Right frontal lobe—precentral gyrus white matter
	26	−28	60	13.03	Right parietal lobe—postcentral gyrus
2393	−20	−58	−32	10.24	Left cerebellum—posterior lobe, tonsil
	−34	−54	−38	6.79	Left cerebellum—posterior lobe, tonsil
	−26	−78	−12	6.72	Left cerebrum—occipital lobe fusiform gyrus
81	38	30	28	5.67	Right frontal lobe—middle frontal white matter

tissue displaced by the lesion, or is it replaced by it? Is the difference in pathological tissue, such as gliotic tissue, and normal tissue affecting the normalization algorithm? Table 1 compares the results of activation after using two normalization techniques for the structural image shown in Figure 4. Table 1 reveals that the exact location of activation for the five highest z values in the activation map is at least partially determined by the normalization method used. For example, the second cluster listed in the table is located either in the frontal lobe or parietal lobe (i.e., precentral or postcentral gyrus) depending on the normalization method used. Differences are also noted in the locations of the third (anterior versus posterior cerebellum) and the fifth (lingual versus fusiform gyrus) areas of activation. In this example, the significance level and cluster size were also affected by the normalization procedure. Thus, the use of separate normalization procedures in the presence of a large frontal lesion may alter the magnitude (size of voxel cluster) and the location of brain activation.

Only recently have investigators begun to develop software that compensates for the abnormal brains that are often the study of clinical investigations. For example, in an analysis of Alzheimer's disease, investigators developed software to handle the difficulties in normalization caused by studying "atrophied brains."[73] These investigators determined that ventricular enlargement commonly observed in Alzheimer's disease[73] was a source of error during normalization, creating a mismatch at the corners of the ventricles between the source and the template. With some success, these investigators developed an alternative software algorithm (NEUROSTAT) for normalization of PET images that may reduce the distorting effects of ventricular enlargement.[73] In cases of TBI, however, there often exist both focal and diffuse brain changes,[32] thus increasing the probability of a mismatch between the source and template during spatial normalization. This also creates problems for software designed to accommodate diffuse brain changes and not changes in local intensities such as those observed after TBI.

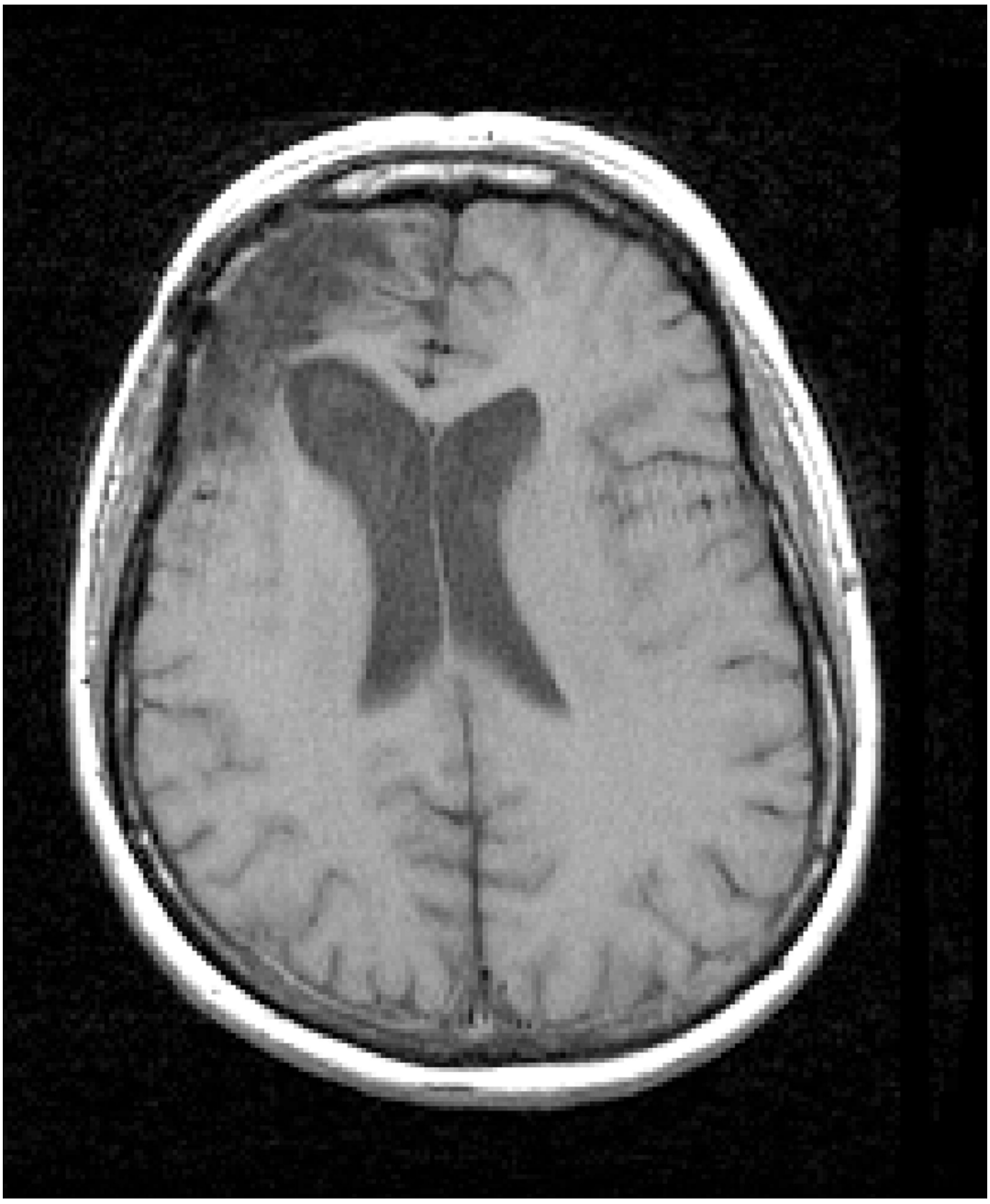

Fig 4. T1 image of an individual with an old right frontal lesion (in radiological convention) and resulting encephalomalacia and ventricular enlargement.

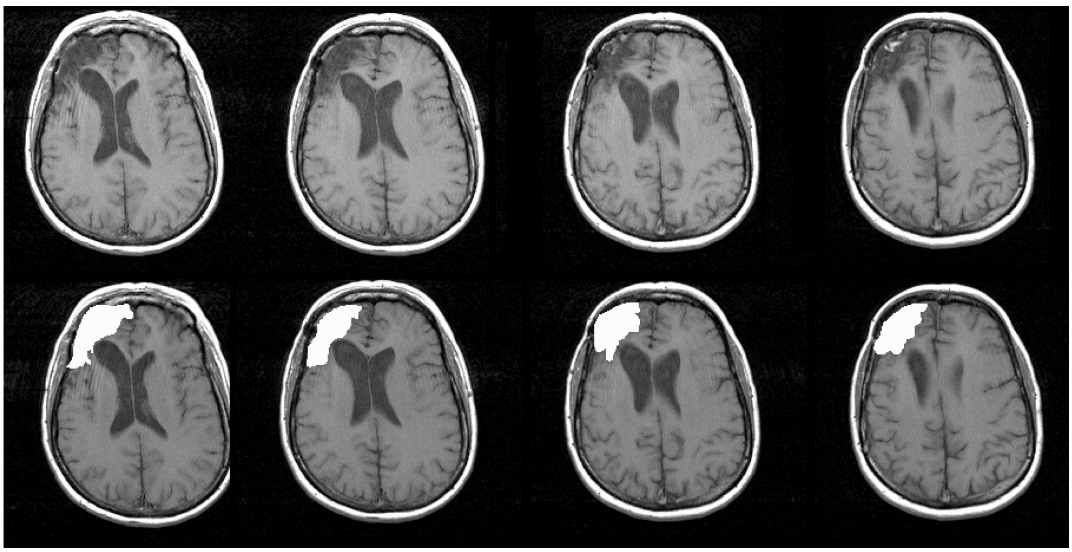

Fig 5. Top panel provides four axial slices of the image provided in Figure 4, and the bottom panel illustrates the lesion masking across slices performed with MRIcro (Rorden and Brett[71]). The nonlinear transform used during spatial normalization will not include the "masked" area when warping the source brain into standardized space.

An alternative method of handling lesioned brains involves lesion masking.[72] What this entails is "masking out," or assigning a zero value to, the areas of lesion for every slice of the patient's T1-weighted image. This process can be performed by loading the T1-weighted (or other structural image to be normalized) image into a program such as MRIcro,[71] which allows for slice-by-slice recognition and masking of the lesion(s). Figure 5 illustrates four slices of a patient's brain with right frontal (in radiological convention) encephalomalacia and ventricular enlargement in the top panel and the lesion masking across four slices in the bottom panel. By masking the lesion, the algorithm in SPM99s nonlinear transform will not attempt to match signal intensity between the source and template images at the lesion site. This masking technique allows for reasonable normalization of lesioned brains, in turn leading to relatively unbiased comparisons of brain activation between individuals with TBI and healthy individuals. This will be important to consider when examining individuals with large brain lesions, and the investigator will need to consider some methodological adjustment in the normalization procedure used (e.g., linear transform only, lesion masking) to minimize the effects of brain lesions on the location and magnitude of brain activation. It should be noted that in cases of large bilateral lesions, lesion masking may not be effective (both hemispheric representatives of a structure will be assigned a zero value, and the morphology of the structure will be unknown), and normalization may be very difficult if not impossible. For these reasons, examination of TBI with fMRI will require a thorough understanding of the imaging data and a comprehensive understanding of the procedures responsible for the resulting images to be interpreted.

CONCLUSIONS

fMRI has the potential to dramatically influence the nature of TBI research. In TBI rehabilitation, fMRI provides researchers with an important opportunity to examine the changes at the level of the cerebral substrate that coincide with behavioral changes that have been linked to therapeutic intervention. However, much work is needed to better understand how to optimally use this technology to examine the effects of TBI on cognition and behavior. Although fMRI has already been used to evaluate clinical interventions after stroke, for example, no such applications have occurred in TBI. Because of this, before fMRI is to be clinically useful in TBI rehabilitation, it will be important to establish reliable and valid research protocols and consistent approaches to data interpretation.

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