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# Rescue of cortical neurovascular functions during the hyperacute phase of ischemia by peripheral sensory stimulation

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ABSTRACT

To investigate the potential therapeutic effects of peripheral sensory stimulation during the hyperacute phase of stroke, the present study utilized electrophysiology and photoacoustic imaging techniques to evaluate neural and vascular responses of the rat cortex following ischemic insult. We employed a rat model of photothrombotic ischemia (PTI), which targeted the forelimb region of the primary somatosensory cortex (S1FL), due to its high reproducibility in creating localized ischemic injury. We also established a hybrid, dual-modality system, including six-channel electrocorticography (ECoG) and functional photoacoustic microscopy (fPAM), termed ECoG–fPAM, to image brain functional responses to peripheral sensory stimulation during the hyperacute phase of PTI. Our results showed that the evoked cerebral blood volume (CBV) and hemoglobin oxygen saturation ($SO_2$) recovered to 84 ± 7.4% and 79 ± 6.2% of the baseline, respectively, when stimulation was delivered within 2.5 h following PTI induction. Moreover, neural activity significantly recovered, with 77 ± 8.6%, 76 ± 5.3% and 89 ± 8.2% recovery for the resting-state inter-hemispheric coherence, alpha-to-delta ratio (ADR) and somatosensory evoked potential (SSEP), respectively. Additionally, we integrated the CBV or $SO_2$ with ADR values as a recovery indicator (RI) to assess functional recovery after PTI. The RI indicated that 80 ± 4.2% of neurovascular function was preserved when stimulation was delivered within 2.5 h. Additionally, stimulation treatment within this optimal time window resulted in a minimal infarct volume in the ischemic hemisphere (4.6 ± 2.1%). In contrast, the infarct volume comprised 13.7 ± 1.7% of the ischemic hemisphere when no stimulation treatment was applied.


## Introduction

Stroke is a neurological deficit caused by a significant reduction in the blood supply to tissue (Lo, 2008) and is a leading cause of death and disability (Lo et al., 2003). Stroke frequently leads to irreversible tissue damage in areas where the cells are subject to necrosis (Kingwell, 2014). The timely redistribution of blood into the ischemic penumbra (salvageable tissue surrounding the ischemic core) could markedly improve the outcome (Lo, 2008; Muir et al., 2006). Currently, the most well-known therapeutic agent for stroke recovery is recombinant tissue plasminogen activator (rtPA) (Azizi et al., 2013), which breaks down clots, resulting in the reintroduction of blood into the ischemic brain region (*i.e.*, reperfusion). Unfortunately, rtPA is viable for only a small portion (approximately 3.6%) of stroke patients (Go et al., 2013) and may cause tissue damage by weakening the blood vessel walls or disrupting the blood–brain barrier (BBB) (Abu Fanne et al., 2010). Therefore, a new

*Abbreviations:* ACA, anterior cerebral artery; ADR, alpha-to-delta ratio; AP, anterior–posterior; AVA, Agri-Food and Veterinary Authority of Singapore; BBB, blood–brain barrier; CBF, cerebral blood flow; CBV, cerebral blood volume; cM1, contralateral motor cortical region; cS1FL, contralateral forelimb region of the primary somatosensory cortex; CW, continuous wave; DOI, diffuse optical imaging; ECoG, electrocorticography; EEG, electroencephalography; fMRI, functional magnetic resonance imaging; fPAM, functional photoacoustic microscopy; IACUC, Institutional Animal Care and Use Committee; LSD, least significant difference; LSI, laser speckle imaging; M1, motor cortical region; ML, medial–lateral; MCA, middle cerebral artery; NACLAR, National Advisory Committee for Laboratory Animal Research; PA, photoacoustic; PBS, phosphate-buffered saline; pMCAO, permanent middle cerebral artery occlusion; PTI, photothrombotic ischemia; RI, recovery indicator; ROI, region of interest; rtPA, recombinant tissue plasminogen activator; S1FL, the forelimb region of the primary somatosensory cortex; S.D, standard deviation; SSEP, somatosensory evoked potential; $SO_2$, hemoglobin oxygen saturation; TTC, 2,3,5-triphenyltetrazolium chloride.

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neuroprotective therapy with minimal side effects (*i.e.*, a noninvasive, non-pharmacological method) is required for ameliorating ischemic insult (Fisher et al., 2009).

Recent studies with permanent middle cerebral artery occlusion (pMCAO) animal models suggested that manipulating sensory or motor functions resulted in a neuroprotective effect during stroke recovery (Frostig et al., 2013; Lay et al., 2010; Lay et al., 2011). Although the response to peripheral sensory stimulation is likely due to remodeling of both cerebral blood flow (CBF) and electrophysiological function (Frostig et al., 1990; Lay et al., 2011), the effects of sensory stimulation on neurovascular coupling and dynamics during the hyperacute phase of ischemia are not fully understood. To address this question, measurement of hemodynamic and neural responses after ischemia induction is essential. Optical imaging techniques, such as diffuse optical imaging (DOI) and laser speckle imaging (LSI), have been used to evaluate stroke physiology (Culver et al., 2003; Luckl et al., 2010). Using these techniques, changes in hemodynamic functions, such as CBF and cerebral blood volume (CBV), can be assessed, as well as changes in oxygen concentration and cerebral oxygen metabolism in the cortical ischemic area. However, the spatial resolution of DOI reconstructed images is low because of the diffusion of light in biological tissues, thereby limiting the ability to observe changes in the fine and deep cerebral blood vessels after ischemia (Gibson and Dehghani, 2009). Compared with DOI, LSI demonstrates a higher spatial resolution of blood flow responses but limited penetration (0.5–0.8 mm only) (Liao et al., 2013). Thus, the perfusion- and metabolism-related data can only be evaluated in the superficial layers of the cortex, which is insufficient for assessing the progressive changes in the ischemic region during the entire hyperacute phase of ischemia (Dehghani et al., 2009; Liao et al., 2013; Luckl et al., 2010; Miao et al., 2010; Zhang et al., 2006). Functional magnetic resonance imaging (fMRI) provides noninvasive large-scale measurements of neural functions and can precisely distinguish both stroke mimics and small lesions in cerebral ischemia (Tatlisumak, 2002; Vymazal et al., 2012). However, this technique does not provide precise penumbra data because the estimation is based only on a difference in perfusion parameters and diffusion-weighted MRI (Vymazal et al., 2012). Furthermore, the suitability of MRI for certain stroke patients is still limited because of several factors, such as claustrophobia and the presence of pacemakers or other ferromagnetic material implants (Tatlisumak, 2002).

Conversely, photoacoustic (PA) imaging is an emerging optical imaging technique that provides intrinsic optical absorption (*i.e.*, hemoglobin), high resolution, deep tissue penetration (Zhang et al., 2006) and high compatibility with other imaging techniques (Liao et al., 2013). For instance, PA imaging technology is especially suitable for evaluating the progressive changes in the penumbra area of focal ischemia because of its attributes such as the deeper tissue penetration depth and intrinsic blood contrast. Recently, our studies have shown that functional photoacoustic microscopy (fPAM) can be used for the contrast agent-free imaging of functional CBV and hemoglobin oxygen saturation ($SO_2$) changes in the rat cortex following peripheral sensory stimulation (Hu et al., 2009; Liao et al., 2012a; Liao et al., 2012b).

In this study, we aimed to combine fPAM with electrocorticography (ECoG) recordings to investigate neurovascular function in a rodent model of photothrombotic ischemia (PTI) (Kao et al., 2014). We demonstrated that hemodynamic responses, including the CBV, $SO_2$, and neural activity, namely, somatosensory evoked potential (SSEP) and resting-state ECoG signals, can be simultaneously measured by the proposed ECoG–fPAM system. We employed peripheral sensory stimulation as a potential treatment for PTI at multiple time points (*i.e.*, 0, 1, or 2 h post-PTI onset) and evaluated the degree of neurovascular function recovery in the ischemic area at the forelimb region of the primary somatosensory cortex (S1FL). Inter-hemispheric coherence and alpha-to-delta ratio (ADR) changes calculated from the resting-state ECoG signals at the bilateral cortical regions were also evaluated. The infarct volume was also assessed for examining the efficacy of the peripheral sensory stimulation treatment. Additionally, to investigate the recovery of neurovascular function in the PTI region, we employed the hemodynamic/ADR interaction as a recovery indicator (RI) for an in-depth assessment of functional recovery. Thus, using the ECoG–fPAM system, we demonstrated that the delivery of peripheral sensory stimulation during an appropriate time window could significantly reduce ischemic lesion severity.

## Materials and methods

### *The electrocorticography-functional photoacoustic microscopy system (ECoG–fPAM)*

Together with the ECoG–fPAM system, we established an experimental environment capable of 1) functional PA imaging, 2) PTI induction at the cortex, 3) peripheral sensory stimulation and 4) ECoG recordings, as shown in Fig. 1. A designed 50-MHz dark-field confocal fPAM system was used to image the functional hemodynamic changes in the selected cortical blood vessels. Two visible wavelengths of laser pulses, 560 and 570 nm ($\lambda_{560}$ and $\lambda_{570}$), were employed for PA wave excitation. These wavelengths were used because the detected photoacoustic signals at $\lambda_{560}$ were sensitive to changes in $SO_2$, whereas those at $\lambda_{570}$ were dominated by changes in CBV (Liao et al., 2010). Please refer to the supplementary material for details regarding the setup of the fPAM system and the data analysis of the relative functional changes in the CBV and $SO_2$ in specific regions performed in this study (Liao et al., 2012a; Tsytsarev et al., 2012). For the ECoG recordings, seven stainless steel epidural electrodes (including one reference electrode) were secured on the skull to acquire SSEPs and resting-state ECoG signals, which were pre-amplified (PZ2-32, Tucker-Davis Technologies, Alachua, FL, USA) and recorded using a bio-signal processor (RZ5D, Tucker-Davis Technologies, Alachua, FL, USA). MATLAB software (MATLAB R12, MathWorks Inc., Natick, MA, USA) was used to analyze related parameters of evoked potentials in response to peripheral sensory stimulation. Please refer to the supplementary material for details concerning the data analysis of the electrophysiological recordings, including the SSEPs, inter-hemispheric coherence and ADR calculations.

### *Animal preparation*

All experimental protocols used in this study were evaluated and approved by the Institutional Animal Care and Use Committee (IACUC) of the National University of Singapore. Animal care and surgical procedures were performed according to the National Advisory Committee for Laboratory Animal Research (NACLAR) guidelines for facilities licensed by the Agri-Food and Veterinary Authority of Singapore (AVA), which is the regulatory body of the Singapore Animals and Birds Act.

Thirty male Wistar rats weighing 250–300 g (InVivos Pte Ltd., Singapore) were divided into four groups; six animals were included in the control group, and eight animals were included in each of the three experimental groups. The animals were anesthetized with a 50 mg/kg bolus of pentobarbital and maintained with 15 mg/kg/h pentobarbital throughout the experiment. The rats were mounted on a custom-made acrylic stereotaxic head holder. Body temperature was measured using a rectal probe and was maintained at 37 ± 0.5 °C using a self-regulating thermal plate (TCAT-2 Temperature Controller, Physitemp Instruments, Inc., Clifton, NJ, USA).

The skin was subsequently removed from the skull to expose the bregma. Six stainless steel epidural electrodes were bilaterally secured to the skull over the motor cortical regions (M1: anterior–posterior (AP) = +4.2 mm, medial–lateral (ML) = ±3 mm) and the S1FL cortical regions (S1FL and S1FL* AP = +1.7 mm and −0.8 mm, respectively; ML = ±4.5 mm) for SSEPs and resting-state ECoG recordings (Fig. 1). One reference electrode was positioned at 3 mm to the right of the lambda landmark. Next, a cranial window of approximately 3 mm (AP) × 8 mm (ML), centered at the bregma, was produced for PA