

UTILIZING MRI TO MEASURE THE TRANSCYTOLEMMAL WATER EXCHANGE RATE FOR THE RAT BRAIN

JAMES D. QUIRK¹, G. LARRY BRETTHORST¹, JEFFREY J. NEIL²,
AND JOSEPH J. H. ACKERMAN^{1,3}

¹ *Department of Chemistry*

² *Department of Neurology, Division of Pediatric Neurology,*

³ *Departments of Internal Medicine and Radiology,*

Washington University Box 8227

4525 Scott Avenue Room 2313

St. Louis, MO 63110

Abstract.

Understanding the exchange of water between the intra- and extracellular compartments of the brain is important both for understanding basic physiology and for the interpretation of numerous MRI results. However, due to experimental difficulties, this basic property has proven difficult to measure *in vivo*. In our experiments, we will track overall changes in the relaxation rate constant of water in the rat brain following the administration of gadoteridol, a relaxation agent, to the extracellular compartment. From these changes, we will utilize probability theory and Markov Chain Monte Carlo simulations to infer the compartment specific water exchange and relaxation rate constants.

Due to the correlated nature of these parameters and our inability to independently observe them, intelligent model selection is critical. Through analysis of simulated data sets, we refine our choice of model and method of data collection to optimize applicability to the *in vivo* situation.

1. Introduction

Investigators have long attempted to characterize water motion in *in vivo* systems as a way of better understanding basic physiological processes (see [1] for a review of some recent work). These efforts continue, as research in numerous fields is hampered by our incomplete knowledge about how water moves within and between the compartments of a living organism. In magnetic resonance, for example, the behavior of contrast agents used in angiography and our ability to detect and understand the mechanism of stroke through diffusion-weighted imaging are just two of the many areas where this knowledge can be applied.

There are numerous difficulties in making these measurements, including our inability to observe the motion of individual water molecules; we see only their bulk

properties. Studies to measure water motion have been successfully carried out in cell culture and perfused organ systems, but suffer from severe limitations. Most of these methods do not work on intact organisms, and extension of *ex vivo* results to the *in vivo* situation is tenuous. The organism’s normal regulatory systems are not functioning and the cells or organs are bathed in artificial solutions, rather than the extracellular or interstitial fluids. The brain, our system of interest, is one of the most difficult to study as it is physically protected by the skull and the blood brain barrier prevents many blood-borne compounds from entering the brain.

Magnetic resonance (MR) has revolutionized our ability to study the brain and other organs *in vivo* and *in situ* by allowing us to obtain structural and chemical information in a non-invasive and non-destructive way. While the signal in MR comes from the individual nuclei of the water (or other) molecules, we still can only directly observe bulk properties across a region of cubic millimeters, or microns, depending upon the method used and the system or animal being studied.

Our goal is to observe the living rat brain with MR and to devise a method to indirectly measure intercompartmental water exchange. As this will require solving a multi-variable inference problem, we will utilize bayesian probability theory with Markov Chain Monte Carlo (MCMC) methods.

2. Exchange and MR

2.1. EXCHANGE THEORY

Presently, we are concerned with transcytolemmal exchange, the process whereby water molecules cross the cell membrane separating the intra- and extracellular spaces. The rate constant governing exchange (K) is dependent upon direction and the relative volumes of the two spaces. Denoting the intracellular space with the subscript a and the extracellular space with b , we define K_{ab} as the exchange rate constant for water moving out of the cell, K_{ba} as the exchange rate constant for water moving into the cell, and P_a and P_b as the intra- and extracellular water volume fractions.¹ At equilibrium, these quantities are related by:

$$P_a K_{ab} = P_b K_{ba} \tag{1}$$

Differences in the chemical and physical properties of the intra- and extracellular spaces of the brain are important, but our ability to measure these properties is degraded in the presence of exchange. Over the course of such a measurement, some water molecules will sample the environments of both compartments. As a result, we can only detect a weighted average of the compartmental values.

Without the ability to look at individual cells or molecules, we are unable to directly measure the exchange rate and so cannot account for its effects. We must rely upon the exchange contamination of other measurements, the very property

¹The water volume fraction considers only the aqueous volume and differs from the “true” volume fraction, which includes the space occupied by the cellular structure. The water volume fraction is the relevant measure for examining physiologic properties. The small vascular space is ignored for these measurements.

that makes exchange important, to infer what the exchange rate constant must have been. The measurement we chose to look at is the MR longitudinal relaxation rate constant (R_1).

2.2. MR THEORY AND RELAXATION

In magnetic resonance, we manipulate the longitudinal magnetization of a sample (M_z), the Boltzmann excess of nuclei with spins oriented along the direction of the magnetic field, to obtain a signal. Once disturbed from its equilibrium alignment, $M_z(\infty)$, the magnetization will recover in a manner described by the phenomenological Bloch-Equation:

$$\frac{dM_z(t)}{dt} = -R_1 [M_z(t) - M_z(\infty)] \quad (2)$$

Integrating Eq. (2) over the proper limits, we obtain:²

$$M_z(t) = M_z(0) + M_z(\infty) (1 - \exp[-R_1 t]) \quad (3)$$

Measuring the relaxation rate constant, R_1 , is a standard MR experiment, usually accomplished by a process called inversion recovery. In this experiment, the longitudinal magnetization is inverted, [$M_z(t) \rightarrow -M_z(\infty)$], and following a time delay to allow the system to partially recover, the magnetization is measured. After waiting for the system to fully return to equilibrium, the experiment is repeated utilizing different time delay values and the results are fit to Eq. (3).

The R_1 's of intra- and extracellular water are unknown, but are expected to be different as the value is dependent upon the chemical environment. We selectively increase the R_1 of the extracellular space of the brain by injecting gadoteridol,³ a gadolinium-based relaxation agent⁴ which is confined to the extracellular space because it does not cross the cell membrane to enter the cell. This increase in the relaxation rate constant is linear in the concentration of gadoteridol over the range of concentrations we will be using, and the slope of the response is referred to as the relaxivity of the relaxation agent.

As it cannot pass through the blood brain barrier, we must administer the gadoteridol directly into the extracellular space of the brain. An anesthetized rat is placed in a stereotaxic headframe and two needles are inserted into the cerebral ventricles through holes drilled in the skull. Gadoteridol, at concentrations up to 500 mM, is infused at 50 μ L per hour for 1-2 hours. After waiting three hours for the agent to evenly distribute throughout the extracellular space, the rat is studied.

²These equations are usually presented as a function of the relaxation time T_1 , utilizing the substitution $R_1 = T_1^{-1}$. However our later equations will have a more compact form if this substitution is avoided.

³Gadolinium 10-(hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate

⁴As an increase in the relaxation rate constant leads to a tissue-specific increase in signal intensity under most MR imaging procedures, these compounds are also referred to as contrast agents. In this paper, we will use the terms relaxation agent and contrast agent interchangeably.

Magnetic resonance imaging (MRI), enables us to obtain an image of the head and to analyze only the signal coming from a specific region of interest within that tissue. By tracking changes in the R_1 of a region of brain upon administration of varying amounts of gadoteridol, we will infer both the exchange and relaxation rate constants of the *in vivo* brain.

2.3. BLOCH-MCCONNELL EQUATIONS

MR relaxation in the presence of exchange is a well studied phenomenon and the necessary modifications to the Bloch equation were made by Harden McConnell in 1958. These equations, known as the Bloch-McConnell two-site exchange equations, dropping the z subscripts on the magnetization, are:[2,3]

$$\frac{dM_a(t)}{dt} = -R_{1a} [M_a(t) - M_a(\infty)] + K_{ba} M_b(t) - K_{ab} M_a(t) \quad (4)$$

$$\frac{dM_b(t)}{dt} = -R_{1b} [M_b(t) - M_b(\infty)] + K_{ab} M_a(t) - K_{ba} M_b(t) \quad (5)$$

These equations have traditionally been applied to the problem of chemical exchange, when atoms physically move between sites. If these sites are distinct chemical environments (are on different molecules or are non-equivalent positions on the same molecule), they can be independently detected and acted upon via MR.⁵ A selective inversion of one exchanging site will cause the other site to lose intensity initially and then recover as inverted atoms exchange between the two compounds and relax to equilibrium at the two different sites. By tracking the signal change at both sites over time, the exchange rate constant can be easily determined.

When solving Eqs. (4,5), we make a few simple assumptions. Water is assumed to be in equilibrium at the start of the experiment and since a compartment's magnetization is directly proportional to the number of water molecules, we can utilize Eq. (1) substituting M_a and M_b for P_a and P_b respectively. As cells are small compared to the region of study in MRI, we assume that the intra- and extracellular spaces are equally affected by the inversion and therefore the magnetizations remain in their equilibrium proportions immediately following inversion (due to imperfections in the experimental setup, we do not assume that the inversion is complete). Integrating under these conditions, Eqs. (4,5) become:

$$M_a(t) = \left[H(t) + G(t) \frac{K_{ab}}{K_{ba}} \right] M_a(0) + \left[1 - H(t) - G(t) \frac{K_{ab}}{K_{ba}} \right] M_a(\infty) \quad (6)$$

$$M_b(t) = \left[I(t) \frac{K_{ab}}{K_{ba}} - J(t) \right] M_a(0) + \left[J(t) + (1 - I(t)) \frac{K_{ab}}{K_{ba}} \right] M_a(\infty) \quad (7)$$

⁵At high temperatures, chemical exchange becomes fast compared to the timescale of the MR measurement and the two signals begin to merge, destroying their independence.

where:

$$G(t) = \frac{\exp[\alpha_1 t] - \exp[\alpha_2 t]}{u - v} \quad (8)$$

$$H(t) = \frac{u \exp[\alpha_2 t] - v \exp[\alpha_1 t]}{u - v} \quad (9)$$

$$I(t) = \frac{u \exp[\alpha_1 t] - v \exp[\alpha_2 t]}{u - v} \quad (10)$$

$$J(t) = \frac{u v \exp[\alpha_1 t] - u v \exp[\alpha_2 t]}{u - v} \quad (11)$$

$$u = \frac{\alpha_1 + K_{ab} + R_{1a}}{K_{ba}} \quad (12)$$

$$v = \frac{\alpha_2 + K_{ab} + R_{1a}}{K_{ba}} \quad (13)$$

$$\begin{aligned} \alpha_{1,2} = & - \frac{1}{2} (R_{1a} + R_{1b} + K_{ab} + K_{ba}) \\ & \pm \frac{1}{2} \sqrt{(R_{1a} - R_{1b} + K_{ab} - K_{ba})^2 + 4 K_{ab} K_{ba}} \end{aligned} \quad (14)$$

2.4. *IN VIVO* EXCHANGE AND RELAXATION MEASUREMENT

With *in vivo* water exchange the situation becomes far more difficult. Water molecules in the intra- and extracellular spaces are chemically identical and thus have the same MR resonance frequency. As there are presently no known “chemical shift agents” which would selectively shift the water resonance in one compartment relative to the other, allowing their independent detection, our measurements can detect only the sum of the two compartments. We also cannot directly measure the compartment specific R_1 values *in vivo* and, as the relaxation is environment-dependent, the *in vitro* values cannot be used. Finally, the exchange rate constants in Eqs. (6,7) are highly correlated with the relaxation rate constants, making it difficult to differentiate their effects upon a recovery curve.

Before infusion of contrast agent, the observed recovery is mono-exponential and can be well modeled by Eq. (3), as exchange is rapid compared to the difference in the relaxation rate constants of the two compartments. Upon addition of gadoteridol, a second exponential relaxation component begins to emerge in the recovery, requiring the addition of a second term to Eq. (3). In both of these cases, the observed recovery rate constants are complicated functions (α_1 and α_2) of the exchange and relaxation rate constants. As we are interested in separating the effects of exchange and relaxation, we must fit the data to Eqs. (6,7). Due to the increased number of parameters, this will require highly accurate and cogent data and prior information to be successful.

As a consequence, we will acquire data at 32 or 64 logarithmically spaced delay times to enable accurate quantification of a rapidly recovering component and of

exchange (which both have a more pronounced effect on the early timepoints of the recovery curve). Our MR images consist of 64 by 64 pixels, representing a 0.5 centimeter thick cross-sectional slab through the rat brain. Utilizing conventional imaging techniques, our accurate R_1 experiment would require many hours. This has led most imaging studies of relaxation times to utilize either 4-8 different delay times and a simplified version of Eqs. (6, 7), or to utilize short-cuts in image acquisition, introducing distortions into the data which we believe are greater than can be tolerated for our analysis. We will utilize echo planar imaging (EPI), which allows us to collect an entire data set in under six minutes, while introducing artifacts which primarily fall only at tissue-air interfaces and the image edges, away from our area of interest deep inside the brain tissue.

Once the images have been acquired, we will identify the pixels which represent the basal ganglia of the brain and determine their intensity. In most imaging studies, these pixels would be averaged and the regional intensity traced over the various delay times. However, as illustrated in Bretthorst[4], this averaging process assumes reproducibility in the pixels intensities across the image and over time. Due to the heterogeneous nature of living tissue these assumptions do not hold, indicating a joint analysis of the pixels would be more appropriate. We require that the pixels have the same relaxation and exchange rate constants (R_{1a} , R_{1b} , and K_{ab} , K_{ba}), but allow for different signal amplitudes. On simulated mono-exponential recovery data, with inter-pixel intensity variations of up to 15%, we obtain improvements in the accuracy of the rate constant estimation approaching the expected square root of the number of pixels by performing a joint pixel analysis rather than averaging. Given the difficulty of our measurement, we cannot ignore this improvement.

3. Models and their Success

3.1. GENERAL EXPERIMENT AND MODEL

Our general model consists of eight independent parameters: four amplitudes (the initial and equilibrium magnetizations for each of the two compartments) and four rate constants (two relaxation and two exchange rate constants). Due to the correlated nature of these parameters and the non-specific nature of the data, we have chosen bayesian probability theory with Markov Chain Monte Carlo methods to infer the parameter values that best explain the data. The inference procedure is simplified by marginalizing the amplitudes, which are equipment dependent and therefore not of interest.⁶ We are left with calculating the joint posterior probability, $P(R_{1a} R_{1b} K_{ab} K_{ba} | D I)$.

Utilizing bayesian methods allows us to rigorously incorporate any outside information we have about the various parameters into our experiments. From other studies, we know that the intracellular space is larger than the extracellular space, and most likely accounts for 70-85% of the volume. Our relaxation agent

⁶While the equilibrium magnetizations are related to the volume fractions, Eq. (1) allows the volume fractions to be calculated directly from the exchange rate constants.

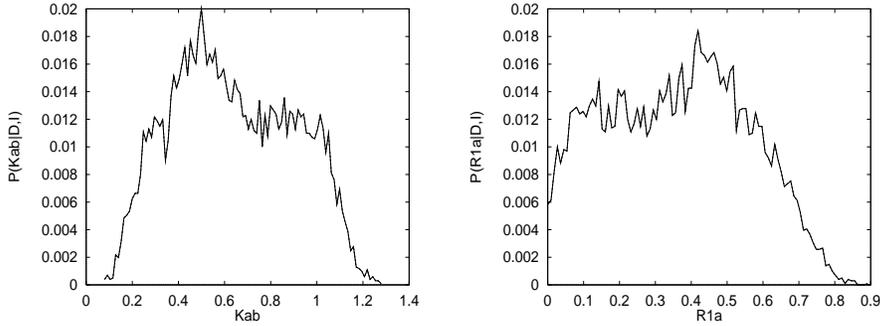


Figure 1. Plots of the posterior probability distributions for K_{ab} and R_{1a} on simulated data utilizing the simple model. Though both plots peak somewhat near the correct values of 0.5, the data supports a wide range of possible parameter values.

can only increase the extracellular relaxation rate constant, R_{1b} , which will be a linear function of gadoteridol concentration. The relaxation rate constant of pure *in vitro* water is approximately 0.25 second^{-1} and the presence of macromolecules *in vivo* will provide additional relaxation pathways, increasing the relaxation rate constant. The relaxation and exchange rate constants are defined to be positive. Finally, our ability to move the system from mono- to bi-exponential recovery upon addition of relaxation agent implies that the exchange rate constants are likely to be small relative to the difference in relaxation rate constants between the two compartments. We therefore place a weak Jeffreys prior on the rate constants and impose a conservative upper bound on the values to save computation time.

We have examined numerous variations on this model and noted where they failed to fit simulated data or gave indeterminate parameter estimates. From these failures, we have improved upon our model and learned more about the types of information we need to acquire in order to obtain a reliable answer. While we have used numerous parameter sets for producing simulated data, all of the results presented here are from data generated utilizing input parameter values of: $P_a = 0.8$, $K_{ab} = 0.5 \text{ seconds}^{-1}$, $R_{1a} = 0.5 \text{ seconds}^{-1}$, $R_{1b} = 1.3 \text{ seconds}^{-1}$, brain gadoteridol concentration = 3 mM , *in vivo* relaxivity of gadoteridol = $4 \text{ seconds}^{-1}\text{M}^{-1}$, and 100 : 1 signal to noise ratio. We will show parameter estimates from only K_{ab} and R_{1a} , as other parameters follow similar trends.

3.2. A SIMPLE MODEL

The simplest experiment of this type involves infusing a known amount of contrast agent into the brain of a rat, collecting a single inversion recovery curve, and analyzing these data with Eqs. (6,7) As might be expected, these data are insufficient to adequately describe the system. Figure 1 illustrates how the parameter values are ill-determined by this analysis and in Figure 2, we see that the parameter values returned by the simulations are highly correlated, indicating an inability to discriminate the relaxation and exchange effects.

Clearly, this simple model is insufficient for our needs and additional infor-

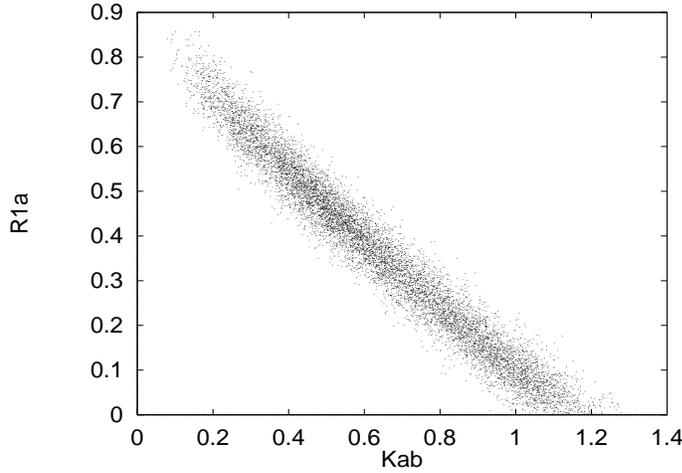


Figure 2. This plot shows the K_{ab} and R_{1a} parameter values chosen by the individual chains in the MCMC simulation for simulated data. The plot illustrates the high degree of correlation between the two parameters, explaining the inability to settle upon a particular value in Figure 1.

mation is required that will break the correlations between the relaxation and exchange rate constants.

3.3. LINEAR MODEL

The underlying assumption that governs our experimental approach is that infusion of contrast agent will only alter the relaxation rate constant of the extracellular space and has no effect upon exchange. By studying rats at a series of contrast agent concentrations, we introduce a dynamic range to one parameter (R_{1b}) while leaving the others constant, possibly breaking their correlation.

In this modified experiment, we infuse different, but known amounts of contrast agent into several rats and collect an inversion recovery curve for each. The data from all of the rats can be jointly analyzed by assuming that each rat has the same parameter values and that they differ only in the concentration of gadoteridol, and therefore R_{1b} . We include the relaxivity of gadoteridol among the model parameters as we are relying on the linearity of changes in R_{1b} upon changes in contrast agent concentration.⁷

Analysis of simulated data with this model appeared promising. Data was generated to represent a single 64 point inversion recovery curve collected from each of 10 rats which had been administered varying doses of gadoteridol. As shown in Figure 3, the posterior parameter estimates are narrowly peaked at the correct values.

⁷Like the relaxation rate constant, the relaxivity of the contrast agent is also dependent upon the chemical environment, so we cannot utilize values obtained *in vitro*.

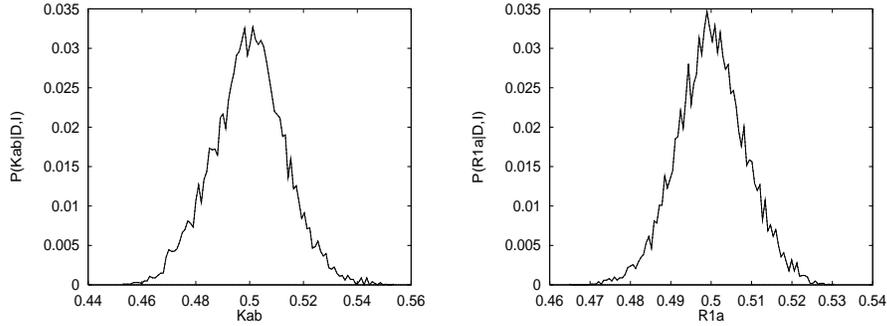


Figure 3. Plots of the posterior probability distributions for K_{ab} and R_{1a} on simulated data utilizing the linear model. Both parameter distributions are centered upon the correct value of 0.5 with a high degree of certainty.

However, when we apply this model to *in vivo* data, it fails to fit the data and returns parameter values that are inconsistent with our outside observations. We believe this is due to the model assumptions that different rats have identical parameter values and that the gadoteridol concentrations are known. As inter-animal variability in physiologic parameters (e.g., heart rate, blood pressure, core temperature, etc.) is well known, it is not unreasonable to assume that variations in the relaxation and exchange rate constants of a few percent could also be present.

Accurate knowledge of the contrast agent concentration is required in this model to enforce the linear response in the relaxation rate constant. Due to difficulties in accurately injecting small volumes into the cerebral ventricles, the final concentration of gadoteridol in the brain is somewhat uncertain. By analyzing cerebrospinal fluid (CSF) withdrawn from the brain, a previous study in our lab noted that following identical injections into different rats, post-experimental variations of up to two-fold were noted in CSF gadoteridol concentrations. By enforcing a linear change in relaxation rate constants in response to our uncertain estimates of gadoteridol concentration, we were assuming a false accuracy in our estimates. It is therefore not surprising that our analysis failed.

To evaluate the effects of unaccounted parameter variability, we generated a data set similar to that used in Figure 3, except that for each of the ten rats, the parameter values used to generate the data were normally distributed about the “true” value with a standard deviation of 2% of the parameter value. Analyzing this data assuming identical parameter values, we obtain parameter estimates which are centered greater than three standard deviations away from the true parameter values, as shown in Figure 4, and were unable to accurately account for all of the data. Residuals from fits to these data were highly systematic and resembled those obtained from the *in vivo* study.

It is possible to incorporate inter-rat parameter variability by allowing each rat to stray by a few percent from the “average” for each parameter value (and for contrast agent concentration). However, this introduces four new parameters for each rat, providing the system with sufficient flexibility that we cannot determine unique, reasonable parameter values, in a similar manner to the simple model.

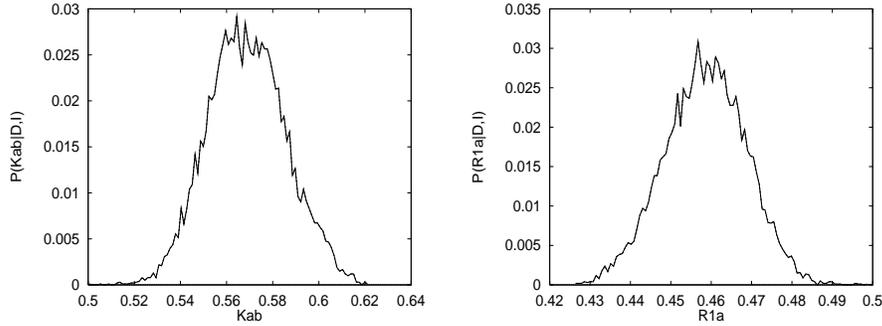


Figure 4. Plots of the posterior probability distributions for K_{ab} and R_{1a} on simulated data with 2% variability utilizing the linear model, which does not account for this variation. The distributions are significantly offset from the true parameter values of 0.5, indicating a failure in the model.

3.4. EXPONENTIAL WASHOUT MODEL

As variation across a dataset of only a few percent can lead to incorrect parameter estimates, we must utilize a model where this variation is minimized. The concentration of gadoteridol in the brain is relatively stable over short periods of time due to the blood brain barrier, which restricts flow out of the brain as well as in. However, over a period of hours, the contrast agent will be slowly eliminated from the brain, reducing its extracellular concentration. This washout should be exponential, as are most biological elimination curves.

By keeping each rat in the MRI and continuously collecting recovery curves over the course of a few hours, we are able to obtain data at a range of gadoteridol concentrations on the same rat. Assuming the rat is physiologically stable while in the magnet,⁸ the various other parameter values should not alter over time. We add to the list of parameters the unknown rate constant for gadoteridol washout, which is well determined as our data are collected at regular and accurately known times.

Data collected from each rat can be analyzed separately, eliminating inter-rat variability in our analysis, and the parameter values obtained can then be averaged across rats to produce overall parameter estimates. Knowledge of the cerebral gadoteridol concentration can improve the accuracy of our estimates, by fixing the beginning or end point of the exponential washout, but is not required for this analysis. Our recovery curves must be collected rapidly compared to the rate of gadoteridol washout, a requirement which was implicitly assumed in the previous models in which contrast agent washout was ignored.

Simulated data from tracking a single rat over two hours were analyzed utilizing this model assuming no information about contrast agent concentration. Results from this analysis are shown in Figure 5 and illustrate that our parameter estimates, though more uncertain than for the linear model, routinely fall within

⁸The rat's temperature and blood gasses can be monitored during this time to verify this stability.

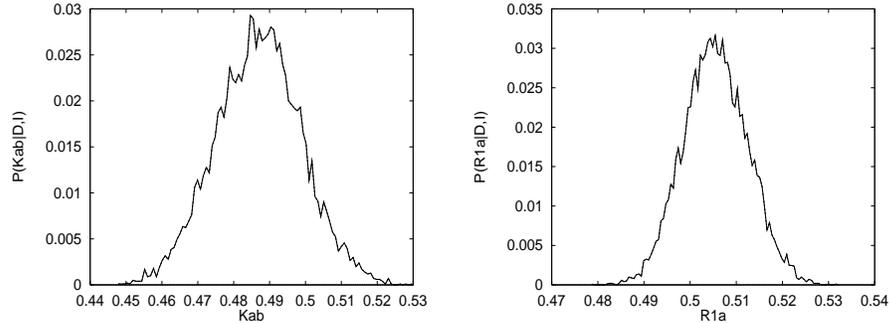


Figure 5. Plots of the posterior probability distributions for K_{ab} and R_{1a} on simulated data from a single rat utilizing the exponential washout model and a typical value of the washout rate. Though not peaked at exactly the input value, the distribution does reasonably cover that value.

a single standard deviation of the correct answer. The accuracy of these estimates is highly dependent upon the washout rate and improves upon averaging across numerous rats and with accurate knowledge of the gadoteridol concentration.

We propose to utilize this model for processing our data. While not perfect, this model suffers neither from the limitations of the simple model nor the inaccurate assumptions of the linear model and avoids difficulty with inter-animal variability.

4. Conclusions

In this paper, we describe our efforts to develop a method for measuring the transcytolemmal water exchange rate in the rat brain utilizing magnetic resonance imaging and Markov Chain Monte Carlo. The problem quickly becomes one of model selection, as we attempt to find an analytical model which is capable of returning accurate parameter estimates while accounting for the variability of *in vivo* measurements. By analyzing simulated data we were able to learn from the failure of simple models to determine the type and accuracy of information needed to perform the real experiment. We have determined that our best chance for success involves a model which analyzes individual rats by tracking the exponential washout of contrast agent from the brain. We hope to soon test this procedure with *in vivo* data.

References

1. A. S. Verkman, A. N. van Hoek, T. Ma, A. Frigeri, W. R. Skach, A. Mitra, B. K. Tamarappoo, and J. Farinas. Water Transport Across Mammalian Cell Membranes. *Am. J. Physiol.*, **270**:C12-C30, 1996
2. H. M. McConnell and D. D. Thompson. Molecular Transfer of Nonequilibrium Nuclear Spin Magnetization. *J. Chem. Phys.*, **26**:958-959, 1957
3. H. M. McConnell. Reaction Rates by Nuclear Magnetic Resonance. *J. Chem. Phys.*, **28**,(3):430-431, 1958
4. G. Larry Bretthorst. *Bayesian Spectrum Analysis and Parameter Estimation*, Volume 48. Springer-Verlag, New York, New York, 1988.