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Designing *in vivo* concentration gradients with discrete controlled release: a computational model

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Abstract

One promising neurorehabilitation therapy involves presenting neurotrophins directly into the brain to induce the growth of new neural connections. The precise control of neurotrophin concentration gradients deep within neural tissue that would be necessary for such a therapy is not currently possible, however. Here we evaluate the theoretical potential of a novel method of drug delivery, discrete controlled release or DCR, to control effective neurotrophin concentration gradients in an isotropic region of neocortex. We do so by constructing computational models of neurotrophin concentration profiles resulting from discrete release locations into the cortex and then optimizing their design for uniform concentration gradients. The resulting model indicates that by rationally selecting initial neurotrophin concentrations for drug-releasing electrode coatings in a square 16-electrode array, nearly uniform concentration gradients (i.e., planar concentration profiles) from one edge of the electrode array to the other should be obtainable. Discrete controlled release therefore represents a promising new method of precisely directing neuronal growth *in vivo* over a wider spatial profile than would be possible with single release points.

Keywords

neuroengineering; tissue engineering; brain repair; drug delivery, discrete controlled release; computational model, neurotrophin; nerve growth factor; NGF

1. INTRODUCTION

The delivery of exogenous growth factors or neurotrophins to induce new neural growth and connectivity represents a promising therapy for some disorders of the central nervous system (Schabitz, Berger et al. 2004; Yasuhara, Borlongan et al. 2006). Neurotrophins induce neurite extension when they are detected by receptors in neuronal membranes (Campenot 1977; Lykissas, Batistatou et al. 2007). Several of these molecules have been used to induce artificial growth of neural processes both *in vitro* (Kimpinski, Campenot et al. 1997; Khademhosseini, Langer et al. 2006) and *in vivo* (Garofalo, Ribeiro-da-Silva et al. 1992; Grider, Mamounas et al. 2005). While sophisticated techniques have been developed for controlling neurotrophin concentration gradients *in vitro* (Bellamkonda, Ranieri et al. 1995; Cao and Shoichet 2001; Kapur and Shoichet 2004), similar techniques are generally lacking *in vivo* except under special geometric constraints (Kemp, Walsh et al. 2007). The development of a technique to establish and maintain arbitrary neurotrophin concentration

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profiles deep within the central nervous system has the potential to enable a new class of neural therapies.

Neurotrophins are proteins used by the central and peripheral nervous systems to promote cellular processes related to development, cellular differentiation, circuit formation, regeneration, repair and neural plasticity (Lewin and Barde 1996; Skaper 2008). Mammalian neurotrophins include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4). These macromolecules achieve their native action by diffusing through the extracellular space and activating cell surface receptors. The spatiotemporal distribution of neurotrophins, therefore, can have tremendous influence over nervous system structure and function. Artificial manipulation of neurotrophins or of other soluble molecules that activate the same receptors has the potential to induce desirable effects on the nervous system and/or prevent undesirable effects.

Under the right conditions, axons will generally extend toward diffusible chemoattractant molecules and away from chemorepellant molecules (Braisted, Tuttle et al. 1999; Tucker, Meyer et al. 2001), even in adults (Isacson and Deacon 1996; Isacson and Deacon 1997; Kimpinski, Campenot et al. 1997; Oudega and Hagg 1999). The axons are able to do so by detecting concentration gradients across their growth cones, which contain a spatially arrayed collection of receptors (Mueller 1999). Axon growth cones presumably perform a spatial differentiation on the concentrations of relevant signaling molecules (Goodhill 1998; Goodhill and Urbach 1999; Mortimer, Feldner et al. 2009). The relative spatial distributions (i.e., concentration gradients) of chemoattractant and chemorepellant signals play an important role in axonal guidance during normal development (Bagnard, Lohrum et al. 1998; Bagnard, Thomasset et al. 2000). By manipulating the concentration gradients of such molecules artificially (and possibly the context in which such signals are interpreted by the target neuronal population), axonal extension may be controllable even in adult brains.

The approximate concentration range and minimal gradient effective at inducing PC12 cell neurite extension has been evaluated *in vitro* with a two-compartment diffusion device capable of establishing linear concentration gradients of NGF (Cao and Shoichet 2001). This device allowed precise experimental control of both total concentration and concentration gradient and revealed that the experimental cells could detect a gradient as small as $133 \text{ ng mL}^{-1} \text{ mm}^{-1}$. Furthermore, the receptors appeared to saturate at a total concentration of 995 ng mL^{-1} , such that no directional cue would be detectable by these cells at higher total concentrations. For this model steady-state system, then, the maximum theoretical range over which NGF could induce directional neurite extension is $995/133 = 7.5 \text{ mm}$. The maximum empirical range of directional neurite extension for this system was only 5 mm , which, although less than the theoretical maximum, was considerably greater than the value that had been previously reported under similar experimental conditions (Goodhill 1997). The likely reason for these relatively long effective distances lay in the well-controlled linear concentration gradient in this study, which allowed for the longest possible distance over which growth could be directed with soluble factors. These findings imply that control of neurotrophin concentration gradients leads to precisely directed neuronal growth over relatively large distances. More recent studies indicate that, in addition to the gradient magnitude, the effectiveness of local concentration profiles in promoting directional growth of neuron can also be dependent upon the absolute concentration of neurotrophin (Li, Liu et al. 2008; Mortimer, Feldner et al. 2009). In such cases the maximum effective distance over which neurite extension could be controlled might be decreased.

Given the potential therapeutic value of creating controlled concentration gradients, other methodologies of creating such profiles have been explored. Microfabricated hydrogels or similar fixed substrates, for example, can be designed with arbitrary concentration gradients

of a wide variety of compounds across their extent (Bellamkonda, Ranieri et al. 1995; Khademhosseini, Langer et al. 2006; Musoke-Zawedde and Shoichet 2006; Li, Liu et al. 2008). Microfluidic systems also have the potential to create user-determined concentration gradients using fluid flow principles (Yang, Yang et al. 2002; Khademhosseini, Langer et al. 2006; Saadi, Rhee et al. 2007). While hydrogels typically immobilize the compounds of interest within a matrix, microfluidic systems rely upon steady-state fluid mixing behavior to produce their desired effects and must be continuously refreshed. Gradient “printing” can also create arbitrary concentration patterns in thin gels (Rosoff, McAllister et al. 2005). Point sources (Utley, Lewin et al. 1996) or single line sources of controlled release (Williams, Holecko et al. 2005) are unlikely by themselves to provide sufficient flexibility and/or scalability to apply tissue-wide without further considerations.

The systems described above for establishing and maintaining a desired concentration gradient *in vitro* all face substantial problems creating a similar effect *in vivo*. While controlling a concentration gradient inside an organ using these methodologies may be possible in certain special instances (i.e., a concentration gradient orthogonal to an accessible surface), a general framework for achieving desired concentration gradients in solid tissue has yet to be developed. Creating concentration gradients parallel to an organ surface appears to be particularly challenging with existing technology. Release of soluble compounds from polymer-coated penetrating microelectrode arrays has been proposed as a means of influencing tissue in predictable patterns (Utley, Lewin et al. 1996; Williams, Holecko et al. 2005, Mark Saltzman, personal communication). If one extends this idea to allow for independent variation of initial soluble compound concentration on each of the electrodes, then manipulation of this parameter in addition to the electrode array geometry should allow systematic concentration gradient manipulation parallel to on organ surface. Penetrating microelectrodes are a mature technology used routinely in the brains of many species, and the effects of arrays of such electrodes on brain tissue is an actively investigated phenomenon (Polikov, Tresco et al. 2005). A controlled-release device with similar or identical characteristics as microelectrodes would therefore have the advantage of creating a relatively well-understood tissue interaction.

In this study we develop the quantitative theory behind *discrete controlled release* or DCR, which we propose as a technique useful for inducing desired concentration gradients deep within tissue with a finite number of geometrically arranged release points. The resulting models suggest that by designing proper initial conditions, it may be possible to achieve directed neuronal growth within the confines of the delivery system.

2. METHODS

2.1. Attractant-Only (AttOn) System

In order to assess the feasibility of establishing desired concentration gradients with a discretely controlled system, we formulated a computational model for an array of slender rods mimicking the shanks of penetrating electrodes releasing nerve growth factor (NGF) *in vivo* along their entire shank. The brain region being modeled is an isotropic region of cortical gray matter, such as within an architectonically uniform region of neocortex. The actual release system being utilized in our laboratory uses ethyl vinyl acetate copolymer (EVAC) loaded with NGF and polymerized directly on the electrode shanks (Hoffman, Wahlberg et al. 1990; Walsh, Kim et al. 1995). The theoretical development here, however, should be generally applicable to a variety of release systems configured according to the indicated geometries. The electrodes modeled are 200 μm diameter tungsten-epoxy metal microelectrodes used daily in our lab for electrophysiology recording and readily available from FHC (Bowdoin, ME). In our model, these electrodes were arranged in a 4 $\times$ 4 square

with an inter-electrode distance of 1000 μm (figure 1). All solutions for NGF concentration are given for the steady-state condition.

NGF serves as a convenient model chemoattractant for this study because it has been shown to promote neurite outgrowth in the direction of its largest concentration gradient (Moore, MacSween et al. 2006). The half-life of NGF delivered directly into the brain has been estimated to be 0.5–1 hour, with overall distribution closely matching the values predicted from first-order kinetics (Krewson, Klarman et al. 1995; Krewson and Saltzman 1996; Stroh, Zipfel et al. 2003; Mahoney, Krewson et al. 2006). All computational models and simulations in this study were formulated under that assumption that the chemical(s) of interest follow first-order kinetics, which would be applicable to NGF as well as a wide variety of other molecules. The model excludes a convection term because convection appears unlikely to be a major contributor to NGF redistribution within the brain (Krewson, Klarman et al. 1995; Krewson and Saltzman 1996). From these studies, the NGF diffusion coefficient is taken to be $4 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ and the elimination rate constant is taken to be $2.5 \times 10^{-4} \text{ s}^{-1}$ for brain parenchyma (Krewson and Saltzman 1996). To simplify the analysis, the flux across the electrode boundary is assumed to be constant with time at steady state, and the brain tissue is assumed to be a spatially infinite sink for diffusion. These assumed conditions suggest that the computational model can be formulated as a steady-state, nonconvective NGF diffusion problem with first-order kinetics, which could represent reasonable assumptions for many molecules besides NGF.

From the generalized statement of conservation of mass with linear elimination we have

$$\nabla \cdot \vec{N}_A + \frac{\partial C_A}{\partial t} = R_A = -kC_A, \quad (1)$$

in which $\vec{N}_A$ represents the molar flux of NGF, C_A represents the concentration of NGF as a function of time and position, and k represents the elimination rate constant of NGF within the target region of the brain. From Fick's law,

$$\vec{N}_A = -D_{Ae} \nabla C_A + C_A \vec{V}_M, \quad (2)$$

where $\vec{V}_M$ is the molar average velocity of the brain fluid and D_{Ae} is the diffusion coefficient of NGF in the brain. The assumptions of steady-state, non-convective flow can be employed to yield

$$-D_{Ae} \nabla^2 C_A = -kC_A \Rightarrow \nabla^2 C_A = \frac{k}{D_{Ae}} C_A. \quad (3)$$

Equation (3) can be expressed using the common definition of the Thiele modulus as

$\phi^2 = \frac{kR^2}{D_{Ae}}$, thus yielding

$$\nabla^2 C_A = \frac{\phi^2}{R^2} C_A. \quad (4)$$

It can be seen from equation (4) that the net concentration profile of NGF is linear and thus may be represented as a superposition of individual concentration profiles established by

each NGF-releasing electrode. Let us make a simplifying assumption that NGF flux along each electrode shank is everywhere equal. The major consequence of this assumption is that the concentration of NGF in the brain would only vary in a plane perpendicular to the axis of the stimulating electrodes (i.e., it would be constant with depth below the brain surface). This condition would be most closely approximated at depths near the midpoint of each electrode, and could be imposed at deeper depths by lengthening electrode shank lengths. In solving the concentration profile established by each electrode, this assumption, combined with the inherent rotational symmetry of the stimulating electrodes, allows us to express the concentration of NGF as a function of distance from the center of an electrode to a point of interest, r , measured in a plane perpendicular to the electrode shaft. Let $\vec{r}$ be a position vector pointing from the origin of the coordinates situated at the center of the electrode array (figure 1B). The net concentration of C_A at an arbitrary location $\vec{r}$ established by N electrodes may then be expressed as

$$C_{A,net}(\vec{r}) = \sum_{n=1}^N C_{A,n}(\vec{r}), \quad (5)$$

where $C_{A,n}(\vec{r})$ represents the concentration profile contributed by the n^{th} electrode at $\vec{r}$. Now let $\vec{r}_n$ represent the positional vector from the origin to the center of the n^{th} electrode in the array. Hence, $|\vec{r} - \vec{r}_n|$ represents the distance between the n^{th} electrode and a point of interest. Solving for the concentration profile established by a single electrode using the boundary conditions $C_{A,n}(|\vec{r}|=R) = C_{A0,n}$ (a constant) and $C_{A,n}(|\vec{r}| \rightarrow \infty) = 0$, as well as equation (4), gives

$$C_{A,n}(\vec{r}) = C_{A0,n} \frac{K_0\left(\frac{\phi}{R}|\vec{r} - \vec{r}_n|\right)}{K_0(\phi)}, \quad (6)$$

where K_0 is the zero-order, modified Bessel function of the second kind. Note that equation (6) represents a generalized concentration profile established by a single NGF-coated electrode with surface NGF concentration of $C_{A0,n}$ and radius R . Let p_n represent the concentration of NGF at the surface of the n^{th} electrode: $p_n = C_{A0,n}$. It follows, then, that

$$C_{A,n}(\vec{r}) = C_A(|\vec{r} - \vec{r}_n|) = p_n \frac{K_0\left(\frac{\phi}{R}|\vec{r} - \vec{r}_n|\right)}{K_0(\phi)}. \quad (7)$$

Finally, combining equation (5) with equation (7) yields

$$C_{A,net}(\vec{r}) = \sum_{n=1}^N p_n \frac{K_0\left(\frac{\phi}{R}|\vec{r} - \vec{r}_n|\right)}{K_0(\phi)}. \quad (8)$$

Equation (8) presents a generalized formulation of concentration profile at location $\vec{r}_n$ established by an electrode with constant surface concentration p_n . Note that both p_n and $\vec{r}_n$ represent potential design parameters of the electrode array that may be modified to establish a desired concentration profile. Because commercially available electrode arrays typically have fixed electrode configurations with limited variation, however, the electrode

surface NGF concentrations $\vec{p} = \langle p_1, p_2, \dots, p_N \rangle$ represent the primary modifiable parameters for the modeled controlled release system. In order to model the NGF release by the 4×4 microelectrode array in our design, all non-design parameters in equation (8) must be specified. Using values of the electrode radius, diffusion coefficient, and elimination coefficient given earlier, the value of the Thiele modulus ϕ was calculated to be 0.25. In order to simplify the modeling process, the length scale was normalized to the radius of the electrode such that, in the model, unit length represents 100 μm . Hence, for all the simulation results to follow, the inter-electrode distance is set to 10 units.

2.2. Attractant-Repellent (AttRep) System

One speculative, but logical extension of the AttOn system presented above is a DCR system that is capable of releasing more than one type of compound simultaneously. Such a release system could, through combinatorial effects on the target cells, amplify or refine the desired behavior. Relevant compounds in the current context may be broadly classified as chemoattractants or chemorepellents that can induce neuronal growth up or down their concentration gradients, respectively. Neurons are naturally exposed to concentration gradients of multiple growth cues during development in a fashion that may be at least partially reproducible by artificial means (Bagnard, Thomasset et al. 2000; Li, Liu et al. 2008). Hence, to fully assess the theoretical potential of a DCR system in directing neurite extension, we also considered a model of a DCR system that utilizes both a chemoattractant, such as NGF, and a hypothetical chemorepellant. The intention of this purely theoretical system was to explore the feasibility of creating effective gradients using DCR, both generally for broad applications but also specifically in the current context for creating smooth, linear growth cues to induce unidirectional neuronal extension.

In formulating a model of the combined effect of a chemoattractant and a chemorepellent, we assumed that the effect of these chemicals at each location combines to give rise to an effective concentration C_{eff} , and it is this concentration profile that neurons ultimately follow. Hence, we assume that C_{eff} is some function of the concentration of chemoattractant C_A and chemorepellent C_R , or $C_{eff} = f(C_A, C_R)$. Under this formulation, the simplest form of interaction between C_A and C_R is if C_{eff} is a linear combination of chemoattractant and chemorepellent, or $C_{eff} = aC_A - bC_R$, where a and b can be taken to be the relative strength of chemoattractant and chemorepellent, respectively. The chemorepellant in this model is assumed to be an idealized chemical that affects neurons in precisely the opposite manner of the chemoattractant. In order to simplify the analysis, we further assumed that the chemorepellant is released from the same electrode surface as the chemoattractant and that neither of the chemicals interferes with the other's mass transfer process. In this attractant-repellent (AttRep) system, the Thiele modulus for the chemorepellant electrode and concentration assignments present $N+1$ additional parameters relative to the AttOn system. In actuality, only the electrode surface concentrations represent modifiable design parameters, and thus the AttRep system will have a total of 32 degrees of freedom.

Let $\vec{q} = \langle q_1, q_2, \dots, q_N \rangle$ represent the chemorepellant electrode surface concentrations. The effective concentration can then be expressed as

$$C_{eff}(\vec{r}) = \sum_{n=1}^N (p_n C_{A,n} - q_n C_{R,n}) = \sum_{n=1}^N \left(p_n \frac{K_0 \left(\frac{\phi_A}{R} |\vec{r} - \vec{r}_n| \right)}{K_0(\phi_A)} - q_n \frac{K_0 \left(\frac{\phi_R}{R} |\vec{r} - \vec{r}_n| \right)}{K_0(\phi_R)} \right), \quad (9)$$

where ϕ_A and ϕ_R represent the Thiele modulus for chemoattractant and chemorepellant, respectively. Note that the coefficients reflecting relative attractant and repellent strength, a

and b , are absorbed by the concentration assignment values p_n and q_n . For the current study the value of ϕ_R was arbitrarily chosen to be 10 times larger than the value of ϕ_A . It should be noted that positive values of C_{eff} indicate net attraction while negative values indicate net repulsion.

2.3. Optimization Scheme

As noted earlier, in order to maximize the effectiveness of the DCR system in directing neuronal extension preferentially in one direction, the established concentration gradient should be as close as possible to a uniformly planar gradient oriented towards the preferred direction of growth. Among various parameters of the model, only electrode surface NGF concentration values will be manipulated to alter the concentration profile established by the system. Hence, the goal of this procedure is to find the set of initial electrode surface NGF concentrations $\vec{p} = \langle p_1, p_2, \dots, p_N \rangle$ that would give rise to the most planar concentration gradient directed toward the preferred direction. To perform a systematic search for the

optimal parameter vector $\vec{p}$, an appropriate cost function $B(\vec{p})$ must be developed that can assess the resemblance of the established concentration profile to the ideal planar concentration gradient. To be useful, the value of the cost function should become smaller as the established concentration gradient approaches a unidirectional, planar concentration gradient, and should yield its absolute minimum value when the concentration gradient is uniformly planar. Once such a cost function is established, the optimal value of $\vec{p}$ can be obtained approximately by minimizing the value of $B(\vec{p})$ under electrode surface concentration adjustments using the method of gradient descent.

Let $\vec{a}_d$ represent the preferred direction of neuronal extension. Since the growth will be preferentially toward the direction of steepest increase in NGF concentration, the direction of growth at location $\vec{r}$ is given by

$$\vec{v}(\vec{r}) = \nabla C_{A,net}(\vec{r}) = \sum_{n=1}^N p_n \nabla C_{A,n}(\vec{r}). \quad (10)$$

Intuitively, the local effectiveness of the concentration profile in directing neural growth towards $\vec{a}_d$ can be quantified by

$$\alpha(\vec{p}, \vec{r}) = \vec{a}_d \cdot \vec{v}(\vec{r}) = \sum_{n=1}^N p_n \vec{a}_d \cdot \nabla C_{A,n}(\vec{r}). \quad (11)$$

Since the value of $\alpha(\vec{p}, \vec{r})$ would increase as more local concentration gradient vectors point toward $\vec{a}_d$, the local cost function, $\beta(\vec{p}, \vec{r})$, is taken to be negative of $\alpha(\vec{p}, \vec{r})$:

$$\beta(\vec{p}, \vec{r}) = -\alpha(\vec{p}, \vec{r}) = -\sum_{n=1}^N p_n \vec{a}_d \cdot \nabla C_{A,n}(\vec{r}). \quad (12)$$

Finally, the local cost function may be spatially summed to yield the net cost function

$$B(\vec{p}) = \iint_D \beta(\vec{p}, \vec{r}) dA = - \iint_D \left(\sum_{n=1}^N p_n \vec{a}_d \cdot \nabla C_{A,n}(\vec{r}) \right) dA, \quad (13)$$

where D represents the region of interest (i.e., within the bounds of the array) over which the planar concentration gradient is to be established. Employing the linearity of the integral operator,

$$B(\vec{p}) = - \sum_{n=1}^N \iint_D (p_n \vec{a}_d \cdot \nabla C_{A,n}(\vec{r})) dA. \quad (14)$$

If we let $d_n = \iint_D \vec{a}_d \cdot \nabla C_{A,n}(\vec{r}) dA$, then equation (14) becomes

$$B(\vec{p}) = - \sum_{n=1}^N p_n d_n = \vec{p} \cdot \vec{d}. \quad (15)$$

The form of equation (15) indicates that $B(\vec{p})$ is effectively minimized simply by maximizing the value of P_n for which $d_n = \max_m d_m$. This result implies that the cost function in equation (15) is minimized by allocating all of the initial NGF load to the electrode(s) with the maximum value of d_n while setting the initial load for the rest of the electrodes to 0. Our aim of establishing a cost function that is minimized by a planar concentration gradient obviously is not achieved in this case, particularly with larger electrode arrays. This problem can be circumvented by normalizing the net gradient by its magnitude. The cost function in equation (15) can be revised in such a way to give

$$\hat{v}(\vec{r}) = \frac{v(\vec{r})}{|v(\vec{r})|} = \frac{\sum_{n=1}^N p_n \nabla C_{A,n}(\vec{r})}{\left| \sum_{n=1}^N p_n \nabla C_{A,n}(\vec{r}) \right|}. \quad (16)$$

Using the redefined direction field specified by equation (16), equations (13) and (14) can then be modified to become:

$$\hat{\beta}(\vec{p}) = - a_d \cdot \hat{v}(\vec{r}) = - \frac{\sum_{n=1}^N p_n \vec{a}_d \cdot \nabla C_{A,n}(\vec{r})}{\left| \sum_{n=1}^N p_n \nabla C_{A,n}(\vec{r}) \right|} \quad (17)$$

$$\widehat{B}(\vec{p}) = \iint_D \widehat{\beta}(\vec{p}, \vec{r}) dA = - \iint_D \left(\frac{\sum_{n=1}^N p_n \vec{a}_d \cdot \nabla C_{A,n}(\vec{r})}{\left| \sum_{n=1}^N p_n \nabla C_{A,n}(\vec{r}) \right|} \right) dA. \quad (18)$$

Finally, the intermediate cost function in equation (18) may be normalized with respect to the area of interest and then shifted, yielding

$$\bar{B}(\vec{p}) = 1 + \frac{\widehat{B}(\vec{p})}{A} = 1 - \frac{1}{A} \iint_D \left(\frac{\sum_{n=1}^N p_n \vec{a}_d \cdot \nabla C_{A,n}(\vec{r})}{\left| \sum_{n=1}^N p_n \nabla C_{A,n}(\vec{r}) \right|} \right) dA. \quad (19)$$

Note that $0 \leq \bar{B}(\vec{p}) \leq 2$, based upon the definition given in equation (19). It should be also be noted, however, that the cost function as given in equation (19), designated as cost function $\widehat{A}$, is only dependent upon the directions of the established concentration gradients and not upon their magnitudes, as would be desired (figure 2A). An infinite number of solutions exists in such a case, including unidirectional yet non-uniform concentration profiles (figure 2B).

Alternatively, this cost function can be normalized with respect to the greatest concentration gradient magnitude to yield

$$\widehat{B}(\vec{p}) = 1 - \frac{1}{A} \frac{\iint_D \left(\sum_{n=1}^N p_n \vec{a}_d \cdot \nabla C_{A,n}(\vec{r}) \right) dA}{\max_{\vec{r}} \left| \sum_{n=1}^N p_n \vec{a}_d \cdot \nabla C_{A,n}(\vec{r}) \right|}, \quad (20)$$

designated as **cost function $\widehat{B}$** . The problem of finding the optimal surface concentration parameter may be reformulated as the problem of finding $\vec{p}$ that minimizes cost function $\widehat{B}$. It should be noted that the scaling of $\vec{p}$ by a constant factor would have no effect on either of the cost functions. That is, $B(k\vec{p}) = B(\vec{p})$. Hence, an additional constraint of the forms $\sum_{n=1}^N p_n = c$ or $\max p_n = c$ may be imposed upon the optimization problem without restricting the solution set further. In formulating the optimal planar concentration profile that can be established by the model, the method of constrained gradient descent will be employed to solve for the value of $\vec{p}$ that would minimize cost function $\widehat{B}$.

All computation and simulations for the current study were performed using MatLab software (Mathworks, Natick, MA) unless otherwise specified. In particular, the optimization was performed with the Optimization Toolbox using the constrained optimization function with a variable step size (Bryoden 1970). Cost function $\widehat{B}$ employed here presents only one cost function, specifically designed to create planar concentration profiles. One could readily extend the DCR system model to be optimized with other and perhaps more complicated cost functions. For example, a cost function for non-planar concentration profile may be readily formulated should a non-planar concentration profile be desired (Li, Liu et al. 2008; Mortimer, Feldner et al. 2009). For each case of optimization performed, at least 30 trials of optimization were performed with random initial points, and the trial that yielded the absolute minimum cost value was reported. Doing so decreases the possibility of inadvertently settling in a local minimum of the cost function instead of the desired absolute minimum.

2.4. Assessment of Directional Preference

Due to the lack of arbitrary rotational symmetry, the DCR system might be expected to exhibit behavior that is a function of the preferred direction of concentration gradient. We assessed the directional preference of the system by observing the change in minimally attainable cost value for a wide range of preferred directions. As can be seen from figure 1, the 4×4 square electrode array has a rotational symmetry around the center point with the angle of 90 degrees. Furthermore, it has a reflective symmetry with respect to the 45° line. Based upon these symmetries, unique minimum cost values exist only for preferred directions ranging between 0° and 45° (or between 45° and 90°, as was actually calculated). The minimum cost values for 30 distinct directions evenly spaced from 45° and 90°, inclusive, were used to generate polar plots of maximal gradients.

2.5. Discretization of Concentration Assignments

In the cost function given in equation (20), the values of assigned concentrations vary on a continuous scale, which may result in as many initial concentration values as there are electrodes. It is more practical, however, to have only a limited number of initial concentrations to limit the number of unique electrode coatings that must be prepared. We therefore discretized the initial concentration value to limit the total number of values necessary and investigated the effect of this quantization upon the steady-state concentration profile by manually selecting specific concentration values and assigning each electrode with one of the selected values. The resulting steady-state concentration profiles were compared directly with profiles that resulted from the optimization process, and the change in the cost value was evaluated to assess the degree of reduction in the effectiveness of the quantized system in establishing a planar concentration profile.

3. RESULTS

The effectiveness of the DCR system in approximating the ideal planar concentration gradient was assessed by running the optimization using cost function $\widehat{B}$ for 16 electrodes ($N=16$) arranged in a square 4×4 grid. Figure 3 shows the attractant-only system optimized with preferred direction of 90° or $\langle 0 \ 1 \rangle$. Unless otherwise stated, optimization was performed at least 30 times for each direction, and the run with the minimum cost value was recorded. As can be seen from figure 3, the optimization under cost function $\widehat{B}$ assigns substantial nonzero concentration values to the top two rows.

3.1. Attractant-Only System

In order to assess the capability of the attractant-only system in establishing a planar concentration profile in a variety of directions, the concentration assignment to each of 16 releasing electrodes was simultaneously optimized using cost function $\widehat{B}$ as above, but this time the preferred gradient direction was varied between 90° and 45° in 15° increments. For each preferred gradient direction tested, the optimization was run 30 times and the run with the minimum cost value was recorded. Once again, only a single chemoattractant molecule is included in the AttOn model. The resulting gradients can be seen in figure 4A–D. It is apparent that the projected steady-state gradient direction rotates along with the target gradient direction. Patterns beyond 45° of rotation are symmetric with those shown and are therefore not depicted. The precise pattern of initial concentrations is dependent upon the desired gradient angle, and a desired angle of 45° results in more electrodes with nonzero initial concentrations (figure 4E–H).

The mean cost of the AttOn system as a function of desired angle is 0.75. The local inhomogeneities in concentration gradient immediately surrounding the electrodes, as seen

in figure 4A–D, contribute substantially to this cost value. The cost of the AttOn system appears to be relatively robust with respect to desired angle, however, implying that such system should do equally well at establishing desired concentration gradients in any orientation with a square electrode array. This capability would be expected to improve with larger electrode array sizes.

3.2. Attractant-Repellent System

The effectiveness of the AttRep system in establishing a planar concentration profile was assessed by observing the form of established concentration profiles as the concentration assignments of attractant and repellent were varied. For the 16-electrode array, 32 total concentration assignments must be made, although there are only 31 free parameters due to the scale independence of the concentration values. The optimization results for several preferred gradient directions are summarized in figure 5. As expected, the resulting effective concentration gradients are more nearly uniform than those of the AttOn system for all orientations of the desired concentration gradient directions. This difference is seen explicitly in figure 6, which compares the cost values for the two systems as a function of desired gradient direction. The mean cost value for the best AttOn system is 0.747 while the mean cost value for the best AttRep system is considerably enhanced at 0.212, an improvement corresponding to the visible decrease in local concentration gradient inhomogeneities in figure 5A–D. The remaining local concentration gradient inhomogeneities apparent in the AttRep system are more variable as a function of desired gradient orientation, however, as reflected in the greater deviation from a circle of the former mean cost as a function of desired direction in figure 6. Nevertheless, the AttRep system overall performed considerably better than the AttOn systems in creating a nearly planar concentration profile at any desired gradient orientation, largely because in this particular formulation it was able to smooth out local concentration gradient inhomogeneities.

3.3. Discretization of Concentration Assignments

The nature of the optimization routine allowed these initial concentration values to vary continuously over a range of values. The disadvantage of this procedure in practice is that it could lead to a large number of distinct initial concentrations needed to achieve the desired steady-state concentration profile. Symmetry arguments alone imply that fewer initial concentrations than the full number of electrodes should be required. In order to determine the smallest number of distinct initial concentration values needed to obtain a reasonable steady-state concentration profile, the distribution of normalized initial concentrations was determined from 60 AttRep concentration assignment vectors. These vectors were sampled uniformly from several desired gradient orientations ranging from 0° to 90°, with the parameter assignments yielding the minimum cost over 30 iterations being recorded for each sampled direction (figure 7). A clustering of initial concentration values is apparent in these histograms near normalized values of 0, 0.4, and 1 for the chemoattractant and 0, 0.15, and 0.4 for the chemorepellant. This finding implies that desired performance might be achievable for a 16-electrode square array with a relatively small number of initial concentration values. The same analysis for the AttOn system reveals clustering around normalized concentration values of 0, 0.5 and 1.

To test this hypothesis, new AttOn and AttRep systems were created with only the two nonzero values of initial chemoattractant concentration/chemorepellant concentrations. An optimized AttOn system with an orientation toward 90° is depicted in figure 8A,B. The cost value of this system is 0.746. A discretized version of this system is depicted in figure 8C,D, with a cost value of 0.778. An optimized AttRep system with an orientation toward 90° is depicted in figure 8A,B. The cost value of this system is 0.239, indicating performance

consistent with the minimum of many runs depicted graphically in Figure 6. Replacing the optimized initial concentration values with only two nonzero values for each molecule results in a qualitatively similar steady-state concentration gradient with a modestly increased cost value of 0.307 (figure 8C,D). Most of this decrement is likely attributable to the identical configurations of rows 2 and 3 of the array, and allowing two additional concentration values results in a nearly equivalent cost function between the optimized and discretized systems (not shown). Additionally, two unique nonzero initial concentrations are sufficient to achieve discretized AttOn system performance on par with optimized systems. Altogether, these findings imply that only a relatively small number of unique electrode coating procedures need be conducted in order to obtain desired concentration profiles.

4. DISCUSSION

The aim of the current study was to assess the plausibility of establishing a unidirectional, uniform concentration gradient in three-dimensional tissue using a novel drug-delivery system we term discrete controlled release (DCR). Essentially, a geometric array of microelectrodes coated with a synthetic polymer releasing neurotrophic factors was simulated computationally as chemical releasing points with constant chemical flux across the polymer surface. In particular, we modeled two types of systems inserted into isotropic neocortex: an attractant-only system (AttOn) and an attractant-repellent (AttRep) system. While the AttOn system was modeled after a particular chemoattractant neurotrophin (NGF), the AttRep system presents a generalization that incorporates a hypothetical chemorepellent in addition to the chemoattractant. The AttOn system is a subset of the AttRep system with all repellent concentrations set to zero ($\vec{q}=0$). The main findings imply that as long as interactions between chemoattractant and tissue are accurately modeled and as long as constant flux exists at the electrode surface, nearly planar concentration profiles (equivalent to nearly uniform concentration gradients) in tissue located within the extent of a coated electrode array can be established. The gradients established represent steady-state solutions independent of the exact methodology of chemical release.

Simulation results for the AttOn system suggest that a relatively planar concentration profile can be established in a desired direction. It is also noteworthy that the performance of the AttOn system was largely indifferent to the desired direction of the concentration gradient. The presence of local concentration gradient inhomogeneities near the releasing electrodes, however, resulted in a substantial increase in the cost value for this system relative to the AttRep system and also reduced the smoothness of the established concentration profile. Local concentration gradient inhomogeneities would likely reduce the distance over which neuronal extension could be directed effectively because as the neural processes approach the vicinity of the electrodes, their growth direction could be deflected toward the center of the nearest electrode. Surprisingly, the overall AttOn system performance was relatively unaffected by the desired direction of the concentration gradient. This directional insensitivity of the AttOn system may be explained by positing that the presence of local concentration gradient inhomogeneities effectively masked any effect of geometrical arrangement of electrodes on the system performance. Not surprisingly, in the AttOn system the most smoothly planar local concentration gradient occurred in the vicinity of electrodes with an initial concentration assignment of zero. Discretization of the initial concentrations revealed that planar gradient similarity was only modestly worsened when the total number of unique initial concentration values for each molecule was confined to two. These findings provide guidelines for how to go about testing steady-state concentrations established by a physical DCR system designed using these parameters.

The AttRep system was shown to be capable of establishing a concentration profile that was much smoother and more nearly planar, yielding a minimum cost value of 0.212 in

comparison to the minimum cost value of 0.747 for the AttOn system. Furthermore, the concentration gradient established by the AttRep system was fairly uniform throughout the area of the electrode array, and local concentration gradient inhomogeneities that could deflect the direction of neural growth were less prevalent. While the AttRep system outperformed the AttOn system in all desired directions, the AttRep system's performance showed greater variation with respect to direction. In particular, the AttRep system tended to yield a more planar concentration profile as the orientation of desired direction transitioned from 45° to 90°. Such directional bias is not unexpected given the square geometry of the electrode array. The directional sensitivity of AttRep system and the lack thereof for the AttOn system suggest that the more capable the system is at establishing a planar concentration profile with less local concentration gradient inhomogeneity, the more sensitive the system is to the exact geometric arrangement of the electrode configurations. While the AttRep system as presented in the study is based on a hypothetical chemorepellent with artificial properties, results from the AttRep system reveal the potential of DCR system to provide more refined control when using multiple molecules with independent effects. Although the geometric configuration of electrodes was omitted from explicit consideration in the model, it would be interesting to assess the effect of varying array geometry on the concentration profile that can be established with DCR. In particular, the scalability of the technique to larger array designs in different geometries is theoretically possible, although we have yet to model such systems. The fundamental limiting factor for a practical neurotrophic DCR system is likely to be the maximum distance over which neurons can be induced to grow with linear concentration distributions using a single molecule. One can imagine extending this theoretical concept to the release of multiple molecules in various combinations to counteract this difficulty (Cao and Shoichet 2003).

One of the simplifying assumptions made in the development of this technique was that the neurotrophin concentration would be constant with respect to the depth dimension. For the relatively short cortical thicknesses involved (no more than 2 mm), assuming similar tissue properties as a function of depth seems reasonable. The mathematical development for DCR could readily be extended from a 2D polar consideration to a 3D cylindrical consideration to model a controlled, nonuniform concentration with respect to depth. The physical interpretation of such a system would be electrodes driven to different depths depending upon their array position. Mathematical derivation of spherically symmetrical concentrations for single points of release such as at the tip of an infusion port (as opposed to lines of release along electrode shafts) would also be possible, although such a method in practice would undoubtedly introduce more local inhomogeneities in the steady-state concentration gradient. Even if an analytical solution is not available for a particular array geometry or release system, the principles introduced here would be readily adaptable to numerical simulation. In order to ensure that actual concentration gradients at a particular depth matched theory as much as possible, one could drive electrodes with longer shafts into the brain to ensure that the depth of interest was far away from the shaft endpoints, thus mitigating edge effects. Although further penetration into the brain by electrodes is not necessarily desirable, it is not likely to cause any additional damage as long as the diameter of the electrode remains small. Alternatively, non-uniform initial concentration assignment may be employed toward the tip of the electrode to mitigate the likely edge effects for shallower electrodes.

Using DCR to design neurotrophin concentration gradients *in vivo* faces potential limitations. The most obvious limitation is the ability to induce the desired neuronal behavior with any particular design. Release systems tend to have unpredictable results on growth *in vivo*, in some cases inducing neuron growth but not in the direction or pattern desired (Romero, Rangappa et al. 2000) or even having a dose-dependent effect on adult neurons (von Bartheld, Kinoshita et al. 1994; Li, Liu et al. 2008; Mortimer, Feldner et al.

2009). Departure from theoretical behavior could be inadvertently brought about by local inhomogeneities in the designed concentration profile or even the effects on unconstrained regions of tissue outside the extent of the electrode array. Neuronal processes might be expected to grow into the array from all sides, for example, without some barrier to growth designed in. It is likely that the model presented here will require refinement once it is tested *in vivo*. Nevertheless, we have presented a model system amenable to adjustment and generalization (e.g., for the creation of a desired nonlinear concentration profile) that provides a theoretical framework for what could be expected when such a system is deployed.

Other biological and technological factors could also prevent an actual system from recapitulating the model. Endogenous factors represent potentially powerful influences on neuronal growth patterns in the central nervous system that may be challenging to overcome by a drug delivery system. If multiple molecules can be released simultaneously from a DCR system, it may be possible to use inhibitors, chelators or antibodies to mitigate the effects of undesirable endogenous factors. Also, the ability of any polymer-based controlled-release system to maintain the intended steady-state concentration profiles long enough to induce the desired neuronal growth behavior could be a limiting factor. Since the theoretical model under study in this paper was built upon a set of geometric constraints that allowed analysis of a closed-form, steady state solution for the established concentration profile, the theoretical model in itself does not provide insight into the time scale of establishing or maintaining the steady-state concentration profile. A crude estimate for the time constant in establishing the steady-state solution can be made as the ratio between the squared length scale of the system and the diffusion coefficient. For the model tested in this study, the time scale of achieving steady-state solution is expected for each millimeter of extent to be

$\tau = \frac{(0.1\text{cm})^2}{4 \times 10^{-7}\text{cm}^2\text{s}^{-1}} = 25000\text{s} \approx 7\text{hours}$. This potential limitation could be overcome by using constant infusion techniques with several release points along a shank or possibly a porous coating allowing diffuse release from the surface. Feasibility studies of an actual DCR system based upon these designs would assist in determining the practicality of concentration profile designs described here. These theoretical systems are intended to represent a general approach for controlling concentration gradients, but the systems being modeled (with the exception of AttRep) are intended to guide directed growth within adult auditory cortex using release from EVAC-coated microelectrodes. It is this particular system in which the theory of DCR is being tested initially in our laboratory.

The novel integrated system of spatially patterned drug delivery described here should be adaptable to a number of applications for invoking neuronal growth and repair. This study presents a first step in testing and establishing the capability of DCR as a framework for establishing a complex controlled concentration gradient below the surface of the brain through controlled release from a small number of releasing points.

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ABBREVIATIONS

AttOn	Attractant-Only System
AttRep	Attractant-Repellent System

BDNF	brain-derived neurotrophic factor
DCR	discrete controlled release
NGF	nerve growth factor
NT-3	neurotrophin factor 3
NT-4	neurotrophin factor 4

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Figure 1.

Geometric Configuration of the Model

(A) Depiction of 4x4 electrode configuration used for simulation. (B) Position vectors from the origin to the center of the n^{th} electrode ($\vec{r}_n$) and to an arbitrary position within the electrode array ($\vec{r}$). Note that $\vec{r}_n$ is shown for the case of electrode on row 3, column 2 or $n=6$. For the simulations the origin was located in the center of the array, and electrodes were located 10 units apart from one another on a vertical and horizontal grid.

Figure 2.**Cost Function Comparison**

Comparison of two different concentration gradients with uniform direction, yielding identical cost value of 0 under cost function A. (A). Ideal planar concentration gradient with uniform gradient vector. (B). Non-ideal concentration gradient with uniform direction but non-uniform gradient magnitude.

Figure 3.
Optimized Attractant-Only system oriented toward 90°
(A) Optimized concentration assignment, graphically depicted as a coating around each electrode. (B) Resultant concentration profile established by the AttOn DCR system.

Figure 4.

Effect of rotating the desired direction of concentration gradient for an Attractant-Only system

(A–D) As the desired direction is rotated from 90° to 45° in 15° increments, the maximal gradient of the steady-state concentration profile also rotates. Overall cost is relatively unchanged, implying that this type of system could produce a concentration gradient of equivalent quality in any orientation relative to the layout of the electrodes. (E–H) Initial electrode concentration assignments for the electrodes reveal, as expected, that different initial concentration patterns across the electrode array are responsible for the observed concentration profiles.

Figure 5.

Effects of rotating the desired direction of concentration gradient for an Attractant-Repellant system.

(A–D) As in Figure 4A–D, rotating the desired direction results in rotated concentration profiles. Fewer local inhomogeneities are apparent at 45° than at 90°, resulting in a lower cost function for the former. While the rotational symmetry of this system is not as great as the Attractant-Only system, the cost values overall are considerably lower, indicating more nearly planar concentration profiles.

Preferred Direction of Planar Gradient vs. Minimum Cost

Figure 6.

The effect of preferred direction on the minimum cost value

To obtain the minimum cost value, the optimization was run at least 30 times for each preferred direction in 15° increments, and the absolute minima recorded. Because of symmetry, experiments were actually conducted only between 90° and 45°. The procedure was performed for both AttOn and AttRep systems. Cost is higher overall for the AttOn system but is also more uniform relative to angle of preferred direction.

Figure 7.

Histograms of chemoattractant and chemorepellent concentration assignments distribution for Attraction-Repellant system.

Each distribution was constructed by taking the assigned concentration values as the preferred direction of concentration gradient was varied uniformly between 90° and 45° . For each direction, the concentration assignment vectors $\vec{p}$ and $\vec{q}$ were normalized such that $\max(\{p_n\} \cup \{q_n\}) = 1$. (A) Histogram of chemoattractant concentration assignments. (B) Histogram of chemorepellent concentration assignments. Both histograms are essentially trimodal with only two nonzero modes.

Figure 8.

Effect of discretizing initial concentration values upon the steady-state concentration profile for the Attractant-Only and Attractant-Repellant systems.

(A) AttOn steady-state concentration profile optimized toward the direction of 90° . (B) Corresponding initial concentration assignments. (C) Discretized AttOn steady-state concentration profile optimized toward 90° . (D) Discretized initial concentration assignments. In this particular case, the attractants were discretized to take on one of the three values in $\{0, 0.5, 1\}$. (E) AttRep steady-state concentration profile optimized toward the direction of 90° . (F) Corresponding initial concentration assignments. Lighter gray bars correspond to the attractant concentrations while the darker gray bars depict repellent concentrations. (G) Discretized AttRep steady-state concentration profile optimized toward 90° . (H) Discretized initial concentration assignments. In this particular case, the attractants were discretized to take on one of the three values in $\{0, 0.4, 1\}$, and repellents were discretized to take one of the three values in $\{0, 0.15, 0.2\}$.