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Fast Optical Tracking of Diffusion in Brain Extracellular Space

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1. Introduction

Extracellular space (ECS) surrounds neurons and glia cells of the brain. This labyrinth filled with cerebro-spinal fluid provides an environment in which signaling and nutrient molecules diffuse. Diffusion is thus very important for the brain's well being and its measurement can provide important clues about the brain function under normal conditions and in certain pathological states such as ischemia, epilepsy, brain injury, or cancer. For an excellent overview of the diffusion in brain, see review [1].

One of the important methods for assessing the ECS diffusion in brain is the Integrative Optical Imaging (IOI) [2]. A small volume of fluorescent dye, approximated by a point source, is released by pressure injection from a glass microelectrode into a brain slice preparation. These marker molecules are too large to enter the cells during the measurement. The diffusing cloud is imaged under an epifluorescent microscope, and the image time series is evaluated to find the ECS tortuosity. In neuroscience, the tortuosity is traditionally defined as $\lambda = \sqrt{(D_0/D)}$, where D_0 is the free diffusion coefficient and D the effective diffusion coefficient.

The original IOI method assumed that D is constant in time. This assumption cannot be made when fast changes take place in the ECS, e.g., during spreading depression (SD) accompanied by a large DC potential shift and cellular swelling [3]. We developed an improved IOI method that allows D to be a function of time, and tested its utility by measuring fast changes in ECS diffusion during SD.

2. Theory

We assume an anisotropic homogeneous environment characterized by an effective diffusion tensor $\mathbf{D}(t) = D_0\,\mathbf{\Lambda}^{-1}(t)$, where D_0 is the free diffusion coefficient and $\mathbf{\Lambda}(t)$ is the tortuosity tensor, and by a time-dependent linear uptake $\kappa(t)$. The diffusion equation for concentration $c(\mathbf{r}, t)$ as a function of position $\mathbf{r}$ and time t is

$$\frac{\partial c(\mathbf{r},t)}{\partial t} = \nabla \cdot (\mathbf{D}(t) \cdot \nabla c(\mathbf{r},t)) - \kappa(t)\, c(\mathbf{r},t) \quad .$$

The initial concentration at time t_0, relevant for the IOI method, is given by a Gaussian distribution. Its shape is governed by a positive constant A and a symmetrical positive-definite matrix $\mathbf{\Sigma}_0$:

$$c(\mathbf{r},t_0) = \frac{A}{(2\pi)^{3/2}\left|\mathbf{\Sigma}_0\right|^{1/2}} \exp\left(-\frac{\mathbf{r}\cdot\mathbf{\Sigma}_0^{-1}\cdot\mathbf{r}}{2}\right) \quad .$$

Applying a 3D Fourier Transform to the spatial coordinates, the solution is found to be again a Gaussian distribution, which is becoming lower and wider with time:

$$c(\mathbf{r},t) = a(t)\,\exp\left(-\frac{\mathbf{r}\cdot\boldsymbol{\Sigma}^{-1}(t)\cdot\mathbf{r}}{2}\right) \quad ,$$

where we used abbreviations

$$\boldsymbol{\Sigma}(t) = \boldsymbol{\Sigma}_0 + 2\int_{t_0}^{t}\mathbf{D}(t')\,dt' \quad \text{and} \quad a(t) = \frac{A}{(2\pi)^{3/2}\left|\boldsymbol{\Sigma}(t)\right|^{1/2}}\exp\left(-\int_{t_0}^{t}\kappa(t')\,dt'\right).$$

Having obtained $\boldsymbol{\Sigma}(t)$ and $a(t)$ by fitting the imaging time series, the effective diffusion and the uptake can be obtained as

$$\mathbf{D}(t) = \frac{1}{2}\frac{d\boldsymbol{\Sigma}(t)}{dt} \quad \text{and} \quad \kappa(t) = \frac{d}{dt}\ln\left(a(t)\left|\boldsymbol{\Sigma}(t)\right|^{1/2}\right) \quad .$$

3. Extracellular diffusion in brain during spreading depression

A small and inert dextran marker molecule (MW 3000, 1 mM in 150 mM NaCl) labeled by the Texas Red fluorescent dye was pressure-injected into stratum radiatum of rat hippocampal slices (400 μm). The SD was induced by a brief injection of 1 M KCl from another microelectrode (Fig. 1). A CCD camera then imaged the diffusing marker molecules approximately once every second. We have found that ECS diffusion of dextran completely stopped during the maximum shift in the DC potential associated with the SD wave. The diffusion movement partly recovered over the next several minutes (Fig. 1).

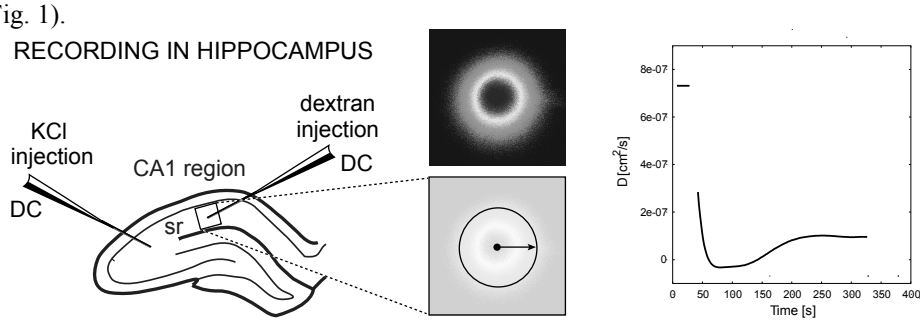

Figure 1. Left - experimental arrangement. Right - diffusion changes during SD.

4. Conclusion

The fast optical tracking of diffusion can achieve time resolution of approximately one second, which is higher by a factor of ten in comparison with the traditional measurement methods [4]. We believe that this technique could be used to study diffusion in many other situations characterized by rapid changes in brain tissue properties, e.g., in epilepsy or during transient neuronal oscillations.

References

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