

Chapter 24

MRI in Experimental Stroke

Timothy Q. Duong

Abstract

Stroke is the third leading cause of death and the leading cause of long-term disability in the United States. Brain imaging data from experimental stroke models and stroke patients have shown that there is often a gradual progression of potentially reversible ischemic injury toward infarction. A central core with severely compromised cerebral blood flow (CBF) is surrounded by a rim of moderately ischemic tissue with diminished CBF and impaired electrical activity but preserved cellular metabolism, often referred to as the “ischemic penumbra.” Re-establishing tissue perfusion and/or treating with neuroprotective drugs in a timely fashion is expected to salvage some ischemic tissues. Diffusion-weighted imaging (DWI) based on magnetic resonance imaging (MRI) in which contrast is based on water apparent diffusion coefficient (ADC) can detect ischemic injury within minutes after onsets, whereas computed tomography and other imaging modalities fail to detect stroke injury for at least a few hours. Along with quantitative perfusion imaging, the perfusion–diffusion mismatch which approximates the ischemic penumbra could be defined non-invasively. This chapter describes stroke modeling, perfusion, diffusion, and some other MRI techniques commonly used to image acute stroke and, finally, image analysis pertaining to experimental stroke imaging.

Key words: MRI, perfusion–diffusion mismatch, ADC, CBF, DWI, PWI, experimental stroke model, rodents, fMRI.

1. Introduction

Stroke is the third leading cause of death and the leading cause of long-term disability. A stroke is caused by a disturbance in the blood supply to the brain, resulting in loss of brain functions. Stroke is a medical emergency. Earlier detection and earlier treatment would mean more brain tissue can be salvaged. There are two types of stroke. Ischemic stroke, which occurs as a result of an obstruction within a blood vessel accounts for

about 85% of all stroke cases. Hemorrhagic stroke which occurs as a result of bleeds into the surrounding brain and subsequent increase intracranial pressure accounts for about 15% of stroke cases. According to the statistics published recently by the American Heart Association (1), someone in the United States suffers a stroke approximately every 40 s, and 5.8 million Americans have permanent neurological deficits from stroke with more than 71% of these stroke survivors unable to return to work. The American Heart Association projected that \$70 billion will be expended on the care of stroke patients in 2009 (2). This cost is steadily rising because the conditions that put people at risk for stroke (such as heart disease, diabetes, and obesity) are also steadily on the rise.

Magnetic resonance imaging (MRI) provides flexible and multiple clinically relevant information to image stroke in a single setting. In particular, diffusion-weighted imaging (DWI) (3) in which contrast is based on water apparent diffusion coefficient (ADC) is widely recognized as a useful imaging modality, because of its ability to detect stroke within minutes after onsets, whereas computed tomography and other imaging modalities fail to detect stroke injury for at least a few hours. Hyperintense regions on DWI correspond to tissues with a reduced apparent diffusion coefficient (ADC) of water. Although the biophysical mechanism(s) underlying ADC reduction remains poorly understood and controversial (3, 4) the ADC decline has been correlated with energy failure and breakdown of membrane potential in animal models (5–7).

CBF can be measured by using an exogenous intravascular contrast agent or by magnetically labeling the endogenous water in blood (8, 9). 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 non-invasive, and the labeled water has a favorable short half-life (\sim 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.

In humans, the “perfusion–diffusion mismatch” is presumed to approximate the “ischemic penumbra.” Although the strict definition of ischemic penumbra requires correlation with energy metabolism (5–7) and such a correlation is not feasible in humans, the “ischemic penumbra” and viability thresholds have been operationally defined based on DWI, PWI, and equivalent modalities. While the “perfusion–diffusion mismatch” is widely observed in acute human stroke (10–14) similar observations in animal stroke models have been limited and the temporal evolution of the perfusion–diffusion mismatch in animal models has yet to be systematically investigated. Animal models where focal

ischemia can be reproducibly studied under controlled conditions would be important for identifying and predicting the severity of ischemic injury and for evaluating the efficacy of therapeutic intervention.

In this chapter, we describe the stroke surgery procedures, a few common MRI protocols used in acute stroke imaging and, finally, image analysis pertaining to experimental stroke imaging in rats.

2. Materials

2.1. Stroke Modeling

1. Rats (200–250 g) (vendor: many)
2. Anesthetics (isoflurane or pentobarbital, etc.) (vendor: many)
3. Common surgical tools and supplies (vendor: many)
4. 4–0 monofilament nylon suture for occlusion (vendor: many)
5. PE-50 tubing (vendor: Fisher Scientific or Cole Palmer)
6. Warm pad, temperature feedback monitoring, and other monitoring equipment to ensure normal animal physiology (vendor: Fisher Scientific or Cole Palmer)
7. TTC (2,3,5-triphenyltetrazolium chloride) for histology (vendor: Sigma)

2.2. MRI

1. Bruker 7 T scanner (Billerica, MA)
2. 40-G/cm BGA12 gradient insert ($ID = 12$ cm, 120- μ s rise time)
3. Animal holder
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, RF coil configurations should also work

2.3. Peripheral MRI Compatible Monitor Equipment and Animal Supports

1. Oximetry (heart rate, arterial oxygen saturation) – (vendor: Mouse Ox)
2. Blood pressure (invasive with artery catheterization) – (vendor: Biopac/Acknowledge)
3. Respiration rate via force transducer – (vendor: Biopac/Acknowledge)

4. Forepaw stimulation device – (vendor: many)
5. Circulating warm water bath (Haack water bath, Cole Palmer)
6. Temperature feedback regulator (Digisense, Cole Palmer)
7. Anesthetic delivery, such as vaporizer – (vendor: many)

3. Methods

3.1. Stroke Surgery

1. Male rats (200–250 g) are anesthetized with isoflurane (~2%). Weights of animals should be within 50 g to ensure consistent lesion volume. Other anesthetics can also be used. All anesthetics have some effects on stroke outcome (such as infarct volumes) and thus studies need to be designed and interpreted with this confound in mind. Male rats are often used to avoid the effects of female hormone on ischemic injury. Female rats are also widely studied and some female hormones have been found to have neuroprotective effects.
2. Aseptic preparations are strongly encouraged as infection and immunological responses could affect outcome.
3. Focal brain ischemia is induced using the intraluminal suture occlusion method, originally described by Koizumi et al. (15) and adapted by our group (16, 17). The right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) are exposed through a midline incision of the neck. The ECA will be permanently ligated distally, severed and flipped 180° around, such that it will be placed parallel to the common carotid artery. The occluder is made of a 4–0 monofilament nylon suture with its tip rounded by flame or coated with silicone (the latter yields less vascular damages in reperfusion studies upon withdrawal of the occluder). Occluder is advanced via the ECA to cause occlusion until a mild resistance is felt. The length is typically 18–20 mm from the CCA and ICA bifurcation. ECA access is preferred, because the occluder can be withdrawn to pass the bifurcation to allow blood flow to resume from the CCA to the MCA during reperfusion.
4. The right femoral artery is catheterized for blood–gas sampling, continuous blood pressure and heart rate monitoring. These physiological parameters are important, because devi-

ations could affect stroke outcome, increasing statistical scatters.

5. Rats are secured in a supine position on an MR-compatible rat stereotaxic headset, anesthesia is reduced to $\sim 1.1\%$ isoflurane. Rats breathe spontaneously. Mechanical ventilation can also be used. Rectal temperature should be maintained at $37.0 \pm 0.5^\circ\text{C}$. 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 data are acquired at 30, 90, and 180 min and again at 24 h post-ischemia.
6. For reperfusion studies, the animals are taken out of the scanner and the suture is withdrawn past the bifurcation to allow blood flow to resume from the CCA to the MCA which can be confirmed visually. Successful reperfusion can be confirmed by MR angiography (MRA) and CBF MRI later. The animal is then placed back in the scanner. Three principle planes of the localizer images and EPI images are carefully aligned with images obtained before taking the animal out the holder. The accuracy of placing the holder and image slices to those before taking out the holder is typically within one pixel or less. The entire procedure typically takes ~ 15 min. *For a permanent occlusion study*, the ECA will be ligated permanently; the occluder will be inserted via the CCA to block blood flow to the MCA.
7. Rectal temperature is maintained at $36.5\text{--}37.5^\circ\text{C}$ and respirations are recorded throughout the study. Body core temperature is critical, because it could affect stroke outcome.
8. At the end of the study, animals are euthanized properly. The brain should be taken out of the skull as soon as possible (within 15 min) and prepared for histology. Brain slices are cut with the same thickness and orientation as the MRI acquisition. Brain slices are incubated in TTC (2,3,5-triphenyltetrazolium chloride, 0.125% w/v) solution at 37°C for about 30 min. The brain slices are then transferred to 10% formalin solution and stored at 4°C for 24 h. For histological analysis, brain slices are photographed and analyzed using the software BioScan OPTIMAS (Edmonds, WA). A typical photoscanner can also be used and analysis can be done using NIH Image (free software). Edema correction needs to be applied (18) to correctly derive infarct volume. Alternatively, transcardial perfusion fixation may also be used for immunohistochemistry.

9. Finally, readers are encouraged to review the series of recommendations on good laboratory practice aimed at preventing the introduction of bias in experimental stroke investigation by MacLeod et al. (19)

3.2. MRI

- Pilot scan
- T_2 -weighted MRI
- DWI
- ASL PWI (alternatives: FAIR PWI or DSC)
- fMRI

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.

Tune and match RF coil: Tune and match RF coil by adjusting the capacitors to ^1H resonance frequency and $50\ \Omega$.

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

Shimming: Run autoshim or manual shim as needed.

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

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 1.5 mm coronal slices to cover the region of interest.

T_2 MRI: T_2 -weighted images are acquired using the fast spin-echo pulse sequence (echo time per echo = 6.5 ms) with two different effective echo times (52 and 104 ms), echo train length 16, and 16 signal averages. Typical parameters are spectral width is 30–50 kHz, $\text{TR} = 2\text{--}3\ \text{s}$ (90° flip angle), pulse shape Gaussian or Sinc3, pulse duration 1–2 ms.

DWI: ADC_{av} can be obtained by averaging three ADC maps with diffusion-sensitive gradients separately applied along the x -, y - or z -direction. An average of at least three axes is preferred to minimize anisotropic effects. Single shot, echo-planar images (EPI) can be acquired with matrix = 64×64 , spectral width = 200 kHz, repetition time $\text{TR} = 2\ \text{s}$ (90° flip angle), echo time $\text{TE} = 37.5\ \text{ms}$, b value = 4 and three directions of $1,170\ \text{s}/\text{mm}^2$, separate between diffusion gradient $\Delta = 24\ \text{ms}$, diffusion gradient duration $\delta = 4.75\ \text{ms}$, field of view $\text{FOV} = 2.56\ \text{cm} \times 2.56\ \text{cm}$, eight 1.5 mm slices, and 16 averages (total time $\sim 2.5\ \text{min}$).

CBF: There are two methods to measure CBF, namely continuous arterial spin labeling (cASL) or dynamic

susceptibility-enhanced 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 stroke, the contrast agent is often trapped and longer wait time may be necessary.

For the cASL technique, single-shot, gradient-echo, echo-planar-imaging (EPI) acquisition is used. Paired images are acquired alternately - one with arterial spin labeling and the other without (control). MR parameters were data matrix = 64×64 , FOV = $2.56 \text{ cm} \times 2.56 \text{ cm}$, eight 1.5-mm slices, TE = 20 ms, and TR = 2 s (90° flip angle). Continuous arterial spin labeling employed a 1.78-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 20–40, depending on the SNR needed.

For the DSE technique, single-shot, gradient-echo, echo-planar-imaging (EPI) acquisition with matrix = 64×64 , FOV = $2.56 \text{ cm} \times 2.56 \text{ cm}$, 3–5 slices of 1.5-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). Start the DSE acquisition of 1 min. About 20 s into the acquisition, deliver the contrast agent in a single bolus flush of saline. Continue DSE acquisition for another 40 s. Note that if DSE is used, Gd-DTPA has a non-negligible intravascular half-life. cASL and fMRI studies cannot be done immediately after Gd-DTPA injection.

fMRI: Combined CBF and BOLD measurements are made using the continuous arterial spin-labeling technique with single-shot, gradient-echo, echo-planar-imaging (EPI) acquisition. Paired images are acquired alternately - one with arterial spin labeling and the other without (control). MR parameters were data matrix = 64×64 , FOV = $2.56 \text{ cm} \times 2.56 \text{ cm}$, eight 1.5-mm slices, TE = 20 ms, and TR = 2 s (90° flip angle). Continuous arterial spin labeling employed a 1.78 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. For each set of CBF and BOLD measurements, 60 pairs of images (4 min) are acquired during baseline and 30 pairs (2 min) during hypercapnic challenge or forepaw stimulation.

- Hypercapnic challenges used a premixed gas of 10% CO₂ with 21% O₂ and balance N₂.
- Forepaw somatosensory stimulation used the previously optimized parameters under identical isoflurane anesthetic condition in normal animals (20): 6 mA current with 0.3 ms pulse duration at 3 Hz. These stimulation parameters did not cause an increase in MABP. Needle electrodes are inserted under the skin of the two forepaws before surgery. The electrodes are connected in a series and the two forepaws are stimulated simultaneously.
- Each trial consists of 4 min of data acquired during baseline and 2 min of data acquired during a hypercapnic challenge or forepaw stimulation. This is for combined BOLD and CBF measurements. If only BOLD fMRI is acquired, 2 min baseline and 1 min “stimulation” would be sufficient.

3.3. Image Analysis

Image calculation and co-registration can be done using codes written in Matlab (MathWorks Inc, Natick, MA) (20, 21). 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.

(a) *Map calculations:* ADC maps with intensity in unit of mm²/s are calculated pixel-by-pixel by using (22).

$$\text{ADC} = -\ln(S_1/S_0)/(b_1 - b_0)$$

where $b_i = \gamma^2 G_i^2 \delta^2 (\Delta - \delta/3)$, \ln is the natural logarithm, S_0 and S_1 are the signal intensities obtained with b_0 and b_1 , respectively. The b -value is proportional to the gradient strength (G), magnetogyric ratio (γ), duration of each gradient pulse (δ), and the time (Δ) between applications of the two gradient pulses. ADC maps are calculated at each time point.

For ASL images, CBF images (S_{CBF}) with intensity in units of mL/g/min are calculated (23, 24) pixel-by-pixel using

$$S_{\text{CBF}} = \lambda/T1 \bullet (S_C - S_L)/(S_L + (2 - 1)S_C),$$

where S_C and S_L are signal intensities of the control and labeled images, respectively. $\lambda = 0.9$ ml/g – is the partition coefficient (25), α is the labeling efficiency which is measured to 0.75–0.9 in animal models.

For DSC-CBF calculation, the transverse relaxation rate ($\Delta R2^*$) is calculated using the equation $\Delta R2^*(t) = -\ln(S(t)/S_0)/TE$, 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 (26, 27). 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.

T_2 maps can be calculated from at least T_2 -weighted MRI with two echo times (TE). $T_2 = -\ln(S_{TE2}/S_{TE1})/(TE_2-TE_1)$, S_{TE2} and S_{TE1} are the signal intensities obtained with $TE_{2\mu}$ and TE_1 , respectively.

Thresholding ADC and CBF maps: To determine the ADC and CBF critical thresholds, ADC or CBF is separately lowered (via a Matlab program) until the CBF- and ADC-defined LV at 3 h numerically equal to the TTC infarct volume at 24 h. This method sets a fixed value below which the pixels within the ADC or CBF map are considered ischemic. The 3-h time point is chosen because the ADC-derived LV of this stroke model is shown previously to stop evolving by this time (28). The same thresholds can then be used to calculate the LV for all time points.

To utilize the critical thresholds, the ADC- and CBF-derived LV are determined using only the thresholds from Group I and without using TTC data. The ADC- and CBF-derived LV at 3 h are independently correlated with TTC-derived infarct volume at 24 h.

Hypercapnic responses in different tissue types: Images obtained during the transition period between baseline and stimulus onset (30 s for CO_2 challenge and 15 s for forepaw stimulation) 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: (1) on a pixel-by-pixel basis, (2) for the ISODATA-derived normal, mismatch and core clusters, and (3) for the ROI of the forepaw somatosensory cortices.

Forepaw-stimulation responses in the forepaw cortices: Cross-correlation analysis associated with the forepaw primary somatosensory cortices is performed. ROIs of the normal LH forepaw cortices are drawn based on the averaged cross-correlation activation maps of all time points with references to the rat brain atlas and MRI anatomical images to avoid bias to any particular time point. The forepaw ROIs on the ischemic RH are obtained by symmetrically reflecting the LH ROIs along the midline to the RH. ADC, baseline CBF, and fMRI signals in the forepaw primary somatosensory cortex ROI are analyzed pixel by pixel, as well as by averaging pixels within the forepaw ROI. Magnitude baseline CBF and CBF changes are computed.

3.4. Additional Advanced Analysis

Evolution of "mismatch" pixels: The temporal and spatial evolution of the "mismatch" pixels, defined at 30 min after occlusion,

is evaluated as they migrated to different clusters. ADC, CBF, and BOLD under baseline and stimulated (CO₂ or forepaw) conditions are analyzed for the pixels that subsequently migrated into the normal zone, core zone, or remained in the mismatch zone at 180 min post-ischemia. For permanent occlusion, ischemia stopped evolving 180 min post-occlusion which is taken as the imaging endpoint as demonstrated previously (17, 16).

Pixel-by-Pixel Analysis: Pixel-by-pixel scatterplots of the CBF and ADC values are analyzed to evaluate the distribution of pixels over time. Only the center four slices are analyzed to minimize the misalignment between gradient-echo and spin-echo images at the ear canals. Four quadrants on the CBF-ADC scatterplots are derived using the TTC-derived ADC and CBF thresholds. The four zones are operationally defined as (i) the “normal” cluster where both ADC and CBF are above the thresholds, (ii) the “core” cluster where both ADC and CBF are below the thresholds, (iii) the “mismatch” cluster where the ADC is above the threshold but CBF is below the threshold, and (iv) “zone 4” where ADC is below the threshold, but CBF is above the threshold. Tissue volumes, means, and standard deviations of the ADC and CBF values of each cluster are evaluated at each time point. The history of the pixels that eventually became infarcted is analyzed. The pixels from where the “core” (red) pixels came at the previous time points are colored blue in the CBF-ADC spaces. Projection profiles of the ADC and CBF distributions are also plotted at each time point.

Iterative self-organizing data analysis (ISODATA): The ISODATA technique is an unsupervised segmentation method based on K -means clustering algorithm with additional iterative splitting and merging steps that allow statistical adjustment of the number of clusters and the cluster centers. Two major improvements based on Jacobs et al.’s algorithm (29) are incorporated, namely the use of Mahalanobis distance measure and spatial contiguity.

In the original ISODATA method (30 31), Euclidean distance is used which did not take into account the variances of each feature parameter. Mahalanobis metric (32) removes several of the limitations of the Euclidean metric, namely (1) it automatically accounts for the scaling of the coordinate axes, (2) it corrects for correlation between the different features, and (3) it can provide curved or linear decision boundaries. Mahalanobis distance r can be written as

$$r^2 = (x - m_x)' C_x^{-1} (x - m_x),$$

where r is the Mahalanobis distance from the feature vector \mathbf{x} to the mean vector \mathbf{m}_x , and C_x is the covariance matrix for \mathbf{x} .

The surfaces on which r is constant are ellipsoids that are centered about the mean \mathbf{m}_x . In the special case where the features are uncorrelated and the variances in all directions are the same, these surfaces are spheres, and the Mahalanobis distance measure reduces to the Euclidean distance measure.

Spatial contiguity incorporates spatial information when assigning clusters. Due to “noise” in the ADC and CBF measurements, a small fraction of (often single) pixels could be mistakenly assigned to another cluster. Consequently, a few scattered pixels of one class could be embedded in another class. The (dis) contiguity at a single pixel (j) is defined as the fraction of its spatial neighbors that are not in the same cluster:

$$D_j = \frac{\text{Number of adjacent pixels } i \text{ for which } k(i) \neq k(j)}{\text{Number of adjacent pixels}}$$

where $k(j)$ is the cluster to which j belongs and $k(i)$ is the cluster to which j 's neighbors that i belongs. Eight neighbors are used in this study. For cluster re-assignment, another contiguity index of pixel j , D_{jl} , is defined as

$$D_{jl} = \frac{\text{Number of adjacent pixels } i \text{ for which } k(i) \neq k(j) \text{ and } k(i) = L}{\text{Number of adjacent pixels}},$$

where $L = 1 \sim n_{\text{cluster}}$ and $L \neq k(j)$ (n_{cluster} is the total number of clusters). Pixels are re-assigned if they had 6 or more out of 8 possible neighbors belonging to another class ($D_j \geq 6/8$) *and* the class to which these pixels are to be re-assigned had to have 5 neighbors out of 8 possible neighbors ($D_{jl} \geq 5/8$). Both conditions needed to be satisfied; otherwise the pixel would not be assigned. The goal is to remove 1 or 2 “noisy” pixels only, avoiding erroneously re-assigning pixels, especially at 30 min post-occlusion, where there are small “islands” of normal tissues embedded in large abnormal ADC lesions.

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