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Short- and long-latency somatosensory neuronal responses reveal selective brain injury and effect of hypothermia in global hypoxic ischemia

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Wu D, Xiong W, Jia X, Geocadin RG, Thakor NV. Short- and long-latency somatosensory neuronal responses reveal selective brain injury and effect of hypothermia in global hypoxic ischemia. *J Neurophysiol* 107: 1164–1171, 2012. First published December 7, 2011; doi:10.1152/jn.00681.2011.—Evoked potentials recorded from the somatosensory cortex have been shown to be an electrophysiological marker of brain injury in global hypoxic ischemia (HI). The evoked responses in somatosensory neurons carry information pertaining to signal from the ascending pathway in both the subcortical and cortical areas. In this study, origins of the subcortical and cortical signals are explored by decomposing the evoked neuronal activities into short- and long-latency responses (SLR and LLR), respectively. We evaluated the effect of therapeutic hypothermia on SLR and LLR during early recovery from cardiac arrest (CA)-induced HI in a rodent model. Twelve rats were subjected to CA, after which half of them were treated with hypothermia (32–34°C) and the rest were kept at normal temperature (36–37°C). Evoked neuronal activities from the primary somatosensory cortex, including multiunit activity (MUA) and local field potential (LFP), were continuously recorded during injury and early recovery. Results showed that upon initiation of injury, LLR disappeared first, followed by the disappearance of SLR, and after a period of isoelectric silence SLR reappeared prior to LLR. This suggests that cortical activity, which primarily underlies the LLR, may be more vulnerable to ischemic injury than SLR, which relates to subcortical activity. Hypothermia potentiated the SLR but suppressed the LLR by delaying its recovery after CA (hypothermia: 38.83 ± 5.86 min, normothermia: 23.33 ± 1.15 min; $P < 0.05$) and attenuating its amplitude, suggesting that hypothermia may selectively downregulate cortical activity as an approach to preserve the cerebral cortex. In summary, our study reveals the vulnerability of the somatosensory neural structures to global HI and the differential effects of hypothermia on these structures.

short-/long-latency somatosensory response; multiunit activity; cardiac arrest

HYPOXIC-ISCHEMIC INJURY (HI) occurs when the brain is deprived of adequate blood flow and oxygen. HI initiates a cascade of molecular and cellular events that impair neuronal function and network properties (Taoufik and Probert 2008), which can lead to severe neurological deficits and multiple organ failures. Common causes of HI include birth asphyxia in neonates and cardiac arrest (CA) in all ages (Busl and Greer 2010; Geraghty and Torbey 2006; Robinson et al. 2003). HI-related neurological complications have been a leading cause of morbidity and disability after CA (Berek et al. 1997; Vaagenes et al. 1996). Research on therapeutic hypothermia shows benefits toward

survival and rehabilitation of neurological function in a population of patients after CA (Chamorro et al. 2010; Holzer 2010). Mild hypothermia in the range of 32–34°C has been shown to effectively improve neurological outcomes in comatose survivors of CA (Bernard et al. 2002; Zeiner et al. 2000). Moreover, immediate administration of hypothermia after injury seems to be important for the hypothermic therapy to be most beneficial (Jia et al. 2008b). Although the neuroprotective effects of hypothermia on hypoxic-ischemic encephalopathy have been well demonstrated (Gunn and Thoresen 2006), the precise mechanism behind these effects remains veiled.

Among the large array of central and peripheral nervous systems affected by CA, the somatosensory pathway is of particular interest to us since 1) HI is preferentially harmful to the primary sensory and motor systems as shown in the pediatric model of HI (Martin et al. 1997) and 2) somatosensory evoked potential (SSEP) monitoring is a well-accepted tool for neurological monitoring and prognosis after CA (Ahmed 1988; Madl et al. 1993; Robinson et al. 2003). The SSEP consists of a series of stereotyped waves reflecting the sequential activation of neural structures along the somatosensory pathways. Short-latency responses (SLRs) in SSEP (e.g., N20 and P25 recorded from human scalp) are directly activated by subcortical input, whereas long-latency responses (LLRs) are generally considered to represent higher cognitive processes involving the cortex (Desmedt et al. 1983; Hillyard and Kutas 1983). Clinically, the short-latency N20 is extensively used as a marker in predicting long-term neurological recoveries after injury (Carter and Butt 2001). Researchers have also looked into long-latency SSEP as a secondary indicator in addition to N20 to improve prognostic accuracy (Zandbergen et al. 2006). However, the underlying neuronal activity that gives rise to the surface-recorded SSEP remains largely unknown.

In this study, a rodent model is used to investigate HI following CA (Geocadin et al. 2008; Jia et al. 2006, 2008b). With this model, our aim is to investigate the vulnerability of somatosensory function as measured by multiunit activity (MUA) and local field potentials (LFP) and the immediate effects of hypothermia on these responses in a rat model of CA-induced HI. We test the hypothesis that the SLR and LLR will show different patterns of evolution in HI, which in turn may suggest different vulnerabilities in their subcortical or cortical generators. Furthermore, we test the hypothesis that hypothermia will have inhomogeneous effects on subcortical and cortical neurons, and thereby provide insights into the possible mechanisms of hypothermic neuroprotection. By de-

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lineating the electrophysiological events in the early recovery stage after CA, we hope to understand selective injury in the cortex and subcortex of the somatosensory pathway, and how hypothermia modulates the injury.

METHODS

Twelve adult male Wistar rats were used in this study and were assigned to normothermia ($n = 6$) or hypothermia ($n = 6$) groups. The experimental protocol was approved by the Animal Care and Use Committee at the Johns Hopkins University School of Medicine. Since we aimed to examine brain function immediately after recovery from CA-induced HI, the experimental procedure only involved acute *in vivo* recording.

Surgery procedures. A 16-channel silicon microelectrode array (CM16, NeuroNexus Technology, Ann Arbor, MI) was used to record multiple neuronal activities. The electrode consisted of four parallel shanks, with four recording sites spread evenly along each shank (Fig. 1, A and B). The recording sites were 200 μm apart longitudinally. With an input impedance of 1 M Ω , we could usually record two to four neurons on each channel.

The microelectrode was implanted in the right primary somatosensory cortex, in the forelimb region (S1FL) (AP, 0; ML, 4), to record MUA and LFP. At the same time, screw electrodes (Plastics One, Roanoke, VA) were implanted epidurally over the left S1FL (AP, -1; ML, -3.8) and the left and right S1 hindlimb regions (AP, -2; ML, ± 1.5) to record SSEPs. A separate screw electrode on the parasagittal frontal lobe (AP, 2; ML, 2) served as the intracranial reference (Fig. 1C).

Rats were fixed on a stereotaxic frame under isoflurane anesthesia (2%). After infusion of local anesthetics at the surgical site, a scalp incision was made above the midline. The skin and underlying tissue layers were dissected to expose the cranium. We used a manual drill to make a burr hole over the right S1FL, which was then expanded to a 1-mm-diameter area with a clip application forceps. Dura was removed to prevent bending of the microelectrode. The microelectrode was adjusted precisely to the target area by carefully tuning the position of the micromanipulator and slowly advanced downward to ~ 1.5 mm from the surface of cortex, where the expected firing pattern was obtained.

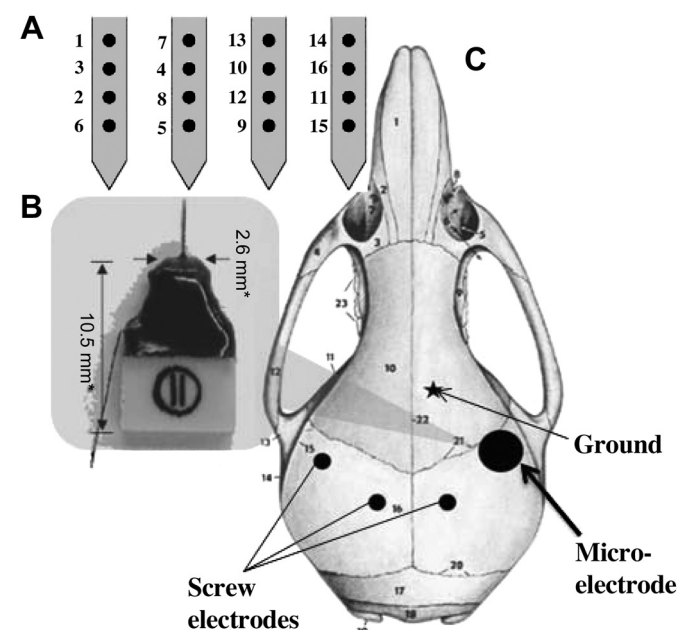


Fig. 1. Schematic of the electrode implantation sites (A), CM16 microelectrode packaging (B), and recording site map (C).

Asphyxia, resuscitation, and hypothermia. Before the surgery, the rat was intubated with a tracheal cannula, mechanically ventilated with 2% isoflurane in a 50%:50% N₂-O₂ gas mixture, and cannulated at the femoral artery and vein to continuously monitor arterial blood pressure (BP), sample arterial blood gas (ABG), and administer fluid and drugs.

After the surgery, a baseline recording was collected under 1.5% isoflurane, followed by a 5-min anesthesia washout. During the final 2 min of anesthesia washout, 2 mg/kg of vecuronium, a muscle paralytic, was administered, and the gas mixture was switched to room air. We stopped the ventilator and clamped the gas supply tube to initiate asphyxial CA; CA was defined to be the time when the pulse pressure was < 10 mmHg. After 7 min of asphyxia, we unclamped the tube and started ventilation with 100% O₂. Cardiopulmonary resuscitation (CPR) was performed with external chest compressions, mechanical ventilation, and boluses of epinephrine and sodium bicarbonate until return of spontaneous circulation (ROSC). BP was monitored via the arterial line, and ABG was checked before and after CA. No anesthetics were applied to the rat after CA, and the animal was allowed to recover spontaneously. The evoked neuronal activities were continuously recorded under a comatose state and without further sedation until there were signs of arousal. After completing the recording during this early recovery period, we sedated the animal and removed all electrodes, uncannulated the artery and vein, and sutured the incision. Afterwards the rat was extubated and observed during recovery from unconsciousness.

Temperature was consistently controlled during the experiment with an automated temperature control system that consisted of a heating pad, a rectal probe, and a monitor (TCAT-2 Temperature Controller, Physitemp). In the normothermia group ($n = 6$) rat temperatures were kept at 36–37°C, whereas in the hypothermia group ($n = 6$) rats were cooled down to a mild hypothermic temperature (32–34°C). Cooling was achieved within 10 min after ROSC by external systemic cooling with a fan and an alcohol-water mist spray, and temperature was maintained within 32–34°C (Jia et al. 2008a, 2008b). After the experiment, the hypothermic rats were gradually warmed up to normal temperature over a period of 60–75 min with an infrared lamp (Thermalet TH-5, Physitemp).

Stimulation and recording. Responses in the somatosensory cortex were evoked by bilateral electrical stimuli to the median nerve with two pairs of stainless steel needle electrodes placed in the distal forelimbs. The stimulation current was controlled through a data acquisition system (TDT System 3, Tucker-David Technologies, Alachua, FL). Stimulation pulses were 200 μs in duration and 6 mA in amplitude, delivered at 0.5-Hz unilateral frequency to the left and right forelimbs in an alternating fashion.

The MUA and LFP signals were sampled at 12.2 kHz. SSEPs were sampled at 6.1 kHz, and ECG and BP were sampled at 324 Hz. Data were recorded continuously with the TDT system for the 10 min prior to CA (baseline), 5 min of anesthesia washout, and 7 min of CA, followed by resuscitation and the recovery period lasting until the rat's arousal.

Data analysis. Low-frequency LFP and high-frequency MUA were recorded from the microelectrode. We used a second-order band-pass Butterworth filter to separate MUA (300–3,000 Hz), LFP (0.5–30 Hz), and high-frequency oscillations (HFO; 80–200 Hz) (Canolty et al. 2006; Ray et al. 2008). Neuronal spike discharges were detected with a threshold set to be two to three times the standard deviation above the mean, depending on the signal-to-noise ratio. LFPs and SSEPs were passed through a notch filter to remove 60-Hz noise.

Peristimulus time histogram (PSTH) (Shimazaki and Shinomoto 2007) was used to visualize the rate and timing of MUA in relation to stimulus (Fig. 2). Within the same neuron population, we defined the response recorded shortly after stimulation (~ 20 ms) as SLR and that recorded later (~ 200 ms) as LLR. The threshold to detect a response in the PSTH was defined as two times the spontaneous firing rate, where the spontaneous firing rate was defined as the averaged firing

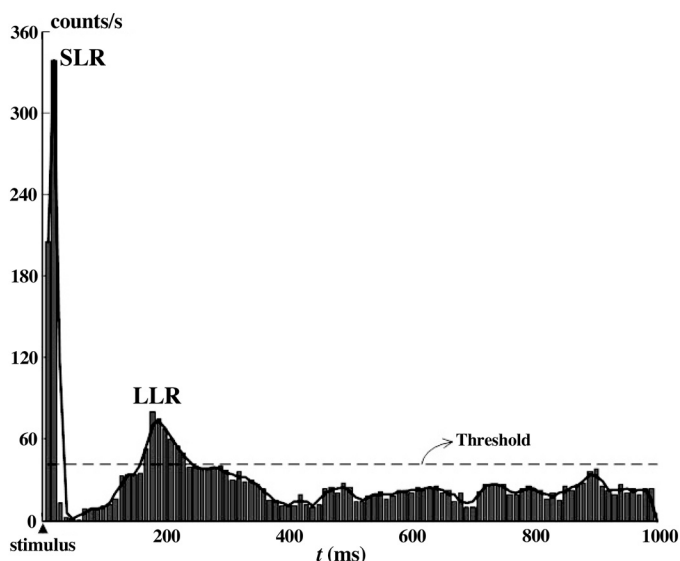


Fig. 2. Peristimulus time histogram (PSTH) of somatosensory neuronal firing. The PSTH includes all spike events from 16 channels and is averaged over 1-min recording (30 trials). The envelope of multiunit activity (eMUA) is outlined on top of the PSTH (bold outline). Short-latency response (SLR) refers to the early peak above threshold, and long-latency response (LLR) refers to the later peak, where the threshold (dashed line) is the firing rate at 2 times the spontaneous firing rate.

rate during the 400- to 1,000-ms interval after stimulus when no known responses would be expected to take place. To obtain the amplitude and latency of the peak response in MUA, we introduced the concept of the envelope of MUA (eMUA) (Choi et al. 2010) so that local maxima were detected as peaks. The eMUA was constructed by 1) assigning spike times to 1-ms bins to obtain a fine-scale PSTH, 2) applying a moving window of 10 ms with a step size of 1 ms to construct a contour of the histogram, and 3) smoothing the contour with a low-pass filter (0.5–30 Hz) to obtain an envelope. The eMUA represents neuronal firing from a local ensemble and enables us to analyze the strength and latency of the collective ensemble response.

RESULTS

Nature of SLR and LLR. The neuronal response to somatosensory stimuli recorded from S1 cortex in our study consists of three components, SLR at ~15 ms after stimulus, a silent period, and LLR around 200 ms after stimulus, as illustrated by the averaged PSTH in Fig. 2. This type of triphasic response not only is reflected in the MUA but can also be seen in LFP and surface-recorded SSEPs. Although we only consider the low-frequency spectra of the LFP, the SLR and LLR are also present in higher-frequency LFPs (80–200 Hz), termed the high-frequency oscillation (HFO) (Kandel and Buzsáki 1997; Pleniz and Kitai 1996; Ray et al. 2008). The HFO presumably reflects cortical processing of incoming sensory information (Curio et al. 1994; Jones et al. 2000) and thalamocortical interactions (Baker et al. 2003a). Plotted in Fig. 3 are the simultaneously recorded multimodality signals after a single stimulus, which correlate well with each other and all show the early and late peaks. SLR is a stronger response and is concentrated within a narrow window, whereas LLR is of lower intensity and is sparsely distributed in time.

The empirical observation of SLR and LLR encouraged us to explore the physiological processes that generate these events. On one hand, as the major somatosensory signals travel

along the spinocortical track and cross at the medulla of the brain stem to the contralateral thalamus and S1 cortex, the SLR and LLR we recorded correspond to the contralateral stimulus. On the other hand, corticocortical interactions take place between hemispheres through the corpus callosum, so we expect to observe some evoked activities from the ipsilateral stimulus as well. Interestingly, we saw LLRs after ipsilateral stimuli but no SLRs (Fig. 4). The contralateral-only presence of SLR suggests that it is the direct output from ascending sensory pathway, whereas the bilateral presence of LLR is an indication that it involves cortical network process between hemispheres, as illustrated in Fig. 4E.

Evolution of SLR and LLR in CA. We investigated the evolution of somatosensory neuronal responses along the experiment timeline at different depths of the cortex using the averaged PSTH. Figure 5 shows the PSTHs aligned at the four depths of a microelectrode array. These are plotted at representative time windows in a typical normothermia CA experiment. Each subplot is the PSTH averaged over 1 min (30 trials) from one channel and then averaged over channels of the same depth. In this recording, the deeper channels showed a slightly larger LLR than the superficial channels. At 2 min after the onset of asphyxia, signals began to weaken. We observed that the LLR disappeared earlier than the SLR, and that deeper channels had a greater decrease in the number of spikes. At 3 min after CA, all channels became isoelectric. There were no neurons firing until ~15 min after CA, which was 8 min after ROSC, when SLR began to emerge. It was not until 20 min after ROSC that LLR appeared, at which time SLR had already increased to baseline level if not higher. At 1 h after CA, SLR remained high, while LLR was slowly increasing. By the end of the experiment, SLR had returned to baseline and LLR had increased to slightly above baseline. A quantitative measurement of SLR and LLR is summarized later in this article.

Effects of hypothermia. We tested whether hypothermia after CA would alter the pattern of somatosensory neuronal response and its evolution in HI. An example of a hypothermia experiment is shown in Fig. 6. While the basic pattern of evolution was similar to that in normothermia, we noticed that under hypothermia LLR reappeared much later and that the ampli-

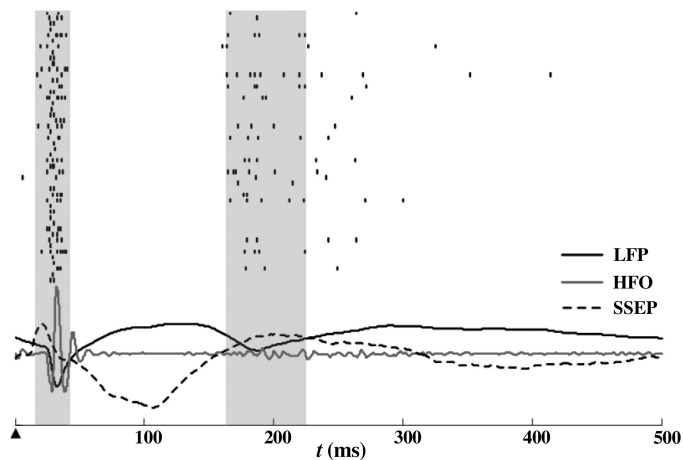


Fig. 3. Sorted single-unit activities of individual neurons (raster plot), local field potential (LFP), high-frequency oscillation (HFO), and somatosensory evoked potential (SSEP) in response to a single stimulus. SLR at 15–20 ms and LLR at ~200 ms after stimulus are present in all signal modalities (shaded areas). Only the first 500 ms of the signal is shown.

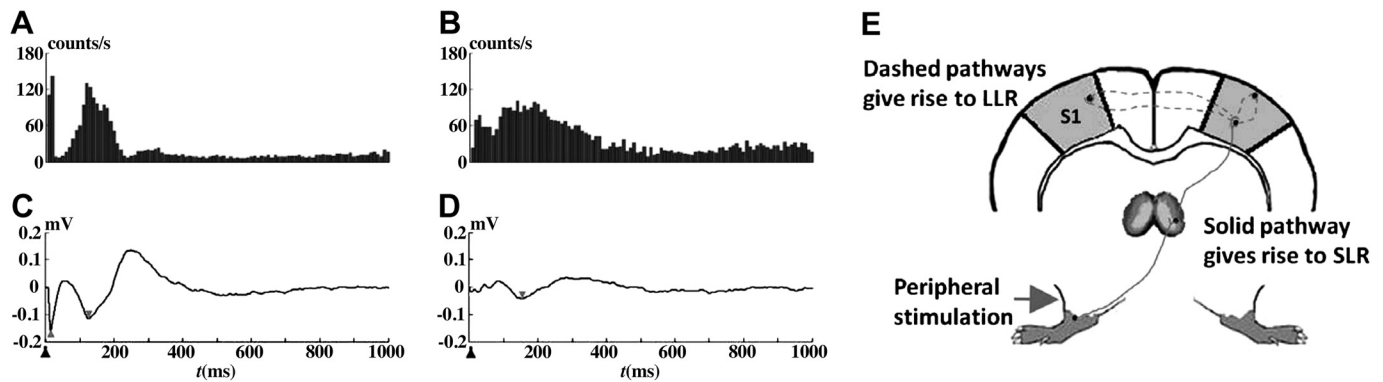


Fig. 4. Somatosensory neuronal responses to contralateral and ipsilateral stimuli at baseline. *A* and *B* are the averaged PSTHs from contralateral and ipsilateral stimuli, respectively, and *C* and *D* are the corresponding LFPs. Signals are averaged over the last minute of baseline (30 trials) and then averaged over the channels. Up-pointing and down-pointing arrowheads indicate SLR and LLR, respectively. *E*: proposed SLR and LLR pathways.

tude of SLR kept increasing and exceeded the baseline toward the end of experiment.

Table 1 summarizes the times for each rat when SLR and LLR disappeared and reappeared after the onset of asphyxia. There were no differences between the normothermia and hypothermia groups in the time periods when SLR (hypo: 3.17 ± 0.31 min, normo: 3.17 ± 0.31 min) and LLR (hypo: 2.17 ± 0.17 min, normo: 2.00 ± 0.26 min) disappeared. There was a slight difference in the time periods when SLR reappeared (hypo: 18.17 ± 1.08 min, normo: 15.38 ± 0.87 min; $P = 0.171$, Wilcoxon rank-sum test) but significant differences in the time periods when LLR reappeared (hypo: 38.83 ± 5.86 min, normo: 23.33 ± 1.15 min; $P = 0.0303$, Wilcoxon rank-sum test). We concluded that hypothermia delayed the time when the somatosensory response began to recover, and that the delay in LLR was more evident than that in SLR. In the end, the hypothermia rats aroused from coma slightly earlier than the normothermia rats (hypo: 60.00 ± 11.83 min, normo: 65.17 ± 14.38 min).

Amplitude and latency of the responses also carried important information about hypothermia effects, but before we compare the difference after injury, it would be necessary to

know the responses under a controlled, noninjured condition. We performed a noninjury hypothermia experiment with temperature variation from 36 to 31°C. We observed that the amplitudes of both SLR and LLR did not show significant change at different temperatures but their latencies increased as the temperature lowered. Generally, it is known that hypothermia delays somatosensory response, but the response amplitude has poor correlation with low temperature (Kottenberg-Assemacher et al. 2003; Markand et al. 1990).

Next, we showed the amplitudes and latencies of SLR and LLR between normal and hypothermia groups after injury (Fig. 7). All values were obtained by dividing real values by the corresponding baseline values. In normothermia SLR amplitude rapidly increased, overshoot slightly, and stabilized around 40 min after CA, whereas in hypothermia rats SLR amplitude increased linearly during recovery, eventually reaching as high as twice the baseline amplitude (Fig. 7A). Latency of SLR in both hypothermia and normothermia groups was significantly increased (Fig. 7B) because of slowed peripheral nerve conduction and central synaptic transmission (de Haan and Kalkman 2001). The difference in LLR was more prominent. In normothermia LLR amplitude approximated baseline level at

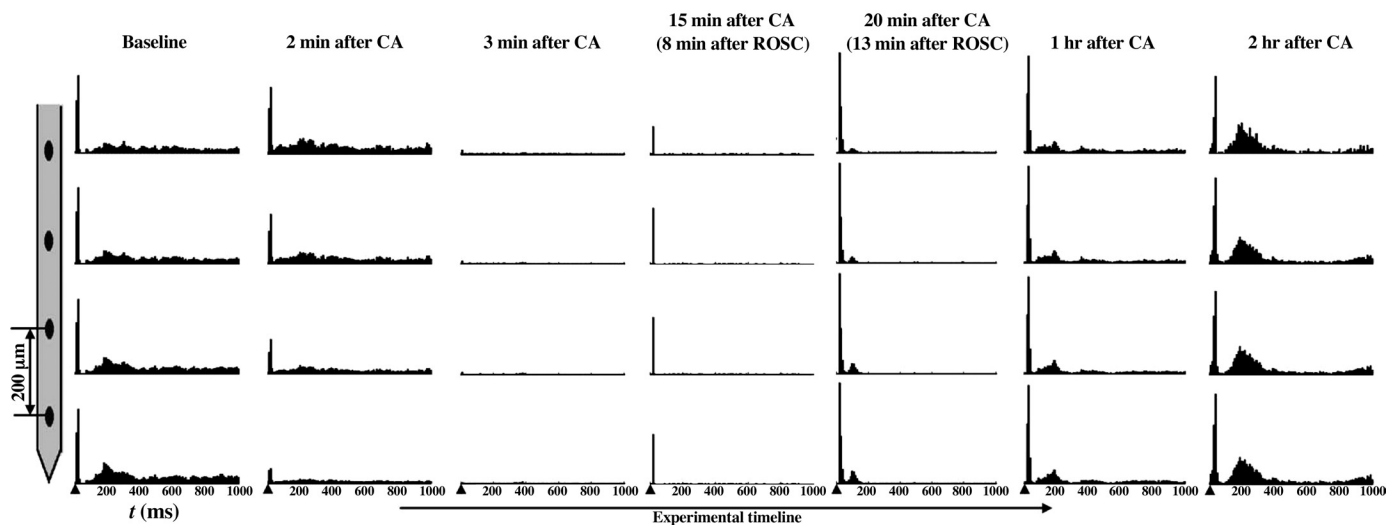


Fig. 5. Evolution of somatosensory neuronal responses at baseline, and 2, 3, 15, and 20 min and 1 and 2 h after cardiac arrest (CA) in a normothermia rat. MUA are presented in the PSTH with 10-ms bins. PSTHs at the 4 depths of the microelectrode array are shown, which are temporally averaged over every 1 min (30 trials) and spatially averaged across the same depth. Note that the LLR disappears earlier than the SLR after onset of CA, and it returns later than the SLR. Signals in deeper channels are suppressed by a greater amount than those in superficial channels upon injury. ROSC, return of spontaneous circulation.

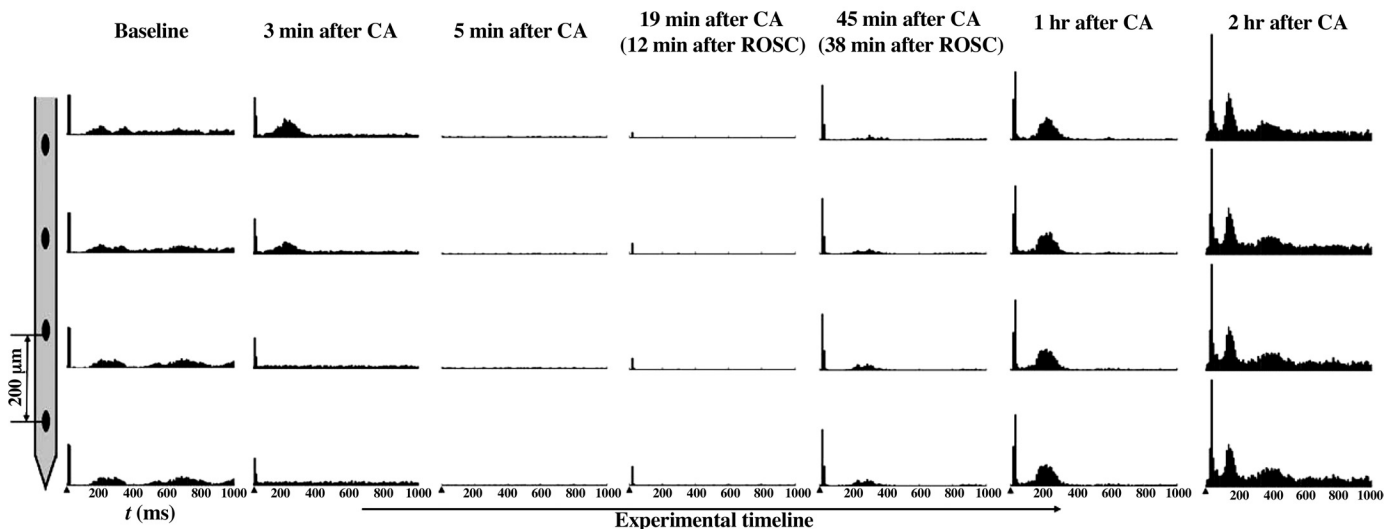


Fig. 6. Evolution of somatosensory neuronal responses at baseline, and 3, 5, 19, and 45 min and 1 and 2 h after CA in a hypothermia rat. MUA are presented in PSTHs with 10-ms bins. PSTHs at 4 depths of the microelectrode are shown as temporally averaged over every 1 min (30 trials) and spatially averaged across the same depth.

the time it reappeared, whereas in hypothermia LLR amplitude remained very low throughout recovery (Fig. 7C) and its latency was increased by a much higher extent compared with that in normothermia (Fig. 7D). There was higher across-subject variation in LLR measures, especially in the hypothermia subjects—not all of them could be stably detected after HI. Data are shown for the first 60 min after CA, in which the most dramatic changes took place.

DISCUSSION

Our overall goal was to study cortical neural activities in the context of HI and hypothermic treatment. The somatosensory system was investigated in view of its evident vulnerability to global ischemic injury and its clinical importance to evaluation of neurological functions after injury. In particular, SSEP recording from the scalp has become a routine procedure for neuromonitoring, and hence understanding the neural correlates of the SSEP signals takes on clinical significance. Our experiments confirmed a tight correlation between the macro-

scopic SSEP and microscopic neural firing (Fig. 2). We demonstrated for the first time in this global HI model that the surface noninvasive SSEPs well approximated the underlying cortical signals at the neuronal level.

In this study, we observed a stereotypical pattern of somatosensory responses to peripheral stimulus, namely, SLR and LLR. It has also been found that whisker pad-induced response in the barrel cortex consists of a short-latency excitation around 5 ms followed by a postexcitatory inhibition and a long-latency excitation around 100 ms (Drouin et al. 2006). Considering the shorter trigeminal pathway, this finding is likely comparable to our observation in the somatosensory pathway. While the SLR is most probably a direct electrical activation from afferent fibers to the cortex via the thalamus, the origin of LLR merits further exploration. In humans, the long-latency peak P300 is commonly recorded in visual, auditory, and somatosensory evoked potentials and is thought to be involved in cognition (Desmedt et al. 1983; Hillyard and Kutas 1983). The LLR at 200 ms in rats could be an equivalent to P300 in humans,

Table 1. Times when SLR and LLR disappear or return after CA

Treatment	SLR Disappearance	LLR Disappearance	SLR Reappearance	LLR Reappearance	Sign of Arousal
Hypothermia (32–34°C)	3	2	16	59	65
	3	2	23	45	55
	4	3	18	50	115
	3	2	17	29	45
	2	2	19	22	55
	4	2	16	28	60
	Mean	3.17	2.17	18.17	38.83
Normothermia (36–37°C)	3	2	13	21	115
	4	2	16	24	41
	2	1	16	20	75
	3	2	14	28	95
	4	3	17	24	35
	3	2	19	23	30
Mean	3.17	2.00	15.83	23.33	65.17
P value	1	1	0.171	0.0303*	1

Values are in minutes after onset of asphyxia. SLR, short-latency response; LLR, long-latency response; CA, cardiac arrest. * $P < 0.05$, Wilcoxon rank-sum test.

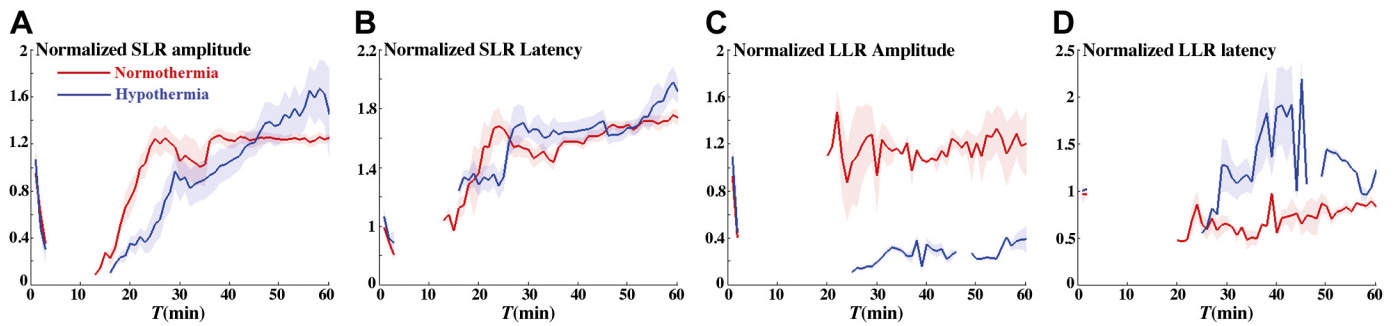


Fig. 7. Amplitude (A) and latency (B) of the SLR and amplitude (C) and latency (D) of the LLR in the normothermia ($n = 6$) and hypothermia ($n = 6$) groups in the first hour after CA. Amplitudes and latencies are normalized to baseline values for each rat, and then mean values (lines) and standard deviations (shaded regions) are calculated within group.

which may reflect information processing in the corticocortical network. Our finding that SLR exists only in the contralateral response while LLR exists both in contralateral and ipsilateral responses (Fig. 4) strongly supports the cortical network hypothesis, in which the stimulus primarily evokes the contralateral cortex and then propagates across a broader cortical network.

It was seen in both normothermia and hypothermia cases (Fig. 5 and Fig. 6) that LLR disappeared earlier than SLR after the onset of CA insult. The average time difference was 1 min (Table 1), and the recovery took place in reverse order. The SLR began to appear very shortly after ROSC (~ 10 min), whereas the reappearance of LLR was significantly delayed ($P < 0.05$). It was evident that the LLR was more vulnerable than SLR, suggesting a greater susceptibility to HI in the cortical network than in subcortical structures. Our earlier work with SSEP measures showed that the N7, representing thalamic activity, recovered much earlier than the cortical response (Xiong et al. 2010). Evidence from histological studies also suggests preferential necrosis in the cerebral cortex, whereas the brain stem and thalamus are minimally affected in global ischemia (Sieber et al. 1995). Therefore, we speculate that basic neural functions in the subcortical regions may be better preserved in HI compared with higher-level cognitive function in the cortex.

Quantitative analysis revealed that the electrophysiological properties of somatosensory neurons after CA are different in normothermia and hypothermia. First, the LLR was substantially reduced and delayed in hypothermia. As it is likely that LLR relates to cortical network activity, the reduced LLR indicates that hypothermia downregulates cortical metabolism (Gunn and Thoresen 2006) as one of the potential strategies of hypothermic neuroprotection (Polderman 2009). Second, hypothermia potentiates the amplitude of SLR after injury. The mechanism for hypothermic potentiation remains unclear, but it has been reported that cooling amplified the size of action potentials in peripheral nerves (Ritchie and Straub 1956), and thus increased the input signal. It is also possible that hypothermia diminishes activities of cortical inhibitory neurons such as the thalamic reticular neurons (Greer 2006) to produce a hyperexcitable state (Ferron et al. 2009). Finally, we want to point out that temperature gradients in the brain due to surface cooling (Iwata et al. 2006; Laptok et al. 2001) could be one of the causes for the greater hypothermic effect in the cortex than subcortical regions. We decided to undertake surface cooling because it has the widest use and support in the clinical

community (Bernard et al. 2002). To evaluate this influence, we could potentially adopt other cooling methods (Hoedemaekers et al. 2007) in the future, e.g., pharyngeal cooling that absorbs heat from cerebral blood flow through a heat exchanger placed at the pharynx to achieve a rapid and uniform cooling within the brain without affecting the core body (Trubel et al. 2004).

While this study has focused on the immediate effects of hypothermia, we are aware that hypothermia needs to be maintained longer in order to achieve a significant therapeutic benefit (Oddo et al. 2006; Peberdy et al. 2010). Potentially, a chronic recording system could be used to monitor the long-term effect of hypothermia on free-moving animals after arousal from coma, along with behavioral tests to examine the neurological outcome. Future work would also involve graded hypothermia studies. It would be informative to show neuron activity under gradient hypothermia and relate it to neurological outcome, as clinically it is shown that moderate ($30\text{--}32^\circ\text{C}$) and mild ($32\text{--}34^\circ\text{C}$) hypothermia is neurologically beneficial, with increasing benefits as the temperature lowers (Wass et al. 1995; Xia and Xia 2011), but deep hypothermia ($27\text{--}30^\circ\text{C}$) is detrimental (Steen et al. 1979). We also would like to link the electrophysiological changes to the metabolic changes as well as altered neurochemical activity, for example, glutamate excitotoxicity. This may entail studies with positron emission tomography (PET) with suitable probes. On the other hand, to better locate the functional activity in the large areas of the brain in this global ischemic model, we may use functional MRI (fMRI) studies to verify the differential functional responses in the cortical and subcortical regions of the brain.

Conclusions. Hypothermia selectively suppresses LLR by delaying its reappearance after CA, increasing the latency and decreasing the amplitude. Evidence indicates that LLR reflects cortical network process, whereas SLR reflects subcortical signal delivery. Therefore, this study provides *in vivo* electrophysiological evidence for the differential vulnerability in the cortex and subcortex during normal and hypothermic interventions. We propose that cortical network functions are preferentially disrupted in HI and that hypothermia protects the brain by selectively downregulating corticocortical activity.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: D.W., W.X., X.J., R.G., and N.V.T. conception and design of research; D.W., W.X., and X.J. performed experiments; D.W. and W.X. analyzed data; D.W., W.X., R.G., and N.V.T. interpreted results of experiments; D.W. and W.X. prepared figures; D.W., W.X., R.G., and N.V.T. drafted manuscript; D.W., W.X., X.J., R.G., and N.V.T. edited and revised manuscript; D.W., W.X., X.J., R.G., and N.V.T. approved final version of manuscript.

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