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ACCURATELY DETERMINING PROPAGATION VELOCITY OF CORTICAL SPREADING DEPRESSION IN RATS BY OPTICAL INTRINSIC SIGNAL IMAGING

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Cortical spreading depression (CSD) is a wave of neuronal and glial depolarization that propagates across the cortex at a rate of 2–5 mm/min accompanied by reversible electroencephalogram (EEG) suppression, a negative shift of direct current (DC) potential, and change of optical intrinsic signals (OIS). Propagation velocity of CSD is an important parameter used to study this phenomenon. It is commonly determined in an electrophysiological way that measures the time required for a CSD wave to pass along two electrodes. Since the electrophysiology technique fails to reveal the spreading pattern of CSD, velocity calculated in this manner might be inaccurate. In this study, we combined the electrophysiological recording and OIS imaging (OISI) for detecting changes in DC potential and OIS during CSD simultaneously. An optical method based on OISI to determine the CSD velocity, which is measured by generating a series of regions of interest (ROI) perpendicular to the advancing wavefront along propagation direction of CSD at different time points and then dividing by the distance between ROIs over time, is presented. Comparison of the accuracy of the two approaches in determining the CSD velocity is made as well. The average rate of 33 CSDs is 3.52 ± 0.87 mm/min by use of the optical method and 4.36 ± 1.65 mm/min by use of the electrophysiological method. Because of the information about spreading pattern of CSD provided optically, the velocity determined by OISI is of smaller deviation and higher accuracy.

Keywords: Cortical spreading depression velocity; optical intrinsic signal imaging; electrophysiology; accuracy.

1. Introduction

Cortical spreading depression (CSD) was first discovered by Leão in 1944.[1] Since then CSD has been characterized by a wave of depolarization of neuron and glia that propagates across the cortex at a rate of 2–5 mm/min.[1] It leads up to a negative shift in direct current (DC) potential accompanied by a reversible electroencephalogram (EEG) suppression,[2] change of OIS as well as significant shifts in intra- and extra-cellular ion concentrations, e.g., sodium, potassium, calcium, and chloride.[3] CSD is also thought to be associated with trauma,[4] cerebral ischemia,[5,6] and migraine.[7] It could be induced by a variety of stimuli such

as mechanical pinprick, electrical stimulation, and chemical stimulation.[8]

Propagation velocity of CSD is an important parameter used to study the phenomenon. Many experiments have indicated that many factors, such as citalopram[9] and electrical stimulations,[10,11] could influence the CSD propagation velocity. In addition, CSD provides a reliable index for cortical excitability. An increased CSD velocity seems to reflect an elevated cortical excitability, which correlates with a decreased threshold for CSD induction, and vice versa.[11] Accurate determination of velocity is thus essential for studying the relationship between CSD and cortical excitability. The traditional way to determine CSD velocity is based on electrophysiological signals. Velocity is indexed by the velocity of propagation of DC potential change and calculated from the time required for a CSD wave to pass along two cortical electrodes.[11–13] As we all know, velocity of wave is theoretically defined as the distance traveled in per unit time on the propagation direction. In electrophysiology, relative position between two electrodes is fixed after electrode implantation. However, propagation direction of CSD remains unpredictable, variable, and not always consistent with the relative position of electrodes. Moreover, spreading pattern of CSD is nonuniform.[14–16] The speed of spread was different along different directions.[16] As a result of these factors, the velocity determined in the electrophysiological way is not always accurate.

Optical intrinsic signal imaging (OISI) is a functional neuroimaging technique that measures the cortical reflectance changes with millisecond temporal resolution and micron spatial resolution. OISI is particularly appropriate for studying CSD because a large region of cortex can be investigated simultaneously and multiple time points can be collected as CSD spreads.[17–19] OISI is able to provide information about spreading pattern of CSD.[20] We hypothesized that OISI could determine CSD velocity accurately, and in order to verify it, we combined electrophysiological recording and OISI to detect simultaneously the changes in DC potential and OIS during CSD. A method based on OISI to determine CSD velocity, which is measured by generating a series of regions of interest (ROI) perpendicular to the advancing CSD wavefront at different time points along propagation direction and then dividing by the distance between ROIs over time, is presented. CSD velocity is calculated by electrophysiological way as well for comparison purposes.

2. Materials and Methods

2.1. *Animal preparation*

All experimental procedures were approved by the Committee for the Care and Use of Laboratory Animals at Huazhong University of Science and Technology. Nine adult male Sprague–Dawley rats weighing between 200 and 300 g were used in this study. They were anesthetized with an intraperitoneal injection of a mixture of α-chloralose (50 mg/kg) and urethane (600 mg/kg). Anesthetic depth was assessed by periodically monitoring the toe pinch withdrawal reflex. The body temperature was maintained at $37 \pm 0.5°C$ with a rectal probe and feedback-controlled heating blanket.

The rats were placed in a stereotaxic apparatus. The head was shaved and a midline incision was made, after which the underlying tissue was dissected and retracted. The skull over the right parietal bone was removed with a dental drill (Fine Science Tools, USA) under constant saline cooling to form an imaging window. A burr hole (2 mm in diameter) with the dura intact was drilled over the right frontal bone for CSD induction. The animals were left to recover for at least 1 h.

2.2. *CSD induction*

CSD was elicited by applying a cotton ball (1–2 mm diameter) soaked with KCl solution (1.5 mol/L) into the burr hole for about 17 min since this substance is effective in causing a massive neuronal depolarization and the origin of each CSD is not fixed, which is quite suitable for studying the accuracy of velocity measurement. CSD was induced at 30 min intervals. After each episode, the burr hole was washed with saline solution.

2.3. *Electrophysiology recording*

To record field potential, two Ag–AgCl electrodes were gently inserted into the cortex to a depth of 0.5–1 mm under high magnification by micromanipulator. A common reference electrode was mounted on the nasal bone. The analog signals were amplified by 100× using a high-input impedance amplifier (A-M Systems 3000, USA), low pass filtered at 100 Hz. The output of the amplified signal was concurrently sampled at 100 Hz with optical imaging by a data acquisition (DAQ) board (PCI-6014, National Instruments).

2.4. *Optical imaging*

Light was provided by a halogen light source (Olympus LG-PS2, Japan) and filtered at 550 ± 10 nm. Image was acquired using a 12-bit charge-coupled device (CCD) camera (480×640 pixel resolution, Pixelfly, PCO Computer Optics, Germany) attached to a microscope (Olympus SZ6045TR Zoom, Japan). The mode of CCD was set to an exposure time of 25 ms and taking an average of 32 frames (0.8 s/frame). Thirteen hundred images were acquired in total. Images and electrophysiological data were collected simultaneously by a workstation running a LabVIEW virtual instrument (National Instruments).

2.5. *Data analysis*

Data analysis was carried out on a Matlab workstation. Images were analyzed using running subtraction to show spreading pattern and detect the wavefront of CSD. Details of running subtraction have been described elsewhere.[20,21] Briefly, running subtraction was the actual mathematical subtraction performed on the corresponding pixels (pixels at identical location) of successive images. Based on previous experiments and theoretical analysis, random noise could be reduced effectively by taking an average of several frames. To get CSD wavefront, a raw spreading pattern image of CSD during 3.2 s (Fig. 1(A)) was first calculated by:

$$\delta F_i = \frac{F(i+7)+F(i+6)+F(i+5)+F(i+4)}{4} - \frac{F(i)+F(i+1)+F(i+2)+F(i+3)}{4}, \quad (1)$$

where $F(i)$ is the ith frame. Then raw spreading pattern image was converted into binary image (0 (black) for pixels with luminance less than a level and 1 (white) for all other pixels) using the thresholding to get a band and superimposed on the white light image (Fig. 1). The band is thought to be standing for the wavefront. Then wavefronts at different time points are obtained in the same way. In this study, wavefronts passing electrode 1 (E1) and electrode 2 (E2) were selected.

We chose ROIs perpendicular to advancing CSD wavefront passing E1 and E2 along propagation direction, calculated the average light intensity in these ROIs for each frame and plotted these reflectance values versus time to get the time lag of corresponding trough of OIS. Values were then divided by the distance between ROIs over time lag to get CSD velocity by OISI way.

In electrophysiological way, we measured the time lag of DC potential to reach trough at the two electrodes. The velocity is calculated by dividing the distance between cortical electrodes over time lag.

All data were expressed in form of mean ± standard deviation. Paired t-tests were used for statistic analysis. $p < 0.05$ was taken as statistically significant.

3. Results

After application of KCl, 2–5 CSDs were induced during our recording. For each CSD, wavefronts passing E1 and E2 were superimposed on the corresponding white light image. Figure 2(A) is a representative mixed image. The red band stands for the wavefront when CSD reaches E1 and the green band

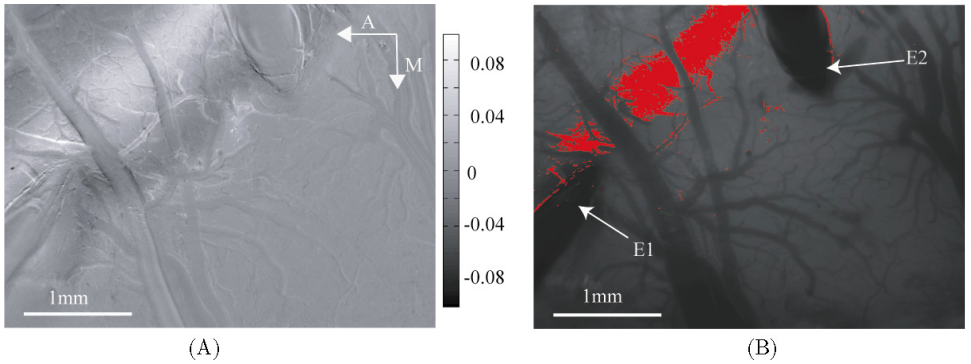

Fig. 1. A typical spreading pattern image of CSD. (A) Raw spreading pattern image get by successive subtraction. (B) Spreading pattern image was superimposed on the corresponding white light image. E1: electrode 1, E2: electrode 2, A: anterior, and M: medial.

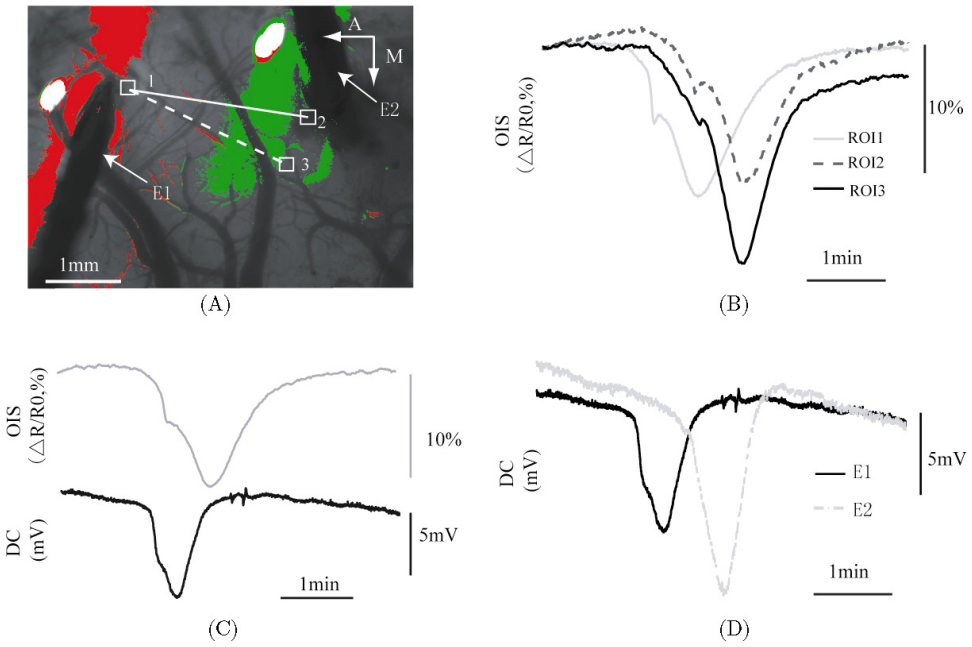

Fig. 2. A typical optical and electrophysiological response to CSD. (A) Wavefronts passing by the two electrodes were superimposed on the white light image. (B) Multiphasic changes in OIS associated with CSD. From these curves, propagation of CSD could be indicated. (C) Relationship between DC potential at E1 and OIS in ROI1 that is adjacent to E1. (D) Negative DC shift in field potential at E1 and E2. E1: electrode 1, E2: electrode 2, A: anterior, and M: medial.

for CSD wavefront reaching E2. Relative position of electrodes is shown by the real line and CSD propagation direction is indicated by the dotted line. Taking the optical reflectance intensity before CSD as control, relative light intensity change of each frame in ROI1 and ROI2, which are adjacent to E1 and E2, respectively, were calculated and plotted versus time (Fig. 2(B)). ROI3 is the region where ROI1 propagated along CSD propagation direction at the time of reaching E2. A multiphasic change in OIS was observed on the entire cortex. ROI2 and ROI3 are of uniform time course and lag behind ROI1. Similar time lag could also be seen in the negative DC potential shift at E1 and E2. Besides, in order to indicate the relationship between electrophysiological and optical response to CSD, DC potential at E1 and optical signals in ROI1 were plotted together (Fig. 2(C)). DC potential correlates well with OIS. Because of this correlation, the time lag of DC potential at the two electrodes was equal to that of OIS trough between ROI1 and ROI2 (or between ROI1 and ROI3), but the distance between ROI1 and ROI2 representing the location of E1 and E2, respectively, differed with that between ROI1 and ROI3. Therefore, the velocity calculated by electrophysiological way deviated from that by optical way.

In total 33 CSDs were recorded in nine rats. We calculated the velocity of each CSD by electrophysiological and OISI methods respectively. Statistic analysis was performed to compare the two methods on their accuracy in determining CSD velocity (Table 1).

As shown in Table 1, the average rate of 33 CSD was 3.52 ± 0.87 mm/min and 4.36 ± 1.65 mm/min by optical and electrophysiological methods, respectively. Velocity determined electrophysiologically was significantly different with that with optical approach. Since the optical method could provide information about spreading pattern of CSD and propagation direction could be shown, CSD velocity determined by OISI way is of smaller deviation and is closer to actual rate compared to that in an electrophysiological way.

Table 1. Statistic analysis of CSD velocity calculated by OISI and electrophysiological way. Values are mean ± s.d. $^*p < 0.05$ compared with OISI method.

Method	CSD velocity (mm/min)
OISI	3.52 ± 0.87
Electrophysiology	$4.36 \pm 1.65^*$

4. Discussion

The results show that CSD velocity determined by OISI had smaller deviation and was more accurate when compared with electrophysiological way. This could be explained theoretically and our experiment verified this view practically.

First, this phenomenon could be explained from theory. In electrophysiology, orientation of CSD propagation is unpredictable and variable. Before the experiment, we have no idea of how CSD will propagate. Relative position of electrodes is not always consistent with the propagation direction. Because of this probable direction inconsistency, the velocity determined by electrophysiological way is not always accurate. While by OISI, we could use running subtraction to see the propagation direction of CSD and then choose ROI along this direction to calculate velocity.

Practically, in our experiment, after using the running subtraction to show the wave propagation, we found that relative position of electrodes is not always consistent with the wave propagation direction. There is kind of difference between the two directions (Fig. 2(A)). The time lag of DC potential at the two electrodes was equal to that of OIS trough at ROI1 and ROI3, but the distance between ROI1 and ROI2 representing the location of E1 and E2 respectively differed with that between ROI1 and ROI3. Therefore, velocity determined by electrophysiological way is inconsistent with that by optical way. In addition, we found that in one trial, propagation direction of CSD induced by KCl varied between different CSD waves (Fig. 3). CSD1 propagates from upper left to lower right quarter, while CSD2 propagates from lower left to upper right square. Just because of this direction variation, sometimes the velocity calculated by electrophysiological way is even beyond the range of CSD velocity (2–5 mm/min) that adds to its inaccuracy. In addition, CSD induced by KCl could be time-varying,[20] which means that CSD will not spread the whole cortex and bypass some areas where electrophysiological signal could not be detected (Fig. 3 CSD3).

Moreover, optical method has several advantages over electrophysiological way. First, for optical way, we use light to illuminate cortex that will not cause damage to cortex; therefore, it is a non-invasive way. In electrophysiology, at least two electrodes need to be inserted. The insertion of electrode will cause damage to cortex and is invasive. Second, electrophysiology could be influenced easily by electric noise and environment noise. Optical way is much stable and less influenced by those factors. Third, electrophysiology can only detect the signal at several points and is local, while optical method can image the whole cortex that helps investigators to better understand the evolution of CSD.

In summary, OISI method was a better way for studying the CSD velocity and cortical excitability in rats because of its efficiency, simplicity, stability, and accuracy compared with traditional methods. Importantly, this method allows for exact propagation situation of the entire cortex. This will permit researchers to better identify the evolution of CSD. Therefore, OISI method could be used to determine CSD velocity accurately.

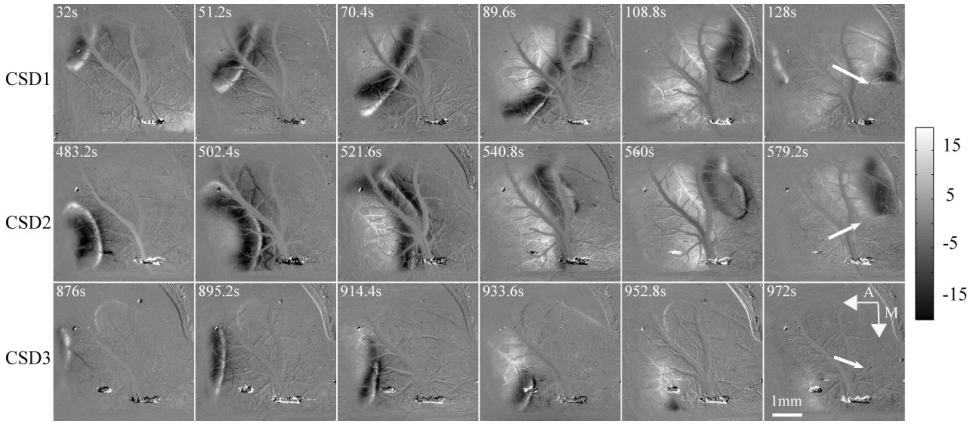

Fig. 3. Spatial evolution showing propagation direction varied and time-varying CSD. Each row denotes one CSD. Propagation direction is indicated by white arrow in the last image of each row. CSD1 propagates from upper left to lower right quarter; CSD2 propagates from lower left to upper right quarter; and CSD3 did not spread fully in the whole cortex. A: anterior; M: medial.

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