Amperometric Microsensors Monitoring Glutamate-Evoked in-situ Responses of Nitric Oxide and Carbon Monoxide from Live Human Neuroblastoma Cells

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Figure S1. Optical microscopic images of the sensors. Top views of (**A**) dual and (**B**) septuple NO/CO microsensors. WE1 in A and Electrode No. 1, 3, 5 and 7 in B were 76 μ m in diameter, and WE2 in A and Electrode No. 2, 4 and 6 in B were 50 μ m in diameter. The average distance between NO and CO electrodes in the dual sensor is 84.34 ± 10.63 μ m (n = 8). The average distance between electrodes in the septuple sensor is 307.36 ± 26.33 μ m (n = 12). The distances between electrodes are measured as lengths between centers of two adjacent disks.



Figure S2. Representative cyclic voltammograms of (**A**) metal deposited and (**B**) bare 50- μ m diameter NO electrodes (blue) and 76- μ m diameter CO electrodes (red) in 10 mM Ru(NH₃)₆Cl₃ + 0.1 M KNO₃ aqueous solution between 0.15 V and -0.4 V (vs. Ag/AgCl) with scan rate of 10 mV·s⁻¹.



Figure S3. (**A–G**) Dynamic response curves of the seven electrodes in a septuple sensor to stepwise increase of NO concentration. The injection of a NO standard solution caused the current fluctuations at CO electrodes, which were returned to the background currents eventually. (**H**) Calibration curves showing the averaged currents and error bars, corresponding to the dynamic response curves of the NO electrodes (No. 2, 4, and 6: solid line) and the CO electrodes (No. 1, 3, 5, and 7: dashed line). The averaged sensitivity of the NO electrodes was $46.0 \pm 3.21 \text{ pA} \cdot \mu \text{M}^{-1}$ with a high linearity (R² = 0.998).



Figure S4. (**A–G**) Dynamic response curves of the seven electrodes in a septuple sensor to stepwise increase of CO concentration. The injection of a CO standard solution caused the current fluctuations at NO electrodes, which were returned to the background currents eventually. (**H**) Calibration curves showing the averaged currents and error bars, corresponding to the dynamic response curves of the NO electrodes (No. 2, 4, and 6: dashed line) and the CO electrodes (No. 1, 3, 5, and 7: solid line). The averaged sensitivity of the CO electrodes was $62.0 \pm 3.53 \text{ pA} \cdot \mu \text{M}^{-1}$ with a high linearity ($\text{R}^2 = 0.998$).



Figure S5. Dynamic current responses of the seven electrodes in a septuple sensor to the sequential additions of 50 µM acetaminophen (AP), 50 µM ascorbic acid (AA), 10 µM dopamine (DA), 50 µM uric acid (UA), and 50 µM nitrite, in order. Although the currents were slightly increased with the additions of AP, AA, and DA particularly at the electrodes No.1, 3, and 5, all the electrodes showed reasonable selectivity to CO and NO, with selectivity coefficients (log $K_{CO(or NO),i}^{amp}$) < -2, where log $K_{CO(or NO),i}^{amp}$ = log($\frac{\text{sensitivity to } i}{\text{sensitivity to } CO(or NO)}$) and i = interfering species. The injection volume for all of the interferents was 30 µL.



Figure S6. Plots of (**A**) Δ NO, (**B**) Δ CO, (**C**) Δ tNO, and (**D**) Δ tCO depending on the relative distances between the glutamate injection site and the location of each electrode in Figure 4. For NO electrodes (No. 2, 4 and 6), shorter distances between the glutamate injection site and electrode No. 2 and 6 were denoted as D1, and the longer distance between the glutamate injection site and electrode No. 4 was denoted as D2. In the same principle, the relative distance between the glutamate injection site and cO electrodes (No. 1, 3, 5 and 7) were denoted as D1, D2 and D3. The numbers on the graphs were matched with the electrode numbers in Figure 4, indicating the electrode locations.