Voltage-sensitive dye based photoacoustic imaging visualization of whole brain electrodynamics

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ABSTRACT

Brain voltage imaging plays a critical role in understanding brain function and diagnosing neurological conditions. In recent years, optical imaging of brain voltage has garnered encouraging progress, however, current techniques are limited by penetration depth, FOV, and the photostability of the voltage indicators. Photoacoustic imaging shows strong potential for whole-brain global imaging and real-time monitoring, especially for deep brain regions. Therefore, we developed a full-field photoacoustic brain detection (WF-PABD) platform adapted with a photostable voltage-sensitive dye (PA-VSD), enabling direct assessment of voltage dynamics across the entire mouse brain with epilepsy. The results illustrate that PA-VSD can resolve the microvasculature of the entire mouse brain at high resolution and present cranial structures with high contrast, even under conditions of low blood background. The monitoring indicates that PA-VSD can visualize seizures over extended periods and precisely locate active epileptic foci. Furthermore, through rapid temporal distribution and brain regions correlation analysis, we identified both the electrical conduction pathway and its directionality, which hold significant value for the long-term monitoring and diagnosis of electroencephalographic-related disorders.

Keywords: Brain voltage dynamic, Voltage-sensitive dye, Photoacoustic imaging, Seizures, Electrical conduction pathway, High spatiotemporal resolution

1. INTRODUCTION

Photoacoustic (PA) imaging, also termed as optoacoustic imaging, is an emerging hybrid imaging technique that can non-invasively identify tissue with high specificity and micron-scale resolution at increased penetration depth.[1,2] The vascular specificity of PA imaging assists in the neurovascular coupling of neural voltage imaging, as evidenced by numerous studies in recent years.[1,3,4] However, these studies typically interrogate neuronal voltage activities through vascular and blood oxygenation fluctuations rather than direct measurement. In this work, we propose a novel strategy, that employs a whole-field photoacoustic brain detection (WF-PABD) platform adapted with a photostable voltage-sensitive dye (PA-VSD), to directly monitor voltage dynamics over 30 minutes in an intact epileptic mouse brain. A methodology is developed to utilize PA data analysis to provide identification and screening of epileptic signals. Regarding system validation, WF-PABD possesses a vast FOV of ~5 cm in diameter for enough covering the whole

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mouse brain, a high spatial resolution of ~110 μm, a fast imaging-rate of 10 Hz, and a deep penetrating depth covering all areas of interest. PA-VSD localized epilepsy foci and screened seizures dynamics, as well as offered guidance on electrical conduction pathways and functional communications across brain regions. Through multiple data processing, it can be discovered that epileptic signals contain more high-frequency components than calm signals have. Moreover, there exhibits a close correlation between the change rates and high-frequency amplitudes of PA signals, showing a peak-to-peak pairing feature. The results highlight the potential of PA-VSD as a high-spatiotemporal-resolution tool for in-depth analysis of brain voltage dynamics. It is expected that the platform might serve as a springboard for further research in photoacoustic neuroscience and neuropathophysiology.

2. RESULTS

A VSD named DiSC3(5) [5] was employed for in-vivo measurements in this work, which has an absorption peak at 656 nm. A simplified schematic of the WF-PABD system is illustrated in **Figure 1a**. In the animal experiments, the platform used a laser with the excitation wavelength of 670 nm, which close DiSC3(5) absorption peak and avoid high blood background. We set the top horizontal section of the mouse cerebral cortex as the 0-depth plane, and non-invasively captured the brain images of the live mouse. The vascular and brain structures at different depths (0-4 mm) were displayed in **Figure 1b**. It was found that the reconstructed PA brain vascular structures were clearly shown, even for the tiny blood vessels at the edges. The results indicate that the PA-VSD can provide sufficient resolution and penetrating depth covering all interested regions of brain.

The epilepsy mouse model was induced by PTZ in advance. We set up two groups for the PA-VSD imaging: a control group that received a saline injection, and an epilepsy group that received a PTZ injection. To allow sufficient time for DiSC3(5) to enter the brain through systemic circulation and achieve stabilized distribution, we initially observed the signals for two hours before inducing acute seizures in mice, then monitoring PA voltage response for 30 mins in PTZ-induced epileptic mouse brain. The epilepsy groups displayed more extreme signal changes than the control group, showing a somewhat significant difference (**Figure 1c**). PA-VSD could detect seizures as early as 2 minutes in advance, making it as sensitive as EEG monitoring.

For further investigation, we chose black zone in figure 1c with obvious seizures for functional connection analysing. As seen, there was a noticeable increase in brain activity start from 5th second, with bright regions demonstrating the exact localization of the seizure's foci. Area 1 in 5th second started to light up and rapidly transmitted to areas 2 as well as 3 at 7 s (**Figure 1d**). Then, the Allen Mouse Brain Atlas was used to identify 58 brain regions corresponding to PA brain images and make them separate ROIs. 210 frames of raw data were extracted, and the time series of the 58 ROIs were computed respectively. A two-by-two correlation analysis was carried out, shown in **Figure 1e**. We were surprised to find that the active areas of the left hemisphere in Figure 1d coincide with the brain regions in set 3 in Figure 1e. Those regions presented certain neighbouring correlations and conductance. For instance, ROI 3-5-9-8-10-13-25 formed as an electrical signal transport channel. Combined with high-temporal-resolution PA time profile, we can see the direction of transmission (red dotted arrow in Figure 1d). Considering that surgical treatment has been effective for some epilepsy cases in the past years, this approach could potentially assist in guiding epilepsy surgery by providing a more precise localization of the active epileptic foci.

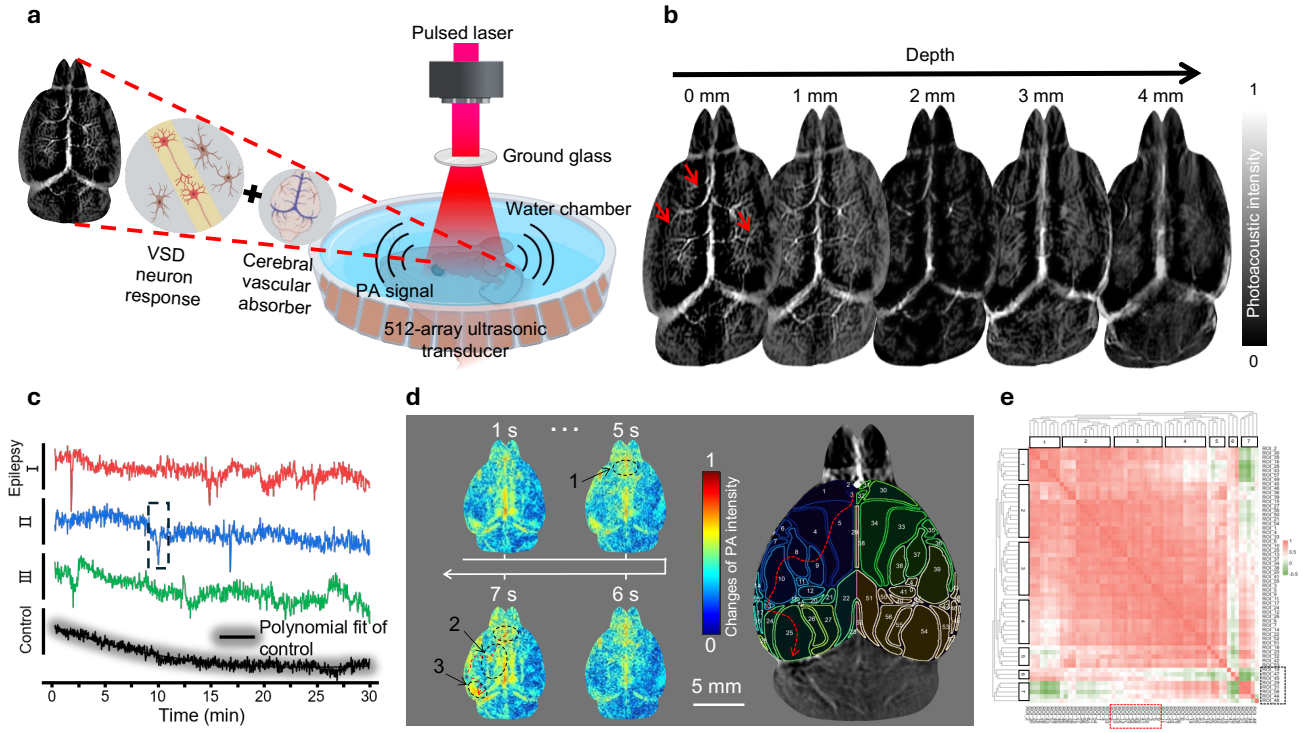

Figure 1. (a) WF-PABD implementations. (b) Intravital visualization of whole mouse brain at different depths. (c) Temporal profiles of brain voltage signals monitored in epilepsy mice. (d) Time-varying photoacoustic reconstructed images and signal conduction pathway. (e) Correlation analysis of two-by-two brain regions.

3. CONCLUSION

This work is the first trial to employ a high-spatiotemporal-resolution WF-PABD for prolonged monitoring and tracking of the VSD response to directly reflect brain activity in vivo. Specifically, a generalized VSD indicator DiSC3(5) was employed to conduct PA imaging under a single wavelength excitation of 670 nm, which is under a noticeably reduced blood absorption background and making the DiSC3(5) signal changes more representative of brain voltage variations. The designed platform can provide an effective FOV of about 5 cm in diameter, which is adequate to image the entire mouse brain and potentially a portion of human brain.[6] Then this platform yields an ultrahigh acquisition speed of 10 μs/frame, which can overcome the motional artifacts and hence allows for monitoring under free motion status. By employing PA-VSD, we can pinpoint the epileptic foci and determine regions with more active epilepsy activity, potentially providing guidance for surgical navigation and treatment. Interestingly, by combining high-temporal-resolution visualization and investigating brain region correlations, we obtained results on electrical conduction pathways and directions in certain regions, which can contribute to the study of nerve function and electrical conduction. Overall, we believe that the proposed PA-VSD framework can provide high-spatiotemporal-resolution visualization, accurate lesion localization, and valuable insights into brain function communication through whole-brain neuronal voltage activities, with far-reaching implications for in vivo neurophysiological investigations.

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