Published in final edited form as:

J Neurosci Methods. 2012 July 30; 209(1): 62–73. doi:10.1016/j.jneumeth.2012.05.024.

Dynamic Contrast-Enhanced MRI of Gd-albumin Delivery to the Rat Hippocampus *In Vivo* by Convection-Enhanced Delivery

Jung Hwan Kim^{1,*}, Garrett W. Astarý^{2,*}, Tatiana L. Nobrega², Svetlana Kantorovich³, Paul R. Carney^{2,3,4}, Thomas H. Mareci⁵, and Malisa Sarntinoranont¹

¹Department of Mechanical and Aerospace Engineering, University of Florida, Gainesville, FL

²J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL

³Department of Neuroscience, University of Florida, Gainesville, FL

⁴Division of Pediatric Neurology, University of Florida, Gainesville, FL

⁵Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL

Abstract

Convection enhanced delivery (CED) shows promise in treating neurological diseases due to its ability to circumvent the blood-brain barrier (BBB) and deliver therapeutics directly to the parenchyma of the central nervous system (CNS). Such a drug delivery method may be useful in treating CNS disorders involving the hippocampus such temporal lobe epilepsy and gliomas; however, the influence of anatomical structures on infusate distribution is not fully understood. As a surrogate for therapeutic agents, we used gadolinium-labeled-albumin (Gd-albumin) tagged with Evans blue dye to observe the time dependence of CED infusate distributions into the rat dorsal and ventral hippocampus *in vivo* with dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). For finer anatomical detail, final distribution volumes (V_d) of the infusate were observed with high-resolution T_1 -weighted MR imaging and light microscopy of fixed brain sections. Dynamic images demonstrated that Gd-albumin preferentially distributed within the hippocampus along neuroanatomical structures with less fluid resistance and less penetration was observed in dense cell layers. Furthermore, significant leakage into adjacent cerebrospinal fluid (CSF) spaces such as the hippocampal fissure, velum interpositum and midbrain cistern occurred toward the end of infusion. V_d increased linearly with infusion volume (V_i) at a mean V_d/V_i ratio of 5.51 ± 0.55 for the dorsal hippocampus infusion and 5.30 ± 0.83 for the ventral hippocampus infusion. This study demonstrated the significant effects of tissue structure and CSF space boundaries on infusate distribution during CED.

© 2012 Elsevier B.V. All rights reserved.

Correspondence to: Dr. Malisa Sarntinoranont, Department of Mechanical and Aerospace Engineering, 212 MAE-A, University of Florida, Gainesville, FL 32611, msarnt@ufl.edu, Tel: (352) 392-8404, Fax: (352) 392-7303.

*These authors contributed equally to this work

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Convection-enhanced delivery (CED) is a drug delivery method in which drugs are directly infused into target tissues under a controlled flow rate or pressure. CED shows promise for treating neurological diseases by circumventing the blood-brain barrier (BBB) to deliver therapeutics directly to the central nervous system (CNS) parenchyma (Bobo et al., 1994; Groothuis et al., 1999; Morrison et al., 1994). A distinct advantage of CED, over diffusion-driven mechanisms of drug delivery, is that agents are delivered by bulk flow through the porous extracellular space in tissue resulting in greater transport distances and nearly homogenous concentration profiles that drop off steeply at infusate distribution boundaries (Groothuis et al., 1999; Krauze et al., 2005a; Lonser et al., 2002; Saito et al., 2006). Although it is an invasive surgical technique, minimal tissue damage was observed by histological analysis (Inoue et al., 2009; Mardor et al., 2009; White et al., 2011), and no significant neurological deficit was found after CED on a rodent and non-human primate (Bruce et al., 2003; Lonser et al., 1999; White et al., 2011). Therefore, CED potentially can deliver therapeutics to specific regions within the CNS with minimal injury, while limiting both exposure of non-targeted regions and systemic toxicity.

CED has been used to deliver therapeutic agents to malignant gliomas (Laske et al., 1997; Lonser et al., 2007; Sampson et al., 2007; Song and Lonser, 2008; Thomale et al., 2009; Voges et al., 2003), viral vectors for gene therapy treatment of Parkinson's disease (Cunningham et al., 2008; Fiandaca et al., 2008) and has been proposed for drug delivery to treat epilepsy (Rogawski, 2009). Understanding the influence of methodological and biological factors on CED infusate distributions is important when targeting a specific region of the CNS. Methodological factors include cannula design, choice of infusion site, infusion rate, and infusate properties such as viscosity (Mardor et al., 2005). Previous studies aimed to improve cannula design to reduce backflow (Krauze et al., 2005b; White et al., 2011; Yin et al., 2010), and to evaluate the performance of chronically implanted cannulae (Foley et al., 2009), while other studies have focused on the influence of infusion site on infusate distribution (Kim et al., 2010; Sampson et al., 2007; Yin et al., 2010). Biological factors include anatomical structure, endogenous fluid flow, substrate binding, efflux of the infusate from tissue (Raghavan et al., 2006). As the infused-region tissue structure becomes more complex, anatomy may have an increasingly greater impact on infusate distributions. The hippocampus is an example of a complex tissue structure with heterogeneous and intricate neuroanatomy that is affected by many disorders, environmental factors, and physiological effects (Astary et al., 2010). A substantial body of literature exists regarding hippocampal injury in both humans and in animal models (Harry and Lefebvre d'Hellencourt, 2003). Given its importance in brain function and its vulnerability and involvement in many disorders, the hippocampus may be an ideal target for the delivery of therapeutics by CED. In fact, CED has already been proposed to deliver therapeutic agents to treat epilepsy (Rogawski, 2009). However, the prediction of CED distribution within the hippocampus requires a proper understanding of bulk flow dynamics within its cytoarchitecture.

The hippocampus is a complex structure comprised of both white matter (WM) and gray matter (GM) regions. The GM regions consist of densely packed neuronal cell bodies in the

cornu ammonis (CA) subfields within the hippocampus proper, and the granule cell bodies within the stratum granulosum of the dentate gyrus. Projections to and from these densely packed cell layers constitute the WM regions of the hippocampus. Major fiber projections include Schaffer collateral fibers that carry information from CA3 to CA1, and perforant path fibers originating from the entorhinal cortex layers II/III that synapse onto apical dendrites of both pyramidal and granule cells. Of course, there is additional WM and GM, such as interneurons and subcortical projections that contribute to the intricacy of the hippocampal formation. Furthering the complexity, the hippocampus is also a rolled structure, surrounded by pial surfaces and containing penetrating fissures. The fissures and pial boundaries communicate with the extracellular spaces and the ventricular compartments. From a transport perspective, the dense GM regions could serve as isotropic domains resistant to flow whereas WM regions may function as anisotropic domains with lower resistance to flow along directions parallel to fiber projections. The hippocampal fissures may further divert infusate into surrounding CSF regions, hindering the tissue penetration of infusate to regions of the hippocampus greater distances from the infusion site. Thus, the combined effect of these anatomical factors may result in a heterogeneous distribution of therapeutic agents when using CED to treat various disorders within the hippocampus. An understanding of the anatomical influences on infusate distribution is needed to appropriately plan therapies to maximize treatment in the affected area and limit exposure of healthy brain regions.

Dynamic images of the temporal evolution of the contrast agent distribution may give more information regarding the influence of tissue architecture on final infusate distribution. For instance, Krauze et al. (Krauze et al., 2008) have used dynamic MR imaging of the primate brainstem to monitor the ratio of distributed volume (V_d) to infused volume (V_i) to assess the safety of long-term CED and to determine the direction of distribution of their contrast agent, Gadoteridol loaded liposomes. Jagannathan et al. (Jagannathan et al., 2008) used dynamic MR imaging of CED to investigate leakage of Gd-DTPA and Gd-albumin across pial surfaces of the caudate by monitoring the ratio of V_d/V_i during infusion into this structure. Bui et al. (Bui et al., 1999) infused gadodiamide into the rat brain ventricular system and used dynamic MR imaging to follow the transport of the contrast agent throughout the ventricular system and diffusion into adjacent brain tissues.

In recent work (Astary et al., 2010), we used Gd-albumin enhanced MRI to investigate the effect of anatomical structure on distribution profiles after the infusate was delivered to the rat dorsal and ventral hippocampus by CED. These images after infusion indicate that distribution was not uniform, with barriers to transport that showed an apparent disconnect between the dorsal and ventral regions of the hippocampus. However from these end-point images, we are only able to infer the factors that might have contributed to determining the final distribution. To further understand these factors influencing CED delivery into the hippocampus, the use of dynamic MR imaging may provide information about the preferential route of tracer transport, the role of the hippocampal fissures and pial boundaries. In addition, the cause and timing of backflow along the cannula tract can be examined. In this study, real-time dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) was used to observe the distribution of an extracellular tracer, Gd-albumin labeled with Evans blue dye, in the rat dorsal and ventral hippocampus during delivery by

CED. Large volumes of the tracer were infused into both structures to further investigate the apparent disconnect between the dorsal and ventral hippocampus (Astary et al., 2010). For some subjects, inline pressure was also measured throughout the duration of the constant-velocity infusion to demonstrate the feasibility of monitoring direct infusions in biological tissue in real time. Finally, post-infusion, high-resolution T₁-weighted imaging and light microscopy of brain sections provided images of infusate distributions with finer anatomical detail that were used to compare the dynamic distribution measures.

MATERIALS AND METHODS

Infusion System

Gadolinium-diethylene-triamine pentaacetic acid (Gd-DTPA) labeled albumin (Gd-albumin, MW ~ 87 kDa with ~35 molecules of Gd-DTPA per albumin molecule, R. Brasch Laboratory, University of California, San Francisco, CA) tagged with Evans Blue dye was prepared as a tracer for contrast enhancement in MR imaging. Gd-DTPA labeled albumin was evaluated using high performance liquid chromatography to verify that it was not aggregating and the covalent bonds attaching the Gd-DTPA molecules to albumin were intact (Ogan et al., 1987). Phosphate buffered saline (PBS) was used to dilute Gd-albumin to a concentration of 10 mg/mL for optimal contrast enhancement. A 100 μ L gas-tight syringe (Hamilton, Reno, NV) driven by a syringe pump (Cole-Parmer 749000, Cole-Parmer, Vernon Hills, IL) was used for CED. The syringe pump, which is not MR compatible, was placed outside of the MR-room containing the magnet. The syringe was connected to six meters of semi-transparent, chemically inert polyaryletheretherketone (PEEK) tubing (ID = 0.254 mm, OD = 1.5875 mm, Valco Instruments, Houston, TX), then the PEEK tubing was connected to a chemically inert silica infusion cannula. The infusate was loaded into the system and flushed until all visible bubbles were removed. Then, the silica cannula (ID = 40 μ m, OD = 104 μ m, Polymicro Technology, Phoenix, AZ) was implanted into the brain. A two-way valve served as a connection between the syringe and a PEEK tubing adapter to prevent undesired infusion during transportation and handling.

For experiments including pressure measurements (n=4), an additional three-way connector (Valco Instrument, Houston, TX) was placed between the two-way valve and PEEK tubing to couple a fiber optic pressure sensor (FISO Technologies, Québec, CA). Prior to infusion, the transducers were zeroed to obtain baseline intracranial pressure values. The transducers operational range was 60–260 kPa (460–1960 mm Hg), with a resolution of less than 130 Pa (1 mm Hg). Data was acquired using a UMI4 signal conditioner (FISO Technologies, Quebec, CA) at a sampling frequency of 20 Hz. In initial calibration experiments, the cannula tip was placed into a beaker of water at a depth of 4 mm below the surface and in-line pressures were measured at constant flow rate to determine the pressure drop across the infusion system from the transducer to the cannula tip under the given infusion parameters. This measured infusion system pressure drop (6.9 kPa or 52 mm Hg) was subtracted from all steady state pressure measurements taken in tissue to estimate the infusion pressure at the cannula tip.

Animal Preparation and Surgical Procedures

Experiments were conducted on male Sprague-Dawley rats ($n = 6$) weighing 250–350 g using protocols and procedures approved by the University of Florida Institutional Animal Care and Use Committee. Animals were initially anesthetized with xylazine (10 mg/kg, SQ) and isoflurane (4%) in 1 L/min oxygen, and then placed in a stereotaxic frame (David Kopf Instruments, CA), where inhalation anesthesia was maintained (1.5% isoflurane in 0.4 L/min oxygen) throughout the surgery via a nose mask. A heating pad was placed under the rat to maintain core temperature. A mid-sagittal incision was made between the eyes and extended caudally to the level of the ears to expose the cranium. Three millimeter diameter burr holes were drilled into the skull above infusion sites determined using stereotaxic coordinates (Paxinos and Watson, 1998). A silica cannula was connected to an infusion system primed with Gd-albumin with Evans Blue dye prior to implantation. The cannula was then stereotactically inserted into the brain 0.25 mm deeper than the targeted dorsal dentate gyrus of the hippocampus (AP = -3.7 mm, ML = -2.2 mm, DV = -2.75 mm). After 5–10 minutes, the cannula was retracted 0.25 mm and then secured in place using skull fixture adhesive (Cranioplastic, PlasticsOne Inc., Roanoke, VA, USA). This cannula retraction method was implemented to reduce delayed infusion and administration of a bolus injection due to cannula clogging. A second silica cannula was also implanted into the ventral CA1 sub-region on the contralateral side of the hippocampus (AP = -5.88 mm, ML = 5.1 mm, DV = -5.2 mm) in the same manner. A sufficiently large distance separated the two location sites so that there was not overlap of tracer spread. Figure 1A shows the surgical setup for implanting cannulas into the brain. Immediately following the infusion surgery (~2 hours), the animals were transported to the 11.1 Tesla magnet and then placed in a MR compatible stereotaxic frame to secure the positioning of the head and support the placement of the RF coil. Saline (2 mL) was administered subcutaneously to animals to avoid dehydration, and anesthesia was maintained (2 % isoflurane in 1 L/min oxygen) throughout MR imaging (see Figure 1B). Respiration and body temperature were also measured (SA Instruments Inc., Stony Brook, NY) to monitor physiological conditions. A proportional-integral-derivative (PID) air temperature controller was used for maintaining body temperature at 37° C. After animals were placed within the magnet bore, 8 μ L and 10 μ L of Gd-DTPA albumin were infused into the dorsal and ventral hippocampus, respectively, with approximately six meters infusion lines extending from the bore of the magnet to a safe operational distance for the syringe pumps, Figure 1C. At the end of the experiment, animals were euthanized and transcardially perfused with fixative. Extracted brains were stored in 10% paraformaldehyde solution overnight.

MR Imaging and Image Segmentation

In vivo MR images were measured on a Bruker Avance console (Bruker NMR Instruments, Billerica, MA) connected to an 11.1 Tesla, horizontal 40 cm bore magnet system (Varina Inc., Magnex Scientific Products, Walnut Creek, CA). A custom-made 130 degree arc, 3.5 cm rectangular linear-surface coil constructed on a 4 cm diameter half-cylinder was used for linear transmission and detection of MR signal. Before infusion, three transverse T_1 -weighted spin echo images were obtained with a $24 \times 24 \times 10$ mm³ (read $\times$ phase $\times$ slice) field of view (FOV) in a matrix 104×104 with 10 slices with read in the mediolateral

direction to determine the pre-contract enhancement baseline signal in the brain tissue. T₁-weighted measurements were performed with a recovery time (TR) of 330 ms, echo time (TE) of 9.4 ms, and number of signal averages (NA) of 6. During the infusion, T₁-weighted spin echo image measurements were repeated serially to capture the evolution of the infusate distribution. Each scan was obtained with a total acquisition time of ~ 3.5 min and a total of 12 scans were acquired over a 42 min period of time during infusion. Following the infusion, high-resolution T₁-weighted spin echo images (TR = 1000 ms, TE = 15 ms, 30 slices at 0.5 mm thickness, NA = 8, 24 × 24 mm² FOV with 200 × 200 matrix) were acquired to confirm the final distribution pattern.

Dynamic distribution volumes during the time of Gd-albumin infusion and final distribution volumes were calculated by performing semi-automatic image segmentation on the T₁-weighted coronal images using routines written in MATLAB (The MathWorks Inc., Natick, MA, USA). Dorsal and ventral hippocampus infusion volumes were segmented separately with the following specific threshold criterion: Voxels were included in the infusion volume if their signal intensity was at least 6 standard-deviations-of-noise higher than the signal intensity in the corresponding region contralateral to the site of infusion. The segmentation output of the MATLAB routine was refined using the ITK-SNAP open-source medical image segmentation tool (Yushkevich et al., 2006). Dynamic and final distribution volumes in the dorsal and ventral hippocampus were calculated by counting the number of voxels included in each segmented region and multiplying by the volume of a single voxel.

The projected areas of the segmented Gd-albumin final distribution volumes were calculated to provide another means of characterizing the distributions by determining the principle planes of transport of the infusate within the dorsal and ventral hippocampus. The projected areas were calculated by collapsing the segmented volume onto the medial-lateral / inferior-superior (ML/IS) plane, the medial-lateral / anterior-posterior (ML/AP) plane, and the inferior-superior / anterior-posterior (IS/AP) plane. The area of the collapsed segmented volume within each plane was then calculated and compared using a student's t-test for heteroscedastic variance in R (Ihaka and Gentleman, 1996).

Brain Tissue Sectioning

The fixed brain was mounted on a vibratome (Leica VT 1000A, Leica Microsystems Inc., Germany) and cut into 300 μm thick coronal sections. The brain was sectioned 2 mm from the infusion site in both the anterior and posterior direction to obtain all sections where infusate was distributed. The sectioned brain tissue was mounted on glass slides that were observed on an Optixcam microscope (Microscope Store, LLC, Wirtz, VA) to view distributions of Evans blue in the brain slices. MR images of the final Gd-albumin distribution were then qualitatively compared to Evans blue dye distributions.

RESULTS

Infusion sites

Direct infusions were performed in the right dorsal hippocampus (n = 6) and in the left side of the ventral hippocampus (n = 6) of the same animal. The exact cannula position within

the hippocampus was not visible in pre-infusion MR scans due to low resolution of fast T₁-weighted imaging. However, the location of the cannula tip was confirmed from high-resolution MR images (see Figure 2). The cannula position was approximately the same in the medial-lateral and anterior-posterior positions for all subjects. For dorsal infusions, the cannula tip was located in the infrapyramidal blade of the dentate gyrus for half of the subjects. For the remaining subjects, the cannula tip was located in the suprapyramidal blade of the dentate gyrus near the granule cell layer border (for anatomical reference see Figure 3D). For ventral hippocampus infusions, the cannula tip was located in the suprapyramidal blade of the dentate gyrus for half of the subjects and in the CA1 subregion of the hippocampus for the remaining subjects.

Dynamic Contrast Agent Distribution in the Dorsal Hippocampus

Contrast agent distribution, visualized with DCE MR images, was highly dependent on underlying tissue structure (see Figure 3A). Colored markers (blue dots = the molecular layer of the dentate gyrus, red squares = the CA1 of the dorsal hippocampus, green diamonds = the corpus callosum in Figure 3A) indicate the evolution of contrast agent distribution based on the measured dynamic images. Initially, the contrast agent was visible in the dentate suprapyramidal blade and the CA1 region of the hippocampus near the targeted infusion site. Subsequently, it traveled medially along the CA1 field and the molecular layer of the dentate. The contrast agent finally spread to the dentate infrapyramidal blade at the end of the infusion. At the end of the infusion, high-resolution T₁-weighted images showed the final infusate distribution (see Figure 3B), while excised coronal brain slice sections confirmed infusate distribution in aforementioned structures (see Figure 3C). Once contrast agent distributed throughout the dorsal hippocampus, a small amount of contrast agent was also observed to spread to the ipsilateral ventral hippocampus toward the end of the infusion (dashed blue circle in Figure 4).

The presence of contrast agent within fluid filled structures such as the velum interpositum was confirmed in final high-resolution MR images (green dots in Figure 4B). This distribution was also seen in light microscopy images of Evans blue dye (Figure 4C) in the dorsal hippocampus with Evans blue dye seen around the third ventricle, within the velum interpositum and even along the midbrain cistern indicating a connection between these fluid-filled structures. High concentration of contrast agent was also seen within and surrounding the hippocampal fissure (orange squares in Figure 4) in high-resolution T₁-weighted images and for Evans Blues dye in microscopy images of excised tissue.

Dynamic Contrast Agent Distribution in the Ventral Hippocampus

For infusions into the ventral hippocampus, contrast agent initially distributed in the suprapyramidal blade of the dentate gyrus (blue dots in Figure 5A) and traveled along this structure. Infusate spread through the CA1 subfield (red squares in Figure 5A), the granular cell layer near the infusion site and distributed medially toward the center of the brain. However, the degree of contrast agent penetration across this cell layer decreased with distance from the infusion site. Backflow was also observed in the corpus callosum and cortex (green diamonds in Figure 5A). T₁-weighted high-resolution images (see Figure 5B) and Evan's blue images of excised brain sections (see Figure 5C) confirmed the final

distribution pattern in these structures of the ventral hippocampus. A small amount of contrast agent was also observed in the ipsilateral dorsal hippocampus towards the end of infusion (dotted red circle in Figure 6).

Greater enhancement of regions surrounding extraventricular CSF-filled spaces such as the velum interpositum, midbrain cisterns (between the hippocampus and thalamus or midbrain) was observed for sufficiently large infusion volumes (Figure 6, green dots – midbrain cistern, orange squares – hippocampal fissure). DCE-MRI showed contrast agent within the midbrain cistern region (Figure 6A) within 20 minutes of infusion. Pre-signal enhancement in the CSF-filled spaces was also observed due to leakage of contrast agent into the CSF-filled space from the contralateral dorsal hippocampus infusion. High-resolution MR images confirm the presence of contrast agent in the midbrain cistern (Figure 6B) and light microscopy images of excised brain slices indicate the presence of Evans blue dye within the midbrain cistern as well as along the inferior border of the midbrain (Figure 6C).

Final Contrast Agent Distribution

The presence of contrast agent in the tissue and ventricular space was visualized as a hyperintense signal in DCE-MR images compared to surrounding regions. Hyperintense signal was seen in the CA1, CA3 subfield, and the dentate gyrus of the dorsal hippocampus in all subjects (see Figure 2A–F). The contrast agent was confined to the dorsal hippocampus with only a small amount observed in adjacent structures, such as the thalamus and the ipsilateral ventral hippocampus. In the ventral hippocampus infusion (see Figure 2G–L), contrast agent was visualized in the CA1-CA3 subfields and dentate gyrus of the ventral hippocampus in four of six subjects. In two of the subjects, contrast agent was primarily observed in the CA1 subfield and alveus of the hippocampus, likely due to the lateral placement of the cannula tip.

Compared to previous CED experiments that were not conducted during MRI, the occurrence of severe backflow was greater. This increased backflow occurrence was likely due to experimental requirements such as the long infusion line and increased waiting periods needed to perform CED in the magnet bore. Some backflow along the cannula tract was observed toward the end of infusion in all subjects. Three of the dorsal hippocampus infusions resulted in contrast agent leakage into the corpus callosum and cortex (see Figure 2A, B, D). The remaining three dorsal hippocampal infusions resulted in leakage into the corpus callosum (see Figure 2C, E, F). Two out of six ventral hippocampal infusions presented minimal backflow into the corpus callosum toward the end of the infusion, but did not disperse into the cortex (see Figure 2G, I). The remaining four ventral hippocampal infusions resulted in mild backflow into the corpus callosum at the start of infusion, which progressed to leakage into both the corpus callosum and cortex at the end of the infusion (see Figure 2H, J, K, L).

Segmentation for Volume Distribution of Contrast Agent

The contrast agent distributions for both the dorsal and ventral hippocampus infusion were reconstructed as three dimensional volumes. Similar to our previously published results (Astary et al., 2010), significant differences were observed between the shape of the dorsal

and ventral hippocampus final distribution volumes, which reflects differences in tissue structure. Transient contrast agent volume distribution in the hippocampus at various time frames, including volume of backflow and leakage into CSF spaces, were calculated for all subjects. Similar linear increases in total distribution volume were observed after contrast agent was visible in MR scans (see Figure 7). The mean V_d/V_i ratios were 5.51 ± 0.55 and 5.30 ± 0.83 for the dorsal and ventral hippocampus infusion, respectively; with a linear regression R-squared value was over 0.98 for each subject. The mean and standard deviation of final volume distributions from DCE-MR scan were 39.37 ± 3.42 μL after 8 μL dorsal hippocampal infusion and 45.91 ± 9.19 μL after 10 μL ventral hippocampal infusion. Slightly larger final volume distributions were obtained from high resolution T1 weighted scans after infusion (41.8 ± 2.3 μL for the dorsal and 47.40 ± 6.25 μL for the ventral hippocampus). An infusion delay up to ~ 10 min of was observed for each subject due to apparent obstruction of the cannula orifice. Severe delay of infusion was seen for one of six subjects. Mild pre-infusions, or infusion of contrast agent during animal transport and preparation for MRI, were shown in four out of six subjects for the ventral infusion. Backflow along the cannula track resulted in distribution of the contrast agent in regions superior to the cannula tip ($\sim 15\%$ of the total tissue distribution volume) such as the corpus callosum and the cerebral cortex. Final distribution volumes from high resolution scans collected over 30 min after the end of infusion were also analyzed and V_d was approximately 2 μL larger than ones from DCE-MR scans collected earlier. This difference was likely due to tracer diffusion in tissue after the end of infusions.

Projected areas were calculated for each final segmentation volume to determine the preferred planes of transport for dorsal and ventral hippocampus infusions. It is anticipated that infusate transport parallel to the dense granule cell layer would be preferred. Therefore, distribution in the ML/AP plane would be greatest for dorsal hippocampus infusions while distribution in the IS/AP plane would be greatest for ventral hippocampus infusions. For dorsal hippocampus infusions, projected areas in the ML/AP (mean: 25.3 mm^2 , sd: 2.8 mm^2) and IS/AP (mean: 25.6 mm^2 , sd: 4.3 mm^2) planes were both larger ($p\text{-value} < .01$) than in the ML/IS plane (mean: 17.2 mm^2 , sd: 1.8 mm^2). No statistically significant difference was seen between projected areas in the ML/AP and ML/AP planes ($p\text{-value} \sim 0.88$). For ventral hippocampus infusions, the only significant difference between projected areas were found to be between the ML/IS (mean: 22.5 mm^2 , sd: 3.8 mm^2) and IS/AP (mean: 29.7 mm^2 , sd: 4.9 mm^2) planes ($p\text{-value} = 0.02$). No significant difference was found between projected areas in the ML/AP (mean: 23.7 mm^2 , sd: 5.2 mm^2) and IS/AP planes ($p\text{-value} = 0.06$) or ML/AP and ML/IS planes ($p\text{-value} = 0.65$).

Pressure measurement

Inline infusion pressure was measured in a few cases during the infusion with simultaneous T₁-weighted imaging. Mild ($n=1$) and severe backflow ($n=3$) were observed (see Figure 8). For the mild case, the contrast agent started to occupy the dorsal hippocampus after the pressure reached ~ 22 kPa (~ 165 mm Hg), see Figure 8B and infusion pressure stabilized after approximately 12 min, reaching a steady-state. Contrast agent distributed in the dorsal hippocampus with minimal backflow into the corpus callosum, see Figure 8C and 8D. For severe backflow, a significant amount of contrast agent was seen in the dorsal hippocampus,

corpus callosum and the surface of the brain after pressure increased to ~33 kPa (250 mm Hg), see Figure 8B and 8C. There was a large pressure build up followed by a drop toward the end of the infusion (Figure 8D). This pressure history indicated the occurrence of extensive backflow and experiments showing this pressure history were not used in tracer distribution studies (previous sections).

DISCUSSION

In vivo CED experiments were performed in the dorsal and ventral hippocampus to explore the effects of tissue structure on distribution profiles. While a previous study by our group used MR to evaluate final distributions after the end of tracer infusions in the rat brain, this is the first study to look at the temporal evolution of the contrast agent distribution in the rat hippocampus. The advantages of dynamic infusion data include the ability to 1) visualize transport routes within the hippocampus, 2) assess the role of the hippocampal fissure and pial boundaries in determining final distribution profiles, and 3) discern the timing and impact of backflow. In addition, the combination of imaging and pressure data provided useful information relating to the cause of backflow and may provide a method of testing new cannula designs in the future.

Overall, dynamic imaging of infusions into the hippocampus gives additional insight into the various factors influencing CED distributions in complex tissue structures. For instance, the contrast agent was observed to distribute primarily parallel to dense cell layers with limited penetration into these high fluid resistance (low hydraulic conductivity) regions. Additionally, previous studies have shown that contrast agents infused into the extracellular spaces of the brain parenchyma are able to cross pial boundaries and enter CSF spaces (Jagannathan et al., 2008). Contrast agent access to CSF spaces was also observed in this study as infusions into the dorsal and ventral hippocampus resulted in contrast agent uptake in the velum interpositum and midbrain cisterns, respectively. Therefore, the orientation and arrangement of cell layers within a complex tissue structure and its proximity to CSF spaces are important features to take into account when planning CED surgeries and to capture in computational models predicting CED distributions.

Distribution Profiles

High-resolution MR images of final distribution profiles revealed the position of the contrast agent relative to the granule cell layer of the dentate gyrus and the hippocampal fissure – anatomical structures within the hippocampus that were too small to resolve with the time-limited, DCE-MRI protocol. Additionally, light microscopy images of the Evans blue dye distribution in excised brain sections revealed the presence of contrast agent within the pyramidal cell layers of the CA subfields, granule cell layer of the dentate gyrus, and hippocampal fissure. It was observed in DCE-MR images, for infusions into both the dorsal and ventral hippocampus, that the distribution of contrast agent was dependent upon tissue structure as well as backflow along the cannula track. For both infusion sites, Gd-albumin was initially distributed in the region of the cannula tip, the CA1 subfield of the hippocampus. Gd-albumin was then observed to preferentially transport along substructures within the hippocampus. In dorsal hippocampus infusions, Gd-albumin was observed to

spread primarily in the medial-lateral and anterior-posterior directions within the CA1 subregion, molecular layer of the dentate gyrus, and polymorphic layer of the dentate gyrus (see Figures 3A and 4). In ventral hippocampus infusions, Gd-albumin was observed to transport primarily in the inferior-superior and anterior-posterior directions (see Figures 5A and 6), within the molecular layer of the dentate gyrus and CA1 subregion of the hippocampus.

The anatomical structure of the hippocampus may explain contrast agent transport in preferential directions for a given position along the anterior-posterior axis, mediolaterally for dorsal hippocampus infusions and superoinferiorly for ventral hippocampus infusions. 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. Furthermore, this form of hippocampal tissue architecture in the coronal plane is extended in the anterior-posterior direction, as the hippocampus forms a banana-shaped structure in three-dimensions. These regions of cell bodies may present a higher resistance to fluid flow (or lower hydraulic conductivity) than regions comprised mainly of neuronal projections. Therefore, the infusate will tend to flow parallel to the borders of pyramidal cell layers, rather than perpendicular or across dense cell layers, resulting in mediolateral transport in the dorsal hippocampus and superior-inferior transport in the ventral hippocampus. This effect is demonstrated in Figure 5A where Gd-albumin is observed to travel 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. Although the pyramidal cell layers are resistant to flow, they are not impermeable: contrast agent was observed within the cell layers in light microscopy images of brain sections (see Figures 5C and 6C). This was observed in the dorsal hippocampus (see Figure 3A at 20 min 45 sec) as contrast agent spread from the suprapyramidal blade of the dentate gyrus to the polymorphic layer of the dentate gyrus by traversing the granule cell layer. The decreased hydraulic conductivity is demonstrated by the difference in transit times across the granule cell layer and along the molecular layer of the dentate gyrus. Gd-albumin travelled the 0.5 mm across the granule cell layer in approximately 7 minutes whereas it travelled a similar distance within the polymorphic layer of the dentate gyrus in approximately 3 minutes and 30 seconds. Therefore, contrast agent distribution patterns in the hippocampus are dictated, in part, by the configuration of high hydraulic conductivity regions (e.g. layers consisting of predominately neuronal projections) and low hydraulic conductivity regions (e.g. dense pyramidal cell layers).

CSF spaces are another factor postulated to influence the distribution of contrast agents within the hippocampus (Astary et al., 2010). CSF spaces effectively acted as mass sinks since tracers could not be tracked once they reached these regions due to dilution and clearance through these interconnected fluid spaces. The hippocampal fissure, velum interpositum, and midbrain cistern all represent additional regions of interconnected fluid-filled cavities that can function as mass sinks. The hippocampal fissure, a sulcus separating the dentate gyrus from the CA1 field in the hippocampus, traverses the dorsal hippocampus along the medial-lateral axis and the ventral hippocampus along the inferior-superior axis. The velum interpositum and midbrain cisterns are fluid-filled spaces located at the border between the thalamus and dorsal hippocampus, and midbrain and ventral hippocampus,

respectively. Each of these three regions is continuous with ventricular compartments and could act as mass sinks to infusate distributing within the hippocampus. DCE-MR images of the contrast agent infusion did not have sufficient spatial resolution to distinguish enhancement in the hippocampal fissure from enhancement in surrounding structures (e.g. the CA1 subfield of the hippocampus, molecular layer of dentate gyrus). High-resolution images of the final distribution show hypointense signal in the region of the hippocampal fissure with respect to surrounding regions of the infused hippocampus, indicating a lack contrast agent uptake in this region. However, histological images show high concentrations of Evans blue dye at the boundaries of these regions. The same effect was observed for contrast uptake in the granule cell layer of the dentate gyrus as well as the corpus callosum.

Resolution in the DCE-MR images was enough to demonstrate the distribution of contrast agent within the velum interpositum and midbrain cistern. Distribution of contrast agent within the velum interpositum typically occurred at later time points of infusion since this structure is inferior to the cannula tip. As mentioned previously, distribution of the contrast agent in the inferior-superior direction occurred more slowly than in other directions within the dorsal hippocampus. In both dorsal and ventral hippocampus infusions, the contrast agent demonstrated access to fluid-filled spaces, within these tissue structures, that are continuous with the ventricles. Therefore, it is probable that the removal of contrast agent from infused tissue through the action of mass sinks demonstrates that these sinks will impact the distribution of agents delivered by CED in the hippocampus. This could explain, in part, the apparent disconnect between the dorsal and ventral hippocampus despite the large infusion volumes used in this study.

Image Segmentation

Segmentation of DCE-MR images indicates that the distributed volume of contrast agent (V_d) increases linearly with the infused volume of contrast agent (V_i). Furthermore, the slope (V_d/V_i) of this curve is, on average, equal in the dorsal and ventral hippocampus. This finding meets expectations since the underlying tissue structure within these two regions is the same, although the orientation of subregions (e.g. polymorphic layer of the dentate gyrus) may differ. Although V_d/V_i are similar for each subject, the initial rise of V_d occurs at variable V_i due to the delays in infusion discussed in the analysis of inline pressure measurements. It would be expected that infusion into a homogenous porous media would result in V_d increasing linearly with V_i and a slope, V_d/V_i , that is equal to the inverse of porosity of the infused media. Calculating porosity from the V_d/V_i measured in the dorsal and ventral hippocampus yields 0.18 ± 0.02 and 0.19 ± 0.03 , respectively, which agree well with reported measures of rat brain porosity (0.2) (Sykova and Nicholson, 2008). However, it should be noted that porosity estimations from V_d/V_i data in the hippocampus simplify the complex interplay between factors that influence V_d . For example, the porosity in the hippocampus is heterogeneous with potentially lower porosity in dense cell regions (e.g. pyramidal cell layers) than in areas constituted primarily of neuronal projections (e.g. CA1 subfield). Additionally, the pressure field at the cannula tip can cause a local tissue swelling (dilation) which alters porosity. Mass sinks, such as the hippocampal fissure, and backflow can also result in smaller increases in V_d for a given increase in V_i .

Other studies have also noted a linear relationship between V_d and V_i (Jagannathan et al., 2008; Lonser et al., 2002). Interestingly, Jagannathan et al. (Jagannathan et al., 2008) found V_d/V_i to decrease, in the primate brain caudate, when the advancing front of the contrast agent reached ependymal surfaces that border the ventricles. Presumably, the contrast agent was able to cross this boundary and enter the mass sink of the ventricles, which resulted in a decrease in V_d/V_i with increasing V_i . Although similar mass sinks exist within the hippocampus, V_d/V_i remained constant for all time points in this study. This indicates that the contrast agent may enter the mass sinks throughout the entire infusion, in contrast to late time points, when reaching a pial surface. The hippocampal fissure and other mass sinks within the hippocampus may therefore be a factor influencing contrast agent distribution throughout the entire infusion process.

In addition to dynamic V_d data, final distribution volumes were also calculated. Projected area calculations were performed on the final V_d to determine preferential planes of transport for dorsal and ventral hippocampus infusion data. Infusion into an isotropic medium would ideally result in a spherical infusion volume with projected areas that are equal in all planes. Therefore, the projected area calculations give some idea of the anisotropic nature of distributions in the hippocampus. For dorsal hippocampus infusions, projected areas in the ML/AP and IS/AP planes were both significantly larger than in the ML/IS plane. The only significant difference in projected areas for ventral hippocampus infusions occurred between the IS/AP and ML/IS with the projected area in the IS/AP plane being greater. This result agrees with the behavior of the contrast agent noted in the qualitative analysis of dynamic distribution profiles. Namely, the contrast agent travels primarily parallel to borders of dense cell regions (ML and AP directions for dorsal hippocampus infusions, IS and AP directions for ventral hippocampus infusions).

Advantages and Limitations of MR

MR provides a non-invasive tool, with excellent soft tissue contrast, to monitor CED throughout the contrast agent infusion period using dynamic imaging, and allow the evaluation of the final distributions using high-resolution imaging at the end of the infusion. Other imaging modalities, such as autoradiography (Geer and Grossman, 1997; Groothuis et al., 1999) and histology may provide higher resolution than what is available with MR; however, they can only be utilized to evaluate final distribution profiles in excised sliced tissue. As mentioned previously, the limited resolution of dynamic MR images ($240 \mu\text{m}^2$ in-plane) did not allow certain substructures in the hippocampus, such as the granule cell layer of the dentate gyrus or hippocampal fissure, to be identified. Furthermore, resolution of small tissue structures was further reduced due to volume averaging introduced by the 1 mm slice thickness used in DCE-MR images. Image resolution is ultimately limited by SNR. In spin echo imaging, SNR is primarily a function of voxel volume and signal averaging for a given field strength, imaging bandwidth, TR, TE, and instrument factors (e.g. coil tuning, noise in amplifiers). The DCE-MR imaging parameters used in this study were designed to achieve adequate resolution of the contrast agent distribution at suitable SNR while maintaining a temporal resolution high enough to capture the evolution of the distribution. The use of high-resolution T_1 -weighted imaging ($120 \mu\text{m}^2$ in-plane, $500 \mu\text{m}$ slice thickness) of final distribution volumes, which is relatively insensitive to slow diffusion of the large

contrast agent, supplements the dynamic imaging data by providing greater anatomical detail of the contrast agent distribution.

When infusing MR contrast agents, another limitation of MR for evaluating CED specifically is the potentially ambiguous contrast agent distribution in select regions of infused tissue. Previously it was mentioned that, in some instances, light microscopy images seemingly contradict high-resolution T_1 -weighted MR images by indicating presence of contrast agent (e.g. Evans blue dye) in regions that are hypointense with respect to surrounding infused tissue. This effect can be explained by the fact that MR contrast agents generate image contrast by reducing the longitudinal (T_1) and transverse (T_2) relaxation times of surrounding water. If the concentration of contrast agent is too low, T_1 will not be significantly reduced and signal enhancement may not be any higher than signal fluctuations due to noise in the image. Conversely, if the concentration of contrast agent is too high, the T_2 in the infused region will be reduced enough to generate signal loss without enhancement from T_1 reduction. In this study, the contrast agent infusion concentration was optimized based on the contrast agent relaxivity, relaxation times in the hippocampus, and the porosity of brain tissue (assumed to be ~0.2) (Sykova and Nicholson, 2008). The contrast agent may enter large, fluid-filled spaces (e.g. the 3rd ventricle) or fluid-filled spaces that communicate with the ventricles (e.g. the hippocampal fissure) resulting in a significant dilution from the infused concentration. In this case, the concentration of the contrast agent may be too low to generate substantial T_1 shortening and concomitant signal enhancement in T_1 -weighted images. Additionally, the infusion concentration of Gd-albumin was optimized based on average relaxation times within the hippocampus, generating signal enhancement in a majority of this structure. If a select portion of infused regions have significantly shorter T_2 than found on average within the hippocampus, the addition of contrast agent may cause T_2 shortening that results in hypointense signal. This could be the cause of hypointense regions within the granule cell layer and corpus callosum. It must be reiterated that the ambiguous distribution of contrast agent was limited to only a few select regions within the hippocampus and, when coupled with information from light microscopy, did not interfere with the aims of this study – to evaluate how tissue structures in the hippocampus impact the temporal evolution of the contrast agent distribution.

Inline Pressure Measurements

The combined use of dynamic imaging and inline pressure measurements indicate that backflow was most likely due to occlusion of the cannula annulus. This may be explained by first an accumulation of pressure within the infusion line, followed by expulsion of any blockage, then a bolus infusion of contrast agent into tissues due to any build-up of fluid within the long infusion line. Presumably, the initially high infusion pressures, observed in cases of severe backflow, served to clear obstructions at the tip of the cannula. In this case, the transient, high-pressure bolus infusion would be substantial enough to overcome tissue stresses that seal tissue to the cannula surface, and open a low-resistance channel for backflow. Experiments exhibiting this pressure history were not used in distribution studies. On the other hand, for the minimal backflow case, inline pressure increased linearly and then reached an approximately steady-state value as contrast agent was observed to enter the tissue. While infusion pressures were measured in only a few data sets, the pressure data

provided a quantitative understanding of infusion in tissue, while dynamic MR images provided insight into the tissue response throughout the infusion period. Additional inline pressure studies looking at the effects of different needle tip geometries and infusion sites are needed to fully characterize the normal and backflow cases for clinical monitoring of CED.

CONCLUSION

In this study, *in vivo* DCE-MRI was used to monitor the evolution of contrast agent distribution in the dorsal and ventral hippocampus after delivery by CED. Additionally, *in vivo* high resolution MR imaging and *ex vivo* light microscopy of brain sections were used to evaluate the final distribution of the contrast agent while obtaining finer anatomical detail of structures within the hippocampus. Dynamic images of the contrast agent distribution suggest that the spatial composition of low hydraulic conductivity regions (e.g. pyramidal cell layers) and high hydraulic conductivity regions (e.g. CA1 subregion) within the hippocampus influences convective transport in this structure. MR and light microscopy images also indicate that contrast agent infused into the dorsal and ventral hippocampus has access to fluid-filled structures that may act as mass sinks. Segmentation of dynamic distribution images reveals a constant, linear relationship between V_d/V_i indicating that the mass sinks may impact the distribution of contrast agent throughout the entire duration of infusion for the infusion sites used in this study. In addition, inline pressure measurements in a few subjects were performed during infusions demonstrating the potential of these measurements for diagnosing the cause of backflow, predicting the severity of backflow and characterizing the performance of new cannula designs. In summary, distribution profiles in the dorsal and ventral hippocampus following CED at a particular infusion site are primarily influenced by 1) the spatial arrangement of high and low hydraulic conductivity regions, 2) the presence of mass sinks and 3) backflow along the cannula.

Acknowledgments

The project described was supported by award number R01NS063360 from the National Institute of Neurological Disorders and Strokes and Wilder Center of Excellence for Epilepsy Research. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Neurological Disorders and Stroke or the National Institutes of Health. We would like to thank William Triplett for advice and assistance with data processing software, and Drs. Mansi Parekh and Rabia Zafar for providing valuable discussions. The MRI data were obtained at the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility in the McKnight Brain Institute at the University of Florida.

REFERENCES

- Astary GW, Kantorovich S, Carney PR, Mareci TH, Sarntinoranont M. Regional convection-enhanced delivery of gadolinium-labeled albumin in the rat hippocampus *in vivo*. *J Neurosci Methods*. 2010; 187:129–137. [PubMed: 20067808]
- Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH. Convection-enhanced delivery of macromolecules in the brain. *Proc Natl Acad Sci U S A*. 1994; 91:2076–2080. [PubMed: 8134351]
- Bruce JN, Muh CR, Piepmeier JM, Rock JP. Prolonged convection-enhanced delivery into the rat brainstem - Comments. *Neurosurgery*. 2003; 52:393–394.

- Bui JD, Nammari DR, Buckley DL, Inglis BA, Silver XS, Mareci TH, Phillips MI. In vivo dynamics and distribution of intracerebroventricularly administered gadodiamide, visualized by magnetic resonance imaging. *Neuroscience*. 1999; 90:1115–1122. [PubMed: 10218810]
- Cunningham J, Pivrotto P, Bringas J, Suzuki B, Vijay S, Sanftner L, Kitamura M, Chan C, Bankiewicz KS. Biodistribution of adeno-associated virus type-2 in nonhuman primates after convection-enhanced delivery to brain. *Mol Ther*. 2008; 16:1267–1275. [PubMed: 18523450]
- Fiandaca MS, Forsayeth JR, Dickinson PJ, Bankiewicz KS. Image-guided convection-enhanced delivery platform in the treatment of neurological diseases. *Neurotherapeutics*. 2008; 5:123–127. [PubMed: 18164491]
- Foley CP, Nishimura N, Neeves KB, Schaffer CB, Olbricht WL. Flexible microfluidic devices supported by biodegradable insertion scaffolds for convection-enhanced neural drug delivery. *Biomed Microdevices*. 2009; 11:915–924. [PubMed: 19353271]
- Geer CP, Grossman SA. Interstitial fluid flow along white matter tracts: A potentially important mechanism for the dissemination of primary brain tumors. *Journal of neuro-oncology*. 1997; 32:193–201. [PubMed: 9049880]
- Groothuis DR, Ward S, Itskovich AC, Dobrescu C, Allen CV, Dills C, Levy RM. Comparison of 14C-sucrose delivery to the brain by intravenous, intraventricular, and convection-enhanced intracerebral infusion. *J Neurosurg*. 1999; 90:321–331. [PubMed: 9950504]
- Harry GJ, Lefebvre d'Helencourt C. Dentate gyrus: alterations that occur with hippocampal injury. *Neurotoxicology*. 2003; 24:343–356. [PubMed: 12782100]
- Ihaka R, Gentleman R. R: A Language for Data Analysis and Graphics. *Journal of Computational and Graphical Statistics*. 1996; 5:299–314.
- Inoue T, Yamashita Y, Nishihara M, Sugiyama S, Sonoda Y, Kumabe T, Yokoyama M, Tominaga T. Therapeutic efficacy of a polymeric micellar doxorubicin infused by convection-enhanced delivery against intracranial 9L brain tumor models. *Neuro-Oncology*. 2009; 11:151–157. [PubMed: 18755917]
- Jagannathan J, Walbridge S, Butman JA, Oldfield EH, Lonser RR. Effect of ependymal and pial surfaces on convection-enhanced delivery. *J Neurosurg*. 2008; 109:547–552. [PubMed: 18759589]
- Kim JH, Mareci TH, Sarntinoranont M. A voxelized model of direct infusion into the corpus callosum and hippocampus of the rat brain: model development and parameter analysis. *Med Biol Eng Comput*. 2010; 48:203–214. [PubMed: 20033788]
- Krauze MT, McKnight TR, Yamashita Y, Bringas J, Noble CO, Saito R, Geletneky K, Forsayeth J, Berger MS, Jackson P, Park JW, Bankiewicz KS. Real-time visualization and characterization of liposomal delivery into the monkey brain by magnetic resonance imaging. *Brain research. Brain research protocols*. 2005a; 16:20–26. [PubMed: 16181805]
- Krauze MT, Saito R, Noble C, Tamas M, Bringas J, Park JW, Berger MS, Bankiewicz K. Reflux-free cannula for convection-enhanced high-speed delivery of therapeutic agents. *J Neurosurg*. 2005b; 103:923–929. [PubMed: 16304999]
- Krauze MT, Vandenberg SR, Yamashita Y, Saito R, Forsayeth J, Noble C, Park J, Bankiewicz KS. Safety of real-time convection-enhanced delivery of liposomes to primate brain: a long-term retrospective. *Exp Neurol*. 2008; 210:638–644. [PubMed: 18295759]
- Laske DW, Youle RJ, Oldfield EH. Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors. *Nature medicine*. 1997; 3:1362–1368.
- Lonser RR, Corthesy ME, Morrison PF, Gogate N, Oldfield EH. Convection-enhanced selective excitotoxic ablation of the neurons of the globus pallidus internus for treatment of parkinsonism in nonhuman primates. *Journal of Neurosurgery*. 1999; 91:294–302. [PubMed: 10433318]
- Lonser RR, Walbridge S, Garmestani K, Butman JA, Walters HA, Vortmeyer AO, Morrison PF, Brechbiel MW, Oldfield EH. Successful and safe perfusion of the primate brainstem: in vivo magnetic resonance imaging of macromolecular distribution during infusion. *J Neurosurg*. 2002; 97:905–913. [PubMed: 12405380]
- Lonser RR, Warren KE, Butman JA, Quezado Z, Robison RA, Walbridge S, Schiffman R, Merrill M, Walker ML, Park DM, Croteau D, Brady RO, Oldfield EH. Real-time image-guided direct convective perfusion of intrinsic brainstem lesions. Technical note. *J Neurosurg*. 2007; 107:190–197. [PubMed: 17639894]

- Mardor Y, Last D, Daniels D, Shneur R, Maier SE, Nass D, Ram Z. Convection-enhanced drug delivery of interleukin-4 pseudomonas exotoxin (PRX321): increased distribution and magnetic resonance monitoring. *J Pharmacol Exp Ther*. 2009; 330:520–525. [PubMed: 19478131]
- Mardor Y, Rahav O, Zauberman Y, Lidar Z, Ocherashvili A, Daniels D, Roth Y, Maier SE, Orenstein A, Ram Z. Convection-enhanced drug delivery: increased efficacy and magnetic resonance image monitoring. *Cancer Res*. 2005; 65:6858–6863. [PubMed: 16061669]
- Morrison PF, Laske DW, Bobo H, Oldfield EH, Dedrick RL. High-flow microinfusion: tissue penetration and pharmacodynamics. *The American journal of physiology*. 1994; 266:R292–R305. [PubMed: 8304553]
- Ogan MD, Schmiedl U, Moseley ME, Grodd W, Paaanen H, Brasch RC. Albumin labeled with gddtpa - an intravascular contrast-enhancing agent for magnetic-resonance blood pool imaging - preparation and characterization. *Invest. Radiol*. 1987; 22:665–671. [PubMed: 3667174]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. 4th ed.. San Diego: Academic Press; 1998.
- Raghavan R, Brady ML, Rodriguez-Ponce MI, Hartlep A, Pedain C, Sampson JH. Convectionenhanced delivery of therapeutics for brain disease, and its optimization. *Neurosurg Focus*. 2006; 20:E12. [PubMed: 16709017]
- Rogawski MA. Convection-enhanced delivery in the treatment of epilepsy. *Neurotherapeutics*. 2009; 6:344–351. [PubMed: 19332329]
- Saito R, Krauze MT, Noble CO, Tamas M, Drummond DC, Kirpotin DB, Berger MS, Park JW, Bankiewicz KS. Tissue affinity of the infusate affects the distribution volume during convectionenhanced delivery into rodent brains: implications for local drug delivery. *J Neurosci Methods*. 2006; 154:225–232. [PubMed: 16472868]
- Sampson JH, Brady ML, Petry NA, Croteau D, Friedman AH, Friedman HS, Wong T, Bigner DD, Pastan I, Puri RK, Pedain C. Intracerebral infusate distribution by convection-enhanced delivery in humans with malignant gliomas: descriptive effects of target anatomy and catheter positioning. *Neurosurgery*. 2007; 60:ONS89-99.
- Song DK, Lonser RR. Convection-enhanced delivery for the treatment of pediatric neurologic disorders. *J Child Neurol*. 2008; 23:1231–1237. [PubMed: 18952590]
- Sykova E, Nicholson C. Diffusion in brain extracellular space. *Physiol Rev*. 2008; 88:1277–1340. [PubMed: 18923183]
- Thomale UW, Tyler B, Renard V, Dorfman B, Chacko VP, Carson BS, Haberl EJ, Jallo GI. Neurological grading, survival, MR imaging, and histological evaluation in the rat brainstem glioma model. *Child's nervous system : ChNS : official journal of the International Society for Pediatric Neurosurgery*. 2009; 25:433–441.
- Voges J, Reszka R, Gossmann A, Dittmar C, Richter R, Garlip G, Kracht L, Coenen HH, Sturm V, Wienhard K, Heiss WD, Jacobs AH. Imaging-guided convection-enhanced delivery and gene therapy of glioblastoma. *Ann Neurol*. 2003; 54:479–487. [PubMed: 14520660]
- White E, Bienemann A, Malone J, Megraw L, Bunnun C, Wyatt M, Gill S. An evaluation of the relationships between catheter design and tissue mechanics in achieving high-flow convectionenhanced delivery. *J. Neurosci. Methods*. 2011; 199:87–97. [PubMed: 21549753]
- Yin D, Forsayeth J, Bankiewicz KS. Optimized cannula design and placement for convection-enhanced delivery in rat striatum. *J Neurosci Methods*. 2010; 187:46–51. [PubMed: 20026357]
- Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC, Gerig G. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage*. 2006; 31:1116–1128. [PubMed: 16545965]

Highlights

- Dynamic images indicate preferential contrast agent distribution in the hippocampus
- Distributed volumes increased linearly with infusion volume with a constant slope
- Significant leakage into adjacent cerebrospinal fluid spaces was observed
- MRI and light microscopy revealed final distributions with high anatomical detail
- Pressure measurements showed cannula blockage led to pressure increases and backflow

Figure 1.

A) *In vivo* surgical setup for direct infusion in the dorsal and ventral hippocampus of the rat brain. B) Experimental setup for MR Imaging. C) Schematic diagram of *in vivo* infusion system.

Figure 2. Final, high-resolution, contrast-enhanced MR images after CED into the dorsal (A~F) and ventral (G~L) hippocampus for six individual subjects where the contrast agent was infused at a rate of 0.3 $\mu\text{L}/\text{min}$.

Figure 3.

A) DCE-MR images in the coronal plane show preferential transport behavior in the hippocampus at different time frames for the dorsal hippocampus infusion. Colored markers indicate the location of the dentate gyrus (blue dots), CA1 (red squares), and corpus callosum (green diamonds). B) Final distribution in high-resolution T_1 -weighted images. C) Evans Blue dye distribution in rat brain section corresponding to MR imaging slice. D) Schematic of rat dorsal hippocampus adapted from (Paxinos and Watson, 1998). The abbreviations indicate the location of CA1, CA3, hippocampal fissure (hf), dentate gyrus (dg), granule cell layer (gc), velum interpositum (vi), corpus callosum (cc), and ventricles (V).

Figure 4.

A) DCE-MR images for different coronal slices (left to right) at different time frames (up and down) throughout infusion in the dorsal hippocampus. B) High-resolution T₁-weighted images post infusion and C) Evans Blue dye images progressing from anterior to posterior (left to right) after infusion corresponding to MR imaging slices. Green dots indicate the location of the velum interpositum and orange squares indicate the location of the hippocampal fissure. Dashed blue circle indicates a small amount of infusate entering the ventral hippocampus.

Figure 5.

A) DCE-MR images in the coronal plane show preferential transport behavior in the hippocampus at different time frames for the ventral hippocampus infusion. Colored markers indicate the location of the dentate gyrus (blue dots), CA1 (red squares), and corpus callosum (green diamonds) B) Final distribution observed in high-resolution T₁-weighted images. C) Evans Blue dye distribution in rat brain section corresponding to MR imaging slice. D) Coronal slice in a brain atlas corresponding to MR images. D) Schematic of rat dorsal hippocampus adapted from (Paxinos and Watson, 1998). The abbreviations indicate

the location of CA1, CA2, CA3, hippocampal fissure (hf), midbrain cistern (mbc), dentate gyrus (dg), granule cell layer (gc), corpus callosum (cc), subiculum (S) and cortex (ct).

Figure 6.

A) DCE-MR images for different coronal slices (left to right) at different time frames (up and down) throughout infusion in the ventral hippocampus. B) High-resolution T_1 -weighted images post infusion and C) Evans Blue dye distribution in rat brain section corresponding to MR imaging slices. Green dots indicate the location of the midbrain cistern and orange squares indicate the location of the hippocampal fissure. The dashed red circle indicates a small amount of infusate entering the dorsal hippocampus.

Figure 7. Measured infusate distribution volumes in (A) the dorsal hippocampus (n=6) for 8 μL infusion and (B) the ventral hippocampus (n=6) for 10 μL infusions.

Figure 8. Inline infusion pressure profiles and corresponding distribution patterns at given time frames (A, B, C, D) for a minimal backflow case (Case 1) and severe backflow case (Case 2).