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Measurement of Local Diffusion Properties in Brain Tissue

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1. Introduction

The extracellular space (ECS) of the brain comprises the narrow connected regions between cells and resembles the water phase of foam. The diffusion of an idealized point molecule within the ECS [1] is described by three parameters. 1) Volume fraction defines the tissue volume accessible to the diffusing molecule ($\alpha = V_{ECS}/V_{tissue}$). 2) Tortuosity measures the average hindrance of the ECS relative to an obstacle-free medium ($\lambda = (D/D^*)^{0.5}$, where D is the free diffusion coefficient and D^* the effective diffusion coefficient in tissue). 3) Irreversible loss of the molecules from the ECS, with kinetic constant k' (s^{-1}). Then if S is a source function, the appropriate diffusion equation is:

$$\frac{\partial C}{\partial t} = \frac{D}{\lambda^2}\nabla^2 C + \frac{S}{\alpha} - k'C \qquad (1)$$

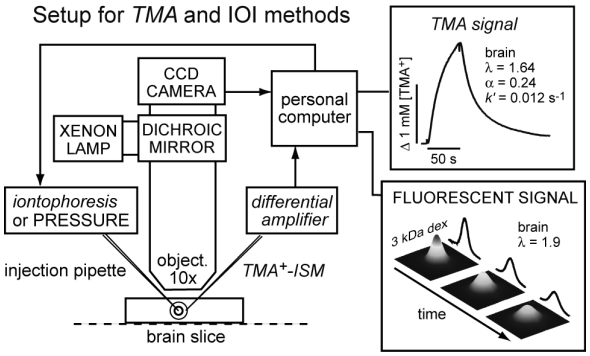

Fig. 1. Setup for combined TMA and IOI methods. A source micropipette releases molecules and these are either detected with an ISM (TMA) or imaged with a camera using an epi-fluorescent microscope (IOI).

Our laboratory has devised two methods to measure these parameters in small brain regions, both based on a 'point-source paradigm'. The first uses tetramethylammonium (TMA, 74 MW) cations to approximate the ideal small marker molecule and measures α, λ and k'. The second method uses integrated optical imaging (IOI) to measure λ for a wide range of macro-molecules. Experiments are made in brain slices or in anesthetized animal models.

2. TMA^+ real-time iontophoretic method (Fig. 1)

A micropipette releases TMA^+ by iontophoresis with source-strength Q for duration t_p. An ion-selective microelectrode (ISM) at a distance r (~ 100 μm) from the release point measures the concentration of TMA^+, C, described by the appropriate solution to Eq (1) [1, 2, 3]:

$$C = G(t),\ t \le t_p;\ C = G(t) - G(t - t_p),\ t > t_p;\ G(u) = \left(Q\lambda^2 / 8\pi D\alpha r\right) \times$$
$$\left[\operatorname{erfc}\left(\frac{\lambda r}{2\sqrt{Du}} + \sqrt{k'u} \right) \exp\left(\lambda r \sqrt{\frac{k'}{D}} \right) + \operatorname{erfc}\left(\frac{\lambda r}{2\sqrt{Du}} - \sqrt{k'u} \right) \exp\left(-\lambda r \sqrt{\frac{k'}{D}} \right) \right]. \quad (2)$$

Experimental data are fitted to Eq (2) using non-linear algorithms written in MATLAB.

3. Integrated optical imaging (IOI) method for fluorescent macromolecules (Fig. 1)

A volume U of macromolecules at concentration C_f with a fluorescent label are released from a micropipette by a very brief pulse of nitrogen. Then C is described by the appropriate solution to Eq (1): [1, 4, 5]:

$$C = \frac{UC_f}{\alpha} \frac{\lambda^3}{(4DT\pi)^{3/2}} \exp\left(-\frac{\lambda^2 r^2}{4DT} - k'T \right);\ T = t + t_0 . \quad (3)$$

The variable t_0 represents a virtual source time-origin (see [5]). For IOI, $k' = 0$. The theory of how the image of the diffusing cloud of molecules maps onto the plane of the CCD (Fig. 1) [4, 6] shows it is legitimate to fit Eq (3) to the image intensity profile to estimate D^* and λ.

4. Conclusions

Measurements with the TMA method show typically $\alpha = 0.2$, $\lambda = 1.6$, $k' = 0.005$ s^{-1}[1]. Measurements with the IOI method show that for globular macromolecules, λ increases with MW, e.g. for epidermal growth factor (MW 6,600) $\lambda = 1.8$ [7], for bovine serum albumin (MW 66,000) [8] and dextran (MW 70,000) [4] $\lambda = 2.3$. In contrast, the linear flexible polymer PHPMA [5] has $\lambda = 1.5$. These data show that we can accurately characterize the diffusion properties of the ECS in living brain tissue using a wide variety of molecules.

References

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