SPREADING DEPOLARIZATIONS OF CEREBRAL CORTEX AFTER BRAIN INJURY: MECHANISM OF INJURY PROGRESSION AND RELEVANCE TO MILITARY NEUROTRAUMA

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ABSTRACT

Based on media reports, neurotrauma has emerged as the signature wound in the Global War on Terrorism (GWOT). Brain trauma is frequently the result of blast injury, producing a unique syndrome with a high incidence of traumatic sub-arachnoid hemorrhage (SAH) and vasospasm. Cortical spreading depolarizations (CSD) are a pathologic short-circuiting of brain function that cause secondary brain damage in animal models of cerebral ischemia. We investigated the role of CSD as a novel pathogenic mechanism in civilians with SAH and traumatic brain injury (TBI). In both diseases, CSD occurs with a high incidence (>50%) and is associated with progressive cerebral metabolic compromise, tissue infarction, and poor neurologic recovery. Results suggest CSD may be mitigated by therapeutic hypothermia. We speculate that CSD underlies delayed deterioration in GWOT casualties who have characteristics of both civilian TBI and SAH. Based on civilian incidence, it is estimated that at least 2,716 U.S. service members in Operation Iraqi Freedom (OIF) have experienced CSD. We consider it a vital obligation to discover the pathophysiologic and therapeutic implications of CSD in order to improve survivability and recovery from military neurotrauma.

1. INTRODUCTION

Historically, 50% of head injured casualties are killed-in-action (KIA), accounting for 31% of all KIA, and 90% of those reaching definitive medical care after

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rapid evacuation will survive, often with severe disabilities (Bellamy, 1984; Bellamy et al., 1996). Craniocerebral trauma in the remaining 10% represents the leading cause of death among those classified as 'died-of-wounds' (DOW), accounting for 40-50%. Recently, brain injury has further emerged as the signature wound of GWOT. Officials report that 67% of casualties suffered by U.S. forces in Iraq have been diagnosed with TBI (Amburn, 2004), and that 20% have been *severe* TBIs (Schlesinger, 2003). At the 31st Combat Support Hospital, head injuries have accounted for up to 56% of KIAs (Holcomb J, personal communication), and 23% of DOWs sustained penetrating brain injury (PBI). Emergency craniotomies are performed to treat these severe injuries in theater, sometimes on a daily basis. Brain injury, however, often results in delayed complications and deterioration of a patient's condition after initial stabilization. Thus, in addition to neurosurgical care in theater, more than 35% of GWOT casualties required neurosurgical treatments even after evacuation to the U.S. (Loveridge et al., 2005).

Improved understanding of the pathophysiology of military neurotrauma is urgently needed to mitigate this serious medical threat. Here we present data on a novel pathophysiologic phenomenon, cortical spreading depolarization (CSD), that has been well-characterized through 60 years' research in animal models of brain injury. Here we describe studies that, for the first time, investigate the role of CSD in a large series of human brain injury patients. In particular, we describe results for aneurysmal sub-arachnoid hemorrhage (aSAH) and TBI and discuss how these results may have particular relevance to the unique type of neurotrauma encountered in GWOT.

1.1 Cortical Spreading Depolarizations (CSD)

CSDs are short-circuiting electrical storms that depolarize neurons and astrocytes *en masse* and interrupt brain function (depressed EEG) for a variable period. Propagating through the cerebral cortex at a rate of ~1-5 mm/min, CSD causes loss of ion homeostasis, with ion fluxes 5-fold greater than those produced by brain seizures, as well as toxic release of excitatory amino acids. Extracellular Ca^{2+}, for instance, decreases 10-fold. CSD also causes tissue acidification and cytoxic edema corresponding to an 80% shrinkage of extracellular tissue volume. Restoration of membrane potentials (and EEG activity) is energy dependent, and if this increased substrate demand is not matched by increased blood flow, as in the case of ischemia, CSD results in progressive tissue infarction (Hartings et al., 2003). In these circumstances, CSDs occur in temporal clusters at short intervals and EEG depression periods are progressively prolonged, leading eventually to EEG flatline. Indeed, volumes of experimental evidence have shown that CSD plays a central causal role in developing brain infarction after stroke and SAH, and it may similarly play a pathogenic role in human brain injury.

The Co-Operative Study on Brain Injury Depolarizations (COSBID; www.cosbid.org) is an international collaboration of neurosurgeons, neurologists, and neurophysiologists investigating the role of CSD in clinical brain injury. In particular, the goals of COSBID are to investigate 1) methods to monitor CSD in human brain, 2) incidence/prevalence of CSD in human cerebral disease, 3) precipitating risk factors and conditions that may trigger CSD, 4) the effects of CSD on neurologic and brain injury outcome. Initial results indeed suggest that CSD is an integral contributor to brain injury after aSAH.

1.2 COSBID Study of Sub-Arachnoid Hemorrhage

Human aSAH can produce a syndrome of delayed ischemic neurological deficits (DIND), defined as fluctuating decline in consciousness and onset of focal neurological symptoms, often accompanied by development of cortical infarcts. The prime mechanism of DIND is vasospasm, or constriction of the cerebral arteries that can occur in both proximal and distal segments. In experimental SAH, CSD is the most potent inductor of arterial spasm in the cerebral microcirculation, resulting in widespread infarction (Dreier et al., 2000). Hence, CSD may be involved as both consequence of proximal and cause of distal arterial spasm underlying DIND.

To monitor for CSD using electrocorticography (ECoG) in civilian patients with aSAH, sub-dural electrode strips were placed on injured, but viable cortex after craniotomy surgery. ECoG recordings were made subsequently for 1-10 days. CSD was identified by rapidly developing depression of ECoG amplitude and simultaneous shifts in the near-DC slow potential, reflecting tissue depolarization (see Methods below).

In aSAH, ECoG revealed 298 CSDs in 13 of 18 patients (72%; Dreier et al., 2006). DIND developed in 7 patients, usually 7-8 days after aSAH. Onset of neurologic symptoms during DIND was time-locked to a cluster of CSDs in every single case (positive and negative predictive values: 86% and 100%, respectively). In 4 patients delayed infarcts developed in the recording area, and in each case, infarction was preceded by progressive prolongation of the depression periods of CSDs to more than 60 minutes. The number of CSDs per day was significantly higher in patients with DIND and/or delayed brain infarct at days 7-9 after aSAH. When depression periods associated with CSD lasted >10 min, patients had worse neurologic outcome at discharge from the acute unit to the rehabilitation unit. Vasospasm occurred in 90% of patients.

1.3 Traumatic Brain Injury

To date, clinical trials to test candidate neuroprotective therapies in TBI have all failed, and there remains no drug that can improve outcome and recovery. Primary brain damage from TBI results from the initial mechanical insult of brain parenchyma and vasculature, but secondary damage can also occur from subsequent pathophysiologic cascades. These are exacerbated by secondary systemic insults, including hyperthermia, hypotension, hypoxia, hypo/hyper-glycemia, and infection. Such insults occur commonly, contributing to ischemia and metabolic crisis, and are associated with poor outcome. After resuscitation, medical management of TBI is therefore mainly supportive, focusing on maintenance of normal physiologic parameters and cerebral substrate delivery. Supportive therapy includes management of hemodynamics, fluid management, volume and inotropic therapy to maintain arterial pressure, ventilatory management, nutrition and metabolism, sedation and analgesia, and infection control. Intracranial insults such as seizures, high intracranial pressure (ICP), and hemmorhage/hematoma also contribute to secondary damage. However, with the exception of surgical evacuation of intracranial mass lesions, brain-specific therapies are inconclusive in their efficacy.

The lack of specific neuroprotective therapies and failure of clinical trials may be partly blamed on 1) incomplete understanding of the evolution of cerebral pathophysiology and 2) lack of selection criteria for patients undergoing trials of therapies to treat specific cerebral pathologies. For instance, therapies targeting recognized intracranial secondary insults such as refractory ICP and sub-arachnoid hemorrhage have demonstrated efficacy. Seizure prophylaxis is also standard of care, as 10-30% of TBI cases have either convulsive or sub-clinical seizures which are argued to worsen outcome. However, seizures, particularly those not developing to status epilepticus (>30 min), may be a minor concern relative to the severe disturbance of cerebral function resulting from the mass tissue depolarizations of CSD. We thus hypothesized that CSD may 1) occur commonly as a fundamental sequela of TBI, 2) be triggered by secondary systemic insults, and 3) contribute to the deterioration of brain function and neurochemistry, thus adversely affecting neurologic recovery.

The medical requirement for neurosurgery to treat patients with moderate-to-severe TBI provides the opportunity to monitor for CSD by ECoG recordings. In these cases, craniotomy is performed as an emergency or semi-elective procedure to evacuate mass intracranial lesions and to relieve or prevent development of life-threatening intracranial hypertension. While the brain is exposed during surgery, a linear ECoG electrode strip is placed on regions of cerebral cortex at risk for subsequent injury and deterioration (Fig. 1). ECoG is then recorded to monitor this tissue through the course of neurointensive care.
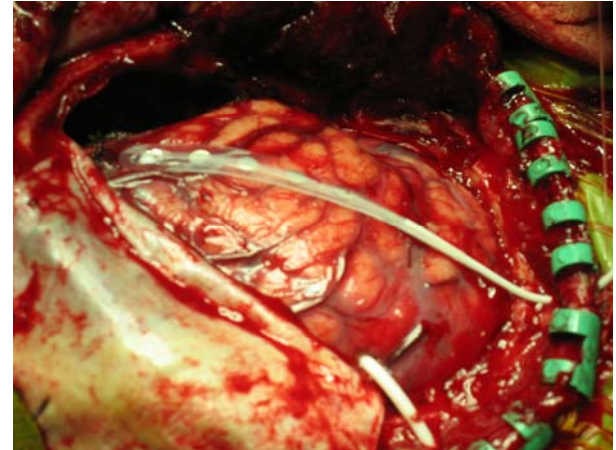

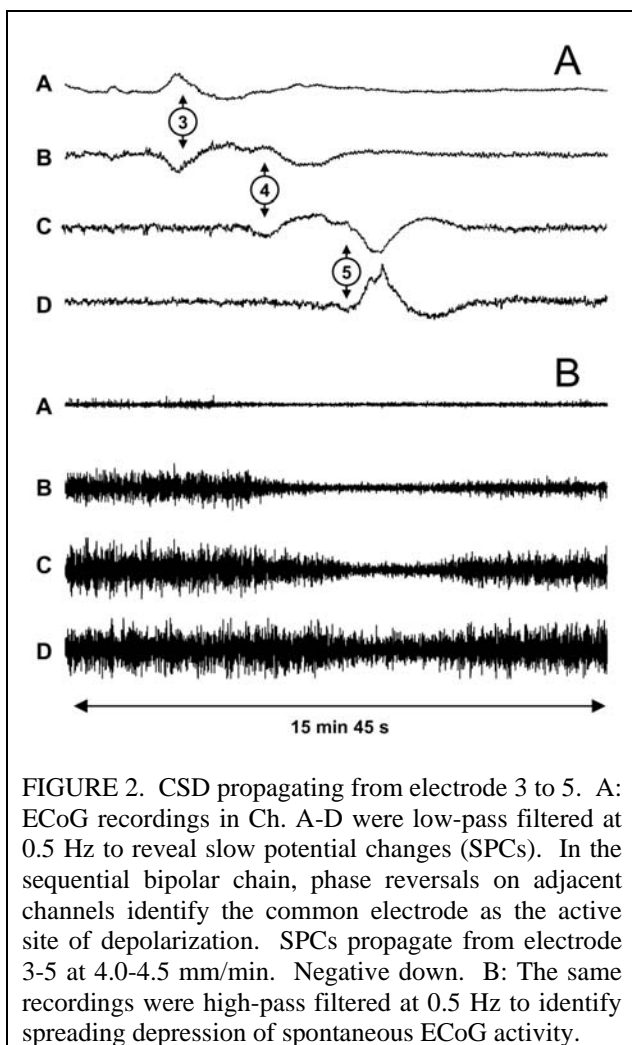FIGURE 1. ECoG recordings are made from electrode strips placed on cerebral cortex during craniotomy surgery.

2. METHODS

2.1 Patient Care

Twenty-nine patients with TBI were prospectively enrolled in the COSBID study. Research protocols were approved by the local ethics committees. After a clinical decision had been made that surgery was required, consent to participate in the study was obtained. After surgery, an ECoG electrode strip was placed on the surface of the cortex and the lead wires were tunneled under the skin and exteriorized through a stab wound to allow later withdrawal of the strip by gentle traction (Fig. 1; Strong et al., 2002). Insofar as possible, the strip was placed along a single gyrus radiating outward from an accessible injured region such that the closest electrode contacts were placed on viable, but often edemic or contused cortex with a low degree of sub-arachnoid blood.

After surgery and transfer to the intensive care unit (ICU), ECoG recordings were initiated and core variables were monitored continuously. All patients were ventilated, at least initially, and 16 patients were immobilized with vecuronium for some duration. Sedation was maintained with either propofol, morphine, or midazolam and fentanyl, and all patients were administered phenytoin for seizure prophylaxis. ICP was monitored by a ventricular drainage catheter (n=15) or an ICP transducer (Codman, n=9). ICP greater than 20 mmHg was treated sequentially by

intravenous vecuronium, ventricular drainage, hyperventilation (pCO2 > 30 mmHg), and mannitol. The target level for cerebral perfusion pressure (CPP) was 60 mmHg, achieved by ICP control, intravenous fluids, and the use of vasopressors. Systolic BP, heart rate, rectal temperature, mean arterial pressure (MAP), arterial oxygen saturation (SaO_2), ICP, and Glasgow Coma Scale were documented hourly, while blood gases, hematocrit, and plasma glucose were documented typically every 3-8 hours (range: 1-12). ECoG was terminated and electrode strips were removed after 5 days of recording or when patients were withdrawn from sedation. No local hemorrhagic or infectious complications of the electrode strip were encountered. Computerized tomography (CT) scans were performed on all patients before and after surgery, and again prior to discharge from the ICU. Clinical outcome was assessed at six months according to the extended Glasgow Outcome Scale (eGOS), which was administered via telephone interview by examiners blinded to ECoG scoring.

FIGURE 2. CSD propagating from electrode 3 to 5. A: ECoG recordings in Ch. A-D were low-pass filtered at 0.5 Hz to reveal slow potential changes (SPCs). In the sequential bipolar chain, phase reversals on adjacent channels identify the common electrode as the active site of depolarization. SPCs propagate from electrode 3-5 at 4.0-4.5 mm/min. Negative down. B: The same recordings were high-pass filtered at 0.5 Hz to identify spreading depression of spontaneous ECoG activity.

2.2 Electrocorticography and Data Analysis

ECoG recordings were made from a linear subdural strip consisting of 6 electrodes, with 10 mm spacing between electrodes and 2.3 mm of exposed platinum per contact (Wyler, Ad-Tech Medical, Racine, WI). Data were acquired continuously in 4 active channels (A-D) from electrodes 2-6, which were connected in sequential bipolar fashion to two Dual Bioamp amplifiers (ADInstruments, New South Wales, Australia). Ground was provided by electrode 1. Band-pass cutoffs for the alternating current (AC) amplifiers were set to 0.01 and 100 Hz. Data were sampled at 200 Hz and recorded and reviewed with the use of a Powerlab 16/SP analog-to-digital converter and Chart-5 software (ADInstruments).

Hallmarks of CSD in animal recordings are the amplitude depression of 'EEG-band' activity (0.5-100 Hz), reflecting the depolarization block of synaptic activity, and a 10-20 mV negative shift of the direct current (DC) extracellular potential, resulting in part from the intracellular flux of cations during depolarization. The use of a low high-pass cutoff setting (0.01 Hz) with alternating current (AC) amplifiers allows the identification of slow potential changes (SPCs) that reflect the DC negativities of CSD. CSDs were thus identified by 1) the simultaneous occurrence of SPCs and rapidly developing depression of 'EEG-band' activity (0.5-100 Hz) at individual electrodes, and 2) the sequential occurrence of SPCs and depressions at adjacent electrodes, evidencing the spread of depolarization events (Figure 2). These methods have been described previously in detail (Fabricius et al., 2006).

3. RESULTS: ALL TBI PATIENTS

By scores on the Glasgow Coma Scale (GCS) at hospital admission, 25 patients (86%) were severe TBI, 2 were moderate, and 2 mild; injuries resulted from falls (n=11), motor vehicle accidents (n=9), assaults (n=7), and firearms (n=2). By the Marshall diagnostic categories, 25 (86%) had surgically evacuated mass lesions, 2 had mass lesions that were not evacuated, and 2 had diffuse swelling. The median age was 41 (range: 18-67) and 23 (79%) patients were male, 6 female.

As in aSAH, a similar 57% of patients exhibited a total of 130 CSDs. The median frequency of CSD was 3.0 (1st, 3rd quart: 1.9, 4.6) events per day. The timing of events showed a bimodal U-shaped distribution, with peak incidence during the first and seventh day post-injury. The speed of CSD propagation was 2.4 (1.2, 3.0) mm/min and maximum depression of 0.5-100 Hz activity was 66 (56, 76)%. Depression lasted 5.9 (4.5, 8.9) min for single CSDs and was often longer on channels closest to the injury. Patients with

parenchymal damage or intracerebral hemorrhage had a greater likelihood (73%) of exhibiting CSD than those with diffuse swelling or sub-dural hemorrhage only (29%; p=0.07).

In many patients CSDs occurred sporadically. In others, however, CSDs occurred in tight temporal clusters (>5 CSDs in 5 hrs) at short, regular intervals of 20 min to 1 hr. In almost every case, these periods were tightly locked to episodes of transient hyperthermia (>38°C; Hartings et al., 2005). Figure 3 shows the disproportionate distribution of high temperature values during CSD relative to all temperatures recorded during ECoG. A similar effect was found for CPP: CSD had a greater likelihood of occurrence when cerebral perfusion was poor. Most of these 8 patients also exhibited progressive prolongation of depression periods following CSDs, indicating worsening metabolic crisis, and four of these patients developed persistent electrical silencing as a consequence of CSD. In these 7 patients, neurologic outcome at 6 months post-injury was worse than in other patients (p=.09).
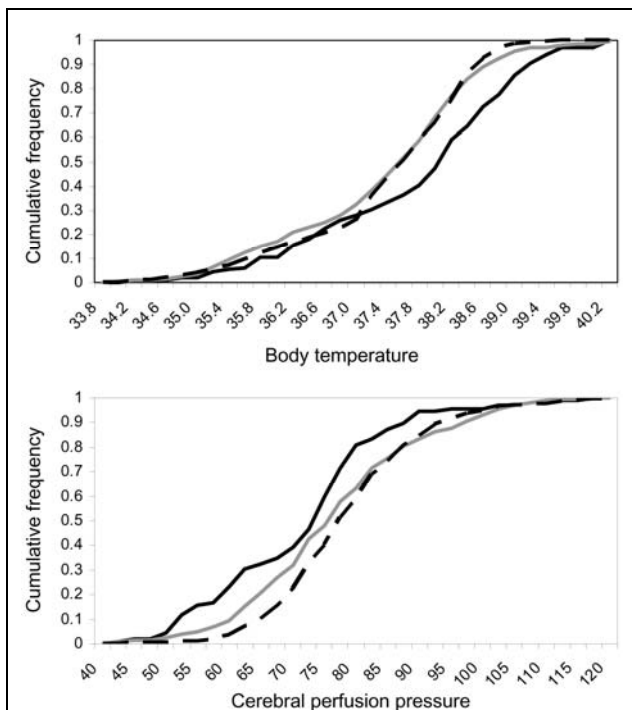

FIGURE 3. CSDs occur disproportionately during times of hyperthermia, low cerebral perfusion pressure (CPP). Plots show the frequency distributions of physiologic values during CSDs (black line) and throughout the entire ECoG monitoring period (dashed) in patients with CSDs. Gray lines show distributions for patients without CSD.

4. RESULTS: CASE REPORT

4.1 Case Presentation

A healthy 20 y.o. male under alcohol intoxication fell and hit his head on a concrete floor. He was taken to a regional medical center at GCS 5 and then transferred to VCU where he was intubated *en route* and was GCS 8 at admission, 5 hr post-injury. The patient was confused and combative, with normal temperature (36.2°C), 100% oxygen saturation and BP 119/57. Pupils were normal. After propofol sedation, initial CT scan revealed a right frontal sub-dural hemorrhage extending from the inferior frontal lobe to the vertex with a large amount of sub-arachnoid blood, 6 mm of midline shift and mass effect (Fig. 4A,B). There was mild effacement of the frontal horns, right frontal intraparenchymal hemorrhage, and extensive skull base fracture. Upon placement of an intraparenchymal ICP probe and obtaining a value of 42 mmHg, it was determined that neurosurgery was required. Informed consent was obtained and the patient was enrolled in the COSBID study. At 12 hr post-injury, a right fronto-temporo-parietal craniectomy, duraplasty, subdural hematoma evacuation, ventriculostomy, and partial frontal lobectomy were performed. Intraparenchymal microdialysis and Licox $P_{ti}O_2$ probes were inserted into the frontal lobe just superior to the area of contusion, separated by 1-2 cm alongside an electrode strip placed on the cortex. The electrode strip extended from the inferior frontal gyrus to inferior parietal cortex (Fig. 4D). After surgery the patient was transferred to the Neuroscience ICU and continuous monitoring was initiated.

During post-operative monitoring, the patient was given 3-6 mg intravenous morphine hourly and intravenous vecuronium was given 4.5-15 mg hourly until hour 66 post-injury. Intravenous phenytoin was administered at 200 mg twice daily for 7 days for seizure prophylaxis. Mannitol (intravenous 50-100 g) and ventricular drainage were used to control ICP. At 18 hours post-injury, 225 ml of Oxycyte were given intravenously. Plasma glucose was measured every 4 hours, ranged 114-178 mg/dL over 53 hours post-injury, and insulin was given sub-cutaneously beginning 71 hr post-injury when glucose reached 212 mg/dL. PO_2 was 217-273 mmHg through 47 hr post-injury, 92-148 through 75 hr, and dropped to 60-70 after ECoG recordings ended. PCO_2 ranged 26-52 mmHg, and pH 7.30-7.47, throughout monitoring. Oxygen saturation was 100%, save for a brief drop to 92% at hour 70.

4.2 Case Findings

At the start of monitoring 13 hr post-injury, peak-to-peak ECoG amplitudes were 500-700 μV in Ch. A-D. Ch. A (nearest injured tissue, electrodes 2 and 3; Fig. 4D) showed recurrent sharp/slow waves at 1-2 sec intervals on a flat background, whereas Ch. B-D showed continuous delta activity. In the first 6 hrs (13-19), the patient was mildly hyperthermic (>38°C), CPP fell below 60 mmHg, and ICP increased from 9 to 20 mmHg. Brain glucose level was initially low (0.69 mmol/L) and fell to <0.1 by hour 16. In this context, a CSD occurred at the start of ECoG monitoring (hr 13), propagating from electrode 2 to 3. From hour 13 to 23, a series of 10 additional CSDs occurred; the first 4
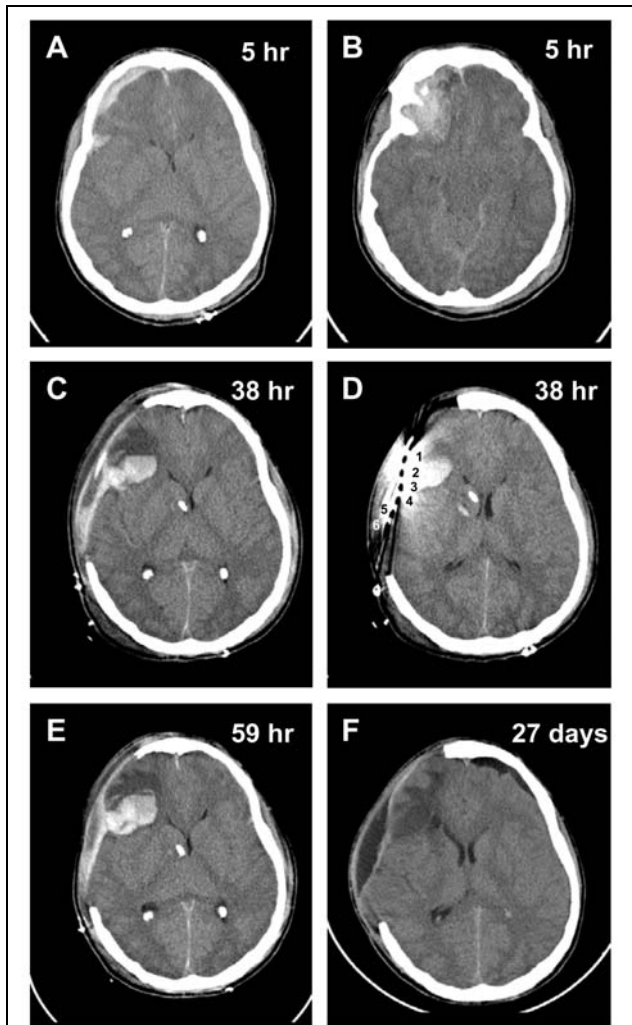

FIGURE 4. CT scans of brain pathology. A-B: Extensive sub-dural and intraparenchymal hemorrhage with mass effect at 5 hr post-trauma. C,E: The same slice as in A shows the progression of intraparenchymal hemorrhage and surrounding edema. D: The positions of electrodes 1-6 are shown adjacent to the hemorrhage. F: CT scan at day 27 shows encephalomalacia in areas that were underlying electrodes 2 and 3 (Ch. A).

occurred on electrodes 2 and 3, while the next 6 were at electrode 3 only. Throughout this period, $P_{ti}O_2$ values dropped from >20 to 8-10 mmHg, brain glutamate (40-80 mmol/L) and lactate (3-7 mmol/L) were elevated, L/P was >40.

After the last CSD of this cluster at hour 23, whole body cooling was initiated (Arctic Sun 2000, Medivance, Inc., Louisville, KY) and core body temperature was decreased to <35.5°C by hour 30. During this time, lactate values normalized (1.3-2.1 mmol/L), L/P fell to <40, glutamate decreased to 8-16 mmol/L, and glucose recovered to >0.3 mmol/L. CPP was >60 mmHg and ICP was maintained at 20. No CSD occurred during this 7 hr period, and recurrent slow/sharp wave activity on Ch. A was gradually attenuated, being replaced by continuous delta. Rhythmic 2 Hz delta activity then evolved and developed at hr 29.5 to a 60 sec seizure of 0.5-1.5 Hz rhythmic polyspike complexes. Beginning 30 min later, over hours 30-32.5 hrs, after glucose dropped again to <100 mmol/L, three additional CSDs occurred at electrode 3. No further CSDs recurred through hour 38, when recordings in Ch. A and B were interrupted. CT scan at this time revealed significant expansion of intraparenchymal hemorrhage to 3.8 x 2.1 cm (Fig. 4C).
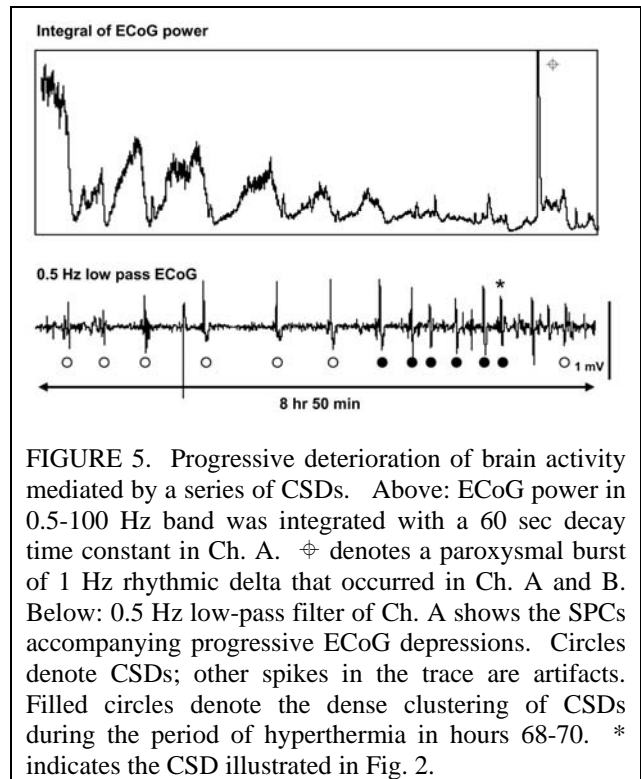

FIGURE 5. Progressive deterioration of brain activity mediated by a series of CSDs. Above: ECoG power in 0.5-100 Hz band was integrated with a 60 sec decay time constant in Ch. A. ⊕ denotes a paroxysmal burst of 1 Hz rhythmic delta that occurred in Ch. A and B. Below: 0.5 Hz low-pass filter of Ch. A shows the SPCs accompanying progressive ECoG depressions. Circles denote CSDs; other spikes in the trace are artifacts. Filled circles denote the dense clustering of CSDs during the period of hyperthermia in hours 68-70. * indicates the CSD illustrated in Fig. 2.

For the next 24 hours, and as previously, no CSDs appeared in Ch. C or D (electrodes 4-6). At hour 59, CT scan revealed further slight increase in the intraparenchymal hemorrhage and surrounding vasogenic edema (Fig. 4E). Physiologic and

microdialysate values remained constant, though $P_{ti}O_2$ had dropped to 6-8 mmHg when recordings were again initiated in Ch. A and B at hour 62. In the next 19 hours (62-81), a series of 20 CSDs were recorded, with intervals ranging 18 min to 2 hr 52 min. Throughout the first 12 CSDs, which occurred on electrodes 2 and/or 3 only, spontaneous ECoG activity in Ch. A progressively deteriorated in a step-wise fashion with each CSD until peak-to-peak amplitude was <200 µV (Fig. 5). At the start of this series, core temperature remained near 35°C, but began increasing 2 hr later when cooling was terminated. In hours 68-70 there was a rebound hyperthemic period (37.9-38.3°C) that corresponded to the most dense clustering of 6 CSDs at 20-30 min intervals (filled circles, Fig. 5). Vecuronium was then turned off and the patient was GCS 15 at hr 68 while temporarily extubated. GCS eye score then fluctuated between 1-3 until stabilizing at 3 at hr 74. From hr 71 to 81, when recordings were terminated, a final 7 CSDs occurred that mainly propagated from electrodes 3 to 6 (e.g. Fig. 2), and L/P again rose above 40 (range: 44-122) where it persisted until microdialysis ended at hour 92. Brain glucose remained at <100 µmol/L throughout this series of 20 CSDs and thereafter.

After monitoring was terminated, the electrode strip and intraparenchymal probes were removed and the patient was weaned from sedation. CT scan on day 27 post-injury showed significant encephalomalacia in the right frontal cortex including areas that were underlying electrodes of channel A (2 and 3) (compare panels D and F, Fig. 4). On follow-up at 3 months, the patient's eGOS was upper moderate and he had returned to his college curriculum. There was no evidence of seizures or complaint of headaches after ICU discharge.

CONCLUSIONS

These results demonstrate that CSD is a common occurrence in militarily relevant brain injury in man, including severe TBI and penetrating injuries requiring neurosurgical treatment. In many of these patients, CSD occurred with signatures of progressive metabolic compromise leading to tissue death: 1) CSD often occurred in clusters, and 2) ECoG depression periods became prolonged, and in some cases, persistent. Microdialysis in the case report supports this conclusion, as glucose values and lactate/pyruvate ratios indicated tissue ischemia during periods of intense CSD activity, and follow-up CT scan showed infarction in areas of ECoG recording exhibiting these signatures. Furthermore, in the TBI population there is a strong trend toward worse 6 mo. outcome in patients developing ECoG flatline in association with CSD.

The strong association of CSD clusters with DIND and poor outcome after aSAH also has implications for the peculiar pathophysiology of blast neurotrauma. SAH is frequently seen as a direct result of blast neurotrauma and is one of the leading causes of mortality and morbidity. Also similar to aSAH, in a study of 119 neurosurgical consults on GWOT casualties at National Naval Medical Center, 47% had traumatic vasospasm (Armonda et al., 2006). Of these, 81% sustained trauma from blast. Vasospasm also occurs in 30-40% of blunt and penetrating brain injuries. Together, these data suggest that ischemic injury is even more common after blast neurotrauma compared to blunt, contusive injuries, and that CSD may underlie DIND-like deterioration after blast.

Based on a 65% overall incidence of CSD in our studies, the total 20,891 U.S. casualties in Iraq as of May, 2006, and a 20% incidence of severe brain injury, it is estimated that at least 2,716 U.S. service members have experienced CSD. We speculate that CSD may underlie delayed deterioration in these casualties and consider it a vital obligation to further elucidate the pathophysiologic and therapeutic implications of CSD in order to improve survivability and recovery from military neurotrauma. The strong effect of body temperature to modulate brain metabolism and CSD incidence may point to induced hypothermia as a possible field-deployable therapy to improve outcome in severe brain injury.

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