Supplementary material

The ECoG-fPAM system

1 Neural activity was monitored by acquiring SSEPs and resting-state (RS) ECoG signals via
2 stainless steel epidural electrodes secured on the rat's skull. The acquired signals were
3 subsequently pre-amplified (PZ2-32, Tucker-Davis Technologies, Alachua, Florida, USA) and
4 then recorded using a BioAmp processor (RZ5D, Tucker-Davis Technologies, Alachua, Florida,
5 USA). The SSEPs, which were elicited via peripheral sensory electrical stimulation, were
6 sampled at 1 kHz, pre-amplified through the PZ2-32 pre-amplifier and band-pass filtered between
7 0.3 and 150 Hz using an RZ5D BioAmp Processor[1]. The stimulus onset time stamps for each
8 trigger pulse were recorded simultaneously with the SSEP signals. MATLAB (R2011a,
9 MathWorks Inc., Natick, Massachusetts, USA) was used for analyses of the ECoG
10 parameters.

11 fPAM imaging was performed to study the functional changes in selected cortical blood
12 vessels using a custom-designed 50-MHz dark-field confocal fPAM system with an axial
13 resolution of 32 μm and a lateral resolution of 61 μm (Acoustic Sensor Co., Ltd., Taiwan). Laser
14 pulses of 4 ns were generated by an optical parametric oscillator (Surlite OPO Plus, Continuum,
15 San Jose, California, USA) at a pulse repetition rate of 10 Hz, and the stimuli were supplied by a
16 frequency-tripled Nd:YAG Q-switched laser (Surlite II-10, Continuum, San Jose, California,
17 USA). For PA wave excitation, two visible wavelengths of the laser pulses, 560 and 570 nm (λ_{560}
18 and λ_{570}, respectively), were employed to monitor the relative functional hemodynamic response

changes[2]. The designed transducer had a -6 dB fractional bandwidth of 57.5%, a focal length of 9 mm and a 6-mm active element. A 1-mm multimode fiber was used to deliver the laser energy. The fiber tip was coaxially aligned with a collimation lens, an axicon, a Plexiglas mirror and an ultrasonic transducer on an optical bench, and this system produced dark-field illumination that was confocal with the focus of the ultrasonic transducer. The transducer was immersed in an acrylic water tank during the imaging process, 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 polyethylene film to ensure good acoustic coupling between the generated PA waves and the transducer through the tank. The PA signals received by the ultrasonic transducer were pre-amplified by a low-noise amplifier (AU-3A-0110, MITEQ Inc., Hauppauge, New York, USA), cascaded to an ultrasonic receiver (5073 PR, Olympus, Center Valley, Pennsylvania, USA), and then digitized by a computer-based 14-bit analog-to-digital (A/D) card (CompuScope 14200, Gage, Lockport, Illinois, USA) at a sampling rate of 200 MHz.

The incident laser energy density on the sample surface was less than 6 mJ/cm^2, which is well below the ANSI safety limit of 20 mJ/cm^2[3,4]. Fluctuations in laser energy were monitored using a photodiode (DET36A/M, Thorlabs, Newton, New Jersey, USA). Before further signal processing, the recorded photodiode signals were applied to compensate for PA signal variations caused by laser energy instability. The achievable penetration depth of the current fPAM setup was estimated to be 3 mm, with an approximately 18-dB signal-to-noise ratio[3], which was defined as the ratio of the peak signal value to the root-mean-square value of the noise. No signal

1 averaging was performed to capture real-time hemodynamic responses for functional imaging

2 analysis[3].

3

Animal preparation

4 A skin incision was made above the skull to expose the Bregma. RS ECoG and SSEP

5 recordings were acquired using six stainless steel epidural electrodes that were secured to the

6 skull bilaterally over the primary motor cortical regions (M1, anterior-posterior (AP) = +4.2 mm,

7 medial-lateral (ML) = ±3 mm) and two areas of S1FL (S1FL and S1FL$^+$, where $^+$ indicates the

8 region closer to the hindlimb primary somatosensory cortex, AP = +1.7 mm or -0.8 mm, ML =

9 ±4.5 mm) (**Figure 1A**). In addition, a reference electrode was positioned 3 mm to the right of

10 Lambda. The electrodes, which were connected with silver wires, were attached to a ZIF-Clip

11 head-stage that was interfaced with the data acquisition system (Tucker-Davis Technologies,

12 Alachua, Florida, USA). To facilitate PA imaging and PTI induction, a cranial window (denoted

13 by the black box in **Figure 1A**) of approximately 3 mm (AP) × 8 mm (ML) that was centered at

14 Bregma was generated using a high-speed drill while keeping the dura intact. The interaural line

15 (indicated by the blue dashed line in **Figure 1A**) and the Bregma reference site (*i.e.,* the red solid

16 line in **Figure 1A**) were used to position the rat's head in the fPAM system for subsequent

17 experiments[3].

18

Photothrombosis technique for focal ischemia induction

1 Focal ischemia was induced using the photothrombosis technique on a targeted cortical
2 arteriole that was a distal branch of the middle cerebral artery (distinctly identified by its
3 morphology as observed under a surgical microscope[5,6]). This model was selected because of its
4 high spatial specificity and reproducibility, which are crucial for mechanistic studies of the
5 effects of rtPA[7]. This model was also highly compatible with our ECoG-fPAM system and
6 enabled simultaneous examination of the changes in multiple physiological parameters after
7 ischemia. The targeted arteriole was located in S1FL of the right hemisphere[3,8]. The
8 photosensitive dye Rose Bengal (Na^+ salt, R3877, Sigma-Aldrich, Singapore) was diluted to 10
9 mg/ml in HEPES-buffered saline and was infused via the tail vein at 0.2 ml/100 g rat body weight
10 over 2 min using an intravenous cannula (Introcan Safety-W PUR 24G, B. Braun, Singapore) to
11 ensure complete administration of the drug. Following the onset of infusion, the single cortical
12 arteriole that was selected for occlusion was illuminated with 5 mW of 532 nm continuous wave
13 (CW) laser light (MGM-20, Beta Electronics, Columbus, Ohio, USA)[9,10] (the MGM-20
14 instrument was coupled to the designed dark-field optical path of the fPAM system, as illustrated
15 in **Figure 1A**), and this light was applied for 20 min until a stable clot formed[10-12]. Because we
16 induced PTI in a cortical arteriole in the right S1FL, this area is referred to as the ipsilesional
17 S1FL (iS1FL), whereas the corresponding region in the opposite hemisphere is referred to as the
18 contralesional S1FL.
19

Peripheral sensory electrical stimulation protocol

1 Peripheral sensory electrical stimulation was applied to evoke neurovascular responses in
2 the ischemic cortical region[1]. Subdermal needle electrodes were inserted into the rat's left
3 forepaw (contralateral to the occlusion), and electrical stimulation was applied using a stimulator
4 (DS3, Digitimer, Hertfordshire, UK) that was controlled and triggered by a multichannel BioAmp
5 processor (RZ5D, Tucker-Davis Technologies, Alachua, Florida, USA). A monophasic constant
6 current of 2-mA intensity with a 0.2-ms pulse width at a frequency of 3 Hz and a 5-sec
7 stimulation duration was used for each block, as shown in **Figure 2A**. A 7-min recording block
8 was employed in this study for functional signal acquisition; each recording block consisted of a
9 1-min RS ECoG recording followed by a 5-sec stimulation period to elicit somatosensory evoked
10 responses while simultaneously recording the SSEPs. To allow adequate time for the brain to
11 return to the RS, SSEP recording was followed by a 3-min resting period prior to the subsequent
12 PA imaging measurement[13]. To evoke a hemodynamic response and to facilitate fPAM imaging,
13 an additional 5 sec of electrical stimulation (*i.e.*, 2-mA intensity with a 0.2-ms pulse width at a
14 frequency of 3 Hz) was then applied to the rat's left forepaw, followed by 3 min of PA imaging.
15 These recording blocks were employed before PTI (baseline) and were repeated once every 30
16 min beginning at 15 min post-PTI and ending at 6 h post-PTI.

17 The PA signals at λ_{560} or λ_{570} were acquired in each block to assess stimulation-induced
18 hemodynamic changes within S1FL. PA B-scan images acquired in the S1FL region were used to
19 assess stimulation-induced relative hemodynamic changes.

20

Analysis of the evoked neural activity recording data

1 Electrophysiological assessments of post-PTI changes including SSEP and RS ECoG as
2 described previously[1]. SSEP components such as the P1 (*i.e.,* the first positive peak after
3 forepaw stimulation), N1 (*i.e.,* the first negative peak directly following P1) and peak (P1)-to-
4 peak (N1) (P-P) amplitudes were extracted to verify the successful induction of PTI, as
5 indicated by significantly diminished SSEP after PTI onset, and to compare the evoked
6 responses induced by forepaw electrical stimulation before and after PTI[1]. To promote
7 thrombus stabilization, the recording protocol in all groups was initiated 15 min post-PTI
8 onset[14,15]. Stimulation at 3 Hz was applied for 5 sec during each block, and 15 corresponding
9 sweeps were extracted to generate an averaged SSEP over a 100 ms epoch of the post-stimulus
10 pulse to evaluate the changes in cortical function[16]. The P1, N1 and P-P amplitudes were
11 extracted from the averaged SSEPs. All SSEP parameters mentioned above were evaluated
12 using only Ch4 because it was located in iS1FL, which was close to the targeted PTI
13 location[16]. SSEPs were evoked by electrical stimulation of the contralateral forepaw (*i.e.*,
14 left forepaw).
15

Spectral analysis of the electrophysiological recording data

16 In addition to the evaluation of evoked neural activity, the variations in the RS ECoG signal
17 across the ischemic and non-ischemic cortical regions were assessed based on analyses of spectral
18 measures (*i.e.*, ADR and BSI) and inter-hemispheric coherence[17-19]. Inter-hemispheric coherence,

1 a measure of the linear relationship between two signals at a specific frequency, is a traditional
2 indicator of synchronization between brain structures; in this study, synchronization between the
3 ischemic and non-ischemic regions was evaluated by performing coherence calculations[1]. Here,
4 coherence was calculated for the delta (*i.e.,* 0.1-4.0 Hz) and alpha (*i.e.,* 8.0-13.0 Hz) frequency
5 bands because they are strong indicators of injury or recovery that are used for the clinical
6 evaluation of recovery from ischemia[20,21]. These coherence calculations were performed using a
7 specific electrode combination (Ch3 and Ch4) because these electrodes were placed near the
8 corresponding PTI location (in S1FL). Although Ch5 and Ch6 were placed at $S1FL^+$, these two
9 channels were located near the border of the forelimb and hindlimb regions; thus, the changes in
10 the ECoG signals recorded by these electrodes were subtler than those recorded by Ch3 and
11 Ch4[16,22]. Additionally, Ch1 and Ch2 were positioned above the M1 region, and the activity in this
12 region does not correspond to the administered forepaw stimulation; therefore, the signals
13 recorded by these electrodes were not significantly affected by focal PTI in the selected location.
14 However, these channels (*i.e.*, Ch1, Ch2, Ch5, and Ch6) were used to evaluate the overall state of
15 neural activity, as explained in detail in the following section.
16 To limit inter-subject variability, coherence analysis was performed relative to the baseline
17 amplitudes considering 20 sec of non-overlapping ECoG signals selected from every recording
18 block[23]. A multivariate autoregressive (MVAR) model was adopted to perform coherence
19 analyses [23]. $X(t)=[x_1(t), x_2(t), ..., x_N(t)]^T$ was used to calculate the *N*-channel ECoG signal, and

$x_n(t)$ denotes the nth channel of the ECoG signal (*i.e.*, n = 1, 2,… N, where N = 6 in this study) at time t. The mth-order MVAR model is given by:

$$X(t) = \sum_{r=1}^{m} A(r)X(t-r) + E(t)\,, \tag{1}$$

where r is the model order (r = 1, 2,…m), $A(r)$ is the $N \times N$ coefficient matrix of model coefficients, $X(t-r)$ is the ECoG signal amplitude at time *t-r,* and $E(t)$ represents white Gaussian noise[23]. The optimal model order m was determined based on Schwarz's Bayesian criterion[24].

The coherence spectrum function, $C_{xy}(f)$, for two given signals was calculated as follows:

$$C_{xy}(f) \equiv \frac{S_{xy}(f)}{\sqrt{(S_{xx}(f)S_{yy}(f))}}\,, \tag{2}$$

where f denotes the frequency (f = 8.0 to 13.0 Hz for alpha coherence and f = 0.1 to 4.0 Hz for delta coherence), $S_{xx}(f)$ and $S_{yy}(f)$ are the respective auto-spectral power densities and $S_{xy}(f)$ is the cross-spectral power density of the electrode pair x, y (here, the electrode channel pair indexes x = 3 and y = 4, where x is situated in the left S1FL and y is located in the right S1FL). Coherence, $Coh_{xy}(f)$, was further defined by the absolute value of the coherence spectrum function $C_{xy}(f)$[25]:

$$Coh_{xy}(f) \equiv \left|C_{xy}(f)\right|. \tag{3}$$

From Eq. (3), we obtained $Coh_{xy}(f)$, a measure of the correlation between the ischemic and non-ischemic hemispheres, which helped us to observe the changes in cortical activation after PTI onset.

Further, to establish clinical correlations of our study results, we calculated the ADR, a widely clinically used quantitative electroencephalographic measure of cerebral injury. In this study, the ADR was utilized to evaluate neural functional changes after PTI. The ADR is defined as the ratio of the alpha power to the delta power, which correspond to states of normal and injured brain activity, respectively, in the specified cortical location[1,26]. Thus, a lower ADR value corresponds to higher delta activity and worse functional outcomes, and a higher ADR reflects functional recovery based on the presence of greater alpha power. Here, to assess recovery and injury in the peri-infarct region, the ADR results were calculated for only electrode Ch4 because this electrode was located in the ischemic region. The mean power in the delta and alpha frequency ranges at Ch4 was measured using fast Fourier transform (FFT) analysis. The percent change in the ADR was then calculated relative to the baseline ADR for the selected electrode, Ch4.

In addition, the BSI was used as a measure of the overall injury status of the cortical regions. The BSI is an established quantitative measure of injury in acute hemispheric stroke patients, and a significant correlation was found between the BSI and the National Institutes of Health Stroke Scale (NIHSS) score[27,28]. Compared to the ADR, the BSI is more sensitive for detecting early ECoG changes that provide prognostic information related to long-term functional outcomes[27]. The BSI value indicated the degree of inter-hemispheric asymmetry between homologous channel pairs among Ch1-Ch6, whereas the ADR reflected the state of recovery or injury at only a single electrode location. As described by de Vos *et al.*[27], the BSI was determined

1 by calculating the power spectral density using Welch's averaged, modified periodogram spectral

2 estimation method with a 2-sec Hamming window and 50% overlap[27]. The BSI was defined as

3 follows[27]:

4

$$BSI(t) = \frac{1}{MN} \sum_{j=1}^{M} \sum_{i=1}^{N} \left| \frac{R_{ij}(t) - L_{ij}(t)}{R_{ij}(t) + L_{ij}(t)} \right|, \quad (4)$$

5 where $R_{ij}(t)$ and $L_{ij}(t)$ are the FFT-based power spectral densities of the RS ECoG signal

6 obtained from electrode channel pairs (**Figure 1A**) in the ipsilesional ($R_{ij}(t)$) and contralesional (

7 $L_{ij}(t)$) hemispheres, respectively, based on Welch's method. The BSI was used to evaluate inter-

8 hemispheric asymmetry among homologous channel pairs (with i = 1, 2,... N) and maximum

9 channel pairs (N = 3) in the frequency range (M) from 1 to 25 Hz (at frequency j Hz, where j = 1,

10 2,... M) separated into the delta, theta, alpha and beta frequency bands[27,29]. Note that the lower

11 bound for the BSI is 0 (perfect symmetry for all channels), indicating normal conditions, and that

12 the upper bound of the BSI is 1, which implies maximal asymmetry and a state of maximal

13 cerebral injury[27].

14

Data analysis of the measured hemodynamic changes

15 Two optimized wavelengths (λ_{560} and λ_{570}) were used to monitor functional CBV and SO_2

16 changes[3]. Note that we assumed that CBV is proportional to the specific cortical region imaged at

17 λ_{570} (*i.e.*, $I_{R(570)}$)[30]. PA cross-sectional B-scan images of specific cortical regions captured at λ_{570}

1 ($i.e.$, $I_{R(570)}$) were used. Functional CBV changes ($i.e.$, R_{CBV}) were calculated according to the

2 following equation:

3
$$R_{CBV}(t) = \frac{A(I_{R(570)}(t))}{A(I_{R(570),baseline})}, \quad (5)$$

4 where t is the time point in each block, $A(I_{R(570)}(t))$ represents the cross-sectional area at the given

5 time for each block, and $A(I_{R(570),baseline})$ is the baseline value for the cross-section estimated from

6 the image acquired immediately before the onset of evoked forepaw electrical stimulation in the

7 block[13]. $A(I_{R(570)})$ was calculated based on the total vessel pixel count of a selected cross-sectional

8 area ($i.e.$, $I_{R(570)}$). A vessel pixel was defined as a pixel that displayed a PA signal that was

9 threefold greater than the background signal[13,31,32].

10 Functional images of SO_2 changes ($\Delta I_{F(560)}(t)$) at a given time point t in each block were

11 assessed according to the following equation:

12
$$\Delta I_{F(560)}(t) = \frac{I_{(560)}(t)}{I_{R(570)}(t)} - \frac{I_{(560),baseline}}{I_{R(570),baseline}} = I_{F(560)}(t) - I_{F(560),baseline}, \quad (6)$$

13 where $I_{(560)}$ ($i.e.$, the PA image acquired at λ_{560}) is normalized to $I_{R(570)}$ on a pixel-by-pixel basis

14 and $I_{(560),baseline}$ is the baseline image at λ_{560} that was acquired immediately before the onset of

15 forepaw electrical stimulation in each block[3]. Note that negative values for $\Delta I_{F(560)}$ ($i.e.$, a

16 positive $-\Delta I_{F(560)}$) indicate an increase in the SO_2 levels and vice versa[3]. The mean functional SO_2

17 changes ($R_{SO_2}(t)$) in a specific cortical region during the stimulation period were determined as

18 follows:

1

$$R_{SO_2}(t) = \sum_{(x,z)\in \text{vessel pixel}} (I_{F(560)}(x,z,t)) / A(I_{R(570)}(t)) - \sum_{(x,z)\in \text{vessel pixel}} (I_{F(560),baseline}(x,z,t)) / A(I_{R(570),baseline}(t)). \quad (7)$$

2 From Eqs. (6) and (7), the fPAM system enabled an independent assessment of the relative
3 changes in CBV and SO_2, in which $I_{R(570)}$ was used as a measure of CBV and $I_{F(560)}$ was used as a
4 measure of SO_2. Please refer to our previous studies for additional details concerning the data
5 analysis of the functional changes in CBV and SO_2 in specific regions[13,31].
6

Measurement of the infarct volume

7 Histological quantification of the extent of infarction was performed using 2,3,5-triphenyl-
8 tetrazolium chloride (TTC, T8877, Sigma-Aldrich, Singapore). At 24 h after successful PTI
9 induction, the rats were deeply anesthetized with 10% chloral hydrate, and their brains were
10 rapidly removed, washed in phosphate-buffered saline (PBS) at room temperature and frozen at -
11 20°C for 10 min[1]. The brain tissue from 4 mm anterior to 6 mm posterior to Bregma was sliced
12 into ten serial 1-mm coronal sections. The sliced brain sections were stained with 2% TTC for 30
13 min at 37°C in the dark, followed by overnight immersion at 4°C in 4% paraformaldehyde in 0.1
14 M PBS, pH 7.4. The infarcted tissue remained unstained (white), whereas the normal tissue was
15 stained red. The extent of ischemic infarction was traced, and the integrated volume was
16 calculated using ImageJ software (NIH Image). The infarct volume was calculated by adding the
17 infarct areas of all sections and multiplying by the slice thickness. To compensate for the effect of
18 brain edema, the corrected infarct volume was calculated as follows: percentage of corrected

infarct volume = {[total lesion volume − (ipsilateral hemisphere volume − contralateral hemisphere volume)]/contralateral hemisphere volume} $\times$ 100.

Determination of a safe and efficient rtPA therapeutic time window in a rat PTI model

To determine the optimal rtPA infusion onset time for recovery in the rat PTI model, in this study, we adopted measures of neural integrity and hemodynamic responses to evaluate hyperacute ischemic neurovascular changes. The values of the aforementioned parameters throughout the post-PTI monitoring period, *i.e.*, from PTI onset to 6 h post-PTI, were compared to the baseline values. Based on the trends of the changes post-PTI, we determined the following distinct time windows for both beneficial and unproductive rtPA administration: (1) the golden time window: the rtPA infusion onset time was associated with an increase in neurovascular functions (*i.e.*, neural integrity and hemodynamic responses), represented by a post-PTI value of at least 80% of baseline at the end of the monitoring period[16,33]; (2) unproductive rtPA administration timing: the rtPA infusion onset time was associated with a post-PTI value (at the end of the 6 h monitoring period) that was worse than the baseline value for at least one of the analyzed parameters (*i.e.*, neural integrity or hemodynamic responses)[16,33]. If the corresponding unproductive rtPA administration timing was earlier than the golden time window, it was classified as early rtPA administration, and if the unproductive rtPA administration timing was later than the golden time window, it was referred to as late rtPA administration.

References

1 1. Liao LD, Bandla A, Ling JM, et al. Improving neurovascular outcomes with bilateral forepaw
2 stimulation in a rat photothrombotic ischemic stroke model. *Neurophotonics*. 2014; 1: 011007.
3 2. Liao L-D, Tsytsarev V, Delgado-Martínez I, et al. Neurovascular coupling: in vivo optical
4 techniques for functional brain imaging. *Biomed Eng Online*. 2013; 12: 38-58.
5 3. Liao LD, Li ML, Lai HY, et al. Imaging brain hemodynamic changes during rat forepaw
6 electrical stimulation using functional photoacoustic microscopy. *Neuroimage*. 2010; 52: 562-70.
7 4. Liao LD, Orellana J, Liu YH, et al. Imaging of temperature dependent hemodynamics in the
8 rat sciatic nerve by functional photoacoustic microscopy. *Biomed Eng Online*. 2013; 12: 120-32.
9 5. Markgraf CG, Kraydieh S, Prado R, Watson BD, Dietrich WD and Ginsberg MD.
10 Comparative histopathologic consequences of photothrombotic occlusion of the distal middle
11 cerebral artery in Sprague-Dawley and Wistar rats. *Stroke*. 1993; 24: 286-92.
12 6. Sigler A, Goroshkov A and Murphy TH. Hardware and methodology for targeting single brain
13 arterioles for photothrombotic stroke on an upright microscope. *J Neurosci Methods*. 2008; 170:
14 35-44.
15 7. Wester P, Watson BD, Prado R and Dietrich WD. A photothrombotic 'ring'model of rat stroke-
16 in-evolution displaying putative penumbral inversion. *Stroke*. 1995; 26: 444-50.
17 8. Paxinos G and Watson C. *The rat brain in stereotaxic coordinates*. San Diego: Academic
18 Press, 2007.

9. Nakase H, Kakizaki T, Miyamoto K, Hiramatsu K and Sakaki T. Use of local cerebral blood flow monitoring to predict brain damage after disturbance to the venous circulation: cortical vein occlusion model by photochemical dye. *Neurosurgery*. 1995; 37: 280-6.

10. Watson BD, Dietrich WD, Busto R, Wachtel MS and Ginsberg MD. Induction of reproducible brain infarction by photochemically initiated thrombosis. *Ann Neurol*. 1985; 17: 497-504.

11. Kao YC, Li W, Lai HY, Oyarzabal EA, Lin W and Shih YY. Dynamic perfusion and diffusion MRI of cortical spreading depolarization in photothrombotic ischemia. *Neurobiol Dis*. 2014; 71: 131-9.

12. Liao L-D, Bandla A, Ling JM, et al. Improving neurovascular outcomes with bilateral forepaw stimulation in a rat photothrombotic ischemic stroke model. *Neurophotonics*. 2014; 1: 011007-.

13. Liao LD, Lin CT, Shih YY, et al. Transcranial imaging of functional cerebral hemodynamic changes in single blood vessels using in vivo photoacoustic microscopy. *J Cereb Blood Flow Metab*. 2012; 32: 938–51.

14. Lay CC, Davis MF, Chen-Bee CH and Frostig RD. Mild sensory stimulation completely protects the adult rodent cortex from ischemic stroke. *PLoS One*. 2010; 5: 11270.

15. Schaffer CB, Tsai PS, Nishimura N, et al. All-optical thrombotic stroke model for near-surface blood vessels in rat: focal illumination of exogeneous photosensitizers combined with real-time two-photon imaging. *High-power lasers and applications*. 2003; 4978: 222-31.

16. Liao L, Liu YH, Lai HY, et al. Rescue of cortical neurovascular functions during the hyperacute phase of ischemia by peripheral sensory stimulation. *Neurobiol Dis*. 2015; 75C: 53-63.

17. Kaneko D, Nakamura N and Ogawa T. Cerebral infarction in rats using homologous blood emboli: development of a new experimental model. *Stroke*. 1985; 16: 76-84.

18. Zhang S-j, Ke Z, Li L, Yip S-p and Tong K-y. EEG patterns from acute to chronic stroke phases in focal cerebral ischemic rats: correlations with functional recovery. *Physiol Meas*. 2013; 34: 423-35.

19. Finnigan S and van Putten MJAM. EEG in ischaemic stroke: Quantitative EEG can uniquely inform (sub-)acute prognoses and clinical management. *Clin Neurophysiol*. 2013; 124: 10-9.

20. Nuwer MR, Jordan SE and Ahn SS. Evaluation of stroke using EEG frequency analysis and topographic mapping. *Neurology*. 1987; 37: 1153-9.

21. Sainio K, Stenberg D, Keskimaki I, Muuronen A and Kaste M. Visual and spectral EEG analysis in the evaluation of the outcome in patients with ischemic brain infarction. *Electroencephalogr Clin Neurophysiol*. 1983; 56: 117-24.

22. Basole A, Kreft-Kerekes V, White LE and Fitzpatrick D. Cortical cartography revisited: A frequency perspective on the functional architecture of visual cortex. *Prog Brain Res*. 2006; 154: 121-34.

23. Schlögl A and Supp G. Analyzing event-related EEG data with multivariate autoregressive parameters. *Prog Brain Res*. 2006; 159: 135-47.

24. Schwarz G. Estimating Dimension of a Model. *Ann Stat*. 1978; 6: 461-4.

25. Nolte G, Bai O, Wheaton L, Mari Z, Vorbach S and Hallett M. Identifying true brain interaction from EEG data using the imaginary part of coherency. *Clin Neurophysiol*. 2004; 115: 2292-307.

26. Claassen J, Hirsch LJ, Kreiter KT, et al. Quantitative continuous EEG for detecting delayed cerebral ischemia in patients with poor-grade subarachnoid hemorrhage. *Clin Neurophysiol*. 2004; 115: 2699-710.

27. de Vos CC, van Maarseveen SM, Brouwers PJ and van Putten MJ. Continuous EEG monitoring during thrombolysis in acute hemispheric stroke patients using the brain symmetry index. *J Clin Neurophysiol*. 2008; 25: 77-82.

28. van Putten MJ and Tavy DL. Continuous quantitative EEG monitoring in hemispheric stroke patients using the brain symmetry index. *Stroke*. 2004; 35: 2489-92.

29. Binienda Z, Beaudoin M, Thorn B and Ali S. Analysis of electrical brain waves in neurotoxicology: gamma-hydroxybutyrate. *Curr Neuropharmacol*. 2011; 9: 236.

30. Roston S. The blood flow of the brain. *Bull Math Biophys*. 1967; 29: 541-8.

31. Liao L-D, Lin C-T, Shih Y-YI, et al. Investigation of the cerebral hemodynamic response function in single blood vessels by functional photoacoustic microscopy. *Journal of biomedical optics*. 2012; 17: 061210-10.

32. Li L, Zemp RJ, Lungu G, Stoica G and Wang LV. Photoacoustic imaging of lacZ gene expression in vivo. *Journal of biomedical optics*. 2007; 12: 020504-1-3.

33. Christou I, Alexandrov AV, Burgin WS, et al. Timing of recanalization after tissue plasminogen activator therapy determined by transcranial Doppler correlates with clinical recovery from ischemic stroke. *Stroke*. 2000; 31: 1812-6.