A Viable Isolated Tissue System: A Tool for Detailed MR Measurements and Controlled Perturbation in Physiologically Stable Tissue

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In vivo magnetic resonance imaging (MRI) assessment of neuronal tissue is prone to artifacts such as movement, pulsatile flow, and tissue susceptibility. Furthermore, stable in vivo scans of over 3 h are difficult to achieve, experimental design is therefore limited. Using isolated tissue maintained in a viable physiological state can mitigate many of these in vivo issues. This work describes the fabrication and validation of an MRI compatible viable isolated tissue maintenance chamber. Parameters measured from maintained rat optic nerves did not change significantly over 10 h: (i) mean axon radius [electron microscopy – 0 h: 0.75 ± 0.46; 5 h: 0.74 ± 0.35; 10 h: 0.76 ± 0.35 mm (P > 0.05, t-test)], (ii) action potentials [grease-gap electrophysiology – 4.89 ± 0.16 mV, (P > 0.05, Pearson test)], and (iii) diffusion tensor imaging parameters [fractional anisotropy: 0.86 ± 0.02 (P > 0.05, Pearson test), mean diffusivity: 1.48E-06 ± 9.74E-08 cm²/ s, (P > 0.05, Pearson test)]. In addition, a thorough diffusion-weighted MR protocol demonstrated the comparable stability of viable isolated and chemically fixed rat optic nerve. This MRI compatible viable isolated tissue system allows researchers to probe neuronal physiology in a controlled environment by limiting in vivo artifacts and allowing extended MRI acquisitions. Magn Reson Med 69:1603–1610, 2013. © 2012 The Authors Magnetic Resonance in Medicine published by Wiley Periodicals, Inc. on behalf of International Society for Magnetic Resonance in Medicine.

Key words: diffusion MRI; physiologically stable; viable; electron microscopy; electrophysiology; validation

In vivo magnetic resonance (MR) assessment of neuronal tissue is confounded by movement, surrounding tissue structure, vascular and susceptibility effects, and restricted scan duration [1]. These factors limit the data acquisition and in turn, the accuracy and stability of quantitative MR data. Attempts to mitigate these issues using (i) artificial phantoms, (ii) chemically fixed tissue, and (iii) viable isolated tissue (VIT) have been made in preclinical studies.

Artificial phantoms representing some features of white matter have been produced using rayon textile filament fibres [2], collections of (water-filled) plastic capillaries [3], and glass micro capillaries [4,5]. However, artificial phantoms are limited as they do not replicate the structural complexity of biological tissue nor its biochemical activity. Chemically fixed tissue does provide this structural complexity and overcomes several issues of imaging in vivo [6], but water populations differ in distribution and behavior [7,8], and fixed tissue also lacks in vivo electrophysiological and biochemical activity. VIT potentially fills the gap between in vivo and fixed tissue. Movement and other artifacts associated with in vivo imaging are removed while maintaining the structural complexity and chemical properties of living tissue [9]. In addition, VIT can maintain electrophysiological activity, and thus allows measurement of physiological responses following tissue perturbation [10]. Importantly, VIT allows extended acquisition periods that are not feasible in vivo, and therefore allows in depth exploration of tissue characteristics.

VIT models have been scanned over long sustained durations [11–14]. Tissue structure is often explored to provide comparative control data for MR microstructure imaging techniques, e.g. in Ref. 15,16. However, tissue integrity and viability at physiological temperature over time have not been explored in a magnetic resonance imaging (MRI) compatible VIT system. For isolated tissue to be useful and reliable as an MR model system, the tissue must remain stable over the time periods required for MR acquisitions. This article describes the validation and assessment of a system for extended scanning durations of an isolated rat optic nerve. We use: (i) electron microscopy for assessment of axonal swelling, degeneration, and secondary demyelination [17]; (ii) electrophysiology to assess maintenance of electrophysiological viability [10]; and (iii) diffusion-weighted MR (DW-MR) measurements to assess MR stability [13]. Using these kinds of measurements, we demonstrate that the MRI compatible VIT chamber maintains rat optic nerve in a stable and viable state for 10 h.

METHODS

Chamber Manufacture, Design, and Function

The chamber (Fig. 1) was designed in a popular computer aided design suite (SolidWorks™ — Dassault Systèmes, Paris, France), and exported as a high-resolution mesh file for production. The chamber was manufactured from Fine Polyamide (PA-2200 nylon), using high-resolution selective laser sintering, on a P100 Formiga selective laser sintering system (EOS, Munich, Germany).
Optic Nerves

Optic nerves were excised from adult Sprague Dawley rats (200–250 g) after decapitation [18]. Sections of optic nerve running from immediately behind the eyeball to just in front of the optic chiasm (about 10 mm in length) were used for all experiments. Average total extraction time was 3.5 min; average optic nerve diameter was 1.1 mm.

Temperature, aCSF, and Chamber Monitoring

During all experiments, the temperature of aCSF in the chamber was assessed using a temperature recording system (Small Animal Instruments Inc. New York, NY, USA). Temperature of aCSF within the chamber was maintained at 37.0 ± 0.2°C. Flow effects, liquid retention, and air bubble formation within the chamber were assessed with multislice gradient echo sequences: repetition time: 30 ms, echo time (TE): 5 ms, matrix: 128 x 128, flip angle: 20 degrees, 3 slices, 1 mm thick, field of view (FOV): 30 by 30 mm. These were also used to set up DW-MR and diffusion tensor imaging (DTI) acquisitions.

Electrophysiology

Grease-gap electrophysiology [18] was used to record compound action potentials from viable rat optic nerves during maintenance within the chamber. The chamber design included inserts to create three electrically isolated compartments (Fig. 1a). Recording electrodes were placed in the aCSF of two adjacent compartments while the stimulus electrode was placed in contact with the end of nerve in the remaining compartment. This allowed stimulation of the nerve in the first, and recording over the second and third compartments. The compound action potential was recorded using Ag/AgCl electrodes following electrical stimulation of the nerve with rectangular voltage pulses: a stimulus rate of 0.01 Hz (70–110 V amplitude, 50 μs width) was maintained for 10 h. To demonstrate the return to electrophysiological baseline of a compromised nerve, oxygen and glucose deprivation (OGD) was imposed for 2 h by using aCSF containing no glucose and gassed with 5% CO₂ in N₂. OGD simulates an ischaemic insult to the nerve and induces irreversible loss of axonal function [19].

Electron Microscopic Histology

Transmission electron microscopy was used to assess visible structural damage to axons within optic nerves over time. Rat optic nerves maintained in the chamber for 5 and 10 h were compared to a freshly extracted and fixed control nerve. To demonstrate the structural effects of metabolic failure, a further nerve was subjected to OGD for 2 h within the chamber. The freshly extracted OGD and maintained nerves were fixed overnight in 4% formaldehyde and 2% glutaraldehyde solution and washed and stored in 0.1 M phosphate buffer (pH 7.4) before postfixation in 2% osmium tetroxide (OsO₄) in phosphate buffer for 1 h. After dehydration, nerves were mounted in Durcupan resin and ultrathin sections (0.7
were taken. Four 1000 \( \mu \text{m}^2 \) fields were taken from the center of each of the four nerves, using a JEM-1010 (Nippon Denshi, Tokyo, Japan) transmission electron microscope (5000\times magnification). Fibres of various diameters are homogeneously distributed across the rat optic nerve [20]. Therefore, sampling axons from the center of the optic nerve (relative to both cut ends and the outer surface of the nerve) provides a representative sample of fibres throughout the nerve. The total internal areas of axons and their perimeters (excluding myelin) were measured using the image analysis software ImageJ (NIH, Bethesda, MD, USA) and its region of interest (ROI) analysis toolbox. Axon radii were calculated by averaging the maximum and minimum internal radii of each axon. Glial cell bodies, glial processes, and blood vessels were excluded from the analysis. Three hundred individual axons were measured in each treated nerve. Detailed 20,000 \times \text{magnification} images were also taken for visual inspection.

**Diffusion MR Measurements**

Repeated DW-MR measurements were acquired, once per hour, over 10 h to assess the MR stability of VIT in the chamber. The stability of the DW-MR signal from a viable nerve was compared to that of a fixed nerve. Fixed samples were immersed in 4% formaldehyde solution for 24 h then washed in 100 \( \text{mL} \) 0.1 M phosphate buffered saline (pH 7.4) for 10 h at 4°C before MR experiments.

We base our DW-MR protocol on Ref. [7], which is able to detect differences between VIT and chemically fixed tissue. We describe the DW-MR protocol in detail in Table 1. Briefly, 44 DW-MR measurements with a range of \( b \)-values to maximize sensitivity to possible microstructural changes, and four unweighted measurements were acquired each hour for 10 h. Diffusion gradients were oriented perpendicular to the nerve. The duration of the diffusion encoding gradients (8) was 3 ms and repetition time was 3700 ms. TE was minimized separately for each \( \Delta \) and an unweighted measurement was included for each TE. Twelve repetitions were taken for each individual measurement.

To acquire this protocol once per hour, a spatially selective signal acquisition [21] with no phase encoding was used. The slice positioning and frequency encoding directions relative to the nerve are shown in Figure 2a. This method acquires a single line of voxels; their signal is gathered only from a cuboid 10 \( \times \) 10 mm encompassing the nerve. A single axial imaging slice, 2 mm thick, FOV: 6 by 6 mm, matrix: 64 by 64, positioned in the center of the nerve

| Hourly acquisition | Set Measurement | \( \Delta \) (ms) | \( b \)-value (\( \mu \text{m}^2 \text{ms}^{-1} \)) | TE (ms) | Set Measurement | \( \Delta \) (ms) | \( b \)-value (\( \mu \text{m}^2 \text{ms}^{-1} \)) | TE (ms) |
|--------------------|----------------|-----------------|-------------------------------|-------|----------------|----------------|----------------|-------|
| Unweighted         | 0              | 0               | 0.00                          |       | Unweighted     | 0              | 0               |       |
| 1                  | 0.07           | 1               | 0.08                          |       | 2              | 0.81           | 2               | 1.46  |
| 3                  | 2.34           | 3               | 4.57                          |       | 4              | 4.71           | 4               | 9.41  |
| 5                  | 7.85           | 5               | 15.98                         |       | 6              | 11.79          | 24              | 24.28 | 34    |
| 7                  | 16.53          | 7               | 34.31                         |       | 8              | 22.06          | 8               | 46.08 |
| 9                  | 28.40          | 9               | 59.57                         |       | 10             | 35.26          | 10              | 74.80 |
| 11                 | 43.47          | 11              | 91.75                         |       | 12             | 52.31          | 12              | 110.44|
| Unweighted         | 0              | 0               | 0.00                          |       | Unweighted     | 0.07           | 2               | 1.84  |
| 2                  | 1.84           | 2               | 1.84                          |       | 3              | 6.00           | 3               | 5.98  |
| 4                  | 12.55          | 4               | 12.50                         |       | 5              | 21.49          | 5               | 21.04 |
| 7                  | 46.52          | 7               | 46.33                         |       | 8              | 62.61          | 8               | 62.37 |
| 9                  | 81.10          | 9               | 80.77                         |       | 10             | 101.96         | 10              | 101.56|
| 11                 | 125.22         | 11              | 124.72                        |       | 12             | 150.86         | 12              | 150.26|

Table 1

**Showing the MR Scan Parameters Used for Each Hourly DW-MR Experiment on Both Viable and Chemically Fixed Optic Nerves**

| Hourly acquisition | Set Measurement | \( \Delta \) (ms) | \( b \)-value (\( \mu \text{m}^2 \text{ms}^{-1} \)) | TE (ms) | Set Measurement | \( \Delta \) (ms) | \( b \)-value (\( \mu \text{m}^2 \text{ms}^{-1} \)) | TE (ms) |
|--------------------|----------------|-----------------|-------------------------------|-------|----------------|----------------|----------------|-------|
| Unweighted         | 0              | 0               | 0.00                          |       | Unweighted     | 0              | 0               |       |
| 1                  | 0.07           | 1               | 0.08                          |       | 2              | 0.81           | 2               | 1.46  |
| 3                  | 2.34           | 3               | 4.57                          |       | 4              | 4.71           | 4               | 9.41  |
| 5                  | 7.85           | 5               | 15.98                         |       | 6              | 11.79          | 24              | 24.28 | 34    |
| 7                  | 16.53          | 7               | 34.31                         |       | 8              | 22.06          | 8               | 46.08 |
| 9                  | 28.40          | 9               | 59.57                         |       | 10             | 35.26          | 10              | 74.80 |
| 11                 | 43.47          | 11              | 91.75                         |       | 12             | 52.31          | 12              | 110.44|
| Unweighted         | 0              | 0               | 0.00                          |       | Unweighted     | 0.07           | 2               | 1.84  |
| 2                  | 1.84           | 2               | 1.84                          |       | 3              | 6.00           | 3               | 5.98  |
| 4                  | 12.55          | 4               | 12.50                         |       | 5              | 21.49          | 5               | 21.04 |
| 7                  | 46.52          | 7               | 46.33                         |       | 8              | 62.61          | 8               | 62.37 |
| 9                  | 81.10          | 9               | 80.77                         |       | 10             | 101.96         | 10              | 101.56|
| 11                 | 125.22         | 11              | 124.72                        |       | 12             | 150.86         | 12              | 150.26|
was acquired every hour for 10 h. Repetition time: 5000 ms, TE: 30 ms, G: 22 G/cm
D: 20 ms δ: 3 ms, six directions: [Gx, Gy, Gz] = [1,1,0], [1,0,1], [0,1,1], [-1,1,0], [0, -1,1], and [1, 0, -1]. A ROI covering the center of the nerve was selected (10 voxels). Fractional anisotropy and mean diffusivity were calculated using the open source CAMINO software package [22].

RESULTS

Chamber Performance

We have observed that the chamber (Fig. 1) is suitable for MR measurements: The nylon construction material of the chamber shows no proton MR signal at the shortest achievable TEs of our system (0.5 ms). The chamber is also suitable for grease-gap electrophysiology recordings: With the inserts (Fig. 1a), three electrically isolated chambers can be created and maintained. From room temperature, the chamber requires 10 min to achieve a stable temperature of 37.0 ± 0.2°C which was maintained for the duration of experiments. The MRI bore temperature (17.5 ± 1°C) did not affect the temperature maintenance of the chamber (data not shown). Leaking of aCSF or heating water was not observed. We have found the chamber to be reliable, consistent, and reusable.

Electrophysiological Recordings of Optic Nerves

Electrophysiological recordings demonstrated that optic nerves in the chamber maintained electrophysiological viability over 10 h (Fig. 3a). The peak amplitude of recorded compound action potentials remains above 4 mV for the duration of the experiment and shows no significant trend over time (P = 0.64, Pearson test). The lack of a Faraday cage around the set-up caused transient electrical interference (outliers in the data shown in Fig. 3a) but these are nonsystematic and so do not affect our conclusions. We have observed that optic nerves produced compound action potentials with amplitudes of 4.0 ± 0.25 mV, up to 15 h post extraction (data not shown). The electrophysiological signal returns to baseline (0 mV) after 2 h OGD (Fig. 3b). This is consistent with previous findings in an ischaemic rat optic nerve model [23]. An example of a typical, compound action potential recorded with this system is shown in Figure 3c.

Assessment of Tissue Structural Integrity

Transmission electron micrographs demonstrated the structural stability of optic nerves over 10 h within the chamber (Fig. 4). Visual inspection of electron micrographs indicated a comparable degree of neuronal survival and structural stability between the control (immediate fixation), and both 5 and 10 h maintained samples (Fig. 4a, b, and c, respectively). A nerve subjected to OGD for 2 h (Fig. 4d) demonstrated structural changes in the tissue due to metabolic failure.
The damaged sample shows reduced organization of microtubules, severely damaged astrocytes, less dense myelin layers, and separation of myelin from the cytoplasm in 100% of observed axons. Averaged parameters calculated from measurements of axons (at 5000× magnification) within each treated nerve are shown in Table 2. Samples (a), (b), and (c) show a similar magnitude and variation in both areas, perimeters, and diameters ($P > > 0.05$, t-test). The OGD nerve (d) shows both a greater variation and a significant increase in axon areas, perimeters, and radii in comparison to the immediate fixation control nerve ($P < 0.05$, t-test).

MR Measurements

DW-MR measurements demonstrated the signal stability of optic nerve tissue maintained in the chamber over 10 h in comparison to fixed tissue (Fig. 5). Variability in averaged signals over time is comparable in both the fixed and live nerves at all b-values and diffusion times. For example: at a b-value of 90 μm$^{-2}$ ms and $\Delta$ of 50 ms, the standard deviation of the averaged measured signals over 10 h = 0.027 (fixed) and 0.035 (VIT). We observe that signal attenuation at high b-values is markedly increased in the fixed nerve; indicating a higher diffusivity in the fixed sample. Gradient echo imaging throughout the 10 h DW-MR experiments demonstrated no air bubble formation, either on the surface of the tissue or on the inner surfaces of the chamber (data not shown).

Table 2

| Treatment               | Area (μm²)   | Perimeter (μm) | Radius (μm) |
|-------------------------|--------------|----------------|-------------|
| Immediate fixation      | 0.86 ± 1.0   | 4.7 ± 2.6      | 0.75 ± 0.46 |
| ACSF 5 h                 | 0.77 ± 0.9$^a$ | 4.4 ± 2.4$^a$ | 0.74 ± 0.35$^a$ |
| ACSF 10 h                | 0.89 ± 1.0$^a$ | 4.7 ± 2.4$^a$ | 0.76 ± 0.35$^a$ |
| Oxygen and Glucose Deprivation | 1.2 ± 1.8$^b$ | 5.1 ± 3.6$^b$ | 0.88 ± 0.54$^b$ |

Axons were sampled over four 1000 μm² fields (5000× magnification) positioned in the centre of each nerve, covering approximately 1000 axons per sample.

$^a$No significantly different to immediate fixation control ($P > > 0.05$, t-test).

$^b$Significantly different to immediate fixation control ($P < 0.05$, t-test).

FIG. 4. Sections of transmission electron micrographs at 20,000 times magnification taken from four differently treated optic nerves. a: immediately fixed upon extraction, b: ACSF for 5 h within the chamber, c: ACSF for 10 h within the chamber, and d: oxygen and glucose deprived for 2 h. Open arrow shows a healthy mitochondrion, solid arrow shows compact myelin layers surrounding axons. Scale bars represent 1 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
DTI data further demonstrated the comparable stability of viable and fixed samples (Table 3). We observed no trend in either fractional anisotropy or mean diffusivity in either sample over the time course ($P = 0.37$ and 0.44, respectively, Pearson test) and variability between samples was comparable.

FIG. 5. Representative semilog DW signal attenuation plots from MR experiments on a viable (left) and a fixed (right) optic nerve. Points represent the average signal along the length of the optic nerve. Both nerves were scanned once per hour at four diffusion gradient separations ($\Delta = 10, 20, 35,$ and $50$ ms) and a range of $b$ values (0–150 $\mu$m$^2$ ms) for 10 h. The range of 12 $b$-values used for each $\Delta$ are shown to the right of each pair of plots. A marked difference in signal intensity at higher $b$-values can be seen between the viable and fixed nerves. The acquisition of VIT measurement 1 (20 ms) failed—this data point has thus been omitted.
DISCUSSION

Validation Methods

Electrophysiological recordings of action potentials from optic nerves maintained within the chamber demonstrated their maintenance of electrophysiological viability over time. In contrast to this, compromised cellular metabolism is detectable as a reduced compound action potential amplitude [10], as demonstrated in the OGD experiment (Fig. 3b).

Electron microscopic images of optic nerves demonstrated the structural stability of the tissue. In both maintained samples and in the fresh control, microtubules are present and evenly distributed throughout the axon [24] and the mitochondria appear healthy. Previous studies on rat optic nerves deprived of oxygen and glucose have demonstrated a characteristic set of structural changes [18]. We note that in the maintained samples, myelin layers surrounding some axons (≈1 in 100) also show a degree of separation from one another. This is seen to an equal degree in both the control and maintained samples and is therefore likely due to histological processing artifacts.

Detailed DW-MR and six direction DTI acquisitions were used to test the microstructural stability of both viable and fixed optic nerve tissue. The attenuation of the DW-MR signal was found to be significantly increased in the fixed nerve compared to that of the viable optic nerve. In addition, mean diffusivity calculated from DTI was increased in fixed tissue. This is consistent with previous findings on VIT at room temperature using a similar DW-MR protocol [7]. We note that previous studies comparing in vivo and fixed tissue, e.g. in Ref. [25], show lower apparent diffusion coefficients in fixed tissue than in vivo tissue. However, unlike this study, the fixed tissue measurements in Ref. [25] are acquired at room temperature rather than physiological temperature. In both the fixed and viable optic nerves, minor variations from the mean signal are seen; these are likely to be due to scanner instability. These data demonstrate that the DW-MR signal stability of viable nerves is comparable to that of fixed optic nerves, which are inherently structurally stable. The spatially selective signal acquisition was chosen to allow repeated detailed DW-MR measurements. The MR volume sampled included an aCSF partial volume of ~5%. The aCSF signal was fully attenuated at low b-values, and this partial volume does not affect our ability to determine temporal stability from this data. DTI data included no aCSF partial volume.

Temperature and Bubble Formation

Temperature within the MRI VIT chamber is stable for 10 h. Temperature control within the chamber is an important consideration. Rat brain tissue oxygen consumption (an indication of metabolic activity) has been shown to increase by 20% with a temperature increase of 2.5°C [26]. Molecular motion is highly sensitive to changes in the environmental temperature and an increase in tissue temperature increases the self-diffusion of water [27]. In addition, a temperature difference of 17°C has been shown to produce marked changes in transmembrane water exchange rates in isolated tissue [28]. Without the use of a surfactant on the inner surfaces of the chamber, gas bubbles form. A nonionic surfactant readily absorbs into the chambers material and prevents bubble formation. We have observed no deleterious effects on the tissue due to surfactant application. Comparisons to preliminary electrophysiology and electron microscopy work without surfactant (data not shown) support the conclusion that the chambers surfactant coating does not damage the tissue.

Time Limits

Previous studies suggest that isolated rat optic nerves maintain electrophysiological activity for longer periods post extraction [29]. However, removal of the cell bodies from the axons during tissue extraction rules out any further protein manufacture by the cell; progressive structural damage therefore accumulates over time. The stability of VIT in the chamber demonstrated here leads us to conclude that, using this system, data can be acquired from a stable sample for up to 10 h: although we suggest that this is a conservative upper limit for stable acquisitions using VIT and even longer acquisition times may well be possible.

Manufacturing, Costs, and Design Considerations

The chamber manufacturing method ensures stability and reproducibility. The chamber consists of homogeneous nylon, this material is ideal for use with MRI: the magnetic susceptibility of nylon is less than 3 ppm away from that of water [30]. Furthermore, identical chambers can be reproduced economically and rapidly using a modern selective laser sintering system (total production cost per unit as of 2012 ≈ 50 USD).

Although the chamber has been designed to fit into a small (26 mm) birdcage coil, the aggregate volume is significantly larger than the sample volume. High signal to noise ratio data can be acquired with this set-up; however, the quality of MR data could potentially be improved by using a surface coil. The current design is

Table 3

Fractional Anisotropy (FA) and Mean Diffusivity (MD) Calculated from Hourly DTI Acquisitions in Both Viable and Fixed Rat Optic Nerves

| Time (h) | VIT FA | Fixed FA | VIT MD (cm²/s) | Fixed MD (cm²/s) |
|---------|--------|----------|----------------|-----------------|
| 1       | 0.85   | 0.80     | 1.58E-06       | 3.08E-06        |
| 2       | 0.84   | 0.81     | 1.61E-06       | 3.04E-06        |
| 3       | 0.89   | 0.83     | 1.43E-06       | 2.86E-06        |
| 4       | 0.86   | 0.86     | 1.38E-06       | 2.90E-06        |
| 5       | 0.84   | 0.84     | 1.51E-06       | 3.00E-06        |
| 6       | 0.84   | 0.85     | 1.57E-06       | 2.97E-06        |
| 7       | 0.88   | 0.82     | 1.33E-06       | 2.82E-06        |
| 8       | 0.90   | 0.84     | 1.35E-06       | 2.94E-06        |
| 9       | 0.87   | 0.85     | 1.50E-06       | 2.94E-06        |
| 10      | 0.86   | 0.85     | 1.55E-06       | 3.15E-06        |
| Mean    | 0.86   | 0.83     | 1.48E-06       | 2.97E-06        |
| σ       | 0.02   | 0.02     | 9.74E-08       | 9.68E-08        |
CONCLUSION

The chamber described here allows the use of MR protocols that can finely sample parameter space and produce artifact-free datasets from physiologically stable isolated rat optic nerve tissue. For example, this chamber is suitable for evaluating and comparing white matter models of diffusion [31] or magnetization transfer [32]. The chamber has been designed to allow access and controlled perturbation of VIT during MRI acquisitions. The chamber is therefore suitable for studies into a range of pathological conditions and could potentially be used to assess MR detectable effects of drug administration. These experiments will be the focus of future work now we have validated the stability of the system.

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