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BRIEF COMMUNICATION

Resistance of optogenetically evoked motor function to global ischemia and reperfusion in mouse *in vivo*

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Recently we have shown that despite reperfusion, sensory processing exhibits persistent deficits after global ischemia in a mouse *in vivo* model. We now address how motor output, specifically cortically evoked muscle activity, stimulated by channelrhodopsin-2 is affected by global ischemia and reperfusion. We find that the light-based optogenetic motor map recovers to 80% within an hour. Moreover, motor output recovers relatively faster and more completely than the sensory processing after 5-minute period of global ischemia. Our results suggest a differential sensitivity of sensory and motor systems to the effects of global ischemia and reperfusion that may have implications for rehabilitation.

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INTRODUCTION

Stroke is characterized as a disturbance of blood flow to the brain, resulting in neuronal death and impairment of sensory,[1] motor, and cognitive function.[2] During recovery, neurons exhibit both short- and long-term plasticity to re-establish lost function.[3] We adapted a model of global ischemia that permits timely and precise bilateral occlusion of the common carotid arteries (CCAO) and reperfusion. This model is relevant to brain insults due to cardiac arrest or other transient ischemic attacks to study its impact on motor function. Previously, we found that reperfusion led to a recovery of dendritic structure from 7- to 9-minute transient global ischemia, and a gradual but partial recovery of sensory response in the cortex after ~1 hour.[1,4] Changes in motor function after stroke have been assessed exclusively in focal ischemia models using intracortical microstimulation (ICMS)[5] or behavior.[6] However, rapid plasticity has been largely unstudied, as a single ICMS map may require up to an hour to perform and the invasive nature of ICMS limits the repeatability of mapping.[7] Recently developed automated motor mapping techniques based on light activation of channelrhodopsin-2 (ChR2) permit non-invasive investigation of both motor output and map integrity with high temporal resolution after stroke. Pairing this method with measurements of cortical electroencephalography (EEG) evoked by direct ChR2 stimulation (ChR2-EEG) and peripheral sensory stimulation of the same limb (forepaw-EEG) where the motor output (ChR2-electromyography (EMG)) was assessed, we compared motor function, cortical neuronal excitability, and sensory function within the same mouse after global ischemia and reperfusion. This study provides new insights into the recovery of motor and sensory processing after global ischemia.

MATERIALS AND METHODS

Animal, Surgical Procedures, and Regulation of Cortical Temperature

Adult male *Thy1*-ChR2-yellow fluorescent protein transgenic mice (B6.Cg-Tg(Thy1-COP4/EYFP)18Gfng/J)[8] aged ~2 to 3 months were used. Experimental protocols were approved by the University of British Columbia Animal Care Committee and conformed to the Canadian Council on Animal Care and Use Guidelines. A 4×4 mm cranial window was made over the somatosensory and motor cortex leaving the dura intact. Body temperature was maintained throughout the surgical and imaging procedures at $37 \pm 0.5^{\circ}$C. In all animals, the cortical surface temperature was maintained at ~36.5°C by attaching a custom-made stainless steel head plate connected to a water pump (Figure 1A).

Global Ischemia Model

CCAO was performed as in previous studies[1] to induce global forebrain ischemia under ketamine/xylazine anesthesia.

Laser Speckle Contrast Imaging

The procedures for laser speckle imaging and analysis were performed as described previously[4] (for details see the Supplementary Information).

EEG and EMG Recording

We employed the same system for EEG recording[1] and forelimb EMG.[9] A single electrode was placed within 1 mm of the forelimb somatosensory cortex (Figure 1, b electrode) for recording direct current-EEG (DC-EEG) signal. The signal was high-pass filtered (0.1 Hz, Bessel filter, 8 pole) to exclude the slow DC shift for EEG studies. EEG signals were sampled at 1,000 Hz. EMG signals were sampled at 1,000 Hz for single-point ChR2 stimulation and at 5,000 Hz for motor mapping.

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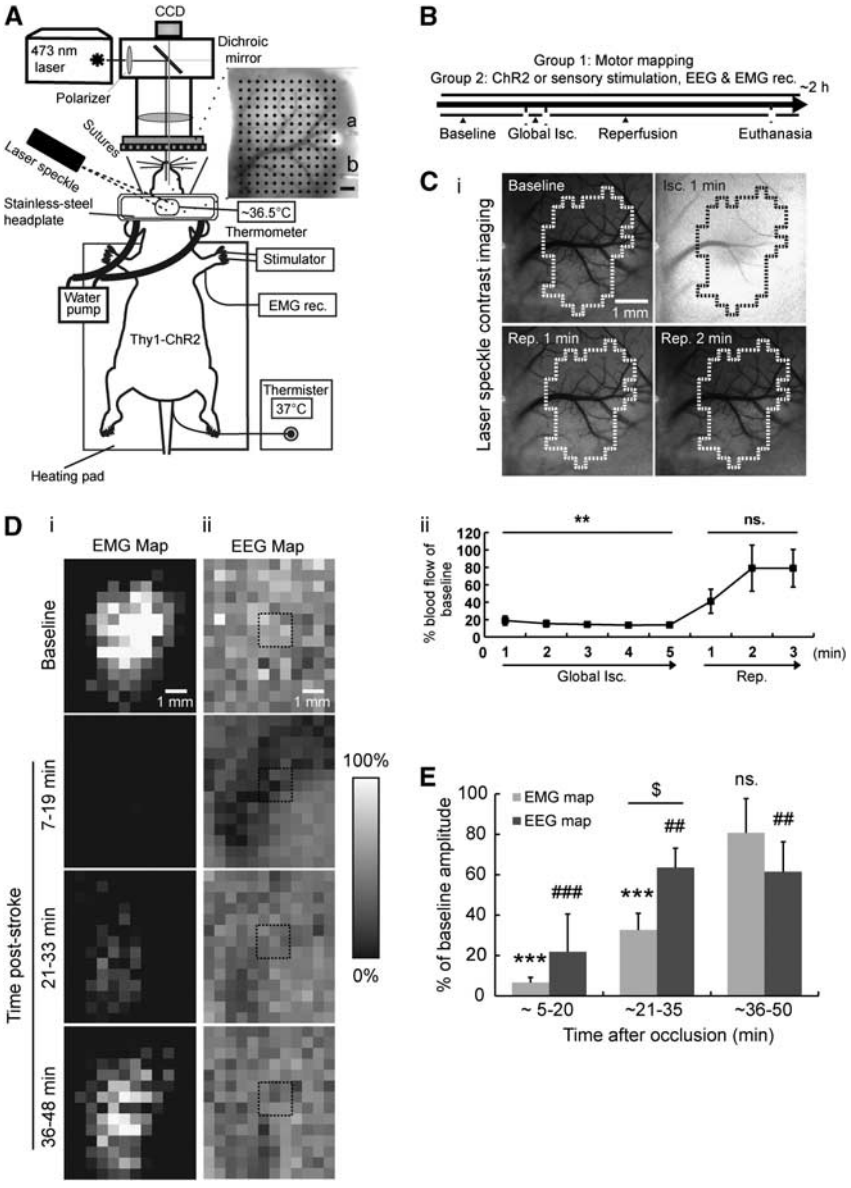

Figure 1. A 5-minute common carotid artery occlusion (CCAO) diminishes the amplitude of the electromyography (EMG) and electroencephalography (EEG) maps evoked by cortical channelrhodopsin-2 (ChR2) stimulation, with rapid recovery after reperfusion. (**A**, **B**) An overview of experimental setup and design. (**A**) Anesthetized, head-fixed mice are placed on a scanning stage and an array of cortical points (insert) is stimulated by a 473 nm collimated laser beam directed through a video microscope objective. Motor output is detected by EMG electrodes in the forelimb muscles. Surface temperature (a—temperature probe) and cortical EEG signal (b—electrode) are collected. Scale bar is 500 μm. (**B**) Experimental design. Two groups of mice are used to conduct either motor mapping or motor output (repetitive center stimulation in the motor map) assessment during baseline, 5-minute CCAO and reperfusion. (**C**) Laser speckle contrast imaging confirms $>80\%$ decrease in blood flow in the forelimb motor representation during CCAO, which recovers to $>80\%$ of pre-CCAO value within 2 minutes after reperfusion. (i) Representative laser speckle contrast images at pre-CCAO, 1 minute after ischemia, 1 and 2 minutes after reperfusion. The region surrounded by white dash line indicates the forelimb motor representation obtained by laser-based motor mapping (threshold at 0%). (ii) Quantitative summary of blood flow in the motor representation after CCAO and reperfusion. ($n=4$, $^{**}P<0.01$, compared with pre-CCAO value, one-way analysis of variance (ANOVA). (**D**) A 5-minute CCAO depresses both EEG and EMG maps (integration of signals) evoked by the cortical ChR2 stimulation. Reperfusion recovers both maps within 1 hour. Representative EMG maps (i) and EEG maps (ii) evoked by the cortical ChR2 stimulation after 5-minute CCAO and reperfusion. Only the center of motor map (3×3 pixels indicated by black dash line) is used to calculate the ChR2-evoked EEG signal. (**E**) Quantitative summaries of EMG maps and EEG maps after CCAO and reperfusion ($n=5$, $^{***}P<0.001$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, compared with the pre-CCAO values, one-way ANOVA; $^{\$}P<0.05$, EMG map versus EEG map, Student's *t*-test). All data are shown as mean $\pm$ s.e.m.

Light-Based Optogenetic Motor Mapping and ChR2 Stimulation

Light-based optogenetic motor mapping methodology has been described previously[9] (for details see the Supplementary Information).

Forepaw Stimulation

Two metal pins in the contralateral forepaw delivered a 1.5-mA 1-millisecond electrical pulse generated by a constant current stimulus isolator (A385, World Precision Instruments, Sarasota, FL, USA) triggered

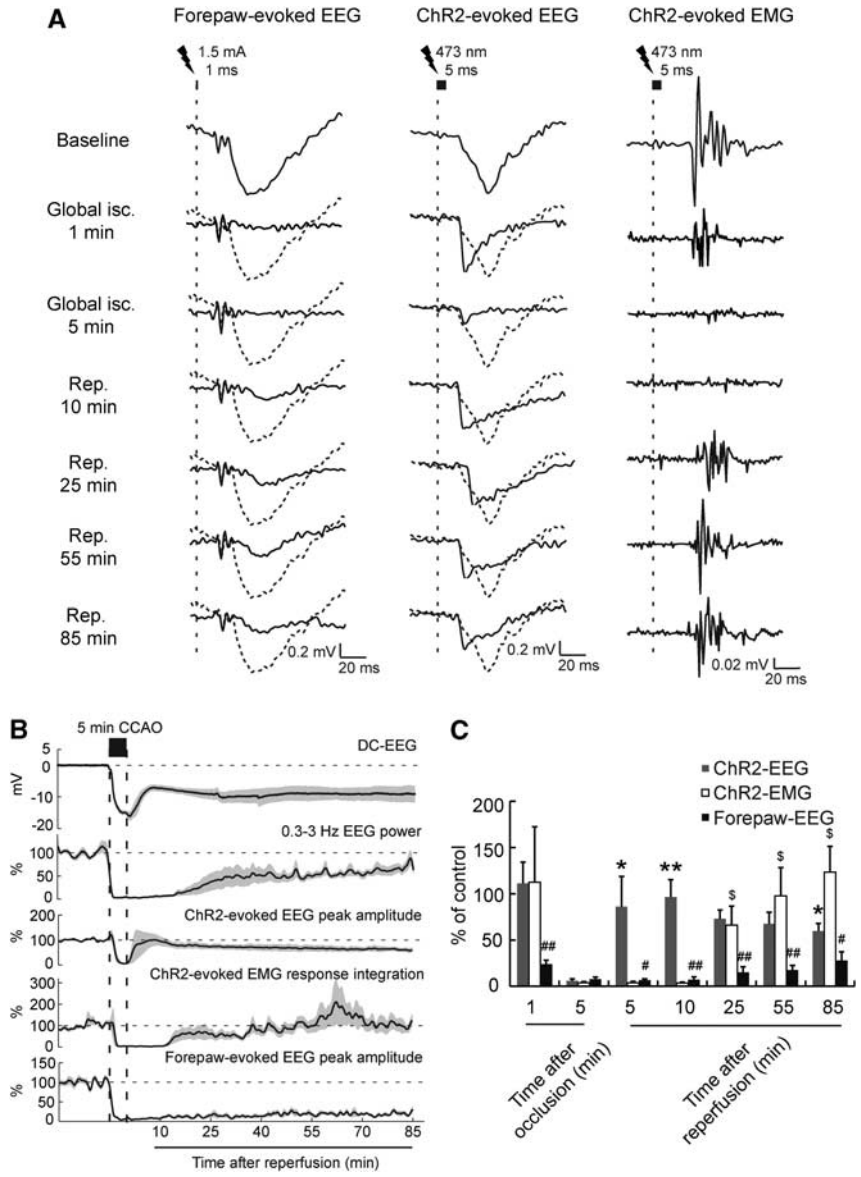

Figure 2. Single-point channelrhodopsin-2 (ChR2) cortical stimulation and electroencephalography (EEG) and electromyography (EMG) recording reveal rapid response recovery after reperfusion despite persistent deficits in forepaw stimulation-evoked cortical EEG responses. Channelrhodopsin-2 and sensory stimulation are interleaved and repeated every 10 seconds. (**A**) Representative traces of forepaw stimulation-evoked EEG (left column), ChR2 stimulation-evoked EEG (middle column), and ChR2-stimulation-evoked EMG (right column). (**B**) Changes of direct current-EEG ($n = 5$), 0.3 to 3 Hz EEG power ($n = 5$), ChR2-evoked peak amplitude ($n = 5$), ChR2-evoked EMG responses integration ($n = 5$), and forepaw-evoked peak amplitude ($n = 5$) are shown from top to bottom. Except for DC-EEG, the other statistical data were based on relative values, which were normalized to pre-common carotid artery occlusion (CCAO) value as 100%. The shaded band indicates s.e.m. These findings are consistent with previous work[1] and use different methods of anesthesia (ketamine/xylazine in the present experiment versus urethane in the previous one) and cortical temperature regulation ($\sim 36.5^{\circ}$C). (**C**) Statistical analysis of ChR2-mediated EEG (ChR2-EEG) and EMG (ChR2-EMG) responses, and forepaw stimulation-mediated EEG responses (forepaw-EEG) after 5 minute CCAO and reperfusion at select time points ($^{*}P < 0.05$, $^{**}P < 0.01$, ChR2-EEG versus ChR2-EMG; $^{\$}P < 0.05$, ChR2-EMG versus forepaw-EEG; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, ChR2-EEG versus forepaw-EEG, Student's *t*-test). All data are shown as mean ± s.e.m.

5 seconds after ChR2 laser stimulation by a 1322 A Digidata system (Molecular Devices, Sunnyvale, CA, USA) with a 10-second interval.

Statistical Analyses

All values except DC-EEG were normalized to percentage of pre-CCAO. A one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* tests was used to compare differences in blood flow, EMG and EEG values at each time point to pre-CCAO baselines in experimental groups. Differences between ChR2-EEG, ChR2-EMG, and forepaw-EEG were analyzed with Student's *t*-test. Statistically, significance was set at $P < 0.05$.

RESULTS

Here, we apply optogenetics to evaluate changes in motor representations while simultaneously generating EEG maps of cortical neuronal excitability during and after a 5-minute period of transient global ischemia (Figure 1A). In a second group

of mice, these changes were assessed with greater temporal resolution by repeatedly stimulating a single point at the center of the forelimb motor cortex while also collecting forepaw-evoked EEG, ChR2-EEG, and spontaneous EEG signals (Figure 1B). Laser speckle contrast imaging within the forelimb motor map confirmed significant decreases in arterial blood flow ($18.9 \pm 5.3\%$ of the baseline value, $P < 0.01$) at 1 minute after the onset of CCAO ($n = 4$, Figure 1Ci). Within 2 minutes of reperfusion, arteriole blood flow in the motor representation rapidly recovered to $79.0 \pm 26.5\%$ of the baseline value ($P > 0.05$).

We performed repeated optogenetic mapping before, during, and after CCAO, with EMG maps and EEG maps collected every $\sim$15 minutes after CCAO and reperfusion. The motor map was diminished to $6.6 \pm 2.6\%$ of the baseline value ($P < 0.001$) in the first mapping session, 5 to 20 minutes after the onset of a 5-minute CCAO, recovered partially by the second map (21 to 35 minutes post stroke, $32.6 \pm 8.4\%$, $P < 0.01$), and reached $80.7 \pm 17.0\%$ of baseline ($P > 0.05$) by the third map 36 to 50 minutes after CCAO onset (Figure 1Di). The sum of integration of ChR2-EEG signal measured from the center of the motor map (3×3 pixels) was suppressed to $21.8 \pm 8.4\%$ of the baseline value ($P < 0.001$) 5 to 20 minutes after occlusion, partially recovered to $63.6 \pm 4.3\%$ ($P < 0.01$) after 21 to 35 minutes, and further recovered to $61.6 \pm 6.6\%$ ($P < 0.01$) 36 to 50 minutes after CCAO and reperfusion (Figure 1Dii). We found that ChR2-evoked EEG map recovered faster than the ChR2-stimulation-generated motor map, indicating that excitability of cortical layer five neurons recovered sooner than motor output.

We next compared recovery of motor processing and sensory processing within animals. We first performed optogenetic mapping, then in a separate group of animals to increase temporal resolution of sensory and motor responses, laser stimulation was restricted to the center of the motor map (while measuring EMG from the contralateral forelimb). In single-point stimulation animals, we also electrically stimulated the contralateral forelimb and measured the resulting cortical EEG response. Cortical motor and peripheral sensory stimulation were interleaved and repeated every 10 seconds before, during, and after CCAO ($n = 5$, Figure 2A). Global ischemia induced ischemic depolarization and suppression of spontaneous 0.3 to 3 Hz EEG power. Reperfusion rapidly, but partially recovered the ischemia-induced DC shift, and slowly recovered spontaneous 0.3 to 3 Hz EEG power (Figure 2B). A > 10 mV DC shift was observed in all five animals, consistent with our previous results, which used both DC-EEG and IOS imaging to determine spreading ischemic depolarization in the same model.[1] Changes in the peak amplitude of ChR2 and forepaw-evoked EEG (the minimum value in a 40-millisecond period after stimulation) were correlated with changes in DC-EEG and 0.3 to 3 Hz EEG power after CCAO and reperfusion, respectively (Figure 2B). Within 1 minute after occlusion, the forepaw-evoked response was blocked while ChR2-EEG and ChR2-EMG were preserved. Responses evoked by ChR2 and forepaw stimulation were suppressed relative to baseline measures at 5 minutes after onset of occlusion (ChR2-EEG: $5.9 \pm 2.2\%$; ChR2-EMG: $3.88 \pm 1.24\%$; forepaw-EEG: $7.6 \pm 2.3\%$). ChR2-EMG recovered faster than the forepaw-EEG ($66.2 \pm 20.2\%$ versus $15.1 \pm 5.9\%$ at 25 minutes after reperfusion, $P < 0.05$). Although motor output recovered more rapidly than peripherally evoked EEG responses, it was still slower than the recovery of ChR2-EEG (ChR2-EEG: $86.1 \pm 32.5\%$ versus ChR2-EMG: $3.9 \pm 1.3\%$, $P < 0.05$, or versus forepaw-EEG: $6.5 \pm 1.5\%$, $P < 0.05$ at 5 minutes after reperfusion) (Figure 2C). We also observed gradual declines in ChR2-EEG after it recovered to the peak value ($96.3 \pm 18.8\%$) at 10 minutes after reperfusion, which may be due to delayed damage induced by the ischemic insult. However, the ChR2-evoked EMG responses recovered further ($97.8 \pm 30.5\%$ at 55 minutes, $123.4 \pm 27.8\%$ at 85 minutes after CCAO and reperfusion) despite the partial suppression of excitability of the neurons, which were mediating the EMG responses.

DISCUSSION

We investigated changes in sensorimotor processing after a transient global ischemia by assessing EMG and EEG responses to cortical optogenetic stimulation and forepaw electrical stimulation. Optogenetics have allowed us to study the acute effects of global ischemia that may be relevant to transient ischemic attacks. We discovered that motor output was suppressed during global ischemia and gradually recovered after reperfusion, though recovery of motor output was relatively slower than that of ChR2-EEG responses. We identified a period of time when the cortex was excitable (by ChR2-EEG) but motor output (by ChR2-EMG) was blocked, which corroborates earlier studies comparing sensory processing with cortical neuronal excitability.[1] The changes in ChR2-EEG waveform after ischemia may be the result of an incomplete recovery of synaptic transmission, as the waveform reflects both direct depolarization of ChR2-excited neurons and a secondary synaptic transmission-induced response.[1,10] Interestingly, after reperfusion, motor output was nearly fully recovered, consistent with previous reports of cortically evoked responses detected in the brain stem after a transient middle cerebral artery occlusion in rats.[11] However, the delayed and incomplete recovery of the sensory processing suggests that rehabilitative manipulations may need to take the relative vulnerability of sensory and motor systems into consideration after transient ischemic attacks. Given that transient stroke can induce persistent deficits in presynaptic transmitter release,[12] the most probable mechanism that results in the difference is that ChR2 is predominately expressed in layer 5B pyramidal neurons, which can project to the spinal cord, whereas sensory processing involves polysynaptic transmission within the cortex,[13] which is more likely to be suppressed by global ischemia, consistent with previous findings.[14] Given recent results showing that mild sensory stimulation could be protective after ischemia,[15] it is possible that the sensory and ChR2 stimulation paradigm could alter damage. However, the short, single pulses employed in either ChR2 or forepaw stimulation is unlikely to produce a significant hemodynamic response, which is proposed to rescue brain function during the acute phase of focal ischemia.[15]

Here, we show that optogenetics can be employed to study rapid plasticity of neuronal excitability and motor function after global ischemia with high temporal and spatial resolution. In future, with chronic preparations and non-invasive motion sensors,[9] we could extend the current study to longitudinal investigation. Potentially, this method may benefit the assessment of both acute and long-term therapeutics for stroke and their potential actions on sensory versus motor processing.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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