

EXPERIMENTAL PAPER

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, $T = 33^\circ\text{C}$, immediately post-resuscitation, maintained 6 h) to conventional hypothermia (CH, $T = 33^\circ\text{C}$, starting 1 h post-resuscitation, maintained 12 h) 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-min duration of asphyxial CA. Four sham rats were included for evaluation of the effect of hypothermia on qEEG.

Results: The 72-h NDS of the IH group was significantly better than the CH group for both 7-min (74/63; median, IH/CH, $p < 0.001$) and 9-min (54/47, $p = 0.022$) groups. qEEG showed greater

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recovery with IH ($p < 0.001$) and significantly less neuronal cortical injury by HDS (IH: $18.9 \pm 2.5\%$ versus CH: $33.2 \pm 4.4\%$, $p = 0.006$). The 1-h post-resuscitation qEEG correlated well with 72-h NDS ($p < 0.05$) and 72-h behavioral subgroup of NDS ($p < 0.01$). No differences in qEEG were noted in the sham group.

Conclusions: Immediate but shorter hypothermia compared to CH leads to better functional outcome in rats after 7- and 9-min CA. The beneficial effect of IH was readily detected by neuro-electrophysiological monitoring and histological changes supported the value of this observation. © 2007 Elsevier Ireland Ltd. All rights reserved.

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 CA.^{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 h.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 h after CA significantly improved neurological outcomes compared to normothermic controls.^{15,24} 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 neuro-monitoring 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-h hypothermia (IH) compared to 12-h conventional hypothermia (CH) initiated at 1 h 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 ± 20 g) were assigned at random to 4 groups based on CH/IH and 7/9-min 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 et al.¹⁵), rats were mechanically ventilated with 1.0% halothane in N₂/O₂ (50/50%). Five minutes of baseline recording was followed by 5-min 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 min. CPR was performed with sternal chest compressions (200 min^{-1}) until return of spontaneous circulation (ROSC). Sedative and anesthetic agents were avoided to minimize confounding effects on EEG.²⁶

CH was induced 1 h 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 min and was maintained between 32 and 34 °C for 12 h.¹⁵ For the IH groups, cooling was initiated immediately (within 15 min) after ROSC and was maintained at 32–34 °C for 6 h. The 6-h 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 h 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 h post-ROSC.

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

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-min EEG recordings were then performed at 24,

48, and 72 h 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.^{15,24} 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 Appendix A. We chose 9 segments from EEG data in each rat and calculated qEEG: baseline, CA, 30-min, 1-h, 4-h, 6-h, 24-h, 48-h, and 72-h.

Neurological evaluation

The Neurological Deficit Scale (NDS) was patterned after the neurological examination in humans¹⁰ 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 A for details). NDS was determined by a trained examiner blinded to temperature groups after the re-warming period at 2 h post-hypothermia on the first day, and then repeated at 24, 48, and 72 h after ROSC. The primary outcome measure was defined as the 72-h 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 atlas³⁰ in each hemisphere under $\times 400$ 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 CA1 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 ($[\text{injured cells}/\text{total number}$

of counted cells] $\times 100$) representing the degree of necrotic cell death per region.³⁵

Statistics

Univariate analysis was performed for parametric data (reported as mean \pm S.E.M.) 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

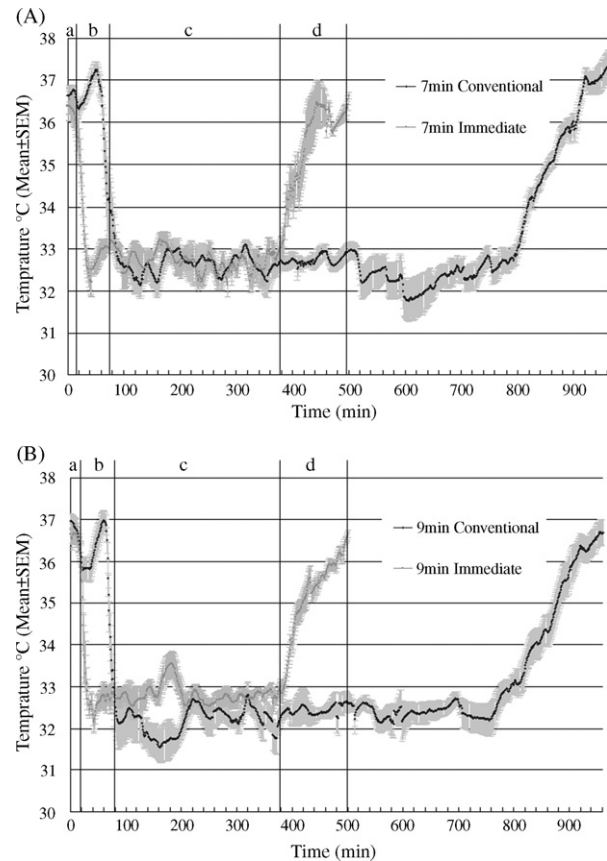


Figure 1 Temperature recording of conventional hypothermia (CH) and immediate hypothermia (IH) rats in the (A) 7-min asphyxia group and (B) 9-min asphyxia group. The dark line is CH and light line is IH. The solid heavy line is mean temperature and the field with lighter shading is S.E.M. For the first period (a), no temperature difference was noted during baseline and cardiac arrest periods ($36.7 \pm 0.0/36.3 \pm 0.0$ (A(a)) for 7-min group (CH/IH, mean \pm S.E.M.) and $36.7 \pm 0.1/36.5 \pm 0.0$ (B(a)) for 9-min group) in (A(a)) and (B(a)). In the second period (b), the mean temperature during the CH group was higher than IH ($36.2 \pm 0.1/33.2 \pm 0.1$ (A(b)) for the 7-min group and $35.8 \pm 0.2/32.9 \pm 0.1$ (B(b)) for the 9-min group) (both $p < 0.001$). The temperature was within the $32\text{--}34^\circ\text{C}$ range during hypothermia (period (c)) ($32.7 \pm 0.0/32.7 \pm 0.0$ (A(c)) for the 7-min group and $32.2 \pm 0.0/32.8 \pm 0.0$ (B(c)) for the 9-min group). In period (d), animals in the IH group were re-warmed while hypothermia was maintained for 6 more hours in the CH group ($32.7 \pm 0.0/35.4 \pm 0.1$ (A(d)) for 7-min group and $32.4 \pm 0.0/35.2 \pm 0.1$ (B(d)) for 9-min group) (both $p < 0.001$). After 12 h of hypothermia in the CH group, normothermia was maintained similar to the IH group.

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-h 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-min

groups during the hypothermia experiment is shown in Figure 1. Cooling to target temperature was achieved in 11.1 ± 4.6 min and re-warming in 116.8 ± 21.4 min to reach the target range of $36.5\text{--}37.5^\circ\text{C}$. All animals were maintained at $37 \pm 0.5^\circ\text{C}$ after re-warming during the first 24 h.

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 min after ROSC). There are significant differences of P_{CO_2} at 10 min ($p < 0.01$) and 20 min ($p < 0.01$) and of blood pressure at 40 min ($p < 0.05$) 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 min (7CH), 20.3 ± 1.7 min (9CH), 16.3 ± 1.2 min (7IH) and 19.6 ± 2.8 min (9IH), respectively, where CA refers to the starting period of asphyxia and the interval

Table 1 Arterial blood gas and MAP data in cardiac arrest experiment among 4 groups

	7CH ^a	7IH ^b	9CH	9IH	<i>p</i> value of 4 groups
Baseline					
pH	7.48 ± 0.03	7.42 ± 0.02	7.53 ± 0.06	7.45 ± 0.01	0.220
P_{CO_2} (mmHg)	36 ± 2	39 ± 4	40 ± 0	38 ± 1	0.929
P_{O_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
10 min post-CA					
pH	7.34 ± 0.03	7.35 ± 0.02	7.35 ± 0.04	7.35 ± 0.03	0.936
P_{CO_2} (mmHg)	42 ± 3	36 ± 2	38 ± 2	30 ± 2	0.009*
P_{O_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
20 min post-CA					
pH	7.36 ± 0.04	7.37 ± 0.01	7.22 ± 0.00	7.38 ± 0.02	0.155
P_{CO_2} (mmHg)	38 ± 2	35 ± 2	50 ± 0	29 ± 2	0.009*
P_{O_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
40 min post-CA					
pH	7.40 ± 0.04	7.31 ± 0.02	7.43 ± 0.04	7.36 ± 0.02	0.164
P_{CO_2} (mmHg)	42 ± 4	44 ± 4	39 ± 3	32 ± 3	0.089
P_{O_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*

* Statistically significant difference was noted but was minimal to cause any significant change in pH. Hypothermia may decrease the P_{CO_2} at 10 and 20 min in 9IH^{48,49} while CO_2 elevation is due to normothermia in 9CH animals.^{49,50} The relative increase in MAP at 40 min in IH group may be a reflection of the ongoing systemic beneficial effect hypothermia.^{5,51,52}

^a Conventional hypothermia.

^b Immediate hypothermia.

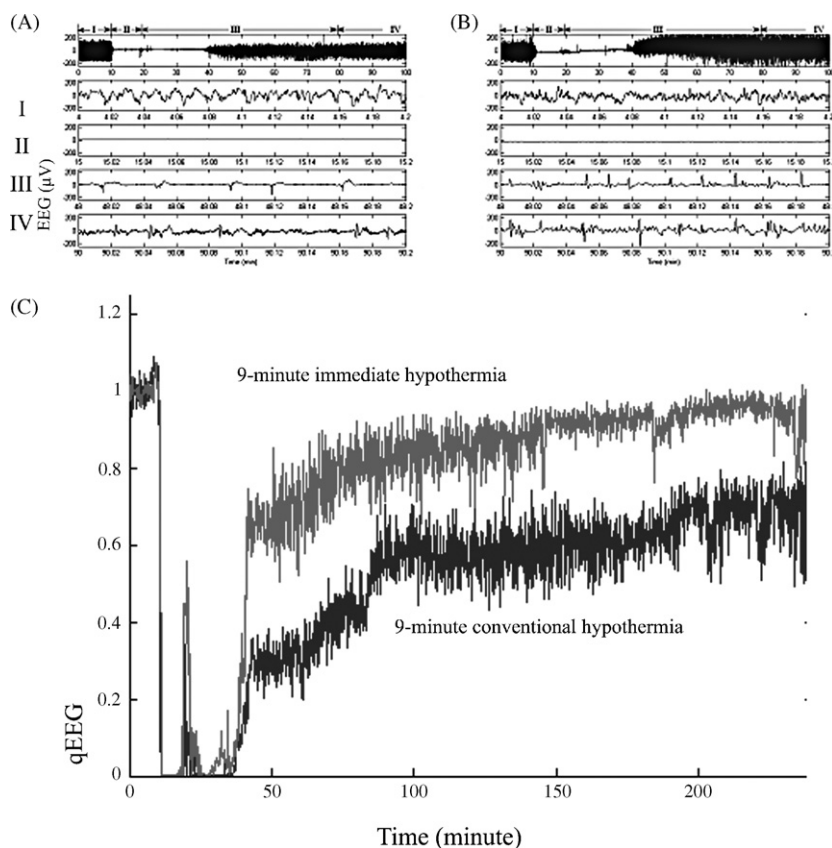


Figure 2 Raw EEG data and qEEG of representative 9-min asphyxia animals in the first 4 h. (A) 9-min conventional hypothermia (9CH); (B) 9-min immediate hypothermia (9IH): (I) Baseline (0 min), (II) Cardiac arrest (CA) period (19 min), (III) 1 h after CA, (IV) 4 h after CA—hypothermia maintenance period; (C) comparison of qEEG in CH and IH, CH started at 1 h and IH started immediately after ROSC.

from CA to the first burst is the duration of isoelectric EEG.

There was a statistically significant difference between 7-min CA (16.3 ± 0.7) and 9-min 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 2A and B). Subtle differences in EEG, however, were readily discerned using qEEG analysis (Figure 2C). 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 3A and 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-h study period ($p < 0.001$).

The predictive capacity of early qEEG for 72-h NDS by bivariate analyses, using aggregate qEEG and NDS data ($n = 28$), revealed significant correlations between 72-h NDS

and qEEG values showing statistical significance at 1, 4, 6 and 24 h ($p < 0.05$). 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 h ($p < 0.05$).

Functional recovery by NDS

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-min groups (IH/CH (median, 25–75th percentile): 74, 60.75–74/63, 50.25–70; $p = 0.001$) and 9-min 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 h) and 7CH (63.8 h) groups or between the 9IH (67.5 h) and

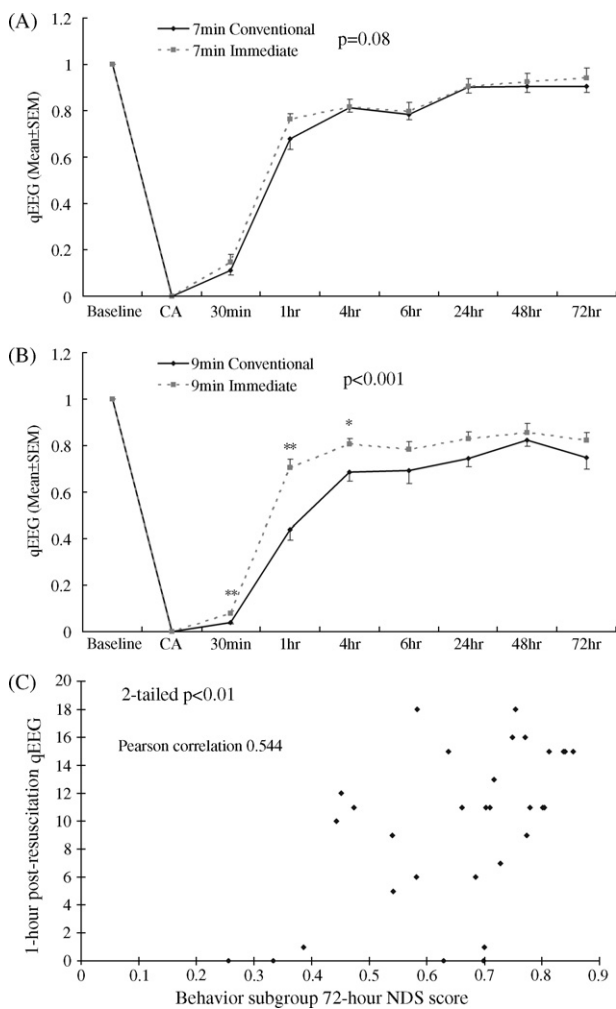


Figure 3 Comparison of qEEG analyses by hypothermia methods in different periods in (A) 7-min and (B) 9-min asphyxia times ($^*p < 0.05$, $^{**}p < 0.01$). Time in X-axis is the period after return of spontaneous circulation (ROSC). CA is cardiac arrest period, 30 min is immediate hypothermia (IH) starting period, 1 h is conventional hypothermia (CH) starting period, 4 and 6 h are hypothermia maintenance periods. The qEEG value correlated well with 72-h NDS as early as 1 h after ROSC. The qEEG in 7-min IH tended to be better than CH but overall statistically similar. qEEG predicts 72-h functional recovery at 1 h, as shown in (C). For temperature recording in Figure 1, baseline and CA is period a; 30 min is period b; 1 h and 4 h is period c; 6 h is period d.

9CH (59.0 h) groups. Compared to survivors, rats that prematurely died within 72 h after ROSC had lower qEEG values at 6 h (dead/survivors: $0.55 \pm 0.10/0.78 \pm 0.02$, $p = 0.002$) and 24 h (dead/survivor: $0.71 \pm 0.06/0.86 \pm 0.02$, $p < 0.05$).

Quantitative brain histopathological damage scoring (HDS)

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

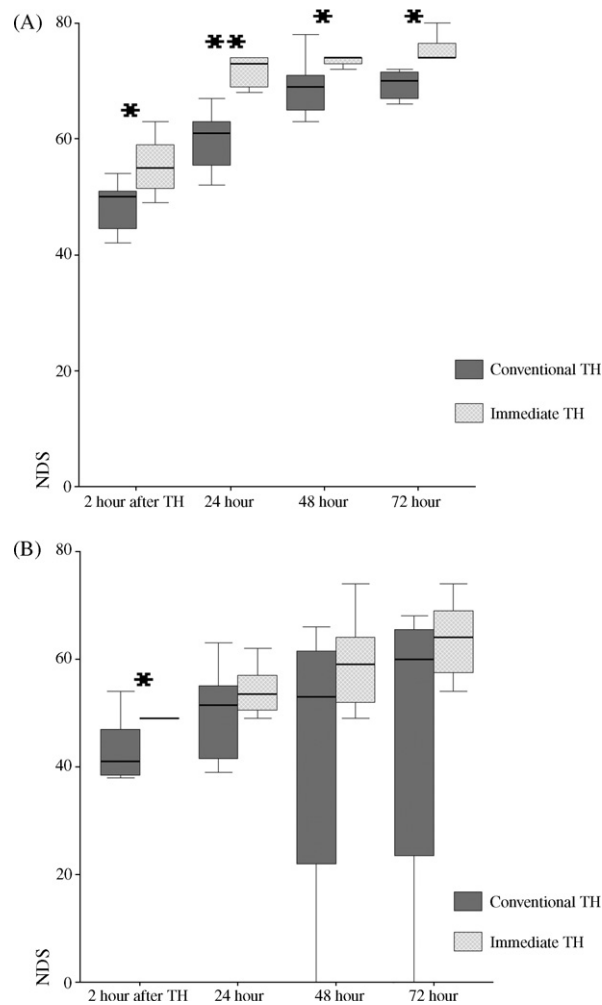


Figure 4 NDS by injury and temperature groups (median (25–75th percentile)), TH: therapeutic hypothermia. (A) significant difference was noted over the 72-h experiment in (A) 7-min immediate hypothermia (7IH) vs. conventional hypothermia (7CH) ($p = 0.001$) and (B) 9-min IH (9IH) vs. CH (9CH) ($p = 0.022$) asphyxial cardiac arrest (CA). Significant differences existed in all periods between the 7-min groups and at 2 h post-hypothermia between the 9-min groups ($^*p < 0.05$, $^{**}p < 0.01$). It was noted that qEEG was able to detect the significant difference as early as 30 min between 9-min groups and qEEG value correlated well with 72-h NDS as early as 1 h after CA.

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

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

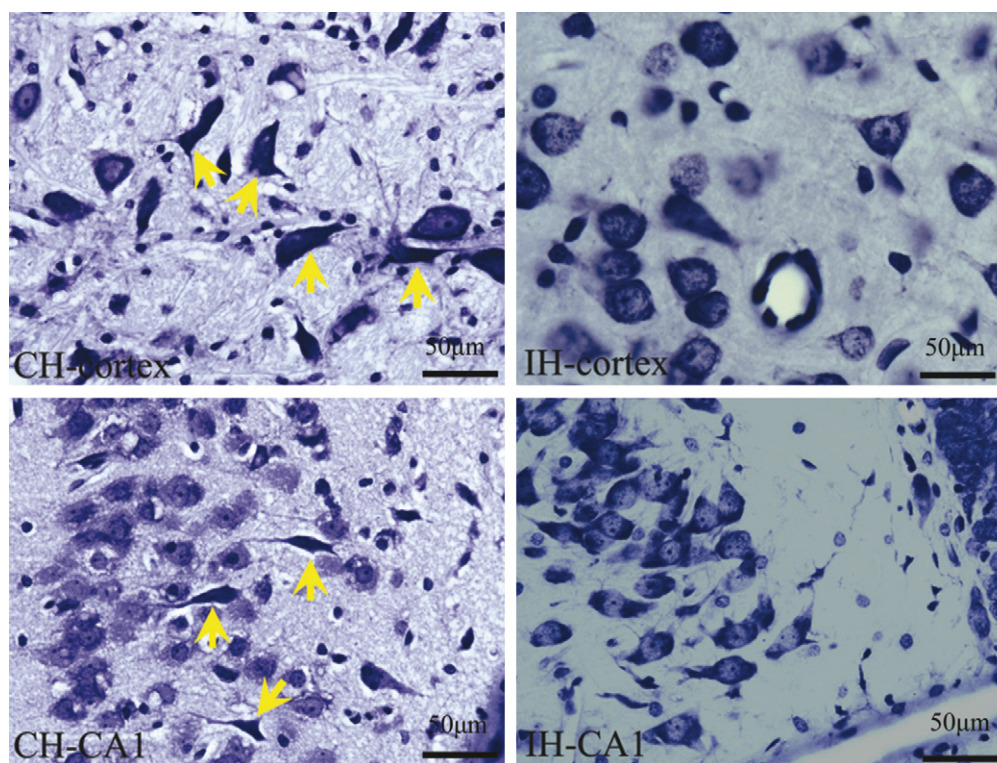


Figure 5 Photomicrograph illustrating of brain injury in CA1 and cortex in 9-min asphyxia rats by hypothermia groups. Greater ischemic neuronal death (\uparrow) was found in conventional hypothermia (CH) rats (Cresyl violet staining, 400 \times). IH: immediate hypothermia.

Table 2 Quantitative comparison of brain injury with histopathological damage scoring (% , mean \pm S.E.M.)

	7-min CH ^a	7-min IH ^b	9-min CH	9-min IH
CA1	20.9 \pm 7.4	12.1 \pm 3.0	28.9 \pm 8.2	17.4 \pm 5.6
Cortex	30.6 \pm 5.3	19.2 \pm 3.6	36.2 \pm 7.7	18.5 \pm 3.8

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 h and were excluded from quantitative brain histopathological damage scoring.

^a Conventional hypothermia.

^b Immediate hypothermia.

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 h versus 12 h). We observed that the better qEEG recovery of the IH group, which was established during 30 min to 4 h after ROSC, was validated by both NDS and HDS (cortex) at 72 h. 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 Association⁴⁰ 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 CA⁴¹ and (b) the challenge to improve outcomes further with earlier yet shorter hypothermia delivery.

Previous experiments of delayed induction of hypothermia (by 1 h 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 ($33 \pm 1^\circ\text{C}$), 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 consensus⁴⁷ showed that histological outcomes do not readily translate into clinical outcomes, as reflected in the 7-min CA group. The significant neuronal injury observed in CA1 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 min 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 min post-CA, all rats showed a decline in pH from the asphyxial cardiac arrest. These values however, are a result of the 10-min post-ROSC hyperventilation period

(tidal volumes 10 ml/kg, respiration rate 65 min^{-1} and positive expiratory end pressure 6 cm H₂O) 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 min while hypothermia has been reached in 9IH group (average cooling time to target is 11.1 min). Hypothermia may decrease the P_{CO_2} in 9IH while CO₂ elevation is due to normothermia in 9CH animals, which is consistent with other recent publications.^{48–50} By 40 min, hypothermia has been ongoing in the IH group for ~ 30 min 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 resistance.^{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 ($32\text{--}34^\circ\text{C}$) 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 10–90 min^{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 h 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.

Conflict of interest

There are no conflicts of interest in this study.

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Appendix A

A.1

The NDS and its components can be found in Table A.1

(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

Table A.1 (Continued)

(C) Motor assessment Strength	Total score: 6 Normal 3/stiff or weak 1/no movement or 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 Gait coordination Balance on beam	Total score: 6 Normal 3/abnormal 1/absent 0 Normal 3/abnormal 1/absent 0
(F) Behavior Righting reflex Negative geotaxis Visual placing Turning alley	Total score: 12 Normal 3/abnormal 1/absent 0 Normal 3/abnormal 1/absent 0 Normal 3/abnormal 1/absent 0 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 × 0.5 m alley); (3) visual placing (the animal is lifted and is able to visually orient itself to objects and depth); (4) negative geotaxis (animal placed on its back on a plane angled at 45° corrects itself and moves up the incline).

A.2. 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.^{15,55,24,56–58}

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.⁵⁹ 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 $0 \leq p(m) \leq 1$ 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 $\{s(i): i = 1, \dots, N\}$ denote the raw sampled EEG signal. Now we define a sliding temporal window $w \leq N$, and the sliding step $\Delta \leq w$. Then sliding windows are defined by

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

where $n = 0, 1, \dots, [N/\Delta] - w + 1$ 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:

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

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

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

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

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

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 $\{s(i): i = 1, \dots, N\}$. In short, IQ is the Shannon entropy of the decorrelated entire EEG data set.⁵⁵ Sub-band IQ (SIQ) is the average value of IQ within different frequencies bands such as 0–2, 2–4, 4–8, 8–16, and 16–32 Hz. SIQ has better distinction capacity and separately characterizes recovery trends in different bands.^{16,60}

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