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TWO-DIMENSIONAL VISUALIZATION OF THE PROPAGATION SPEED OF CORTICAL SPREADING DEPRESSION IN RAT CORTEX

TINGTING XU, PENGCHENG LI, SHANGBIN CHEN and WEIHUA LUO*
Britton Chance Center for Biomedical Photonics
Wuhan National Laboratory for Optoelectronics
Huazhong University of Science and Technology
Wuhan 430074, P. R. China
**whluo@mail.hust.edu.cn*

Cortical spreading depression (CSD), which is a significant pathological phenomenon that correlates with migraines and cerebral ischemia, has been characterized by a wave of depolarization among neuronal cells and propagates across the cortex at a rate of 2–5 mm/min. Although the propagation pattern of CSD was well-investigated using high-resolution optical imaging technique, the variation of propagation speed of CSD across different regions of cortex was not well-concerned, partially because of the lack of ideal approach to visualize two-dimensional distribution of propagation speed of CSD over the whole imaged cortex. Here, we have presented a method to compute automatically the propagation speed of CSD throughout every spots in the imaged cortex. In this method, temporal clustering analysis (TCA) and least square estimation (LSE) were first used to detect origin site where CSD was induced. Taking the origin site of CSD as the origin of coordinates, the data matrix of each image was transformed into the corresponding points based on the polar-coordinate representation. Then, two fixed-distance regions of interest (ROIs) are sliding along with the radial coordinate at each polar angle within the image for calculating the time lag with correlating algorithm. Finally, we could draw a two-dimensional image, in which the value of each pixel represented the velocity of CSD when it spread through the corresponding area of the imaged cortex. The results demonstrated that the method can reveal the heterogeneity of propagation speed of CSD in the imaged cortex with high fidelity and intuition.

Keywords: Cortical spreading depression; least square estimation; propagation speed; cross correlogram.

1. Introduction

The phenomenon of cortical spreading depression (CSD) has been discovered by Leão for more than half a century.[1,2] CSD is of significant importance for neuroscience researches because of its pathophysiological correlation with migraine and cerebral ischemia.[3] Characterized by EEG depression, temporary disruption of ion homeostasis, DC potential shift, and the multi-phase change of optical intrinsic signals, CSD spreads like a wave over the cortex from a focal point toward the periphery at a speed of 2–5 mm/min. Undoubtedly, understanding

*Corresponding author.

the propagation characteristics of CSD will promote the elucidation of the mechanisms of CSD, which might provide a therapy way for neural diseases like migraine aura, ischemia, and epilepsy.[4]

Optical intrinsic signal imaging (OISI), which detects neuronal activity-related changes in light reflectance, is an ideal technique for mapping neuronal activity over broad cortical regions with providing high spatial resolution of up to 50 μm and high temporal resolution.[5–8] Enduing with these advantages, brain activity over large area such as CSD can be observed by OISI to investigate its propagation characteristics.[9,10]

Propagation speed is one of the important parameters for characterizing the CSD phenomenon.[11] Previous studies have focused on the propagation speed of CSD by randomly and manually choosing several points in the CSD-invaded cortex to estimate its average speed. However, by multi-sites electrical physiological recording, Godukhin *et al.* found that the propagation pattern/speed of CSD in the cortex at different directions were not the same.[9] Eiselt *et al.* also discovered the heterogeneous propagation pattern of CSD using imaging method.[5] Therefore, estimation of propagation speed of CSD based on several sites cannot be impartial enough to represent the heterogeneous pattern of the speed of CSD over the whole cortex.[12] In our study, we aim to provide a method for visualizing the propagation speed of CSD in two dimensions over the imaged cortex. As a result, we can draw a jet image, in which the value of each pixel represented the velocity of CSD through the corresponding area of the imaged cortex. The visualized result is potential to distinguish variations of CSD speed over time and region with high fidelity.

2. Materials and Methods

2.1. *Animal experiment*

The procedure of optical imaging of CSD was described in detail in our previous works.[12] CSD was elicited by pinpricks in adult male Sprague–Dawley rats (weighing 250–350 g) under α-chloralose/urethane anesthesia (50 and 600 mg/kg, respectively, i.p.). The body temperature of the anaesthetic animal was monitored with a rectal temperature probe and maintained at 37.0 ± 0.5°C using a heating regulator pad. The fixed wavelength (550 ± 10 nm) light source was used to illuminate the thinned parietal cortex. A 12-bit CCD camera (Pixelfly, PCO Computer Optics, Germany) was attached to a microscope (Olympus SZ6045TRCTV, Japan) to record images. Images were acquired from an area of about 4.8×6.4 mm (25 ms exposure time, 64 frames online average, 4×4 binning, and 120×160 pixel array). In each trial, 300 frames were collected and stored into a personal computer hard disk for off-line analysis.

2.2. *Propagation speed calculation*

The velocity of CSD wave is defined as the distance traveled in unit time along the propagation direction. To quantify the CSD velocity for optical imaging study, two pixels (pixel 1 and pixel 2) are selected and the speed of CSD propagation between them can be defined as:

$$\text{Speed} = \text{distance/time}. \tag{1}$$

In this study, we select automatically the pair of pixels with a fix distance of 10 pixels (corresponding to 0.4 mm), and then calculate the time lag of CSD response between these two pixels with two methods mentioned in the following subsection.

2.2.1. *Origin site estimation of CSD*

Temporal clustering analysis (TCA) and least square estimation (LSE) are combined to determine the location of the origin of CSD (also the pinprick point) according to our previous works.[12–14] Early response pattern of CSD, which is relatively more uniform than the later pattern, are used in calculation for the pinprick location, which is presented as (a, b) in Fig. 1.

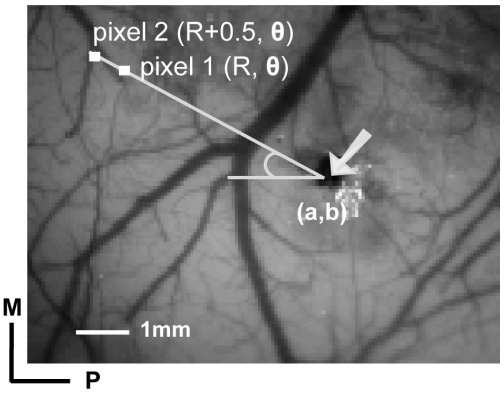


Fig. 1. Coordinate transformation is illustrated in a raw optical image of the rat cortex. The white arrow indicates the pixel (a, b) at site of the pinprick point, which is regarded as the origin with polar coordinate. On the longer line lies two pixels (pixel 1 (R, θ) and pixel 2 $(R + 0.4, \theta)$) with white square dot are selected for speed calculation. M: medial and C: caudal.

2.2.2. *Coordinate transformation*

Among each pair of pixels, the pixel 1 $(x1, y1)$ can be converted to the corresponding representation (R, θ) in polar coordinate according to the following equations:

$$\sqrt[2]{(x1-a)^2+(y1-b)^2} = R, \tag{2}$$

$$\tan^{-1}(y1-b)/(x1-a) = \theta, \tag{3}$$

where (a, b) is the pinprick point as shown in Fig. 1.

Then, another pixel is automatically determined as $(R + 10, \theta)$ along the same polar radius across pixel 1, and then is converted to pixel 2 $(x2, y2)$ by the inverse transformation from $(R + 10, \theta)$,

$$x2 = (R+10) \times \cos\theta + a, \tag{4}$$

$$y2 = (R+10) \times \sin\theta + b, \tag{5}$$

2.2.3. *Temporal lag recognition*

Two methods were introduced to estimate the time delay of CSD propagation between the two selected pixel points with a fix distance. One approach was to use the cross correlogram analysis. First, we chose a temporal window (n time points) along the time course curve of optical reflectance changes in pixel 1 which contained the CSD dynamic phase as a template, meanwhile the whole time course of optical changes in pixel 2 was considered as the target. The template stepped point by point along the target curve for calculating the correlation coefficient $\widehat{r}_j$ as following (Fig. 2(b)).

$$\widehat{r}_j = \frac{L_{xy}}{L_{xx}L_{xy}} = \frac{\sum_{i=1}^{n}(x_i-\bar{x})(y_{i-1+j}-\bar{y})}{\sqrt[2]{\sum_{i=1}^{n}(x_i-\bar{x})^2}\sqrt[2]{\sum_{i=1}^{n}(y_{i-1+j}-\bar{y})^2}} \tag{6}$$

where x_i is the reflectance intensity of pixel 1 in the ith image, y_{i-1+j} is the reflectance intensity of pixel 2 in the $(j + n - 1)$th image.

The maximum $\widehat{r}_j$ along the time course of pixel 1 can be obtained by:

$$\widehat{r}_m = \max\{\widehat{r}_1, \widehat{r}_2, \ldots, \widehat{r}_{300-n+1}\}. \tag{7}$$

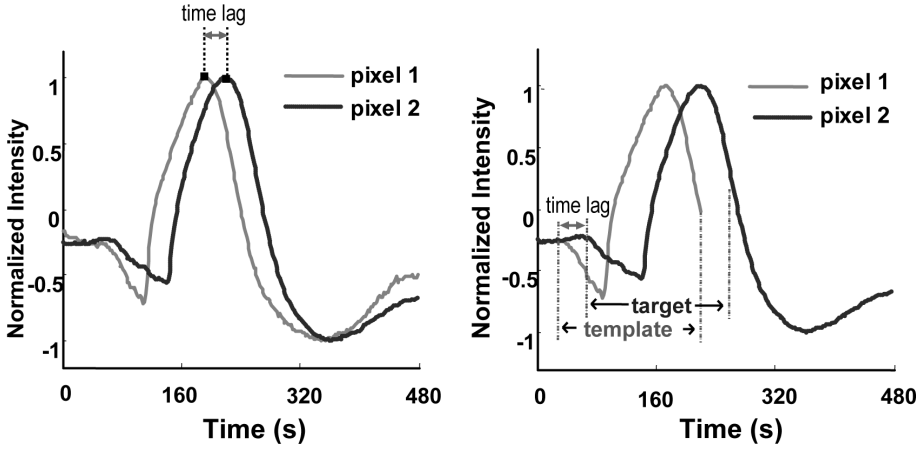
Then the time lag of CSD between two pixel points (pixel 1 and pixel 2) is m frames, which can be converted to the real time interval t as following:

$$t = m(\text{frames}) \times 1.6(s/\text{frame}). \tag{8}$$

For another approach, we intended to track the extreme response peak along the time dimension for each pixel. For pixels 1 and 2, the maximum point of reflectance intensity $I_j(1)$ and $I_j(2)$ was obtained as following:

$$I_j(i) = \max\{I_1, I_2, \ldots, I_{300}\}, \quad i = 1, 2, \tag{9}$$

where I_j is the reflectance intensity of one pixel in the jth image. Thus, the subtraction of j for the two pixels we can easily calculate the time interval.



(a) (b)

Fig. 2. Using OISI signal to estimate the time lag for CSD propagation between two pixel points with a fix distance. (A) Normalized time course of optical reflectance in site of pixels 1 and 2. The black squares indicate the peak points of the time course. The time lag between the two peak time points is used to estimate the speed of spreading of CSD wave. (B) Using the running correlation coeffient method for time interval calculation. The red line shows the template part from time course curve of pixel 1, stepping along the time course curve of pixel 2, which is the target.

3. Results

For one representative CSD imaging trial, we used our method for propagation speed calculation (Fig. 3). At an early time (about 25 s) after pinprick in the cortex, the propagation of CSD appeared like a standard wave circle. Then these frames in the early period after CSD induction can be used to calculate the origin of CSD. Afterwards, the spreading of CSD was not significantly uniform.

Based on this dataset including an complete episode of a CSD wave throughout the imaged cortex, we obtained two images (120×160 pixels) representing the propagation velocity of CSD at each pixel by the methods mentioned above. It had been smoothed with Gaussian kernel by using a sliding 3 by 3 window. The scale indicated speed value, in which red represented a higher value and blue stood for a lower speed.

Except for the vein compartment, the velocity in both figures (Fig. 4) was about 2–5 mm/min either in the pinprick surrounding area or the periphery place, which was in accordance with that observed by previous researches. To verify the accuracy of the visualization, we chose three regions of interest (ROIs) in regions that have different speed levels to confirm whether the speed varied from one to other. In each ROI, a pair of pixels with a fixed distance (0.4 mm, 10 pixels in image). As demonstrated in Fig. 5, the upper left time courses of pixels 1 and 2 in ROI 1 had the lowest time lag since the lines were almost overlapping. The upper right normalized changes in light reflectance of ROI 2 possessed a higher time interval. However the ROI 3 had the largest time lag between the paired time courses within it. From the time courses of light reflectance variance in ROI 1, 2, and 3, we knew that CSD had the fastest speed in ROI 1 and the speed in ROI 3 was the slowest, which is consistent with the visualization level in these two figures (Fig. 4).

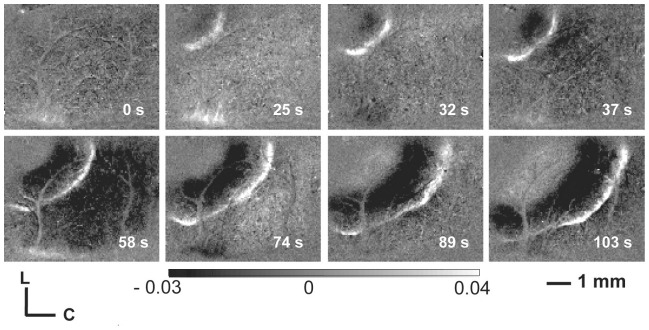


Fig. 3. Spatiotemporal evolution of a CSD wave in a representative experiment. Spreading patterns at different time point are chosen to show the spatiotemporal evolution. From the evolution of the wave, it is obvious that the propagation velocity is not uniform that in later frames the CSD wave appeared like a contorted wave circle. M: medial and C: caudal.

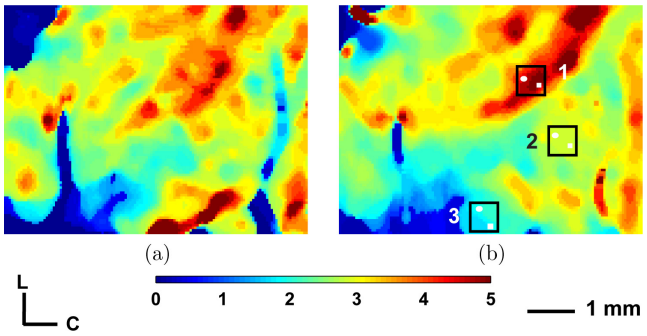


Fig. 4. The two-dimensional visualization of the spreading speed of CSD on the imaged cortex are presented. The left figure is obtained using peak response recognition method, while the right figure was calculated by using cross correlogram analysis.

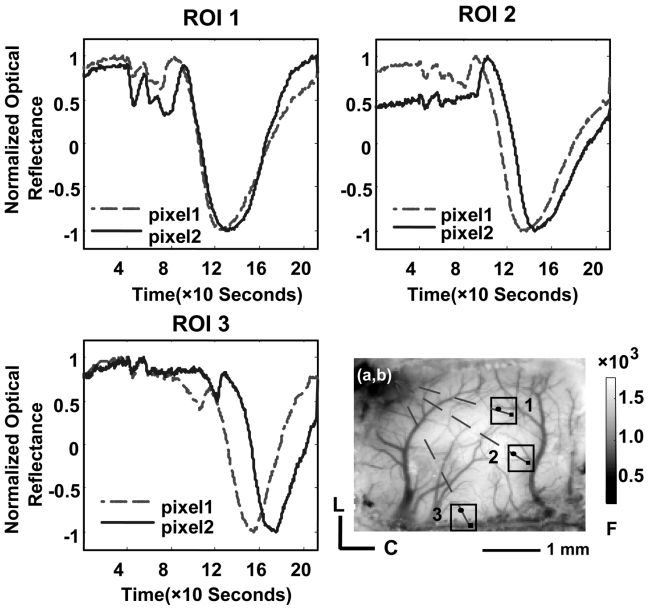


Fig. 5. The normalized optical reflectance during the time course of pixels 1 and 2 in ROI 1, 2, and 3 region are presented, respectively. The right down figure is the raw picture of the imaged cortex. The black box with black number stand for the chosen ROI regions and two black dots represent pixels 1 and 2, respectively. The dot which is nearer to the pinprick pixel (a, b) is pixel 1 and the other is pixel 2.

4. Discussion

In physiological condition, propagation speed of CSD is a reliable index of tissue excitability in the

cortex. An increase in CSD velocity seems to reflect an elevated cortical excitability accompanying with a decreased threshold for CSD induction and vice versa.[11] Previous studies have indicated that CSD propagation can be affected by additional factors, like some drugs, i.e. citalopram,[15] direct cortical electrical stimulations,[11,16] and so on, which might provide a intervening way for neural diseases like migraine aura, ischemia, and epilepsy.[4] Determining the variance of CSD propagation speed over the whole cortex accurately is essential for studying cortical excitability and underlying mechanism. The traditional way to determine CSD velocity is based on electrophysiological signal while a CSD wave passing two or several electrodes.[11,17,18] During monitoring, the position of the electrodes is fixed while the propagation direction of CSD is unpredictable and variable. So, the relative position of electrodes is hard to be consistent with CSD propagation direction. OISI is an idea technique for the study of CSD because of its characteristics endowed with full-field imaging mode.[19–21] Based on the abundant information involved in CSD process acquired by OISI, the propagation speed of CSD over the whole invaded cortex could be determined accurately.

In this study, we have used the transformation of coordinate to compute the propagation speed of CSD through every pixel in the images. Furthermore, two approaches for detecting the CSD spreading time lag were applied. Although both images visualized speed variances clearly in detail, among these two approaches, the cross correlogram approach seems better to determine the time lag between two pixels. This was mainly attributed that the maximum determination could be easily influenced by noises and regional heterogeneity, yet the cross correlogram is relatively robust for the variance in time course of CSD over different regions.

In previous OISI studies, some ROIs over the imaged cortex were randomly chosen to routinely compute a mean value of propagation speed for a CSD wave. However, the calculated results could only represent the velocity in the limited regions and neglect the heterogeneous pattern of the speed of CSD over the whole cortex. No such problem exists in the two-dimensional visualization of CSD propagation velocity because we calculate the spreading speed in the whole imaged cortex and demonstrate it in one two-dimensional image, in which the value of each pixel represents the velocity value that CSD wave propagates. This two-dimensional visualization of CSD propagation speed can help us to differentiate the abnormal regions over the cortex, and further elucidate the underlying mechanism of the heterogeneity of the CSD propagation speed by being combined with other physical or chemical intervening means.

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