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Temporal and spatial control of neural effects following intracerebral microinfusion

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Abstract
Spatial and temporal control of neural drug delivery is critical for many therapeutic applications and analyses of brain patterns and behavior. Specifically, for localized injections that serve to deliver drug or inactivate an isolated tissue region in order to observe changes in neural activity at that site, excess distribution into surrounding regions may confound analysis or adversely affect healthy tissue. Here, we develop a mass transport model that simulates a short period of initial infusion of inactivating drug, followed by a successive convective wash with artificial cerebrospinal fluid (aCSF), while tracking the regions of tissue that are above a certain threshold concentration of inactivating agent. We analyze the effect of parameters such as effective diffusion coefficient, extracellular volume fraction, and injectate concentration upon spatiotemporal distribution profiles. Further, we observe the effects of following the initial injection with a wash-out period with aCSF upon the breadth of the volume affected by the injectate. These simulations indicate that, by injecting small volumes of drug at low concentrations and following them with an aCSF flush, a well-delineated region of tissue can be altered for a controlled duration.

Keywords: Convection-enhanced delivery, neural inactivation, mass transport, mathematical model

Introduction
Spatial and temporal control of drug delivery to the brain is important for many therapeutic applications. For example, convection-enhanced delivery for cancer therapy requires a concentrated solution of drug injected directly into a tumor site, thus maintaining high concentration in the tumor area but low concentration away from the tumor (Hall et al. 2003; Kioi et al. 2006). Intracerebral microinjection of pharmacologically active agents is also a powerful tool in researching the organization of neural circuits. The behavioral effects of local inactivations produced either by iontophoresis or pressure injection have been used in this way for several decades (Martin 1991; Kurata and Hoffman 1994; Malpeli 1999; Martin and Ghez 1999; Wallace et al. 2001; Edeline et al. 2002; Kita et al. 2006; Wardak et al. 2006). These “temporary lesions” have been used to study the removal or alteration of specific circuits without the confounds of accommodation and adaptation which are known to follow permanent changes in the central nervous system.

The usual aim of an injection is to inactivate a discrete and well-localized section of neural tissue and then to observe changes either in behavior or neural activity at sites far from the injection. The spread of substance outside the intended region complicates the interpretation of effects observed following the injection; thus, spatial and temporal control over the location of effective dose of the drug is critical for well-characterized experiments. Quantitative analyses of the spread of substances in neural parenchyma have indicated that typical injections of as little as a microliter can have a much broader spatial distribution than anticipated, with drug and physiological action of injected muscimol (a commonly injected GABA-A agonist) identified up to 3 mm from the site of an initial injection (Martin 1991; Martin and Ghez...
1999; Arikan et al. 2002; Edeline et al. 2002). This is substantially larger than many of the target zones of injections, thus it is crucial to interpretation to know how far in the brain an effective dose of an injected substance spreads. It is equally important to know the time span over which the agent is exerting a physiological effect: the complications introduced by the unknown spatial distribution of a substance are compounded by variability in the duration of action.

While typical drug delivery to the brain consists of a localized injection of drug causing changes in the activity indefinitely until the drug is inactivated or removed via diffusion into capillaries, a recent attempt to control the temporal duration of drug activity has been shown to produce rapid control of the effect of a pharmacological agent (Kliem et al. 2004). Kliem et al. inserted a recording electrode combined with an injection cannula into the globus pallidus pars interna (GPI). They then injected muscimol at a rate of 0.5 μl/min for 2 min while recording neuronal activity. Immediately following this injection period, neuronal activity in GPI at the recording electrode was completely silenced. After 2 min of infusion, the muscimol injectate was changed to artificial cerebrospinal fluid (aCSF), and muscimol was cleared from the injected zone with a flush of aCSF (0.5 μl/min). Over the next 3 min, the neuronal activity resumed normal levels, demonstrating that careful control can in fact be exerted over the effects caused by a pharmacological agent. However, Kliem et al. did not address changes at sites more distant from the injection site, where it is possible that the injection continued to cause reduced neural activity after the aCSF wash.

In this paper, we model an injection followed by aCSF washout and show that, in principle, it should be possible to pharmacologically alter small areas of tissue for a controlled time interval. We begin with a straightforward series of simulations using well-established equations describing flow and mass transport in biological tissues (Baxter and Jain 1989; Chen et al. 1999). Our approach is to model a similar delivery scheme consisting of an initial injection administered for 2 min, a post-injection diffusion-only period for 2 min, and a subsequent convective aCSF wash-out for up to 150 min. We explored the distribution of injected agent with a particular focus on the region for which the concentration of solute exceeded a threshold concentration. Our simulations indicate that, by injecting small volumes at low concentrations and following these injections with a timed flush, it will be possible to alter the activity of a well-delineated region of tissue for a fixed duration.

**Methods**

The distribution of any substance following injection into tissue such as the brain is governed by standard mass transport and fluid dynamics equations (Baxter and Jain 1989; Morrison et al. 1994; Mak et al. 1995; Walker and Cook 1996; Kalivas et al. 1997; Nicholson 2001; Sarrin and others 2003). These equations describe the changes in concentration arising from (1) convective flow of a solution, (2) diffusion resulting from Brownian motion of solute in the solvent, and (3) irreversible removal or degradation of a solute. Convective flow and diffusion following injection are impeded by cells, such as neurons and glia, and extracellular matrix, thereby limiting transport through the tissue. To account for this effect, we have included a coefficient (α) representing the extracellular volume fraction, which is defined as the fraction of the total volume available to the extracellular fluid (the remaining fractions are cellular volume and extracellular matrix volume) (Nicholson 2001). Here, we model the mass transport in porous media to describe the effect of diffusion coefficient (D_{eff}), extracellular volume fraction (α), injected concentration (C_0), and delivery times on the spatiotemporal distribution of drug injected and followed by aCSF washout.


$$\frac{\partial C}{\partial t} = \frac{\alpha D}{\lambda^2} \nabla^2 C - \alpha v_i \nabla C - \alpha kC. \quad (1)$$

This equation describes the change in drug concentration, C, with respect to time and space, due to the three mechanisms mentioned above: diffusion, convection, and first order degradation. The extracellular volume fraction, α, is reflected in this equation because only a fraction of the overall volume is accessible to the solution as discussed above. Common values for volume fraction in a variety of neural tissues range from 0.18 to 0.3 (Nicholson 2001, Table V). By dividing the entire equation by α and setting $\theta = C/C_0$, a simplified version of the Equation (1) is rendered that is not dependent on extracellular volume fraction except in the velocity as discussed below.

$$\frac{\partial \theta}{\partial t} = \frac{D}{\lambda^2} \nabla^2 \theta - v_i \nabla \theta - k\theta. \quad (2)$$

The first term on the right side of Equation (2) accounts for the change in concentration due to diffusion. Due to the exclusion of solute transport to the extracellular space, a molecule must also traverse a longer path length, thereby affecting the effective diffusion coefficient of a substance. This concept is related by the dimensionless variable tortuosity, λ, and is formally defined by a relation between the diffusion constant of the substance in the solvent and the effective diffusion constant, D_{eff}, observed in the porous media: $\lambda = \sqrt{D/D_{eff}}$ (Nicholson and Phillips 1981; Nicholson 1985; Walker and Cook 1996; Kalivas et al. 1997; Nicholson 2001; Sarrin and others 2003).
Values for tortuosity for a variety of brain regions have been measured by several authors and have been reported by Nicholson (2001). The diffusion coefficient, $D$, is more difficult to measure directly in situ; thus, values are typically measured in agar or agarose gels. Examples of these values are in Table I for a variety of low-molecular weight compounds. For purposes of illustration, we assume that the injectate is muscimol which has a generally accepted $D$ in the brain of $8.7 \times 10^{-6} \text{cm}^2 \text{s}^{-1}$ (Wallace et al. 2001).

The second term on the right side accounts for convective transport of the solute due to bulk fluid flow through the element at a rate $v_i$. The radial velocity $v_i$ in compartment $i$ at a distance $r$ from an injection site is given by $v_i = q_i/4\pi r^2 \alpha$, where the solute is being injected with a volumetric flow rate, $q_i$, over a cross-sectional area dependent on location with respect to the injection point $(4\pi r^2)$. The third term on the right side describes first order degradation/removal, $kC$, of the solute from the solvent at a concentration-dependent rate. This degradation term describes to a first-order approximation such processes as enzymatic degradation, sequestration into glia, binding to membrane proteins, or transport across the blood brain barrier with subsequent removal by capillary flow. This parameter was estimated first using values obtained for the permeation reaction rate constant, $k$, in the literature of 0.008 min$^{-1}$ or $1.33 \times 10^{-4}$ s$^{-1}$ (Martin 1991; Heiss et al. 2005). To confirm these values for our simulation, $k$ was iteratively varied by observing the length of time necessary to obtain a minimally effective dose of muscimol in experiments reported in the literature (Heiss et al. 2005). The value determined using this method ($1.30 \times 10^{-4}$ s$^{-1}$) is almost identical to the literature estimate ($1.33 \times 10^{-4}$ s$^{-1}$), so $1.30 \times 10^{-4}$ s$^{-1}$ is used for $k$ in these simulations.

The Peclet number ($Pe$) can be calculated to determine whether both the diffusion and convection terms are necessary in the model. Since $Pe$ is dependent on the fluid velocity, it is a function of radial distance from the injection site (Welty et al. 2001).

$$Pe = \frac{q_r}{4\pi \alpha D}.$$  \hspace{1cm} (3)

In this study, we model a volumetric flow rate of $0.5 \mu l \text{min}^{-1}$ with values of $\alpha = 0.2$ and $D = 5.0 \times 10^{-6} \text{cm}^2 \text{s}^{-1}$ as a base case. For these conditions, $Pe$ is between 10 and 0.1 at radial positions 0.66 and 66 mm from the center of the injection site, respectively. In this regime ($Pe \sim 1$), both diffusion and convection are important contributors to the mass transport of the drug in the tissue, so both the terms are needed in the equation.

Each of the analyses that follow systematically alters one of the primary variables to illustrate the dependence of solute distribution on that factor. The following values are used for the variables unless otherwise mentioned: $\lambda = 1.6$, $\alpha = 0.2$, and $D = 5.0 \times 10^{-6} \text{cm}^2 \text{s}^{-1}$. These analyses consist of an initial injection of solute $(0 < t < t_1)$, which is followed with a convective flush of aCSF ($t_2 \leq t \leq t_f$). This results in the following initial and boundary conditions:

At $t = 0$: \hspace{0.8cm} $C = 0$ \hspace{0.8cm} $0 \text{mm} < r < 10 \text{mm}$

At $t = 0$ mm (injection site) \hspace{0.8cm} $C = C_0, \quad q_r = 0.5 \mu l/\text{min}$ \hspace{0.8cm} $0 < t < t_1$

At $t = 0$ mm \hspace{0.8cm} $C = C_{1,r-1}, \quad q_r = 0$ \hspace{0.8cm} $t_1 \leq t < t_2$

At $t = 0$ mm \hspace{0.8cm} $C = 0, \quad q_r = 0.5 \mu l/\text{min}$ \hspace{0.8cm} $t_2 \leq t \leq t_f$

where $C_{1,r-1}$ is the concentration in the node adjacent to the injection site at the time step previous to the current time step, and $C_{100,r-1}$ is the concentration in the node adjacent to the outer boundary at the previous time point (thereby establishing no-flux boundary conditions). The time at which solute injection ceases is represented by $t_1$, aCSF infusion begins at $t_2$, and $t_f$ is the final time point in the simulation. In this simulation $t_1$, $t_2$, and $t_f$ are 120, 240, and 9000 s, respectively. At the outer boundary ($r = 10$ mm, chosen to represent the brain size of small animal models), a no-flux boundary condition is established to maintain an accurate prediction of physiological conditions. The number of nodes chosen for the simulation was 100. A sensitivity analysis was used to verify that less than 2% error resulted between this and simulations with 300 nodes. This simulation was solved using the ordinary differential equation solver in Matlab 7.1 (The Mathworks, Inc., Natick, MA, USA), and the spatial derivatives were estimated for the convection and diffusion terms using first- and second-order Taylor series expansions, respectively.

### Results

The overall goal of this work is to develop a customizable injection regime that allows a fine
control over the duration and extent of post-injection inactivation of surrounding healthy brain tissue. The model presented here first predicts the spatiotemporal distribution of locally injected drug. We present many of the results in terms of the extent of tissue for which the concentration of solute is above the threshold for physiological effect. These are the regions where tissue physiology is altered after delivery. Thus, we are analyzing the impact of microinjection in terms of the threshold concentration. To this end, we first analyze the sensitivity of the spatiotemporal distribution and corresponding threshold concentration profiles to changes in $D_{\text{eff}}$ and extracellular volume fraction ($\alpha$). We then assess the effect of varying the initially injected drug concentration ($C_0$) on the transport profile. Based on this analysis, we predict an optimal in vivo injection regime that should allow physiological alteration of tissue in a restricted zone for a predefined time interval.

**Dependence of drug distribution on $D_{\text{eff}}$ and $\alpha$**

For the purposes of illustration, we will be using muscimol injections as an example. The generally accepted threshold for inactivation of tissue by muscimol is 150 nM, a conservative value estimated from five times the IC$_{50}$ measured in vitro (Arikan et al. 2002; Heiss et al. 2005). In the Discussion, we describe the effect on these results if a lower value of activity threshold is set or if response is dose dependent and not a simple threshold. To relate model predictions to this estimation, results in this section are displayed using iso-concentration curves of the pharmacological threshold limit plotted as a function of radial distance from the injection site and time. Typical experiments involve muscimol injections of 0.5–2 μl in a 1 mg/ml solution, or about 8.8 mM (Martin 1991; Kurata and Hoffman 1994; Malpeli 1999; Martin and Ghez 1999; Wallace et al. 2001; Kita et al. 2006; Wardak et al. 2006). Therefore, unless stated otherwise, parameter variation studies were conducted using the following parameters: $\lambda = 1.6$, $\alpha = 0.2$, $k = 1.3 \times 10^{-4} \text{ s}^{-1}$, $D = 5 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, and $C_0 = 8.8$ mM.

The first parameter varied is the $D_{\text{eff}}$. While this value has not been measured for many of the commonly injected pharmacological agents, $D_{\text{eff}}$ tends to scale inversely with molecular weight. The compounds shown in Table I span the ranges of many such agents (e.g. the molecular weight of muscimol is 114, lidocaine = 234, bicuculline = 367). Therefore, the diffusion coefficient ($D$) in Equation (1) was varied between $(1 \times 10^{-5}$, $5 \times 10^{-6}$, $1 \times 10^{-6}$, and $1 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$), corresponding to the range of typical estimated values (Table I). In Figure 1(A), the iso-concentration curves of the pharmacological threshold limit are plotted against radial distance from the injection site and time for this set of diffusion constants. For purposes of illustration, 150 nM was chosen as a threshold for muscimol-driven inactivation based on the results of Heiss et al (2005). A horizontal slice through this figure would show the times at which the concentration first exceeded the threshold, and then again would show when it dropped below threshold at a particular location. A vertical slice would show the region within which the concentration is greater than threshold at a particular time. The area within each iso-concentration trend represents regions at the corresponding times that have a concentration of solute greater than 150 nM, while the area outside of each trend depicts areas where the concentration of solute is less than the threshold concentration.

Figure 1(B) displays the corresponding overall distribution of drug in the tissue region at 2.5 h as a function of radial distance from the injection site. As the diffusion coefficient increases, the drug occupies an increased amount of tissue as measured by the maximum radial extent of the above threshold concentration. Similarly, the region of pharmacologically deactivated ($C > 150$ nM, indicated by the
horizontal line) area at 2.5 h increases with \(D\). The greater diffusion coefficients produce broader overall distributions of drug with corresponding increases in the breadth of peak concentration. In the case of the greatest diffusion coefficient, \(1 \times 10^{-6} \text{cm}^2 \text{s}^{-1}\) (solid gray line), a concentration greater than the threshold limit of 150 nM occurs 1.09–6.93 mm from the catheter tip, producing 1.39 cm\(^3\) of physiologically altered tissue.

As the diffusion coefficient decreases, the overall spatiotemporal distribution decreases, and the size of inactivated regions generally decreases as well. For instance, when the diffusion coefficient is \(1 \times 10^{-6} \text{cm}^2 \text{s}^{-1}\), the distribution of drug resulting in inactivation (\(C > 150 \text{nM}\)) fills a spherical shell 2.57–6.34 mm from the injection site (volume = 0.996 cm\(^3\)). At a lower diffusion coefficient, \(D = 1 \times 10^{-7} \text{cm}^2 \text{s}^{-1}\), inactivation occurs between 3.36 and 5.64 mm, resulting in an overall inactivated volume of 0.592 cm\(^3\). At diffusion coefficients this low, the dominating force becomes convection (\(Pe > 10\) at all radii less than 33 mm), so diffusion has little impact on the transport of the substance both to and from the delivery region; therefore, not as great of a volumetric difference is observed by further decreases in the diffusion coefficient. Furthermore, it is important to note that, due to the high initially injected concentration of 8.8 mM, even 2.5 h post-injection there is a large region above threshold concentration (\(C > 150 \text{nM}\)) for all cases.

The variation in effective diffusion coefficient is related by the path length available for the solute to traverse, expressed by the dimensionless variable tortuosity (\(\lambda\)), described above. In this case, varying \(\lambda\) between typical experimentally determined values from the literature of 1.5, 1.6, and 1.7 results in a variation of effective diffusion coefficient between \(2.22 \times 10^{-6}\), \(1.95 \times 10^{-6}\), and \(1.73 \times 10^{-6}\), using a baseline value of \(D = 5 \times 10^{-6} \text{cm}^2 \text{s}^{-1}\) (Nicholson 2001). Since smaller tortuosity values correspond to a shorter path length for the molecules to traverse through tissue, as expected, variations in this parameter lead to slightly more rapid distribution of the solute as \(\lambda\) decreases (data not shown). Mainly due to the negligible effect on the effective diffusion coefficient, values for \(\lambda\) spanning the expected values in most areas of central nervous system (Table I) do not have a major impact on the overall behavior of the injected solute.

The effect of extracellular volume fraction, \(\alpha\), on the distribution of diffusible agents is shown in Figure 2. When this value varies with a two-fold increase in available volume for the movement of the solute (i.e. \(\alpha = 0.15, 0.2, 0.3\)), the distributions after 2.5 h for the greater values of \(\alpha\) exhibit a decrease in radial distance traversed. This suggests that errors in measurement of volume fraction will have a slight impact on the overall behavior of a solute in the extracellular environment. For values spanning 0.15, 0.20, and 0.30, the volume for which concentration is greater than 150 nM spans 1.68–7.92, 1.39–7.33, and 1.09–6.34 mm, respectively. In terms of the volume of tissue expected to be inactivated, this translates to 2.06, 1.64, and 1.06 cm\(^3\) for these values of \(\alpha\). As the porosity of the network increases, more space is available for the solute to distribute, thereby lessening the region, where concentration is greater than the threshold.

**Dependence of drug distribution on initial concentration**

Figure 3(A) presents the overall transport profile through the tissue at time points during injection, immediately post injection during the diffusion-only time period, and during the extended infusion of aCSF only. After the initial injection of drug (120 s), a typical infusion profile is seen: a concentration equal to \(C_\circ\) near the injection site which rapidly decreases to zero concentration at greater radii. At 240 s, after two minutes of diffusion between injection and washout, a slight decrease in the concentration near the injection site is observed due to diffusion and removal. Infusion of aCSF begins at 240 s and, by 260 s, the concentration near the injection site decreases rapidly to zero. The bell-shaped curve shows the movement of drug further away from the injection site. At subsequent times (300, 600 s), the amplitude of this bell shape (fraction of initial concentration) decreases as drug is driven through the tissue. Eventually (600 s), the amplitude of the bell shape falls below the threshold required for drug efficacy. Figure 3(B) shows this information on a logarithmic \(y\)-axis (fraction of initial concentration) to better show the relationship of the actual concentration to the threshold concentration required for drug activity. This view of the data shows that the time required for the concentration to fall below the 150 nM threshold is heavily dependent on the initial concentration. The maximal concentration decreases below...
the threshold after 600 s (black diamonds) for $C_0 = 0.88 \, \mu$M, after 3000 s (black circles) for $C_0 = 8.8 \, \mu$M, but never falls below the threshold for $C_0 = 8.8 \, \mu$M.

This relationship can be also seen in Figure 4, which depicts the iso-concentration curves for radial distance and time as a function of initial concentration. In this case, initial concentration was varied among 0.88, 8.8, and 8.8 mM. When the initial concentration is 8.8 mM (as used in the above simulations), the volume of tissue that remains inactivated (greater than the 150 nM threshold) after 2.5 h is maximal, occurring between 1.39 and 7.33 mm, corresponding to a volume of 1.64 cm³. In comparison, when the initial injection concentration is 8.8 or 0.88 μM, there is no tissue remaining inactivated after 34 and 8 min, respectively. However, the maximum distance of inactivated tissue for 8.8 μM occurs from 1.39 to 2.48 mm (1.09 mm) and from 1110 to 1160 s. For the 0.88 μM concentration, maximum inactivation occurs from 0.99 to 1.39 mm (0.4 mm) at 400 s. The corresponding maximum inactivated volumes for the 8.8 or 0.88 μM concentrations are 0.054 and 0.007 cm³, respectively.

It should be especially noted that, while sites close to the injection site are reactivated and resume neuronal activity, sites further away can remain inactivated for long periods, especially at high initial concentrations (e.g. 8.8 mM). Therefore, the lower initial injection concentration cases, where activation resumes after a short time period, are desirable as all of the tissue resumes normal neuronal activity after a short washout time. These results provide a delivery scheme by which, by varying the initial concentration and combining a convective wash, it is possible to constrain the pharmacological impact of the injected agent (in this case, inhibition induced by injected muscimol) both in time and space.

**Discussion**

Microinfusion of pharmacological agents is a common tool in neuroscience, yet control is lacking over both the spatial distribution and the timing of changes induced by the infusions. Precise interpretation of the results of local inactivations will require much finer control over both parameters. Our simulations have suggested first that typical injection protocols are likely to inactivate substantial volumes of tissue and to leave them inactivated for a long time. Many injections of muscimol reported in the literature involve between 0.5 and 2 μl at 1–10 mM [for representative cortical and subcortical injection regimes (Kurata and Hoffman 1994; Kita et al. 2006; Wardak et al. 2006)]. Our simulations indicate that even with a flush, this regime could leave 1.64 cm³ of tissue inactivated. Thus, we expect that more dilute injections will provide finer control over both the spatial and temporal inactivation profiles resulting from microinfusion.
As with all modeling, there are several simplifying assumptions made in this analysis. Most notable among these is the assumption that mass transport in the tissue is isotropic. Diffusion coefficients are often isotropic in brain tissue, even in structures like the corpus callosum (for detailed listings from various structures and species, see Nicholson (2001)). However, convection is often anisotropic as is observed, for example, when backflow from striatal microinfusions tends to follow fiber tracts in white matter (Chen et al. 1999). Thus, while convection is anisotropic in white matter, diffusion remains largely symmetrical. This is in agreement with previous observations of spherical distribution of many agents after infusions on the order of single microliters (Hupe et al. 1999; Malpeli 1999; Nicholson et al. 2000; Edeline et al. 2002; Heiss et al. 2005). It would be straightforward to incorporate anisotropy into the velocity term by setting a pressure boundary condition at the injection site, rather than a flow rate, and define an anisotropic permeability for the tissue. For the purposes of this study, however, we seek to create a general model that is applicable to drug delivery in general rather than a specific injection site. Thus, we compute the model assuming radial symmetry with 1-dimensional (radial) convection and diffusion while neglecting mass transport in the dimensions perpendicular to the radial direction, which should be accurate in isotropic tissue.

Tortuosity has limited effect until the pore sizes controlling diffusion begin to approach the size of the diffusing particles. The motion by which particles diffuse is Brownian, and thus it tends to follow tortuous paths in any case. In the case of nonisotropic media in the brain such as white matter tracts, the resistance to bulk flow is much smaller along the tracts than across them, but the pore size is still several orders of magnitude larger than the molecules themselves in both directions. Thus, the anisotropy controlling permeability to bulk flow is unlikely to have much effect on tortuosity, thus little effect on $D_{eff}$.

Another notable assumption is that of the threshold concentration for muscimol activity. The effective concentration that we chose for these analyses (150 nM) is derived from a study in which muscimol was infused steadily over a period of 5 days and animals were assessed for neurological symptoms (Heiss et al. 2005). The animals in that study, rhesus macaques, showed no symptoms at concentrations below 150 nM. By contrast, in a study that combined electrophysiology with direct visualization of bound muscimol, changes in neural activity were observed for concentrations as low as 10 nM (Arikan et al. 2002). Since the study of Arikan et al. assessed only bound muscimol concentrations, we used the more conservative infused concentration estimate of 150 nM for the threshold value. This observation, however, does raise questions about the use of a threshold concentration and a classification of effects as binary. It is clear from Arikan et al. that infusions will produce more subtle effects than simply turning off neuronal activity.

In this study, however, the assumption of a threshold for drug activity does not change the underlying data from the model. As shown in Figures 1(B), 3(A) and 3(B), the simulations provide concentrations of drug at all points and times. It is only in the interpretation of these data that it was helpful to set a threshold concentration to generate iso-concentration plots and inactivated tissue volumes, so simulations could be compared. Thus, treating the effect as having a simple threshold effect provides a good first approximation, but the fluid modeling used in this effort provides all of the concentration with respect to space and time information necessary to analyze the data with dose-dependent activity rather than a simple threshold.

A final notable assumption is the treatment of the infusion point as a point source with uniform spread in the radial direction. In actual fact, the initial convective flow is likely to align somewhat with the cannula for two reasons: the injectate leaves the cannula as a vector flow and the path of least resistance tends to be back along the cannula (see Heiss et al. (2005) for elliptical inactivation fields caused by these effects). While our model does not capture these elements of flow, the general principles (much wider spread of effective concentration than expected and limited ability to control timing of effects) remain for the typical use of microinjection as a means of introducing inactivations in the central nervous system.

With all of these considerations, it remains that, as a general model, current methods for pharmacologically altering the activity of neuronal systems by microinfusion are likely to have much more widespread effects over a much longer time period than is generally acknowledged. This caution has been raised previously (Hupe et al. 1999; Malpeli 1999; Edeline et al. 2002; Heiss et al. 2005), but here we are able to quantify this phenomenon and predict a simple solution: dilute the infused agent to a concentration much closer to the physiological threshold. This should have two effects. First, since the concentration falls rapidly with the infusion radius, the area affected by the infusion can be more carefully limited spatially. Second, for the same reason, washing out the highly diluted material reduces the concentration of the solute to a value below threshold concentration much more quickly. Thus, this method also provides a means to control the timing of a pharmacological manipulation.

**Conclusion**

In this study, we find that, while the spread of substances has a reasonable degree of dependence on the diffusion constant and thus varies from one
substance to the next, for most substances the diffusion (although not the convection) is relatively independent of the geometric constraints that are known to vary from one cerebral region to another. Second, we found that, with a few simple assumptions, we could estimate the time-course and spatial extent of tissue that will experience altered physiology following a microinfusion. Speaking generally, the most commonly applied microinfusion regimes produce regions of altered physiology much larger than expected, and these alterations tend to persist for a very long time, i.e. many hours. Finally, based on these results, we have predicted an injection scheme that can be used to inactivate a well-demarcated region of neuronal tissue for as little as a few minutes.

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