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Voxelized Computational Model for Convection-Enhanced Delivery in the Rat Ventral Hippocampus: Comparison with In Vivo MR Experimental Studies

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Abstract

Convection-enhanced delivery (CED) is a promising local delivery technique for overcoming the blood–brain barrier (BBB) and treating diseases of the central nervous system (CNS). For CED, therapeutics are infused directly into brain tissue and the drug agent is spread through the extracellular space, considered to be highly tortuous porous media. In this study, 3D computational models developed using magnetic resonance (MR) diffusion tensor imaging data sets were used to predict CED transport in the rat ventral hippocampus using a voxelized modeling previously developed by our group. Predicted albumin tracer distributions were compared with MR-measured distributions from *in vivo* CED in the ventral hippocampus up to 10 μ L of Gd-DTPA albumin tracer infusion. Predicted and measured tissue distribution volumes and distribution patterns after 5 and 10 μ L infusions were found to be comparable. Tracers were found to occupy the underlying landmark structures with preferential transport found in regions with less fluid resistance such as the molecular layer of the dentate gyrus. Also, tracer spread was bounded by high fluid resistance layers such as the granular cell layer and pyramidal cell layer of dentate gyrus. Leakage of tracers into adjacent CSF spaces was observed towards the end of infusions.

INTRODUCTION

High potential therapeutic agents, such as immunoglobulins, enzymes, micro-particles and polynucleotides, have been introduced by recent advances in pharmaceuticals for the treatment of neurological conditions such as epilepsy, Alzheimer's disease, and malignant brain tumors.^{16,19,33} However, the delivery of some of these therapeutic agents to the central nervous system (CNS) is problematic due to low penetration of macromolecular therapeutic agents from blood capillaries into interstitial spaces through the blood–brain barrier (BBB). Convection-enhanced delivery (CED) is an investigative delivery technique by which an

infusion cannula is introduced directly into the brain parenchyma. With controlled infusion, bulk fluid motion in the extracellular space delivers therapeutic agents over larger distribution volumes in the brain compared with other diffusion-driven delivery methods. CED bypasses the BBB, provides targeted drug delivery, and minimizes systemic toxicity. Previous CED transport studies have been performed to better understand transport mechanisms and distribution patterns of agents in brain tissue.^{6,9,24,28,35,39,49} These studies have found CED tissue distributions to be sensitive to choice of infusion site, infusion rate, and cannula size. Tissue distributions also vary with therapeutic agent, depending on the size, charge and surface characteristics of these compounds.⁸

Development of computational CED models that predict interstitial flow and tissue distributions within the CNS would facilitate treatment planning so as to avoid undesired drug spread, such as leakage into cerebrospinal fluid (CSF) regions, which can lead to toxicity or secondary effects in untargeted regions. These brain transport models need to incorporate realistic anatomical boundaries and tissue properties to correctly account for delivery within complex tissue structures consisting of white matter, gray matter, and adjacent CSF regions all of which have varying transport characteristics based on their underlying structure. Gray matter, which mainly consists of neuronal cell bodies, neuropil, and glial cells, has an approximately isotropic structure. Within gray matter, diffusivity and hydraulic conductivity (a measure of how easy it is to drive fluid within a porous media under a pressure gradient) may be considered to be the same in all directions.²⁰ On the other hand, white matter is composed of bundles of myelinated axons. Preferential diffusional transport has been measured to take place along the direction of aligned fiber tracts.^{36,47} Previous CED studies also indicate that preferential convective transport also occurs along fiber track directions.^{6,14,50} Diffusion weighted imaging which measures the effective diffusivity of water in tissues can be used to determine preferential transport directions in white matter. Preferential directions of water diffusion have been found to correspond to average aligned axonal bundle directions within an imaging voxel, and this data has been used to calculate fiber tract trajectories within the brain.^{3,12} Our group^{21,23,42} as well as others^{26,40,46} have used diffusion tensor images (DTI) to account for anisotropic extracellular transport properties in CED transport models of the CNS.

Our group has developed a voxelized modeling approach²¹ which avoids time consuming slice-by-slice segmentation and tissue volume reconstruction procedures often required when building image-based models. Developed porous media transport models account for spatially varying transport properties and realistic boundaries between white matter, gray matter, and CSF. This methodology has been recently applied to CED transport in the rat brain hippocampus and corpus callosum to demonstrate feasibility of this modeling scheme in complex CNS structures.²³ Distinct distribution patterns were predicted for each infusion site based on differences in underlying tissue microstructure and anatomical boundaries. Correspondingly, CED tracer distributions were found to be sensitive to modeling parameters which changed anatomical structures including tissue segmentation thresholds, image voxel resolution, and tissue porosity.

It has proven difficult to validate DTI-based brain transport models in experimental CED studies. Sampson *et al.* have previously compared CED model predictions (BrainLab, Brainlab Inc., Westchester, IL) with measured distributions of a tumor targeted cytotoxin coinjected with radiolabeled albumin in patients with malignant gliomas using single-photon emission computed tomography (SPECT).⁴⁰ However, discrepancies >25% were observed between predicted and measured distribution volumes, possibly due to the low resolution of SPECT imaging. Also, this study focused on distributions within tumors rather than the effect of surrounding neuroanatomical structures which is a potentially important factor when using CED for other neurological diseases. Other comparative studies have focused

primarily on tissue phantoms.^{10,26,27} Given the scarcity of comparative studies, there is a need for additional studies that use different modeling approaches and experimental conditions including different animal models and infusion sites.

In this study, tissue distributions of an MR-visible albumin tracer were investigated during and following controlled-infusions into the ventral hippocampus of the rat. This infusion site was selected because recent studies indicate the potential usefulness of hippocampal infusions for treating epilepsy and other neurological disorders.^{4,17,37} For example, the ventral hippocampus site is where electrical status epileptic produces the most functional injury,⁵ and future studies aimed at reversing such injury would target this site. Information about how infused compounds distribute within this convoluted and heterogeneous structure is lacking. Gd-DTPA labeled albumin (M_w 87 kDa) was selected as the macromolecular tracer. It acts as a surrogate for protein-based therapeutic compounds (e.g., immunoglobulins, enzymes, and polynucleotides^{16,19,52}) and has been shown to collocate with such compounds when coinfused.³⁰ CED tracer distributions were predicted using our voxelized modeling approach, and these patterns were compared with MR-measured distributions. Voxelized models were developed using multiple DTI data sets from fixed rat brain tissue samples. These models accounted for the effect of extracellular flows within underlying tissue structures of white matter and gray matter with special care taken to correctly render connected CSF spaces. CED experiments conducted on living rats used dynamic contrast-enhanced MR imaging (DCE-MRI) and high resolution T1-weighted MR images to determine tracer distribution patterns *in vivo*. Simulation and experimental comparisons of general distribution patterns, tissue distribution volumes and projected areas of these distributions were conducted.

MATERIALS AND METHODS

Animal Preparation and Diffusion Weighted Imaging of Fixed Tissue Samples

High-resolution DTI was used to develop computational models. For small rat brains, long acquisition times were required, and fixed rat brains were used. All animal procedures followed guidelines by the NIH and regulations of the University of Florida Institutional Animal Care and Use Committee. Five male Sprague–Dawley rats weighing ~250–350 g were anesthetized, and perfusion fixation was performed with a 4% paraformaldehyde solution. Brains were removed from the skull and kept in 4% paraformaldehyde solution. Brains were soaked in phosphate buffered solution (PBS) for 24 h prior to imaging to remove the fixative since it has been shown to reduce T2 times.⁴⁴ During imaging, brains were placed in fluorinert (a fluorinated oil with no MR signal) to allow reduction in the field of view (FOV) without aliasing for faster scan times.

MR images were measured using a Bruker Advance imaging console with a 17.6 T, 89 mm vertical bore magnet system (Bruker NMR Instruments, Billerica, MA). DTI for excised brains were collected using a diffusion-weighted spin echo sequence. Two horizontal DTI data sets were measured with recovery time (TR) of 4000 ms and echo time (TE) of 28 ms and eight averages using 40 slices of 0.3 mm thickness in a FOV of 27 mm × 18 mm with a matrix of 180 × 120. Also two coronal DTI data sets were measured with a FOV of 18 mm × 12 mm in 0.3 mm slices in a matrix of 120 × 80 in 32 slices using TR = 1400 ms, TE = 25 ms, and four averages. Imaging parameters for the remaining DTI data set were same as those used in a previous study.²³ 32 horizontal slices of 0.3 mm thickness, FOV of 30 mm × 15 mm in a matrix of 200 × 100, TR = 1400 ms, TE = 25 ms, and five averages. For all DTI data, images with low diffusion-weighting (100 s mm⁻²) and higher diffusion-weighting (1250 s mm⁻²) were measured in 6 gradient-directions and 46 gradient directions respectively. Bilinear interpolation by a factor of two in the slice direction was performed

for diffusion-weighted images. The resolution for all data sets was 0.15 mm^3 . A rank-two diffusion tensor of water, $\mathbf{D}_e$, was fit for each voxel using multiple-linear regression.³

Brain Transport Modeling

3D brain transport models were developed from DTI data using a voxelized modeling approach as previously described in Kim et al.^{21,23} However, a different segmentation method was implemented in this study as described below. Additionally, the subarachnoid cavity surrounding the brain was included in the segmentation. For a full description of transport modeling procedures, assumptions, and parameters the reader is referred to Kim et al.²³

Porous Media Transport—CNS tissue was assumed to behave as rigid porous media for which the continuity equation is

$$\nabla \cdot \mathbf{v} = \beta \quad (1)$$

where $\mathbf{v}$ is the tissue-averaged extracellular fluid velocity, and β is the volumetric flow rate source term for endogenous fluid flow ($\sim 0.3 \mu\text{L min}^{-1} \text{ g}^{-1}$, Ref. 1). It should be noted that this porous media formulation does not account for tissue swelling due to edema or perivascular transport. (While concentrated perivascular distributions have been noted in other studies, especially for larger liposomes and nanoparticles,^{13,25} the majority of smaller albumin tracer has been found to distribute throughout tissues following CED in the hippocampus²). Fluid flow in a porous medium is governed by Darcy's law,

$$\mathbf{v} = -\mathbf{K} \cdot \nabla p \quad (2)$$

where p is the pore fluid pressure, and $\mathbf{K}$ is the hydraulic conductivity tensor. Darcy's law was also employed within CSF and subarachnoid spaces instead of the Navier–Stokes equation. This assumption ignores boundary layer effects and lumps viscous effects into the hydraulic conductivity term. Tracer transport through porous media is governed by the convection–diffusion relation

$$\phi \frac{\partial \tilde{c}}{\partial t} + \nabla \cdot (\mathbf{v} \tilde{c}) = \nabla \cdot (\phi \mathbf{D}_t \cdot \nabla \tilde{c}) \quad (3)$$

where t is time and ϕ is tissue porosity (or pore fraction). $\tilde{c}$ is the normalized tracer concentration in tissue and $\mathbf{D}_t$ is the diffusivity tensor of the tracer in tissue.

Tissue Transport Properties—Hydraulic conductivity, $\mathbf{K}$, and albumin tracer diffusivity, $\mathbf{D}_t$, in isotropic gray matter voxels and anisotropic white matter voxels were assigned values as previously described in our previous CED modeling paper.⁴² Within white matter the principal direction of $\mathbf{D}_e$ as measured by DTI was assumed as the maximum transport direction of $\mathbf{K}$ and $\mathbf{D}_t$. Eigenvectors of the water diffusion tensor, $\mathbf{D}_e$, were combined with eigenvalues to assign transport properties in directions perpendicular and parallel to underlying fiber directions for each voxel using the following equations,

$$\mathbf{K} = \mathbf{V} \begin{bmatrix} K_{\perp} & 0 & 0 \\ 0 & K_{\perp} & 0 \\ 0 & 0 & K_{\parallel} \end{bmatrix} \mathbf{V}^T$$

$$\mathbf{D}_t = \mathbf{V} \begin{bmatrix} D_{\perp} & 0 & 0 \\ 0 & D_{\perp} & 0 \\ 0 & 0 & D_{\parallel} \end{bmatrix} \mathbf{V}^T \quad (4)$$

$$\mathbf{V} = [\mathbf{v}_1 \mathbf{v}_2 \mathbf{v}_3]$$

where $\perp$ and $\parallel$ subscripts describe hydraulic conductivity and tracer diffusivity eigenvalues in directions perpendicular and parallel with white matter fibers, respectively. $\mathbf{v}_3$ is the water diffusion eigenvector in the direction of maximum diffusion, and $\mathbf{v}_1$ and $\mathbf{v}_2$ are eigenvectors in transverse directions to fibers. Diffusivities of albumin tracer in brain tissue and for tracers in varying directions of white matter have been previously measured,^{36,48} see Table 1A. Few studies have measured hydraulic conductivity in brain tissue, especially relative gray and white matter and anisotropic values. Model estimations for hydraulic conductivity from our previous spinal cord tracer study were used.^{41,42} To account for less resistance to flow in CSF and subarachnoid spaces, hydraulic conductivity in fluid regions was assigned to be three orders of magnitude higher than the maximum value in white matter. Hydraulic conductivity of impermeable skull tissue was assigned to be five orders of magnitude lower than that of gray matter values. The baseline porosity value, $\phi = 0.26$, was estimated from CED tracer distributions in the spinal cord.^{41,50}

Tissue Segmentation and Voxelized Modeling Approach—Computational models were developed directly from DTI data sets. A semi-automatic segmentation scheme was employed to assign transport properties on a voxel-by-voxel basis within regions of isotropic gray matter, anisotropic white matter, and free water (CSF regions). In our previous studies, only fractional anisotropy (FA), a measure of the degree of anisotropy of water diffusion within a voxel, was used for tissue segmentation.²³ However, this segmentation technique did not accurately account for CSF regions within the brain. In this study, average diffusivity (AD), the mean of the diagonal elements of $\mathbf{D}_e$, was used to differentiate between tissues and fluid-filled spaces. AD was used because it provides information about the overall mobility of water molecules within the voxel and tends to be higher in free water (CSF regions) than in tissues. AD threshold values were adjusted until water regions were delineated and matched with an anatomical atlas,³⁴ see Table 1B. Tissue regions were segmented as either white matter or gray matter regions using FA thresholds values. To provide a realistic fluid layer around the brain, the subarachnoid space was also incorporated as an additional improvement upon previous brain models. A 0.15 mm thick layer of fluid (one voxel layer) was manually assigned on the surface of the brain (Fig. 1b). Regions outside this layer were assigned as skull.

For computational models, only one side of the brain hemisphere was considered to reduce calculation times. This volume covered coronal slices from bregma 0.0 to −9.0 mm anterior. A 3D rectangular volume mesh was created for this partial volume using 8-node brick elements where each mesh element corresponded to a diffusion weighted imaging voxel element (0.15 mm³). Two infusion sites corresponding to experimental infusion sites (see “Brain Transport Modeling” section) were selected to observe the effect of variation of the infusion site on final distribution patterns. Cube-shaped infusion sites (single voxel, 0.15 mm³) were placed in the (1) ventral CA1 subfield of the hippocampus (AP = −5.88 mm, ML = 5.1 mm, DV = 5.2 mm) and (2) at the interface between the alveus of the hippocampus and the corpus callosum (AP = −5.04 mm, ML = 5.6 mm, DV = 5.2 mm). FA maps were compared with a brain atlas³⁴ to obtain approximately the same target infusion coordinate for each brain based on neuroanatomical structures. A computational fluid dynamic package,

FLUENT (v.6.3.26 Fluent, Lebanon, NH) was used to solve for 3D porous media transport using tissue transport properties based on segmentation results. Due to the rigid tissue assumption, steady-state flow conditions were established within less than one second. Therefore, transient concentration profiles were solved using Eq. (3) with steady-state velocity profiles solved using Eqs. (1) and (2). For infusion of albumin tracer, constant infusion rate of $0.3 \mu\text{L min}^{-1}$ and normalized concentration of $\tilde{c}=1$ was assigned at the infusion site boundaries. A symmetric (zero normal flux) boundary condition was applied on the surface of the internal brain boundary. $p=0$ and $\tilde{c}=0$ were assigned on external brain boundaries. Initial concentration of the albumin tracer in tissues assumed $\tilde{c}=0$ at time zero.

Tissue concentration values of 5% of the maximum concentration were used as the threshold cut-off for contour profiles to calculate tissue distribution volumes. Projected areas of predicted tracer distribution volumes were also computed to quantify dominant transport directions. The projected areas were calculated by collapsing predicted tissue distribution volumes onto a medial–lateral/inferior–superior (ML/IS or coronal) plane, a medial–lateral/anterior–posterior (ML/AP or horizontal) plane, and an inferior–superior/anterior–posterior (IS/AP or sagittal) plane.

In vivo CED Distribution Experiments

We evaluated the accuracy of this modeling approach by comparing predicted distributions to previously published *in vivo* distribution data collected by our group^{2,22} (not the same rats tested in “Animal Preparation and Diffusion Weighted Imaging of Fixed Tissue Samples” section). In both of these studies, CED experiments were conducted on male Sprague–Dawley rats weighing 250–350 g using protocols and procedures approved by the University of Florida Institutional Animal Care and Use Committee. Animals were initially anesthetized, and then placed in a stereotaxic frame, where inhalation anesthesia was delivered via a nose mask. A mid-sagittal incision was made exposing the cranium, and 3 mm diameter burr holes were drilled into the skull above the infusion sites. A silica cannula (OD = $147 \mu\text{m}$), connected to the infusion system primed with Gd-DTPA albumin with Evans Blue dye (~35 molecules of Gd-DTPA per albumin molecule, 10 mg mL^{-1} , R. Brasch Laboratory, University of California, San Francisco, CA), was then stereotactically implanted in the ventral CA1 subregion of the hippocampus. The infusion system consisted of syringe driven by a syringe pump connected to non-compliant polymer tubing (ID = 0.381 mm , OD = 0.794 mm , length ~0.5 m) coupled to the silica infusion cannula. In the first of these studies², $5 \mu\text{L}$ of Gd-albumin tagged with Evans blue dye was infused at a rate of $0.3 \mu\text{L min}^{-1}$ *in vivo* ($n=7$). Final distributions were visualized using high resolution spin echo T1-weighted imaging [TR = 1000 ms, TE = 10 ms, number of averaged signal NA = 8, $20 \times 20 \times 10 \text{ mm}^3$ (read $\times$ phase $\times$ slice) FOV in a matrix 160×160 with 20 slices] at 11.1 T. These experiments were separated into two infusion site groups corresponding to CA1 ($n=4$) and alveus ($n=3$). It should be noted that imaging data was acquired approximately ~30 min after CED infusions during which tracers could diffuse further into tissue (~1 mm).

In the second study,²² transient tracer transport during *in vivo* CED was investigated using similar surgical and CED protocols ($n=4$). Since infusions were performed in the magnet, the syringe pump which is not MR-compatible was placed outside of the room containing the magnet, and the infusion syringe was connected to longer length (6 m) non-compliant tubing. An MR-compatible infusion cannula was implanted in the CA1 subfield, and CED was initiated and observed while the rat was imaged. T1-weighted DCE-MRI (TR = 330 ms, TE = 9.4 ms, 10 slices at 1 mm thickness, NA = 6, $24 \times 24 \text{ mm}^2$ FOV with 104×104 matrix) provided transient tracer distribution. High resolution spin echo T1-weighted images (TR = 1000 ms, TE = 15 ms, 30 slices at 0.5 mm thickness, NA = 8, $24 \times 24 \text{ mm}^2$ FOV with 200×200 matrix) were acquired to determine final infusate distributions at the end of

infusions. The total infusion volume was 10 μL for this series of experiments. Since backflow and infusion delay (a delay in appearance of infusate) was reported in two of the data sets, four distribution data sets were compared with our model results which does not account for backflow. Tracer distribution volumes were calculated using a semi-automated segmentation routine in MATLAB and open-source medical image segmentation software, ITK-SNAP.⁵¹ Contrast enhanced voxels were included in the total distribution volume if their signal intensity was at least six times the standard deviations of noise in the corresponding region contralateral to the site of infusion.

RESULTS

Segmentation of Brain Structures

Figure 1 shows the improved segmentation method implemented in this study. CSF regions, i.e., ventricles, velum interpositum (between the dorsal hippocampus and thalamus) and midbrain cisterns (between the hippocampus and mid brain) were assigned in accordance with atlas boundaries, Fig. 1a. Some CSF regions in the brain such as the midbrain cistern were connected to subarachnoid spaces. Volumetric percentages occupied by each brain structure (white matter—blue, gray matter—green and CSF regions—yellow) were calculated for each rat brain model. Average percentages for white matter and gray matter were 32.75 ± 2.89 and $56.10 \pm 2.69\%$, respectively. The relative tissue proportion of white and gray matter was consistent between animals. CSF spaces comprised $11.07 \pm 1.01\%$ of the total model volume. These percentages are similar to values in the human brain.¹⁵

Predicted Tracer Distributions and Variation Between Rats

Predicted tracer distributions in the ventral hippocampus for the two infusion sites are shown in Fig. 2 for the five excised rat brains. Figure 2a shows predicted distribution contours after 5 μL infusions into the CA1 subfield. For this location and infusion volume, preferential distributions into white matter and more limited penetration into neighboring gray matter were predicted. This resulted in more hippocampal coverage for infusion in the CA1 site, see Fig. 2a. Tracers occupied the majority of the hippocampus proper and the dentate gyrus (see Fig. 1c), as well as, CSF regions. Figure 2b shows predicted tracer distributions after 5 μL infusions at the interface between the alveus of the hippocampus and the corpus callosum. Small differences in cannula placement (~ 1 mm) resulted in different infusate distribution patterns. Greater preferential distributions were determined along the fiber tract directions of white matter compared to ventral CA1 infusions, see Fig. 2b. Most of the tracer was confined within the hippocampal alveus, corpus callosum and fimbria of the hippocampus. Minimal amounts of tracer entered into the CSF space.

For each infusion site, similar tracer distributions were predicted for the five different DTI data sets. Some variations between brain models resulted from differences in segmented anatomical boundaries and tissue composition. The average ratio of white matter to gray matter distribution volume was 0.80 ± 0.08 for four of the rat brain models. In one model this ratio was larger, 1.71, and greater tracer penetration into white matter was predicted for CED into the CA1 subfield due to a larger white matter region adjacent to the infusion site. For the alveus/CC infusion site, some variation in the degree of preferential transport along fiber tracts in the corpus callosum, and the extent of the infusate penetration into adjacent tissue were also observed across different models (see coronal plane distributions in Fig. 2b). In the horizontal plane, varying tracer penetration into the fimbria was also observed between brain models.

For the alveus/CC infusion site, computational models predicted an average total tracer distribution volume of $41.1 \pm 0.8 \text{ mm}^3$ after 5 μL infusion. Predicted distribution volumes in

gray matter, white matter and CSF spaces were 12.92 ± 1.97 , 26.67 ± 2.11 and 1.5 ± 0.64 mm³, respectively. This corresponds to tracer distributions in white matter which were approximately twice that predicted in gray matter tissue. Only ~3% of the total distribution volume was predicted to distribute within CSF regions. Tracer distributions in tissue were predicted to be greater in the IS/AP plane, with predicted tracer distributions of 25.180 ± 2.80 , 33.6 ± 5.10 , and 22.2 ± 2.10 mm² in ML/AP, IS/AP, and ML/IS planes, respectively.

For the CA1 infusion site, computational modeling was used more extensively to predict transient tracer behavior for CED infusion up to 10 μ L. Predicted tissue distributions for increasing infusion volumes are presented for white matter, gray matter and CSF filled regions in Fig. 3. Overall, total tissue distribution volume increased approximately linearly, see Fig. 3a. Tracer distribution volumes were closely matched between brain models up to an infusion volume of approximately 5 μ L; then differences became greater with increasing infusion volumes. It should be noted that these volumes include tracer distributions within CSF spaces, and CSF distributions were determined to increase approximately linearly with increasing infusion volumes, see Fig. 3b. Distributions within white matter and gray matter were similar and the percentage tracer distribution in gray matter (range of 36.5–50.5%) closely matched the percentage distributions in white matter (38.9–46.3%), as shown in Fig. 3c. Predicted CSF distribution volumes were similar for all subjects with infusions <5 μ L, but for larger infusion volumes, the differences between subjects increased (see Fig. 3b). After ~4 μ L infusion, the percentage of the total tissue distributions in CSF approached a constant value of ~20%, see Fig. 3c.

Projected tracer distributions on three orthogonal planes are presented in Fig. 3d for infusions at the CA1 infusion site. With increasing infusion volumes, projected areas were predicted to increase, and differences between projected areas reached a maximum of 33% difference between projected areas in the IS/AP and ML/IS planes after 10 μ L infusion. Overall, average projected areas in the IS/AP plane were higher than projected areas in ML/AP and ML/IS planes throughout the infusion. Projected area in the ML/IS and ML/AP planes were more closely matched.

Comparison Between Predicted and Measured Tracer Distributions After 5 μ L Infusions

For both ventral infusion sites, representative distributions for predicted and measured high-resolution MR CED distributions after a 5 μ L infusion² are compared in Figs. 4 and 5. Three dimensional contours for predicted and measured infusion distributions show similarity of overall tracer distribution shape, see Figs. 4c and 5c. Also model predictions were able to capture certain transport characteristics seen in MR images. For infusion in the CA1 subfield, infusate was confined in the ventral hippocampus with tracer occupying the molecular layer of the dentate gyrus (blue dots), CA1 subfield (green dots), and interspersed gray matter in the hippocampus. A significant amount of infusate was predicted to enter CSF space and this was also observed in MR images (yellow dots). In addition, minimal tracer spread was found in the dorsal hippocampus (dashed circle), as shown in Figs. 4a and 4b. For infusions near the alveus interface, preferential tracer distributions along fiber tracts of the corpus callosum (see green dots in Fig. 5a) and limited penetration into adjacent gray matter were observed in both simulations and experiments. However, there were differences between simulated and measured distributions in the fimbria of the hippocampus. In computational models, a considerable amount of tracer was predicted to enter the fimbria (see yellow dots in Fig. 5a) with an additional small amount in the internal capsule. However in MR images, only a small spread of tracer was observed in the fimbria, and no tracer was found in the internal capsule (see yellow dots in Fig. 5b).

For 5 μ L infusions, predicted and measured distribution volumes for the alveus boundary infusion were 41.10 ± 0.76 mm³ ($n = 5$) and 32.59 ± 0.05 mm³ ($n = 3$), respectively. These

volumes were found to be statistically different (t -test, $p < 0.01$) and the predicted distribution volumes were 20.7% higher than measured volumes. Projected areas of tracer distribution at this site were also consistently greater than experimental measures, as shown in Fig. 6a. The model did capture the trend of increased distribution in the IS/AP plane. For the CA1 infusion site, predicted and measured tracer distribution volumes were $29.37 \pm 1.94 \text{ mm}^3$ ($n = 5$) and $33.22 \pm 0.76 \text{ mm}^3$ ($n = 4$), respectively. A statistically significant difference was not found (t -test, $p = 0.10$) between these volumes. Projected areas of distribution along different planes were also found to be closely matched, as shown in Fig. 6b. Predicted distributions in the IS/AP plane were also slightly greater than experimental measures.

Comparison Between Predicted and Measured Transient Distributions Up to 10 μL Infusions

For infusions into the CA1 site, predicted results were compared with *in vivo* dynamic distribution data for infusion volumes up to 10 μL ,²² as shown in Fig. 7. *In vivo* tracer was observed to travel superiorly from the infusion site and then inferiorly along the molecular layer of the dentate gyrus. Predicted tracer distributions matched the measurements well when there was minimal premature leakage, no cannula tip clogging, or no infusate backflow along the cannula track. In simulation and experiments, posterior tracer distributions appear to be equivalent. Tracers gradually filled the posterior dentate gyrus with hippocampal spread directed in the ventral direction, see first columns in Figs. 7a and 7b. There was almost full coverage of the posterior hippocampus and dentate. More anteriorly (2nd and 3rd columns), simulations and experiments show spread into the CA1 and CA2 with some differences. Simulations predicted an initial distribution and spread within the molecular layer of the dentate gyrus before filling the rest of the hippocampus; experimentally, anterior spread of tracer initiated and filled in the striatum radiatum of the CA1 and CA2 before distributing in the molecular layer of the dentate gyrus. Overall, simulations predicted tracer transport to initiate in the molecular layer of the DG then spread through the DG into the hippocampus; experiments showed tracer initiated within the hippocampus (stratum radiatum or CA1 and CA2) then spread laterally into the DG. These differences between simulations and experiments may be attributed to differences in the infusion site placement. The tracer was predicted and observed to enter CSF spaces once tracer reached the cistern and the CSF space between the ventral hippocampus and thalamus.

Predicted and measured tracer distribution volumes were also compared for 10 μL infusions into CA1 as shown in Fig. 8a. Predicted distribution volumes were slightly higher than measured distribution volumes. For small infusions ($<3 \mu\text{L}$), simulated distribution volumes did not match experimental distribution volumes due to a delay in tracer appearance and spread. These time delays ($<7 \text{ min}$) were attributed to slight tissue clogging of the needle tip which may have occurred during needle insertion. Tissue clogging was not accounted for in our model and resulted in underprediction of tracer spread at early time points. However after 10 μL infusion, predicted ($53.48 \pm 8.02 \text{ mm}^3$) and measured ($51.50 \pm 3.42 \text{ mm}^3$) distribution volumes were similar with no statistical differences between the simulation and experiments (t -test, p -value: 0.719). Projected areas in each plane after 10 μL infusions were also compared, as shown in Fig. 8b. For both simulations and experiments, projected areas in the ML/AP plane was higher than projected areas in the IS/AP and ML/IS planes. Projected areas showed good agreement with no statistical differences between simulations and experiments in all planes.

DISCUSSION

CSF fills the ventricular cavities of the CNS and bathes the external surface of the brain and spinal cord within the subarachnoid space. This tracer distribution study shows that a large

portion of infusate can reach and spread into adjacent CSF spaces, such as the midbrain cisterns, during CED infusions into the hippocampus. This finding is similar to other CED studies which have also noted diversion of tracers into adjacent CSF regions.¹⁸ Once tracer enters CSF regions, it can easily transport through fluid since fluid resistance is lower than in tissues. Through these channels, infusate may reach untargeted brain regions. For example, once infusate enters into the midbrain cistern, it could travel into adjacent subarachnoid spaces. Therefore, it is important to account for anatomically realistic CSF spaces in computational models to properly predict distributions within these potentially important transport pathways.

In this study, an improved tissue segmentation scheme using AD values was introduced to model more realistic CSF spaces. AD of D_e was able to distinguish between tissue and fluid-filled regions in brain scans. Corresponding computational models developed using this segmentation scheme predicted tracer distributions within CSF regions similar to leakage patterns observed in MR tracer experiments. It should be noted that DTI-based thresholds used for segmentation were not the same for each brain model. This variation was likely due to differences in signal-to-noise ratios, and other instrument factors, in addition to inherent variability between different rat brains. Overall, the improved segmentation allowed the model to account for transport into adjacent CSF spaces, such as the third ventricle, lateral ventricle, and midbrain cisterns which may not otherwise be captured.

The voxelized modeling approach also allows rapid development of 3D models, and computational models for five rat brains were created to compare variations between different animals. Two different infusion sites only a small distance apart were simulated, and significantly different distribution patterns were predicted for each site. However, predicted tracer distribution volumes, shapes and projected areas were similar between brain models for each of the hippocampal infusion sites selected. Thus, certain common underlying structures and tissue boundaries were predicted to provide the same transport pathways across animals. Distribution patterns began to vary with increasing infusion volumes, since some variation of tissue structures, including the size and shape of the hippocampus and the degree of alignment of fiber tracts, existed between different rat brains. Also, infusate distributions were found to be highly dependent on adjacent neuroanatomical structures and tissue boundaries near the infusion site.

Direct comparison of predicted and MR-measured tracer concentrations was not possible since MR-measured concentrations were not calculated (the relaxivity and relaxation of the Gd-albumin contrast agent were not known). This resulted in some error in comparing tracer distribution volumes since the 5% concentration threshold used in models is not necessarily equivalent to the signal threshold used in MR-measures, and predicted distributions volumes are sensitive to choice of concentration threshold values. (For example, 2% changes in thresholds resulted in ~9% changes in volume after 5 μ L infusions). Additionally, the MR image slice resolution was sacrificed to increase in-plane resolution while maintaining a voxel volume resulting in a suitable signal-to-noise ratio (SNR). The increased in plane resolution was important for comparing MR and computational model distribution patterns with respect to anatomical features in the ventral hippocampus. The decreased MR resolution in the slice-encoding direction may have led to over or underestimation of distribution volumes in the anterior-posterior direction; however, tracer transport in the ventral hippocampus was observed to occur primarily in the dorsal-ventral and medial-lateral directions reducing this source of error. Given these limitations, predicted and MR-measured tracer distribution volumes after 10 μ L infusions were determined to be similar with no statistical differences found between the simulation and experiments. For tissue distributions measured after the end of 5 μ L infusions, predictions matched MR-measured distributions more closely; however, it should also be noted that these experimental

measures slightly overestimate CED tissue spread since additional diffusion occurred after the end of infusion before MR scans were taken.

MR images allowed comparison of tracer distribution patterns. Predicted and measured tracer distribution patterns were found to be qualitatively similar for both infusion sites, and albumin tracer was predicted to primarily occupy the same structures as those shown in the measurements. Overall, 3D transport models were able to capture certain major transport characteristics: (1) Tracers were confined in ventral hippocampus structures, such as the dentate gyrus and CA1 subfield, with limited penetration into adjacent gray matter. Gray matter structures may serve as effective boundaries while white matter regions, such as the corpus callosum and mossy fibers within the molecular layer of dentate, behave as preferential fluid pathways with relatively less fluid resistance. These boundaries transported tracers primarily in the inferior-superior and anterior-posterior directions. (2) A significant amount of infusate entered into CSF spaces depending on the site of infusion. For infusions greater than 4 μL , approximately 20% of the tracer distribution volume was predicted to be within CSF spaces for the CA1 infusion site, while less than 5% of the tracer distribution volume was predicted to be within CSF spaces for the corpus callosum/alveus infusion. Thus, infusion distribution patterns were sensitive to overall access to CSF regions as well as other surrounding neuroanatomical structures adjacent to an infusion site.

The preferential inferior-superior transport directions observed for ventral hippocampus infusions can be explained by underlying anatomical structure. The molecular layer of the dentate gyrus, the polymorphic layer of the dentate gyrus, and the CA1 subfield of the hippocampus are all bordered by pyramidal cell layers characterized by densely-packed cell bodies. The structure of the hippocampus is extended in the anterior-posterior direction, as the hippocampus forms a banana-shaped structure in three-dimensions. Densely-packed gray matter regions present higher resistance to extracellular fluid flow. Therefore, tracer flow may be confined and bounded by pyramidal cell layers resulting in superior-inferior transport. Moreover, while there are longitudinal and other more diffuse projections travelling to and from the hippocampus, unidirectional connectivity is a distinctive feature of the hippocampus that can guide tracer transport along the transverse plane of the hippocampus. This effect is demonstrated in Fig. 7 where predicted and experimental distributions show tracer traveling superiorly from the infusion site, within the molecular layer of the dentate gyrus, around the granule cell layer, and then inferiorly along the molecular layer of the dentate gyrus. Matching these observations, computational modeling predicted greater projected areas in the IS/AP plane. However, these predictions slightly over predict those seen in MR-measurements which may be due to the presence and expansion of hippocampal fissures, which may act as mass sinks.

As mentioned previously, CSF regions can act as mass sinks that dilute and carry away tracer from surrounding tissues. Darcy's law for porous media, the same relation as used in adjacent tissues, was used for rapid model generation of these CSF spaces. One limitation of this approach is that boundary layers and viscous effects within fluid-filled regions are neglected. High hydraulic conductivity (low resistance) values were assigned which may have led to possible over prediction of fluid and tracers into these regions. However, underprediction of tracer distributions was not noted. In addition to the third ventricle, lateral ventricle, and cistern regions which were included in our computational model, hippocampal fissures are internal structures which traverse the ventral hippocampus along the inferior-superior axis and may separate and expand with infusion providing fluid connections to ventricles. DTI and DCE-MR images did not have sufficient spatial resolution to distinguish enhancement in the hippocampal fissure from enhancement in surrounding tissue structures. However, tracer may be diverted into this space, and in our previous study² histological images show high concentrations of infused dye at the

boundaries of these regions following CED. Fissure diversion may explain why computational models slightly over predict tissue distributions at the CA1 infusion site. The linear relation between the volume of distribution and volume of infusion, which was both predicted and measured, suggests that tracers enter CSF through the entire infusion. In addition, alveus infusion models predicted infusate to occupy the hippocampal fimbria while little infusate was observed to enter this region experimentally. This resulted in an overprediction of the total infusate distribution volume of up to 20.7%. Again this difference may be due to incorrectly capturing the connectivity between the fimbria of the hippocampus and the corpus callosum, possibly due to the limited image resolution available or inadequate segmentation. The model may also impose tissue classifications which may not hold for all brain regions. For example, axons near the alveus emerge from the pyramidal cell layer of the CA1 resulting in a structure that is a combination of both white and gray matter. As a result of differences in tissue composition between animals, differences in tracer distributions were observed. This tissue variation resulted in inconsistent segmentation, with some of these regions characterized as white matter, and some characterized as gray matter, between brain models. Thus, small length-scale neuroanatomical structures and boundaries can be factors affecting infusate transport in the brain, and high resolution images and proper tissue segmentation are important for accurate models.

Computational CED models are dependent on tissue transport properties including tracer diffusivity, tissue hydraulic conductivity and porosity. While physiological ranges for tracer diffusivity and porosity have been better established,^{32,36,48} fewer studies have measured hydraulic conductivity due to difficulties in measuring interstitial velocities and pressures *in vivo*.³¹ As a result, there is greater variation in hydraulic conductivity values used in previous CNS transport models. Porosity and hydraulic conductivity values used in this study were estimated for a lower flow rate ($0.1 \mu\text{L min}^{-1}$) study in the spinal cord.^{41,42} At other flow rates, the anisotropy ratio and tissue porosity may diverge from the presented values because of edema and tissue deformation effects. Previous parameter analysis studies by our group²³ showed tracer distributions to be sensitive to changes in tissue porosity. Predicted tracer distributions were less sensitive to anisotropy of white matter hydraulic conductivity (ratio of $K_{\text{wm}/}$ to $K_{\text{wm}\perp}$); however, distributions were sensitive to relative white matter and gray matter values ($K_{\text{wm}\perp}$ to K_{gm}). Further quantitative analysis comparing spatial distribution patterns would provide a better idea of the accuracy of computational models and the need to establish improved transport properties.

Variation between *in vivo* and *ex vivo* data sets also introduces sources of error related to matching infusion site locations, anatomical variation between animals, and fixation. In practice, it was difficult to place cannulas in the same anatomical site with each surgery and image resolution also limited determination of the location of the cannula tip. Thus, there was some placement uncertainty (up to $\sim 300 \mu\text{m}$) when assigning infusion sites within the model. For the *ex vivo* data set, there was also tissue shrinkage with fixation ($\sim 8\%$)⁴³. Axonal fiber orientation is not expected to change with fixation, and assignment of white matter properties is not expected to be affected significantly. However, tissue boundary changes may account for differences between predicted and MR-measured distributions. For example, differences between data sets were large enough to make simple superposition of *ex vivo* and *in vivo* tracer distributions difficult to interpret, not shown. It should also be noted that computational models were based on rigid porous media and did not account for tissue swelling or edema near the point of infusion.^{7,11,45} Neither does the model account for brain pulsatility which may enhance transport through dispersion within the extracellular space or through perivascular transport.

Experimental CED studies were designed to minimize backflow. However, MR scans revealed backflow of the infusate along the cannula track that extended tracer spread superiorly into the corpus callosum, especially during dynamic CED experiments. While backflow models which account for elastic expansion of tissue around the needle due to infusion pressure have been developed by Morrison *et al.* and Ragavan *et al.*,^{29,38} these models do not account for the variable backflow distances seen in our experiments which may be due to transiently high infusion pressures²² that occur during expulsion of tissue clogs. The effect of such needle tip obstructions are difficult to model and were not included. For this infusion system, reduction of the infusion line length and other sources of fluid compliance sources may reduce the occurrence of tissue clogging and backflow.

In order to further test this modeling approach, additional CED studies are needed comparing predicted and MR-measured tracer infusion patterns *in vivo* (e.g., different locations, flow rates, and pathologies). Also, contrast agent relaxivity quantification will allow *in vivo* measures of contrast agent concentration profiles. In addition, collection of *in vivo* DTI scans before infusions would allow direct comparison within the same subject and eliminate errors associating with variation between animal brains.

CONCLUSIONS

Comparative studies provide insight into the effects of underlying transport mechanisms and tissue structure on extracellular CED transport. However, few studies have previously compared model predictions of CED to actual tissue distribution measurements, and we know of no studies have done so within the hippocampus. In this study, computational transport models were able to capture certain major transport characteristics; including albumin tracer confinement in the hippocampus and diversion into adjacent CSF spaces, especially near the end of larger infusions. Predicted and distribution volumes after 10 μ L infusion were found to be similar. Similarity of transport predictions between different rat brains also showed the consistency of the modeling approach. The ability of future models to reliably predict distribution volumes will depend on investigating CED within other brain regions, as well as, improved imaging methods, segmentation, backflow prediction, and the addition of drug-specific interaction. The development of predictive models allows consideration of subject-dependent structural changes and with further development may be used to predict changes in distribution due to structural changes in disease states or to select the ideal infusion site which maximizes tissue coverage and minimizes systemic and neurologic toxicity.

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FIGURE 1.

(a) Improved segmentation map of the rat brain using FA and AD thresholds: blue = white matter, green = gray matter, yellow = free water regions (CSF, ventricle, and subarachnoid spaces), and red = impermeable regions (skull). A coronal slice is presented. (b) Segmented CSF spaces matched corresponding regions in a rat atlas.³⁴ (c) Labeled schematic of brain regions of interests: cc = corpus callosum, ct = cortex, alv = hippocampus alveus, gc = granular cell layer of the dentate gyrus, MoDG = molecular cell layer of the dentate gyrus, hf = hippocampal fissure, and mbc = midbrain cistern.

FIGURE 2.

Predicted albumin tracer distribution patterns after 5 μL CED into two sites in the ventral hippocampus: (a) between the molecular layer of the dentate gyrus and CA1 subfield and (b) at the interface of the alveus and corpus callosum. Normalized concentration contours were overlaid on FA images in coronal, horizontal and sagittal planes images through point of infusion. Distributions from 5 different computational models are compared.

FIGURE 3.

Predicted albumin distributions for 10 μL CED into the CA1 infusion site: (a) total tracer distribution volume in tissue, (b) tracer distribution in CSF regions, (c) percentage tracer distributions in tissues, and (d) projected areas of tissue distributions in varying planes. Bars correspond to 61 standard deviation.

FIGURE 4.

(a) Predicted and (b) *in vivo* T1 measures² of albumin tracer distribution for 5 μ L infusion in the CA1 infusion site. Five adjacent coronal slices are shown where yellow dots = leakage into the CSF space, blue dots = molecular layer of the dentate gyrus, green dots = CA1 subfield of the ventral hippocampus and dashed orange circle = infusate in the dorsal hippocampus. (c) 3D contours of predicted (left & red) and measured (right & green) tissue distributions. These volumes were found to be statistically different (*t*-test, $p < 0.01$) and the predicted distribution volumes were 20.7% higher than measured volumes.

FIGURE 5.

(a) Predicted and (b) *in vivo* T1 measures 2 of albumin tracer distribution for 5 μ L infusion in the alveus/CC infusion site. Five adjacent coronal slices are shown where yellow dots = fimbria of the hippocampus. (c) 3D contours of predicted (left & red) and measured (right & green) tissue distributions. A statistically significant difference was not found (*t*-test, $p < 0.10$) between these volumes.

FIGURE 6.

Projected areas of albumin tracer distribution in tissue along various planes following 5 μ L CED in (a) alveus/ CC and (b) CA1 infusion sites. Simulated distributions were compared with *in vivo* tracer distributions measured in our previous study, Astary *et al.*² For the alveus/CC site, measured and predicted areas were statistically different in all planes (*t*-test, *t*-value: 3.766 (ML/AP), 4.046 (IS/AP), 2.330 (ML/IS), $t_{0.05,6} = 1.943$). For the CA1 site, no statistical difference was found in all planes (*t*-test, *t*-value: 0.508 (ML/AP), 1.823 (IS/AP), 1.203 (ML/IS), $t_{0.05,7} = 1.895$).

FIGURE 7.

(a) Predicted and (b) *in vivo* T1 images²² of transient tracer distribution in the rat brain for CED at the CA1 infusion site. Three coronal slices are shown for each infusion volume time point (infusion rate = $0.3 \mu\text{L min}^{-1}$). Red regions are predicted tracer distributions (normalized concentration >5%) overlaid on FA images.

FIGURE 8.

Predicted and MR-measured tracer distributions for the CA1 infusion site: (a) tissue distribution volumes and (b) projected areas of tracer distribution after 10 μL CED. In (a) simulated distributions are presented as an average volume with standard deviation. After 10 μL infusion, predicted ($53.48 \pm 8.02 \text{ mm}^3$) and measured ($51.50 \pm 3.42 \text{ mm}^3$) distribution volumes were similar with no statistical differences between the simulation and experiments (t -test, p -value: 0.719). In (b), no statistical difference was found between simulated and measured areas in all planes (t -test, t -value: 0.200 (ML/AP), 1.611 (IS/AP), 1.394 (ML/IS), $t_{0.05,7} = 1.895$).

TABLE 1

(A) Tissue properties used in the extracellular transport simulations. (B) Fractional anisotropy (FA) and normalized average diffusivity (AD) ranges used in tissue segmentation of the rat brain.

A		
Parameter	Value	Reference
ϕ	0.26	34,42
D_{tgm}	$1.60 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$	41
D_{twmll}	$2.29 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$	32,41
$D_{\text{twm}\perp}$	$1.34 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$	32,41
D_{csf}	$1.34 \times 10^{-11} \text{ m}^2 \text{ s}^{-1}$	32,41
K_{wmll}	$6.75 \times 10^{-12} \text{ m}^4 \text{ N}^{-1} \text{ s}^{-1}$	34,46
$K_{\text{wm}\perp}$	$4.22 \times 10^{-13} \text{ m}^4 \text{ N}^{-1} \text{ s}^{-1}$	34,46
K_{gm}	$4.22 \times 10^{-15} \text{ m}^4 \text{ N}^{-1} \text{ s}^{-1}$	34,46

B	
Tissue region	Range
White matter	δ to 1.0
Gray matter	0.0 to δ
Non-tissue (free water region)	η to 1.0

	Mean	SD
FA threshold (δ)	0.233	0.015
Normalized AD threshold (η)	0.244	0.045