Integrated Electroosmotic Perfusion of Tissue with Online Microfluidic Analysis to Track the Metabolism of Cystamine, Pantethine and Coenzyme A

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Quantitative explanation of cysteamine being treated as the final product. According to Michaelis–Menten kinetics

$$k = \frac{V_{max,ADO}[\mathrm{CSH}]_0}{K_{M,ADO} + [\mathrm{CSH}]_0} \quad \text{[Eq. S-1]}$$

Where $K_{M,ADO}$ and $V_{max,ADO,}$ are Michaelis constant and maximum reaction rate of cysteamine dioxygenase (ADO, EC 1.13.11.19) with cysteamine as the substrate, respectively, $[\mathrm{CSH}]_0$ is the initial cysteamine concentration and k is the initial reaction rate. The reported K_m of ADO at pH 8.0 with cysteamine as the substrate is around 3.8 mM,[1] while the highest concentration of cysteamine generated in experiments of CoA or pantethine metabolism is around 1 μM. Because $[\mathrm{CSH}]_0 << K_{M,ADO}$, Eq. S-1 can be reduced to

$$k = \frac{V_{max,ADO}}{K_{M,ADO}}[\mathrm{CSH}]_0 = c[\mathrm{CSH}]_0 \quad \text{[Eq. S-2]}$$

Where $c = V_{max,ADO}/K_{M,ADO}$. In the absence of ADO, the measured plateau peak height, H, is the function of the concentration of cysteamine as follows

$$H = a[\mathrm{CSH}]_0 + b \quad \text{[Eq. S-3]}$$

where a and b are slope and intercept of the calibration curve, respectively.

Adding the effect of ADO, the relationship between peak height and the concentration of cysteamine becomes

$$H = a([\mathrm{CSH}]_0 - t_S \cdot c[\mathrm{CSH}]_0) + b = a(1 - t_S \cdot c)[\mathrm{CSH}]_0 + b \quad \text{[Eq. S-4]}$$

Where t_S is the average reaction time that the substrate spends interacting with OHSCs during electroosmotic perfusion. It is clearly that peak height is still directly proportional to $[\mathrm{CSH}]_0$ but with a modified slope in comparison to Eq. S-3. Therefore, if the calibration curve is established using the same conditions as that for the actually measurement (e.g. both are affected by the ADO), the analyte concentration read from the calibration curve reflects the value before a small portion of this compound is oxidized by ADO.

Determination of the sampled tissue volume and the average time that the analyte spends inside OHSCs. Assuming that the shape of the sampled tissue is a frustum of a cone as shown in Figure S-2 with the top diameter equal to that of a sampling capillary, 50 μm, and the bottom diameter is 1 mm (estimated from the tissue geometry). The thicknesses of the whole OSHC and an element slice of the tissue culture are L and dl, respectively. The volume of that element slice dV is a function of l (l is the distance between the element slice

and the bottom of the tissue). According to the law of mass conservation, the sample flow rates F (m^3/s) at each any element slice are same. Therefore the time the analyte will be retained in the element tissue slice (undergo enzyme reaction) is given by

$$dt = \frac{\Phi}{F} dV \qquad \text{[Eq. S5]}$$

Therefore the total reaction time t_S (analyte will be exposed to the enzyme) is

$$t_S = \int_{l=0}^{l=L} \frac{\Phi}{F} dV = \frac{\Phi V_{OHSC}}{F} = \frac{V_{eff}}{F} \qquad \text{[Eq. S6]}$$

where V_{OHSC} is the volume of the sampled tissue and Φ is the porosity of the brain tissue. According to the given parameters, $V_{OHSC} = 4.7\times10^{-11}$ m^3. Assuming that the hippocampal tissue culture has a porosity of 0.4,[2] the effective volume of the sampled tissue would be V_{eff} = 0.4 × V_{OHSC} = 19 nL. Applying the flow rate obtained from the PSH migration data, 0.34 nL/s, t_S is 55 s.

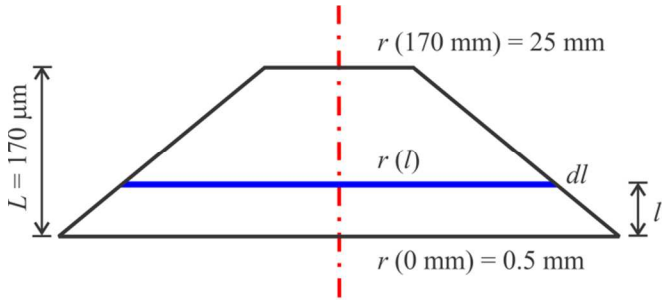

Figure S-1. A schematic side view of the cross section of the sampled region in OHSCs. For illustration purposes, the shape is not drawn to scale.

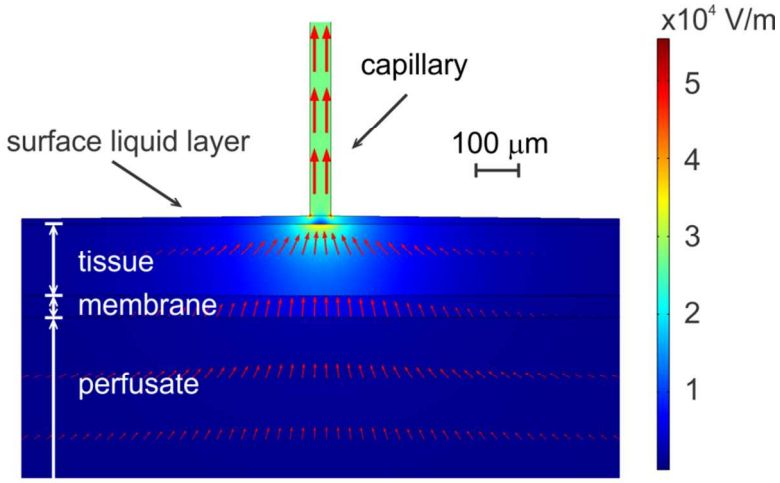

Figure S-2. Simulated electric field and current density in both OHSC and sampling capillary during online electroosmotic perfusion experiment. Parameters are based on the laboratory conditions described in the Experimental Section. Simulation is carried out for the entire region, but only a portion of the capillary, tissue, membrane, and perfusate is shown in the plot for clarity. The red arrows indicate the direction and magnitude of the current density (arrow length is logarithmically proportional to the magnitude of the current density) and the color in the background represents the magnitude of electric field, which is much higher inside the sampling capillary than that in OHSCs. Due to the stronger electric field near the capillary lumen, the magnitude of the velocity in that region is highest in the tissue below the sampling capillary.

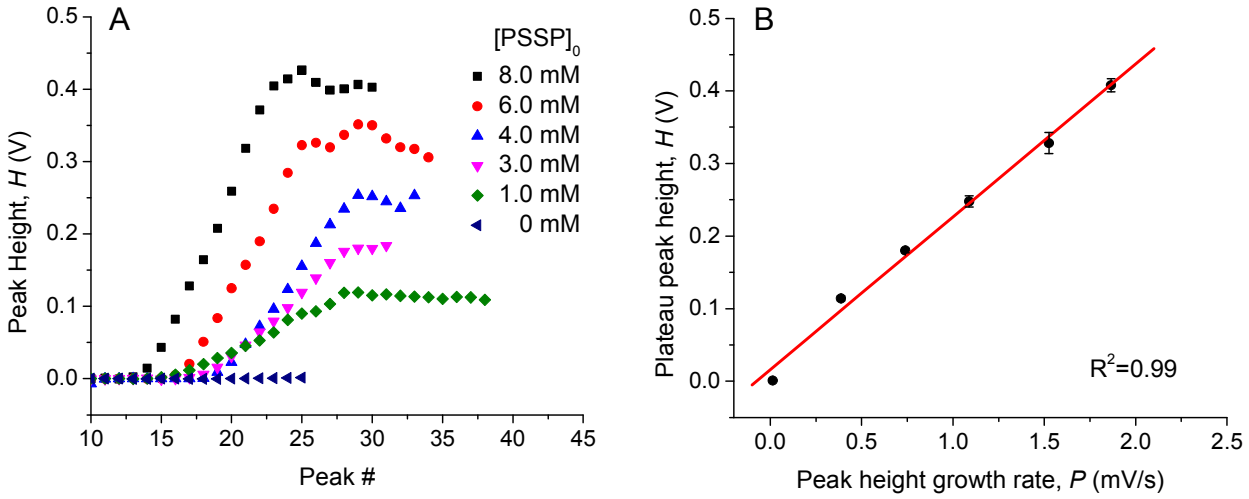

Figure S-3. Plot of plateau peak height, *H*, as a function of peak height growth rate, *P*, around the inflection point of the sigmoid progress curve obtained when the peak heights of pantetheine from consecutive electropherograms are plotted against the time points of sample injection. Pantethine was added into the perfusate ggACSF in the Petri dish with concentration, $[PSSP]_0$, ranging from 0 mM to 8.0 mM. The peak height of pantetheine was obtained by subtracting the contribution from ThioGlo-1 that was extrapolated from the linear regression of ThioGlo-1 peaks observed before pantetheine appeared on the electropherogram to the electropherograms that pantetheine was eluted out. Other conditions were the same as that of online perfusion experiments described in Experimental Section. (*A*) Plateau peak height of pantetheine is plotted vs. the peak #; (*B*) plateau peak height, *H*, is plotted as a function of *P* around the inflection point of the sigmoid progress curve. Data is shown as mean ± SD ($n \geq 4$).

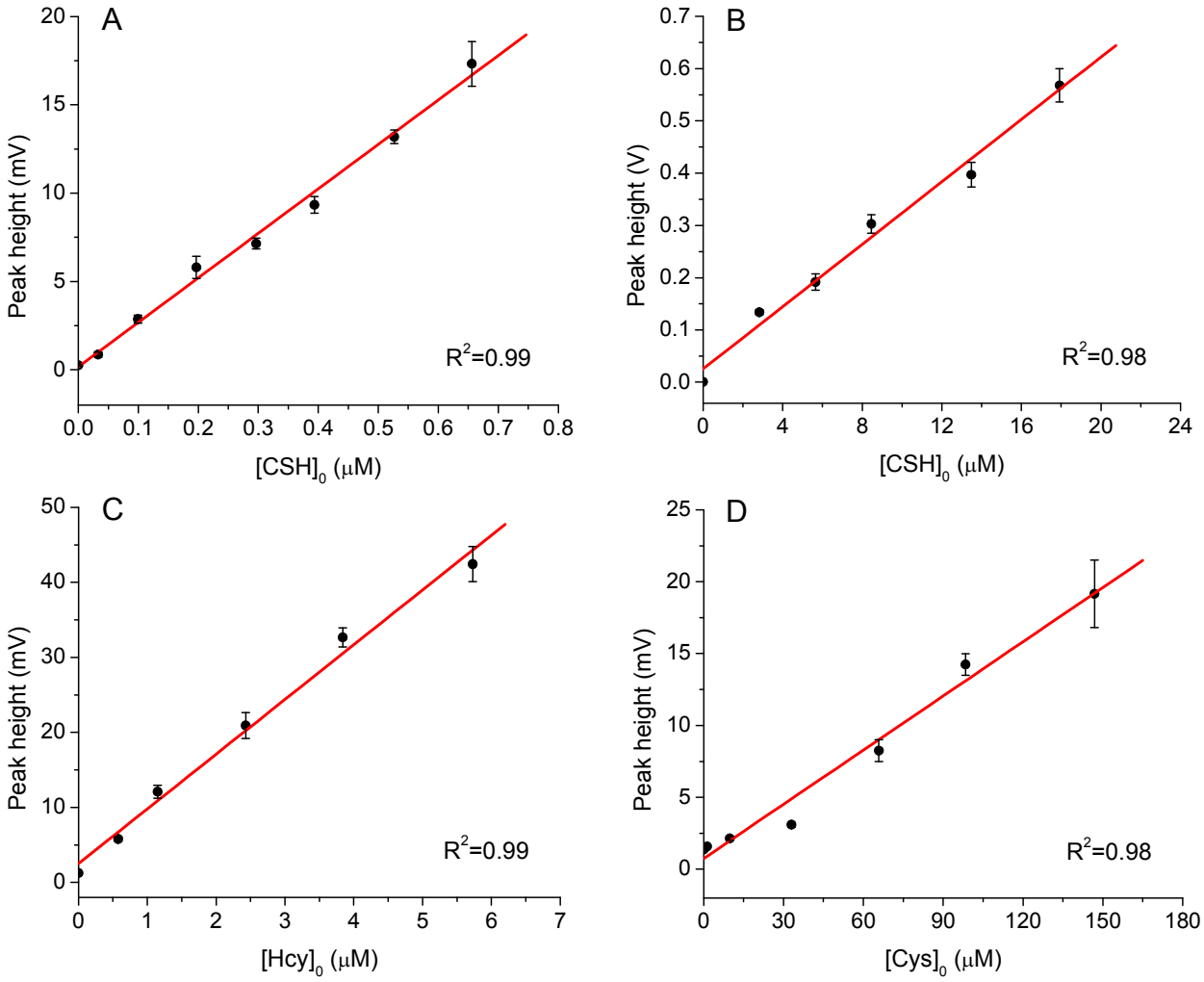

Figure S-4. Calibration curves of aminothiols obtained using microfluidic chip coupled with electroosmotic perfusion in the CA3 region of the OHSCs. (*A*) Cysteamine at low concentration; (*B*) cysteamine at high concentration; (*C*) homocysteine; (*D*) cysteine. Petri dish contained ggACSF spiked with aminothiols at various concentrations. Other conditions were the same as that of online perfusion experiments described in experimental section. Data is shown as mean ± SD ($n \geq 5$).

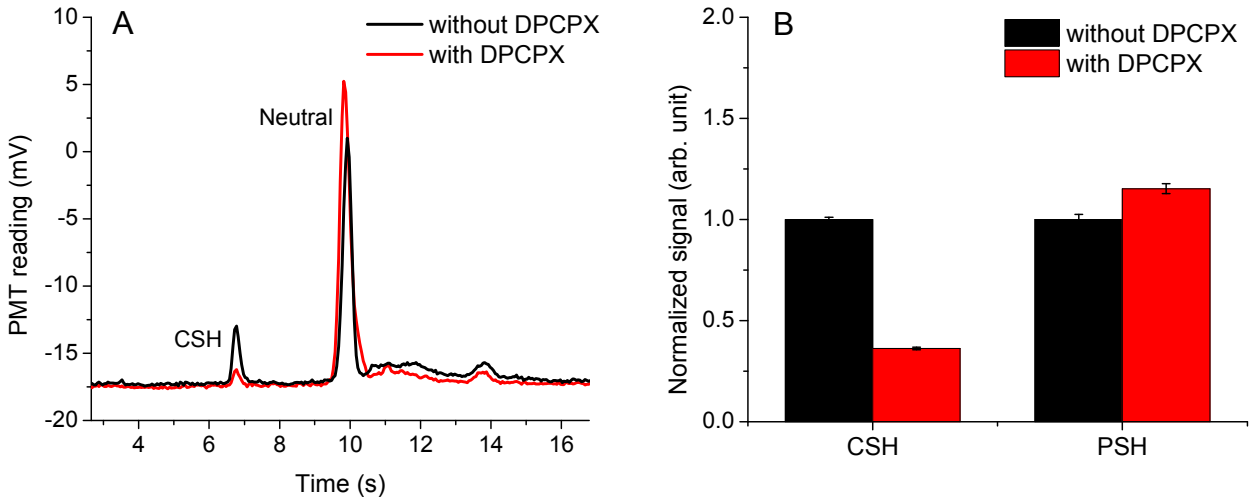

Figure S-5. Inhibition of the pantetheinase by 8-cyclopentyl-1, 3-dipropylxanthine (DPCPX). Petri dish contained 0.12 mM CoA and 0.11 mM of DPCPX or without DPCPX in the perfusate ggACSF. Other conditions were the same as that of online perfusion experiments described in Experimental Section. (*A*) Representative electropherograms from online perfusion experiments with or without inhibitor. (*B*) The peak height, *H*, (for cysteamine) and the peak height growth rate, *P*, around the inflection point of the sigmoid curve (for pantetheine) obtained with or without inhibitor are normalized for comparison. Data is shown as mean ± SEM ($n \geq 16$). Two sample t-test of CSH peak height and ANCOVA analysis of the pantetheine peak growth rate indicated there were significant differences between results obtained with and without DPCPX treatment at $p = 0.04\%$.

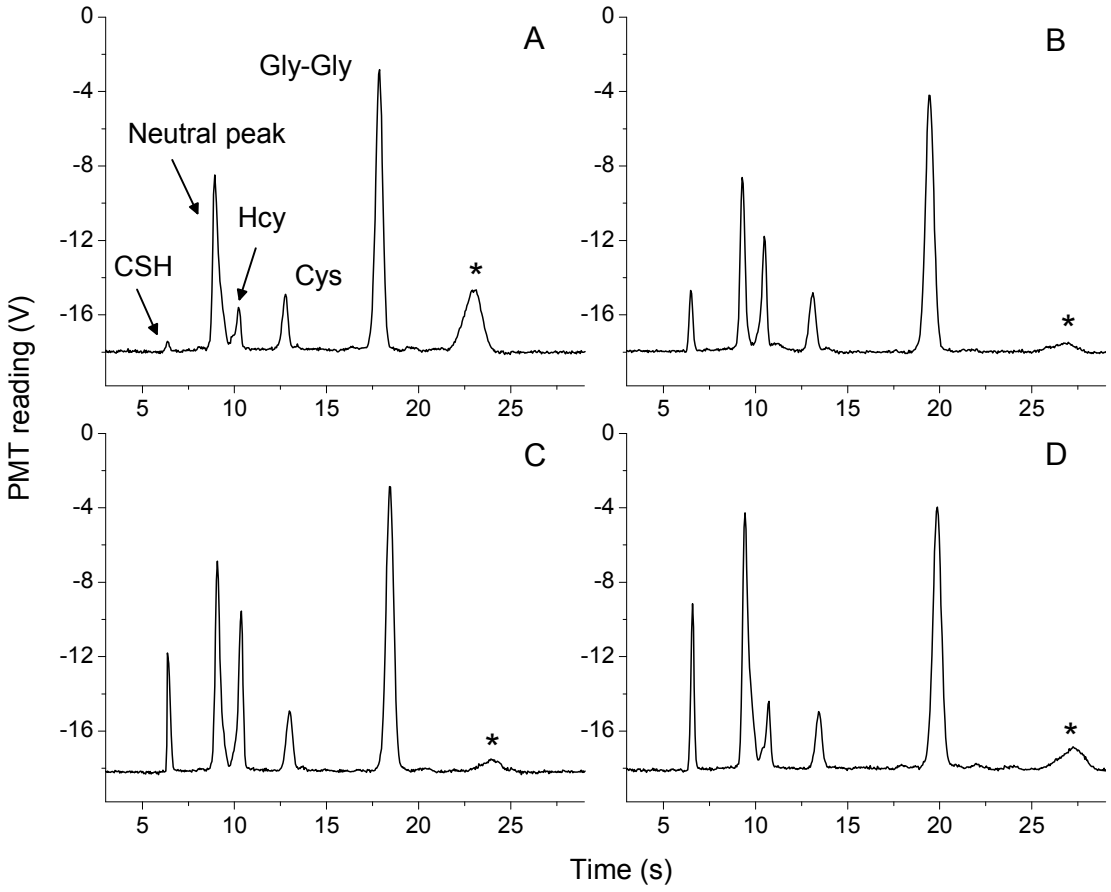

Figure S-6. Representative electropherograms from microfluidic analysis coupled with online electroosmotic perfusion from the CA3 region of the OHSCs. All conditions are the same except that the CoA concentrations in the ggACSF are (*A*) 0 μM; (*B*) 7.3 μM; (*C*) 22 μM; (*D*) 58 μM. Cysteamine, homocysteine, cysteine and Gly-Gly peaks are labeled in plot (*A*). Peaks marked with '*' are impurity in the dye. Neutral peak is contributed from ThioGlo-1 and pantetheine. CSH: cysteamine; Hcy: homocysteine; Cys: cysteine.

REFERENCES

(1) Dominy, J.; Simmons, C. R.; Hirschberger, L. L.; Hwang, J.; Coloso, R. M.; Stipanuk, M. H. *J. Biol. Chem.* **2007**, *282*, 25189-25198.

(2) Sykova, E.; Nicholson, C. *Physiol. Rev.* **2008**, *88*, 1277-1340.