Second-Derivative-Based Background Drift Removal for Tonic Dopamine Measurement in Fast-Scan Cyclic Voltammetry

Seongtak Kang, Jeongrak Park, Yunho Jeong, Yong-Seok Oh, and Ji-Woong Choi*

ABSTRACT: The dysregulation of dopamine, a neuromodulator, is associated with a broad spectrum of brain disorders, including Parkinson's disease, addiction, and schizophrenia. Quantitative measurements of dopamine are essential for understanding dopamine functional dynamics. Fast-scan cyclic voltammetry (FSCV) is the most widely used electrochemical technique for measuring real-time *in vivo* dopamine level changes. Standard FSCV has only been used to analyze "phasic dopamine" (changes in seconds), because the gradual generation of background charging current is inevitable, and acts as the main noise source in the low-frequency band. Although "tonic dopamine" (changes in minutes to hours) is key for understanding the dopamine system, an electrochemical technique capable of simultaneously measuring phasic and tonic dopamine in an *in vivo* environment has not been established. Several modified voltammetric techniques have been developed for measuring tonic dopamine, but the sampling rates (0.1-0.05 Hz) are too low to be useful. Further investigation of the *in vivo* applicability of previously developed background drift removal methods for measuring tonic dopamine levels is required. We developed a second-derivative-based background removal (SDBR) method for simultaneously measuring phasic and tonic neurotransmitter levels in real-time. The performance of this technique was tested via *in silico* and *in vitro* tonic dopamine experiments. Furthermore, its applicability was tested *in vivo*. SDBR is a simple, robust, post-processing technique that can extract tonic neurotransmitter levels from all FSCV data. As SDBR is calculated in individual-scan voltammogram units, it can be applied to any real-time closed-loop system that uses a neurotransmitter as a biomarker.

Dopamine is a neuromodulator that conveys important information such as cognition, reward and pleasure, and voluntary motor movements.[1, 2] Dysregulation of the dopamine system is associated with a broad spectrum of brain disorders such as Parkinson's disease, Tourette's syndrome, addiction, and schizophrenia.[1, 3, 4] Dopamine levels in the target areas of the brain display highly dynamic changes, with fluctuations on different timescales. These changes include rapid transients, which are ramps that may last for several seconds (phasic), and oscillations on the timescale of minutes to hours (tonic).[2] Quantitative analysis of dopamine levels is crucial for learning about the functional role of dopamine dynamics in the normal brain, as well as studying brain-disorder pathology in pre-clinical and clinical studies.

Fast-scan cyclic voltammetry (FSCV) with a carbon fiber microelectrode (CFM) is a well-established electrochemical technique that can effectively measure dopamine-level changes in the brain.[5] FSCV measures faradaic current changes based on the dopamine oxidation peak voltage exhibited in a voltammogram, after subtracting the background current.[6] The high scan rate of FSCV is sensitive enough to measure rapid changes in dopamine levels (phasic dopamine), but it also generates a progressively large background charging current (capacitive current), making it difficult to analyze voltammetry beyond 2 min.[7, 8] The steady rise in the amplitude of the dopamine peak in FSCV due to this background charging current is called background drift. FSCV background drift makes it difficult to measure slow changes in dopamine levels (tonic dopamine).

Attempts to measure tonic dopamine levels in the brain in real-time are still challenging. Modified voltammetric techniques have been proposed for measuring tonic dopamine *in vivo*.[9-12] These modified voltammetric techniques measure tonic dopamine levels, but the low temporal resolution (10–20 s) makes it difficult to analyze detailed dopamine signaling for understanding neuropsychiatric disorders. The high-pass filtering technique can measure phasic dopamine with background drift removed, but also removes tonic dopamine levels, which have a similar frequency band to that of the background drift.[13] Recently, tonic dopamine measurement using background drift removal was attempted using modified FSCV.[14, 15] These methods attempted to estimate the background with an additional voltage waveform; however, estimating the background of the *in vivo* system with an electrode-specific training set still needs further investigation. An integration-based method estimates tonic dopamine by integrating around the dopamine peak of the background-subtracted voltammogram.[16] In this integration-based method, the user must set the integration potential range, causing potential bias in the estimated dopamine level, which can lead to inadequate estimates of tonic dopamine levels.

In this study, we developed a second-derivative-based background drift removal (SDBR) method for measuring phasic and tonic dopamine levels using standard FSCV. SDBR extracts tonic dopamine information by applying a second derivative from the dopamine oxidation peaks in the background-subtracted voltammogram. SDBR uses voltammetry measured with standard FSCV waveforms, so it provides additional tonic dopamine information to all FSCV

studies that measure phasic dopamine. Since the second derivative is applied to individual voltammograms generated every 0.1 second, it can be applied to real-time systems.

Materials and Methods

Data acquisition and analysis. CFM and Ag/AgCl reference electrodes (Pinnacle Technology Inc., Lawrence, KS) were used for FSCV data acquisition. Voltametric scans were electrochemically performed using a triangular waveform that ranged from -0.4 to +1.3 V, with a scan rate of 400 V/s and a waveform frequency of 10 Hz. The data acquisition was performed using High Definition Cyclic Voltammetry software (HDCV, University of North Carolina at Chapel Hill) in conjunction with a WaveNeuro FSCV system (Pine Research Instrumentation, Durham, NC).[17] All data were analyzed in MATLAB R2020b (Mathworks, Natick, MA).

***In vitro* experiment.** CFM and Ag/AgCl reference electrodes were placed in a beaker filled with 0.05 M phosphate-buffered saline (PBS) with a pH of 7.4. All experimental procedures were performed in Faraday cages to ensure environmental stability. Voltammetric scans were then performed, as previously described. One drop of dopamine (dopamine hydrochloride, Sigma-Aldrich) solution of 200 nM was added to the beaker three times, and immediately after each drop, the solution was mixed with a stirrer for 2 min. After mixing, the power supply of the stirrer was turned off to eliminate noise.

Surgery and *in vivo* dopamine measurements. Adult C57BL/6J 35 g male mice were purchased from Charles River Laboratories (Yokohama, Japan) and used for the *in vivo* experiments. All animal care and experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Daegu Gyeongbuk Institute of Science and Technology (DGIST-IACUC-21091701-0003). To evaluate the effectiveness of SDBR *in vivo*, tonic dopamine levels in the striatum of healthy and Parkinsonian mice were measured for 50 min using standard FSCV. In Parkinsonian mice, 15 min after baseline recording, levodopa was directly infused into the lateral ventricle. The surgical details are provided in the Supporting Information.

Second-derivative-based background drift removal (SDBR) method. We modeled the background-subtracted voltammogram around the dopamine oxidation peak generated for each scan based on the following characteristics. First, the voltammogram near the dopamine oxidation peak after background subtraction is symmetrical and has a Gaussian shape.[7] Second, the amplitude current of the dopamine oxidation peak of the background-subtracted voltammogram has a linear correlation with the dopamine concentration.[7, 18] Third, we assumed that the background charging current generated in a narrow range around the dopamine oxidation peak (dopamine oxidation peak voltage ± 40 mV) varies with time, but is independent of voltage. Furthermore, we modeled a background-subtracted voltammogram at specific scan times (t) and voltages (V) around the dopamine oxidation peak voltage (Equation 1).

$$Voltgram_{BS}(V,t) = e^{-\frac{(V-peak_v)^2}{2}} Conc_{DA} + Charg_c(t), \quad (1)$$

where $Voltgram_{BS}$ denotes a background-subtracted voltammogram, $peak_v$ is the dopamine oxidation peak, $Conc_{DA}$ is the dopamine concentration, and $Charg_c$ is the background charging current. If V is set to $peak_v$ to observe the current of the dopamine oxidation peak of the voltammogram:

$$Voltgram_{BS}(peak_v,t) = Conc_{DA} + Charg_c(t). \quad (2)$$

In Equation 2, which represents the current of $peak_v$ in general background-subtracted voltammogram, the $Charg_c$ remains constant. We eliminated the $Charg_c$ in the proposed model and quantified the intrinsic curvature of the dopamine oxidation peak by applying the second derivative to each background-subtracted voltammogram (Equation 3).

$$\begin{aligned} Voltgram_{SDBR}(V,t) &= \frac{-d^2}{dV^2} Voltgram_{BS}(V,t) \\ &= (1-(V-peak_v)^2) e^{-\frac{(V-peak_v)^2}{2}} Conc_{DA}, \end{aligned} \quad (3)$$

where $Voltgram_{SDBR}$ denotes the SDBR-applied voltammogram. If we observe the dopamine peak current after the second derivative of the modeled voltammogram by setting V to $peak_v$:

$$\frac{-d^2}{dV^2} Voltgram_{BS}(peak_v, t) = Conc_{DA}. \quad (4)$$

Note that this second derivative, the tonic dopamine level, can be obtained irrespective of the time-varying charging current level. Additional details are provided in the Supporting Information (Figure S1). The dopamine oxidation peak voltage of each sensor was defined as the voltage with a maximum SDBR value in the range of 0.4 to 0.7 V in the *in vitro* test. To improve the signal-to-noise ratio of the SDBR result, the SDBR values of the five voltage channels adjacent to the dopamine peak voltage were averaged.

Results and Discussion

SDBR *in vitro* experiments. It was confirmed *in vitro* that SDBR can remove the capacitive current, which is the main noise source in the conventional background subtraction method. Figure 1 shows the *in vitro* test results of the background subtraction method and the SDBR. Dopamine (200 nM) was dropped into the PBS solution every 20 min and stirred for 2 min. Standard FSCV measures the faradaic current caused by dopamine around the CFM, and the capacitive current change is gradually generated owing to the high scan rate (Figure 1A). In previous studies, a background subtraction technique was applied to observe phasic dopamine levels (Figure 1B).

However, capacitive currents that cause a continuous current rise, even at the same dopamine concentration, make it difficult to analyze changes in tonic dopamine levels. This continuous increase in the capacitive current with time is described by Equation 2. In contrast, SDBR results were flat with similar values at the same concentration during the 1-h experiment without any background drift (Figure 1C, Equation 4). Figure 1D shows the SDBR calibration plot. The SDBR signal correlated with tonic dopamine concentrations (62.5-1000 nM; n=5 electrodes; R^2=0.996).

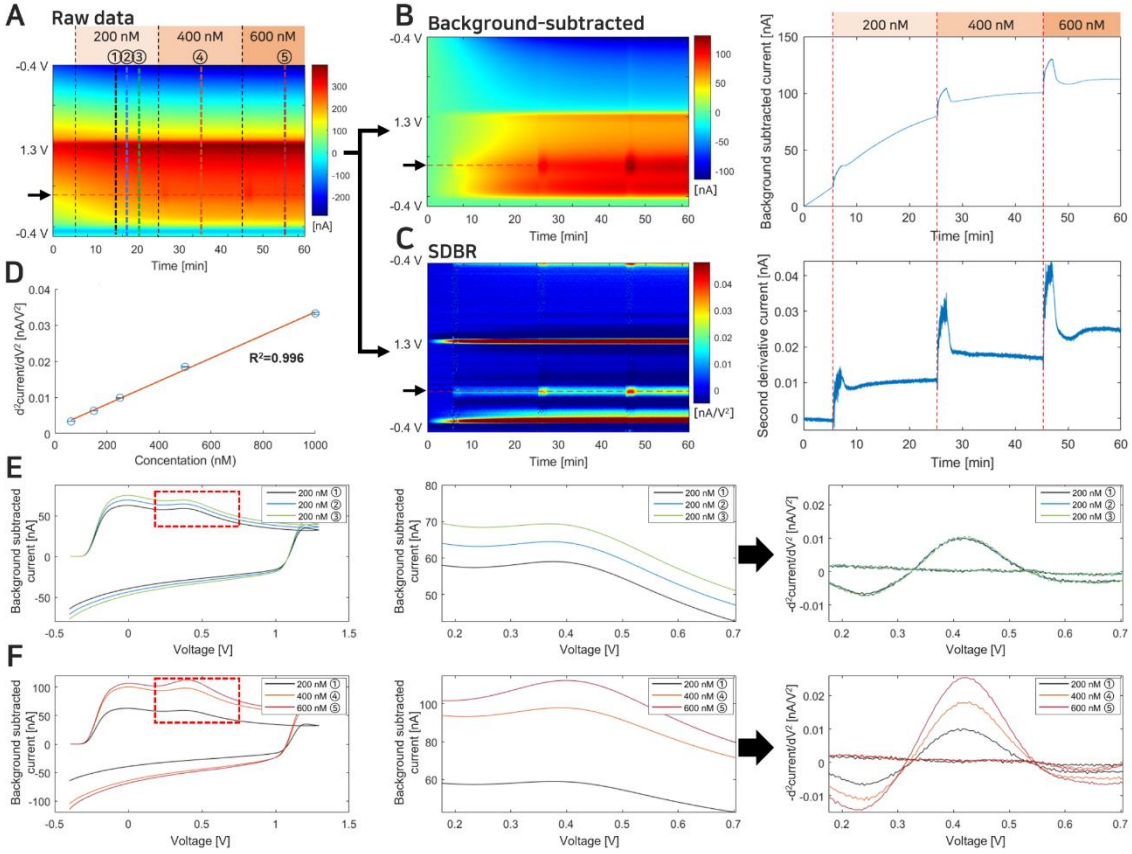

Figure 1. *In vitro* test of SDBR to record the tonic and phasic dopamine with standard FSCV. (A) Raw FSCV color plot *in vitro* test. Black dotted line denotes the timing of the 200 nM dopamine drop. Each circled number and the corresponding colored-line are the voltammogram at a specific time, detailed in (E, F). (B) Background-subtracted color plot and current change of dopamine peak over time. (C) SDBR applied color plot and current change of dopamine peak over time. (D) A calibration plot obtained by SDBR (R^2=0.996), slope=0.0319 $\pm$ 0.0003 pA/V^2 nM^{-1}, limit of detection=8.16 $\pm$ 0.08 nM (n=5 electrodes). (E) Comparison of background subtraction and SDBR for three voltammograms measuring the same concentration at 2-minute intervals. (F) Comparison of background subtraction and SDBR for three voltammograms measuring the different concentrations 10 min after each drop of dopamine solution. (E, F) Left: the background-subtracted voltammogram, middle: the zoomed-in voltammogram of the red dotted box in the left image, right: the result of applying SDBR to the raw voltammogram.

The limit of detection was 8.16 $\pm$ 0.08 nM which is sufficient for dopamine measurements *in vivo*. Figure 1E shows the voltammogram changes at 2-minute intervals (①, ②, and ③ in Figure 1A) for the same dopamine concentration (200 nM). The voltammograms at 2-minute intervals had a similar shape near the dopamine oxidation peak, but the amplitude steadily increased owing to the capacitive current. Despite the passage of time, the SDBR values of the dopamine oxidation peaks were almost the same when they had the same concentration (right image of Figure 1E). Figure 1F shows the voltammograms 10 min after each drop of 200 nM dopamine (different concentrations of dopamine) (①, ④, and ⑤ shown in Figure 1A). The peak currents expressed in the three background-subtracted voltammograms were not linearly correlated to the dopamine concentration, because they were contaminated by the capacitive current. SDBR linearly expressed three different dopamine level changes with the amplitude of the dopamine oxidation peak (right image of Figure 1F).

SDBR *in vivo* experiments. It was confirmed through *in vitro* experiments that SDBR can measure changes in tonic dopamine levels without any background drift. To confirm the practicality of SDBR in the *in vivo* environment, the FSCV results were measured in the striatum of a normal mouse and the striatum of a Parkinson's disease (PD) model (6-OHDA) mouse following levodopa infusion. These results were then analyzed through background subtraction and SDBR (Figure 2). In the background-subtracted FSCV results measured in the striatum of normal mice, the amplitude of the dopamine peak steadily increased (upper images in Figure 2A). However, when SDBR was applied, the estimated dopamine concentration fluctuated within 10 nM for approximately 50 min (lower images in Fig. 2A). Figure 2B shows the results measured by FSCV in the striatum 15 min after levodopa was directly injected into PD model mice. The background-subtracted technique showed that the amplitude of the dopamine peak steadily increased independent of the drug injection (upper images in Figure 2B). However, SDBR showed a flat signal for approximately 15 min,

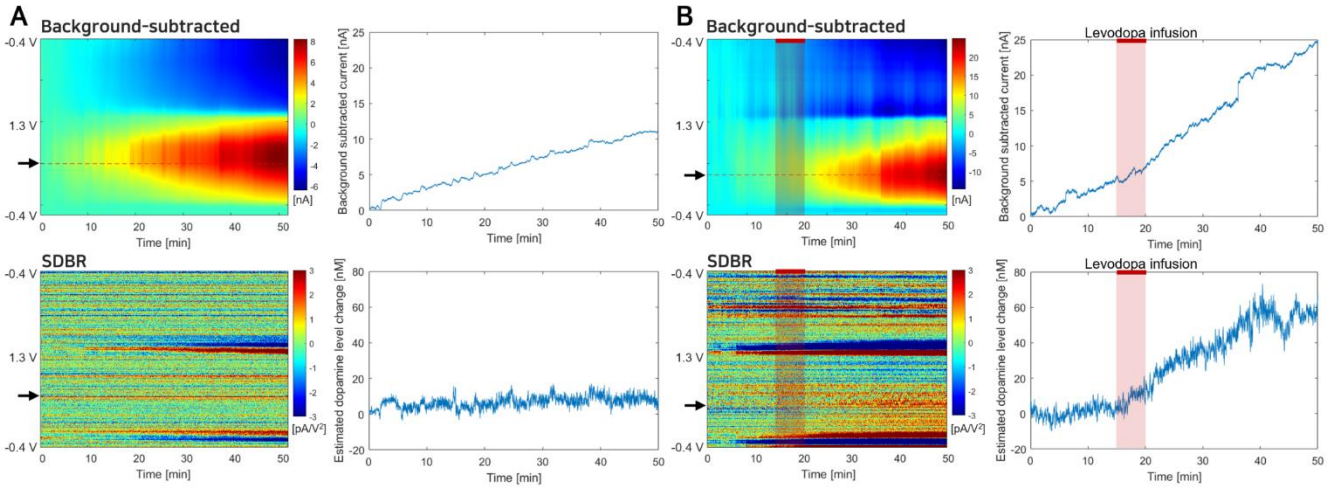

Figure 2. SDBR *in vivo* experiments to record tonic dopamine with standard FSCV. (A) Comparison of background-subtracted FSCV and SDBR results measured in the striatum of healthy mice. (B) Comparison of background-subtracted FSCV and SDBR measured in the striatum of PD model mice following levodopa infusion.

Table 1. A comparison between SDBR and the tonic dopamine measurement methods using FSCV

Method	Temporal resolution	Limit of detection (nM)	Simultaneous availability of phasic dopamine	Need to modify the waveform of standard FSCV?	Reference
FSCAV	20 s	3.7 ± 0.5	Partially	Yes	[9]
CBM-FSCV	10 s	5.8 ± 0.9	No	Yes	[11]
Convolution-based current removal	1 s	<40	Yes	Yes	[14]
M-CSWV	10 s	0.17 ± 0.03	No	Yes	[10]
SWV	15 s	2.03 ± 0.09	No	Yes	[12]
SDBR	0.1 s	8.16 ± 0.08	Yes	No	Proposed

Some of the contents of Table 1 referred to the previously reported review article. [1]

and immediately after levodopa injection, the estimated dopamine concentration increased by 72.4 nM for approximately 25 min and was re-saturated (lower images of Figure 2B). Thus, two types of *in vivo* experiments showed that SDBR can reliably extract changes in tonic dopamine concentration *in vivo*.

As a summary, a comparison between SDBR and the tonic dopamine measurement methods described in this paper is summarized in Table 1. Because SDBR is a post-processing technique applicable to standard FSCV, it can measure phasic and tonic dopamine with high temporal resolution. Also, since SDBR uses standard FSCV as it is, it has the versatility to extract tonic dopamine information from all FSCV data measured with standard FSCV. SDBR has a sufficient limit of detection to measure the tonic dopamine variation. Data from Figure 1 and Figure 2 are freely available online.[22]

Conclusions

SDBR is a novel technology that extracts tonic dopamine levels while maintaining the high temporal resolution of FSCV. This is achieved by applying the second derivative to the voltammogram measured with standard FSCV. Simultaneous measurement of phasic dopamine by FSCV and extracted tonic dopamine through SDBR will contribute to a better understanding of all dopamine systems and brain diseases related to dopamine signaling. SDBR effectively extracts tonic dopamine information by applying a simple second-derivative operation to individual voltammograms. SDBR has the potential to be applied to real-time closed-loop therapy systems that use dopamine levels as a biomarker.[19-21] In addition, because SBDR is a post-processing technology that does not require any modification to the standard FSCV system, it can be universally applied to existing FSCV measurement data. SBDR will provide tonic-level data that can be used to accelerate advances in FSCV-related research.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Author contributions, surgery and *in vivo* dopamine measurement experiment, and *in silico* test (PDF)

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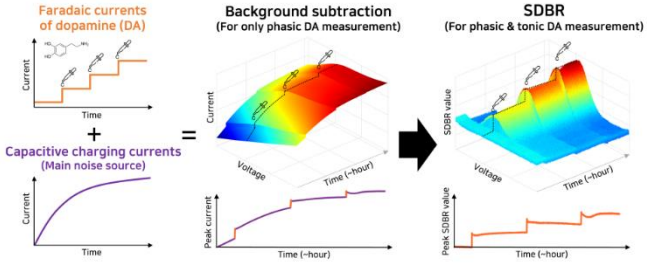

Supporting Information for

Second-Derivative-Based Background Drift Removal for Tonic Dopamine Measurement in Fast-Scan Cyclic Voltammetry

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Author contributions

All authors have given approval to the final version of the manuscript. Seongtak Kang, Conceptualization, Data curation, Software, Formal analysis, Validation, Investigation, Visualization, Methodology, Writing-review and editing; Jeongrak Park, Resources, Validation, Writing-review and editing; Yunho Jeong, Resources; Yong-Seok Oh, Supervision, Validation, Writing-review and editing; Ji-Woong Choi, Supervision, Validation, Methodology, Writing-review and editing.

Surgery and *in vivo* dopamine measurement experiment

Adult C57BL/6J male mice were housed under a 12-h light/dark cycle with *ad libitum* access to food and water. For 6-OHDA-induced Parkinsonian mice, 20 min prior to surgical operation, desipramine hydrochloride (2.5 mg/ml, Sigma-Aldrich) and pargyline hydrochloride (0.5 mg/ml, Abcam) dissolved in sterilized saline were intraperitoneally injected. The mice were anesthetized with 1.5-2.5 % isoflurane and placed in a stereotaxic instrument frame. 6-hydroxydopamine hydrobromide (6-OHDA·HBr, Sigma-Aldrich) was dissolved in sterilized saline and delivered to the left medial forebrain bundle (MFB) at the following coordinates (from bregma): anterior-posterior (A/P) = -1.20; medio-lateral (M/L) = -1.10; dorso-ventral (D/V) = -5.00 (1μg total). After 1-week of post-operative recovery, the CFM was implanted in the left dorsolateral striatum for *in vivo* dopamine measurement experiments at the following coordinates: A/P = +1.00, M/L = -2.20, D/V = -3.00, and the Ag/AgCl reference electrode was positioned in the contralateral hemisphere. For dopamine brain infusion, an infusion guide cannula (C315GMN, Plastics One) was implanted in the right lateral ventricle at the following coordinates: A/P = -0.46; M/L = +1.15; D/V = -2.20. On the day of the FSCV measurements, the mice were anesthetized and connected to an infusion tube filled with levodopa (1 mg/ml, pH 7.4, Sigma-Aldrich) solution. After a 15-minute baseline measurement, the infusion was performed at 200 nl/min.

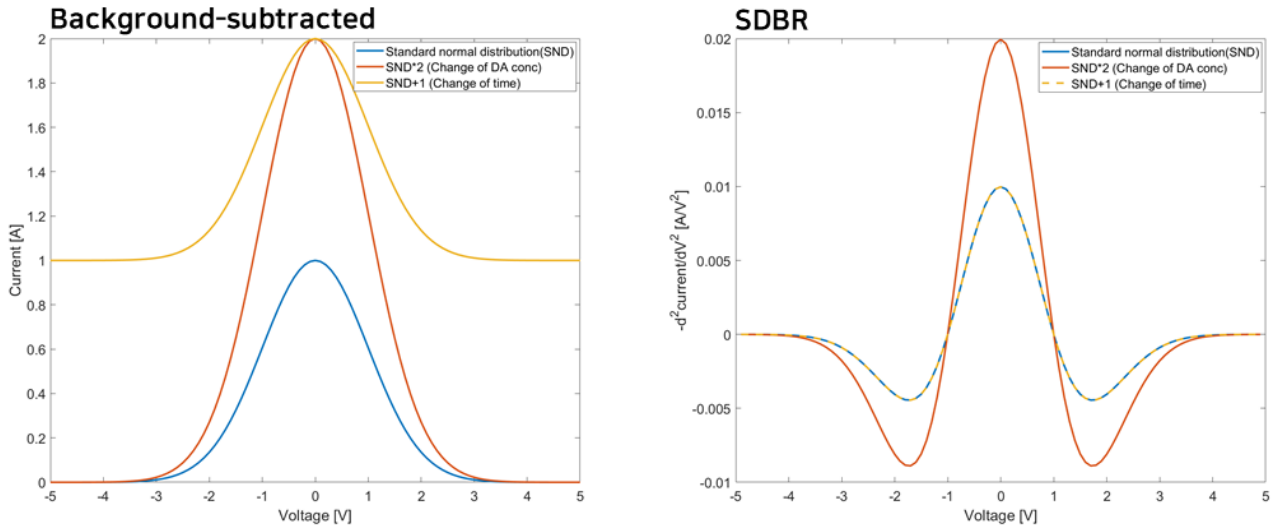

Figure S1. Background subtraction and SDBR results according to dopamine concentration and charging current in standard normal distribution (SND) shape voltammogram model. In the Gaussian voltammogram model, the increase in charging current with increasing time is expressed by addition (yellow line), and the increase in dopamine level is expressed by multiplication (orange line). The two images show the background-subtracted voltammograms (left image) and SDBR results (right image) according to the charging current and dopamine level.

In silico test of second derivative-based background drift removal (SDBR) method

SDBR was mathematically tested after modeling using Equations (1-4) in the main manuscript. Figure S1 shows the changes according to two parameters (dopamine concentration and time) when background subtraction and SDBR were applied to the Gaussian-modeled voltammogram. In Equation (1), the change in concentration is expressed as a linear multiplication of the Gaussian model, and the charging current owing to the change in time is expressed as an addition. In a typical background-subtracted model, the peak current measurement cannot distinguish between an increase in the charging current due to a change in time and an increase in current due to a change in dopamine concentration (left image of Figure S1). However, in the voltammogram model to which the SDBR was applied, the same peak current appeared at the same concentration regardless of the passage of time by eliminating the effect of the charging current. The increased dopamine concentration is expressed as a linear increase in the SDBR peak current.