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Effect of Hypothermia on Cortical and Thalamic Signals in Anesthetized Rats

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***Abstract*— Beneficial effects of hypothermia on subjects with neuro-pathologies have been well demonstrated in both animal studies and clinical trials. Although it is known that temperature significantly impacts neurological injuries, the underlying mechanism remains unclear. We studied the effect of temperature modulation on neural signals in the cortex and the thalamus in uninjured brains of anesthetized rats. Six rats were divided into a hypothermic (32 to 34 ºC, n=3) and a hyperthermic group (38.5 to 39.5 ºC, n=3). EEG, and extracellular signals from somatosensory cortex and the ventral posterolateral nucleus of thalamus were recorded at different temperature phases (normothermia (36.5 to 37.5 ºC) and hypothermia or hyperthermia). During hypothermia, similar burst suppression (BS) patterns were observed in cortical and thalamic signals as in EEG, but thalamic activity was not completely under suppression when both EEG and cortical signals were electrically silent. In addition, our results showed that hypothermia significantly increased the burst suppression ratio (BSR) in EEG, cortical and thalamic signals by 3.42, 3.25, 7.29 times respectively (P<0.01), and prolonged the latency of neuronal response in cortex to median nerve stimulation from 9 ms to 16 ms (P<0.01). Furthermore, during normothermia, the correlation coefficient between thalamic and cortical signals was 0.35±0.02 while during hypothermia, it decreased to 0.16±0.03 with statistical significance (P<0.01). These results can potentially assist in better understanding the effects of hypothermia.**

I. Introduction

Animal studies [1–3] and clinical trials [4, 5] have demonstrated the therapeutic benefits of hypothermia for survival and functional outcomes after brain injury. Hypothermia has been suggested as a method of neuroprotection in the context of many neuro-pathologies such as hypoxic ischemia, stroke, brain trauma and spinal cord injury [6, 7]. Hyperthermia has been shown to increase neural injury and worsen neurological outcomes [1, 8]. Although the beneficial effect of hypothermia has been well demonstrated and hypothermia is becoming a therapeutic intervention after brain injury, the underlying mechanism has not been well understood and the effect of temperature on uninjured brain remains unclear. Therefore, a study of an uninjured brain's response to temperature changes is necessary.

Electrophysiological signals are the reflection of neural activity. Therefore, recordings of such signals can provide a direct measure of the neural response to temperature modulation. Previously, we have quantitatively analyzed the changes in EEG and somatosensory evoked potentials (SEP) with three different temperature phases: normothermia, hypothermia and hyperthermia [7]. In this study, we aim to measure how temperature affects the neural activity in the somatosensory pathway. Our focus is to further investigate the effect of temperature as measured by multiunit activity (MUA) and local field potentials (LFPs) in the cortex and the thalamus.

Here, we measure the effect of temperature on uninjured brain in anesthetized rats. Specifically, we simultaneously recorded extracellular signals from the somatosensory forelimb cortex (S1FL) and the ventral posterolateral (VPL) nucleus of thalamus in a hypothermia (32 to 34 ºC) group and a hyperthermia (38.5 to 39.5 ºC) group of rats. These S1FL and VPL were specifically chosen because of the anatomic projections exiting between them [9]. We then quantify the MUA and LFPs to determine the electrophysiological changes in the uninjured brain with temperature modulation.

II. Methods

Six Wistar rats were used in this study and were assigned to the hypothermia (n=3) or the hyperthermia (n=3) group. The experiment protocols were carried out with the approval of the Johns Hopkins Animal Care and Use Committee.

A. Experimental Procedures

Rats were fixed on a stereotactic frame under a controlled flow of isoflurane anesthesia (2.5%) in 1:1 N_2/O_2 via a tight-fitting facemask and were placed on a heating pad (TCAT-2 Temp Controller, Physitemp, Clifton, NJ) immediately to keep the temperature within normothermic range (36.5 to 37.5 ºC) [7]. After infusion of local anesthetics, a scalp incision was made to expose the cranium. Two screw electrodes (Plastics One, Roanoke, VA) were implanted epidurally over the left S1FL (AP: -1; ML: -3.8) and S1 hindlimb (S1HL) regions (AP: -2; ML: -1.5) to record EEG; a separate screw electrode on the parasagittal frontal lobe (AP: 2; ML: 2) served as the intracranial reference. A Thermocouple Probe (IT-24P, Physitemp, Clifton, NJ) for brain temperature monitoring was inserted 1-2 mm into the brain through a small hole with a diameter of 0.5 mm above the left VPL (AP: -3.48; ML: -3.2). We made the size of the hole as small as possible to reduce the exposure of the brain

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to increase the accuracy of brain temperature measurement. Afterwards, a craniotomy was performed over the right hemisphere, above the areas of S1FL (AP: 1; ML: 4) and VPL (AP: -3.48; ML: 3.2), exposing two portions of the brain with diameters of 3 mm. The dura was carefully removed to prevent bending of the microelectrode. Two pairs of two channel electrodes (1-2 MΩ, Microprobe, Gaithersburg, MD) were then slowly advanced into layer IV of S1FL (800 to 1000 μm from surface of the cortex) and the VPL nucleus of the thalamus (5900 to 6500 μm from surface of the cortex), where immediate evoked firing was observed when the rats' forelimbs were stimulated with soft touch. The technique of electrophysiological recording was described previously [10–12].

Before the baseline recording of electrophysiological signals, anesthesia was lowered to 1.5 % and maintained throughout the entire experiment to maintain an acceptable level of consciousness and cardiopulmonary stability [7]. After the rats stabilized at normothermic temperature (36.5 to 37.5 °C) for 30 mins, spontaneous baseline signals were recorded for 10 mins. Over an additional 10 mins, we recorded evoked activity from median nerve stimulation (two pairs of stainless steel needle electrodes placed in the distal forelimbs, stimulation pulses were 200 μs in duration, 6 mA in amplitude and 0.5 Hz in frequency.). Afterwards, the rats were either cooled down to the hypothermic range (32 to 34 °C) [1, 2] by external cooling using a fan and alcohol-water mist spray or warmed up to the hyperthermic range (38.5 to 39.5 °C) using an infrared lamp (HL-1, Physitemp, Clifton, NJ) [7]. The transition to hypothermia or hyperthermia was achieved within 10 mins and 20 mins respectively. After stabilization at hypothermia or hyperthermia for 30 mins, signals were recorded for 20 mins as described above. Finally, rats were transitioned to normothermic range. Brain temperature was recorded every 2 mins. The rats' blood pressure and ECG were continuously monitored non-invasively (Kent Scientific, Torrington, CT) throughout the experiment.

B. Data Analysis

Two channels of EEG were recorded at a sampling rate of 1220 Hz. A band-pass filter (0.5 to 150 Hz) was used to remove high frequency noise. Extracellular signals were recorded from the cortex and the thalamus at a sampling rate of 24414 Hz using two pairs of two-channel microelectrodes. LFPs and MUA were separated from the raw signals using two band-pass filters (0.5 to 150 Hz and 300 to 3000 Hz). 60 Hz noise was removed using a notch filter from all signals.

We adopted a common time-domain parameter, burst-suppression ratio (BSR), to quantitatively measure the change in the burst-suppression (BS) patterns with temperature modulation. BSR was defined as the percentage of suppression period in a segment of signals [13]. A suppression period was detected if the absolute amplitude of signals was below the standard deviation of baseline signals, for a period longer than 0.5 seconds. BSR was calculated over every one-minute interval without overlap and averaged over the duration of recordings and channels.

The peristimulus time histogram (PSTH) is a powerful tool to analyze the response of neurons to an external stimulus or event. From the PSTH, we extract the rate and timing of neuronal firing in relation to a periodic stimulus. To get the PSTH, we assigned spike times to 1-ms bins. The concept of the envelope of MUA (eMUA) [14] was introduced previously to determine the latency of the peak response in MUA [10]. To get the envelope, we used a low-pass filter at a cutoff frequency of 150 Hz to smooth the contour of the PSTH. Latency of neuronal response was then defined as the time when eMUA reached its maximum value within 40 milliseconds after stimulation.

Previously, Pearson's correlation coefficient was used to calculate the coherence between thalamic and cortical signals during arousal from coma after hypoxic-ischemic brain injury [11]. Here, we computed the correlation coefficient between cortical and thalamic LFPs to determine how temperature affects thalamocortical coherence. The correlation coefficient between two variables X and Y, is defined as

$$\rho_{X,Y} = \frac{\mathrm{cov}(X,Y)}{\sigma_X \sigma_Y} = \frac{E((X-\mu_X)(Y-\mu_Y))}{\sigma_X \sigma_Y}$$

where μ_X and μ_Y, σ_X and σ_Y are the expected values and the standard deviations of X and Y.

III. Results

Brain temperature was well controlled for all 6 rats and the three target temperature phases, normothermia (36.5 to 37.5 °C), hypothermia (32 to 34 °C) and hyperthermia (38.5 to 39.5 °C) were successfully achieved and maintained as shown in Fig. 1.

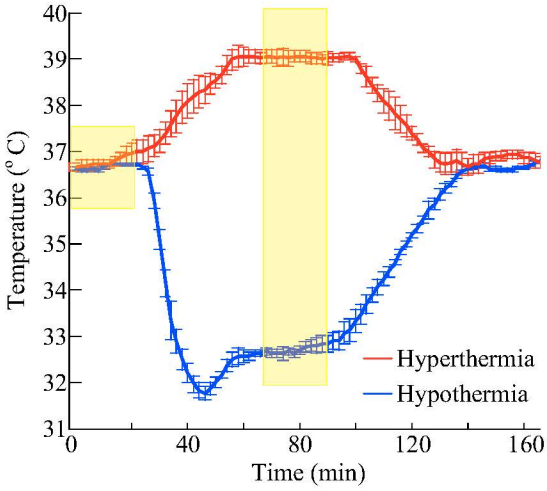

Fig. 1. The brain temperature recordings (Mean±SEM) of the hypothermic (blue) and hyperthermic (red) groups of rats. The period of stabilization before the recoding during normothermia was not shown in this figure. The highlighted areas (yellow square) represent the recording periods at different temperature phases. The temperature was well controlled and the three different temperature phases, normothermia (36.5 to 37.5 °C), hypothermia (32 to 34 °C) and hyperthermia (38.5 to 39.5 °C), were successfully achieved.

The BS pattern in EEG has been commonly observed and thoroughly investigated during arousal from coma after brain injury [8, 15] as well as in deep anesthesia [16]. In this study, we observed not only the BS pattern in EEG, but also a similar pattern in cortical and thalamic signals (Fig. 2). The burst sequences, which consists of high voltage slow waves

intermingled with sharp waves, showed very high synchronization among EEG, cortical and thalamic signals. However, during the silent periods of EEG, similar and synchronous periods of depressed background activity in cortical signals and not thalamic signals, can be recognized (Fig. 2). In addition, we noticed that the degree of BS was correlated with temperature. In Fig. 3, it can be seen that BSR in all three types of signals were greatly increased by hypothermia while they were decreased by hyperthermia. During normothermia, BSR was less than 20 % in both EEG and cortical signals and less than 10 % in thalamic signals. Hypothermia increased the BSR in EEG, cortical and thalamic signals to 76±6% (Mean±SEM), 77±6% and 67±6% respectively (P<0.01). During hyperthermia, BSR were close to zero in EEG and thalamic signals and around 10 % in cortical signals.

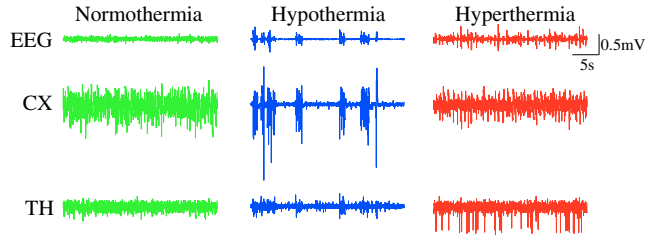

Fig. 2. Example traces of raw EEG, cortical and thalamic signals recorded from two anesthetized rats (one from the hypothermic group and the other from the hyperthermic group) during normothermia (green), hypothermia (blue) and hyperthermia (red). Note that the burst sequences, which consists of high high-amplitude activity, showed very high synchronization among EEG, cortical and thalamic signals. When both EEG and cortical signals were under electrical silence, however, thalamic activity was not completely under suppression. In addition, hypothermia led to a higher degree of burst suppression (BS).

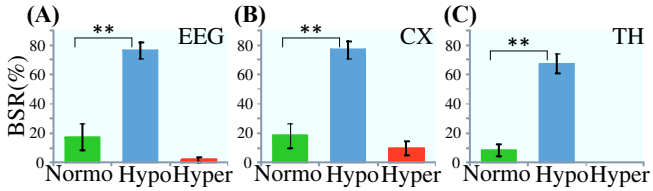

Fig. 3. Burst suppression ratio (BSR) (Mean±SEM) in EEG (A), cortical (B) and thalamic (C) signals for hypothermic (n=3) and hyperthermic (n=3) group of rats. Note that compared with normothermia, BSR significantly increased during hypothermia (**, P<0.01, t test) while decreased during hyperthermia.

We have previously shown the effect of temperature modulation on the latency of SEP [7]. Here, we used PSTH to calculate the latency of the response in cortical and thalamic neurons to peripheral stimulation. An example of the PSTH of cortical and thalamic MUA in three different temperature phases was plotted in Fig. 4. The shape of PSTHs for cortical and thalamic MUA revealed that there was an immediate response in MUA to the stimulation. The shift of the PSTHs showed that the latency, which is the time when PSTHs reached the maximum values, changed with temperature. In Fig. 5, it can be clearly seen that during normothermia, the latency of the response was 9±1 ms (Mean±SEM) in cortical MUA and 5±0 ms in thalamic MUA, while during hypothermia, the latency of the response in cortical and thalamic MUA was delayed to 16±1 ms (P<0.01) and 6±1 ms respectively. Compared with latency during normothermia, the latency of the response in cortical MUA slightly decreased and there was almost no change in the latency of thalamic MUA during hyperthermia.

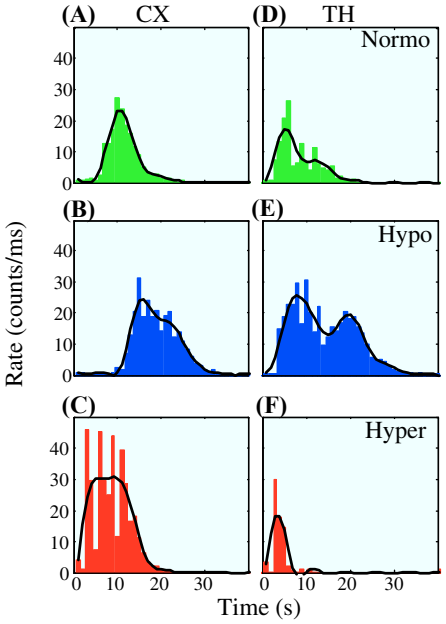

Fig. 4. Peristimulus time histograms (PSTHs) of neuronal firing in cortex (A-C) and thalamus (E-F) during normothermia (green), hypothermia (blue) and hyperthermia (red). Each PSTH included spike events over every 1-min recording, and was averaged over the entire 10-min recording and 2 channels. The envelope of multiunit activity (eMUA) is outlined on top of the PSTH (black outline). The shift of shape of PSTH along time line with temperature phases showed that the latency of neuronal response in cortex and thalamus to peripheral stimulation changed with temperature modulation.

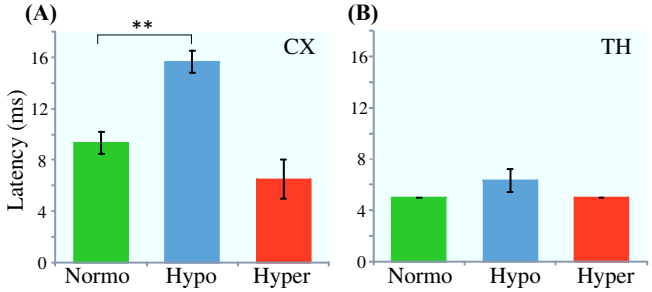

Fig. 5. Latency of neuronal responses (Mean±SEM) in cortex (A) and thalamus (B) to peripheral stimulations during three different temperature phases. The latency was significantly delayed during hypothermia in cortex (**, P<0.01, t test).

The effect of temperature on coherence between brain regions was reflected in the correlation coefficient. Hypothermia was characterized by an increased correlation coefficient between two channels of EEG (0.53±0.05 (Mean±SEM) during normothermia versus 0.75±0.05 during hypothermia, P<0.05), and a decreased correlation coefficient between thalamic and cortical signals (0.35±0.02 (Mean±SEM) during normothermia versus 0.16±0.03 during hypothermia, P<0.01). There is no significant change in correlation coefficient between EEG and cortical signals with temperature modulation.

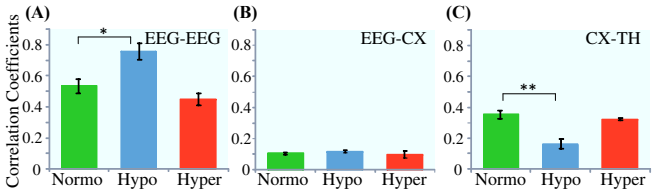

Fig. 6. The correlation coefficient (Mean±SEM) between two channels of EEG (A), EEG and cortical signals (B), and thalamic and cortical signals (C) during normothermia, hypothermia and hyperthermia. Note that compared with normothermia, the correlation coefficient between two channels of EEG and between thalamic and cortical signals significantly decreased during hypothermia (*, P<0.05; **, P<0.01, t test).

IV. Discussion

This study demonstrated the effects of hypothermia (32 to 34 ℃) and hyperthermia (38.5 to 39.5 ℃) on EEG, cortical and thalamic signals in uninjured brains of anesthetized rats. Each rat underwent hypothermia or hyperthermia instead of going through hypothermia and hyperthermia sequentially [7] so as to avoid a residual effect from temperature.

We observed similar BS patterns in cortical and thalamic signals as in EEG, Nonetheless, neuronal activity was not completely suppressed in the thalamus when EEG and cortical signals were electrically silent. This might be an indication that the cortex is disconnected from the input of the thalamus during silent epochs. Steriade et al. [17] showed that almost all (95%) cortical neurons were under electrical silence while 30-40 % of thalamic continued firing during flat EEG epochs. Hypothermia increased BSR in cortical and thalamic signals by about 3 and 7 times respectively, which might result in a higher degree of disconnection between the thalamus and the cortex and lead to a decrease in correlation coefficient between thalamic and cortical signals. The observed increase in correlation coefficient between two channels of EEG may result from hypothermic potentiation of isoflurane-induced EEG synchronization. Another possible reason could be that hypothermia increases the synchronization of EEG. In addition, prolonged latencies of neural responses in the thalamus and the cortex to stimulation were consistent with our previous findings on SEP under hypothermia [7].

Our study showed that temperature has a significant effect on both cortical and thalamic signals as well as on EEG while having a slight regional difference. As we adopt the intervention of hypothermia as a treatment for injured brains, it is important that we completely understand the response of the uninjured brain to temperature modulation. More experiments will be needed to further validate these results. Our results could potentially help determine critical parameters for hypothermia delivery, such as the rate of cooling, the depth of temperature change and the optimal time and duration of treatment delivery.

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