Magnetic Resonance Imaging in Experimental Traumatic Brain Injury

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Abstract

Traumatic brain injury (TBI) is a leading cause of death and disability in the USA. Common causes of TBI include falls, violence, injuries from wars, and vehicular and sporting accidents. The initial direct mechanical damage in TBI is followed by progressive secondary injuries such as brain swelling, perturbed cerebral blood flow (CBF), abnormal cerebrovascular reactivity (CR), metabolic dysfunction, blood–brain-barrier disruption, inflammation, oxidative stress, and excitotoxicity, among others. Magnetic resonance imaging (MRI) offers the means to noninvasively probe many of these secondary injuries. MRI has been used to image anatomical, physiological, and functional changes associated with TBI in a longitudinal manner. This chapter describes controlled cortical impact (CCI) TBI surgical procedures, a few common MRI protocols used in TBI imaging, and, finally, image analysis pertaining to experimental TBI imaging in rats.

Key words MRI, Traumatic brain injury, ADC, CBF, DWI, PWI, BBB, Experimental TBI model, Rodents, fMRI

1 Introduction

Traumatic brain injury (TBI) is a leading cause of death and disability, affecting 3.5 million American civilians, with an annual cost exceeding $60 billion [1]. In addition, more than 270,000 US service members have been diagnosed with TBI since the beginning of the global war on terrorism (http://dvbic.dcoe.mil). Common causes of TBI include falls, violence, injuries from wars, and vehicular and sporting accidents. The initial direct mechanical damage in TBI is followed by progressive secondary damage such as brain swelling, perturbed cerebral blood flow (CBF), abnormal cerebrovascular reactivity (CR), metabolic dysfunction, blood–brain-barrier disruption, inflammation, oxidative stress, and excitotoxicity, among others [2, 3]. Ischemic-like events (such as membrane depolarization, ion dysregulation, oxidative stress, excitotoxicity, and inflammation, among others) subsequently lead to apoptotic and necrotic cell death. The multidimensional cascades...
of secondary brain injury in TBI offer many potential targets for therapeutic intervention.

Magnetic resonance imaging (MRI) offers the means to non-invasively probe many of these secondary injuries. MRI has been used to image anatomical, physiological, and functional changes associated with TBI in a longitudinal manner [4, 5]. $T_1$ and $T_2$ anatomical MRI allows for visualization of gross structural damage, possible hemorrhage, and vasogenic edema in TBI [6, 7]. Diffusion-weighted imaging, in which contrast is based on the apparent diffusion coefficient (ADC) of water, can detect ischemic brain injury within minutes. ADC reduction is often attributed to cytotoxic edema, a common feature of TBI [8–10]. Diffusion tensor imaging (DTI) provides both magnitude and directional information about water diffusion and thus has been used to detect changes in fiber tracts following TBI [11]. Fractional anisotropy (FA), an index of anisotropy of water motion from DTI measurements, is sensitive to white-matter injury, microstructural changes [12, 13], and diffuse axonal injury [13–17] in TBI.

Disruptions in CBF and CR could be the result of either direct mechanical injury to vessels or indirect reduction of local perfusion pressure by elevated intracranial pressure. CBF can be measured using an exogenous intravascular contrast agent or by magnetically labeling the endogenous water in blood [18, 19]. The former is efficient, but it is incompatible with dynamic CBF fMRI as the long half-life of the contrast agent allows only one CBF measurement per bolus injection. Arterial spin labeling (ASL) techniques, on the other hand, are totally noninvasive, and the labeled water has a short half-life (~blood $T_1$), making it possible to perform multiple repeated measurements that can be used to augment spatial resolution and/or signal-to-noise ratio.

Blood–brain-barrier (BBB) disruption is common following TBI [20, 21]. Traditional histological Evans Blue extravasation has been widely used to measure BBB leakage following TBI [20, 22, 23]. However, this method requires the sacrifice of the animals and does not allow for longitudinal assessments. Dynamic contrast-enhanced (DCE) MRI can longitudinally measure the transport coefficient $K_{\text{trans}}$ which reflects BBB permeability [24].

Rodent models have been widely utilized to study TBI; they include the controlled cortical impact (CCI), fluid percussion, acceleration-impact or weight drop, Marmarou, Feeney, and blast injury models [25, 26]. The common areas of impact included somatosensory/motor, auditory, parietal, and visual cortices. Outcomes and lesion sizes are highly variable due to the use of different experimental models and/or different injury parameters [25, 26].

In this chapter, we describe the controlled cortical impact (CCI) TBI surgery procedures, a few common MRI protocols used in TBI imaging, and, finally, image analysis pertaining to experimental TBI imaging in rats.
2 Materials

2.1 TBI Modeling

1. Rats (200–250 g) (Taconic Farms, Hudson, NY; Charles River, Wilmington, MA).
2. Anesthetics (isoflurane or pentobarbital, etc.) (VetOne, MWI, Boise, ID).
3. Common surgical tools and supplies (Fine Science Tools, Foster City, CA; Integra Miltex, Plainsboro, NJ; World Precision Instruments, Sarasota, FL; Ethicon, Somerville, NJ).
4. Pneumatic controlled cortical impactor (Precision Systems and Instrumentation, Fairfax Station, VA).
5. Stereotaxic frame equipped with tooth and ear bars (myNeurolab.com, St. Louis, MO).
7. Antibiotic ointment (McKesson, Richmond, VA).
8. Analgesic (Buprenex) (Henry Schein, Melville, NY).
9. Warm pad, temperature feedback monitoring, and other monitoring equipment to ensure normal animal physiology (Fisher Scientific, Pittsburgh, PA; Cole-Palmer, Vernon Hills, IL).
10. Cresyl violet acetate for Nissl staining (Sigma, St. Louis, MO).

2.2 MRI

1. Bruker 7 T scanner (Billerica, MA).
2. 40-G/cm BGA12 gradient insert (ID = 12 cm, 120 μs rise time).
3. Animal holder (custom-made).
4. Custom-made RF transmitter and receiver coils for brain imaging.
5. Custom-made RF transmitter coil for arterial spin labeling.
6. Actively decoupled switch box to detune RF coils.
7. Other magnet, gradient, and RF coil configurations should also work.

2.3 Peripheral MRI-Compatible Monitor Equipment and Animal Supports

1. Oximetry (heart rate, arterial oxygen saturation) (Mouse Ox, STARR Life Sciences, Oakmont, PA).
2. Blood pressure (invasive with artery catheterization) (Biopac/Acknowledge, Goleta, CA).
3. Respiration rate via force transducer (Biopac/Acknowledge, Goleta, CA).
4. Forepaw stimulation device—home-made device or Grass stimulators.
5. Circulating warm water bath (Haake water bath, Rheology Solutions, Bacchus Marsh, Victoria, Australia; Cole Palmer, Vernon Hills, IL).
6. Temperature feedback regulator (Digi-Sense, Cole-Palmer, Vernon Hills, IL).

7. Anesthetic delivery, such as vaporizer (Universal Vaporizer Support, Foster City, CA).

3 Methods

3.1 TBI Surgery

[24, 27–32]

1. Male rats (250–350 g) are anesthetized with isoflurane (~2%). Other anesthetics can also be used. Male rats are often used to avoid the effects of female hormones on outcome. Female rats are also widely studied; some female hormones have been found to have neuroprotective effects.

2. Aseptic preparations (Betadine and ethanol washes) should be performed to prevent infection and immunological responses that could affect outcome.

3. The animal is secured in a stereotaxic frame with ear and tooth bars; an incision is made at the level of the cerebellum as posterior from the impact site as possible to prevent artifacts during MRI acquisition. The periosteum is removed over the impact site. A Ø5mm craniotomy is created over the left S1FL (+0.25 mm anterior and 3.5 mm lateral to bregma), exposing the dura matter. The intact dura matter is impacted using a pneumatic-controlled cortical impactor (Precision Systems and Instrumentation, LLC, Fairfax Station, VA) fitted with a Ø3mm tip (5.0 m/s, 250 μs dwell time, 1 mm depth) to produce a mild focal TBI. Following the impact, the cranial opening is sealed with bone wax, the scalp sutured closed, and antibiotic ointment applied. Saline is injected under the skin to facilitate the removal of air pockets between the scalp and the skull, thus minimizing artifacts during MRI acquisition (see Note 1). Buprenex (0.05 mg/kg) is given subcutaneously every 12 h for 3 days as needed for pain relief.

4. The right femoral artery is catheterized for blood-gas sampling, continuous blood pressure, and heart-rate monitoring. These physiological parameters are important, because deviations could affect TBI outcome causing an increase in statistical scatters.

5. Rats are secured in a supine position on an MR-compatible rat stereotaxic headset, and anesthesia is reduced to ~1.1% isoflurane. Rats breathe spontaneously. Mechanical ventilation can also be used. Rectal temperature should be maintained at 37.0 ± 0.5 °C. It is strongly suggested that heart rate, respiration rate, mean arterial blood pressure, and oxygen saturation (from oximetry) are monitored. Blood gas should be sampled once during a break between imaging scans. All recorded physiological parameters are within normal physiological ranges. MRI is acquired on the day of the TBI procedure.
(1–3 h post-TBI), and again on days 1, 2, 7, and 14 after TBI onset.

6. Rectal temperature is maintained at 36.5–37.5 °C and respirations are recorded throughout the study. Body core temperature is critical, because it could affect the outcome.

7. Nissl staining is used to measure morphological changes within and around the impact following TBI [27, 33]. Briefly, anesthetized rats are perfused on day 14 post-TBI with ice-cold heparinized phosphate-buffered saline, followed by ice-cold 4% buffered paraformaldehyde (PFA). Brains are removed and post-fixed with 4% PFA and subsequently cryopreserved (30% sucrose) for 48 h at 4 °C. Coronal sections measuring 25-μm are affixed to gelatin-coated slides and dried overnight at 37 °C. Slides are hydrated through a series of graded alcohols to distilled water, followed by 0.1% cresyl violet acetate for 7 min. Brain sections are then dehydrated, cleared in Histo-clear, xylene and cover-slipped with Permount mounting medium. Images are acquired on an Olympus BX60F microscope equipped with an Olympus DP70 camera. A 5× objective is utilized to acquire images for mosaic full brain images assimilated MRI using Microsoft ICE software. Intact neurons are defined as nonbasophilic neurons with pale nuclei and discrete nucleoli and that had an intact neuronal body. Dark-stained neurons are defined as neurons with abnormal morphology, such as hyperbasophilic neurons and neurons with shrunken morphology.

3.2 MRI

1. **Position of RF coil**: Position the RF coil as central to the region of interest as possible. For a surface coil, avoid pressing the coil too hard on the animal’s head as it would increase “loading,” which decreases SNR.

2. **Tune and match RF coil**: Tune and match RF coil by adjusting the capacitors to 1 H resonance frequency and 50Ω.

3. **Position scan**: Position the scan on x, y, and z to ensure that the subject is centered. Open up the FOV if needed.

4. **Shimming**: Run autoshim or manual shim as needed.

5. **Calibrate RF pulses**: Calibrate RF pulses for given pulse shapes and durations. This can be set up to be done automatically.

6. **Pilot scan**: Perform a pilot scan, using a 2D gradient echo FLASH or RARE sequence (10–30 s). Based on the pilot scan, plan 5–8 1.0 mm coronal slices to cover the region of interest.

7. **T2 map (9.5 min)**: T2-weighted images are acquired using a fast spin-echo sequence with four effective echo times (18, 54, 90, and 126 ms), and four echo train lengths, where the center of the echo train is taken as the effective TE for T2 calculation. Typical parameters are spectral width 30–50 kHz, TR=2–3 s (90° flip angle), pulse shape Gaussian or Sinc3, and pulse duration 1–2 ms.
8. **Diffusion tensor-weighted MRI (3.5 min):** DTIs are obtained with five $b=0$ and 30 directions with a 1200 s/mm$^2$ $b_{\text{max}}$-value. Echo planar imaging (EPI) scans with partial Fourier (3/4) are also acquired using the following settings: seven 1.0 mm coronal images, field of view (FOV) = 2.56×2.56 cm, matrix 96×96 and reconstructed to 128×128, single shot, repetition time (TR) = 3 s, echo time (TE) = 32 msec, separate between diffusion gradient $\Delta = 14$ ms, diffusion gradient duration $\delta = 5$ ms, and 2 transients for signal averaging.

9. **CBF:** There are two methods to measure CBF, namely continuous arterial spin labeling (cASL) or dynamic susceptibility contrast (DSC) MRI with Magnevist (Gd-DTPA) or Omiscan (another contrast agent). With the latter, measurement can only be made once every hour or so because intravascular half-life of MRI is on the order of 6 min. In TBI, the contrast agent is often trapped; a longer wait time may be necessary.

   For the cASL technique, single-shot, gradient-echo, and echo-planar-imaging (EPI) acquisition is used. Paired images are acquired alternately—one with arterial spin labeling and the other without (control). MR parameters are as follows: data matrix = seven 1.0 mm coronal images, field of view (FOV) = 2.56×2.56 cm, matrix 96×96 and reconstructed to 128×128, single shot, repetition time (TR) = 3 s, and echo time (TE) = 10.2 msec. Continuous arterial spin labeling employed a 2.7-s square radiofrequency pulse to the labeling coil in the presence of 1.0 G/cm gradient along the flow direction, such that the condition of adiabatic inversion is satisfied. The sign of the frequency offset is switched for control (non-labeled) images. Number of averages is typically 60–100 depending on the SNR needed.

   Parameters for the DSC technique are single-shot, gradient-echo, echo-planar-imaging (EPI) acquisition with matrix = 64×64, FOV = 2.56 cm×2.56 cm, 3–5 slices of 1.0 mm, TE = 20 ms, and TR = 0.333 s (22° flip angle). Preload the IV line with 0.15–0.2 ml of Magnevist or Omiscan (typically 3 ft long of PE-50 tubing will hold such volume). About 20 s into the acquisition, deliver the contrast agent in a single bolus flush of saline. Continue DSC acquisition for another 40 s. Note that if DSC is used, Gd-DTPA has a non-negligible intravascular half-life. cASL and fMRI studies cannot be done immediately after Gd-DTPA injection (see **Note 2**).

10. **fMRI:** Combined CBF and BOLD measurements are made using the continuous arterial spin-labeling technique with single-shot, gradient-echo, and echo-planar-imaging (EPI) acquisition. Paired images are acquired alternately—one with arterial spin labeling and the other without (control). MR parameters are as follows: seven 1.0 mm coronal images, field
of view (FOV) = 2.56 \times 2.56 \text{ cm}, matrix 96 \times 96 and reconstructed to 128 \times 128, single shot, repetition time (TR) = 3 \text{ s}, and echo time (TE) = 10.2 \text{ ms}. Continuous arterial spin labeling employed a 2.7-s square radiofrequency pulse to the labeling coil in the presence of 1.0 \text{ G/cm} gradient along the flow direction, such that the condition of adiabatic inversion is satisfied. The sign of the frequency offset is switched for control (non-labeled) images. For each set of fMRI measurements, 4 min of baseline data acquisition, 3 min of data acquisition during hypercapnic challenge, and 3 min of data acquisition during the post-stimulation surveillance period. Hypercapnic challenges use a premixed gas of 5% CO$_2$ with 21% O$_2$ and balance N$_2$.

11. $K_{trans}$-MRI (19.4 min): $K_{trans}$ MRI data are acquired using a 2D multi-slice FLASH sequence. A prescan module is used to determine the flip angle and $M_0$ distribution, which includes 3 FLASH scans with different TRs: 64 ms (scan 1), 200 ms (scan 2), and 3000 ms (scan 3). The rest of imaging parameters are as follows: five 1.0 mm coronal slices, TE = 2 ms, FOV = 2.56 \times 2.56 \text{ cm}^2, 128 \times 128 data matrix, and 30° nominal flip angle. The prescan takes 7.4 min. Dynamic scans use a TR of 64 ms, and otherwise identical sequence parameters. After baseline data are acquired for 2 min, a bolus (0.2 ml/kg) of gadodiamide (GE Healthcare, USA) is injected intravenously through the tail vein, during which the dynamic scan is continued. A total of 90 dynamic images are acquired with a temporal resolution of 8 s, lasting 12 min total. Because of using gadodiamide, cASL scan cannot be performed after $K_{trans}$ scan (see Note 2).

12. **Quantitative susceptibility mapping (QSM):** QSM data are acquired using a 3D multi-gradient echo (MGE) sequence with the following parameters: FOV = 2.56 \times 2.56 \times 2.56 \text{ cm}^3, data matrix = 256 \times 192 \times 128, TE1 = 4.4 \text{ ms}, echo spacing = 6.1 ms, 4 echoes, and TR = 28 ms.

### 3.3 Image Analysis

Image calculation and co-registration can be done using codes written in Matlab (MathWorks Inc, Natick, MA) [34, 35]. In addition to Matlab programs, we also use the STIMULATE (University of Minnesota) software for display and plotting. There are also many other free software programs available to calculate and display MRI images.

#### 3.3.1 Map Calculations

1. **ADC**

ADC maps with intensity in unit of mm$^2$/s are calculated pixel by pixel by using [36, 37],

$$
\text{ADC} = -\ln(S_i / S_0) / (b_i - b_0)
$$
where \( b_t = \gamma^2 G^2_i \delta^2 (\Delta - \delta/3) \), \( \ln \) is the natural logarithm, and \( S_0 \) and \( S_t \) are the signal intensities obtained with \( b_0 \) and \( b_1 \), respectively. The \( b \)-value is proportional to the gradient strength \( (G) \), magnetogyric ratio \( (\gamma) \), duration of each gradient pulse \( (\delta) \), and the time \( (\Delta) \) between applications of the two gradient pulses. ADC maps are calculated at each time point.

2. Fractional anisotropy (FA):

FA can be calculated using either Matlab code or other MRI analysis toolboxes (e.g., DTIStudio, BrainVoyager). Three eigenvalues \( (\lambda_1, \lambda_2, \text{and} \lambda_3) \) are derived from DTI data and FA is calculated using

\[
FA = \sqrt{\frac{3\left( (\lambda_1 - \mathbb{E}[\lambda])^2 + (\lambda_2 - \mathbb{E}[\lambda])^2 + (\lambda_3 - \mathbb{E}[\lambda])^2 \right)}{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}
\]

3. CBF

For ASL images, CBF images \( (S_{\text{CBF}}) \) with intensity in units of ml/gram/min are calculated \([38, 39]\) pixel by pixel using

\[
S_{\text{CBF}} = \frac{1}{T_1} \left( S_c - S_L \right) / \left( S_L + (2a - 1) S_c \right),
\]

where \( S_c \) and \( S_L \) are signal intensities of the control and labeled images, respectively. \( \lambda = 0.9 \text{ ml/g} \)—is the partition coefficient \([40]\). \( a \) is the labeling efficiency which is measured to 0.75–0.9 in animal models.

For DSC-CBF calculation, the transverse relaxation rate \( (\Delta R_2^*) \) is calculated using the equation \( \Delta R_2^* (t) = -\ln(S(t)/S_0) / TE \), where \( S(t) \) is the signal intensity at time \( t \), \( S_0 \) is the precontrast baseline signal intensity, and TE is the sequence echo time. A CBF map is then generated by deconvolving the change in tissue concentration over the first pass of contrast agent with an arterial input function using singular value decomposition \([41, 42]\). Mean transit time and cerebral blood volume can also be obtained with this analysis and they may be useful for stroke analysis. This software can be obtained from many sources.

4. \( T_2 \)

\( T_2 \) maps can be calculated from \( T_2 \)-weighted MRI with two or more echo times (TE). When using two echoes, \( T_2 \) can be calculated directly using

\[
T_2 = -\ln \left( \frac{S_{TE2}}{S_{TE1}} \right) / (TE_2 - TE_1),
\]

where \( S_{TE2} \) and \( S_{TE1} \) are the signal intensities obtained with \( TE_2 \) and \( TE_1 \), respectively. If using more than two echoes, a linear fit needs to be used to fit

\[
\ln(S_{TE}) = \ln(S_0) - TE / T_2,
\]

where \( S_0 \) is the maximum signal from the sample.
5. $K^{\text{trans}}$ mapping [24]

The steady-state spoiled gradient echo (GRE, acquired using the 2D FLASH sequence) signal amplitude for a given TR ($M_{\text{TR}}$) can be related to $M_0$, $R_1$, and $\alpha$ as follows:

$$M_{\text{TR}} = M_0(r, s_{\text{coil}}) \cdot \sin \alpha \frac{1 - \exp[-TR \cdot R_1]}{1 - \exp[-TR \cdot R_1] \cdot \cos \alpha} \cdot \exp(-TE/T_2^*) \quad (1)$$

where $M_0$ is a function of both spatial location $r$ and coil sensitivity $s_{\text{coil}}$. To minimize the $R_2^*$ signal decay, a single-echo GRE scan is used with minimum TE and a low dose of gadolinium. As an approximation, $\exp(-TE/T_2^*) \approx 1$ is used in the subsequent calculation. A prescan module composed of three GRE scans is used to determine flip angle ($\alpha$) and tissue magnetization ($M_0$).

For flip-angle mapping, simulations using Eq. 1 show that the ratio of GRE magnitudes at TRs of 200 and 64 ms depends strongly on $\alpha$, but very weakly on $T_1$. Given the narrow $T_1$ distribution of brain tissue, a raw $\alpha$ (or $B_1^+$) map can be obtained from the ratio map with a fixed $T_1$ value. Assuming that $\alpha$ is a smooth function of location, the final $\alpha$ map can be obtained after smoothing.

For $M_0$ determination, according to Eq. 1, the GRE signal becomes less dependent on $T_1$ with increasing TR. With a long TR of 3000 ms, GRE magnitude depends primarily on $\sin(\alpha)$ for small to medium flip angles ($\alpha < 45^\circ$), but very weakly on $T_1$. Given the normal flip angle of $30^\circ$, the actual flip angle of the brain tissue is within the range of 15–45°; therefore, $M_0$ can be determined from the long TR scan and the flip angle distribution as follows:

$$M_0 = M_{3000\text{ms}} / \sin \alpha \quad (2)$$

Given the smooth $\alpha$ distribution, high-SNR $M_0$ map, and dynamic scans using sensitive surface coil at high field, dynamic $R_1$ map can be obtained using Eq. 1 with sufficient SNR.

The AIF is determined using the data and the scaling approach by Ewing and colleagues [43, 44]. Briefly, the mean AIF ($AIF_{\text{mean}}$) is measured in a group of male rats (approximately 300 g) using the custom-synthesized radiolabeled Gd-DTPA [43]. Assuming that the plasma volume is 1% and there is no BBB leakage in the contralesional side of caudate-putamen (CPU), the AIF is determined using the following scaling:

$$AIF(t) = AIF_{\text{mean}} \cdot \left[ 100 \cdot \int_{t=3\text{min}}^{9\text{min}} R_{t,\text{CPU}}(t) \, dt \right] / \left[ \int_{t=3\text{min}}^{9\text{min}} AIF_{\text{mean}} \, dt \right] \quad (3)$$
The $R_1$ and the AIF are then used to fit the extended Kety model:

$$C_t(t) = K_{\text{trans}} \int_{\tau=0}^{t} C_p(\tau)e^{-k_{\text{ep}}(t-\tau)} d\tau + v_p C_p(t) \quad (4)$$

where $C_t(t)$ and $C_p(t)$ are tissue and plasma gadolinium concentrations, and $k_{\text{ep}}$ is the reversible mass transfer coefficient. In this study, the $R_1$ and scaled AIF values are directly used for data fitting without further conversion to concentrations using the relativity of contrast agent. Since it is difficult to obtain acceptable $K_{\text{trans}}$ maps by simultaneously fitting all three parameters, we use the model selection approach by Ewing and colleagues [44] to select a simpler model that could sufficiently describe the dynamic contrast change. Data is fit to the extended Kety model with the two following assumptions as described by Ewing and colleagues [44]:

Irreversible leakage (two-parameter model, or Model 2):

$$K_{\text{trans}} > 0, k_{\text{ep}} = 0 \quad (5)$$

Reversible leakage (three-parameter model, or Model 3):

$$K_{\text{trans}} > 0, k_{\text{ep}} > 0$$

For model selection, the irreversible model is selected, unless the reversible model yields a statistically significant better fit. The cutoff criteria use the F-statistic, which can be calculated using the summed squared residues (SSE) and the number of samples ($N$) as follows:

$$F = \left[ \frac{\text{SSE}_3 - \text{SSE}_2}{\text{SSE}_3 / (N-3)} \right]$$

where the subscript represents the corresponding model. The final selection mask is determined as

$$M_{\text{select}} = F > F_0 \quad (7)$$

The threshold for F-statistic ($F_0$) is set as 10 ($P < 0.05$). The final $K_{\text{trans}}$ map is then determined as

$$K_{\text{final}} = K_{\text{Model1}} \cdot (1 - M_{\text{select}}) + K_{\text{Model2}} \cdot M_{\text{select}} \quad (8)$$

All the calculations are performed using Matlab R2011b (Mathworks, Natick, MA).

6. Quantitative susceptibility mapping:

QSM is performed using the STI Suite [45]. Briefly, the brain image is reconstructed with 3D fast Fourier transform. The complex data is separated into magnitude and phase. The magnitude image is used for the extraction of the brain
tissue. The phase images are unwrapped using Laplacian-based unwrapping [46] and summed. The background phase is removed using the V-SHARP method [47]. The tissue susceptibility is determined using the LSQR method [48]. The resulting magnetic susceptibility values are directly used for comparison [49].

3.3.2 Abnormal Tissue Volume Analysis

Abnormal tissue volume can be determined using either manually ROI drawing or threshold methods. Manually region of interest (ROI) drawing method usually needs more than two data analyzers to manually delineate the border of the abnormal tissue and then count the number of pixels inside the ROI. Commonly used threshold method is using the mean value ± 2 standard deviations of the homologous contralesional region (see Note 3). Depending on the data, it could be two or three standard deviations (see Note 4). In TBI abnormal tissue volume analysis, ADC, CBF, FA, \( T_2 \), and \( K^{\text{trans}} \) can all be analyzed using these two methods.

3.3.3 Hypercapnic Responses

Images obtained during the transition period between baseline and stimulus onset (30 s for \( \text{CO}_2 \) challenge) are discarded. BOLD images are obtained from the control (non-labeled) images of the CBF measurements. BOLD and CBF magnitude and percent changes relative to baselines are calculated on a pixel-by-pixel basis.

3.4 Additional Advanced Analysis

3.4.1 Data Co-registration

3.4.2 Spatial-temporal Changes of MRI Measures (ADC, FA, CBF, \( T_2 \), \( K^{\text{trans}} \), and Hypercapnic Responses)

Three regions of interest (ROIs) are defined along the ipsilesional cortex, along with three ROIs in homologous regions of the contralesional hemisphere. The same ROIs are used to tabulate the MRI measures across all time points using Stimulate software (University of Minnesota). Values of the ipsilesional ROI are normalized with respect to those of the contralesional ROI.

3.4.3 Quantification of White Matter Damage

To evaluate MRI contrast along the white matter tracts and to eliminate the subjectivity of manual region of interest selection, we project the maximum values of magnetic susceptibility, and the minimum values of DTI FA along the corpus callosum. Such an approach is used to minimize the potential partial volume effect due to the small fraction of white matter in rodent brains. The projected values are compared across different time points.
4 Notes

1. Following the TBI impact, the cranial opening needs to be sealed with bone wax and then the scalp sutured closed. The blood, if any, needs to be cleared. Saline should be injected under the skin to facilitate removal of air pockets between the scalp and the skull to minimize artifacts during MRI acquisition. These steps (using bone wax and saline) are very important to avoid MRI artifacts.

2. Gd-DTPA or gadodiamide will change the relaxation time ($T_1$ and $T_2$) of blood and tissue. Therefore CBF will not be accurately obtained using cASL after using Gd-DTPA or gadodiamide.

3. In TBI, some parameters can change in the positive or negative direction at the same time due to heterogeneity within the tissue. For example, both decreased and increased ADC can be observed in acute TBI. When calculating abnormal ADC tissue volume, both types of tissues need to be counted.

4. The variances of different parameters are different. For small variance data, the threshold needs to use mean value plus higher times of standard deviations (SD). For example, for $T_2$ maps, two SDs would be good. For ADC, which has small variance, it may be needed to use mean plus three SDs as the threshold.

Acknowledgements

This work is supported in part by NIH/NINDS R01 NS45879, a TL1 and KL2 TR001118, and Mike Hogg Fund via the Clinical Translational Science Awards (CTSA, parent grants UL1TR000149, TL1TR001119, and KL2TR001118). We thank our former and current colleagues who have participated on these TBI projects.

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