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Dual use of rectangular and triangular waveforms in voltammetry using a carbon fiber microelectrode to differentiate norepinephrine from dopamine

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ABSTRACT

While dopamine (DA) may be clearly detected by voltammetric techniques such as fast-scan cyclic voltammetry (FSCV) in the heavily-innervated striatum, the differentiation of DA from other monoamines, including norepinephrine (NE) and serotonin (5-HT), is crucial for further applications outside of the striatum. We show that using normal pulse voltammetry (NPV) with 0.1 V to 0.3 V rectangular pulses can differentiate between NE and DA. While the major electrochemical current of NE and DA is obtained on 0.2 V rectangular pulse of NPV, the relative current on 0.1 V pulse was higher for DA than NE. It was possible to differentiate NE from DA by electrochemical current on three NPV pulses using simple mathematics of sequential equation. Since FSCV obtains a larger current value and less noisy results than NPV, we alternately recorded both. The electrochemical current data of NPV was combined with FSCV for principal component regression (PCR) analysis. The estimated value was evaluated by percent error and correlation coefficient from the true concentration, and the slope of the linear regression line. Estimation by sequential equation using NPV results could differentiate NE from DA, while PCR without NPV current data could not. PCR including NPV current data also could differentiate NE from DA. In this study, we compared the performance of electrochemical recordings with rectangular and triangular waveforms under the same conditions, and the advantages and disadvantages became very clear. Together with our previous finding of significant differentiation of pH changes from monoamines on rectangular pulses of NPV, the combined use of rectangular and triangular pulses would improve the molecular identification of monoamines in the brain using voltammetric recording.

1. Introduction

The usefulness of fast-scan cyclic voltammetry (FSCV) for the detection of dopamine (DA) release in the rat striatum has been established [1–3]. It is not only widely used today in rats [4,5], but it is also capable of detecting the reward-associated DA release in behaving mice [6] and monkey striatum [7]. Important cognitive roles of DA in the prefrontal cortex (PFC) have been suggested [8–10], particularly in primates. In addition to the PFC, the rapid DA release in various locations of the brain, such as the hippocampus [10,11], are awaiting a means of detection. For its application in the PFC or the hippocampus, however, DA should be distinguished from other monoamines like norepinephrine (NE) and serotonin (5-HT). In the striatum, the monoamine-like signal has simply been assumed to be DA because of its overwhelming concentration. However, the relative concentration of DA is close to those of NE and 5-HT in the PFC or the hippocampus [12].

The segregation of NE from DA is impossible with FSCV because the voltage-current waveforms of NE and DA are identical [3]. The voltammetric detection of NE on a carbon fiber microelectrode has been performed by FSCV [13–15], along with other voltammetric techniques [16–18]. Efforts to record NE and DA in the different part of the brain has also been tried [15], but the differential recording of two monoamines at the same recording location has not been possible by standard techniques.

While FSCV applies triangular potential scan, rectangular potentials are also used for voltammetric techniques, including normal-pulse voltammetry (NPV), differential pulse voltammetry and chronoamperometry. We have shown the usefulness of voltammetry using rectangular pulse for fine chemical differentiation [19–24]. Rectangular pulse is useful for the segregation of changes in 5-HT from DA [19,20,24]. The voltage dependency of an electrochemical reaction is the basic principle behind chemical identification by voltammetric recordings, and NPV is more effective for finer identifications of voltage

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dependencies, because the NPV waveform has a period with constant voltage. With fast voltage sweeping in FSCV, the voltage selectivity can be temporally skewed due to electrochemical kinetics, although the peak current amplitude is enhanced. When fine chemical identification is required, NPV would be helpful, and it may assist in differentiating NE and DA. During our previous study (Yoshimi 2014 [20]), we noticed a minor difference in the responses of NE and DA on a 0.1 V rectangular pulse (unpublished observation). In the present study, we sought to use this method for the quantitative estimation of NE and DA. In this study, to take advantage of the high sensitivity of fast-scan cyclic voltammetry (FSCV) and the fine chemical identification of normal pulse voltammetry (NPV), we attempted to combine both techniques on a single carbon fiber. Here, we show the differentiation of NE from DA using an alternative dual recording of FSCV and NPV.

2. Materials and methods

2.1. Electrodes

Carbon-fiber microelectrodes were prepared based on our previous procedures [6,7,19–24]. Individual 7 μm diameter carbon fibers (HTA-7, Toho Tenax Co., Tokyo, Japan) were sealed in pulled glass capillary tubes with an epoxy-resin such that 0.25 mm of the carbon fiber protruded from the capillary tube. The reference and counter (auxiliary) electrodes were Ag/AgCl wires.

2.2. Chemicals

Chemical reagents including dopamine HCl (DA), norepinephrine (NE), serotonin HCl (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were purchased from Sigma Aldrich (St. Louis, MO, USA). Ascorbate, uric acid and other special-grade reagents were purchased from Wako Pure Chemicals (Tokyo, Japan). Artificial cerebrospinal fluid (ACSF) for medical use (ARTCEREB, Otsuka, Tokyo, Japan: 145 mM Na +, 129 mM Cl −, 2.8 mM K +, 1.1 mM Mg2 +, 1.15 mM Ca2 +, 23.1 mM HCO3, 1.1 mM phosphate, and 0.61 g/L glucose) was purchased commercially and bubbled with 5% CO2/95% O2 gas for over 0.5 h until measurement to stabilize the pH, as described previously [20].

2.3. Electrochemistry

Two types of electrochemical recordings were performed alternatingly in this study: FSCV and NPV (Fig. 1A). The carbon fiber microelectrode was switched between channel 0 and channel 1 of the multichannel potentiostat (Model HECS-9139, Huso Electrochemical Systems, Kawasaki, Japan). Although other sophisticated method has been used to combine triangular and rectangular waveforms previously [25], we found that switching by simple relay functioned well in our recording system. The Ag/AgCl wires for reference and counter electrodes were common for FSCV and NPV recordings. For pulse control and data acquisition, a commercial control/recording system (TH-1; ESA Biosciences, Inc., MA USA) with two multifunction boards (NI-PCI-6221, National Instruments, TX, USA) integrated with a Windows PC was used. The voltage waveform for FSCV was generated by the TH-1, while the three-step rectangular pulses were generated by a pulse generator (MASTER-9, AMPI, Israel). Since the amplitude of the background current was much higher in FSCV, the gain of the amplifier was switched between the FSCV and NPV recordings. The gain of the amplifier was 200 nA/V with a low-pass filter (LPF) of 0.2 ms time constant for

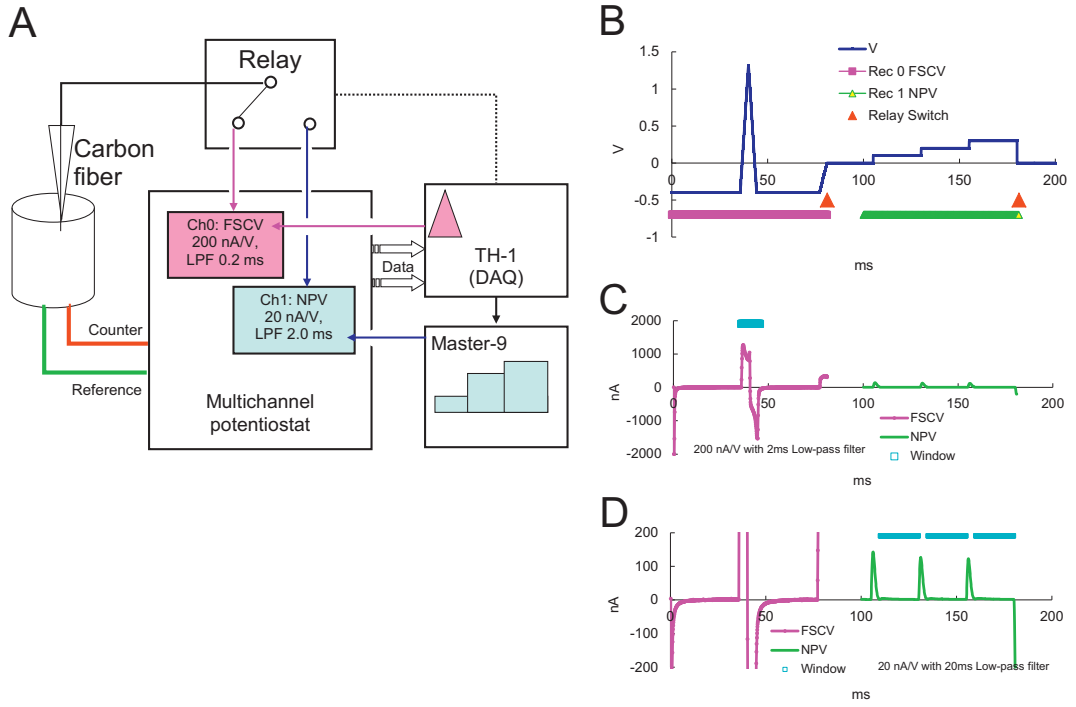

Fig. 1. Alternating dual voltammetry method.
(A) Switching circuit for the alternating dual voltammetric recording. The two channels of a multi-channel potentiostat with different settings were switched alternatingly. A triangular FSCV waveform was directly applied from TH-1 DAQ, while rectangular pulses for NPV were generated by the MASTER-9 triggered by the DAQ. (B) Combined waveform for the alternating dual recording of FSCV and NPV. The timings of the relay switch are indicated with red triangles. The thick horizontal bars indicate the recording duration of FSCV by Ch0 (magenta) and NPV by Ch1 (green). The FSCV part was recorded with a 200 nA/V amplifier with a 0.2 ms LPF, while 20 nA/V with 2.0 ms LPF was applied for the NPV part. The combined waveform was applied at 5 Hz. (C and D) The background currents of the carbon fiber in the PBS. The thick light-blue horizontal bar indicates the duration of current analysis for FSCV (C) and NPV (D). The small electrochemical current was isolated after a background subtraction for both FSCV and NPV. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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