

**EVALUATION OF ELECTROPHYSIOLOGICAL PROGNOSTIC
TOOLS TO TRACK BRAIN RECOVERY WITH TEMPERATURE
MANAGEMENT AFTER CARDIAC ARREST**

by
Leanne Moon Young

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ABSTRACT

Cardiac arrest (CA) occurs in over 347,000 adult Americans annually and is a leading cause of morbidity and mortality. While targeted temperature management (TTM), a neuroprotective treatment, is often implemented following resuscitation, CA typically results in an ischemic brain injury that is detrimental to functional outcome. Prognosis shortly following CA is crucial as it may guide subsequent treatment. Although multiple prognostic tools exist, many have not been verified under TTM or have limitations in early prognostication. Thus, a reliable tool that predicts functional outcome during the early recovery period after CA is required. For this project, it was necessary to first review current literature detailing the existing prognostic tools and their limitations.

Many of the prognostic tools that have been employed have limitations including subjective results interpretation and the confounding effects of the sedatives required for TTM. However, somatosensory evoked potentials (SSEP) and electroencephalogram (EEG) have potential to track recovery. The bilateral absence of the human N20 SSEP peak is currently the most reliable predictor of poor outcome, however, the signal interpretation is subjective and limited by the dichotomous categorization. Thus, the next step in this work included quantitative analyses of SSEP signals to identify objective prognostic markers.

First, the peak amplitude and latency of SSEP signals were calculated. The amplitude of N10 peaks and latency of N7 and N10 peaks in rats were measured objectively and were distinct among temperature and outcome groups. A more complex

and novel calculation of the SSEP phase space area (qSSEP-PSA), which considers multiple SSEP characteristics, was then performed. Animals treated with hypothermia tended to have higher qSSEP-PSA values and better qSSEP-PSA recovery over the early recovery period. These analyses demonstrate that quantitative SSEPs can track brain recovery shortly after resuscitation.

EEG is a common brain-monitoring tool. The information quantity (IQ), a quantitative measure of the EEG information content, was calculated in post-CA rats that underwent laser speckle contrast imaging (LSCI), which measures relative cerebral blood flow (rCBF). IQ was significantly and negatively correlated with rCBF during the early recovery, suggesting that electrical activity recovery can be maintained by lower rCBF shortly after CA.

Thesis Committee: Dr. Xiaofeng Jia, Dr. Kevin Yarema, Dr. Zeng-Jin Yang

THESIS COMMITTEE

Dr. Xiaofeng Jia, M.D., Ph.D. **Project Advisor**

Associate Professor, Department of Neurosurgery, Orthopedics

University of Maryland School of Medicine;

Department of Biomedical Engineering, Anesthesiology and Critical Care Medicine

Johns Hopkins University School of Medicine

Dr. Kevin Yarema, Ph.D. **Committee Chair**

Associate Professor, Department of Biomedical Engineering

Johns Hopkins University

Dr. Zeng-Jin Yang, M.D., Ph.D. **Committee Member**

Assistant Professor, Department of Anesthesiology and Critical Care Medicine

Johns Hopkins University School of Medicine

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CHAPTER 1: TRACKING EARLY BRAIN RECOVERY FOLLOWING CARDIAC ARREST WITH TARGETED TEMPERATURE MANAGEMENT

1.1 Cardiac Arrest

1.1.1 Prevalence and Impact of Cardiac Arrest

An estimated 320,200 adults suffer from out-of-hospital cardiac arrest (CA) in the United States annually [1]. Survival to discharge rate among adults is a mere 10.6% while the rate of good functional outcome is only 8.3% [1]. While the treatments to improve outcome after CA are progressing, it remains one of the leading causes of unexpected death and many patients remain comatose following resuscitation, die before discharge or have poor functional outcome after awakening. CA is one of the primary causes of morbidity and mortality [2]. In addition to the physical impacts of CA, there are significant economic impacts resulting in societal burdens, defined by the productive years of life lost, equal to or greater than other leading causes of death in the US [3]. Thus, the major societal impact of CA underlies the importance of improving prognostication and treatment of post-CA patients to increase survival rates and neurologic recovery.

1.1.2 Cerebral Ischemia

During CA, cerebral ischemia occurs such that the brain fails to receive enough blood to maintain metabolic processes, causing a hypoxic-ischemic cerebral injury. While the injury due to the cardiac event may last for hours or days, it is believed that the primary damage occurs during the ischemic episode and reperfusion [4]. It is well documented and accepted that the neurotoxicity caused by cerebral ischemia is, at least in part, due to increases in extracellular glutamate during cerebral ischemia [5] and reperfusion [6, 7], leading to excitotoxicity [8-10]. The hyperexcitability seen in ischemic injury is likely a combination of both increased excitation and decrease in inhibition, as it is suggested that impairment to GABAergic neurons, associated with inhibitory functions, may play a role in overexcitation [11]. However, the neurotoxic biochemical cascade that occurs during and after cerebral ischemia is complex and may be initiated by a number of factors [12, 13]. The detailed mechanisms are further explained in multiple reviews [14-17].

In addition to the ischemic event itself, injury continues to occur during and after reperfusion. Thus, the cerebral blood flow following resuscitation is of great importance. There are a number of studies that examined reperfusion injuries, including the hypothesis that an increase in glutamatergic transmission during reperfusion, which is supported by *in vitro* work [18], may intensify the excitotoxic injury caused during ischemia [19]. Another widely suggested explanation of reperfusion injury involves oxygen radicals [20, 21], suggesting that the return of oxygen to the brain allows harmful enzymatic oxygenation reactions to occur [22].

Neurologic injury is a crucial contributor to the morbidity and mortality of post-CA patients [23], thus, it is important to understand the mechanisms underlying the ischemic/reperfusion injury and how to assess and monitor the level of injury following CA.

1.1.3 Functional Outcome and Neurological Recovery Assessment

The cerebral cortex is among the most vulnerable regions of global cerebral ischemia [24]. Extensive bilateral cortical or thalamocortical damage may result in problems with arousal and consciousness [25], which is likely why many post-CA patients remain comatose for some time. The brainstem is more tolerant to ischemic damage, resulting in the preservation of sensory motor reflexes in many patients, even in a comatose state [4]. It is important that physicians assess the injury level and neurological recovery of post-CA patients in terms of functional outcome, however this is complicated when patients remain comatose.

The Glasgow coma scale (GCS) is a clinical scale that was developed to evaluate the depth and duration of comatose patients or patients with impaired consciousness [26], however, it has also been used in the assessment of comatose post-CA patients [2, 27-33]. The scale measures three behavioral features – motor responsiveness, verbal performance and eye opening. Each category is given a score from 1 to 5. Often the scores are summed to give a total score from 3 (worst) to 15 (best), however, the original creators of

the scale suggest that each category should be assessed on an individual basis for clinical use [33].

The cerebral performance category (CPC) scale was created [34] and modified [35] to uniformly evaluate cerebral performance. The scale has 5 levels, with CPC 1 representing the best performance and 5 representing the worst (Table 1.1). Many groups have used CPC to evaluate functional outcome after CA [27, 34, 36-39] where in most cases, but not all [40], CPC scores of 1 or 2 represent patients with good outcome and 3-5 are poor outcome [23, 41, 42].

Table 1.1 Cerebral Performance Categories

CPC Level	Cerebral performance description
CPC 1	Good cerebral performance
CPC 2	Moderate cerebral disability
CPC 3	Severe cerebral disability
CPC 4	Persistent vegetative state
CPC 5	Brain death or clinical death

The neurologic deficit scale (NDS) is used to assess functional recovery of post-CA rats, and was developed based on human neurological examination and animal functional outcome scales for global ischemia models [43-45]. The scale evaluates performance in a number of behavioral tests including gait coordination, righting reflex and pupillary light reflex (Table 1.2). The 72 hour NDS score is generally used to determine final functional outcome in asphyxia CA rat models. An NDS ≥ 60 is generally defined as good functional outcome and NDS < 60 is defined as poor functional outcome [46]. Animals with good functional outcome are mobile and have appropriate responses to stimuli whereas animals with poor functional outcome are immobile and have minimal stimuli reactions. This scale has been validated in post-CA rat models [47, 48].

Table 1.2 Neurological Deficit Scale Scoring

<p><u>Arousal:</u></p> <p>Alerting: Normal (0) / Stuporous (5) / Comatose (0)</p> <p>Eye Opening: Open spontaneously (3) / Open to pain (1) / Absent (0)</p> <p>Spontaneous Respiration: Normal (6) / Abnormal (3) / Absent (0)</p> <p>Total Score : 19</p>
<p><u>Brainstem Function:</u></p> <p>Olfaction: Present (3) / Absent (0)</p> <p>Vision: Blinks to threat (3) / Absent (0)</p> <p>Pupillary Light Reflex: Present (3) / Absent (0)</p> <p>Corneal Reflex: Present (3) / Absent (0)</p>

Startle Reflex: Present (3) / Absent (0)

Whisker Stimulation: Present (3) / Absent (0)

Swallowing: Present (3) / Absent (0)

Total Score: 21

Motor Assessment:

Strength: Normal (3) / Weak movement (1) / No movement (0)

(Each side tested and scored separately)

Total Score: 6

Sensory Assessment:

Pain: Brisk withdrawal (3) / Weak withdrawal (1) / No movement (0)

(Each side tested and scored separately)

Total Score: 6

Motor Behavior:

Gait coordination: Normal (3) / Abnormal (1) / Absent (0)

Balance Beam Walking: Normal (3) / Abnormal (1) / Absent (0)

Total Score: 6

Behavior:

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)

Total Score: 12

Seizures:

Seizures: No seizure (10) / Focal seizure (5) / Generalized seizure (0)

Total Score: 10

1.2 Targeted Temperature Management

1.2.1 Hypothermia

Targeted temperature management (TTM), which involves cooling the body to a hypothermic state, has been regarded as an effective treatment following out-of-hospital CA to improve survival and functional outcome [49]. While the specific mechanisms underlying the neuroprotection benefits of TTM are not fully elucidated, it is believed that the cooling reduces the functional requirements of the cells, thereby protecting them. Normothermic cerebral neurons cannot last more than 5 mins in an ischemic anoxic state, thus, TTM is believed to improve neurological functional outcome likely by reducing the cerebral oxygen requirements, preventing free-radical injury and cell membrane damage, or inhibiting the release of damaging neurotransmitters [50].

The ischemic brain has been shown to be highly sensitive to temperature fluctuations [51], which has a significant impact on treatment during the early recovery following resuscitation. The temperature sensitivity of brain injury is likely modulated by increased

levels of excitatory amino acids and free radicals, ischemic depolarization, increased ion flow, pathological events, and post-ischemic microvascular abnormalities [52-58]. Mild hypothermia, which decreases cerebral metabolic rate of oxygen (CMRO₂) consumption [59], has been shown to improve survival and functional outcome following CA in human [23, 41, 60-67] and animal studies [68-73].

Though it is well-established that mild hypothermia improves survival and functional outcome following CA, there is still controversy regarding the various induction factors, including start time, length of treatment, degree of treatment, and method of cooling. Various studies indicate that hypothermia should be initiated immediately after resuscitation, beginning in the field and that a delay of even 15 min could reduce or eliminate the neuroprotective effect [71], while others suggest that a delay before cooling may also be beneficial [74-78]. Additionally, there has been uncertainty regarding the degree of hypothermia and length of application that are most beneficial. The International Liaison Committee on Resuscitation recommended TTM of 32-34°C for 12-24 hours [79]. However, one study has demonstrated that TTM of 36°C is no less beneficial than TTM of 33°C [80]. Thus, while the beneficial effects of TTM have been thoroughly demonstrated, the details of treatment are not unanimously agreed upon.

1.2.2 Hyperthermia and Pyrexia

Hyperthermia has been shown to worsen brain damage in animals [54, 55, 57, 81] and humans [52, 56, 58, 82, 83] in post-CA recovery. Though hypothermia is now commonly used as a means of neuroprotection, it often results in rebound hyperthermia (body temperature $> 38.5^{\circ}\text{C}$) within 24h following rewarming from TTM [83], with a reported prevalence ranging from 22-74% of patients treated with TTM [84, 85]. Studies have shown that in patients who experienced rebound hyperthermia, outcome was worsened [83] and a higher maximum temperature was associated with worse outcome [56]. Despite efforts to finely control temperature during recovery, pyrexia ($\geq 37.6^{\circ}\text{C}$) is common in both TTM and non-TTM patients [86] and also tends to worsen outcome in non-TTM patients [58]. Thus, whether pyrexia occurs spontaneously from a normothermic condition during recovery or as a result of TTM rewarming, it is well documented that fever is harmful to recovery [49].

1.3 Current Prognostic Tools

1.3.1 Importance of Early Prognostication

Early prognostication shortly after resuscitation from CA is essential as it can help guide treatment, reasonably distribute resources and counsel family regarding likely outcome. Current prognostic markers have been verified in normothermic patients however, their reliability in hypothermic patients has not been thoroughly studied and verified.

Overall, many CA patients remain comatose during early recovery, which makes prognostication of functional outcome and treatment planning difficult. It is crucial to properly identify those patients in a comatose state following CA that have a chance to recover versus those who will never awaken. Ideally, reliable prognostication would be possible immediately after resuscitation, while most patients are still comatose and under TTM. Currently, there is no tool that can reliably achieve this prediction under TTM. Early prognostication would be a major step towards optimizing treatment for each individual patient.

1.3.2 Ideal Prognostic Marker Features

The prognosis tools need to reliably predict outcome following CA during the early recovery period in an objective manner under TTM conditions. This primarily means that the tool cannot be largely confounded by sedatives or paralytics or by temperature. Additionally, the prognostic tool should robustly predict both good and poor outcome with high sensitivity and specificity. Further, the prognostic test must be objective, simple to implement in a wide range of medical centers (i.e. does not require highly specialized training or equipment), and time and cost effective. Ideally, the prognostic tool would generate a quantitative value for the patient, which could be compared to a numeric cutoff point to predict good or poor outcome.

1.3.3 Current Tools and Limitations

Awakening from a coma is the best predictor of good outcome, however, a well verified early recovery prognostic indicator for good outcome that can be measured while the patient remains in a coma, has not yet been established. Conversely, there are a number of methodologies to predict poor outcome for normothermic comatose patients, with somatosensory evoked potentials (SSEP) being the most widely accepted.

A number of prognostic tools have been developed to predict outcome following CA under normothermic conditions including bedside clinical assessment, electroencephalogram (EEG) monitoring, biomarkers and somatosensory evoked potentials (SSEP). However, many of these techniques have major limitations such as requiring highly subjective manual pattern recognition and impedance from the sedation that is necessary for TTM [87, 88]. Additionally, the prognostic value of these techniques has not been convincingly verified under TTM.

Clinical Examination

Clinical examination is one of the oldest prognostic tools for post-CA comatose patients. These exams are beneficial as they are universally available and relatively simple for physicians to perform. One common clinical examination is coma or cerebral performance scoring using scales such as GCS [2, 89-91]. Reflex tests such as brainstem reflexes that test various cranial nerves [92, 93] like the pupillary reflex (nerves II and

III), corneal reflex (nerves V and VII), and gag and cough reflex (nerves IX and X) are also common. Vestibular signs may also be tested using oculoccephalic or oculovestibular reflex tests [94]. Finally, observance of seizures or myoclonus status (sudden muscular contractions) [31, 95-97] has been used in the assessment of patient prognosis.

Based on 5 studies [29, 31, 91, 96, 98], the most useful clinical tests at 24 hours post-CA in predicting poor outcome are absent corneal reflexes, absent pupillary reflexes, absent motor response and absent withdrawal to pain [94].

However, clinical examination has major downsides, particularly under TTM. One major drawback is that sedatives and paralytics must be temporarily stopped to successfully perform clinical examinations. Additionally, no clinical tests are able to predict good outcome of comatose patients or accurately predict outcome immediately after resuscitation [94].

Electroencephalogram (EEG)

EEG is one of the most widely used tools in post-CA patient monitoring [99, 100]. EEG represents the summation of neuronal electrical activity within a spatial region and has distinct frequency bands, which have been distinguished based on clinically relevant values: delta (< 4 Hz), theta (4-8 Hz), alpha (8-15 Hz), beta (16-30 Hz), and gamma (> 30

Hz), each representing different cerebral responses and activities [101, 102]. EEG is useful in assessing ischemic damage post-CA due to the typical changes in EEG amplitude and frequency of these relevant bands [103].

Amplitude integrated EEG (aEEG), a processed EEG that weighs the signal based on amplitude, has been shown to have prognostic value when applied during the normothermia state after rewarming from TTM, approximately 37h after CA [104]. In the first 4-8h after beginning the EEG recording, continuous aEEG was correlated with good outcome [104, 105], however, it is flat for most patients, which held no prognostic value [105] so the usefulness of this association is limited.

Continuous EEG (cEEG) is another form of EEG measurement. cEEG has been shown to hold prognostic value during TTM (approximately 12 hours after CA), suggesting that background reactivity of cEEG is not impacted by sedation or temperature [106]. However the cEEG interpretation is still done manually and is therefore subjective and laborious. Another study of cEEG in post-CA TTM patients demonstrated that when cEEG was graded on a scale of 1-3 (1=benign, 3=severe), scores of 1 and 3 were associated with good and poor outcome, respectively, during hypothermia and normothermia [107]. However, there were major cost and labor requirements to employ this cEEG monitoring method.

Thus, there are various drawbacks to the aforementioned EEG prognostic methods, including the confounding effects of sedation, ineffectiveness during the early recovery period, and the subjective and laborious nature of the signal interpretation. Further, it has been suggested that EEG is most useful for prognostication at 24h+ after resuscitation [108, 109], which eliminates its value in tracking early recovery while under TTM. To circumvent these limitations, quantitative EEG measurements that do not require manual pattern recognition or interpretation have been developed.

Based on the notion that cerebral recovery following CA is represented by the EEG information content, an objective, quantitative EEG marker, information quantity (IQ), was developed to track post-CA recovery [110]. The IQ measure is representative of the entropy of the EEG signal. However, since EEG contains multiple frequency bands that represent various brain functions, a modified IQ value was developed, sub-band IQ (SIQ), which represents the IQ value within each clinical sub-band (delta, theta, alpha, beta, and gamma) [111]. Both of these quantitative measures have been shown in numerous animal studies to reliably track neurological function in the early post-CA recovery period under TTM [46, 110-115].

Biomarkers

Following CA, biomarkers such as the concentration of neuron-specific enolase (NSE) from serum or cerebral spinal fluid (CSF) change, and thus have been recognized as

possible predictors for functional outcome [116-123]. Increased levels of CSF NSE, which is primarily located in neurons and neuroectodermal cells, has been associated with poor neurologic outcome in patients with anoxic encephalopathy [117, 122, 124], however, serum NSE is predominantly used for CA patients, for convenience and safety reasons. Multiple studies have demonstrated that higher levels of serum NSE are associated with poor neurologic outcome following CA [116, 125, 126], however, at least one study was unable to reproduce this finding [124]. Additionally, the NSE cutoff level and ideal sample time have not been validated in larger studies [127, 128]. Thus, the serum NSE biomarker presents limitations in the prognosis of post-CA patients.

Somatosensory Evoked Potentials (SSEP)

SSEP following CA involves nerve stimulation and non-invasive measurement of the corresponding responses from the somatosensory cortex, which evaluates the somatosensory pathway and transmission within the central nervous system and brainstem [50]. The technique measures the initial cortical responses after repetitive and alternating stimulation of the median nerves of both wrists, though other stimulation and recording sites may also be used. The negative cortical response 20ms after stimulation, named the N20 peak, is commonly used in post-CA prognostication. The bilateral absence of the N20 peak robustly predicts poor functional outcome [42, 88, 129-136]. Further, the bilateral absence of N20 has been shown to predict nonawakening from coma following conditions other than cardiac arrest [137]. Thus, SSEP has been shown to hold great prognostic value for poor outcome cases following CA.

1.4 Somatosensory Evoked Potentials (SSEP)

While a number of studies have been conducted to validate existing prognostic markers under TTM, there have been inconsistent results across studies. Further, progress in the development of a quantitative and objective prognostic method for post-CA recovery has been limited. While a quantitative SSEP (qSSEP) algorithm has been developed, it has not been validated under temperature management conditions.

1.4.1 SSEP is one of the Best Predictors for Poor Outcome

Following cardiac arrest, patients are often in a state of comatose, making previously standard clinical examinations difficult and unreliable. Electroencephalogram (EEG) has been a common technique to observe brain activity in comatose patients for a long time, however, more recently somatosensory evoked potentials (SSEPs) have been proven to be more reliable in predicting outcome [133, 134]. Further, with TTM becoming an increasingly standard treatment following out-of-hospital CA, EEG and clinical examination are becoming less reliable, as the sedation and muscle relaxants required for hypothermia confound the results of these methodologies. Thus, SSEP has come to the forefront as a better prognostic measurement, as it has a simple bedside setup, is relatively non invasive, and is not confounded by sedative drugs [88].

SSEP has been suggested as one of the most reliable tools to predict functional outcome following CA in normothermic human patients [133, 134]. While there are numerous

features of SSEP that can be extracted, the bilateral absence of the short-latency N20 peak (the negative cortical peak occurring approximately 20ms after stimulation) has been regarded as a reliable indicator for poor functional outcome following CA [133, 134, 136] with TTM [42, 88, 129-132, 135]. However, its reliability has recently been challenged, as a few patients have been reported to have positive functional outcomes after having bilaterally absent N20 peaks [138, 139], though some experts have deemed these cases insufficient to counter the prognostic value of SSEP [140]. Further, while the bilateral absence of N20 peaks indicates poor outcome, the presence of N20 peaks does not suggest good outcome. Thus, it is important to develop a threshold for present SSEP peaks to predict good outcome. Additionally, SSEP is currently used for poor outcome assessment by the bilateral absence of N20 peaks, however, this evaluation is very subjective, even among highly trained experts [141, 142].

Additionally, there are conflicting views among different groups regarding the ideal window of time in which SSEP measurements have the highest prognostic value. Some groups suggest that SSEPs should be measured after 24 hours post-CA [143, 144], while others suggest that 1 hour post-ROSC leads to the best predictive ability [145]. It has been argued that SSEP recordings before 24 hours are not useful because SSEPs may be confounded by impaired cerebral perfusion and reperfusion damage [145]. However, since excitotoxic-ischemic cascades begin shortly after injury, many treatment plans, namely TTM, are most effective when applied immediately after resuscitation, so early evaluation is crucial.

Yet, SSEP measurements still hold great benefits in prognosis following CA as these measurements are repeatable, non-invasive, unimpeded by sedation, and present multiple characteristics that can be quantified, such as latency, amplitude, and shape [88, 146].

1.4.2 Reliability of SSEP Under Targeted Temperature Management

The validation of SSEP under TTM, an increasingly standard treatment, is necessary. Some studies have suggested that hypothermia decreases the prognostic value of the standard SSEP tests in the early stages of recovery after cardiac arrest [128, 139], while others have demonstrated that SSEP remains reliable for poor outcome prediction under hypothermia [88], though there is inconsistent and relatively limited data in these areas to be reliably conclusive.

Most studies comparing SSEP in hypothermic patients to normothermic patients have found that bilaterally absent N20 remains reliable in predicting poor outcome. In one study comparing 14 hypothermic to 27 normothermic patients who received SSEP days 1-3 after CA, bilaterally absent N20 was an invariable predictor of poor outcome [132]. In another study comparing 30 hypothermic and 27 normothermic patients, 3 hypothermic and 8 normothermic patients had bilaterally absent N20, none of whom regained consciousness [135]. Further, a study of 46 hypothermic patients who received SSEP showed that 47.4% of the patients with poor outcome had bilaterally absent N20 responses [129]. In another study with 75 patients undergoing hypothermia, all patients

had SSEPs recorded during hypothermia and again in 34 patients during normothermia after rewarming. During hypothermia, 13 patients had bilaterally absent N20, all of whom had poor outcome. This study also demonstrated that bilateral absence of N20 during hypothermia is a good predictor for bilateral absence of N20 after rewarming [130], which is an important finding, as earlier testing can improve the titration of the hypothermia treatment. Finally, in another CA study, 30 patients had SSEPs recorded approximately 72hrs after being rewarmed from hypothermia. Fourteen of these patients had bilaterally absent N20 and all died without regaining consciousness [147].

However, there are studies that have rare false positive cases that have caused concern regarding the method's reliability under TTM. In a study comparing SSEP of 110 hypothermic patients (33°C) to 94 normothermic patients (36°C), there was a false positive rate of 2.6% for bilaterally absent SSEP N20 [148]. Similarly, in a meta-analysis by Sandroni et al, there were no patients in the included studies that recovered when the N20 was bilaterally absent during TTM but in 538 patients that had SSEP recordings following rewarming, there was 1 false positive [149].

Overall, while SSEP results have been shown to aid the prognostic process after resuscitation, the results have led experts to caution against relying on bilaterally absent N20 responses in making withdrawal of life support treatment decisions.

1.4.3 Prognostic Value of SSEP for Good Outcome Prediction

While the bilateral absence of N20 SSEPs robustly predicts poor outcome in post-CA patients, the presence of unilateral or bilateral N20 peaks does not predict good outcome. Currently, there are no verified SSEP measures that predict good functional recovery of comatose patients. It would be useful to have a quantitative measure of SSEP with a defined cutoff point, which could robustly dichotomize both good and poor outcome.

1.4.4 Quantitative SSEP is an Objective Prognostic Indicator

As previously mentioned, an objective and reliable measurement of SSEP is necessary to eliminate the variability in data interpretation and to simplify the results. A quantitative measure of SSEP could achieve these requirements, if verified to predict final outcome under temperature management.

Amplitudes and Latencies

Perhaps the most obvious method of quantification of SSEP signals is the calculation of peak amplitude and latency. Multiple groups have studied the amplitude and latency of SSEP under temperature management in non-CA patients and animals [150-159] and under normothermic and hypothermic conditions in post-CA patients [135]. Overall, these studies demonstrate that hypothermia increases peak latency, however, there were inconsistent results regarding the effect of hypothermia on SSEP amplitude. The

mentioned studies observed increases, decreases or no change in amplitude, based on the recording site and condition of the subject. Additionally, one study found that under hyperthermic conditions, higher temperatures decrease peak latency and have no significant effect on peak amplitudes [154].

In post-CA patients, SSEP amplitude and latency have been studied by a few groups, but their prognostic values have not been extensively examined. One group found that post-CA patients treated with hypothermia had significantly prolonged cortical N20 peaks compared those treated with normothermia [135, 160]. Additionally, the same study found that N20 amplitudes were not significantly different between temperature groups and was not associated with functional outcome. However, this study lacked the statistical power to analyze the correlation of N20 latency or amplitude with outcome to determine their prognostic value. Another study found that normothermic patients with hypoxic-ischemic damage, defined by CPC > 2 at 1 year, had significantly prolonged N20 latency and lower N20 amplitude compared to those without hypoxic-ischemic damage [160]. Finally, a study of post-CA patients treated with TTM found a threshold amplitude, 0.62uV, at which lower amplitudes were associated with poor outcome [161]. Thus, SSEP amplitude and latency hold possible prognostic value, though they need to be more rigorously verified.

Phase Space Area

A novel quantitative SSEP (qSSEP) technique has been developed and tested in rats [145]. This qSSEP metric is important, not only because it helps to eliminate the subjective nature of SSEP interpretation, but it has also been shown to predict good outcomes in post-CA rats, whereas standard manual interpretation of absent N20 responses (N10 responses in rats) only predicts poor outcome.

The quantitative analysis uses the phase space curve (PSC), a plot of the first derivative against magnitude, to compute the phase space area (PSA), which is indicative of signal power and quantified to obtain a single PSA value that represents the functionality of the somatosensory pathway [145]. The PSC differs from standard SSEP waveform analysis in that it incorporates multiple peak characteristics including peak amplitudes, slopes, and interpeak latency [145]. In the initial study by Madhock et al, PSA increased over the early recovery period (4 hours after ROSC) and while the animals with good and bad outcome (defined by NDS scores) were not differentiable by PSA in the first hour after ROSC, they did have significantly different PSA values in the following 3 hours of early recovery (85-190 min post-ROSC) with an outcome prediction accuracy of 80-93% ($p < 0.05$) and 78% sensitivity to good outcomes with 83-100% specificity [145]. Further, the early recovery PSA (within the first 4 hours after ROSC) successfully predicted 72hr neurologic outcomes, which suggests that early recovery SSEP measurements hold significant prognostic value [145]. To compare this method to standard SSEP analysis,

N10-P15 peak-to-peak amplitude was shown to have similar trends as the PSA, but they were less separated between the outcome groups and had a higher variability.

Thus, this qSSEP method provides a technique that doesn't require sophisticated peak detection but can still track brain injury and is a useful predictor to differentiate between good and poor outcome [145]. One major limitation of the current information on this method is that this study was done using normothermic animals, and needs to be repeated with temperature management to validate the qSSEP method in under TTM. While qSSEP still needs to be validated in animals under hypothermia after CA, it shows promise as an objective early recovery prognostic indicator.

1.4.5 Conclusion

Prognosis of functional outcome while patients are comatose following resuscitation after CA remains an important area of clinical research. Due to the subjective nature of SSEP interpretation, current prognosis markers are inadequate in providing reliable information to dictate treatment options and medical decisions, particularly with TTM becoming a widely used treatment post-CA. While many groups have demonstrated that bilaterally absent N20 responses remain reliable in predicting poor outcome with TTM treatment, the results are not convincing to all experts. Further, development of a quantitative SSEP measurement has been initiated, however, its validation under temperature management is necessary. Overall, the prognostic value of SSEP can be greatly improved by the

validation of an objective and reliable method under TTM administration following resuscitation.

CHAPTER 2: QUANTITATIVE SOMATOSENSORY EVOKED POTENTIALS DURING EARLY RECOVERY ARE ASSOCIATED WITH FUNCTIONAL OUTCOME AFTER CARDIAC ARREST WITH TEMPERATURE CONTROL

2.1 Introduction

Despite advances in pre-hospital care including cardiopulmonary resuscitation (CPR) technique, out-of-hospital cardiac arrest (CA) continues to result in major public health implications [1]. Targeted temperature management (TTM) is currently recommended as a standard neuroprotection method for comatose patients following CA and has been shown to improve survival and functional outcome [49]. Fever is common during recovery [86] and tends to worsen outcome [49, 58]. Overall, many CA patients remain comatose during early recovery, which makes prognostication of functional outcome and treatment planning difficult. Thus, there is a great need for an objective prognostication method to predict functional outcome post-CA during the early recovery period.

A number of prognostic tools have been developed to predict outcome following CA under normothermic conditions, however, many techniques are impeded by the sedation

that is necessary for TTM [87, 88]. The bilateral absence of the short-latency somatosensory evoked potential (SSEP), N20, has been regarded as a reliable indicator for poor functional outcome following CA [133, 134, 136] with TTM [42, 88, 129-132, 135], though still with controversy [138-140]. Further, while the bilateral absence of N20 peaks indicates poor outcome, the presence of N20 has low specificity for good outcome. Thus, it is important to develop a threshold for existing SSEP peaks to predict good outcome. SSEP hold great prognostic benefits following CA as these measurements are repeatable, non-invasive, unimpeded by sedation, and present multiple quantifiable characteristics such as latency, amplitude, and contour [88, 146].

The N7 and N10 rat SSEP peaks, which represent the negative cortical responses at 7 and 10ms after stimulation, are analogous to the N18 and N20 peaks in humans [145]. The rat N7 peak has been shown to recover following CA though its prognostic value has not been identified [162]. Despite recent controversy regarding the value of various degrees of TTM [80, 163], the prognostic value of the presented SSEP markers under TTM of 33°C is important, as TTM of 33°C remains a current standard treatment for post-CA patients, as recommended by international guidelines [49, 79] and has been extensively studied by many groups [23, 41, 51, 67, 164, 165], including our own [46, 112-114, 166].

In this study, we used an established asphyxial-CA rat model to test the hypothesis that SSEP latency and amplitude can be quantified during early recovery from CA as

objective markers to predict functional outcome following resuscitation under TTM conditions.

2.2 Materials and Methods

2.2.1 Animals

A total of 21 adult male Wistar rats ($378\pm 8\text{g}$) were randomly assigned to one of three temperature groups ($n=7$ per group): hypothermia ($33\pm 1^\circ\text{C}$), normothermia ($37\pm 0.5^\circ\text{C}$), or hyperthermia ($39\pm 0.5^\circ\text{C}$). Each animal underwent 7 min asphyxial-CA followed by immediate temperature manipulation, according to their assigned group. An additional 14 rats were assigned to a sham group, which underwent the same experimental procedures except for CA and resuscitation. All experiments were approved by the Institutional Animal Care and Use Committee at Johns Hopkins University.

2.2.2 Electrode Implantation

Approximately 1 week prior to the date of CA, 5 screw electrodes (Plastics One, Roanoke, VA) were cortically implanted in each rat's brain to record the SSEPs [145, 162]. Four electrodes were placed over the somatosensory cortex in the regions that are topographically associated with the forelimbs and hindlimbs, and one ground electrode was placed near the parasagittal right frontal lobe. The electrodes were placed above the dura mater such that they did not penetrate the brain. The electrodes were held in place

with a plastic pedestal and dental cement and the skin was closed around the implant with sutures.

2.2.3 Cardiac Arrest, SSEP and Early Recovery

The rats underwent CA and resuscitation as previously described [46, 110, 113, 145]. Briefly, the rats were anesthetized with 1.5% vaporized isoflurane in 1:1 O₂:N₂ and then a tracheal intubation was performed. A mechanical ventilator (Harvard Apparatus Model 553438) continued to administer the isoflurane mixture with the following ventilation parameters: 50 breaths/minute respiratory rate, 8ml/kg tidal volume, and 3cm H₂O positive expiratory end pressure. The femoral artery and vein were then cannulated (Intramedic Non-Radiopaque Polyethylene Tubing PE-50 catheters, PE 50, Becton Dickinson) to monitor the mean arterial pressure (MAP) and obtain blood samples, and to deliver drugs, respectively. Baseline arterial blood gases (ABGs) were measured via the arterial cannulation. A baseline SSEP was recorded for 15 min followed by a 5 min isoflurane washout. The washout period consisted of 2 min of 100% oxygen followed by 3 min of room air, during which a bolus injection of vecuronium (2mg/kg) was given intravenously to induce muscle paralysis in the rats. The ventilator was then disconnected and the tubes of the breathing circuit were clamped to induce global asphyxia. CA was achieved when pulse pressure < 10mmHg. Immediately following 7 min of CA, cardiopulmonary resuscitation (CPR) was performed with resumption of ventilation (respiratory rate: 40 breaths/min, tidal volume: 8ml/kg, positive expiratory end pressure: 0cm H₂O), 100% oxygenation, sternal chest compressions (200 compressions/min),

intravenous epinephrine (0.005 mg/kg) and intravenous sodium bicarbonate (1mmol/kg) until the return of spontaneous circulation (ROSC), which was defined as pulse pressure > 60mmHg. Hyperventilation was induced for 10 min using the ventilation parameters (respiratory rate: 65 breaths/min, tidal volume: 8ml/kg, positive expiratory end pressure: 0cm H₂O), at which point the respiratory rate was switched to 55 breaths/min for 10 min and subsequently 50 breaths/min. ABGs were measured at 10, 20 and 40 mins after ROSC. Approximately 2 hours following ROSC, animals were extubated. All animals were closely monitored throughout the duration of the experiment for any signs of distress or pain. Stimulation and SSEP recordings were restarted at 30 minutes after ROSC and were maintained in 15 min intervals until 4 hrs after ROSC. During the recovery period, up to 0.5% isoflurane was used when SSEP recordings were restarted (30 min post-ROSC), dependent on the rat's recovery, due to the potential discomfort of the nerve stimulation [145].

2.2.4 Temperature Management

For the hypothermia group, cooling began immediately after ROSC and the target temperature was reached within 10 mins. Cooling was achieved using an alcohol/water mist and a small electric fan. The core temperature was maintained at 32-34°C for 4 hrs. Rewarming began after 4 hrs of hypothermia until normothermia was reached over a period of 2 hrs. For the hyperthermia animals, warming began immediately after ROSC until the target temperature was reached, within 15 minutes. Warming was done using a heating lamp and heating pad (Thermalet TH-5, model 6333, Physitemp Instruments,

Clifton NJ). Hyperthermia, 38.5-39.5°C, was maintained for 4 hrs after ROSC and passive cooling occurred over a period of 2 hrs until normothermia was reached. The core temperature of the normothermia rats was maintained at 36.5-37.5°C for 4 hrs after ROSC using a heating pad. A neonatal incubator was used for the following 24 hrs to prevent spontaneous hypothermia [114].

2.2.5 Neurologic Evaluation

The neurological function of rats following CA was determined using the Neurological Deficit Scale (NDS), which has been well verified in the evaluation of motor, sensory and brainstem function [46, 113, 114]. Animals were given an NDS score ranging from 0 (worst) to 80 (best). The NDS was recorded by a trained and blinded researcher 2 hrs after temperature modulation, and at 24, 48 and 72 hrs following ROSC, where the 72 hr score represents the endpoint functional outcome [154, 162]. Based on previous publications, the animals were grouped into good and poor outcome groups based on final NDS score such that good outcome animals represented those with some mobility and quick response to stimuli [46, 113, 114, 145, 162]. Thus, the 72 hr NDS score was used to define the final functional outcome as either good (72 hr NDS \geq 60) or poor (72 hr NDS < 60) [46]. Animals that died before 72hrs were given a 72hr NDS score of 0.

2.2.6 SSEP Signal Sampling and Analysis

The median nerves in the forelimbs of the rats were stimulated using subdermal needle electrodes and the SSEPs were recorded from the screw electrodes in the respective contralateral hemisphere. A pulse generator provided 200-usec 6mA pulses to the needle electrodes at a frequency of 0.5Hz. The SSEP signals were recording using the TDT System3 data acquisition system (Tucker-Davis Technologies, Alachua, FL). The SSEP data was sampled at a frequency of 6.1kHz and was passed through an amplifier before reaching the computer. SSEPs were recorded on the day of the experiment for 15 min before CA (baseline) and for 15 min intervals, beginning 30 min post-ROSC until 4 hrs post-ROSC. The SSEPs were also recorded for 15 minutes at 24, 48 and 72 hrs post-ROSC, with 1.5% isofluorane.

The SSEP signals were averaged over 450 sweeps during the peak detection process. An algorithm in MATLAB was used to calculate the peak amplitudes and latencies. Specifically, the N10 (N10-P15 peak-to-peak) amplitude and N7 and N10 latencies were determined. The N7 and N10 peaks represent the negative responses that occur approximately 7 and 10ms after stimulation, while the P15 peak represents the positive response 15ms after stimulation. The N7 and N10 latencies were measured as the time to the N7 or N10 peak, respectively, from the time of stimulation. The amplitudes and latencies were normalized by the baseline values recorded before CA. Animals with abnormal baseline SSEPs (bilaterally distorted N10 and P15 peaks) were excluded from the analysis.

2.2.7 Statistical Analysis

All statistical analyses were done using the SPSS Statistics computer package (IBM SPSS Statistics v22, Armonk, NY). A nonparametric Kruskal-Wallis test was used to compare the 72 hr NDS between temperature groups, which are reported as (median (25th, 75th percentiles)). The N7 and N10 amplitudes and latencies were compared among outcome groups using a general univariate analysis, while they were compared among temperature groups using repeated measures analysis of variance (ANOVA). Pearson correlation coefficients were determined using bivariate analyses to determine the relation between SSEP markers and 72 hr NDS. Receiver operating characteristic curves were generated to identify N10 amplitude cut-off points at 100% specificity and maximum sensitivity with an area under the ROC curve that is significantly different from the 0.5 reference line. Aggregate latency or amplitude considered all values at each time point over the entire 4 hr early recovery period for all animals within a particular group. A $p < 0.05$ was considered statistically significant.

2.3 Results

2.3.1 Temperature Management, ABG Monitoring and NDS

The core temperature of all animals was well controlled throughout experiments (hypothermia: $33.8 \pm 0.1^\circ\text{C}$, normothermia: $36.9 \pm 0.1^\circ\text{C}$, hyperthermia: $38.5 \pm 0.05^\circ\text{C}$). The target temperature for each group was reached within 12 ± 2 mins and maintained for the 4 hr early recovery period (Fig. 2.1). Baseline arterial blood gases (ABG) were not

significantly different among temperature groups ($p>0.05$) (Table 2.1). The baseline animal weight was not significantly different between temperature groups ($p>0.05$) (Table 2.1). The 72 hr NDS score was significantly higher in the hypothermia group [74 (74,77)] than the normothermic group [68 (49,72)] ($p<0.01$), which was significantly higher than the hyperthermic group [0 (0, 58)] ($p<0.01$), in which less than half the animals survived to 72hrs post-ROSC. The 72hr survival rates for normothermia, hypothermia and hyperthermia animals were 100% (7/7), 100% (7/7), and 43% (3/7), respectively.

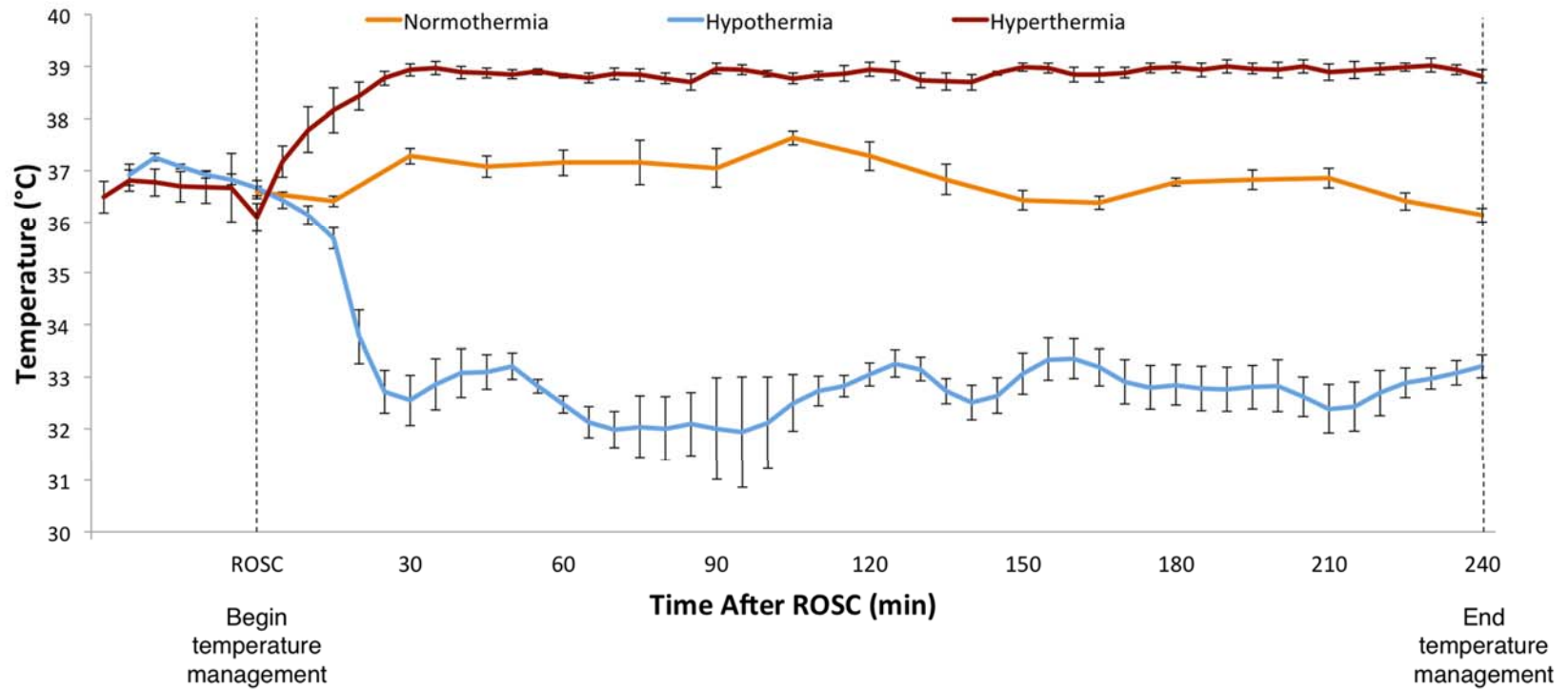


Figure 2.1. The animal temperature (mean±S.E.M.) was well maintained throughout the duration of the experiment. The 4 hr temperature management period is indicated between the dashed lines.

Table 2.1 Baseline ABG and Weight Data

	Hypothermia	Normothermia	Hyperthermia
pH	7.42±0.02	7.43±0.01	7.39±0.02
pCO ₂	46.8±3.6	42.6±3.0	48.5±1.9
HCO ₃ ⁻	29.7±0.9	28.1±1.6	29.6±1.0
SO ₂	99.7±0.3	99.7±0.3	100±0.0
Weight (g)	366±7	370±4	399±20

The evolution of the SSEP pattern was similar in animals, such that the peaks recovered towards the original baseline shape over time during the early recovery period (Fig 2.2A-C). The SSEP recordings showed similar shape but variable peak latencies and amplitudes under different temperature conditions (Fig. 2.2D).

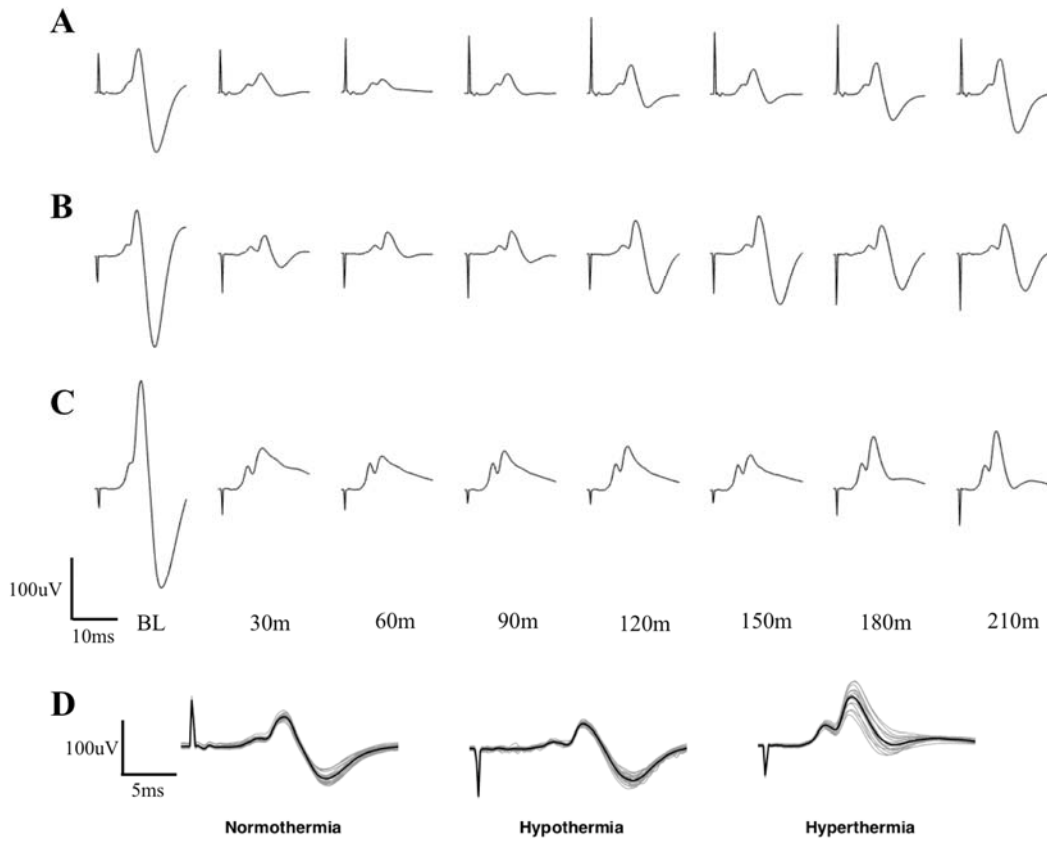


Figure 2.2. Representative time evolution of SSEP recording throughout the experiment in (A) Normothermia (B) Hypothermia and (C) Hyperthermia CA animals. (D) Representative SSEP recordings at 210min post-ROSC for each CA temperature group. The lighter grey lines are the multiple sweeps during the 15 min interval. The superimposed black line is the average of all the sweeps for the time period.

2.3.2 SSEP Marker Changes from Baseline in Sham and CA Animals

The sham animals had significantly higher N10 amplitudes (79% increase, $p < 0.05$) and significantly longer N7 and N10 latencies (35%, 27% increases, respectively, $p < 0.01$ for

both) under hypothermia compared to normothermia. Additionally, the sham animals had significantly shorter N7 and N10 latencies (8%, 7% decreases, respectively, $p < 0.05$ for both) and similar N10 amplitudes (14% decrease, $p > 0.05$) under hyperthermia compared to normothermia. One sham animal was excluded from the statistical analysis due to abnormal baseline waveforms.

The hypothermic CA animals had significant increases in N7 and N10 latency (13%, 21% increases, respectively, $p < 0.01$ for both) from pre-CA baseline and a non-significant decrease in N10 amplitude (22% decrease, $p > 0.05$). The hyperthermic CA animals had significant decreases in N7 latency and N10 amplitude (19%, 71% decreases, respectively, $p < 0.01$) and no change in N10 latency (0% change) compared to pre-CA baselines. The normothermic CA animals similarly had significant decreases in N7 latency and N10 amplitude (8%, 78% decreases, respectively, $p < 0.01$ for both) and a non-significant increase in N10 latency (3% increase, $p > 0.05$). The hyperthermia sham animals had significant decreases in N7 and N10 latency compared to normothermia, however the hyperthermia CA animals had a larger decrease in N7 latency and no change in N10 latency compared to pre-CA baseline values.

2.3.3 N10 Amplitude was Higher and had Better Recovery Over Time in Hypothermic CA Animals

The comparison of normalized aggregate N10 amplitudes between temperature groups showed that hypothermic animals had significantly higher peaks than normothermic and hyperthermic animals over the first 4 hrs of recovery ($p < 0.01$ for both) (Fig. 2.3A). In the time evolution of the N10 amplitude during recovery, hypothermic animals had significantly higher amplitudes than normothermic animals at all time points ($p < 0.05$ at all time points) and than hyperthermic animals at all time points ($p < 0.05$ at all time points) (Fig. 2.3B). The N10 amplitudes (aggregate and time evolution) are shown in Table 2.2.

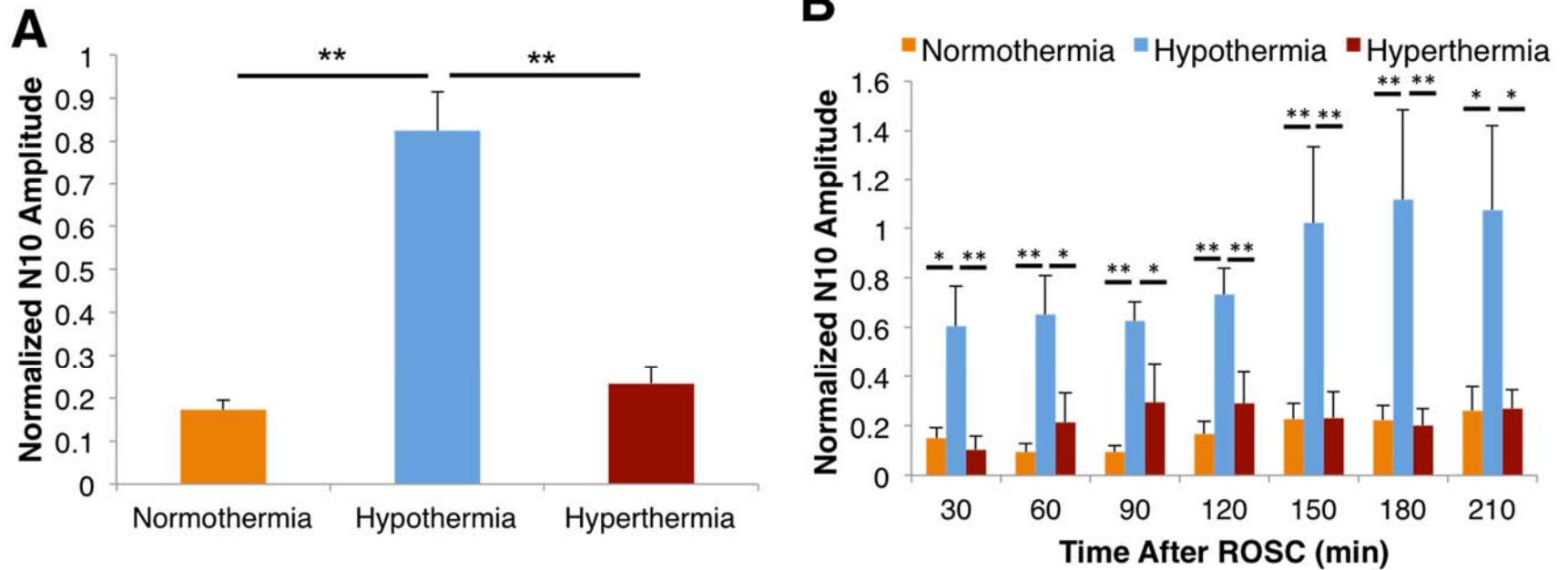


Figure 2.3 (A) Aggregate N10 amplitude was significantly higher in hypothermic CA animals than normothermic and hyperthermic CA animals. (B) Time evolution of N10 amplitude by CA temperature group. The amplitude was significantly greater in hypothermic CA animals than both normothermic and hyperthermic CA animals at all time points. * $p < 0.05$, ** $p < 0.01$

Table 2.2 Normalized N10 Amplitude (mean±S.E.M.) for temperature groups

	Aggregate	30min	60min	90min	120min	150min	180min	210min
Normothermia	0.173±0.022	0.150±0.044	0.093±0.034	0.095±0.024	0.166±0.053	0.228±0.062	0.224±0.056	0.262±0.097
Hypothermia	0.824±0.090	0.604±0.165	0.651±0.158	0.626±0.076	0.732±0.110	1.021±0.312	1.112±0.366	1.076±0.342
Hyperthermia	0.233±0.040	0.104±0.052	0.212±0.123	0.294±0.159	0.290±0.128	0.233±0.105	0.201±0.069	0.270±0.078
Significance	** ‡‡	* ‡‡	** ‡	** ‡	** ‡‡	** ‡‡	** ‡‡	* ‡

* p<0.05, ** p<0.01 between normothermia and hypothermia

† p<0.05, †† p<0.01 between normothermia and hyperthermia

‡ p<0.05, ‡‡ p<0.01 between hypothermia and hyperthermia

2.3.4 Animals with Good Outcome had Better Recovery of N10 Amplitude

Following all CA experiments, animals were grouped based on their functional outcome, as defined by their 72hr NDS score. The animals with good outcome had a significantly higher aggregate N10 amplitude in the first 4 hrs post-ROSC compared to those with poor outcome ($p < 0.01$) (Fig. 2.4A). While the time evolution of the N10 amplitude during recovery did not have significant differences between outcome groups, there is a clear trend showing that poor outcome animals mostly had decreasing N10 amplitude over time while the good outcome group had increasing N10 amplitude over time (Fig 2.4B).

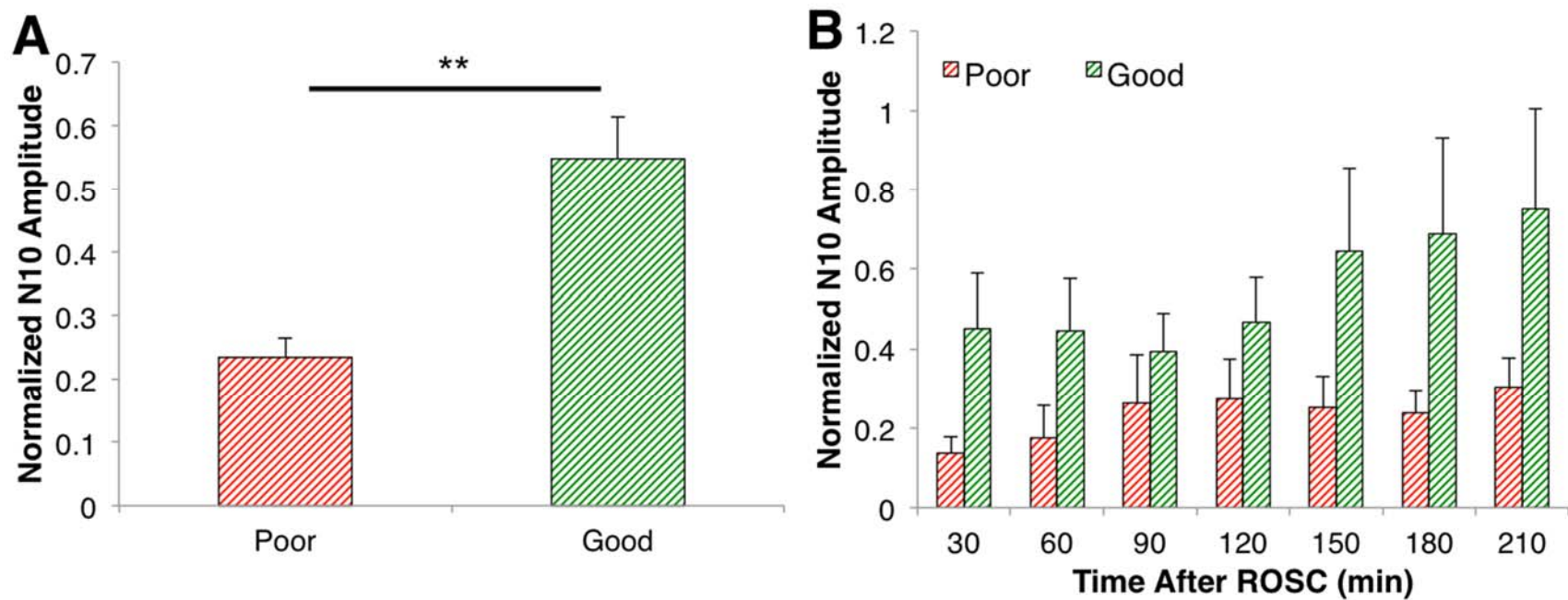


Figure 2.4 (A) Aggregate N10 amplitude was significantly higher in CA animals with good outcome compared to those with poor outcome. (B) CA animals with good outcome had increasing N10 amplitude over time while animals with poor outcome mostly had decreasing amplitude. * $p < 0.05$, ** $p < 0.01$

2.3.5 Predictive value of N10 amplitude

Bivariate analyses were performed to determine the correlation between N10 amplitude and 72hr NDS score. The aggregate N10 amplitude correlated to the 72hr NDS (Pearson correlation coefficient: 0.231, $p < 0.01$) (Table 2.3).

The receiver operating characteristic (ROC) curve was used to determine the predictive value of good outcome of N10 amplitude. The predictive value was deemed good when the area under the ROC curve (AUC) was significantly different than the 0.5 reference curve, which indicates 50% accuracy. Since it is crucial in prognostication following CA to minimize incorrectly identifying a poor outcome patient as good, the cutoff point for these markers were determined at the point of 100% specificity for poor outcome and maximum sensitivity. In the prediction of good outcome, the N10 amplitude at 30min post-ROSC had a prediction accuracy of 79% ($p < 0.05$) at which time point a normalized amplitude > 0.346 had 50% sensitivity and 100% specificity (Table 2.3).

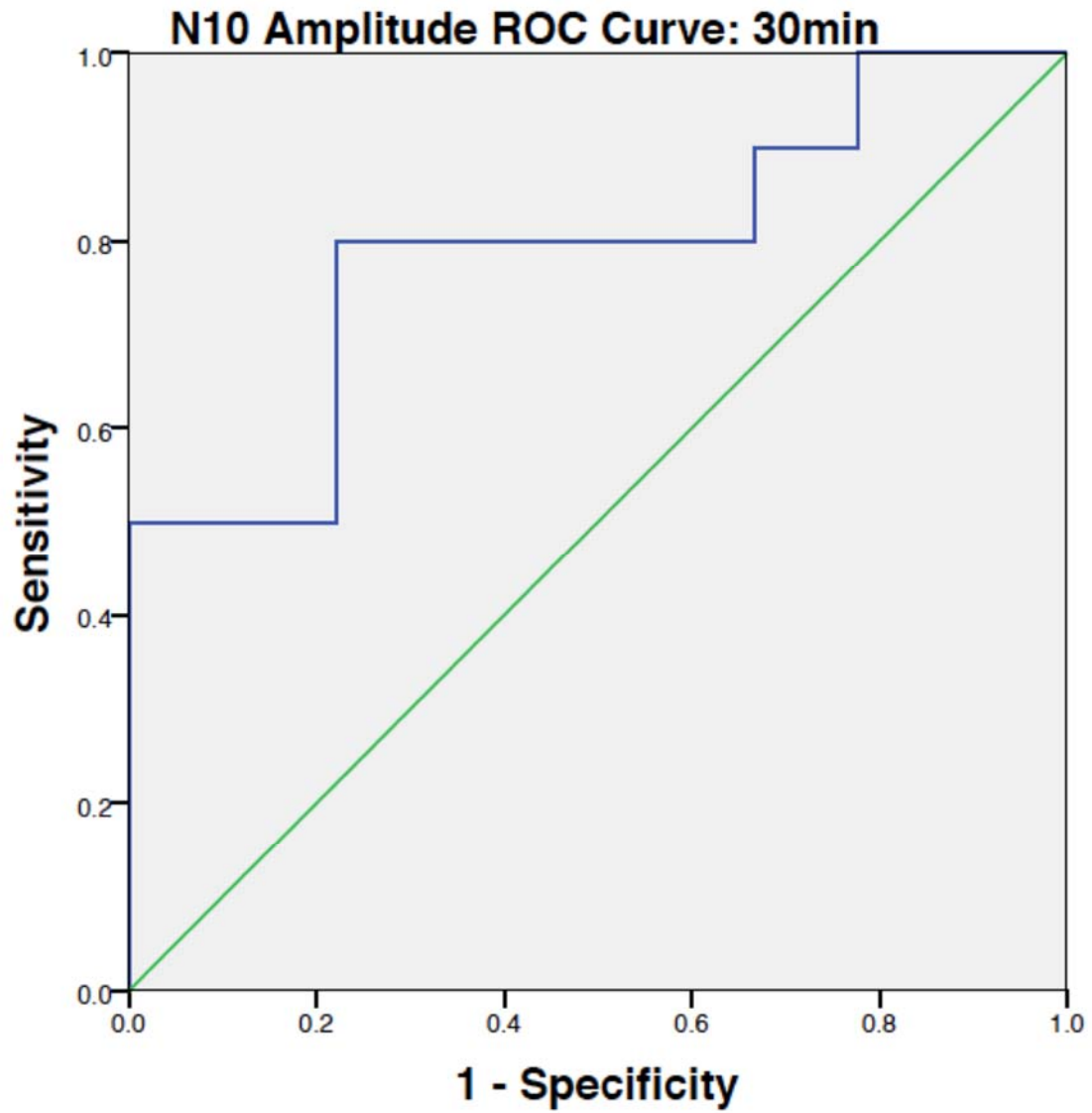


Figure 2.5. ROC curve for the N10 amplitude at 30min after resuscitation has an accuracy of 79% ($p < 0.05$) with 50% sensitivity and 100% specificity.

Table 2.3 Predictive value of N10 amplitude

	Aggregate	30m	60m	90m	120m	150m	180m	210m
<i>Pearson correlation coefficients between SSEP markers and 72 hr NDS</i>								
N10 Amplitude	0.231**	0.399	0.206	-0.034	0.093	0.264	0.338	0.313
<i>Normalized latency cut-off points for good outcome (sensitivity, accuracy) with 100% specificity</i>								
N10 Amplitude	1.032	0.346	-- ^a	-- ^a	-- ^a	-- ^a	-- ^a	-- ^a
	(0.104,	(0.500,						
	69%**)	79%*)						

* p < 0.05, ** p < 0.01, ^a > 0.05

2.3.6 N7 Amplitude

During the N7 amplitude analysis, the N7 peak was consistently concealed by the much larger N10 peak, particularly in the baseline measurements, before the N10 peak was suppressed due to the hypoxic injury. Thus, the N7 amplitude could not be reliably measured throughout the experiment. However, although the N7 amplitude could not be reliably measured, the location of the N7 peak was evident in most animals, thus, the N7 latency could still be reliably measured (normothermia, n=4; hypothermia, n=5; hyperthermia, n=5).

2.3.7 N7 and N10 Latency in Cardiac Arrest Animals

Both N7 and N10 aggregate latencies were significantly longer in hypothermic animals than both normothermic and hyperthermic animals ($p<0.01$) (Fig. 2.6A-B). The aggregate N7 latency was also significantly different between normothermic and hyperthermic animals ($p<0.01$) such that latency increased with decreasing temperature (Fig. 2.6A). The time evolution of the N7 latency showed that the hypothermic animals had significantly longer values than both normothermic and hyperthermic animals at all time points ($p<0.01$) (Fig. 2.6C). The N7 latency was also significantly different between normothermic and hyperthermic animals at all time points after resuscitation except at 60min ($p<0.05$) (Fig. 2.6C). The N10 latency was significantly longer in hypothermic animals than hyperthermic animals beginning at 60min after ROSC ($p<0.05$) (Fig. 2.6D) and than normothermic animals at all time points ($p<0.01$)(Fig. 2.6D). The aggregate and time evolution N7 and N10 latencies are shown in Tables 2.4 and 2.5, respectively.

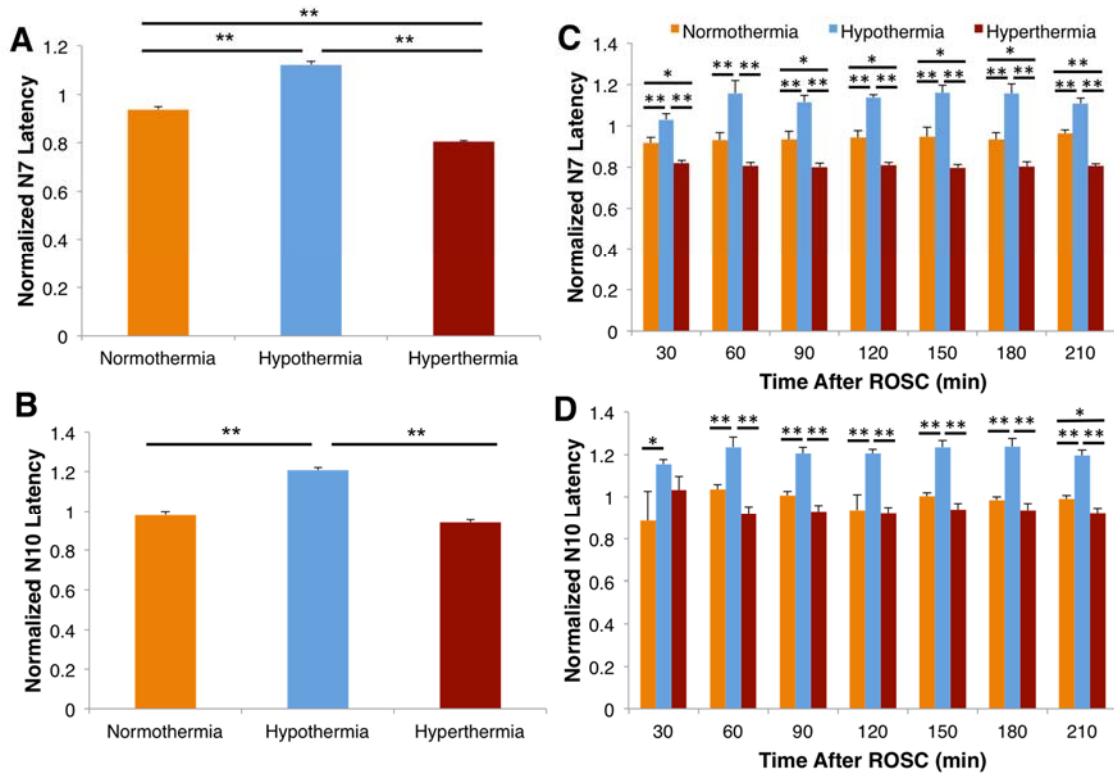


Figure 2.6. (A) Aggregate N7 latency was significantly greater in hypothermic CA animals than normothermic CA animals, which was significantly greater than hyperthermic CA animals (B) Aggregate N10 latency was significantly greater in hypothermic CA animals than both normothermic animals and hyperthermic CA animals. (C) Normalized N7 latency was significantly higher in hypothermic CA animals than normothermic CA animals, which was significantly higher than hyperthermic CA animals at all time points except 60min. (D) Normalized N10 latency was significantly greater in hypothermic CA animals than normothermic CA animals at all time points and than hyperthermic CA animals from 60min until 4hr after ROSC. * $p < 0.05$, ** $p < 0.01$

Table 2.4 Normalized N7 Latency (mean±S.E.M.) for temperature groups

	Aggregate	30min	60min	90min	120min	150min	180min	210min
Normothermia	0.937±0.012	0.917±0.026	0.929±0.037	0.932±0.040	0.943±0.034	0.947±0.046	0.932±0.034	0.962±0.017
Hypothermia	1.121±0.015	1.030±0.029	1.158±0.061	1.116±0.033	1.136±0.014	1.160±0.035	1.156±0.046	1.109±0.025
Hyperthermia	0.805±0.005	0.818±0.014	0.804±0.016	0.799±0.018	0.808±0.014	0.797±0.016	0.802±0.022	0.804±0.010
Significance	** †† ‡‡	** ‡‡	** † ‡‡	** † ‡‡	** † ‡‡	** † ‡‡	** † ‡‡	** †† ‡‡

* p<0.05, ** p<0.01 between normothermia and hypothermia

† p<0.05, †† p<0.01 between normothermia and hyperthermia

‡ p<0.05, ‡‡ p<0.01 between hypothermia and hyperthermia

Table 2.5 Normalized N10 Latency (mean±S.E.M.) for temperature groups

	Aggregate	30min	60min	90min	120min	150min	180min	210min
Normothermia	0.978±0.022	0.884±0.141	1.035±0.024	1.006±0.021	0.938±0.073	1.004±0.017	0.986±0.014	0.991±0.016
Hypothermia	1.209±0.012	1.155±0.023	1.235±0.048	1.204±0.029	1.205±0.019	1.235±0.033	1.238±0.038	1.196±0.024
Hyperthermia	0.941±0.013	1.032±0.065	0.920±0.034	0.930±0.031	0.923±0.026	0.940±0.029	0.937±0.031	0.924±0.023
Significance	** ‡‡	*	**‡‡	** ‡‡	** ‡‡	** ‡‡	** ‡‡	** †‡‡

* p<0.05, ** p<0.01 between normothermia and hypothermia

† p<0.05, †† p<0.01 between normothermia and hyperthermia

‡ p<0.05, ‡‡ p<0.01 between hypothermia and hyperthermia

The aggregate N7 and N10 latencies were both significantly longer in animals with good outcome compared to those with poor outcome ($p < 0.01$ for both) (Fig. 2.7A-B). Similarly, the time evolution of latencies showed that N7 latency was significantly greater in good outcome animals compared to poor outcome animals at all time points in the early recovery period ($p < 0.01$ for all time points) (Fig 2.7C). The N10 latency was significantly greater in animals with good outcome compared to animals with poor outcome beginning at 60min until 4 hrs post-ROSC ($p < 0.01$) (Fig 2.7D).

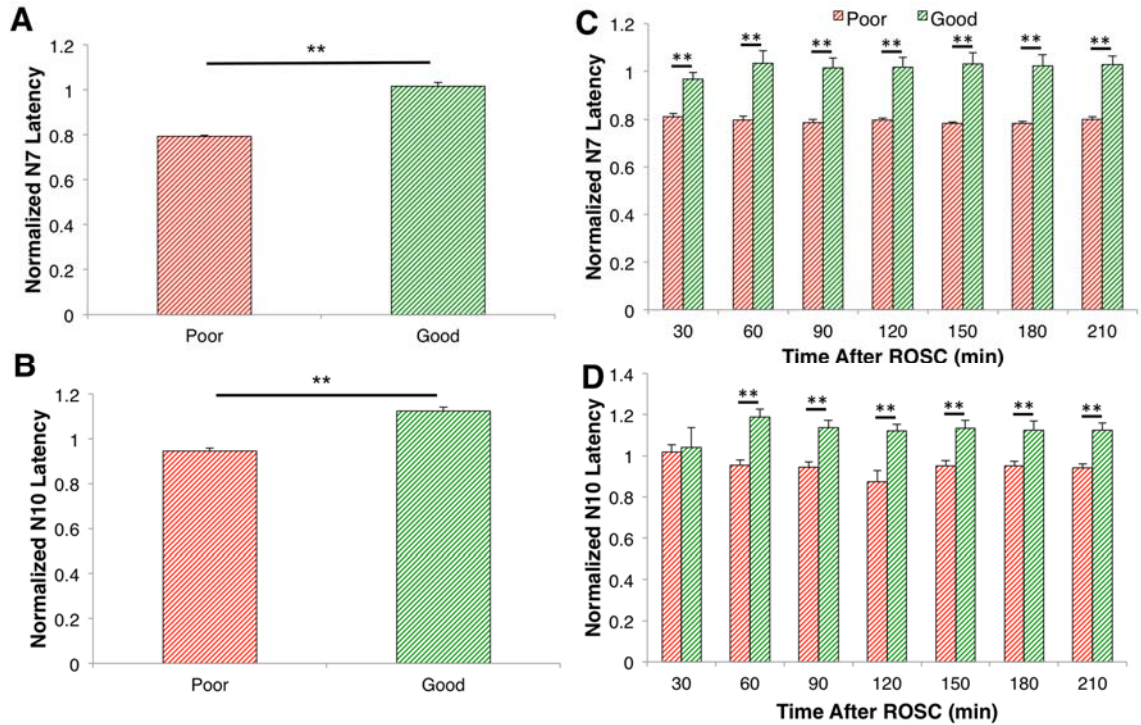


Figure 2.7. (A) Aggregate N7 latency was significantly greater in CA animals with good functional outcome than those with poor outcome. (B) Aggregate N10 latency was significantly greater in CA animals with good functional outcome than those with poor outcome. (C) Normalized N7 latency was significantly higher in good outcome CA animals than poor outcome CA animals at all time points. (D) Normalized N10 latency was significantly higher in good outcome CA animals than poor outcome CA animals beginning at 60min post-ROSC. * $p < 0.05$, ** $p < 0.01$.

2.4 Discussion

In this study, we discovered that short-latency SSEP amplitude, specifically the N10 amplitude, holds potential prognostic value following CA with TTM. The experiment demonstrated that normalized N10 amplitude during the early recovery is significantly greater in rats under hypothermia and in animals with good functional outcome following CA. Similarly, the peak analyses showed that larger N10 amplitudes are strongly associated with good functional outcome. Specifically, the aggregate N10 amplitude of the first 4 hours of recovery was significantly correlated with 72 hour NDS. We have shown for the first time that the N10 amplitude at 30min after ROSC, when all animals were still comatose, serves as an accurate predictor of good outcome at 72 hrs after ROSC.

This study is among the first to demonstrate that SSEP signals recorded during the early recovery period under TTM can be quantified as objective measures to predict outcome following CA. This finding is significant as it not only validates the benefits of SSEP under TTM and during the early recovery period, but it also eliminates the subjective nature of current SSEP methods (determining whether N20 peaks are present or not), which has been shown to have only moderate interobserver reliability [141, 142].

The present study establishes the value of SSEP amplitudes in predicting good functional outcome during the early recovery after CA. The bilateral absence of N20 at 12-24 hrs

after resuscitation has been fairly well established as one of the most reliable predictors of poor outcome after CA in humans [133, 134, 136, 146]. However, the studies supporting this notion in most cases also emphasize that the presence of N20 does not predict that the patient will have good functional outcome. Thus, the present study is crucial as it identifies threshold values of existing peaks to identify subjects with good outcome. It is important to note that the N10 amplitude markers identified in this study are associated with predicting good outcome, whereas all established markers, namely the bilateral absence of the N20 peak, only reliably predict poor outcome.

A recent prospective cohort study by Endisch et al. has demonstrated that SSEP amplitude holds prognostic value in post-CA patients treated with TTM [161]. The authors determined that bilaterally absent or very low amplitude ($<0.62\mu\text{V}$) short-latency cortical SSEPs had a sensitivity of 57% in the prediction of poor outcome while amplitudes above $2.5\mu\text{V}$ rule against severe hypoxic encephalopathy and have a sensitivity of 65% for good outcome. However, the recordings in this study all occurred after 24 hours post-ROSC, after TTM had been stopped, and therefore cannot be used to track recovery or for prognostication in the early recovery period. In the present study, we demonstrated that the rat N10 amplitude in the early recovery correlates with final outcome. We also found that normalized N10 values above 0.346 at 30min post-ROSC can predict good outcome with a sensitivity of 50% and with significant accuracy. This finding that higher amplitude is related to better outcome is consistent with a human study of normothermic post-CA patients, which found that patients with poor outcome

tended to have significantly lower N20 amplitudes compared to those with good outcome [160].

The N10 SSEP amplitude marker in the first 4 hours after CA was distinct among temperature and outcome groups and was associated with good functional recovery. Prognosis during the early recovery period following CA is crucial as it allows better titration of hypothermia and other treatments. Multiple groups have suggested waiting 24 hrs before taking SSEP measurements in clinical settings, as some patients with absent SSEP peaks before 24 hrs eventually regained consciousness [144], or because SSEP signals tend to improve over the first 24 hrs [143]. However, in this rat study we have shown that the differences in early recovery are associated with good outcome, while temperature management is still in progress.

All three temperature groups of CA animals had a decrease in N10 amplitude from their respective pre-CA baselines, which differs from the sham data, which showed that N10 amplitude is increased by hypothermia and unchanged by hyperthermia, suggesting that CA leads to an additional decrease in N10 amplitude. This is a trend that was seen in multiple studies of SSEP during cardiac surgery with cardiopulmonary bypass, in which all studies saw a decrease in the cortical peak amplitude [156-159, 167, 168]. However, the effect of hypothermia on SSEP amplitude is not fully elucidated as various groups have observed increases, decreases, or no change in amplitude depending on the recording site and the condition of the subject [135, 151, 153, 154]. One study found that

hyperthermic temperatures have no significant effect on peak amplitudes in rats [154].

The cohort study by Endisch et al. found that temperature did not impact the relation between SSEP amplitude and outcome, when comparing SSEPs that were recorded above and below 35°C.

Although SSEPs are less vulnerable to the effects of anesthetics than EEG, isoflurane has been shown to decrease the P40-N50 SSEP amplitude in humans [169]. In the present study, the isoflurane administered during the recovery period was minimal. Thus, we do not believe that this influenced the amplitude differences between groups. Moreover, in a clinical CA setting, anesthesia is generally not restarted during the very early recovery period. Further, the Endisch et al. study found that sedation did not largely impact the relationship between SSEP amplitude and functional outcome.

The sham data compared to the CA data demonstrates the effect of CA on SSEP signals under temperature management. There were significant increases in both N7 and N10 latencies with and without CA, however the percent increase in the latencies is smaller in the CA animals. The sham data corroborate previous studies that unanimously demonstrate that hypothermia increases SSEP latency [150-154, 156-159, 167, 168] and that hyperthermia decreases latency [154]. Thus, the SSEP peak latency appears to be confounded by temperature, which consequently impacts the prognostic value while the subject is under TTM.

Our study is limited in that it uses baseline SSEP measurements to normalize the post-CA recordings. It is unrealistic to expect baseline measurements for CA patients in a clinical setting, which is the application goal for these quantified SSEP markers. To address this, we expect that generalized baseline values could be generated from a standardized control group. Additionally, a larger study, perhaps with a more severe injury, is necessary to further elucidate the temperature effect on CA animals with SSEP. All hypothermia animals with moderate brain injury after 7min CA in the present study had good outcome, so the cutoff points generated may not account for the temperature effect of hypothermic animals with poor outcome after severe brain injury. Finally, while we were able to examine the relationship between SSEP characteristics and functional outcome, histological analyses were not performed in the present study to examine the relationship between SSEPs and cerebral injury level. However, a previous study used a rodent CA model to demonstrate that electrical stimulation of the median nerves during the early recovery period does not amplify the neurologic damage at 48 hrs post-ROSC [170]. The overall goal of this project is to develop quantitative markers that hold prognostic value during the early recovery period following CA that can be translated to clinical settings and to demonstrate feasibility.

2.5 Conclusion

The present study demonstrated that SSEP measurements are valid under TTM and may hold more prognostic value as quantitative markers than the dichotomous distinction of

present/absent peaks. Specifically, we were able to show that the rat N10 amplitude has a better recovery under hypothermia than normothermia or hyperthermia in the early recovery following CA and has prognostic value in the prediction of good outcome following CA with temperature management in a rat model. The ultimate goal of this study is to translate the quantitative markers to a clinical setting to improve prognostication during early recovery after CA, as SSEP is already a common tool in this application.

CHAPTER 3: EVOLUTION OF QUANTIFIED SOMATOSENSORY EVOKED POTENTIALS AFTER CARDIAC ARREST WITH TARGETED TEMPERATURE MANAGEMENT

3.1 Introduction

Although the resuscitation and treatment protocols for cardiac arrest (CA) victims are being continuously improved, the functional outcome of survivors is still often poor. Among those patients who survive the cardiac event, morbidity and mortality are primarily caused by poor neurological recovery [31, 34, 171]. Targeted temperature management (TTM), specifically induced hypothermia of 32-34°C for 12-24 hours, is a recommended treatment in the guidelines provided by the American Heart Association (AHA) [49], and has been shown to improve survival and functional outcome [23, 41]. Early prognostication, ideally in the first few hours following resuscitation while the patient is still under TTM, could crucially impact the subsequent treatment and resource allocation, which ultimately could help to improve final functional outcome of post-CA patients.

The challenges in monitoring and predicting cerebral recovery shortly after resuscitation from CA have been well documented [99, 100, 132, 154, 172]. Somatosensory evoked

potentials (SSEP) are used in post-CA patients to assess the recovery of evoked neural activity. Thus, evaluation of SSEP, such as changes in waveform amplitude, latency, or shape, while the CA patient is comatose following resuscitation could help assess the cerebral damage and potential recovery.

The prognostic value of SSEP, even under TTM, has been well verified, as the bilateral absence of the N20 SSEP peak is currently the best prognostic indicator of poor functional outcome [42, 88, 129-136]. However, not only is this method highly subjective, even among highly trained experts [141, 142], it is also temporally limited, as it has been suggested to be most reliable at least 12-24 hours after resuscitation [42], at which point much of the ischemic damage by CA has already begun. The current SSEP signal interpretation requires highly experienced personnel and since CA survivors are often monitored by physicians and nurses with little experience in neurological examination, the implementation of this method is complicated. Further, the small changes in cerebral recovery that may have a more substantial impact on functional outcome may not be accounted for with the dichotomous categorization of SSEP peak presence. An early quantitative SSEP marker would simplify the analysis, remove the subjectivity of the interpretation, and account for varying levels of injury, which could allow for earlier prognostication and better assist treatment. Thus, a quantitative SSEP measurement would address the limitations of the current gold-standard method, allowing for earlier and objective prognostication.

Our recently developed quantitative SSEP (qSSEP) metric that objectively captures the morphologic information of the SSEP waveform, represented by a single numeric value termed the qSSEP phase space area (qSSEP-PSA), was previously shown to hold predictive value for functional outcome following CA during the early recovery period in rats [145]. Here, for the first time, we test the robustness of the qSSEP-PSA metric in tracking cerebral recovery following CA under three temperature conditions: hyperthermia, normothermia and hypothermia.

3.2 Materials and Methods

3.2.1 Animals

In these experiments, we recorded the SSEPs in a rodent CA model with temperature management. Twenty-one animals underwent 7min asphyxia-CA and resuscitation followed by immediate temperature management of one of three temperatures: hypothermia, normothermia, or hyperthermia (n=7/group), which was randomly assigned prior to CA. An additional 14 sham operation rats underwent the same experimental procedures except for CA and resuscitation. All experiments were approved by the Institutional Animal Care and Use Committee at Johns Hopkins University.

3.2.2 Animal Preparation

All animals had 5 screw electrodes (Plastics One, Roanoke, VA) implanted in the skull to record the SSEPs [145, 162] from the somatosensory cortex. Once implanted, the screws were secured with a plastic pedestal and dental cement. The animals were then returned to their home cage and allowed 1 week of recover before the CA experiment.

3.2.3 Cardiac Arrest, Resuscitation and Temperature Management

The CA and resuscitation procedures were performed as previously described [46, 110, 113, 145]. On the day of CA, rats were mechanically ventilated and anesthetized with 1.5% isoflurane in 1:1 O₂:N₂ following intubation. The femoral artery and vein were cannulated to measure arterial blood gases (ABG) and to administer drugs. A baseline SSEP measurement was recorded for 15 min followed by a 5 min anesthetic washout period, during which vecuronium (2mg/kg) was administered. Asphyxial CA was induced as previously described, lasting for 7min. Resuscitation was performed by administering cardiopulmonary resuscitation (CPR) along with epinephrine and sodium bicarbonate until the return of spontaneous circulation (ROSC).

SSEP recordings resumed 30 min after ROSC and were continued in 15 min intervals until 4 hours after ROSC. Isoflurane was delivered as needed, not exceeding 0.5%, during SSEP recordings in the recovery period as the nerve stimulation may cause discomfort to the rats [154, 162].

Temperature was measured rectally and was recorded every 10 min. Temperature management began immediately after ROSC was achieved. The hypothermia group was cooled to 32-34°C within 15 min, which was maintained for 4 hours followed by rewarming to normothermia (36.5-37.5°C) over a period of 2 hours. The hyperthermia animals were warmed to 38.5-39.5°C within 15 min, which was maintained for 4 hours followed by passive cooling to normothermia over a period of 2 hours. Normothermia animals were maintained at 36.5-37.5°C for 4 hours after ROSC using a heating pad. After temperature management, all animals were then placed in a neonatal incubator at 28°C to maintain normothermia (36.5-37.5°C) until 24 hours after ROSC.

3.2.4 Functional Outcome

The neurologic recovery of the rats was determined using the Neurologic Deficit Scale (NDS). Animals were evaluated by a trained and blinded assistant at 6, 24, 48 and 72 hrs after ROSC. A 72hr NDS score greater than 60 was designated as good functional outcome, while less than 60 was determined to be poor outcome [46].

3.2.5 SSEP Measurement

SSEP signals were recorded from the cortical screw electrodes while stimulating (200µsec, 6mA 0.5Hz) the median nerve in each forelimb of the rat with subdermal needle electrodes. SSEPs were acquired by the TDT System3 data acquisition system (Tucker-Davis Technologies, Alachua, FL) at a frequency of 6.1kHz. SSEP

measurements were taken for 15min prior to CA (baseline) and beginning at 30min after ROSC, continuing in 15 min intervals until 4 hr post-ROSC.

3.2.6 qSSEP-PSA Calculation

The qSSEP-PSA metric calculation is explained in detail by the developing authors [145]. Briefly, the SSEP waveforms within each 15 min interval (450 sweeps) were averaged. Then, the phase space curve (PSC) was generated for each time interval by plotting the first derivative against the magnitude. The PSC represents the morphologic information of the waveforms. The area bound by the PSC was then calculated, which is referred to as the qSSEP phase space area (qSSEP-PSA) and represents the power of the SSEP signal. Specifically, the area of the PSC is determined by the Quickhull algorithm, which fits a convex hull to the PSC [173]. The convex hull is determined using the point-index based method, which identifies the PSC indices that lie along the boundary of the convex hull. The qSSEP-PSA is the area within this boundary. Thus, the algorithm captures extensive information of the waveform shape, providing a more encompassing representation of the SSEP than merely amplitude or latency. A custom MATLAB (MathWorks, Natick, MA) algorithm was used for all computations.

Once the qSSEP-PSA was calculated for each 15 min interval, the values were normalized to the baseline qSSEP-PSA. The aggregate qSSEP-PSA considers the

normalized value of all 15 min time periods. Waveforms with abnormal baselines (bilaterally distorted N10 and P15 peaks) were excluded from the analysis [162].

3.2.7 Statistics

The commercial SPSS statistics computer package (IBM SPSS Statistics v22, Armonk, NY) was used for all statistical analyses. The 72 hour NDS score, reported as the [median (25th, 75th percentiles)] was compared between temperature groups using a nonparametric Kruskal-Wallis test. The qSSEP-PSA metric was compared between temperature groups using a repeated measures analysis of variance (ANOVA) and between outcome groups using a one tailed student's t-test, assuming unequal variances. The correlation between qSSEP-PSA and 72 hr NDS was determined using a bivariate analysis to obtain the Pearson correlation coefficients. A p value less than 0.05 was considered statistically significant.

3.3 Results

3.3.1 Baseline Data, Temperature Monitoring, and NDS

The core temperature of all animals was closely monitored and maintained at their designated temperature (normothermia: $36.9 \pm 0.10^\circ\text{C}$, hypothermia: $32.8 \pm 0.10^\circ\text{C}$, hyperthermia: $38.9 \pm 0.03^\circ\text{C}$). The target temperatures were reached within 15mins following ROSC (Fig. 3.1). The baseline weight and ABG data prior to CA were not

significantly different between temperature groups ($p > 0.05$, data not shown). The hypothermia animals had the highest 72 hr NDS score [74 (74,77)] compared to the other temperature groups ($p < 0.01$). The normothermia animals also had significantly higher 72 hr NDS scores [68 (49,72)] compared to hyperthermia animals [0 (0, 58)] ($p < 0.01$). Using a 72 hr NDS score of 60 as the cutoff, there were 9 animals with poor outcome [median (25th, 75th percentiles)] [45 (0, 51)] and 12 animals with good outcome [74 (71, 74)].

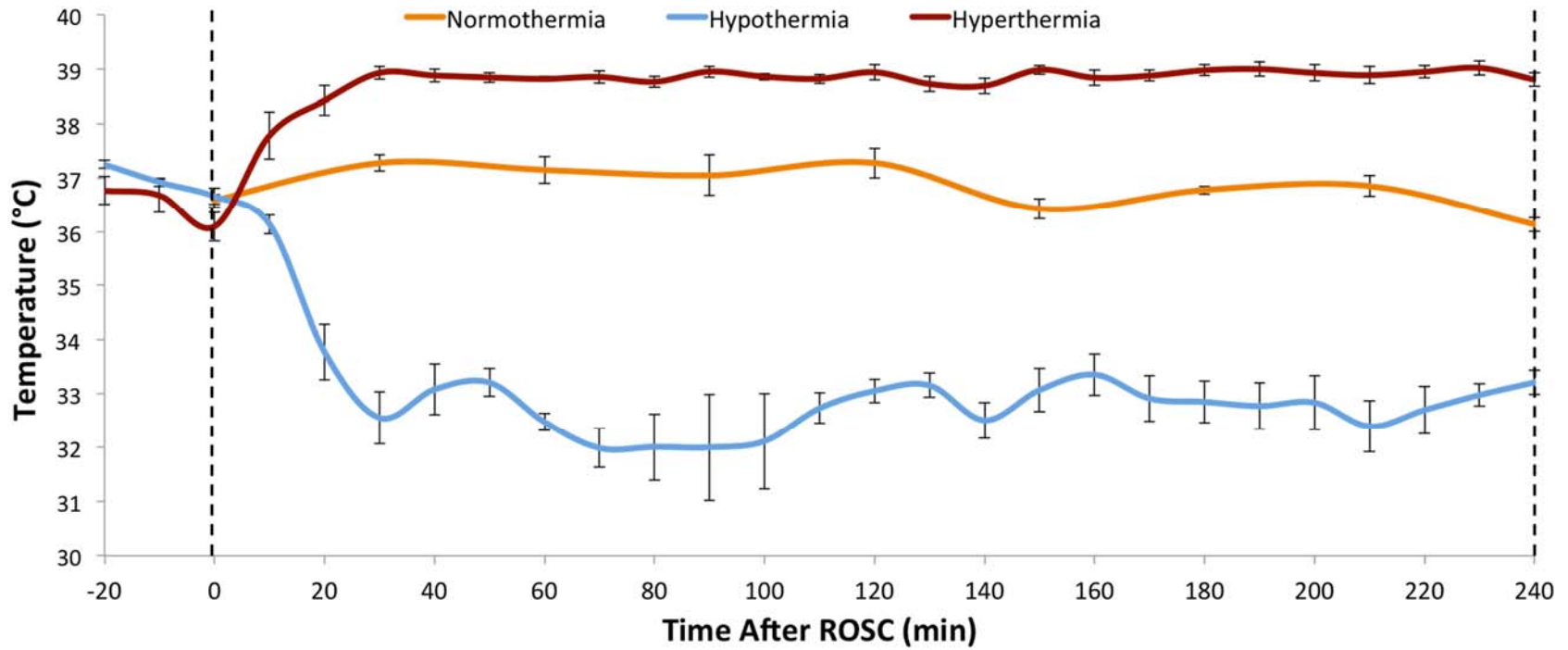


Figure 3.1 Temperature was well monitored throughout the experiment. Temperature management occurred between the dashed lines, beginning at ROSC (time 0 min) and ending at 4 hours post-ROSC (time 240min).

3.3.2 Sham Animal Data

For the sham animals, the percent change in qSSEP-PSA from a normothermia baseline was determined under hypothermia and hyperthermia (Table 3.1). The sham animals had a 37% increase ($p>0.05$) in qSSEP-PSA under hypothermia and a 32% decrease ($p>0.05$) under hyperthermia, compared to normothermia.

3.3.3 Change in qSSEP-PSA from Baseline Following CA

The CA animals of all three temperature groups had a decrease in qSSEP-PSA compared to the pre-CA baseline (Table 3.1). The normothermia CA animals had the largest percent decrease (89%, $p<0.01$), while the hypothermia CA animals had the smallest percent decrease (31%, $p>0.05$). Hyperthermia resulted in a decrease in qSSEP-PSA in sham animals ($p>0.05$), however, the decrease was even larger and significant in hyperthermia CA animals ($p<0.05$). Hypothermia resulted in a decrease in CA animals, though the change was not significantly different from baselines ($p>0.05$).

Table 3.1 Percent changes in qSSEP-PSA in sham and CA animals

Temperature	Intervention	PSA
Hypothermia	Sham	+37%
	CA	-31%
Hyperthermia	Sham	-32%
	CA	-59% *
Normothermia	Sham	--
	CA	-89% **

* p < 0.05, ** p < 0.01 compared to pre-CA baseline

3.3.4 Recovery of qSSEP-PSA Following Cardiac Arrest

SSEP recordings were obtained for each animal and followed a general trend of decreased SSEP peak amplitude (qualitative) during the recovery period compared to baseline. In general, the hypothermia animals had the best recovery of SSEP peaks to baseline size and shape while hyperthermia animals tended to have the worst recovery, resulting in smaller size peaks with abnormal shape (Fig. 3.2A). The corresponding PSCs for the representative SSEP waveforms are displayed in Fig. 3.2B.

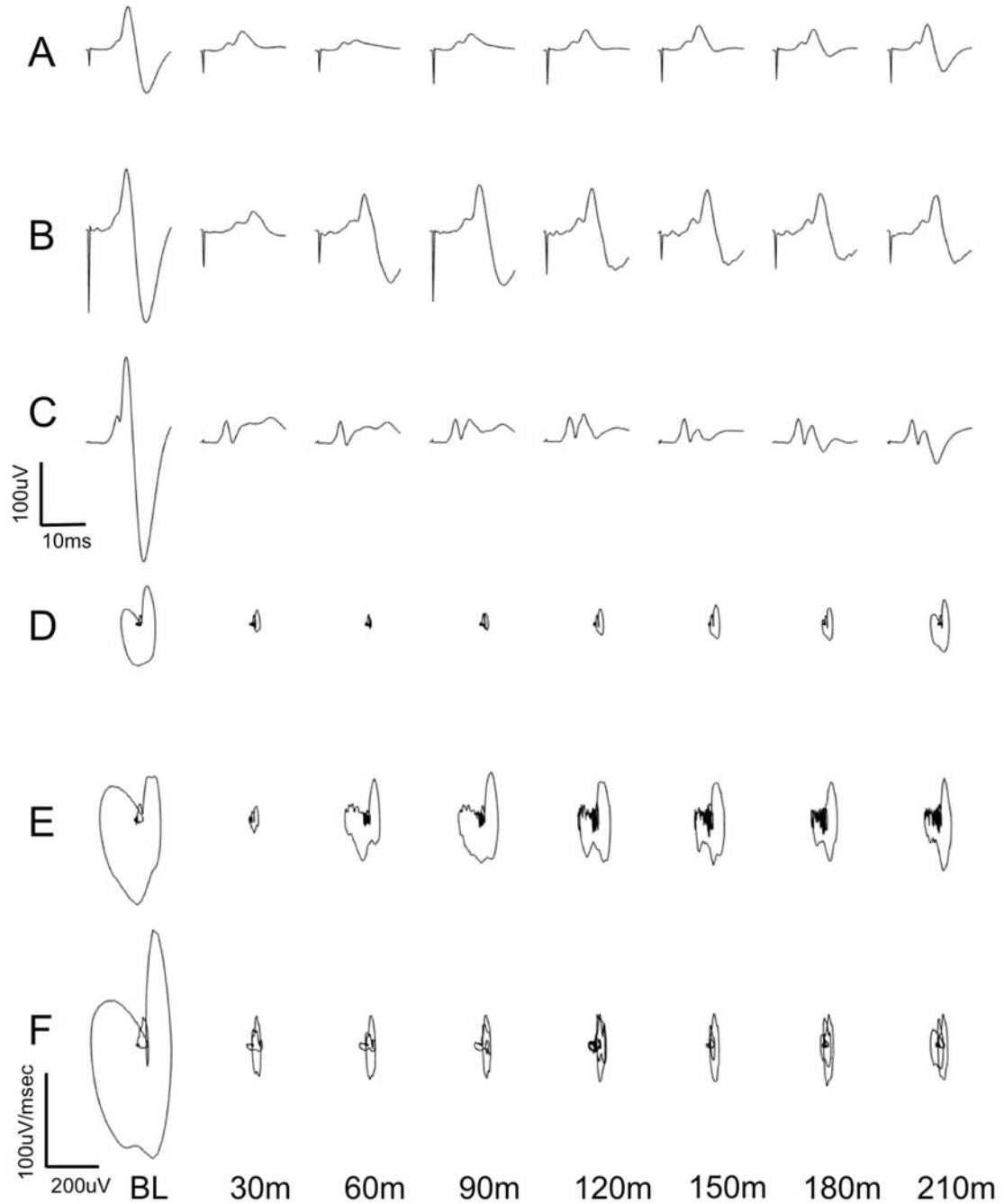


Figure 3.2 Representative SSEP waveforms for (A) normothermia, (B) hypothermia, and (C) hyperthermia animals throughout the experimental period. The lower panels display the corresponding phase space curves for (D) normothermia, (E) hypothermia, and (F) hyperthermia animals.

3.3.5 Aggregate qSSEP-PSA is Higher in Hypothermic Animals

The aggregate qSSEP-PSA was calculated for each temperature group by considering all the normalized qSSEP-PSA values over the 4 hour early recovery period following ROSC. The aggregate qSSEP-PSA was significantly higher in the hypothermia group than both the hyperthermia group ($p < 0.05$) and the normothermia group ($p < 0.01$) (Fig 5.3). The aggregate qSSEP-PSA was also significantly greater in the hyperthermia group compared to the normothermia group ($p < 0.05$) (Fig. 5.3).

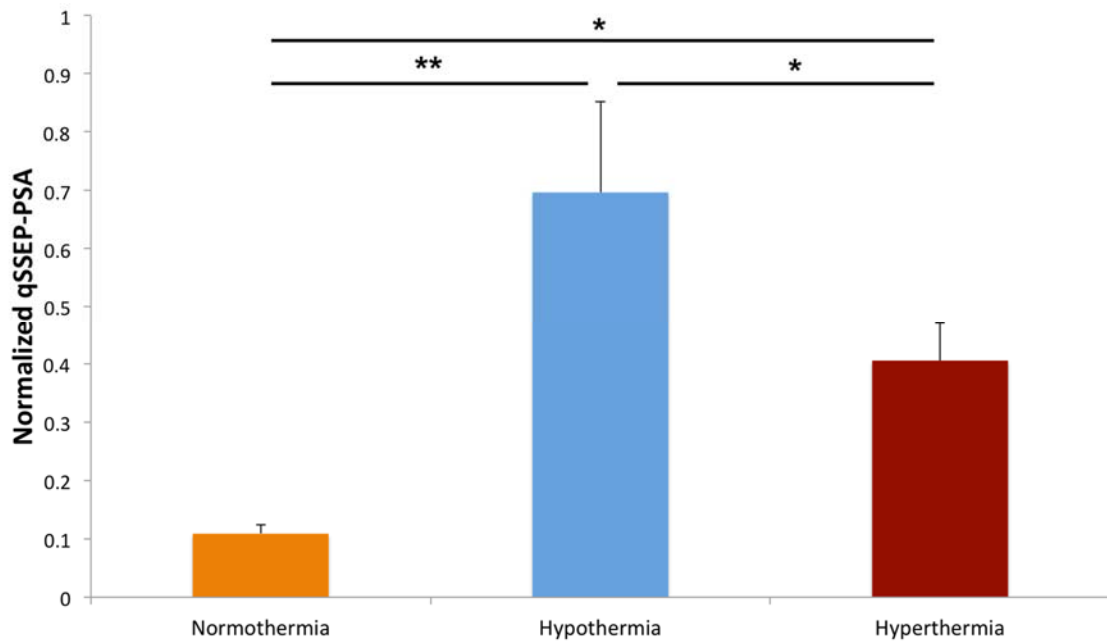


Figure 3.3. Aggregate normalized qSSEP-PSA was significantly larger in hypothermia animals compared to both normothermia and hyperthermia animals. * $p < 0.05$, ** $p < 0.01$.

3.3.6 qSSEP-PSA Increases More in Hypothermic Animals During Early Recovery

The comparison of the time evolution of the normalized qSSEP-PSA values between temperature groups showed that hypothermia values tended to increase throughout the early recovery period (Fig. 5.4). Normothermia qSSEP-PSA values tended to increase slightly but stayed relatively the same over time (Fig. 5.4). The hyperthermia animals showed a slight increase in qSSEP-PSA values during the early stages of recovery (30-90min) followed by a decrease in values during the remainder of the early recovery period. The hyperthermia animals had a significantly higher qSSEP-PSA than normothermic animals at 60 and 90min post-ROSC ($p < 0.05$) (Fig. 5.4). One animal in the hypothermic group had an SSEP peak that was much larger in the recovery period than at baseline, which is unusual for animals that have undergone ischemic injury due to CA, however, the baseline data for this animal was normal. This phenomenon caused a very large S.E.M. value in the hypothermic animals. The aggregate and time evolution qSSEP-PSA values are shown in Table 3.2.

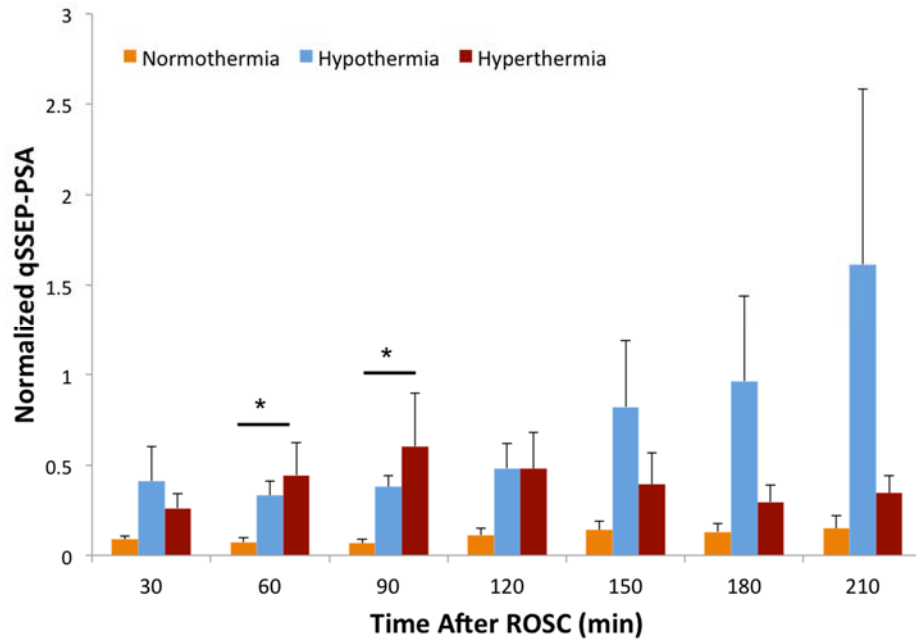


Figure 3.4. Time evolution of normalized qSSEP-PSA between temperature groups. qSSEP-PSA in hypothermia animals trended upwards over time while qSSEP-PSA in hyperthermia animals increased slightly then decreased further into the recovery period. * $p < 0.05$.

Table 3.2 Normalized qSSEP-PSA (mean±S.E.M.) for temperature groups

	Aggregate	30min	60min	90min	120min	150min	180min	210min
Normothermia	0.109±0.015	0.088±0.021	0.073±0.025	0.068±0.023	0.112±0.039	0.142±0.047	0.130±0.046	0.148±0.071
Hypothermia	0.697±0.155	0.412±0.189	0.332±0.081	0.379±0.064	0.482±0.137	0.821±0.368	0.965±0.475	1.616±0.967
Hyperthermia	0.406±0.065	0.261±0.080	0.443±0.181	0.604±0.294	0.481±0.199	0.392±0.175	0.295±0.095	0.345±0.098
Significance	** † ‡		†	†				

* p<0.05, ** p<0.01 between normothermia and hypothermia

† p<0.05, †† p<0.01 between normothermia and hyperthermia

‡ p<0.05, ‡‡ p<0.01 between hypothermia and hyperthermia

3.3.7 Animals with Good Functional Outcome Have Better Early qSSEP-PSA

Recovery

The aggregate qSSEP-PSA of the first 4 hrs post-ROSC for animals with good functional outcome (72hr NDS \geq 60) was higher than that of animals with poor functional outcome (72hr NDS $<$ 60) (Fig. 3.5A), though the difference was not significant with the current animal cohort ($p > 0.05$). The time evolution of the qSSEP-PSA shows that animals with good functional outcome had increasing qSSEP-PSA over the first 4 hours post ROSC (Fig. 3.5B). Conversely, the animals with poor functional outcome had increasing qSSEP-PSA values from 30-90 min post-ROSC followed by decreasing values for the remainder of the early recovery period (Fig. 3.5B). Overall, there was better recovery of the qSSEP-PSA metric in animals with good functional outcome compared to those with poor functional outcome.

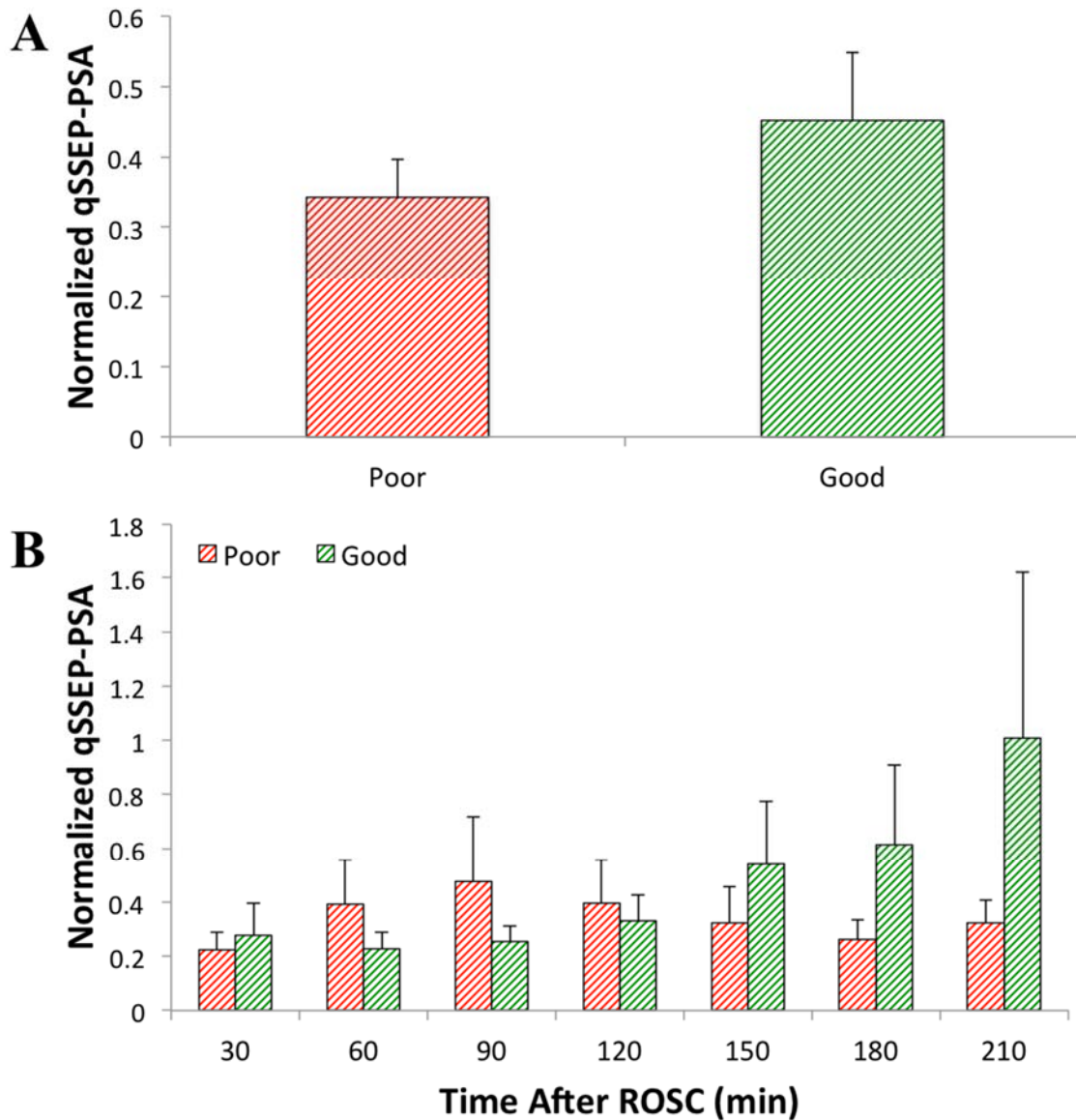


Figure 3.5. A) Animals with good functional outcome had higher aggregate qSSEP-PSA than animals with poor outcome. B) Animals with good functional outcome had better recovery of qSSEP-PSA over the early recovery period compared to animals with poor outcome.

3.3.8 Predictive and Tracking Values of qSSEP-PSA with Temperature

Management

The qSSEP-PSA was correlated with 72hr NDS at 60 and 90 min post-ROSC (Pearson correlation coefficients: -0.528, -0.543, respectively, $p < 0.05$) (Table 3.3). Receiver operating characteristic (ROC) curves were generated for the prediction of good outcome using the qSSEP-PSA metric for both aggregate and time evolution values. The AUC was not significantly different from the 0.5 reference for the aggregate qSSEP-PSA and the qSSEP-PSA at each time point during the early recovery ($p > 0.05$). The accuracy of the metric is deemed good when the area under the ROC curve (AUC) is significantly different ($p < 0.05$) from the standard of 0.5, indicating that it is a good predictor of good functional outcome.

Table 3.3 Pearson correlation coefficients for qSSEP-PSA and 72 hour NDS score

	Aggregate	30m	60m	90m	120m	150m	180m	210m
Pearson correlation coefficient	-0.067	-0.096	-0.528*	-0.543*	-0.400	-0.017	0.125	0.175

* $p < 0.05$

3.4 Discussion

In this study, we applied the recently developed qSSEP-PSA algorithm to SSEP signals of post-CA rats treated with TTM, and determined that the quantitative marker shows distinct trends among both temperature groups and functional outcome groups over the early recovery period. We have demonstrated that the qSSEP-PSA marker holds the potential to objectively track early recovery on a wide scale to account for multiple recovery statuses. More specifically, this marker provides a potential improvement on the current SSEP standard of present/absent dichotomous classification, as it provides a continuous scale of possible values that may represent various levels of recovery or injury, which is both objective and available during the early recovery period. We have shown that there are distinct trends of the qSSEP-PSA marker within the first 4 hours of recovery, importantly suggesting that early SSEP tracking and prognostication may be valid and beneficial. Furthermore, the qSSEP-PSA marker provides an objective measurement that does not require sophisticated training to interpret, in contrast to the current SSEP method of classification.

Our results show that there are significant differences in aggregate qSSEP-PSA between all three temperature groups during the early recovery such that hypothermia animals had significantly larger qSSEP-PSAs than the other temperature groups. Although the hypothermia and hyperthermia groups had similar recovery in the first 120min after ROSC, the hypothermia animals had distinctly better improvement, particularly from 150-240min after resuscitation. A similar observation was seen in the burst frequency in

post-CA rats, where both hypothermic and hyperthermic treated animals had increased bursting during the early recovery [166]. This observation supports the notion that the evolution of SSEP can track the improved recovery that is expected of hypothermia treated subjects. The sham data demonstrates that the distinct evolution among temperature groups is a result of the varying levels of injury, as hypothermia and hyperthermia caused nonsignificant increases and decreases from baseline, respectively, in sham animals.

The recovery of qSSEP-PSA between functional outcome groups was similar to that among temperature groups for the first half of the recovery period (i.e. not much distinction between groups), however, from 150-240min post-ROSC, the animals with good functional outcome distinctly trended upwards while the poor outcome animals had decreasing qSSEP-PSA. This corroborates a previous study that demonstrated that SSEPs evolve at different rates depending on the injury level [162].

Although the N20 absent/present assessment has been proven to be robust in predicting poor outcome, it is still limited by the dichotomous standard, as the method does not have the ability to guide treatment. While the method has repeatedly shown a specificity of 100% for poor outcome, the sensitivity is often low, around 45% [94, 144]. The low sensitivity is most often due to a small proportion of patients that end up with poor outcome actually having bilaterally absent N20s [174]. Additionally, the presence of N20 peaks does not indicate good outcome – almost half of patients with present N20s will

have poor outcome [175]. Therefore, it is important to consider the entire waveform morphology and create a continuous quantitative SSEP marker to track the recovery. As each of the SSEP peaks originate from varying brain structures, it is reasonable that a holistic quantification of the SSEP waveform may help maximize prognostic value. We have demonstrated here that the qSSEP-PSA marker achieves quantification of the entire SSEP waveform. Quantitative SSEP markers allow for prognostication during the early recovery period when peaks tend to have very low amplitudes but may have the potential to increase over time. These low amplitude peaks would likely be categorized as absent using the existing classification method, but a quantitative marker provides a continuous scale to assess the various levels of recovery. The qSSEP-PSA provides this continuous scale that eliminates the need for an abnormal observation (i.e. absent N20s) to be useful in prognostication.

The current method of identifying absent N20 peaks is subjective and requires sophisticated training. One study examined the interobserver variability in the classification of N20 peaks and showed that the interobserver agreement between 5 experts was only moderate [142]. Further, there is a possibility of misclassification when distinguishing between an absent peak and a highly attenuated peak, as is often the case during the early recovery period. The qSSEP-PSA marker presented here is an objective and quantitative representation of the SSEP signal, and therefore completely eliminates the subjective interpretation of SSEP waveforms, addressing a major limitation of the standard N20 absent/present method.

It has been suggested that SSEPs should not be measured before 24 hours, as they may be unreliable [137], and the American Academy of Neurology (AAN) recommendation states that the N20 bilateral absence on days 1-3 after CA should be used to assess poor prognosis [176]. However, it has also been documented that TTM has the greatest protective benefits when applied immediately after resuscitation [71]. This may be explained in part by the damaging molecular cascades that occur during hypoxia and reperfusion [4], resulting in the ischemic injury experienced in CA patients. Thus, early tracking and prognostication is important to help guide treatment and management during the period of secondary injury. Our study demonstrates that the qSSEP-PSA metric is valid during the early recovery, which is an important improvement on the dichotomous N20 classification that requires physicians to wait 24 hours before the method is useful.

One limitation of our study is that it uses pre-CA baseline qSSEP-PSA values for normalization, which is not practical in clinical applications. This could be solved by generating standardized baseline values using typical representative waveforms.

Additionally, the study is limited by the outcome distribution within temperature groups, specifically, the hypothermia group. In the present study, all hypothermia animals had good outcome, which is not representative of clinical outcomes. It may be necessary to induce a more severe injury so that the performance of the qSSEP-PSA marker can be further evaluated with temperature management.

3.5 Conclusion

We have demonstrated that the qSSEP-PSA marker evolves differently among temperature and functional outcome groups in post-CA rats. Hypothermic animals and animals with good functional outcome had better qSSEP-PSA recovery during the early recovery period. This quantitative marker provides many benefits over the current standard of N20 absence/presence distinction. Specifically, we presented a marker that objectively quantifies SSEPs on a continuous scale, which may hold potential prognostic value. Ultimately, the goal of this study is to generate quantitative markers that can be easily translated to a clinical setting to improve the early prognostication of CA patients.

CHAPTER 4: QUANTITATIVE ANALYSES OF SOMATOSENSORY EVOKED POTENTIALS AFTER CARDIAC ARREST WITH GRADED HYPOTHERMIA

4.1 Introduction

Targeted temperature management (TTM), hypothermia of 32-34°C, has been shown to improve survival and neurological outcome following CA [23, 41]. However, it has not been clearly demonstrated which level of hypothermia is most beneficial to outcome. Multiple grades of hypothermia have been studied in both humans and animals [23, 41, 73, 80], but it is unclear which provides the best neuroprotection following CA.

Reliable prognostication during the early recovery period following CA would positively impact the subsequent treatment. While multiple prognostic tools exist, their reliability under hypothermia has been questioned [42, 88, 132, 177, 178]. However, it has been suggested that somatosensory evoked potentials (SSEP) maintain prognostic value under TTM [50].

Although SSEP have proven to be a useful prognostic tool for comatose patients in post-CA recovery [133, 134], the major need for a reliable and objective prognostic tool to

track recovery has been established. In this preliminary study, we describe multiple quantitative analyses of SSEP – N10 amplitude and latency and quantitative SSEP phase space area (qSSEP-PSA) – with the potential to track early cerebral recovery following severe brain injury in a 9 min CA rat model with graded hypothermia. We tested the potential prognostic value of these markers under graded hypothermia and discovered that SSEPs hold prognostic value in the form of graded quantitative markers that extend beyond the dichotomous categorization of peaks.

4.2 Materials and Methods

4.2.1 Animals

In this preliminary study, a total of 16 adult male Wistar rats (371±12 g) underwent 9 min asphyxial CA. The animals were randomly assigned to normothermia or one of three grades of hypothermia (n=4/group): N0 (36.5-37.5°C), H1 (30-32°C), H2 (32-34°C), H3 (34-36°C). All procedures were approved by the University of Maryland Animal Care and Use Committee.

4.2.2 Cardiac Arrest and Temperature Management

The asphyxia-CA and resuscitation were performed as previously described [46, 110, 113, 145, 166]. Rats were intubated and continuously anesthetized with 1.5% isoflurane in 1:1 O₂:N₂, delivered by a mechanical ventilator. The femoral vein and artery were

cannulated and 15 min baseline SSEPs were recorded followed by a 5 min anesthetic washout period, during which Vecuronium (2 mg/kg) was administered. Following the washout, asphyxia was initiated. After 9 min of CA (MAP < 10 mmHg), cardiopulmonary resuscitation (CPR) was performed using sternal chest compressions, 100% oxygen, epinephrine and sodium bicarbonate until return of spontaneous circulation (ROSC, MAP > 60 mmHg). SSEP recordings were taken in 15 min intervals from 30 min until 4 hrs after ROSC. Isoflurane was administered as needed (up to 0.5%) once SSEP recordings began during the recovery period due to the potential discomfort of the stimulations.

Temperature management began immediately after ROSC was achieved. The hypothermic animals were immediately surface cooled and were maintained at their appropriate degree of hypothermia (H1 30-32°C, H2 32-34°C, H3 34-36°C) for 6 hours after ROSC after which the animals underwent rewarming to normothermia (36.5-37.5°C) over 2 hours. The normothermia animals were maintained at 36.5-37.5°C for 6 hours after ROSC using a heating pad. A rectal probe was used to measure temperature, which was closely monitored and recorded every 5min.

4.2.3 SSEP Acquisition and Analysis

Approximately 3 days before the date of CA, rats had 4 screw electrodes (Plastics One, Roanoke, VA) cortically implanted over the somatosensory cortex with 1 ground

electrode over the parasagittal right frontal lobe. The screws were held in place with a plastic pedestal and dental cement [145, 162]. The SSEP signals were recorded from the skull electrodes following stimulation of the median nerves. Stimulation pulses (200usec, 6mA) were delivered to subdermal electrodes at a frequency of 0.5Hz. The subsequent SSEPs were recorded with the TDT System3 data acquisition system (Tucker-Davis Technologies, Alachua, FL) at a frequency of 6.1kHz.

The SSEPs were recorded for 15 min prior to CA (baseline) and beginning 30 min after ROSC, continuing in 15 min intervals until 4 hrs after ROSC. The sweeps within each 15 min interval were averaged (450 sweeps) and the quantitative analyses were performed on the averaged waveform for each time period and then normalized to baseline values. The normalized values for each time period were then averaged to generate the aggregate value for each quantitative marker. Animals with abnormal baseline waveforms (bilaterally distorted N10 and P15) were excluded from the analysis.

N10 amplitude and latency

The rat N10 peak is equivalent to the N20 peak in humans. The N10 amplitude was measured as the peak-to-peak amplitude between the N10 and P15 peaks. The N10 latency was measured as the time from stimulation to the N10 peak. The amplitude and latency were measured using a custom MATLAB (MathWorks, Natick MA) algorithm.

qSSEP-PSA

The qSSEP-PSA marker was calculated as previously described [145]. Briefly, the phase space curve (PSC) of an SSEP waveform was generated by plotting the first derivative against the magnitude, thus capturing the morphologic information of the peak. The phase space area (PSA), a representation of the signal power, was calculated by determining the area bound by the PSC. The qSSEP-PSA was determined by fitting a convex hull to the PSC using the Quickhull algorithm [179]. The point-index based convex hull was calculated by identifying the indices of the PSC that exist along the boundary of the convex hull and the qSSEP-PSA is the area encompassed by this boundary. The algorithm selects points within the waveform, including transitional slopes and smaller peaks, as to capture the extent of the signal, therefore encompassing more information than merely an amplitude or latency. These analyses were performed using MATLAB [145].

4.2.4 Neurologic Recovery and Assessment

The neurologic recovery of rats was assessed using the neurologic deficit scale (NDS) at 6, 24, 28 and 72 hrs after ROSC. The 72 hr NDS was used to determine the final functional outcome such that good functional outcome was defined as 72 hr NDS \geq 60 and poor functional outcome as 72 hr NDS $<$ 60 [46].

4.2.5 Statistics

All statistical analyses were performed using a commercial computer package (IBM SPSS Statistics v22, Armonk, NY). The N10 amplitude and latency, and qSSEP-PSA (mean±S.E.M.) were compared between temperature groups using a repeated measure of analysis of variance (ANOVA) and compared between outcome groups using a student's t-test. Bivariate analyses were used to generate the pearson correlation coefficients between 72 hr NDS and quantitative markers. A p value < 0.05 was considered statistically significant.

4.3 Results

4.3.1 Temperature, NDS, Baseline Data

The temperature was well monitored throughout the duration of the experiment. The groups were maintained at their respective temperature ranges: H1 (31.3±0.06°C), H2 (33.0±0.05°C), H3 (34.7±0.04°C), N0 (37.1±0.03°C). Target temperatures were reached within 11±2min (H1: 18±3min; H2: 10±2min; H3: 5±0min). The 72 hr NDS (median (25th, 75th)) of each temperature group was as follows: H1 (58 (0, 68)), H2 (59 (14, 70)), H3 (39 (0, 77)), N0 (22 (0, 61)). Based on the 72 hr NDS, 7 animals had poor outcome (0 (0,0)) and 9 animals had good outcome (66 (57,71)) (p<0.01). The baseline rat body weight was not significantly different among temperature groups (p>0.05, data not shown).

4.3.2 N10 Amplitude

The N10 amplitude of the early recovery period (first 4 hours after ROSC) showed a decreasing trend among increasing temperature groups (Fig. 4.1A). All three hypothermia groups (H1, H2 and H3) had significantly larger N10 amplitudes than the normothermia (N0) group (all $p < 0.05$). The H1 group also had significantly larger N10 amplitudes than the H3 group ($p < 0.01$). Animals with good outcome also had better N10 amplitude recovery compared to those with poor outcome, though the difference is not significant with the current animal cohort ($p > 0.05$) (Fig. 4.1B).

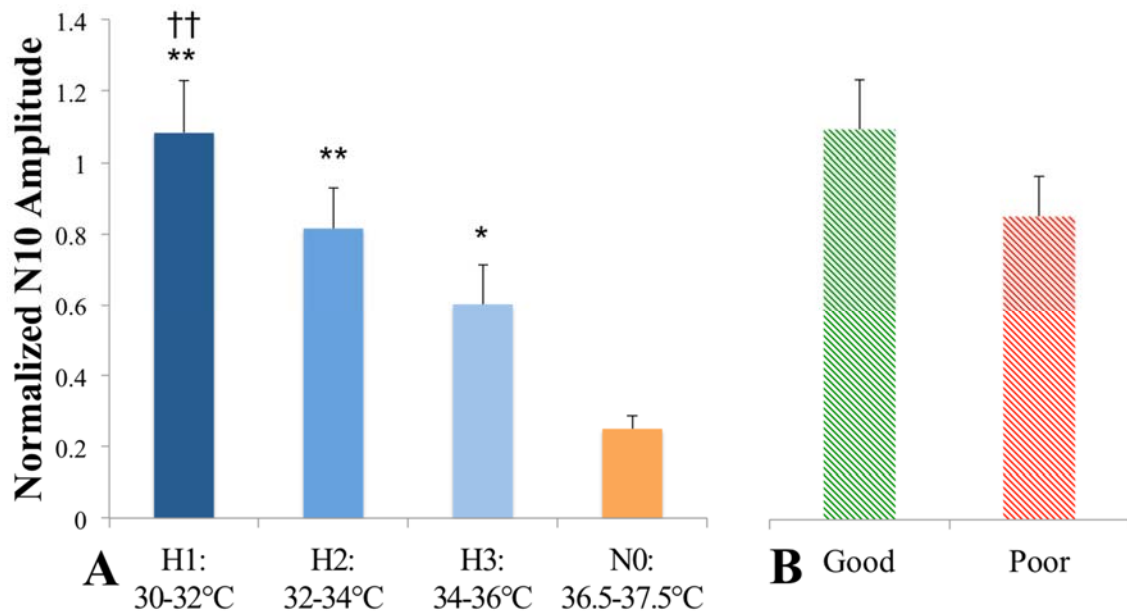


Figure 4.1 A) Normalized aggregate N10 amplitude among four temperature groups. All three hypothermia groups (H1, H2, H3) had significantly larger N10 amplitudes than N0. H1 also had significantly larger amplitude than H3. B) Normalized aggregate N10 amplitude was higher in animals with good functional outcome. * $p < 0.05$, ** $p < 0.01$ compared to N0. †† $p < 0.01$ compared to H3.

4.3.3 N10 Latency

The early recovery N10 latency showed a decreasing trend among increasing temperature groups (Fig. 4.2A). All hypothermia groups (H1, H2, and H3) had significantly longer N10 latencies than the normothermia group (N0) (all $p < 0.01$). The H1 and H2 groups also had significantly longer latencies than the H3 group ($p < 0.01$) and the H1 group had significantly longer latencies than the H2 group ($p < 0.01$). The N10 latency was very similar between animals with good outcome and those with poor outcome ($p > 0.05$) (Fig 4.2B).

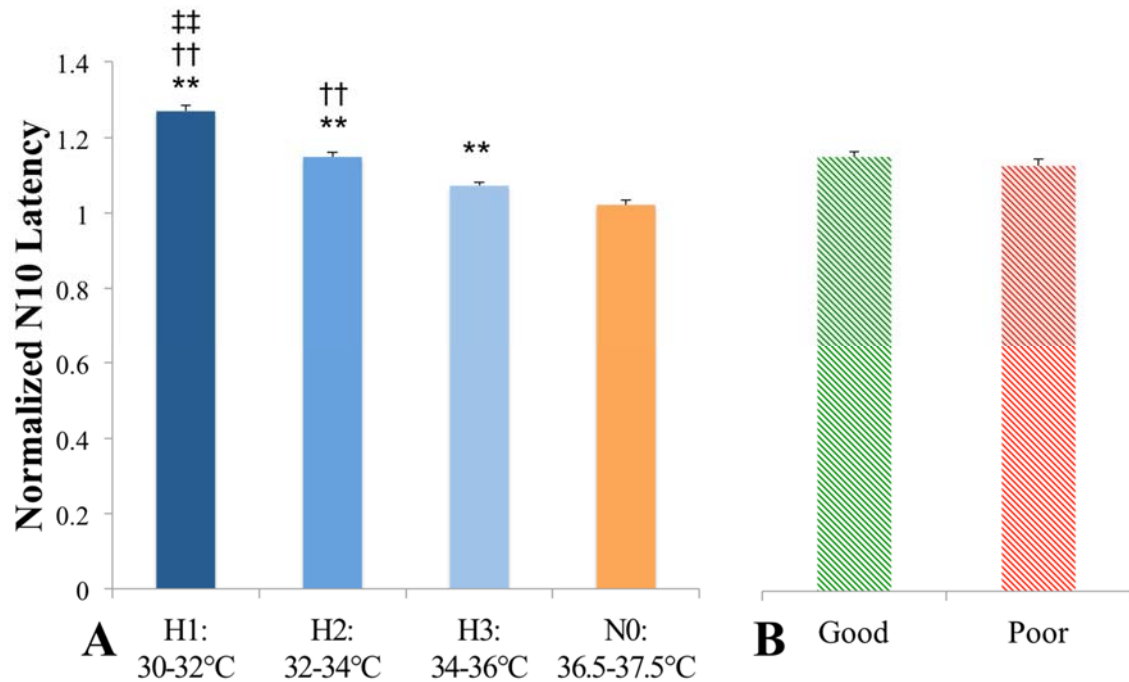


Figure 4.2 A) Normalized aggregate N10 latency among four temperature groups. All three hypothermia groups (H1, H2, H3) had significantly longer N10 latencies than N0. H1 also had significantly longer latency than H2 and H3 while H2 had significantly longer latency than H3. B) Normalized N10 latency similar between the outcome groups. ** $p < 0.01$ compared to N0. †† $p < 0.01$ compared to H3. †† $p < 0.01$ compared to H2.

4.3.4 qSSEP-PSA

The qSSEP-PSA during the early recovery was significantly larger in the H1 and H2 groups than the N0 group ($p < 0.01$) (Fig. 4.3A). The H3 qSSEP-PSA was also lower than the H1 and H2 groups and larger than N0, though the differences were not significant ($p > 0.05$). qSSEP-PSA was also higher in animals with good outcome compared to those with poor outcome, although the difference was not significant ($p > 0.05$) (Fig. 4.3B).

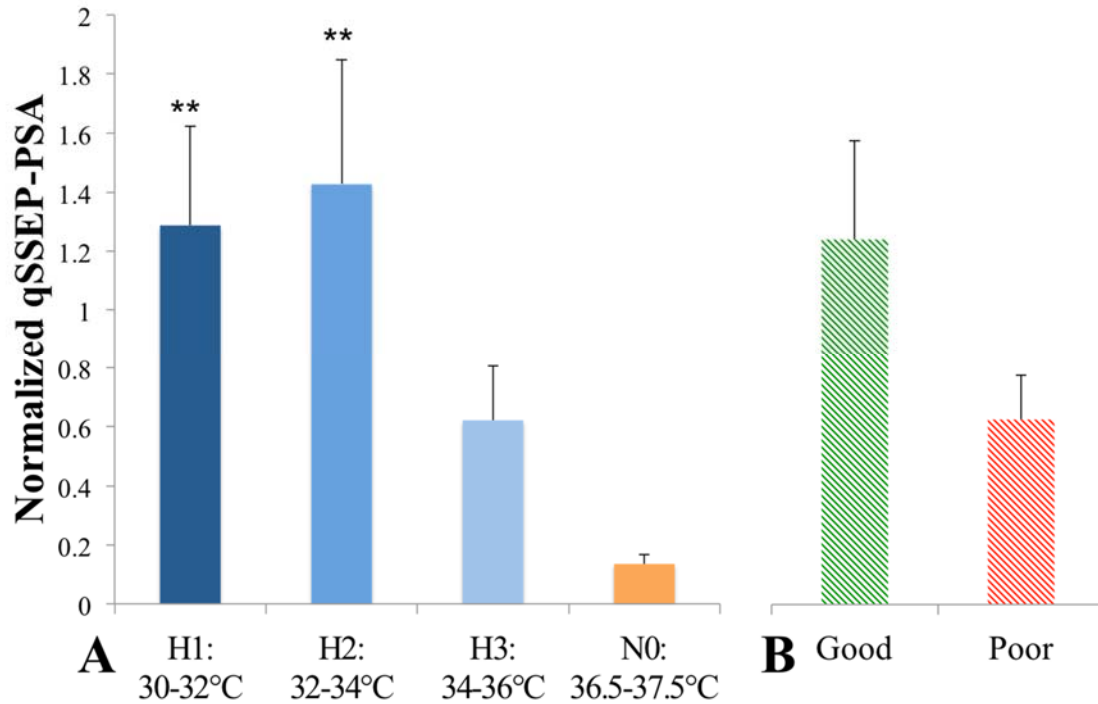


Figure 4.3 A) Normalized aggregate qSSEP-PSA among four temperature groups. Both H1 and H2 had significantly larger qSSEP-PSA values than N0. B) qSSEP-PSA was higher in animals with good outcome. ** $p < 0.01$ compared to N0.

4.3.5 Correlation Between Quantitative Markers

Among the quantitative markers, the N10 amplitude was significantly correlated with both N10 latency (pearson correlation coefficient: 0.400, $p < 0.01$) (Fig. 4.4A) and qSSEP-PSA (pearson correlation coefficient: 0.904, $p < 0.01$) (Fig. 4.4B).

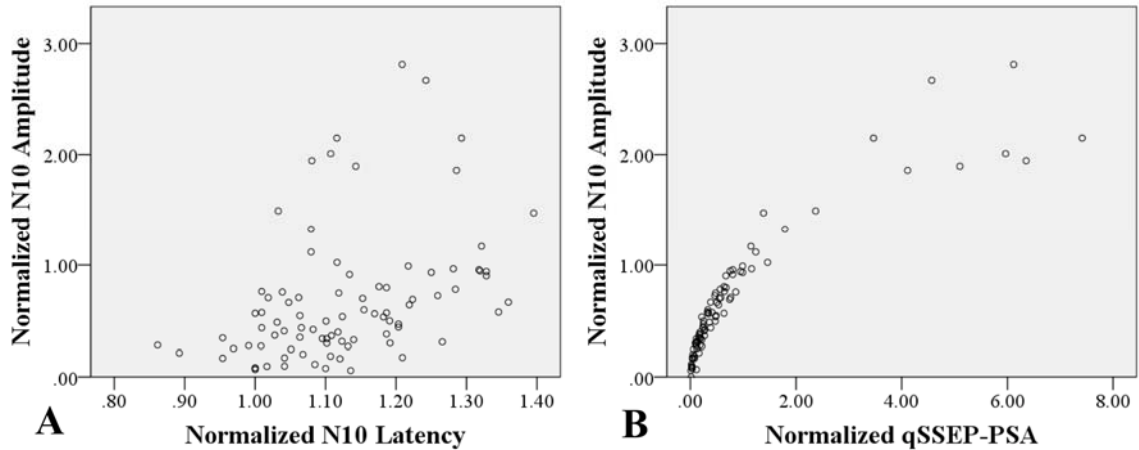


Figure 4.4 N10 amplitude was significantly correlated to a) N10 latency and B) qSSEP-PSA.

4.4 Discussion

In this study, the integrity of the somatosensory pathway following severe brain injury by asphyxial-CA was examined using multiple quantitative markers under graded hypothermic conditions, for the first time. We discovered that quantified SSEPs hold great potential to track recovery following CA with TTM by providing continuous quantitative criteria, thereby accounting for multiple conditions as opposed to dichotomous categorization. All three markers, N10 amplitude and latency, and qSSEP-PSA, demonstrated clear trends among both temperature and functional outcome groups. This experiment demonstrates the potential prognostic value of these objective and novel quantitative markers for the early recovery after severe CA with graded TTM.

The majority of studies evaluating the prognostic value of SSEP following CA are focused on the bilateral absence of N20 [42, 88, 129-136] rather than the relationship between quantitative SSEP measures and outcomes. Only one human study has examined the relationship between SSEP amplitude and outcome, suggesting a threshold amplitude voltage to distinguish bad outcomes [161]. However, the study does not use specific response peaks in the evaluation of amplitude. Here we provide three quantitative, objective and repeatable SSEP markers that hold potential prognostic value following CA with temperature management.

Our previous work developed the qSSEP-PSA marker and demonstrated the potential prognostic value of both qSSEP-PSA and N10 amplitude in normothermic animals with moderate and severe brain injuries following CA [145]. This study showed that animals with moderate injury had better PSA recovery than those with severe injury. Here we demonstrated that qSSEP-PSA had better recovery with deeper hypothermia (H1 and H2) compared to normothermia and in animals with good outcome compared to poor outcome. The qSSEP-PSA marker also mirrored the median 72 hr NDS among temperature groups (H2 > H1 > H3 > N0). The PSA marker is a strong measure of SSEP waveforms as it considers multiple features of the entire signal rather than a single response peak characteristic.

The N10 amplitude and latency have been previously studied in both moderate and severe brain injury (7 and 9 min CA, respectively) under normothermic conditions [162]. This

study demonstrated that amplitude recovery is better after moderate injury rather than severe injury and that latency decreases towards baseline over the early recovery period at both injury severity levels. The present study demonstrated better recovery of N10 amplitude in animals treated with lower temperature grades and with good functional outcome and that N10 latency was overall longer than baseline in animals with deeper hypothermia. Although it has been demonstrated that hypothermia increases N10 amplitude and N10 latency compared to normothermic groups in an uninjured rat model [154], it has been suggested that the effect of mild hypothermia (33°C) does not have a significant effect on the relationship between SSEP amplitude and outcome in a human study [161].

SSEP amplitude has been shown to be related to functional outcome [161]. Here we demonstrate that N10 amplitude is correlated with both N10 latency and PSA during the early recovery from severe brain injury with graded hypothermia. Thus, the prognostic potential of these quantitative SSEP markers, N10 amplitude, N10 latency, and qSSEP-PSA, is further demonstrated.

Although clear trends of the quantitative SSEP markers were demonstrated in this study, the results must be interpreted with caution, as this is a preliminary study with small animal numbers. The use of 72 hr NDS among temperature groups to evaluate the effect of graded hypothermia was not our focus in the present study due to the low power of the

small animal number, though the trend generally followed that of the quantitative markers (higher in the H1 and H2 groups and lowest in N0).

Thus, the data we present here corroborates previous studies of N10 amplitude and qSSEP-PSA under normothermic conditions and it is necessary to extend the present study with larger animal numbers, including a sham group. The quantitative SSEP markers presented here demonstrated prognostic potential following severe CA with graded hypothermia.

CHAPTER 5: ASSOCIATION OF CEREBRAL BLOOD FLOW AND QUANTIFIED EEG AFTER CARDIAC ARREST

5.1 Introduction

Cardiac arrest (CA) frequently causes cerebral ischemic injury by reducing or completely halting blood flow to the brain. This lack of cerebral perfusion is critical in the poor outcomes that result from CA. In addition to the damage done by the anoxic ischemic state, it has been suggested that reperfusion injury occurs following resuscitation [180] by free radicals [181, 182] or diapedesis of red blood cells [183]. Additionally, it has been suggested that autoregulation of CBF is absent or right-shifted in the early recovery period following CA [184]. Thus, it is important to understand the dynamics of CBF following CA.

Cortical electrical activity has been widely studied in anoxic ischemic injury cases and specifically in regards to CA applications. Continuous electroencephalogram (EEG) has become an extremely common tool to measure cortical electrical activity in comatose patients [185, 186], including those recovering from CA. Certain EEG characteristics have been associated with unfavorable outcomes such as burst suppression, low voltage activity and epileptiform pattern [42]. Many studies have classified EEG wave patterns

into groups corresponding to different levels of activity for prognostic purposes or to elucidate the EEG pattern recovery following CA in human studies [29, 42, 97, 99, 104-107, 132, 147, 187-193]. Thus, the cortical electrical activity holds significant prognostic value in the recovery following CA.

Our group previously developed novel methods to quantify EEG signals and measure rCBF with high spatial and temporal precision. In order to reduce the laborious and subjective interpretation of EEG signals, here we use a quantitative measure of the entropy within EEG signals, information quantity (IQ) [110]. Previous studies have shown that the IQ metric successfully tracks brain recovery and predicts functional outcome after CA in rats [46, 110]. To measure rCBF in CA rats, our group developed a laser speckle contrast imaging (LSCI) system to continuously track the rCBF changes during and after CA with high temporal and spatial resolution. LSCI is an emerging imaging tool primarily used for blood flow imaging, which quantifies the resulting pattern of coherent light interference, termed the speckle pattern, due to blood flow [194].

Both rCBF and cortical electrical activity play an important role in recovery in the early stages following CA. Hypothermia is known to decrease rCBF in uninjured brains [195, 196], however, it is not well understood how rCBF correlates with the electrical activity under hypothermic conditions following CA. In this study, LSCI and IQ analyses were applied to CA rats to characterize the relationship between the average rCBF of the

arteries, veins and capillaries of the primary motor cortex and cortical electrical activity during the early recovery after CA with targeted temperature management (TTM).

5.2 Materials and Methods

5.2.1 Animals

A total of 28 adult Wistar rats (322±8g) were used for these experiments. Each animal was randomly assigned to one of four groups (n=7 per group): 7min CA or 9min CA under normothermia (36.5°C-37.5°C, group name 7N or 9N, respectively), or 7min or 9min CA under hypothermia (32°C-34°C, 7H or 9H, respectively). All animals underwent asphyxia-CA and immediate temperature management following resuscitation according to their assigned group. The rats were housed in a controlled temperature environment with a standard dark/light cycle with food and water *ad libitum*. All experimental protocols were approved by the University of Maryland Institutional Animal Care and Use Committee.

5.2.2 Experiment Preparation

All rats underwent an implantation procedure under sterile conditions one day prior to CA. A heating pad was used to control animals' rectal temperature (36.5-37.5°C) throughout the procedure. The animals were placed in a stereotactic frame (David Kopf instruments, Tujunga, CA) with continuous 1.5% isoflurane anesthetization. An incision

was made in the skin over the scalp and a left hemisphere cranial window was prepared (7mm x 5mm, centered at AP -1, ML -2.5) [197]. A cylinder base (height: 4.2mm, radius: 5.5mm, thickness: 0.5mm) was fixed by dental cement encircling the cranial window and connected to the imaging system during acquisition. Three screw electrodes (Plastics One, Roanoke, VA) were cortically implanted in the right hemisphere to record the EEG [114].

5.2.3 Cardiac Arrest, Resuscitation and Temperature Management

CA and immediate temperature control during the early recovery period with EEG monitoring were performed as previously described in detail [46, 110, 113, 145]. Briefly, the rats were intubated, mechanically ventilated, and anesthetized with 1.5% isofluroane in 1:1 O₂:N₂. Arterial blood gases (ABG) and blood pressure were monitored, and drugs were delivered via the cannulated femoral artery and vein, respectively. A 5 min baseline EEG measurement was recorded, followed by a 5 minute anesthesia washout period, during the final three minutes of which the ventilation was switched to room air and vecuronium (2mg/kg) was administered. Asphyxial CA was induced until pulse pressure < 10 mmHg. After 7 or 9 mins of asphyxia depending on the rats' group, mechanical ventilation was resumed and cardiopulmonary resuscitation (CPR) was performed using sternal chest compressions and epinephrine until the return of spontaneous circulation (ROSC) was achieved. Sodium bicarbonate was administered with the epinephrine during CPR to prevent acidosis.

The rats' rectal temperature was monitored using a rectal probe and recorded every 5 min. Normothermia animals were maintained at 36.5-37.5°C using a heating pad for 8 hrs after ROSC. Hypothermia animals were cooled beginning immediately after ROSC using a misted water and alcohol solution and fan to reach a target temperature of 33°C ± 1°C within 15 min. Hypothermia was maintained for 6 hours, after which the rats were rewarmed to normothermia (36.5-37.5°C) using a heating pad and thermal heating lamp (Thermalet TH-5, model 6333, Physiotemp, NJ, USA) over a period of 2 hours. To maintain normothermic body temperature, all animals were kept in a neonatal incubator at 28°C for the first 24 hours after ROSC.

5.2.4 Laser Speckle Contrast Imaging

The CBF was recorded for each rat using the LSCI system. An 8-bit COMS camera (DCC1240C, Thorlabs) was connected to the cylinder base previously implanted and was used to capture the images. A laser diode (780nm; 10mW; L780P010, Thorlabs), powered by a driver module (LDC220C, Thorlabs) was the coherent light source illuminating the region of interest (ROI). Each trial acquired 320 consecutive frames of speckle pattern images (640x640 pixels) at 50 frames/sec and 5ms exposure time. LSCI data were obtained beginning at the 5min anesthetic washout and ending at 90min after ROSC. LSCI analysis was performed to calculate rCBF. The individual cortical artery and vein rCBFs were calculated in the ROI and normalized to the washout period baseline. By selecting a 1mm x 1mm ROI centered at the primary motor cortex (M1, AP - 1.5; ML -0.5), the capillary rCBF was obtained by eliminating cortical arteries and veins

from the images [197]. The overall rCBF used in the results of this chapter was calculated by averaging the vein, artery and capillary rCBFs.

5.2.5 EEG Acquisition and Analysis

Following the baseline measurement prior to washout, continuous EEG was recorded during the anesthetic washout, CA and for 6 hours post-ROSC. Noise was removed in the frequency domain using custom MATLAB algorithms (Mathworks, Natick, MA) and artificial signals were manually eliminated prior to the final analysis. The EEG signals were then quantified using an entropy-based value, IQ [110]. Briefly, the EEG signals were divided into equal lengths using a sliding temporal window technique (window length $\omega=8s$, sliding step $\Delta=8s$, number of magnitude levels $M=20$). Then a discrete wavelet transform (decomposition scale $r=5$) was applied to each temporal window to generate decomposition coefficients, c_n^k , where $k=1,2,\dots,r+1,m$ which represents the k^{th} frequency subband. Since the Shannon entropy is probability-based, the distribution of wavelet coefficients was determined in each time window by finding the probability, $p_n^k(m)$, of each coefficient. We used M bins, I_m , to determine the occurrence frequency of each coefficient in each bin (Eq. 1).

$$[c_n^1, c_n^2, \dots, c_n^{r+1}] = \cup_{m=1}^M I_m \quad (1)$$

Finally, the IQ of the EEG samples was calculated using the entropy formula (Eq. 2).

$$IQ(n) = -\sum_{m=1}^M p_n(m) \cdot \log_2(p_n(m)) \quad (2)$$

The IQ was calculated at 10, 20, 30, 60, and 90 min post-ROSC and normalized to the baseline value.

5.2.6 Neurological Evaluation

The neurological function of each animal was assessed at 6, 24, 28 and 72 hrs after CA using the neurological deficit scale (NDS). The 72hr NDS was used to define functional outcome as either good (72hr NDS \geq 60) or poor (72hr NDS $<$ 60) [46].

5.2.7 Statistics

All statistical analyses were performed using a commercial statistical computer package (IBM SPSS Statistics, version 22, Armonk, NY). Baseline data, IQ, and rCBF were compared between groups using an analysis of variance with repeated measures (ANOVA). The NDS was compared between the four animal groups using a Kruskal-Wallis test and between temperature and CA groups using a Mann-Whitney U test. The correlation between rCBF and IQ was determined using the Pearson correlation coefficient test. A p value $<$ 0.05 was considered significant.

5.3 Results

5.3.1 Baseline Data and Temperature Maintenance

The baseline animal body weight, rectal temperature and anesthetic exposure during preparation were not significantly different among groups (Table 5.1). The temperature was well monitored throughout the duration of the experiment for all animals. The normothermic groups were maintained at $36.81 \pm 0.02^\circ\text{C}$ for 7minCA and $36.85 \pm 0.02^\circ\text{C}$ for 9min CA while hypothermic groups were maintained at $33.85 \pm 0.03^\circ\text{C}$ for 7min CA and $33.41 \pm 0.04^\circ\text{C}$ for 9min CA during the periods of temperature management (Fig. 5.1).

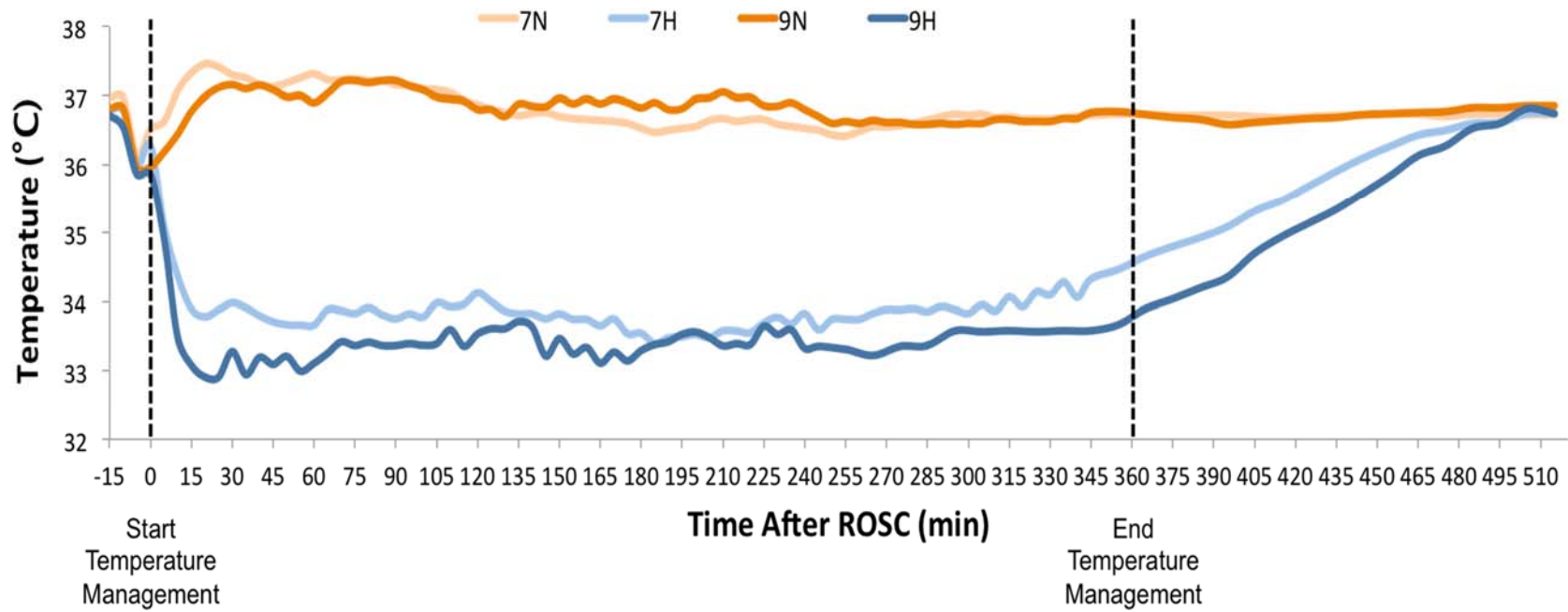


Figure 5.1 Body temperature of each animal group was maintained at their respective value throughout the temperature management period.

Table 5.1 Baseline data for each animal group

	7N	7H	9N	9H	P value
Body weight (g)	340±10	341±8	293±17	315±20	0.09
Body temperature (°C)	37.0±0.0	36.8±0.1	36.8±0.1	36.7±0.1	0.1
Anesthetic exposure (min)	79±2	82±3	73±2	74±3	0.07

5.3.2 NDS Scores

The 72 hour NDS was not significantly different between the four animal groups or between temperature or CA time groups ($p > 0.05$). However, the combination of NDS scores at all time point for each animal were significantly different between temperature groups ($p < 0.01$) and CA time groups ($p < 0.01$) such that hypothermia animals had higher median NDS (66 (49, 74)) compared to normothermia animals (47 (35, 70)) and 7min CA animals had higher median NDS (70 (47, 77)) compared to 9min CA animals (47 (35, 66)).

5.3.3 Changes in Relative Cerebral Blood Flow

The normalized rCBF was greatly decreased during the final 4 min of CA, and then increased back to or above baseline levels at 10 min after ROSC, representing the

hyperemia phase (Fig. 5.2). The rCBF then decreased below baseline from 20-90 min after ROSC, representing the prolonged hypoperfusion phase. This trend was generally observed in all four animal groups (Fig. 5.2). The rCBF values for each group within each time period are shown in Table 5.3.

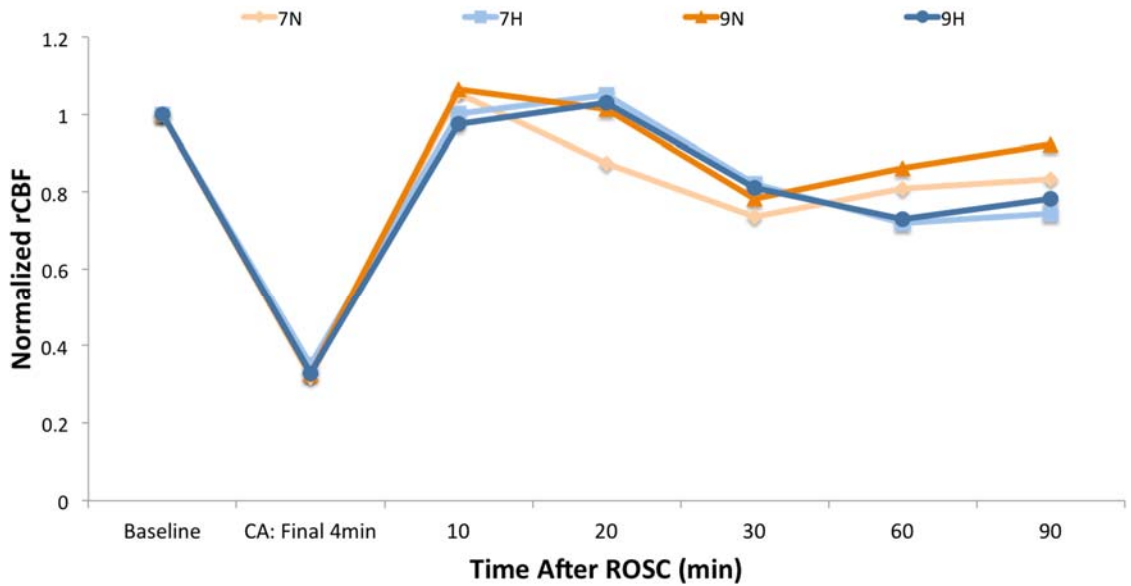


Fig 5.2. Time evolution of the normalized rCBF of all vessels throughout the experiment. The rCBF decreased greatly during the final 4 min of CA, then increased back to or above baseline at 10min after ROSC, then overall gradually decreased at 90min after ROSC.

5.3.4 Changes in Quantitative EEG

The IQ of each of the four animals groups increased over the early recovery period from 10-90min after ROSC (Fig 5.3). The 7H animals showed better recovery of IQ by 90min post-ROSC while the 9N showed the worst recovery (Fig 5.3). The IQ values for each group within each time period are shown in Table 5.3.

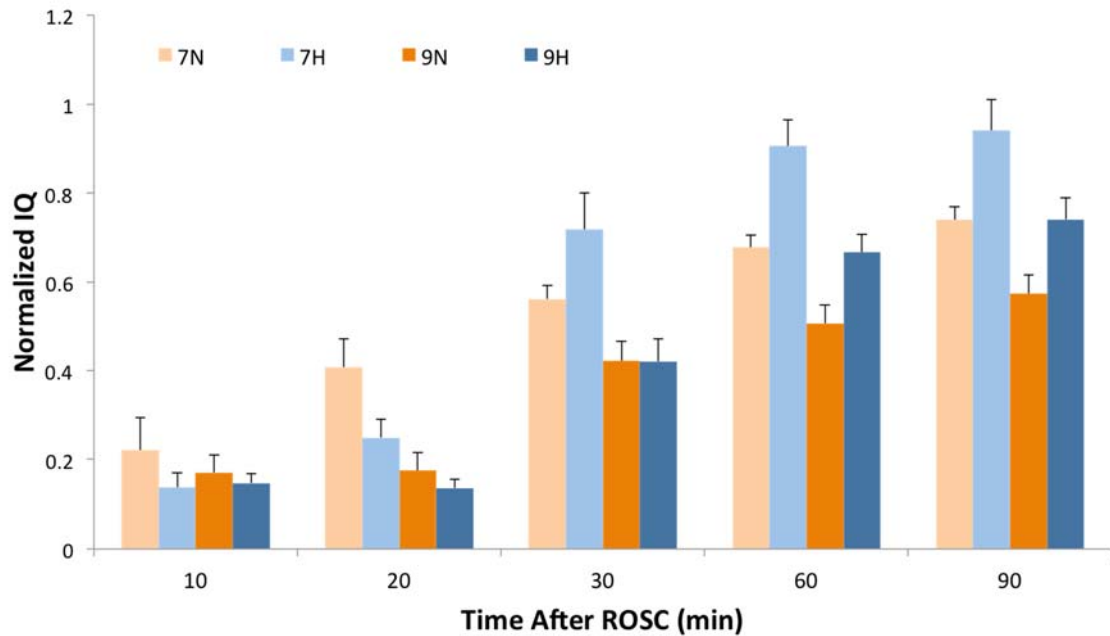


Fig 5.3. Time evolution of the EEG information quantity (IQ) throughout the experiment. The IQ generally increased over time for each animal group, with the 7H group showing the best recovery.

5.3.5 Correlation Between rCBF and IQ

As shown in Figure 5.4, the rCBF and IQ of all four animal groups had a significant negative correlation during the first 90min after ROSC (Pearson correlation coefficient: -0.680, $p < 0.01$). The negative correlation was also significant at 20min (Pearson correlation coefficient: -.550, $p < 0.01$) and 90min (Pearson correlation coefficient: -0.473, $p < 0.05$) after ROSC (Table 5.4).

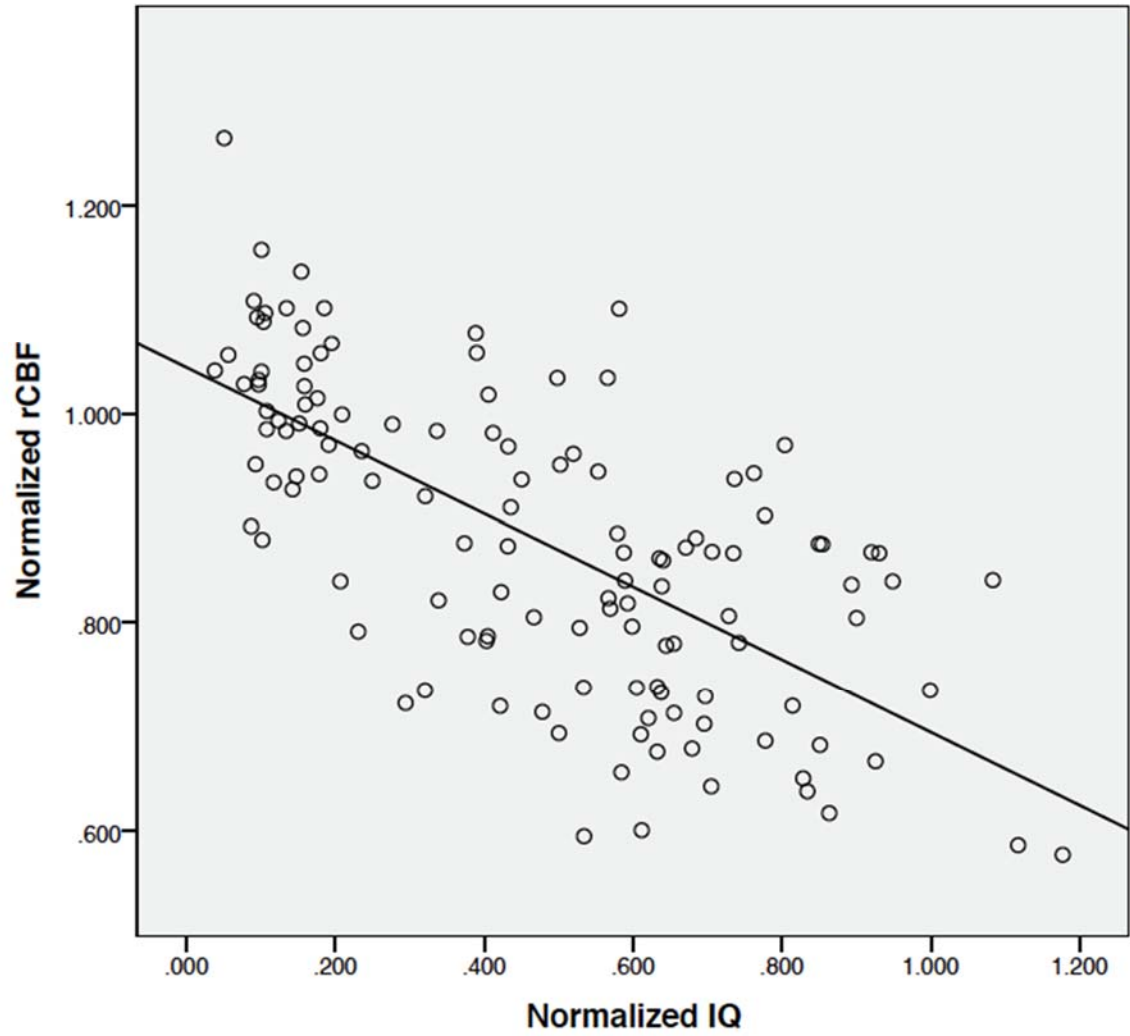


Fig 5.4. rCBF and IQ of all animal groups during the first 90min after ROSC are significantly correlated.

Table 5.2 Pearson correlation coefficients for rCBF and IQ

	Aggregate	10min	20min	30min	60min	90min
Pearson						
Correlation						
Coefficient	-0.680**	0.062	-0.550**	-0.246	-0.360	-0.473*

* $p < 0.05$, ** $p < 0.01$

Table 5.3. rCBF and IQ (mean±S.E.M.) Early Recovery Period Data

Group	Variable	Aggregate	10min	20min	30min	60min	90min
7N	IQ	0.522±0.038	0.220±0.074	0.407±0.064	0.563±0.031	0.678±0.027	0.740±0.031
	rCBF	0.858±0.026	1.054±0.057	0.874±0.038	0.736±0.034	0.808±0.033	0.833±0.046
7H	IQ	0.590±0.067	0.137±0.032	0.249±0.041	0.719±0.082	0.906±0.059	0.941±0.070
	rCBF	0.858±0.029	1.001±0.026	1.050±0.031	0.821±0.053	0.719±0.043	0.745±0.041
9N	IQ	0.366±0.034	0.170±0.039	0.175±0.040	0.421±0.044	0.508±0.041	0.575±0.041
	rCBF	0.929±0.021	1.065±0.033	1.013±0.017	0.783±0.016	0.862±0.026	0.923±0.040
9H	IQ	0.415±0.049	0.147±0.020	0.136±0.019	0.420±0.051	0.667±0.041	0.741±0.049
	rCBF	0.866±0.024	0.976±0.021	1.030±0.018	0.812±0.038	0.729±0.039	0.783±0.035

5.4 Discussion

We have importantly demonstrated, for the first time, that the rCBF is negatively correlated with IQ during the early recovery period (first 90min post-ROSC) following CA, and more specifically, at 20min and 90min post-ROSC. The IQ metric is representative of the cortical electrical activity. The EEG electrical activity, visibly shown as a bursting pattern, is indicative of the recovery of various cortical and subcortical regions following CA [103, 198] and is predictive of final outcome [104-107]. The cortical CBF and cerebral metabolic rate of oxygen (CMRO₂) both decrease in the early period following CA due to increases in cerebral vascular resistance [199, 200], thereby impacting the neurological recovery [201]. Additionally, it has been shown that there is a linear relationship between electrical activity and CMRO₂ [202]. Under standard physiological conditions, increases in electrical activity result in adjusted increases in CBF, generally within seconds [203-208]. However, as shown in the present study, under pathological conditions following CA, electrical activity, represented by IQ, tends to increase over the recovery period while rCBF tends to decrease, after a brief hyperemia phase, indicating an uncoupling of the EEG and rCBF relation, which is supported by previous studies [209]. Therefore, the negative correlation between IQ and rCBF suggests that a lower rCBF can still support increasing electrical during the recovery period. The correlation also importantly indicates that rCBF may hold prognostic value for post-CA patients.

Further, the relationship between rCBF and electrical activity may help uncover the mechanisms of the influence that rCBF has on post-CA recovery. One study found that patients who regained consciousness following resuscitation all had normal CBF values while those who died before regaining consciousness had increased CBF within 24 hours after resuscitation [210]. This generally supports the observation in the present study that the positive relation between electrical activity and rCBF is disrupted by CA, resulting in a negative correlation. Further, one study determined the relationship between electrical activity, as measured by somatosensory evoked potential (SSEP) amplitude, and CBF during and after acute middle cerebral artery occlusion, and identified two relationships: time-dependent and steady state [201]. Ultimately, due to the steepness of the steady state relationship, the authors explain that a small increase in CBF may result in the electrical recovery that is seen after the occlusion, which may be similar to the observations in the present study.

5.5 Conclusion

This study demonstrated that the increasing relation between electrical activity, measured here by IQ, and rCBF under physiological conditions is disrupted following CA, resulting in a negatively correlated relation. Specifically, we verified that a rCBF lower than baseline is able to support electrical recovery and subsequent functional recovery in the early post-CA period. Additionally, this relation suggests that rCBF, as obtained by our novel LSCI system, may hold potential prognostic value in CA applications, though this

was not directly addressed in the present study. Ultimately, the relationship between IQ and rCBF may help uncover the mechanisms and dynamics of post-CA recovery.

CHAPTER 6: SUMMARY AND FUTURE DIRECTIONS

6.1 Summary

The work in this thesis was primarily focused on the evaluation of novel quantitative markers in the prognostication of post-CA subjects, using a rodent model. We determined that SSEP signals hold prognostic value in the form of continuous, quantitative markers, and may provide more benefits than the current binary present/absent standard.

Specifically, we found that N10 amplitude and N7 and N10 latencies of rat SSEPs under TTM following CA have different evolution patterns over the early recovery period, dependent on final functional outcome with both moderate and severe injury. Further, we demonstrated that the qSSEP-PSA marker is distinct among temperature and outcome groups in the early recovery following CA. We examined the relation between rCBF and EEG electrical activity, and found a negative correlation during the early recovery period. Ultimately, we have demonstrated that a multimodal approach using numerous quantitative measures such as SSEP peak amplitudes and latencies, qSSEP-PSA, IQ and rCBF, may optimize prognostication during the early recovery period following CA.

To summarize, in this work we:

- Reviewed the benefits and limitations of existing prognostic tools that are used in post-CA patients in a clinical setting.
- Acknowledged the benefits of SSEP in prognostication and reviewed the benefits and limitations of the standard SSEP analysis, which involves the dichotomous

classification of N20 peaks as either present or absent, where bilaterally absent N20 peaks predict poor outcome with 100% specificity.

- Evaluated the prognostic value of N10 amplitude and N7 and N10 latency of post-CA rat SSEPs during the first 4 hours of recovery under targeted temperature management. We found that the N10 amplitude has a better recovery in hypothermia animals compared to other temperature groups and predicts good outcome following CA.
- Assessed the evolution of the qSSEP-PSA marker in post-CA rats with temperature management. We discovered that the qSSEP-PSA marker evolves differently depending on temperature and outcome groups, such that hypothermic animals and animals with good functional had upward trending qSSEP-PSA values towards the end of the early recovery period, while the other groups tended to trend down or stabilize at lower values.
- Performed a multimodal analysis of SSEPs following severe brain injury by CA under graded hypothermia in a pilot study. We discovered that N10 amplitude and latency and qSSEP-PSA all demonstrated distinct trends among both temperature and functional groups during the early recovery period under graded hypothermia. We also demonstrated that N10 amplitude is correlated with both N10 latency and qSSEP-PSA during the recovery period. Importantly, we established that these markers hold the potential to track recovery following severe cerebral injury.
- Examined the relation between cerebral electrical activity, measured by IQ, and rCBF during CA and throughout the subsequent early recovery period. We discovered that the increasing relation that exists between IQ and rCBF under

physiological conditions is disrupted following CA, and ultimately results in a negative correlation between markers. We showed that lower than baseline rCBFs can support electrical recovery following CA.

6.2 Future Directions

The work presented in this thesis provided the foundation for future work to further develop quantitative markers to improve prognostication during the early recovery period from CA. Possible future work includes:

- Larger animal studies with severe cerebral injury (by 9min asphyxia-CA) to better evaluate the performance of N10 amplitude, N7 and N10 latency, and qSSEP-PSA in tracking recovery under temperature management.
- Validation and optimization of cut-off points of the quantitative markers presented, to predict functional outcome.
- Development of a multimodal approach including short-latency SSEP amplitude and latencies, qSSEP-PSA, IQ and rCBF.
- Further examination of rCBF using LSCI, in terms of distinct vessel rCBF and the respective relations with electrical activity during CA and the early recovery period following resuscitation. This work may contribute to uncovering the mechanisms behind therapeutic hypothermia.

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CURRICULUM VITAE

Born: August 15, 1992 in Calgary, Alberta, Canada

EDUCATION

Johns Hopkins University; Baltimore, Maryland

August 2014-May 2016

- M.S.E. Biomedical Engineering

University of California-Irvine; Irvine, California

September 2010-June 2014

- B.S. Biomedical Engineering, *magna cum laude*
- B.A. Business Administration, *cum laude*
 - Specialization in Accounting
- Dean's Honor List for Engineering and Business
 - Fall 2010/11/12/13, Winter 2011/12/13/14, Spring 2011/12/13/14
- Tau Beta Pi – National Honors Engineering Society

RESEARCH AND DESIGN EXPERIENCE

Johns Hopkins University; Baltimore, Maryland

August 2014-present

- Master's Thesis Research – Dr. Xiaofeng Jia Lab
 - Applied multiple quantitative analyses to somatosensory evoked potentials (SSEP) to develop prognostic markers in an asphyxia cardiac arrest rodent model with targeted temperature management
 - Quantified electroencephalogram (EEG) signals using information quantity (IQ) algorithm of various cardiac arrest rodent models with temperature management
 - Helped debug graphical circuits and data acquisition systems to record brain signals and vital signs from rodent cardiac arrest models

University of California-Irvine; Irvine, California

September 2013-June 2014

- Biomedical Engineering Senior Design Project (*Helico Optics*)
 - Aimed to design a low-cost, on-site, rapid *H. pylori* (stomach bacteria) infection diagnostic device targeted towards developing nations
 - Applied Diffuse Optical Spectroscopic Imaging to use optics to detect low concentrations (ppb) of ammonia in a patient's breath sample to non-invasively detect *H. pylori* presence
 - Designed multiple prototypes of a highly reflective cuvette to be used in the diagnostic system
- Awards
 - Undergraduate Research Opportunities Program (UROP) Fellowship (Winter 2014)

University of California-Irvine; Irvine, California

September 2013-June 2014

- Undergraduate Research Assistant – Dr. Yama Akbari Lab
 - Contributed to the early establishment of a pharmacologic neurostimulation setup for an *in vivo* rat coma model
 - Designed preliminary neurostimulation experimental protocols and materials list (optogenetic and pharmacologic approaches)
 - Completed various histology techniques and troubleshooting experiments

University of Calgary-Hotchkiss Brain Institute; Calgary, Alberta

Summer 2012 & 2013

- Undergraduate Summer Studentship – Dr. Patrick Whelan Lab
 - Implemented optogenetics *in vivo* to explore the descending modulation of dopamine on spinal motor networks
 - Performed successful brain and spinal cord injection surgeries on non-terminal mice
 - Acquired data from *in vivo* optogenetic behavioral experiments
 - Contributed to modification of *in vivo* behavioral protocols
 - Analyzed and presented data at student symposiums and lab meetings

- Performed validation techniques (perfusions, tissue processing, immunostaining)
- Built trial EMG electrodes to be chronically implanted in mice
- Modified Spike2 coding scripts to analyze *in vitro* data
- Awards
 - UCVM NSERC Undergraduate Student Research Award (Summer 2013)
 - AIHS Summer Studentship Research Award (Summer 2013)
 - NSERC Undergraduate Student Research Award (Summer 2012)

University of California-Irvine; Irvine, California

March 2013-June 2013

- Undergraduate Research Assistant – Dr. Gary Lynch Lab
 - Designed an algorithm in ImageJ for image processing and automated cell counting
 - Developed required programming skills mainly through self-directed learning

PRESENTATIONS • POSTERS • PAPERS

Young L.M., Deng R., Jia X. (Feb 2016) Somatosensory Evoked Potentials Are Associated with Post-resuscitation Recovery with Hypothermia. *Society of Critical Care Medicine 45th Annual Critical Care Congress*. Orlando, Florida. Presentation – Selected Research Snapshot Presentation.

Deng R., **Young L.M.**, Jia X. (Nov 2015) Early Quantitative Somatosensory Evoked Potentials Are Associated with Neurological Outcomes After Cardiac Arrest and Therapeutic Hypothermia. *XXII World Congress of Neurology*. Santiago, Chile. Poster.

Deng R., Ma Y., **Young L.M.**, Jia X. (Oct 2015) Effects of Transcranial Direct Current Stimulation on Somatosensory Evoked Potentials in Uninjured Rats. *Biomedical Engineering Society 2015 Annual Meeting*. Tampa, Florida. Poster.

Deng R., **Young L.M.**, Jia X. (Aug 2015) Quantitative EEG Markers in Severe Post-Resuscitation Brain Injury with Therapeutic Hypothermia. *37th Annual International Conference of IEEE Engineering in*

Medicine and Biology Society. Milan, Italy. Paper.

He J., Lu H., Deng R., **Young L.M.**, Tong S., Jia X. (Aug 2015) Real-Time Monitoring of Cerebral Blood Flow by Laser Speckle Contrast Imaging after Cardiac Arrest in Rat. *37th Annual International Conference of IEEE Engineering in Medicine and Biology Society. Milan, Italy. Paper.*

Deng R., Koenig M., **Young L.M.**, Jia X. (Oct 2015; E-pub July 2015) Early Quantitative Gamma-Band EEG Marker is Associated with Outcomes after Cardiac Arrest and Targeted Temperature Management. *Neurocritical Care. Journal Paper.*

Deng R., **Young L.M.**, Jia X. (June 2015) The Subband-Based EEG Marker and Neurological Outcome with Temperature Manipulation After Cardiac Arrest. *XXVII International Symposium on Cerebral Blood Flow, Metabolism and Function. Vancouver, British Columbia. Poster.*

Koblinger K., Fuzesi T., Ejdrygiewicz J., Krajacic A., **Young L.M.**, Bains J., Whelan P.J. (Nov. 2014) A11 neurons in the mouse project to the spinal cord, are dopaminergic and lack expression of dopamine transporter. *Society for Neuroscience Meeting, Washington, DC. Poster.*

Young L.M., Campbell C., Dave K., Kwan E., Matlock A. (June. 2014) Optori: A Diagnostic Tool for H. pylori. *UC Irvine Samueli School 2014 Spring Design Review, Irvine, California. Poster*

Young L.M., Campbell C., Dave K., Kwan E., Matlock A. (June. 2014) Optori: A Diagnostic Tool for H. pylori. *UC Irvine UROP, Irvine, California. Team presentation.*

Young L.M., Campbell C., Dave K., Kwan E., Matlock A. (Mar. 2014) Optori: A Diagnostic Tool for H. pylori. *UC Irvine Samueli School 2014 Winter Design Review, Irvine, California. Poster*

Young L.M., Campbell C., Dave K., Kwan E., Matlock A. (Dec. 2013) Design of a Low-Cost, Portable

Diffuse Optical Spectroscopic Imaging System for Helicobacter Pylori Detection. *UC Irvine Samueli School 2013 Fall Design Review*, Irvine, California. Poster

Koblinger K., **Young L.M.**, Fuzesi T., Ejdrygiewicz J., Bains J., Whelan P.J. (Sept. 2013) Light induced enhancement of locomotion - What is the role of A11 for walking? *Alberta Motor Control Meeting*, Jasper, Alberta. Oral presentation.

Koblinger K., **Young L.M.**, Fuzesi T., Ejdrygiewicz J., Bains J., Whelan P.J. (Sept. 2013) Light induced enhancement of locomotion - What is the role of A11 for walking? *Campus Alberta Neuroscience Symposium*, Calgary, Alberta. Poster presentation.

Young L.M., Koblinger K., Whelan P.J. (Aug. 2013). What is the role of A11 for walking? *Hotchkiss Brain Institute Annual Summer Student Symposium*, Calgary, Alberta. Presentation.

Young L.M., Koblinger K., Whelan P.J. (Aug. 2013). What is the role of A11 for walking? *University of Calgary Veterinary Medicine Annual Undergraduate Research Day*, Calgary, Alberta. Presentation.

Koblinger K., **Young L.M.**, Fuzesi T., Krajacic A., Bains J., Whelan P.J. (Feb. 2013) Light induced enhancement of locomotion - What is the role of A11 for walking? *Hotchkiss Brain Institute, University of Calgary Department of Neuroscience Research Day*, Calgary, Alberta. Presentation.

Young L.M., Koblinger K., Whelan P.J. (Aug. 2012). Optogenetic stimulation of A11 may increase locomotor activity. *Hotchkiss Brain Institute Annual Summer Student Symposium*, Calgary, Alberta. Presentation.

Young L.M., Koblinger K., Whelan P.J. (Aug. 2012). Optogenetic stimulation of A11 may increase locomotor activity. *University of Calgary Veterinary Medicine Annual Undergraduate Research Day*, Calgary, Alberta. Presentation.

COMPUTER SKILLS

- Proficient in MATLAB, Stata, SPSS
- Experience with SolidWorks, Python

TEACHING EXPERIENCE

Teaching Assistant*August 2014-present*

- BME Freshmen Modeling and Design
- Systems Bioengineering I Lab
- Systems Bioengineering II Lab

Grader*January 2015-May 2015*

- Systems and Controls