Neurobiology of Disease 82 (2015) 455–465

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Assessment of neurovascular dynamics during transient ischemic attack by the novel integration of micro-electrocorticography electrode array with functional photoacoustic microscopy

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ARTICLE INFO

Article history:
Received 9 March 2015
Revised 11 June 2015
Accepted 24 June 2015
Available online 3 July 2015

Keywords:
Functional photoacoustic microscopy (fPAM)
Micro-electrocorticography (μECoG)
Neurovascular function
Photothrombotic ischemia
Transient ischemic attack (TIA)

ABSTRACT

This study developed a novel system combining a 16-channel micro-electrocorticography (μECoG) electrode array and functional photoacoustic microscopy (fPAM) to examine changes in neurovascular functions following transient ischemic attack (TIA) in rats. To mimic the pathophysiology of TIA, a modified photothrombotic ischemic model was developed by using 3 min illumination of 5 mW continuous-wave (CW) green laser light focusing on a distal branch of the middle cerebral artery (MCA). Cerebral blood volume (CBV), hemoglobin oxygen saturation (SO_2), somatosensory evoked potentials (SSEPs) and alpha-to-delta ratio (ADR) were measured pre- and post-ischemia over a focal cortical region (i.e., 1.5×1.5 mm^2). Unexpectedly, the SO_2, peak-to-peak amplitude (PPA) of SSEPs and ADR recovered and achieved levels greater than the baseline values at the 4th hour post-ischemia induction without any intervention, whereas the CBV value only partially recovered. In other words, transient ischemia led to increased neural activity when the relative CBV was reduced, which may further compromise neural integrity or lead to subsequent vascular disease. This novel μECoG-fPAM system complements currently available imaging techniques and represents a promising technology for studying neurovascular coupling in animal models.

1. Introduction

Transient ischemic attack (TIA) refers to a brief episode of neurological dysfunction that typically lasts from a few minutes up to 24 h (Easton et al., 2009; Purroy et al., 2004). It produces temporary stroke-like symptoms but typically does not result in cerebral infarct (Easton et al., 2009). TIA was once regarded as benign; however, recent functional magnetic resonance imaging (fMRI) studies have shown

Abbreviations: ADR, alpha-to-delta ratio; ANOVA, analysis of variance; ANSI, American National Standards Institute; AP, anterior–posterior; A/D, analog to digital; CBF, cerebral blood flow; CBV, cerebral blood volume; CW, continuous wave; EEG, electroencephalography; FFT, fast Fourier transform; fMRI, functional magnetic resonance imaging; fPAM, functional photoacoustic microscopy; LSD, least significant difference; MCA, middle cerebral artery; MCAO, middle cerebral artery occlusion; MRI, magnetic resonance imaging; μECoG, micro-electrocorticography; MEG, magnetoencephalography; MEMS, micro-electromechanical systems; ML, medial–lateral; PA, photoacoustic; PBS, phosphate-buffered saline; PPA, peak-to-peak amplitude; S1FL, forelimb region of the primary somatosensory cortex; S.D., standard deviation; SEM, scanning electron microscopy; SNR, signal-to-noise ratio; SO_2, hemoglobin oxygen saturation; SSEPs, somatosensory evoked potentials; TIA, transient ischemic attack; TTC, 2,3,5-triphenyl-tetrazolium chloride.

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http://dx.doi.org/10.1016/j.nbd.2015.06.019
0969-9961/

resting-state neural activity abnormalities in patients who have experienced a TIA (Fazekas et al., 1996; Guo et al., 2014). In addition, TIA increases the risk of stroke to the extent that approximately 10 to 15% of patients who experience a TIA will have a stroke within three months (Easton et al., 2009). Although further understanding the pathophysiology of TIA is required, a suitable animal model is still lacking for studying TIA *in vivo* (Giles and Rothwell, 2007; Hoshino et al., 2013; Purroy et al., 2004). Therefore, we developed a rat TIA model to study the changes in neurovascular coupling during the ischemic-reperfusion process.

The photothrombotic model induces ischemic damage within a given brain area/blood vessel through photo-activation of an injected light-sensitive dye — Rose Bengal (Watson et al., 1985). Rose Bengal is activated in response to illumination and produces singlet oxygen that damages components of endothelial cell membranes, resulting in subsequent platelet aggregation and thrombus formation and the eventual interruption of local blood flow (Watson et al., 1985). In this study, we modified and optimized the parameters of the photothrombotic ischemia model to generate transient ischemia in rats. In particular, we reduced the intensity and exposure duration of continuous-wave (CW) laser light to create ischemia at a focal region such that spontaneous reperfusion could be observed within 4 h. This spontaneous reperfusion was accompanied by the recovery of cerebral blood flow (CBF) and somatosensory evoked potentials (SSEPs) (Liao et al., 2014, 2015).

In this study, functional photoacoustic microscopy (fPAM) was applied to study changes in regional cerebral blood volume (CBV) and hemoglobin oxygen saturation (SO_2) following a TIA over time. In addition, a novel high-density 16-channel electrode array was developed to record micro-electrocorticography (μECoG) signals over a small focal cortical region (i.e., 1.5×1.5 mm^2) (Rubehn et al., 2009; Viventi et al., 2011). We further combined this μECoG electrode array with fPAM to concurrently record the changes in hemodynamics and neural activity (i.e., SSEPs and the alpha-to-delta ratio (ADR)) during the course of transient ischemia. That is, employing the proposed rat TIA model and the μECoG-fPAM system, the goals of this study are two-fold. First, we tested the hypothesis that TIA, which alters cerebrovascular microcirculation and electrophysiological responses, can be induced by adjusting the induction parameters of photothrombosis. Second, we hypothesized that our novel μECoG-fPAM system could capture both the hemodynamic and electrophysiological responses of this rat TIA model, with microscopic vascular and electrophysiological resolution. Using the μECoG-fPAM system, we expect to show the acute evolution of neurovascular functions in a highly resolved manner in proximity to a focal ischemic region.

2. Methods

2.1. Design of micro-electrocorticography (μECoG) electrode array

A novel μECoG electrode array was designed and fabricated for this study. This device contains an open window in the center (i.e., 1.5×1.5 mm^2) surrounded by an array of 16 epidural electrodes. The open window at the center was designed to allow access for both laser exposure to induce transient ischemia and photoacoustic (PA) imaging. These μECoG electrodes were designed with a diameter of 200 μm and an electrode–electrode spacing of 400 μm, as shown in Figs. 1A and B. The electrode array was fabricated using gold, a material that exhibits excellent biocompatibility and does not induce an inflammatory response, making it suitable for bio-signal acquisition (Lago et al., 2007; Viventi et al., 2011). Parylene C, which offers superb biocompatibility (i.e., ISO 10993 and USP Class VI), great flexibility, chemical inertness and low permeability (Metallo et al., 2011), was used as the prime structural and packaging polymer of this electrode array. Please refer to Fig. S1 and Table S1 in the Supplementary material for more details on the fabrication process and corresponding impedance testing of the μECoG electrode array.

2.2. Comparison between the μECoG electrode array and traditional screw electrodes

The recorded ECoG signal variations using the μECoG electrode array and traditional screw electrodes (Liao et al., 2013a, 2014) were compared by targeting the same event (i.e., ischemia). Then, the difference in peak-to-peak amplitude (PPA) between two selected channels was calculated for each electrode type. For the 16-channel μECoG electrode array over the forelimb region of the primary somatosensory cortex (S1FL) of the right hemisphere, the two electrodes of the 16-electrode array with the largest difference in PPA were identified to investigate the percentage difference. For the screw electrodes, two stainless steel screw epidural ECoG electrodes were secured to the skull over the S1FL region in the right hemisphere at the following coordinates using the bregma as the reference: AP: +1.7 mm, ML: +4.5 mm and AP: −0.8 mm, ML: +4.5 mm (Liao et al., 2015).

2.3. Micro-electrocorticography-functional photoacoustic microscopy (μECoG-fPAM) system

The experimental structure and the integrated μECoG-fPAM system, including the μECoG recording system, fPAM system, rat TIA model and forepaw electrical stimulation (i.e., as the trigger for evoking neurovascular responses), are illustrated in Fig. 1C. The μECoG electrode array was placed over the S1FL region in the right hemisphere of rat brain, with 1 stainless steel epidural electrode secured to the skull (i.e., 3 mm to the right of the lambda landmark) as a reference for acquiring SSEPs and resting-state μECoG signals pre- and post-ischemia. The μECoG signals were pre-amplified (PZ2-32, Tucker-Davis Technologies, FL, USA) and recorded using a bio-signal processor (RZ5D, Tucker-Davis Technologies, FL, USA) in subsequent experiments.

The designed 50 MHz darkfield confocal fPAM system was used to image functional hemodynamic changes in selected cortical blood vessels via the open window at the center of the μECoG electrode array. The 4 ns laser pulses were generated at a pulse repetition rate of 10 Hz using an optical parametric oscillator (Surlite OPO Plus, Continuum, CA, USA), which was pumped by a frequency-tripled Nd:YAG Q-switched laser (Surlite II-10, Continuum, CA, USA). Two visible wavelengths of the laser pulses, 560 and 570 nm (i.e., λ_{560} and λ_{570}), were employed for PA wave excitation (Liao et al., 2010). The 50 MHz ultrasonic transducer used in the current fPAM system was custom-made by the Acoustic Sensor Co., Ltd. in Taiwan. This system has a −6 dB fractional bandwidth of 57.5%, a focal length of 9 mm and a 6 mm active element, offering an axial resolution of 32 μm and a lateral resolution of 61 μm.

Laser energy was delivered via a 1 mm multimodal fiber. The fiber tip was coaxially aligned with a collimation lens, an axicon, a plexiglass mirror and an ultrasonic transducer on an optical bench, which formed darkfield illumination and was confocal with the focus of the ultrasonic transducer. The laser exposure on the sample surface was safely within the American National Standards Institute (ANSI) safety limits. During the PA imaging process, the transducer was immersed in an acrylic water tank, and the hole at the bottom of the tank was sealed with a 15-μm-thick polyethylene film. A thin layer of ultrasonic gel was applied to the rat's head, which was then attached to the thin film to ensure proper acoustic coupling of the generated PA waves to the tank. The PA signals received by the ultrasonic transducer were pre-amplified by a low-noise amplifier (AU-3A-0110, MITEQ, NY, USA), cascaded to an ultrasonic receiver (5073 PR, Olympus, MA, USA) and then digitized by a computer-based 14 bit analog to digital (A/D) card (CompuScope 14200, GaGe, Lockport, IL, USA) at a 200 MHz sampling rate.

Fluctuations in the laser energy were monitored using a photodiode (DET36A/M, Thorlabs, NJ, USA). Before any further signal processing, the recorded photodiode signals were applied to compensate for PA