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Anisotropic Diffusion of Flexible Random-Coil Polymers Measured in Brain Extracellular Space by Integrative Optical Imaging

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1. Introduction

In brain regions containing numerous parallel fibers, extracellular diffusion is anisotropic, i.e. faster along the fibers than across them, implying a preferential pathway for the transport of molecules in the extracellular space (ECS) [1,2]. To date, anisotropic diffusion in the ECS was measured with real-time iontophoretic method employing the small ion, tetramethylammonium (*MW* 74) [1,3,4] or with a FRAP-based technique using fluorophore-labeled dextran (*MW* 70,000) [5]. However, the ECS accommodates the diffusion of many endogenous macromolecules, and it is the exclusive route for interstitial transport of polymer-based drug carriers, therapeutic proteins, and virus-enclosed genes. To study how polymers of different sizes behave in an anisotropic ECS, we used integrative optical imaging (IOI) [6] to measure the diffusion of flexible random-coil dextran polymers (*MW* 3,000-525,000) in the molecular layer (ML) of the isolated turtle cerebellum (Fig. 1).

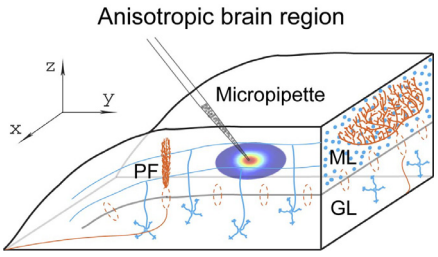

Fig. 1. Schematic of turtle cerebellum (*ML* molecular layer, *GL* granular layer, *PF* parallel fibers).

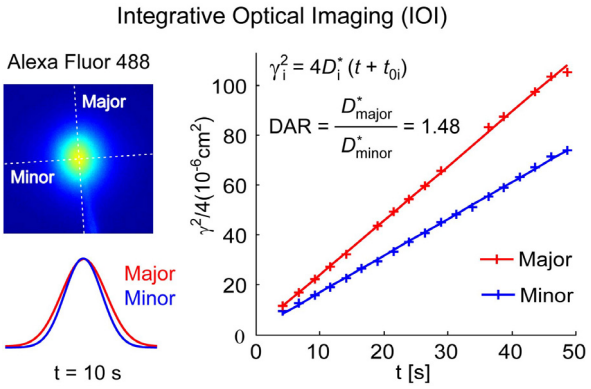

Fig. 2. Anisotropic diffusion of Alexa Fluor 488 (*MW* 547) measured in the anisotropic ML of turtle cerebellum by IOI.

2. Methods and Results

The application of IOI to an anisotropic brain region was first validated using the small fluorophore Alexa Fluor 488 (*MW* 547). The fluorophore was pressure injected into the ML from a glass micropipette (Fig. 1) and a time series of images taken. The series was analyzed to calculate the effective diffusion coefficient (D^*, cm^2s^{-1}) for major and minor axes of the elliptical 2-dimensional (2-d) projection of the 3-d diffusion cloud (Fig. 2). The diffusion anisotropic ratio (DAR=D^*_{major}/D^*_{minor}) was calculated. The measurements were then repeated using fluorophore-labeled dextran polymers (*MW* 3,000, 75,000, 282,000, 525,000). We found that D^* decreased for AF488→dex525

(Table 1). The DAR increased for AF488, dex3, and dex75 but reached a plateau value at 1.78 for dex75, dex282, and dex525 (Fig. 3).

Molecule	MW	Agar gel		Molecular layer		DAR
	(kDa)	D (x10^{-7} cm^{2}s^{-1})	d_H (nm)	D^*_{major} (x10^{-7} cm^{2}s^{-1})	D^*_{minor} (x10^{-7} cm^{2}s^{-1})	
Alexa Fluor 488 (AF488)	0.547	43.69 ± 2.71 (28)	1.1	21.23 ± 1.15 (12)	14.36 ± 1.09	1.48 ± 0.07
dextran (dex3)	3	23.25 ± 1.46 (20)	2.1	8.95 ± 1.31 (32)	5.65 ± 0.83	1.58 ± 0.13
dextran (dex75)	75	4.16 ± 0.16 (11)	11.9	1.24 ± 0.11 (10)	0.70 ± 0.09	1.77 ± 0.17
dextran (dex282)	282	2.34 ± 0.13 (10)	20.9	0.49 ± 0.09 (13)	0.28 ± 0.06	1.81 ± 0.20
dextran (dex525)	525	1.54 ± 0.09 (11)	31.8	0.23 ± 0.05 (9)	0.13 ± 0.04	1.76 ± 0.18

Table 1. Diffusion of Alexa Fluor 488 and dextran polymers in free media (*Agar gel*) and anisotropic ML (*Molecular layer*). Free diffusion coefficients (D) obtained in agar gel were used to calculate hydrodynamic diameters of molecules (Stokes-Einstein equation $d_H = RT/3\pi\eta DN$). Data are expressed as means ± SD; number of measurements are in the parentheses; D and D^* values are at 25°C.

3. Conclusion

As in previous applications of the IOI method in isotropic brain regions [6,7], D^* decreased as the size of the dextrans increased. Surprisingly, the DAR reached a plateau for dex75, dex282, and dex525. This finding contrasts with results from modeling the diffusion of hard spheres between parallel rods, which predicts a monotonic rise in DAR with molecular size [8]. We hypothesize that the two largest dextran polymers approach the dimensions of the ECS, compelling them to deform from a spherical shape in order to traverse the narrow spaces; in fact, large dextran polymers are known to diffuse through pores that are smaller than their diameter [9]. Our findings have implication for the transport of endogenous macromolecules in the ECS and for the design of effective drug carriers.

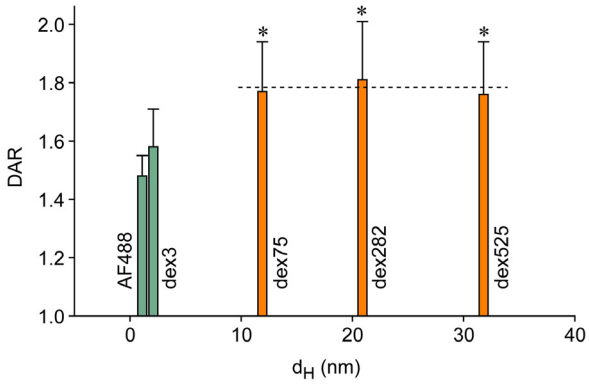

Fig. 3. The DAR measured with AF488 and dextran polymers. Dotted line shows the plateau at 1.78. The DAR for a group of dex75, dex282, and dex525 is significantly larger (*asterisks*) than for AF488 and dex3 (ANOVA, $P < 0.001$).

References: [1] M. Rice et al, J. Neurophysiol. 70 (1993) 2035-44. [2] B. Bielke et al, NeuroReport (1995) 1005-9. [3] I. Vorisek, E. Sykova, J. Neurophysiol. 78 (1997) 912-9. [4] S. Prokopova et al, NeuroReport 8 (1997) 3527-32. [5] M.C. Papadopoulos et al, Biophys. J. (2005) 3660-8. [6] C. Nicholson, L. Tao, Biophys. J. 65 (1993) 2277-90. [7] R.G. Thorne, C. Nicholson, PNAS 103 (2006) 5567-72. [8] J. Han, J. Herzfeld, Biophys. J. 65 (1993) 1155-61. [9] M.P. Bohrer et al, Macromolecules (1984) 1170-3. **Acknowledgement:** Supported by NIH grant NS04775 (to S.H.).