Tuning Sensitivity to Ectopeptidase Rates in the Rat Hippocampus Using Numerical Simulations

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INTRODUCTION

Ectopeptidases are membrane-bound enzymes whose catalytic domains face the extracellular space (ECS) (Figure 1, right). They have traditionally been accepted to inactivate peptides from the ECS[1]. Recently, not only can these enzymes activate peptides, some have also been shown to modulate peptides critical to many important physiological processes, including Alzheimer's and stroke[2,3]. Conventionally, measurements of ectoenzyme activity in tissue or cell are done by incubating whole cells or membrane fractions isolated from homogenization in media with exogenously added substrates. Our group has successfully developed two generations of sampling techniques to study enzyme activity in *intact tissues* (Figure 1, left), which, as far as we know, was the first time it has been done[4,5].
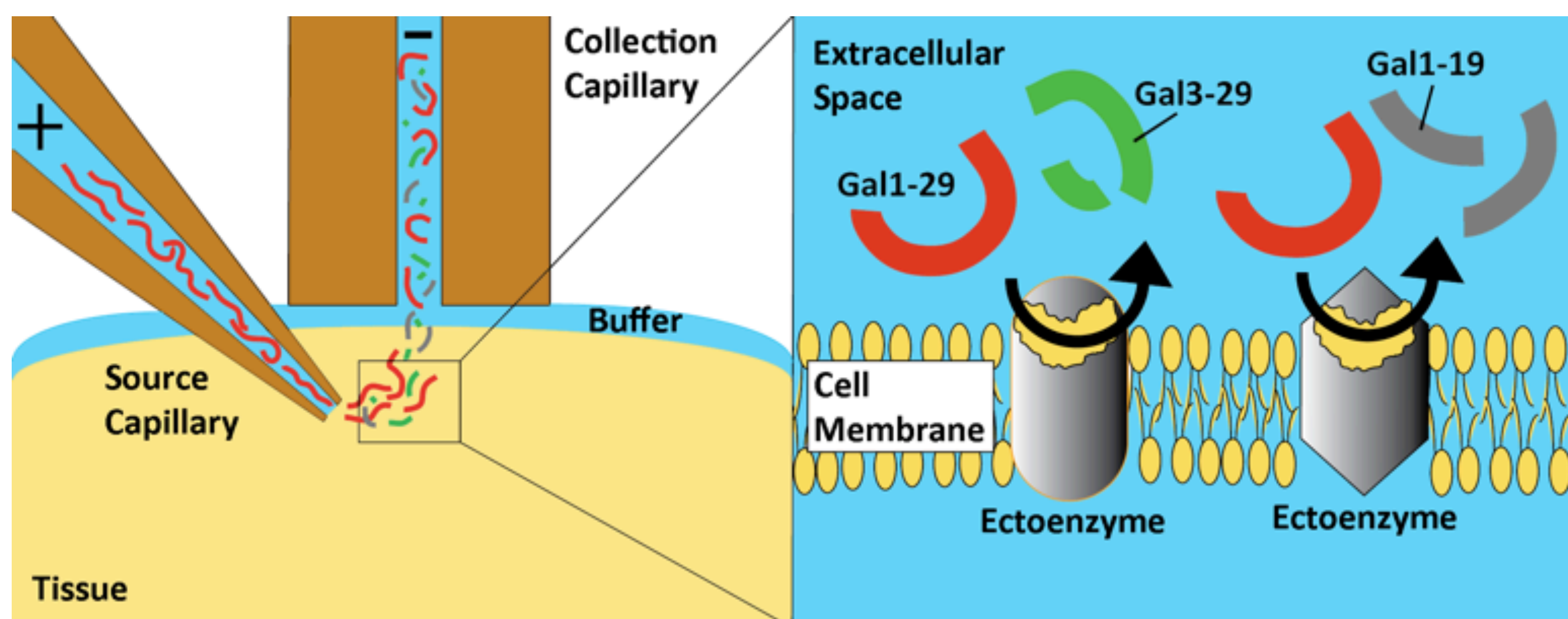

Figure 1. Electroosmotic push-pull perfusion (EOPPP), left, can be used to study ectopeptidase activity in the extracellular space, right. In this specific example, the neuroprotective peptide galanin is cleaved by a couple of different enzymes [5].

COMPUTATIONAL METHODS

The setup consists of two electrolyte-filled capillaries in electrical contact with an organotypic hippocampal slice culture (Figure 1, left) and is recreated in COMSOL (Figure 2). Applied field is the primary driving force for bulk fluid movement through the tissue and capillaries in a process called electroosmosis. The combination of this process with diffusion determines the resulting species velocity, residence time, and, consequently, degree of hydrolysis in the tissue. The four physics modules utilized are Electric Currents, Free and Porous Media Flow, Species Transport in Porous Media, and Particle Tracing. The Free and Porous Media Flow module uses equations that apply most straightforwardly to a homogeneous porous medium. Because we direct fluid through the brain tissue from open capillary tubes, the coefficients in these equations must be adjusted to account for changes in porosity as well as tortuosity. We do this by providing COMSOL with volume-averaged parameters derived from known microscopic parameters and adding an effective charge density term in the medium.
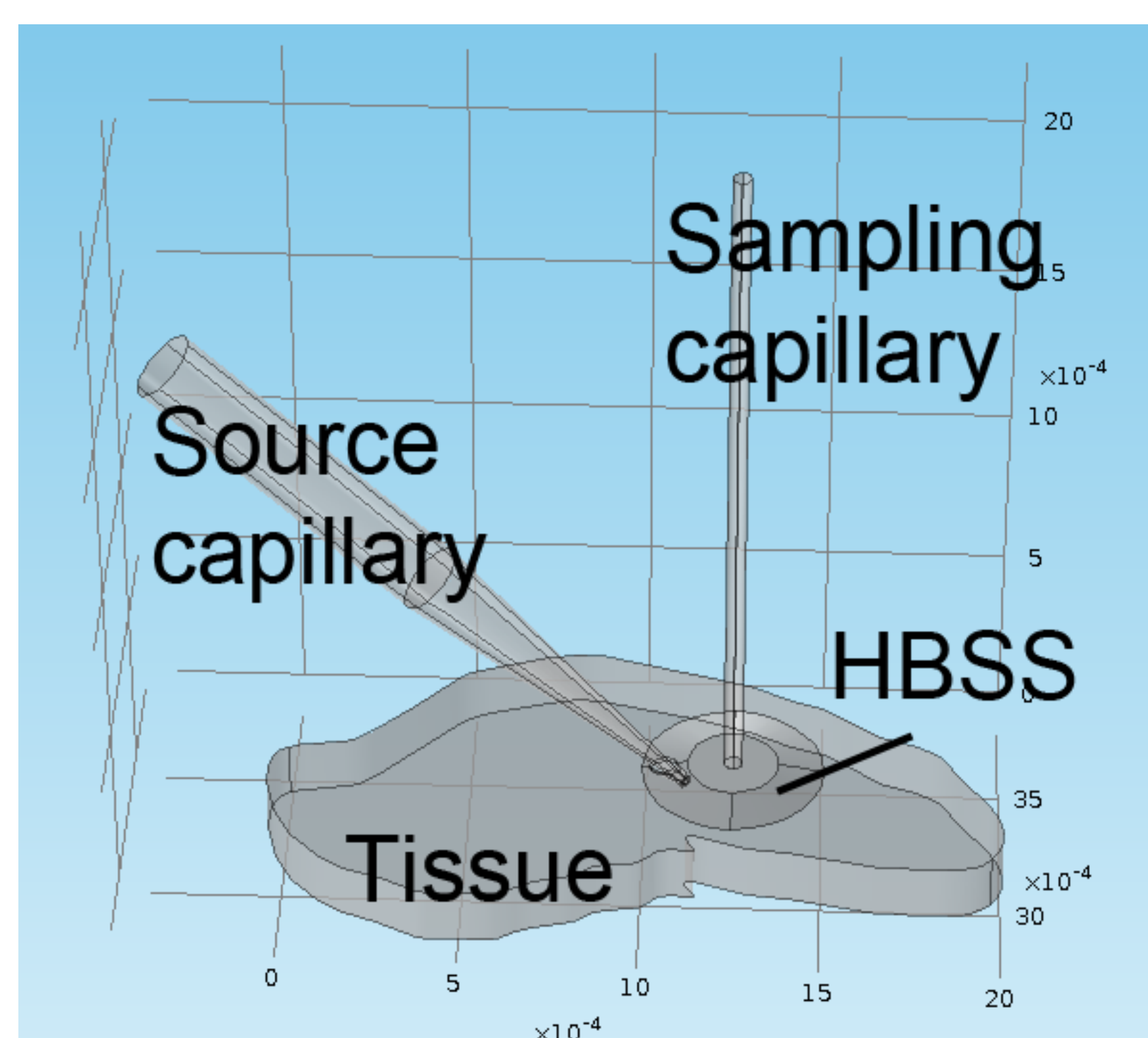

Figure 2. Electroosmotic push-pull perfusion setup recreated in COMSOL Multiphysics v4.4.

RESULTS

Using the calculations as a guide, we varied sampling i.d. and current of our second-generation technique EOPPP (Figure 1, left) to tune the residence time of the substrate. This residence time allows us to "view" a specific range of hydrolysis rates. Longer residence times are optimal for slow kinetic processes while shorter residence times are for rapid ones. We used the model to achieve a better understanding of the physics of our technique, such as mapping out where transport is diffusion- or advection-dominated (Péclet number, Figure 3), quantifying the collection efficiency of our technique (~50-65% at 7-16 µA), and understanding the effect of porous properties (e.g. porosity, permeability, tortuosity, etc) on transport efficiency, to name a few. Through a combination of calculations and experiments, we were able to determine the Vmax for membrane-bound aminopeptidases that hydrolyzes Leu-enkephalin, an important endogenous opioid neurotransmitter, in the subregions of the rat hippocampus (Figure 4).
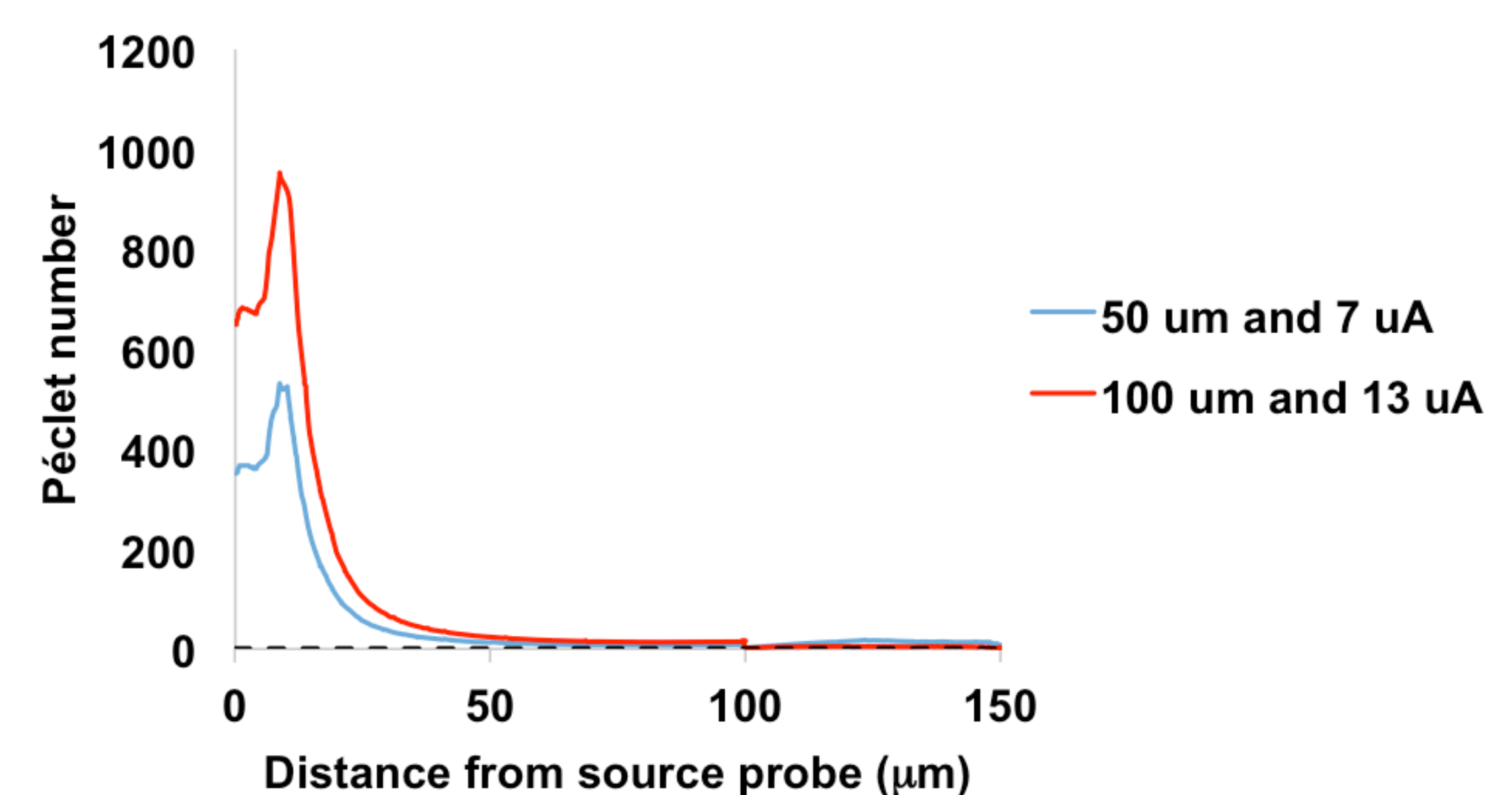

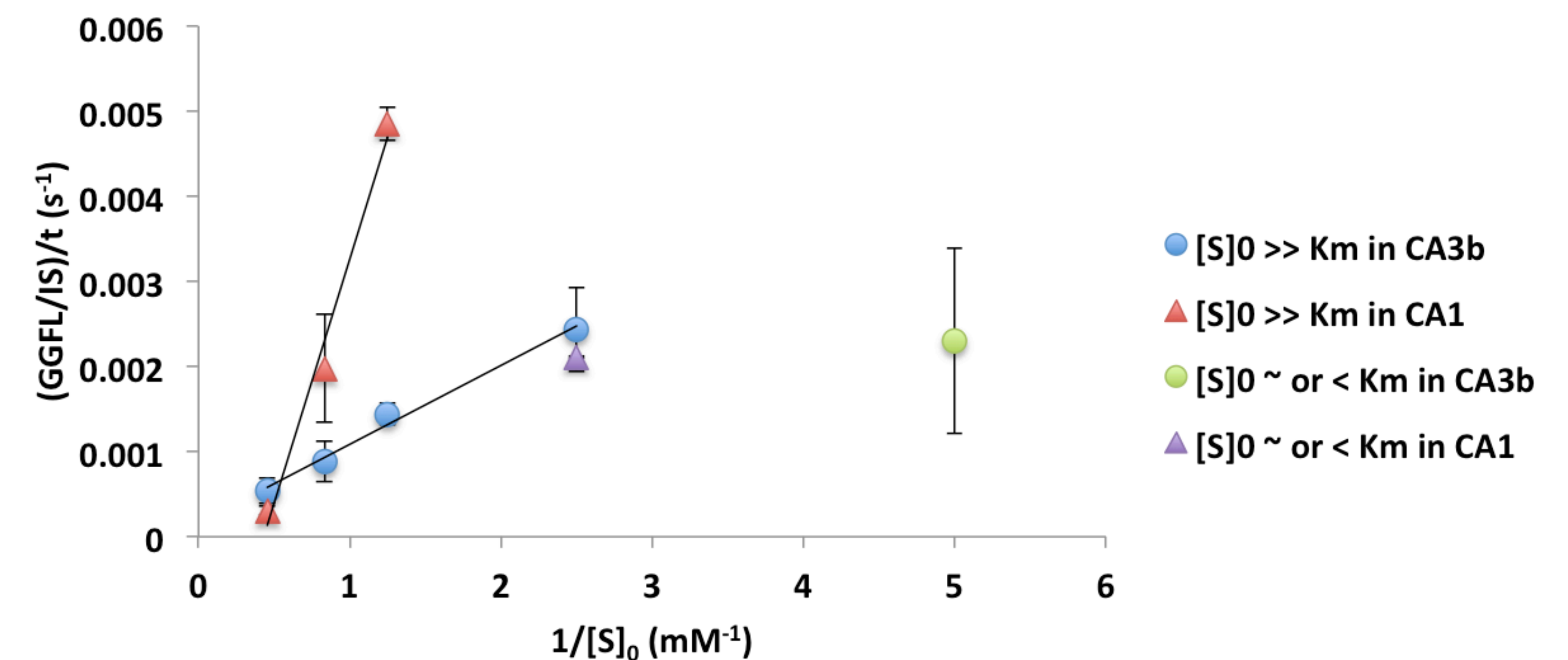**Figure 3.** Plot of Péclet number vs distance from the source probe.

Figure 4. Fitting to integrated zeroth-order Michaelis Menten equation.

CONCLUSION

A combination of simulations and experiments has allowed for significant quantitation of differences in enzyme rates in distinct subregions of the rat hippocampus in a spatially resolved manner. The calculations are an important tool in determining not only residence time of substrates in a complicated system for studies of enzyme kinetics but also in achieving a deeper understanding of our novel sampling technique as a whole.

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