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Optimization of Paired Pulse Voltammetry Using Sawhorse Waveform

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The influence of the reduction process on paired-pulse voltammetry (PPV), which consists of two identical triangle-shaped pulses, was studied in this study by increasing peak potential duration. We propose sawhorse waveform as an optimized PPV waveform for enhancing the responses of PPV on dopamine and serotonin (5-HT). To investigate the influence of the scanning parameters on the reduction process and response of PPV, the responses of dopamine were measured by changing the switching potential (from 1.0V to 1.5V), and scan rates (from 400V/s to 1000V/s) based on triangle shaped PPV. As a result, the oxidation and reduction peak ratio in the response of the primary pulse in PPV decreased in a linear fashion with increases to the duration between oxidation and reduction potential, which lead us to obtain more defined features in the subtracted voltammograms (primary-secondary) in PPV. Based on these results, sawhorse waveform PPV with prolonged switching potential duration was suggested to increase the sensitivity of PPV while maintaining its own advantages, and it is also confirmed in an *in vivo* experiment. In addition to the dopamine experiments, we also conducted an experiment with serotonin utilizing the N-shaped sawhorse waveform PPV. The subtracted voltammogram in the N-shaped PPV with 1.0V peak and 4ms peak potential duration clearly showed the characteristics of serotonin. In conclusion, PPV waveforms could be optimized to improve the sensitivity of PPV responses by the control of the reduction process.

Keywords: Fast-scan cyclic voltammetry (FSCV); Paired pulse voltammetry (PPV); Sawhorse waveform; Dopamine; Serotonin

1. INTRODUCTION

The fast-scan cyclic voltammetry (FSCV) technique with carbon-fiber microelectrodes (CFMs) has been widely used for detecting electroactive neurotransmitters *in vitro* and *in vivo* [1, 2]. Unlike other neurochemical monitoring methods, FSCV offers sub-second time resolution, high spatial

resolution, and great chemical selectivity. With these advantages, many studies have been attempted to elucidate neurological and psychiatric disorders by measuring the extracellular concentration change of neurochemical substances [3-5]. At the same time, FSCV techniques have been improved to unravel underlying neuropathological mechanisms by electrode fabrication [4, 6], signal processing [7, 8], instrumentation [9, 10], and developing specific voltammetric waveforms [11, 12]. Despite all of these contributions for improvement, applying FSCV in vivo still has drawbacks due to complex voltammograms, influence by pH transients, a changing non-faradaic background current, and other environmental changes at the CFM surface.

The authors have previously reported paired-pulse voltammetry (PPV) as a modified FSCV technique to isolate neurotransmitters from other environmental changes [13, 14]. PPV is able to differentiate analytes in mixture environment and minimizes the confounding factors under rapidly changing circumstances at the surface of CFM based on the analytes' adsorption characteristics. The scan waveforms are paired in doublets, two identical triangle-shaped waveforms, separated in time by a short interval at the holding potential. When the voltammogram of the secondary pulse within the doublet is subtracted from the voltammogram of the primary pulse, the effects of the confounding factors like pH change can be eliminated. Recently, we also found that the differences in dopamine (DA) responses between primary and secondary pulses in PPV may be attributed to the different amounts of reduction occurring during the holding potential time between scans as well as characteristics of DA adsorption on CFMs [15]. This suggested that the difference voltammogram (primary pulse minus secondary pulse voltammogram) could be controlled by changing the scanning parameters that affects the dopamine-o-quinone (DOQ)'s reduction process.

We hypothesized that it could be possible to optimize the PPV waveform to obtain more defined responses of PPV on metabolites by controlling the scanning parameters. For example, an enhancement in the DA responses of paired-pulse subtracted voltammograms could be obtained by simply prolonging the duration of the switching potential. The larger amount of DOQ that instantly desorbs after production at the primary pulse during the prolonged switching potential is likely due to the longer duration between oxidation peak potential and reduction peak potential. The less DA reduced from DOQ at the primary pulse (-0,2V) leads to a decrease in the response at the secondary pulse, thus resulting in a greater magnitude and clearer features of the primary minus secondary voltammograms of DA oxidation. In this study, we investigated the influence of the reduction process on PPV by manipulating the scanning parameters and then proposed a new type of PPV waveform for enhancing the responses of PPV on DA and 5-HT.

2. MATERIALS AND METHODS

2.1. FSCV waveform

All experiments were done by changing the scanning parameters of two basic PPV waveforms: triangle shaped PPV [13] and N-shape PPV [14]. Triangle shaped PPV consists of triangle-shaped (sweeping from -0.4 V to +1.0 V and back to -0.4 V) pairs of voltammetric pulses with a specific time delay between the two pulses, repeated with a negative holding potential between the paired pulses (Fig. 1A, solid line). N-shape PPV also consists of a paired N-shape pulse (sweeping from +0.2 V to

+1.0 V to -0.1 V and back to +0.2 V) with a positive holding potential between the paired pulses (Fig. 5A, solid line). The primary pulse is defined as the first pulse and the secondary pulse is the following pulse. This allows two different voltammograms (primary (P) and secondary (S) voltammograms) to be obtained, each corresponding to the two pulses in the binary scans. With these two voltammograms, the difference (P-S voltammogram) is calculated.

2.2. Data Acquisition

Data were acquired by use of a commercial interface (NI USB-6251, 16 bit, National Instruments) with a home computer and software in the LabVIEW programming environment (National Instruments, Austin, TX). NI USB-6251 was also used for synchronization of the applied waveform and flow injection analysis control. After data collection, background subtraction, signal averaging, and digital filtering were all done under software control. Custom software controls waveform parameters and operations, such as data acquisition and transmission, the applied potential waveform, and data sampling rate. Data, in the form of a sequence of unsigned 2-byte integers, are saved to the base-station computer hard drive for offline processing using MATLAB (MathWork Inc., Natick, MA). The acquired data are displayed in several graphical formats by custom software for analysis.

2.3. Fabrication of carbon fiber microelectrodes

The carbon fiber microelectrode (CFM) was constructed by inserting a single carbon fiber (d = 7μm, Cytec Thornel® T300) into a silica capillary and insulated with polyamide. The CFM was connected to the Nitinol wire (Fort Wayne Metals, Indiana, USA, Nitinol #1) with a silver-polyamide mixed paste. The nitinol wire was insulated with polyamide tubing. The exposed carbon fiber was trimmed to a final length of around ~100μm using a scalpel [4].

2.4. Flow injection apparatus

A flow-injection analysis system that consisted of a syringe pump (Harvard Apparatus, Holliston, MA) that directed a buffer solution through a Teflon tube to a 6-port injection valve (Rheodyne, Rohnert Park, CA) was used for in vitro measurements of DA and 5-HT. The injection valve was controlled by a 12V solenoid and was used to transport the analyte from an injection loop to an electrochemical flow cell at a rate of 2 ml/min. A CFM was placed in a flowing stream of buffer and analyte was injected as a bolus.

2.5. Chemicals

DA and 5-HT were dissolved in distilled water at a stock concentration of 1mM diluted to the tris buffer (15mM tris(hydroxymethyl) aminomethane, 3.25 mM KCl, 140mM NaCl, 1.2 mM $CaCl_2$,

1.25 mM NaH_2PO_4, 1.2 mM $MgCl_2$, and 2.0 mM Na_2SO_4, with the pH adjusted to 7.4) and kept in 0.1M perchloric. Then dilute solutions were made in TRIS buffer for desired final concentration before the flow injection experiments. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

2.6. in vivo experiment

Adult male Sprague-Dawley rats weighing 250-450 g were used for this study. The rats were sheltered with a 12:12 hr light and dark cycle (lights on at 0600 hr) with *ad libitum* access to food and water. NIH guidelines were practiced for the care of animals and the Hanyang University Institutional Animal Care and Use Committee approved the experimental procedure. The rats were injected with urethane (1.6 g/kg i.p.) for anesthesia and stabilized in a commercially available stereotaxic frame (David Kopf Instruments, Tujunga, CA) for the surgery. Three burr holes (0.5-1.0 mm diameter) were made on the skull of each rat for the implantation of a CFM, a twisted bipolar stimulating electrode (Plastics One, MS 303/2, Roanoke, VA, USA), and an Ag/AgCl reference electrode. Electrode coordinates were referenced by a rat brain atlas [16] based on flat-skull position using bregma and dura as reference points with coordinates anteroposterior (AP), mediolateral (ML), and dorsoventral (DV). The CFM was placed in the right hemisphere in the caudate putamen (CPu) (AP +1.2 mm; ML +2.0 mm; DV -4.5 to -6.0 mm), and the stimulation electrode was inserted ipsilaterally just above the medial forebrain bundle (MFB) (AP -4.6; ML +1.3; DV -8.0 to -9.0). 4ms bipolar electrical stimulation is delivered for 2 seconds at 150μA with 130Hz parameter. The reference electrode was positioned superficially in the cortical tissue of the left hemisphere.

2.7 Statistics

GraphPad Prism 5 (GraphPad Software, San Diego, CA) was used for statistics. All error bars are given as mean ± standard error of the mean (SEM) for n number of electrodes. A one-way ANOVA with Dunns post-tests was performed to compare effects of parameters to redox ratio or oxidation ratio between primary and secondary pulses. Significance is defined as $p = 0.05$.

3. RESULTS AND DISCUSSION

3.1. The optimization of PPV waveform for the detection of dopamine

In a previous study, we described the differences in DA responses between the primary and secondary pulses based on the characteristics of DA adsorption on CFMs [13]. It was suggested that the possibility of these differences may be due to the effect of the amounts of reduction occurring in the primary pulse influencing the amount of redox in secondary pulse [15]. In this study, in order to investigate the influence the switching potential and scan rate has on the responses of PPV, the responses of DA were measured by changing the switching potential (from 1.0V to 1.5V, fig. 1A), and scan rate (from 400V/s to 1000V/s, fig. 2A) based on the triangle shaped PPV consisting of -0.4V

holding potential, 10Hz repetition time and 2ms interval between two triangle waveforms with a flow cell injection of 3uM DA. The ratio of the reduction peak current over the oxidation peak current at the primary pulse was calculated to examine the influence of the experimental parameters on the reduction process. As a result, increased switching potential significantly decreased the redox peak ratio in the response of the primary pulse (Fig. 1C; one-way ANOVA, $F=3.004$, $p=0.0303$). As shown in Fig. 1C, the redox peak ratio in the response of the primary pulse decreased in a linear fashion with the increases in switching potential. 1.0V vs 1.5V and 1.0V vs 1.4V showed significantly different ratios of redox. The decreasing redox ratios of the primary pulse could be affected by both surface change and time between oxidation and reduction. It is known that extending waveform to 1.5V changes the electrode surface, which leads to the increase of DA sensitivity [11, 17]. In our result, correspondently, the oxidation peak in 1.5V extending waveform was higher than the one in 1.0V waveform (Fig. 1B), whereas the discrepancy of reduction peaks of two waveform was comparably not large. In addition, the time between oxidation and reduction might effect on the redox ratio. A percentage of DOQ could instantly desorb from the CFM surface after production, which means that, the amount of DOQ desorption is proportional to the duration from +0.6 V (oxidation potential) of the anodic sweep to -0.2V (reduction potential) of the cathodic sweep in a primary pulse. Under the present conditions, the time between oxidation and reduction peaks while utilizing a 1.5V switching potential pulse is 6.5ms compared to 4.0ms when using a 1.0V switching potential, a difference of 2.5ms. In PPV, the oxidation peak current of the secondary pulse is influenced by an amount of reduction occurring in the primary pulse as well as DA adsorption during the interval between pulses at the holding potential. The larger amount of DOQ that instantly desorbed after production at the primary pulse using a 1.5V switching potential, rather than a 1.0V switching potential, is likely caused by the longer duration between the oxidation peak potential and reduction peak potential. According to Bath *et al.*, the redox ratios could be affected by DOQ desorption after production and electrostatic force [21]. Since the electrostatic contribution at holding potential (-0.4V) does not exist between oxidation and reduction potential, DOQ desorption is confirmed. This leads to a decrease in the oxidation response at the secondary pulse compared to the one at the primary pulse as depicted in Fig. 1D (one-way ANOVA, $F=8.837$, $p<0.0001$). Similar results were also shown by changing the scan rate from 400V/s to 1000V/s (Fig. 2A). By changing the scan rate from 400V/s to 1000V/s, the duration from +0.6V to -0.2V decreased from 4ms to 1.6ms (Fig. 2A). When the scan rate was increased (decreasing the duration from +0.6V to -0.2V), the redox peak ratio in the response of primary pulse increased in a linear fashion. The primary pulse redox ratio of 400V/s is significantly lower than 1000V/s (Fig. 2C, one-way ANOVA, $F= 4.085$, $p=0.0248$). Secondary versus primary oxidation peak ratios showed identical results to the switching potential experiment (Fig. 2 D, one-way ANOVA, $F= 16.21$, $p<0.0001$). This result is a re-verification of previous research about the reduction process [15]. Because of the rate limited process of reduction in DA, the redox ratio does not change with more oxidation current by higher DA concentration but duration between oxidation peak potential and reduction peak potential [15]. This implies that the redox ratio is changed not because of more oxidation peak current but instant desorption of DOQ after oxidation [21].

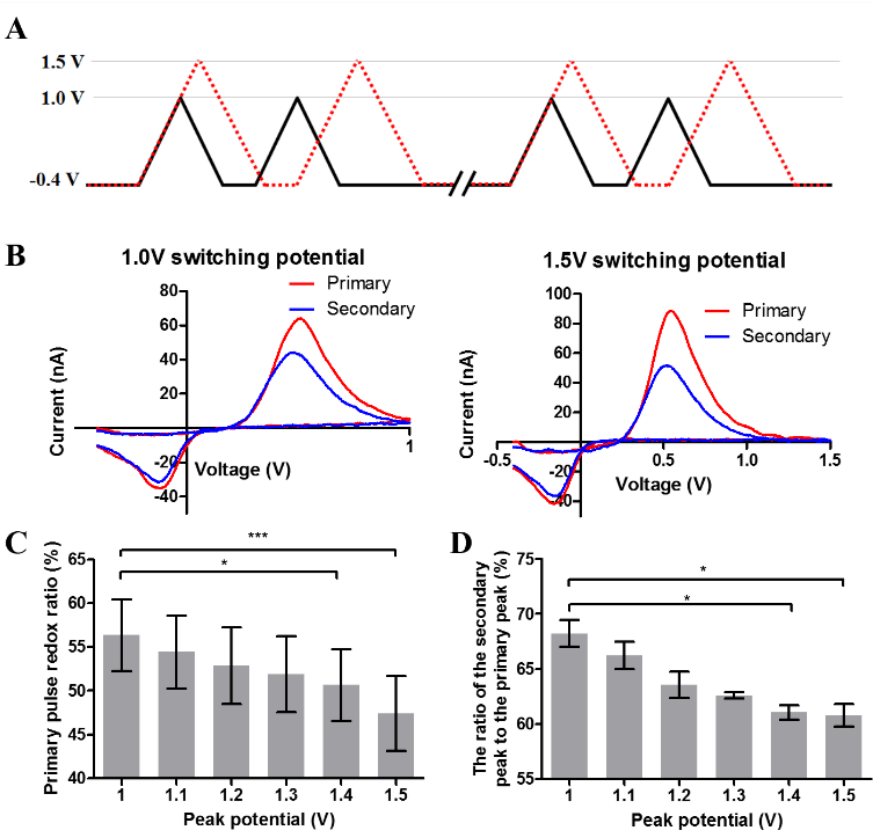

Figure 1. Responses of PPV according to switching potentials at 400V/s scan rate. (A) PPV waveform changing switching potential from 1.0V (black solid line) to 1.5V (red dotted line). (B) The examples of primary P and secondary S voltammograms at 1.0V and 1.5V switching potential. (C) Comparison of different switching potentials on the ratio of reduction peak current over oxidation peak current (redox ratio) in P voltammogram of PPV. The redox ratio of 1.0V switching potential waveform showed significantly different from 1.4V and 1.5V switching potential waveform. (*$p < 0.05$, *** $p < 0.0001$, n=5, One-way ANOVA with Dunn's post-test) (D) The ratio of secondary oxidation peak current over primary oxidation peak current (oxidation peak current ratio) in PPV responses depending on switching potentials. Also 1.4V and 1.5V switching potential waveform has significantly lower oxidation peak ratio than 1.0V peak waveform. (*$p < 0.05$, n=5, One-way ANOVA with Dunn's post-test)

To evaluate the effects of the duration between oxidation and reduction potentials without surface change, we carried out an additional experiment by keeping the switching potential the same (1.0V), but with different switching potential durations in PPV like a sawhorse waveform PPV as depicted in Fig. 3A [18]. The switching potential duration was prolonged in order to control the duration time from +0.6 V (oxidation potential) of the anodic sweep to -0.2V (reduction potential) of the cathodic sweep. With a 5ms switching potential duration, the time between oxidation potential and reduction potential is 6.6ms compared to 1.6ms for no switching potential duration. In this experiment, repetition time is fixed for 92.42ms which is in case of 0ms duration waveform by altering scan parameters from 10Hz (0ms duration) to 9.09Hz (5ms duration).

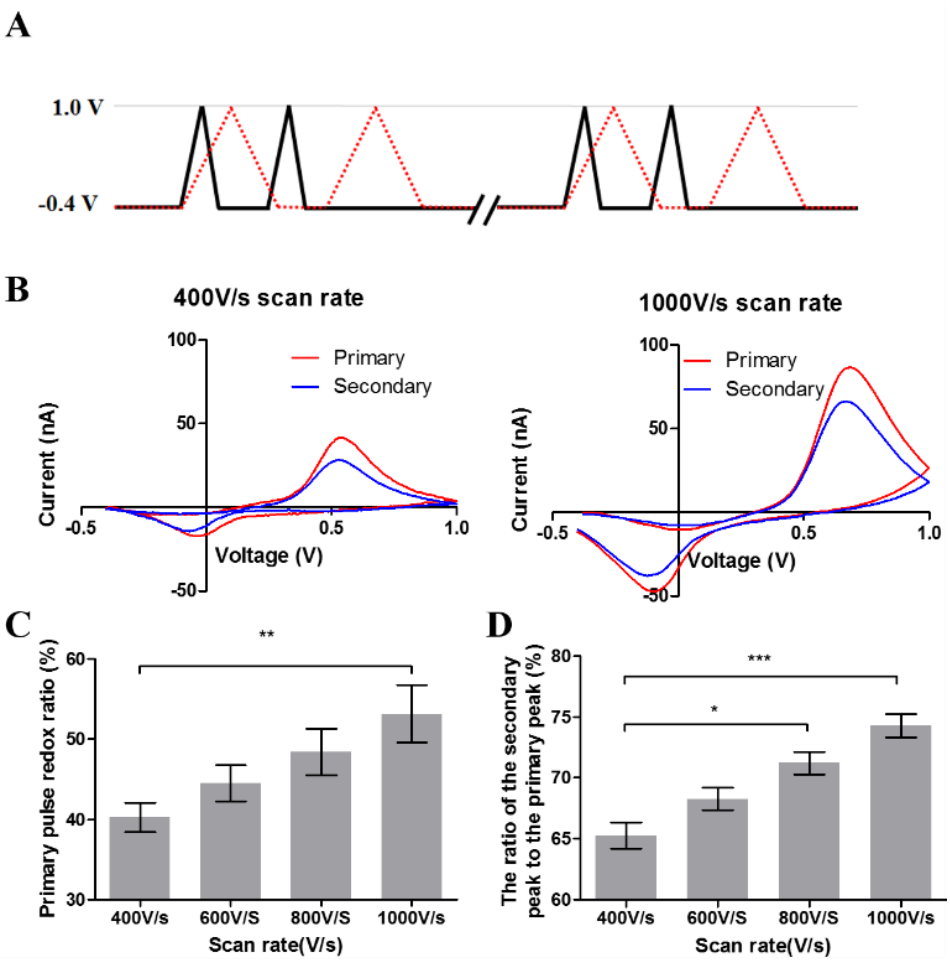

Figure 2. Responses of PPV according to scan rates. (A) PPV waveform changing scan rate from 400V/s (red dotted line) to 1000V/s (black solid line) (B) The examples of P and S voltammograms at 400V/s and 1000V/s scan rate (C) Comparison of different scan rate on the ratio of reduction peak current over oxidation peak current in P voltammogram of PPV. The redox ratio of 400V/s scan rate waveform is showed significantly lower than 1000V/s scan rate waveform. (** $p < 0.005$, n=6, One-way ANOVA with Dunn's post-test) (D) The oxidation peak current ratio depending on scan rates. 400V/s scan rate waveform showed significantly lower peak ratio than both 800V/s and 1000V/s scan rate waveform. (*$p < 0.05$, *** $p < 0.0001$, n=6, One-way ANOVA with Dunn's post-test)

As expected, the redox peak ratio in the response of primary pulse decreased with increases in peak potential duration from 0ms to 5ms (Fig. 3C), which corresponds to the influence of the switching potentials and scan rates in previous experiments. The amount of DOQ desorption is proportional to the time from oxidation potential to reduction potential. As a consequence of the decreased redox peak ratio, the oxidation response at the secondary pulse were decreased as well in longer peak potential durations because less DOQ has reduced at the reduction potential(-0.2V) of primary pulse (Fig. 3D, one-way ANOVA, F= 24.71, p<0.0001). This could lead us to obtain higher sensitivity of subtracted voltammogram (primary-secondary) in PPV (Fig. 3B, black line).

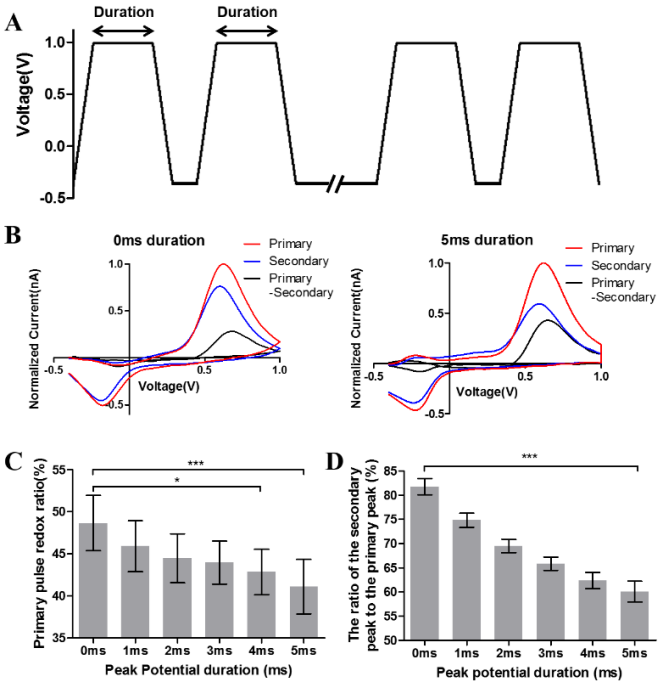

Figure 3. PPV DA responses according to peak potential durations at 1000V/s scan rate. (A) Waveform of PPV with various peak potential duration. (B) Normalized primary and secondary voltammograms (red line and blue line, respectively) with 0ms and 5ms peak potential duration. Black line indicates the subtraction of primary from secondary voltammogram (C) The DA redox ratio of primary pulse with variations of duration. The redox ratio is decreased as peak potential duration from 0ms to 5ms. PPV waveforms with 4ms and 5ms duration are significantly lower than 0ms duration. (*$p < 0.05$, ***$p < 0.0001$, n=5, One-way ANOVA with Dunn's post-test) (D) The oxidation peak current ratio with variaions of holding duration. Decreasing pattern is appeared as duration is increased because of decreasing pattern of redox ratio in primary voltammogram. 5ms duration waveform has significantly lower ratio than 0ms duration waveform. (***$p < 0.0001$, n=5, One-way ANOVA with Dunn's post-test)

In order to increase DA sensitivity higher within fixed repetition time (10Hz), we adapted a sawhorse waveform PPV with a high scan rate as well as a long peak potential duration (Fig. 4A). It is already known that the DA peak current linearly corresponds to the scan rate due to adsorption properties, while capacitive background current varies with the square root of scan rate [19]. It means we can increase the subtracted DA signal to noise ratio in PPV by two factors which are scan rate and switching potential duration. Keithley *et al.* were able to improve the DA sensitivity stably while maintaining the rapid temporal response by increasing scan rate into a sawhorse design [18]. So we evaluated the DA responses of the PPV waveforms with a fixed total pulse length by increasing the scan rate by increments of 200 from 400, 600, 800, to 1000V/s and also by adding a peak potential duration of 0.0ms, 2.4ms, 3.5ms, and 4.2ms, respectively. As a result, the oxidation peak current of the primary pulse (Fig. 4D, red line) showed approximately a 2-fold increase in PPV waveform with a 1000V/s scan rate and 4.2ms peak potential duration compared to the PPV waveform with a 400V/s scan rate and no peak potential duration, whereas the oxidation peak current of the secondary pulse

increased approximately only 20%. The reason that low oxidation peak current of secondary pulse is less DOQ remain to be reduced at the surface of electrode as well as Fig. 3(Fig. 4B). Thus, the oxidation peak current ratio of the secondary over primary pulses significantly decreased by increasing the scan rate and peak potential duration (Fig. 4C, one-way ANOVA, F= 43.67, p<0.0001). The standard deviation of the noise in the current versus time graph remains at around 0.2 in both 400V/s and 1000V/s with a sawhorse waveform (0.19±0.03 and 0.19±0.02, not significantly different, n=5, *p*=0.9166). Therefore, the combination of changing both the scan rate and peak potential duration with a fixed pulse length enabled us to obtain higher response of dopamine as well as clearer DA features in the difference voltammogram between primary and secondary as shown in Fig. 4D.

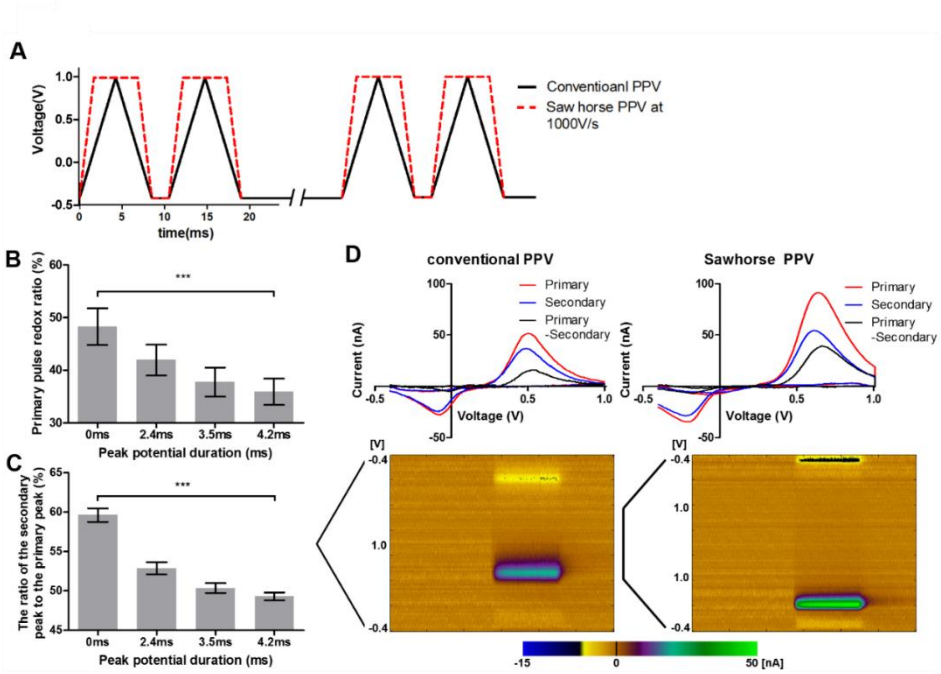

Figure 4. Optimization of sawhorse PPV with fixed duration of PPV. (A) Conventional PPV (dark solid line), and sawhorse PPV (red dashed line). By increasing peak potential holding duration 2.4ms, 3.5ms, and 4.2ms, scan rate is also increased by 600V/s, 800V/s, and 1000V/s respectively. (B) The DA redox ratio of primary pulse with increased pattern of holding duration. The redox ratio is decreased as peak potential duration from 0ms to 4.2ms. 4.2ms and 0ms duration showed significant difference. (***p < 0.0001, n=6, One-way ANOVA with Dunn's post-test) (C) The oxidative peak current ratio with variations of holding duration. The ratio is decreasing trend and 0ms and 4.2ms duration is significantly different. (***p < 0.0001, n=6, One-way ANOVA with Dunn's post-test) (D) Examples of subtracted voltammogram and P-S pseudo-color plot of 3μM dopamine using 0ms holding duration PPV (conventional PPV, left) and 4.2ms sawhorse PPV (right).

3.2. The optimization of PPV waveform for the detection of serotonin

N-shape waveforms with a 0.2V holding potential have been used to detect 5-HT in previous studies due to 5-HT's higher sensitivity compared to triangular waveform [12]. Despite adopting N-shape waveform for 5-HT, the detection of 5-HT *in vivo* is difficult because the reactant after oxidation

reduces the sensitivity and because of complex electrochemical environments [22]. N-shape waveform PPV is able to differentiate the complex analytes *in vivo* [13], however, when N-shape waveform PPV was applied to detect 5-HT as shown in Fig. 5A (black line), the difference voltammogram between the primary and secondary pulses has only around a 20% magnitude of the oxidation peak of the primary oxidation peak current. This is caused by a higher reduction over oxidation ratio in the primary pulse [14]. Thus, the oxidation peak ratio of the primary versus secondary pulse was around 80%, which lead to the reduced response in subtracted voltammogram. In order to maximize the PPV response to 5-HT, we modified the N-shape PPV waveform with an increased peak potential duration and higher scan rate as described in the previous section (Fig. 5A, red dashed line). As a peak potential duration increased from 0ms to 4ms, the oxidation peak current of the primary pulse increased more than the oxidation peak current of the secondary pulse, which lead to a decreased in the oxidation peak ratio (Fig. 5C, one-way ANOVA, F= 10.81, $p<0.0001$). As depicted in Fig. 5D, the subtracted voltammogram in the N-shape PPV with 4ms peak duration showed twice higher signal to noise ratio than conventional N-shape PPV.

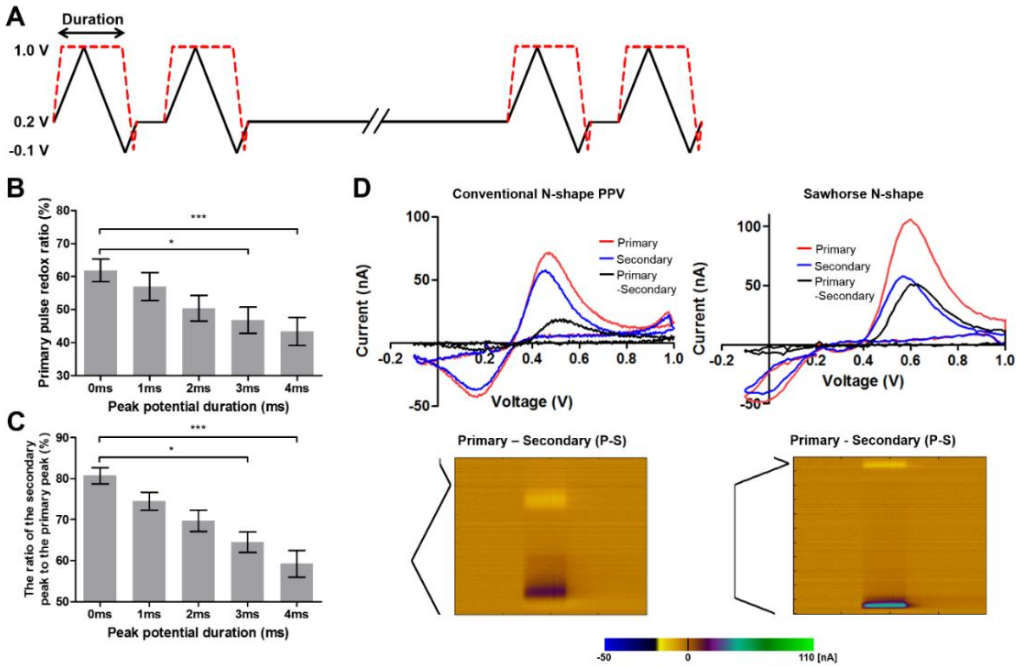

Figure 5. Optimization of N-shape PPV for 5-HT (A) N-shape PPV (dark solid line), and N-shape sawhorse PPV (red dashed line). As holding duration has increased from 0ms to 4ms, scan rate also increased from 400V/s to 1467V/s. (B) The 5-HT redox ratio of primary pulse with increased pattern of holding duration. The redox ratio is decreased as peak potential duration from 0ms to 4ms. 4ms and 0ms duration showed significant difference. (***$p < 0.0005$, n=5, One-way ANOVA with Dunn's post-test) (C) The oxidative peak current ratio with variations of holding duration. The ratio is decreasing trend and 0ms and 4ms duration is significantly different. (***$p < 0.0005$, n=5, One-way ANOVA with Dunn's post-test) (D) Examples of subtracted voltammogram and pseudo color plot of 3μM 5-HT using 0ms holding duration (conventional N-shape PPV, left) and 4ms sawhorse PPV (right).

3.3. The evaluation of sawhorse PPV in in vivo experiment

For evaluating the performance of the sawhorse PPV *in vivo*, we applied a sawhorse PPV (Fig. 4A, red dashed line) for DA detection while utilizing a similar animal preparation to that described in Paek et al.'s study [20]. This consisted of the implantation of a CFM into the CPu of anesthetized rats (urethane, 1.6 g/kg, i.p.) and FSCV recordings during an electrical stimulation of the MFB. When applied with a conventional PPV, compared to the DA response of the primary pulse, the DA response of the secondary pulse was slightly lower, which leads to unclear voltammogram of primary minus secondary pulse (Fig 6A). By applying a sawhorse PPV, the difference between primary and secondary response was increased and the DA features were clearly seen at the voltammogram of the primary minus secondary pulse voltammogram (Fig. 6B). In addition, background current changes due to the surface or pH change as shown in Fig. 6 were not seen in the voltammogram of primary minus secondary pulse, which is one of the merits of PPV as described in our previous study [13]. The oxidation peak of Primary-Secondary voltammogram has shown 3-folded increasement (Fig. 6D).

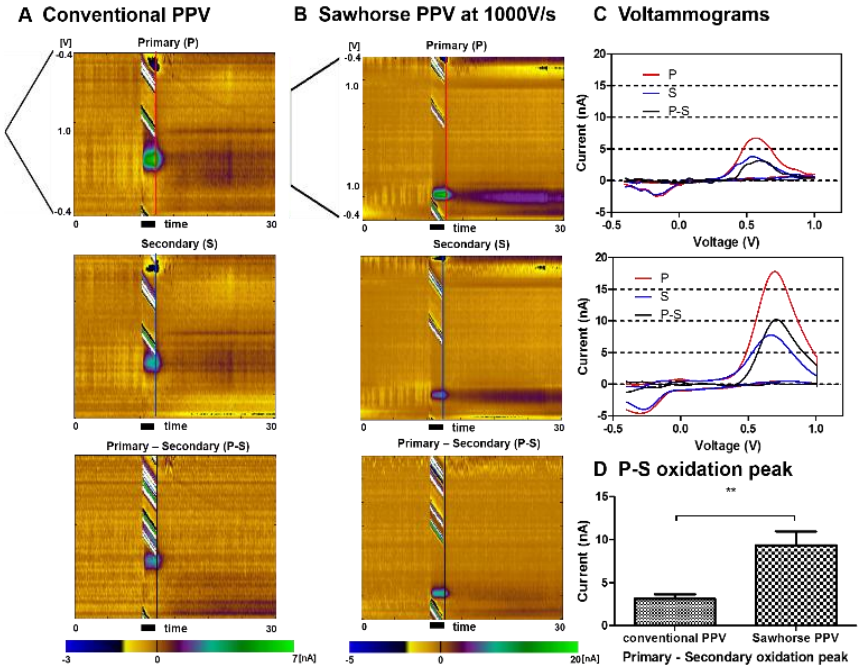

Figure 6. *in vivo* experiment of sawhorse PPV in an anesthetized rat. (A, B) Color plot representation of stimulated dopamine release with conventional PPV (primary waveform, secondary waveform, primary-secondary waveform in order), black bar represents electrical stimulation. Voltammetric sweep waveforms are plotted left side of each primary color plot. (C) Vertical lines in each color plot are extracted as cyclic voltammograms (conventional PPV-above, sawhorse PPV-bottom). (D) P-S oxidation peak current is significantly increased when sawhorse PPV is applied (n=5, paired t-test, p <0.005, t = 5.919, df = 4)

4. CONCLUSIONS

In summary, an optimized PPV waveform was developed by adjusting the scan rate and peak

potential duration, which affected the reduction mechanisms shown in previous research. The proposed waveforms are demonstrated by adjusting switching potential and scan rate in PPV. Each waveform showed approximately a 2-fold increase in sensitivity for both dopamine and 5-HT in difference voltammogram between the primary and secondary pulse. In conclusion, PPV waveform could be optimized to improve the sensitivity of PPV responses by changing the peak potential duration, scan rate, and switching potential voltage for DA and 5-HT measurements due to the control of the reduction process.

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