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Improving neurological outcomes post-cardiac arrest in a rat model: immediate hypothermia and quantitative EEG monitoring

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Summary

Objectives—Therapeutic hypothermia (TH) after cardiac arrest (CA) improves outcomes in a fraction of patients. To enhance the administration of TH, we studied brain electrophysiological monitoring in determining the benefit of early initiation of TH compared to conventional administration in a rat model.

Methods—Using an asphyxial CA model, we compared the benefit of immediate hypothermia (IH, T=33°C, immediately post-resuscitation, maintained 6 hours) to conventional hypothermia (CH, T=33°C, starting 1 hour post-resuscitation, maintained 12 hours) via surface cooling. We tracked quantitative EEG using relative entropy (qEEG) with outcome verification by serial Neurological Deficit Score (NDS) and quantitative brain histopathological damage scoring (HDS). Thirty-two rats were divided into 4 groups based on CH/IH and 7/9-minute duration of asphyxial CA. Four sham rats were included for evaluation of the effect of hypothermia on qEEG.

Results—The 72-hour NDS of the IH group was significantly better than the CH group for both 7-minute (74/63; Median, IH/CH, $p<0.001$) and 9-minute (54/47, $p=0.022$) groups. qEEG showed greater recovery with IH ($p<0.001$) and significantly less neuronal cortical injury by HDS (IH: 18.9 ±2.5% versus CH: 33.2±4.4%, $p=0.006$). The 1-hour post-resuscitation qEEG correlated well with 72-hour NDS ($p<0.05$) and 72-hour behavioral subgroup of NDS ($p<0.01$). No differences in qEEG were noted in the sham group.

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Conflict of interest statement There are no conflicts of interest in this study.

Conclusions—Immediate but shorter hypothermia compared to CH leads to better functional outcome in rats after 7- and 9- minute CA. The beneficial effect of IH was readily detected by neuro-electrophysiological monitoring and histological changes supported the value of this observation.

Keywords

Cardiac arrest; Electroencephalography; Hypothermia; Functional outcome; Brain ischemia

Introduction

Approximately 400,000 out-of-hospital cardiac arrests (CA) occur in the United States each year[1]. Despite advances in cardiopulmonary resuscitation (CPR) and critical care, overall survival from out-of-hospital CA remains poor, averaging only 5–8% in most centers[2]. Among survivors, neurological complications represent the leading cause of morbidity and disability[3, 4].

Two recent clinical trials demonstrated that induced hypothermia to 32–34°C improves survival and functional outcomes in comatose survivors of CA5[5,6]. While a breakthrough treatment for brain injury after cardiac arrest, its full beneficial therapeutic effect has not yet been fully realized. A meta-analysis showed that the number-needed-to-treat to allow one additional patient to leave the hospital with favorable neurological recovery was 4–13[7]. The optimal initiation time, duration of therapy, and depth of hypothermia have not been defined. Early initiation of cooling has been suggested but it appears to be successful even if delayed by 4–6 hours[8]. Most clinical studies delay the initiation of hypothermia by 2 or more hours after resuscitation[5, 6, 9, 10].

Recent animal studies have shown that mild to moderate hypothermia (33–34°C) mitigates brain injury when induced before[11], during[11, 12], or after resuscitation[12–14]. We demonstrated previously that initiation of hypothermia 1 hour after CA significantly improved neurological outcomes compared to normothermic controls[15, 16]. Despite the use of hypothermia to ameliorate brain injury, no direct monitoring is undertaken to assess the brain's response to therapy. We have previously validated quantitative EEG (qEEG) monitoring in a rodent model after resuscitation from CA [17, 18]. This entropy-based qEEG provides real-time, objective tracking of neurological injury, recovery and early prognostication after CA[17–23]. This method is also sensitive to temperature-related modulation of brain injury, with rapid and sustained improvement of qEEG measures in those animals treated with hypothermia compared to normothermic controls[15, 16, 24].

In this study, we tested the hypothesis that neuromonitoring techniques track brain recovery and response to brain-directed therapy in real time. Clinical translation of these techniques may optimize the delivery of hypothermia to a neuro-electrophysiological endpoint. We examined the impact of immediate initiation of 6-hour hypothermia (IH) compared to 12-hour conventional hypothermia (CH) initiated at 1 hour post-resuscitation with real-time qEEG tracking followed by functional outcome assessment and histological assessment of the brain cortical injury.

Material and Methods

The experimental protocol was approved by the Johns Hopkins Animal Care and Use Committee and all procedures were compliant with NIH guidelines.

Experimental asphyxia-CA model

Thirty-six adult male Wistar rats (360±20g) were assigned at random to 4 groups based on CH/IH and 7/9-minute duration of asphyxial CA (7CH, 7IH, 9CH, 9IH, n=8). Four rats were included as a sham control group for evaluation of the effect of hypothermia on qEEG in the absence of CA injury.

Our group and others have used this rodent model to study calibrated brain injury after asphyxial CA [15, 18, 25]. In brief (detail described by Jia[15]), rats were mechanically ventilated with 1.0% halothane in N_2/O_2 (50%/50%). Five minutes of baseline recording was followed by 5-minute washout to ensure no significant residual effect of halothane on qEEG[17]. CA was initiated via asphyxia with cessation of mechanical ventilation for periods of 7 or 9 minutes. Cardiopulmonary resuscitation (CPR) was performed with sternal chest compressions (200/min) until return of spontaneous circulation (ROSC). Sedative and anesthetic agents were avoided to minimize confounding effects on EEG[26].

CH was induced 1 hour after ROSC by surface cooling with cold mist to achieve the target core temperature of 33°C monitored by an intraperitoneal temperature sensor within 15 minutes and was maintained between 32–34°C for 12 hours[15]. For the IH groups, cooling was initiated immediately (within 15 minutes) after ROSC and was maintained at 32–34°C for 6 hours. The 6-hour duration was chosen based on a pilot group of rats showing rapid recovery from unresponsiveness to exploring behavior in IH animals. Then rats were gradually re-warmed from 33.0 to 37.0°C over 2 hours using a warming blanket. To ensure no post-resuscitation spontaneous hypothermia[27], all animals were then kept inside a neonatal incubator (Isolette infant incubator, Air-shields Inc, Pennsylvania) for the first 24 hours post-ROSC.

Continuous EEG was undertaken in four anesthetized sham rats which underwent identical surgical preparation and subjected to normothermic baseline for 30 minutes, and then induced hypothermia for 1 hour.

EEG recording and qEEG analysis

We have used qEEG analysis previously to track brain recovery after CA[15, 16, 20–24]. EEGs were recorded from baseline through re-warming periods using DI700 Windaq system[15]. Serial 30-minute EEG recordings were then performed at 24, 48, and 72 hours after ROSC in each group. In this study we employed entropy to analyze nonstationary EEG signals. The information quantity (IQ) is calculated using a sliding temporal window technique from a window block of EEG signal for the entire data set[15, 16]. Sub-band IQ (SIQ, referred below as qEEG) is the average value of IQ within different frequencies bands. The details of this analysis method are provided in the appendix. We chose 9 segments from EEG data in each rat and calculated qEEG: baseline, CA, 30-min, 1-hour, 4-hour, 6-hour, 24-hour, 48-hour, and 72-hour.

Neurological evaluation

The Neurological Deficit Scale (NDS) was patterned after the neurological examination in humans[10] and functional outcome scales for global cerebral ischemia in animals[11, 13, 25]. The previously validated[15, 18] NDS ranges from 80 indicating a functionally normal rat to a score of 0 for brain or cardiac death prior to conclusion of the study. Behavioral tests including gait coordination, balance beam walking, righting reflex, negative geotaxis, visual placing and turning alley tests were analyzed as a subgroup of NDS[17, 18] (see appendix for details). NDS was determined by a trained examiner blinded to temperature groups after the re-warming period at 2 hours post-hypothermia on the first day, and then repeated at 24, 48, and 72 hours after ROSC. The primary outcome measure was defined as the 72-hour NDS score.

Quantitative brain histopathological damage scoring (HDS)

Ten μm paraffin-embedded sections were stained with Cresyl violet[28, 29] which was used to quantify ischemic changes using a standard rat brain atlas[30] in each hemisphere under ×400 magnification with an Olympus BX51 microscope (Olympus, Center Valley, PA). Cresyl violet staining was chosen based on its reliability and consistency in the evaluation of cytoplasmic and nuclear morphological changes as compared with H&E, and its better suitability for stereological techniques to identify nuclear and nucleolar structures that are required as part of the assessment of cell viability[31–34]. Ischemic neurons were identified using standard criteria:[29, 35–37] pyknosis, karyorrhexis, karyolysis and cytoplasmic changes in form and color.

After de-identifying the histologic slides of clinical data, two investigators, supervised by a neuropathologist, quantified ischemic neurons in a standardized region of temporal cortex layers 4 and 5 anterior to the rhinal fissure and adjacent to the hippocampus, approximating the temporal cortex areas Te1 and Te3[30], along with CA1 of the hippocampus. These areas were chosen because CA-1 is selectively vulnerable to global cerebral ischemia in animals and humans studies and the cerebral cortex is closely related to functional outcome and EEG changes[11, 18, 26, 38, 39]. Stereologic technique was performed in the predefined set of random, non-overlapping microscope fields using Adreas Stereo Investigator software 5.05.4 (MicroBrightField, Williston, VT) to estimate the degree of neuronal injury. In addition to ischemic neurons, normal-appearing neurons were counted in each field. These data were used to calculate a percentage of injury ([injured cells / total number of counted cells] × 100) representing the degree of necrotic cell death per region[35].

Statistics

Univariate analysis was performed for parametric data with the use of the Student's t-test for continuous variables and the chi-square test for categorical variables. Non-parametric analysis of variance was used to test for differences in rank order NDS as a repeated measure. Multivariate General Linear Model was used for advanced comparison of aggregate data to control for influencing factors such as hypothermia method and asphyxia time. The mortality rate was analyzed by Fisher's exact test (crosstabs) and survival was analyzed by a Kaplan-Meier test. Pearson correlation of bivariate analysis was used to analyze the correlation between 72-hour NDS score with serial qEEG and HDS. Statistical significance was set at $p<0.05$. Statistical analysis was performed with SPSS 14.0 (SPSS Inc., Chicago, IL).

Results

Baseline characteristics, temperature monitoring and ABG Data

There are no significant difference between groups of the baseline characteristics including body weights, anesthesia duration for preparation, heart rates and blood pressure ($p>0.05$). The temperature recording for 7- and 9-minute groups during the hypothermia experiment is shown in Figure 1. Cooling to target temperature was achieved in 11.1±4.6 (Mean±SEM) minutes and re-warming in 116.8±21.4 minutes to reach the target range of 36.5–37.5 °C. All animals were maintained at 37±0.5 °C after re-warming during the first 24 hours.

ABG data including arterial pH, HCO_3^-, PO_2 and O_2 saturation, were similar between groups at all time points (baseline, 10, 20, and 40 minutes after ROSC). There are significant differences of PCO_2 at 10 minutes ($p<0.01$) and 20 minutes ($p<0.01$) and of blood pressure at 40 minutes ($p<0.05$) existed between groups (Data shown in table 1).

EEG-bursting analysis and qEEG analysis

Within seconds of CA, the normal EEG signal was reduced to an isoelectric tracing, which was followed by periodic activity resembling burst-suppression of variable duration. The interval between CA and the first burst in the 4 groups was 16.3±0.7 (7CH), 20.3±1.7 (9CH), 16.3±1.2 (7IH) and 19.6±2.8 (9IH) minutes respectively, where CA refers to the starting period of asphyxia and the interval from CA to the first burst is the duration of isoelectric EEG.

There was a statistically significant difference between 7-minute CA (16.3±0.7) and 9-minute CA groups (20.0±0.9) (p=0.004) while no significant difference existed between IH and CH rats. No significant difference was observed in qEEG of sham rats between the periods of hypothermia (0.59±0.02) and normothermia (0.60±0.02) (p=0.921).

Using visual assessment by comparing the evolution of burst frequency and amplitude from baseline of the raw EEG signal alone, qualitative differences between the groups were not evident (Figure 2 A, B). Subtle differences in EEG, however, were readily discerned using qEEG analysis (Figure 2 C). Similar to the raw EEG data, the aggregate qEEG decreased from baseline (qEEG=1) to the lowest point rapidly after CA, then gradually recovered close to baseline.

Mean qEEG relative entropy values over the study period were not different (p=0.08) between 7IH rats (0.78±0.03) and 7CH rats (0.75±0.04); while 9IH rats (0.73±0.03) had significantly greater mean qEEG entropy than 9CH rats (0.63±0.04) (p<0.001) (Figure 3 A, B). Aggregate analysis showed mean qEEG entropy was greater in the IH rats (0.76±0.02) compared to CH controls (0.70±0.03) over the 72-hour study period (p<0.001).

The predictive capacity of early qEEG for 72-hour NDS by bivariate analyses, using aggregate qEEG and NDS data (n=28), revealed significant correlations between 72-hour NDS and qEEG values showing statistical significance at 1, 4, 6 and 24 hours (p<0.05). The value of early qEEG to predict recovery of behavioral and cognitive function, evaluated by comparing qEEG against the behavioral subgroup of NDS, showed a significant correlation at 1 and 4 hours (p<0.05).

Functional Recovery by NDS

Survival NDS analysis results are shown in Figure 4. IH animals had persistently better functional recovery by NDS scores at all time periods with statistical significance in the 7-minute groups (IH/CH (Median, 25^{th}–75^{th} percentile): 74, 60.75–74/63, 50.25–70; p=0.001) and 9-minute groups (IH/CH: 54, 49–61.75/47, 39–60; p=0.022). Aggregate analysis showed statistically significant differences (p=0.001) between CH group (54, 42–66) and IH group (61.5, 51.25–74).

Mortality and qEEG

The mortality rates were 0% (0/8) in 7IH, 12.5% (1/8) in 7CH, 12.5% (1/8) in 9IH, and 25% (2/8) in 9CH group, with an odds ratio (OR) of mortality in the CH group of 3.667 (95%CI: 0.3–42.9). No statistically significant differences in mean duration of survival hours existed between the 7IH (72.0 hours) and 7CH (63.8 hours) groups or between the 9IH (67.5 hours) and 9CH (59.0 hours) groups. Compared to survivors, rats that prematurely died within 72 hours after ROSC had lower qEEG values at 6 hours (dead/survivors: 0.55±0.10/0.78±0.02, p=0.002) and 24 hours (dead/survivor: 0.71±0.06/0.86±0.02, p<0.05).

Quantitative brain histopathological damage scoring (HDS)

There were more ischemic neurons in cortex in the CH group compared to the IH group after 9-minute CA (p=0.04) while this difference was not noted between the 7-minute CA groups.

While significant neuronal injury was noted in CA-1 of the hippocampus as expected with this injury model, the HDS was not different in IH and CH groups subjected to 7- and 9-minute CA (Table 2 and Figure 5). Aggregate analysis showed HDS was significantly higher in the cortex in the CH group (33.2±4.4%, Mean±SEM) compared to the IH group (18.9±2.5%) ($p=0.006$). No statistically significant of injury in CA1 in IH animals (14.4±2.9%) than CH controls (24.6±5.4%) ($p=0.112$).

Bivariate analyses revealed significant correlations between 72-hour NDS and HDS of cortex ($p<0.05$) as well as CA1 ($p<0.01$). HDS of cortex correlated well with HDS of CA1 ($p<0.01$).

Discussion

Our study shows that qEEG analysis methods readily tracked and differentiated the enhanced brain recovery provided by the IH over the CH treatment groups. We also noted that the earlier administration of therapeutic hypothermia after CA not only leads to better functional outcome compared to conventional hypothermia administration, but allowed for reduction of treatment duration by half (6 hours vs. 12 hours). We observed that the better qEEG recovery of the IH group, which was established during 30 minutes to 4 hours after ROSC, was validated by both NDS and HDS (cortex) at 72 hours. These findings showed that the beneficial effects of hypothermia optimization on the brain can be tracked using an advanced but easily interpretable monitoring technique.

While the beneficial effect of therapeutic hypothermia has been a breakthrough in the care of CA patients, its real time effect on brain recovery remains unclear. With the International Liaison Committee on Resuscitation (ILCOR)[8] and the American Heart Association[40] recommendation for the use of hypothermia in appropriate patients, the absolute mortality benefit was 16% in both studies, with a number needed to treat of 6 in order to save 1 life[5, 6]. This therapy therefore has the potential to benefit more patients if better understood and delivered appropriately. The findings of this study seek to address 2 issues: a) to respond to the call of the National Heart Lung and Blood Institute (NHLBI)-Post-resuscitative and initial Utility in Life Saving Efforts (PULSE) initiative to prioritize efforts to improve and monitor neurological recovery after CA[41] and b) the challenge to improve outcomes further with earlier yet shorter hypothermia delivery.

Previous experiments of delayed induction of hypothermia (by 1 hour post-ischemia) after CA have demonstrated improvements in functional outcome and histological markers of injury in rats treated with hypothermia compared to normothermic controls[14, 42, 43], which was consistent with our previous findings[15]. Separate work by Hicks *et al.* [44] compared histological and functional outcomes between immediate hypothermia and hypothermia delayed by an hour using an experimental design similar to the present manuscript. Some differences in technique, methods and statistical powering may account for the difference in findings between the Hicks study and ours. While the paper by Hicks *et al*. did not specifically compare the duration of hypothermia as we did, the potential advantage of earlier administration was suggested with the immediate hypothermia group showing a trend toward better outcomes in the NDS, histological score, and heat shock protein levels. Our experiment lends further support to the theory that cooling should begin as soon as possible after ROSC, similar to other animal models[12, 13, 45].

With electrophysiological markers, this study suggests that earlier initiation of hypothermia may have a greater impact during the early period of recovery and injured neurons that are immediately treated have a better chance of recovering. These observations, as supported by qEEG and histology, preclude the need for longer treatment duration and suggest the need to re-evaluate the timing of hypothermia initiation in human subjects. Given the risks of

coagulopathy and immune suppression, need for sedation and paralysis, expense, and high resource use associated with hypothermia[5, 6, 9, 10], these results are potentially important.

As human studies have shown, with this degree of hypothermia (33±1°C), the detrimental effects have not been significantly different than normothermia. Given the identical temperature range in IH and CH, we did not expect significant differences in detrimental effects, so we did not look at the detrimental effects in much detail. However, considering the commonly reported detrimental side effects, we observed no significant hemorrhage, seizure activity, or arrhythmias. We did not look specifically for pneumonia or renal failure. The enhanced recovery of shorter IH over longer CH is most likely the result of rapid onset rather than a decrement in adverse effects.

Translational research verification may justify changing resuscitation strategies such that paramedics begin cooling in the field or ambulance en route to the hospital. The cost of longer periods of hypothermia centers on need for intensive nursing care. And the cost of rapid inductions may be dependent on technologies; however chilled saline infusion is definitely an economical and effective way to achieve rapid hypothermia induction[46].

While functional outcome by NDS was our primary outcome measure and qEEG was a tracking tool, we also employed histology for additional verification of outcomes. We acknowledge that previous consensus[47] showed that histological outcomes do not readily translate into clinical outcomes, as reflected in the 7-minute CA group. The significant neuronal injury observed in CA-1 of the hippocampus reflects the high susceptibility of this area to global ischemia, but its lack of direct influence on arousal and cortical activity may account for the lack of concurrence with EEG findings. Histological injury in the cortex, a significant site for EEG generation and modulation, demonstrated stronger correlation with qEEG differences. The minor difference between qEEG in the 7IH and 7CH groups despite NDS differences may be due to the fact that 7 minutes is a minimal injury for cortex (represented by EEG), despite significant subcortical injury (reflected in NDS).

As part of our protocol[15], we attempted to normalize the ABG variables, especially pH to limit the injury primarily to CA. In order to achieve this, we adjusted ventilator rates as needed[17]. At 10 minutes post-CA, all rats showed a decline in pH from the asphyxial cardiac arrest. These values however, are a result of the 10-minute post-ROSC hyperventilation period (tidal volumes 10 ml/kg, respiration rate 65/min and positive expiratory end pressure 6 cm H_2O) that we employed for all animals in this model[15]. Although, at some point, 9CH group appears to have a larger metabolic acidosis compared to 9IH, there were no statistically significant differences noted in pH. Hypothermia has not been started for the 9CH group at 20 minutes while hypothermia has been reached in 9IH group (average cooling time to target is 11.1 minutes). Hypothermia may decrease the PCO2 in 9IH while CO2 elevation is due to normothermia in 9CH animals, which is consistent with other recent publications[48–50]. By 40 minutes, hypothermia has been ongoing in the IH group for ~30 minutes while it has not been started in CH group. The relative increase in MAP is probably a reflection of the ongoing systemic beneficial effects of hypothermia and may be due to an increase in systemic vascular resistence[5, 51, 52].

From a neuromonitoring perspective, this study highlights the importance of the immediate post-resuscitation period when brain injury may be most amendable to therapeutic interventions. We have shown previously that qEEG analysis detected the therapeutic benefit of conventional hypothermia compared to normothermic controls[15, 16]. This observation is carried over into the present study. Additionally, we also showed that it is not the temperature (32–34°C) itself that causes the change in EEG but the response of the injured brain to hypothermia as manifested in the qEEG.

As in our previous studies, we observed that animals that proceed more quickly from a flat EEG to a continuous EEG pattern and have higher qEEG values achieved better functional outcomes. We have reported previously the rapid EEG response within 10 to 90 minutes[18, 19, 38] and this reflects the ability of EEG to be a sensitive real-time measure of injury and recovery. The qEEG as a reflection of brain injury was also further crystallized by the ability of our algorithms to prognosticate functional outcomes and mortality within the first few hours after resuscitation. Using aggregate data that included the entire animal population, the qEEG measure was able to accurately predict neurological outcome defined by the NDS and cognitive-behavioral tests as early as 1–4 hours after ROSC, a time in which the animal remains comatose. As a measure of coma recovery, the NDS is weighted toward brain stem function, which is highly preserved in all but the most severe global ischemic injury[53]. The principle improvement in animals with higher qEEG, however, was seen in more advanced behavioral and coordination tests, such as balance beam walking, gait coordination, and righting reflexes. These functions, especially the neuro-behavioral assessment, may require more focused study to fully document the long-term effects of hypothermia on these animals.

While the manual interpretation of continuous raw EEG is laborious, subjective, and requires specialized experience, entropy-based qEEG can be readily used to track brain recovery. Our results suggest that early qEEG monitoring may assist clinicians in tracking recovery after CA and the therapeutic response to hypothermia. The development of accurate neuromonitoring techniques during hypothermia is particularly important for the evaluation of therapeutic response, because clinical neurological assessment is obscured by sedative and paralytic medications. As a continuous, real-time, and non-invasive methodology, qEEG monitors the response to potential neuroprotective strategies by translating complicated and subjective waveform analysis into an objective measure. Similar use of qEEG analysis has been successfully incorporated in hypothermia treatment in neonates with global brain ischemia[54].

Conclusions

In conclusion, our qEEG analysis method is able to detect the brain's response to therapeutic benefits of hypothermia, and it is able to predict recovery of arousal, functional outcome, and survival. The neurological recovery appears to be better under immediate induction, but shorter duration, of hypothermia after resuscitation. These experiments have the potential to develop brain monitoring and guide the optimum effect of therapeutic hypothermia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix

The NDS and its components can be found in Table 1

Table 1

NEURODEFICIT SCORING FOR RATS (Normal = 80; Brain dead = 0)

A)General Behavioral deficit	Total Score : 19
Consciousness	Normal 10/ Stuporous 5 / Comatose or unresponsive 0
Arousal:	Eyes open spontaneously 3/ Eyes open to pain 1/ No Eye Opening 0
Respiration:	Normal 6/ Abnormal (hypo or hyperventilation) 3/ Absent 0
B) Brain-stem Function:	Total Score : 21

Olfaction: response to smell of food	Present 3/Absent 0
Vision: head movement to light	Present 3/Absent 0
Pupillary Reflex: pupillary light reflex	Present 3/Absent 0
Corneal Reflex:,	Present 3/Absent 0
Startle Reflex:	Present 3/Absent 0
Whisker Stimulation:	Present 3/Absent 0
Swallowing: swallowing liquids or solids	Present 3/Absent 0
C) Motor Assessment: Strength: Total Score : 6	Normal 3/ Stiff or Weak 1 / No movement/Paralyzed 0. Left and Right side tested and scored separately.
D) Sensory Assessment: Pain: Total Score : 6	Brisk Withdrawal with pain 3/ Weak or abnormal response (extension or flexion posture) 1 /No Withdrawal 0. Left and Right side tested and scored separately.
E) Motor behavior:	Total Score : 6
Gait coordination:	Normal 3 / Abnormal 1 / Absent 0
Balance on Beam:	Normal 3 /Abnormal 1 / Absent 0
F) Behavior:	Total Score : 12
Righting reflex:	Normal 3 / Abnormal 1 / Absent 0
Negative Geotaxis:	Normal 3 / Abnormal 1 / Absent 0
Visual Placing:	Normal 3 / Abnormal 1 / Absent 0
Turning Alley:	Normal 3 / Abnormal 1 /Absent 0
G) Seizures(convulsive or non-convulsive): Total Score : 10	No Seizure 10 / Focal Seizure 5 / General Seizure 0

Balance beam testing is normal if the rat can cross a 2 cm wide by 1 m long beam suspended 0.5 m above the floor. Abnormal is scored if the rat attempts and does not continue or stays momentarily and falls. Absent is scored when the rat falls off immediately upon placement on the beam. Other behavior reflex subscores evaluated the following: (1) righting reflex (animal placed on its back is able to correct to upright position); (2) turning alley (the animal is made to walk and turn back at the end of a 15 cm by 0.5 m alley); (3) visual placing (the animal is lifted and is able to visually orient itself to objects and depth); and (4) negative geotaxis (animal placed on its back on a plane angled at 45° corrects itself and moves up the incline).

Technique detail of qEEG analysis

The development of this novel, entropy-based EEG analysis, which has shown promising results in objectively tracking the EEG recovery under hypothermia and normothermia after cardiac arrest, has been previously reported[1–6].

Entropy is a method to quantify the order/disorder of a time series. It is calculated from the distribution of one of the signal parameters, such as amplitude, power, or time-frequency representation. The Shannon entropy (SE) gives useful criteria for analyzing and comparing probability distribution and provides a good measure of the information content[7]. The classical Shannon entropy is expressed in:

$$SE = -\sum_{m=1}^{M} p(m)\log_2 p(m)$$

where $P(m)$ is the probability of finding the system in the m^{th} microstate with $0 \le p(m) \le 1$ and $\sum_{m=1}^{M} p(m)=1$. To analyze nonstationary EEG signals, the temporal evolution of SE was determined by an alternative time-dependent SE measure based on application of a sliding temporal window technique.

Let $\{ s(i): i = 1,..., N \}$ denote the raw sampled EEG signal. Now we define a sliding temporal window $w \le N$, and the sliding step $\Delta \le w$. Then sliding windows are defined by

$$W(n;w;\Delta)=\{s(i),i=1+n\Delta,\ldots,w+n\Delta\}$$

where $n = 0,1,...,[n/\Delta] - w + 1$ and $[x]$ denotes the integer part of x. To calculate the probability, $p_n(m)$ within each window $W(n;w;\Delta)$, we introduce intervals such that

$$W(n;w;\Delta)=\bigcup_{m=1}^{M} I_m.$$

Next, wavelet analysis of the signal is carried out to decompose the EEG signals into wavelet subbands, which can be interpreted as frequency subbands. The probability $p_n(m)$ that the sampled signal belongs to the interval I_m is the ratio between the number of the signals found within interval I_m and the total number of signals in $W(n;w;\Delta)$. Using $p_n(m)$, $SE(n)$ is defined as

$$SE(n)=-\sum_{m=1}^{M} P_n(m)\log_2 p_n(m)$$

Based on the above arguments, the information quantity (IQ) can be defined. First the discrete wavelet transform (DWT) coefficients within each window are obtained as:

$$WC(r;n;w;\Delta)=DWT\,[W(n;w;\Delta)].$$

Next, wavelet coefficients are obtained from the DWT. To calculate $p_n^{wc}(m)$ within each transformed window $WC(r;n;w;\Delta)$, intervals in $W(n;w;\Delta)$, are modified

$$WC(r;n;w;\Delta)=\bigcup_{m=1}^{M} I_m^{wc}.$$

Similar with $p_n(m)$ in SE, the probability, $p_n^{wc}(m)$ within each window $WC(r;n;w;\Delta)$ is calculated. and finally the IQ is obtained as:

$$IQ(n)=-\sum_{m=1}^{M} p_n^{wc}(m)\log_2 p_n^{wc}(m).$$

where $p_n(m)$ is an estimated probability that the wavelet-transformed signal belongs to m_{th} bin and M is the number of bin. IQ is calculated from a temporal sliding window block of EEG signal. Thus we explore the IQ evolution of the whole data $\{s(i) : i = 1,..., N\}$. In short, IQ is the Shannon entropy of the decorrelated entire EEG data set[2]. Sub-band IQ (SIQ) is the average value of IQ within different frequencies bands such as 0–2 Hz, 2–4 Hz, 4–8 Hz, 8–16 Hz, and 16–32 Hz. SIQ has better distinction capacity and separately characterizes recovery trends in different bands[3, 8, 9].

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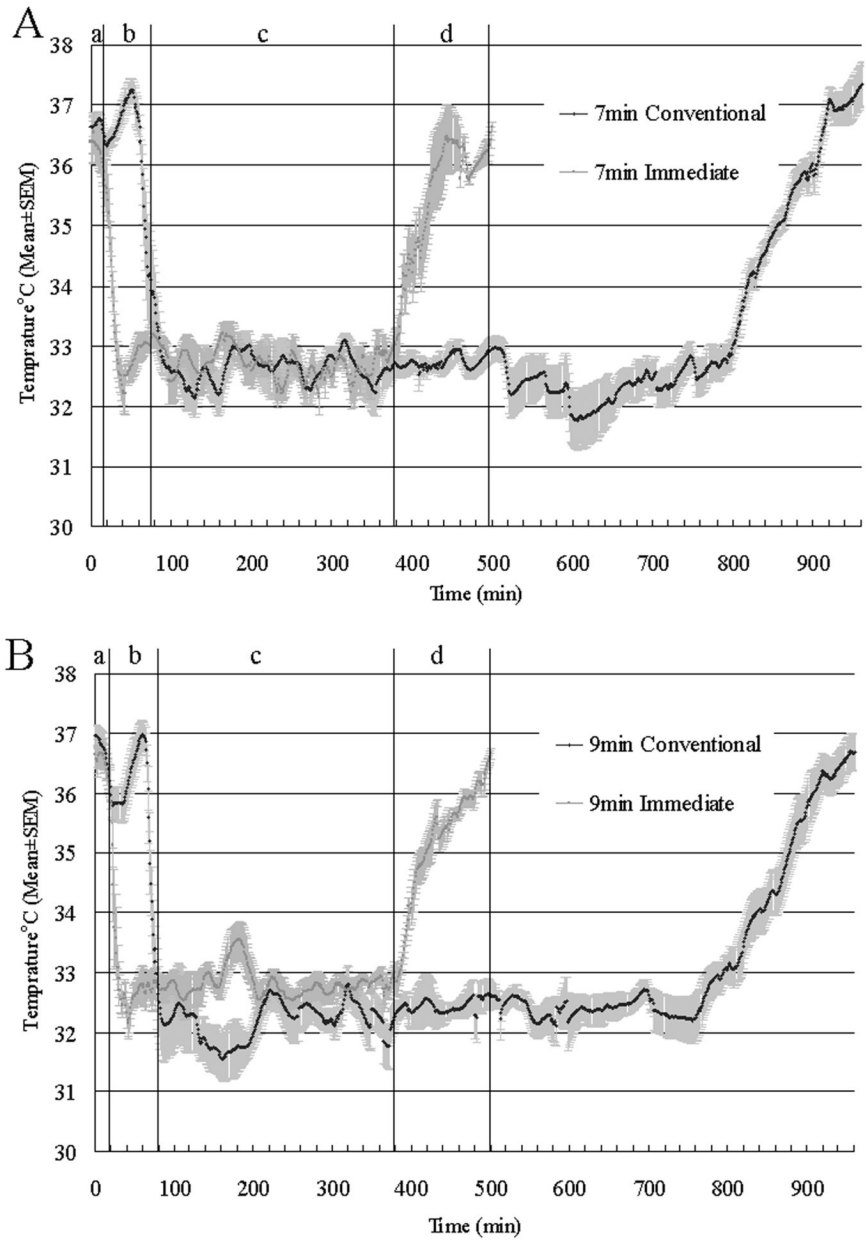

Figure 1.

Temperature recording of conventional hypothermia (CH) and immediate hypothermia (IH) rats in the (A) 7-minute asphyxia group and (B) 9-minute asphyxia group. The dark line is CH and light line is IH. The solid heavy line is mean temperature and the field with lighter shading is SEM. For the first period (a), no temperature difference was noted during baseline and cardiac arrest periods (36.7±0.0/36.3±0.0 (Aa) for 7-minute group (CH/IH, Mean±SEM) and 36.7 ±0.1/36.5±0.0 (Ba) for 9-minute group) in Aa and Ba. In the second period (b), the mean temperature during the CH group was higher than IH (36.2±0.1/33.2±0. 1 (Ab) for the 7-minute group and 35.8±0.2/32.9±0.1 (Bb) for the 9-minute group) (all $p<0.001$).The temperature was within the 32–34°C range during hypothermia (period C) (32.7±0.0/32.7±0.0 (Ac) for the 7-

minute group and 32.2±0.0/32.8±0.0 (Bc) for the 9-minute group). In period d, animals in the IH group were rewarmed while hypothermia was maintained for 6 more hours in the CH group (32.7±0.0/35.4±0.1 (Ad) for 7-minute group and 32.4±0.0/35.2±0.1 (Bd) for 9-minute group) (all $p<0.001$). After 12 hours of hypothermia in the CH group, normothermia was maintained similar to the IH group.

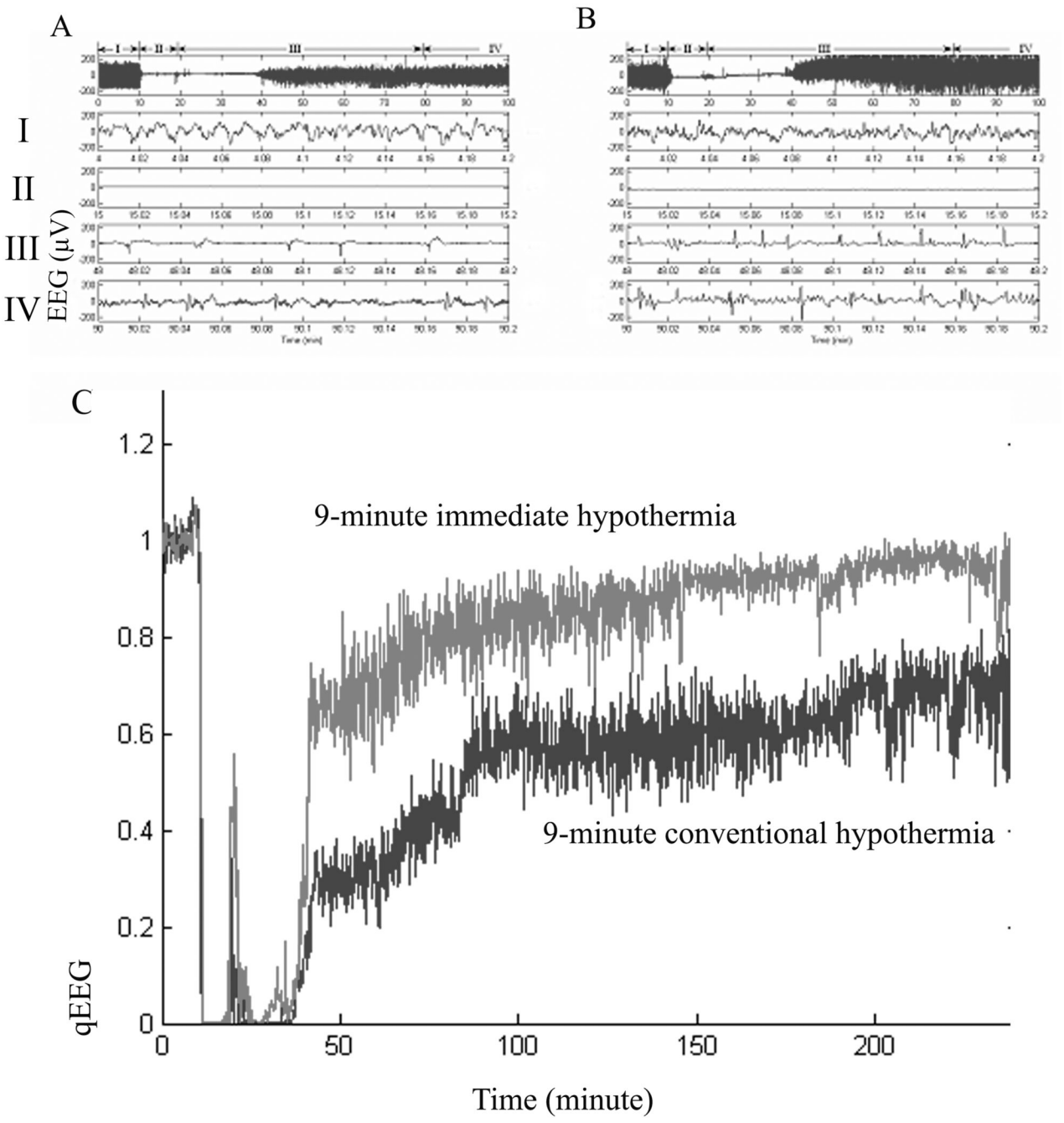

Figure 2.

Raw EEG data and qEEG of representative 9-minute asphyxia animals in the first 4 hours A: 9-minute conventional hypothermia (9CH), B: 9-minute immediate hypothermia (9IH): (I) Baseline (0 minutes), (II) Cardiac arrest (CA) period (19 minutes), (III) 1 hour after CA, (IV) 4 hours after CA - Hypothermia maintenance period; C: comparison of qEEG in CH and IH, CH started at 1 hour and IH started immediately after ROSC.

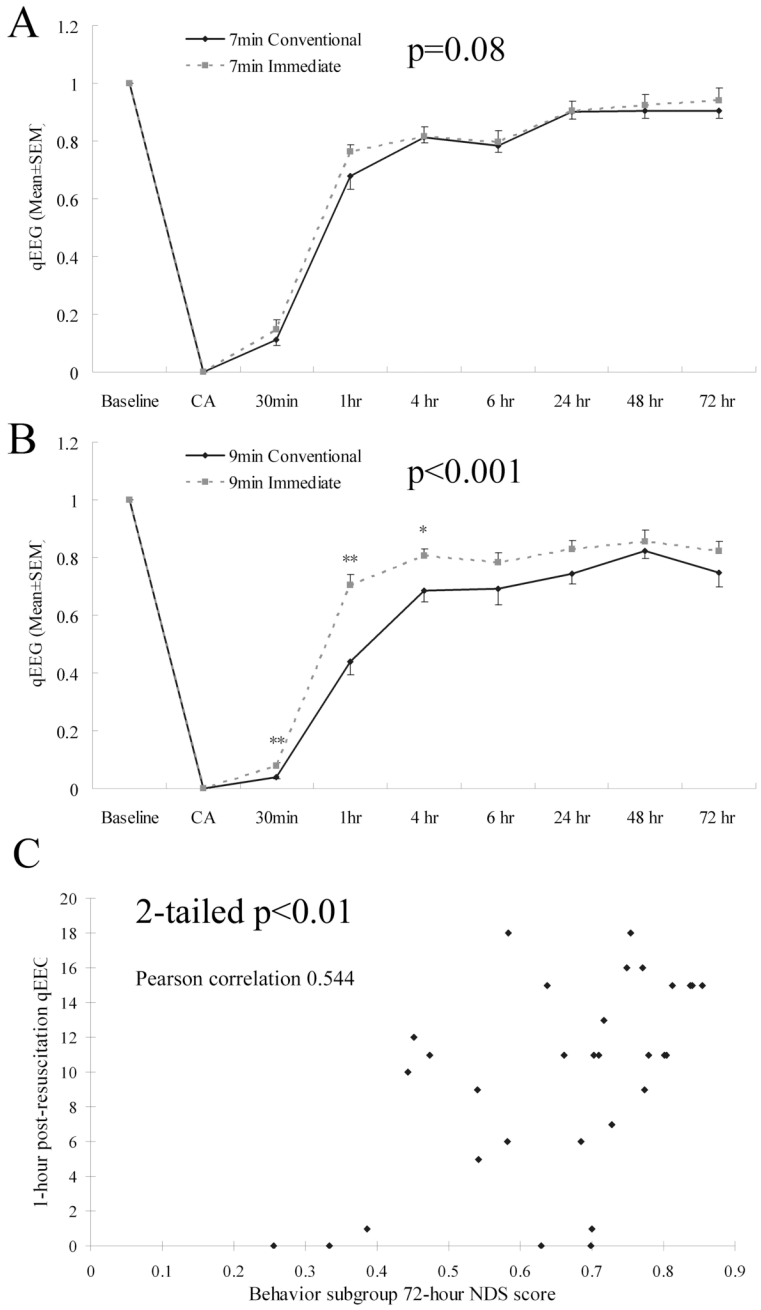

Figure 3.

Comparison of qEEG analyses by hypothermia methods in different periods in A. 7-minute and B. 9-minute asphyxia times (*p<0.05, **p<0.01). Time in X-axis is the period after return of spontaneous circulation (ROSC). CA is cardiac arrest period, 30min is immediate hypothermia (IH) starting period, 1hr is conventional hypothermia (CH) starting period, 4hr and 6hr are hypothermia maintenance periods. The qEEG value correlated well with 72-hour NDS as early as 1 hour after ROSC. The qEEG in 7-minute IH tended to be better than CH but overall statistically similar. qEEG predicts 72-hour functional recovery at 1 hour, as shown in C. For temperature recording in Fig. 1, baseline and CA is period a; 30min is period b; 1hr and 4hr is period c; 6hr is period d.

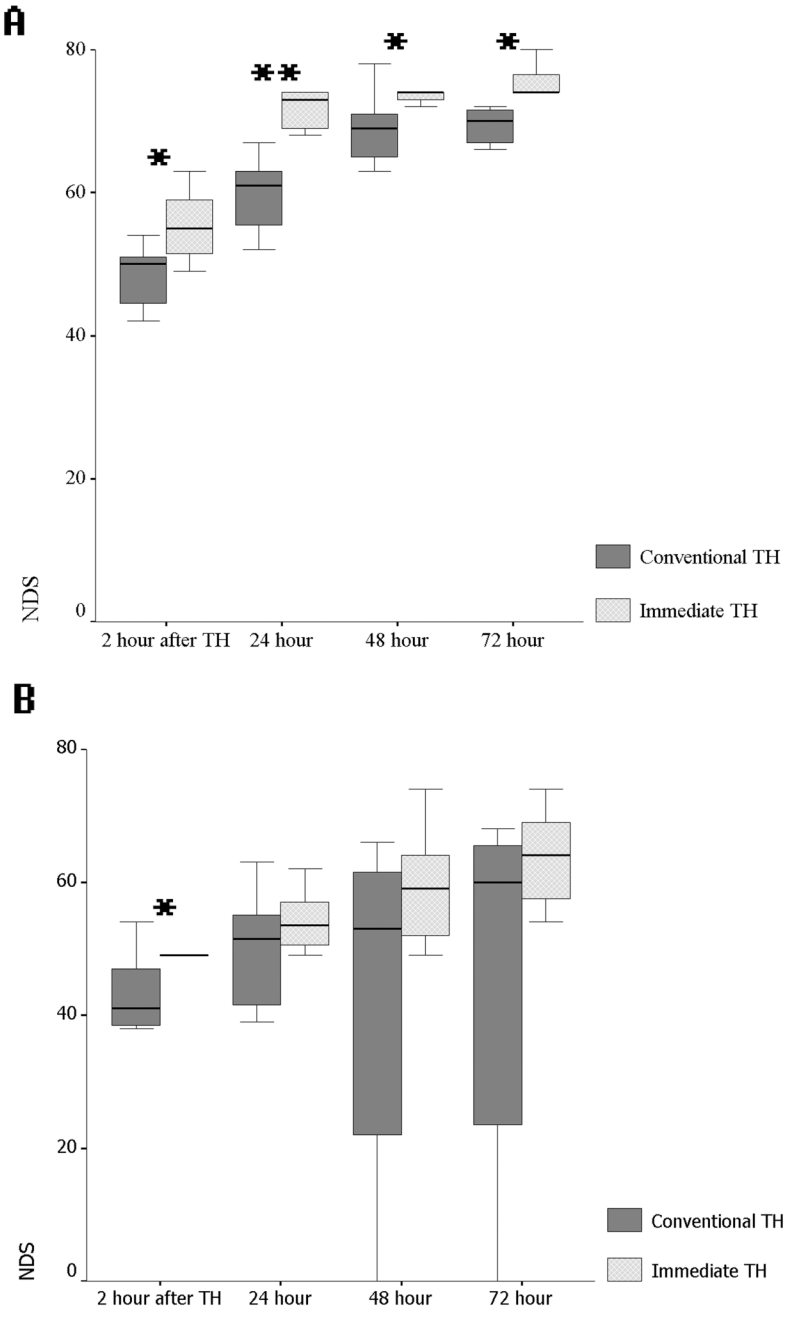

Figure 4.

NDS by injury and temperature groups (Median (25th – 75th percentile)), TH: therapeutic hypothermia. A significant difference was noted over the 72-hour experiment in A. 7-minute immediate hypothermia (7IH) vs. conventional hypothermia (7CH) (p=0.001) and B. 9-minute IH (9IH) vs. CH (9CH) (p=0.022) asphyxial cardiac arrest (CA). Significant differences existed in all periods between the 7-minute groups and at 2 hours post-hypothermia between the 9-minute groups (*p<0.05, **p<0.01). It was noted that qEEG was able to detect the significant difference as early as 30 minutes between 9-minute groups and qEEG value correlated well with 72-hour NDS as early as 1 hour after CA.

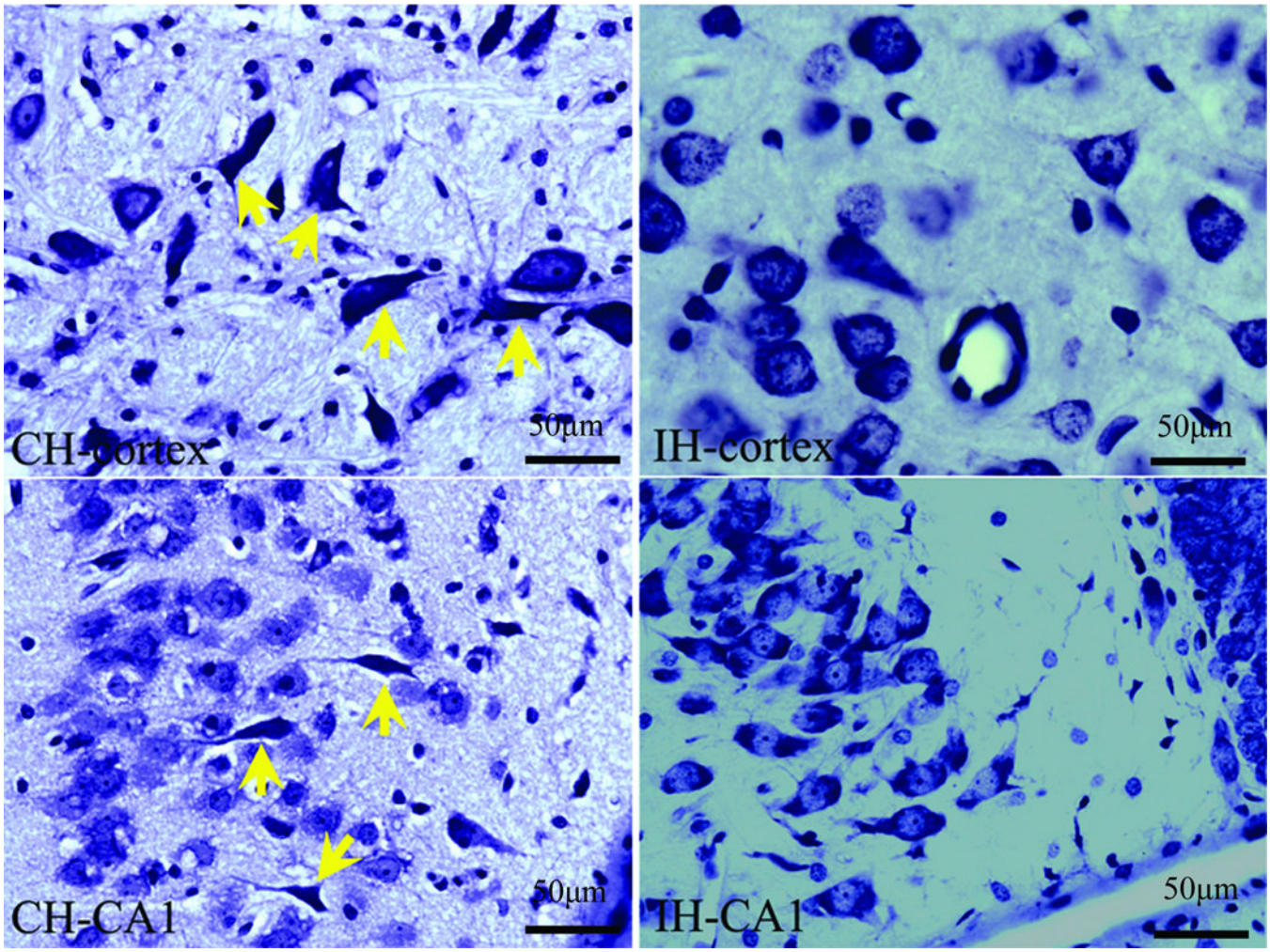

Figure 5.
Photomicrograph illustrating of brain injury in CA1 and cortex in 9-minute asphyxia rats by hypothermia groups. Greater ischemic neuronal death (↑) was found in conventional hypothermia (CH) rats (Cresyl violet staining, 400X). IH: immediate hypothermia.

Table 1

Arterial blood gas and MAP data in cardiac arrest experiment among 4 groups

	7CH [a]	7IH [b]	9CH	9IH	*p* value of 4 groups
Baseline					
pH	7.48±0.03	7.42±0.02	7.53±0.06	7.45±0.01	0.220
PCO_2 (mmHg)	36±2	39±4	40±0	38±1	0.929
PO_2 (mmHg)	323±51	383±25	323±26	391±46	0.598
HCO_3 (mmol/L)	26±1	24±2	28±0	25±1	0.683
O_2SAT (%)	100±0	100±0	100±0	100±0	0.762
MAP (mmHg)	100±7	90±11	88±5	91±7	0.644
10min post CA					
pH	7.34±0.03	7.35±0.02	7.35±0.04	7.35±0.03	0.936
PCO_2 (mmHg)	42±3	36±2	38±2	30±2	0.009*
PO_2 (mmHg)	442±39	403±52	434±49	491±20	0.387
HCO_3 (mmol/L)	22±1	19±1	20±2	17±0	0.022*
O_2SAT (%)	100±0	100±0	100±0	100±0	0.522
MAP (mmHg)	123±9	123±10	119±12	150±12	0.191
20min post CA					
pH	7.36±0.04	7.37±0.01	7.22±0.00	7.38±0.02	0.155
PCO_2 (mmHg)	38±2	35±2	50±0	29±2	0.009*
PO_2 (mmHg)	434±25	496±19	595±0	451±26	0.082
HCO_3 (mmol/L)	21±1	20±1	20±0	18±1	0.165
O_2SAT (%)	100±0	100±0	100±0	100±0	0.862
MAP (mmHg)	85±10	99±8	88±16	102±15	0.753
40min post CA					
pH	7.40±0.04	7.31±0.02	7.43±0.04	7.36±0.02	0.164
PCO_2 (mmHg)	42±4	44±4	39±3	32±3	0.089
PO_2 (mmHg)	483±29	455±39	424±59	440±30	0.841
HCO_3 (mmol/L)	25±1	22±1	24±1	19±1	0.003*
O_2SAT (%)	100±0	100±0	100±0	100±0	0.474
MAP (mmHg)	90±9	110±10	62±5	93±15	0.020*

[a] conventional hypothermia

[b] immediate hypothermia

* Statistically significant difference was noted but was minimal to cause any significant change in pH. Hypothermia may decrease the PCO2 at 10 and 20 minutes in 9IH[48, 49] while CO2 elevation is due to normothermia in 9CH animals[49, 50]. The relative increase in MAP at 40 minutes in IH group may be a reflection of the ongoing systemic beneficial effect hypothermia[5, 51, 52].

Table 2

Quantitative Comparison of Brain injury with histopathological damage scoring (%, Mean±SEM)

	7-minute CH[a]	7-minute IH[b]	9-minute CH	9-minute IH
CA-1	20.9±7.4	12.1±3.0	28.9±8.2	17.4±5.6
Cortex	30.6±5.3	19.2±3.6	36.2±7.7	18.5±3.8

[a] conventional hypothermia

[b] immediate hypothermia

One rat in the 9-IH group, 1 rat in the 7-CH group, and 2 rats in the 9-CH group died before the endpoint of 72 hours and were excluded from quantitative brain histopathological damage scoring.