Inverse neurovascular coupling to cortical spreading depolarizations in severe brain trauma

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Cortical spreading depolarization causes a breakdown of electrochemical gradients following acute brain injury, and also elicits dynamic changes in regional cerebral blood flow that range from physiological neurovascular coupling (hyperaemia) to pathological inverse coupling (hypoperfusion). In this study, we determined whether pathological inverse neurovascular coupling occurred as a mechanism of secondary brain injury in 24 patients who underwent craniotomy for severe traumatic brain injury. After surgery, spreading depolarizations were monitored with subdural electrode strips and regional cerebral blood flow was measured with a parenchymal thermal diffusion probe. The status of cerebrovascular autoregulation was monitored as a correlation between blood pressure and regional cerebral blood flow. A total of 876 spreading depolarizations were recorded in 17 of 24 patients, but blood flow measurements were obtained for only 196 events because of technical limitations. Transient haemodynamic responses were observed in time-locked association with 82 of 196 (42%) spreading depolarizations in five patients. Spreading depolarizations induced only hyperaemic responses (794% increase) in one patient with intact cerebrovascular autoregulation; and only inverse responses (−24% decrease) in another patient with impaired autoregulation. In contrast, three patients exhibited dynamic changes in neurovascular coupling to depolarizations throughout the course of recordings. Severity of the pathological inverse response progressively increased (−14%, −29%, −79% decrease, P < 0.05) during progressive worsening of cerebrovascular autoregulation in one patient (Pearson coefficient 0.04, 0.14, 0.28, P < 0.05). A second patient showed transformation from physiological hyperaemic coupling (44% increase) to pathological inverse coupling (−30% decrease) (P < 0.05) coinciding with loss of autoregulation (Pearson coefficient 0.19 → 0.32, P < 0.05). The third patient exhibited a similar transformation in brain tissue oxygenation, a surrogate of blood flow, from physiologic hypoxic responses (20% increase) to pathological hypoxic responses (−14% decrease, P < 0.05). Pathological inverse coupling was only observed with electrodes placed in or adjacent to evolving lesions. Overall, 31% of the pathological inverse responses occurred during ischaemia (<18 ml/100 g/min) thus exacerbating perfusion deficits. Average perfusion was significantly higher in patients with good 6-month outcomes (46.8 ± 6.5 ml/100 g/min) than those with poor outcomes (32.2 ± 3.7 ml/100 g/min, P < 0.05). These results establish inverse neurovascular coupling to spreading depolarization as a novel mechanism of secondary brain injury and suggest that cortical spreading depolarization, the neurovascular response, cerebrovascular autoregulation, and ischaemia are critical processes to monitor and target therapeutically in the management of acute brain injury.
Keywords: spreading depression; peri-infarct depolarization; pressure reactivity index (PRx); hemedex
Abbreviations: CBF = cerebral blood flow; CSD = cortical spreading depolarization; PRx = pressure reactivity index; PmO2 = brain tissue partial pressure of oxygen; rCBFx = cerebrovascular autoregulation index; TBI = traumatic brain injury

Introduction

Cortical spreading depolarization (CSD) describes a class of pathological waves characterized by a near-complete sustained depolarization of neurons and astrocytes that propagate through the cortex at a rate of 1–8 mm/min (Leão, 1944; Grafstein, 1956; Hansen and Zeuthen, 1981). As a consequence of CSD there is an electrical silence of neuronal activity, termed spreading depression, caused by the depolarization block of ionic membrane channels and adenosine-mediated suppression of synaptic transmission (Leão, 1944; Lindquist and Shuttleworth, 2012). CSD is mediated by release of glutamate into the extracellular space, activation of glutamatergic ionotropic receptors, [K+] efflux, and [Na+] and [Ca2+] influx that overwhelm adenosine triphosphate-dependent pumps leading to a complete breakdown of ionic gradients (Kraig and Nicholson, 1978; Hansen and Zeuthen, 1981; Aiba and Shuttleworth, 2012; Zhou et al., 2013). Beyond electrical disturbances, CSD leads to morphological changes as water follows the cation influx to produce cellular oedema, dendritic beading, and ~70% shrinkage of the extracellular space (Kraig and Nicholson, 1978; Somjen, 2004; Risher et al., 2012). As energy-dependent ionic pumps restore ionic equilibrium, metabolic substrates are depleted rapidly (Mies and Paschen, 1984; Feuerstein et al., 2010).

In the uninjured brain, CSD induces a wave of spreading hyperaemia (physiological neurovascular coupling) that supplies the tissue with the necessary energy to restore ionic equilibrium (Lauritzen et al., 1982; Strong et al., 1988; Kocher, 1990; Mayevsky and Weiss, 1991; Strong, 2005; Fabricius et al., 2006). The spreading hyperaemia shares similar mechanisms to classic neurovascular coupling wherein neuronal activation leads to vasodilation through glutamate release, ionic influx, and production of nitric oxide (Attwell and Iadecola, 2002; Devor et al., 2007). In injured tissue where neurovascular coupling is compromised, CSD induces a microvascular constriction that leads to a transient hyperperfusion (pathological inverse coupling) (Dreier et al., 1998; Shin et al., 2006). Although the exact mechanisms involved in the inverse haemodynamic response are not completely understood, increased release of vasoconstrictors, [K+] and haemoglobin, and decreased nitric oxide (due to increased scavenging and/or inhibition of nitric oxide synthase) play pivotal roles (Dreier et al., 2000).

Inverse neurovascular coupling to CSD reduces the energy available for the restoration of electrochemical membrane gradients, and thus creates a mismatch between the tissue energy demand and supply (Shin et al., 2006; Koide et al., 2013). As a result, the duration of CSD is prolonged (Dreier et al., 1998, 2002; Sukhotinsky et al., 2008). In animal models, prolonged CSDs with inverse neurovascular coupling are sufficient to produce widespread necrosis (Dreier et al., 2000). Inverse neurovascular coupling to CSD has been detected in multiple animal models including subarachnoid haemorrhage, hypotension and hypoxia, and middle cerebral artery occlusion (Dreier et al., 1998; Shin et al., 2006; Strong et al., 2007; Sukhotinsky et al., 2008). Furthermore, the inverse response has been detected in patients with subarachnoid haemorrhage and malignant hemispheric stroke, thus identifying a novel pathophysiological mechanism to target with therapeutics (Dreier et al., 2009; Bosche et al., 2010; Wotizik et al., 2013).

Inverse neurovascular coupling to CSD has never been reported in experimental traumatic brain injury (TBI). However, known secondary brain injury mechanisms nonetheless imply that the inverse neurovascular coupling to CSD may be a pathogenic mechanism in patients. First, CSD occurs in 50–60% of patients with severe TBI and is significantly associated with worse patient outcomes (Hartings et al., 2011). Second, 30–67% of patients with severe TBI exhibit impaired cerebrovascular autoregulation (Bouma et al., 1992; Czosnyka et al., 2001; Vavilala et al., 2004; Zweifel et al., 2008). Third, cerebral blood flow reaches ischaemic levels (<18 ml/100 g/min) that are capable of causing neuronal loss in 30–45% of patients (Obrist et al., 1984; Marion and Bouma, 1991; Marion et al., 1991; Bouma et al., 1992; Muizelaar and Schroder, 1994; Schroder et al., 1996).

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Injury Doppler technology represents the gold standard for investigating neurovascular coupling to CSD both in animal models and in patients (Dreier et al., 1998, 2009). In a study of combined monitoring of CSD and blood flow in patients with subarachnoid haemorrhage, laser Doppler probes integrated into an optoelectrode strip detected both physiologic and inverse coupling that corresponded to changes in brain tissue oxygenation (PmO2) (Dreier et al., 2009). However, limitations of this technique included artefact from patient movement, the delicate nature of the integrated optical fibres, and measurement of relative flow values (Dreier et al., 2009). In this study, we investigated the use of a parenchymal thermal diffusion probe (Hemedex®) to monitor regional cerebral blood flow (CBF) alongside subdural electrode strip recordings in patients with TBI. The parenchymal thermal diffusion probe samples a focal spherical volume of 4–5 mm of tissue surrounding the distal tip of the probe for real-time absolute measure of perfusion that has been previously validated with xenon CT (Vajkoczy et al., 2003; Jaeger et al., 2005). The aim of this study was to evaluate neurovascular coupling to CSD to determine whether pathological inverse coupling is a mechanism of secondary injury in patients with TBI. Despite some technical limitations, we observed haemodynamic responses to CSD in 5/24 (21%) patients with severe TBI. Inverse neurovascular coupling was more prevalent (60/82 CSDs) than physiological coupling (20/82 CSDs) and was associated with impairments in cerebrovascular autoregulation.
Materials and methods

Patient enrolment

Twenty-four patients with acute TBI were prospectively enrolled at three participating centres of the Co-Operative Studies on Brain Injury Depolarizations (COSBID): six patients at King’s College Hospital (London, UK), four patients at University of Pittsburgh Medical Centre (Pittsburgh, PA), and 14 patients at University of Cincinnati Medical Centre (Cincinnati, OH). Inclusion criteria were the clinical decision for neurological surgery for lesion evacuation and/or decompression, and age ≥ 18 years. Patients with fixed, dilated pupils were excluded. Research protocols were approved by institutional review boards, surrogate informed consent was obtained for all patients, and research was conducted in accordance with the Declaration of Helsinki.

Neuromonitoring procedures

At the conclusion of surgery, an electrode strip was placed on the surface of the cortex for subsequent electrocorticography monitoring of spreading depolarizations. The strip was targeted to perilesional cerebral cortex to monitor viable tissue at highest risk for secondary injury (Strong et al., 2002; Fabricius et al., 2006; Hartings et al., 2009, 2011). Close to the recording strip (<1 cm), a CBF monitor (QFlow 500 Probe, Hemedex, Inc.) was implanted ~25 mm below the dural surface (Fig. 1). In three patients at the University of Cincinnati Medical Centre, a PbrO2 probe (Licox, Integra) was also placed near the regional CBF probe. The wire leads from all sensors were tunnelled beneath the scalp to exit 2–3 cm from the craniotomy margin and secured to the skin.

After transfer to the neuro-intensive care unit, patients underwent continuous neuromonitoring. During this period, patients were ventilated and pharmacologically immobilized as required. Sedation was maintained with propofol or midazolam, and analgesia was provided with fentanyl. Phenytoin or levetiracetam was administered for seizure control or prophylaxis in all patients; lorazepam was given in five patients for management of seizures or alcohol withdrawal. A full list of medications for each patient is provided in Supplementary Table 1.

Sedation and paralysis were adequate to maintain intracranial pressure control or prophylaxis in all patients; lorazepam was given in five patients for management of seizures or alcohol withdrawal. A full list of medications for each patient is provided in Supplementary Table 1.

Drug known to reduce the occurrence of CSDs (ketamine) or alter the haemodynamic response (nimodipine) were not used (Dreier et al., 2012). Based on the institution’s practice and clinical indication, intracranial pressure was monitored by a ventricular drainage catheter (n = 5), parenchymal transducer (n = 14), or subdural probe (n = 2). Depressive craniectomy together with sedation and paralysis were adequate to maintain intracranial pressure <20 mmHg in most patients; ventricular drainage, mannitol or hypertonic saline, and barbiturates were seldom required. The target level for cerebral perfusion pressure of >60 mmHg was achieved by intracranial pressure control, intravenous fluids, and vasopressors. Neuromonitoring was terminated and devices were gently removed at the patient’s bedside when invasive neuromonitoring was no longer clinically required or after a maximum of 7 days. No haemorrhagic or infectious complications were associated with the neuromonitoring devices.

Data acquisition

Electrophysiological recordings were made from a linear subdural strip, which consisted of six platinum contacts with 4.2 mm² exposed surface spaced at 10 mm along the strip (Wyler, Ad-Tech Medical). Ground was provided by a self-adhesive Ag/AgCl patch electrode on the shoulder. In 12 patients, electrodes were connected in a sequential bipolar fashion to AC-coupled amplifiers (0.01 Hz high-pass cut-off, GT205, Guger Technologies); data were digitized and recorded with Powerlab 16/SP and LabChart software version 7.2 (ADInstruments, Inc.). In the other 12 patients, monopolar signals were recorded with a direct current (DC)-coupled amplifier (g.USBamp, Guger Technologies) using a platinum subdermal needle (Grass Technologies) at the mastoid as reference. Data were displayed and acquired using a custom multi-modal monitoring program based on BCI2000, a brain-computer interface tool written to support a variety of data acquisition systems, and exported to LabChart software for analysis (Hartings et al., 2013; Wilson et al., 2013). When monitored, the analogue signals of intracranial pressure, mean arterial pressure, and PbrO2 were acquired by the same data acquisition systems.

Data processing and analysis

Electrophysiological data were analysed for CSD according to established methods using bipolar channel derivations and 0.01 Hz high-pass filtering (Fabricius et al., 2006; Hartings et al., 2009, 2011). Briefly, CSDs were identified by (i) the simultaneous occurrence of a slow potential change and depression of high frequency spontaneous activity (0.5–50 Hz) in individual channels; and (ii) the sequential occurrence of slow potential changes and depressions of high frequency activity on adjacent channels, demonstrating spread across the cortex. Depression durations were calculated for each CSD based on the integral of the power of the spontaneous activity. If CSD was evidenced only by a slow potential change with no depression of spontaneous activity, baseline electrical silence, the CSD was termed isoelectric (Hartings et al., 2011). After identifying CSDs, regional CBF data were examined for associated changes. Measurement of perfusion in a single location precluded assessment of the spread of haemodynamic changes. Therefore, the criterion to determine that blood flow changes were correlates of CSD and not spurious coincidental changes was a consistent temporal relationship between electrophysiological and haemodynamic responses for multiple CSDs. We recorded the time (Δt) from detection of CSD on bipolar electrode 3/4 to the start of the haemodynamic response; given the distance was fixed between the electrode strip and blood flow probe, the temporal relationship (Δt) should be consistent for CSD waves propagating in the same direction. The maximum percent increase or decrease in regional CBF was calculated from
a 2-min baseline before the haemodynamic response. Finally, $T_{50}$ was defined as the duration of the change measured at 50% of its maximum amplitude.

The statuses of cerebrovascular autoregulation during the haemodynamic responses to CSD were determined by examining the cerebrovascular autoregulatory index ($rCBF_x$), a moving Pearson correlation coefficient between regional CBF and mean arterial pressure. First, consecutive 8 s averages were calculated for each to eliminate high-frequency noise. Second, a continuous moving Pearson correlation coefficient was calculated for every 40 data points yielding a rCBFx, (Zweifel et al., 2008). Third, rCBFx was averaged hourly to determine the status of cerebrovascular autoregulation during the period of the haemodynamic responses. The $rCBFx$ represents a mathematical approach to quantify the relationship between spontaneous fluctuations of mean arterial pressure to regional CBF. Based on previous studies, correlation coefficients of $<0.3$ indicate intact cerebrovascular autoregulation (Czosnyka et al., 1996; Lang et al., 2002). Correlations were limited to regional CBF because intracranial pressure and $P_{bO_{2}}$ data were acquired with limited amplitude resolution, which precluded analysis of intracranial pressure waveforms. Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc.). Data are reported as mean ± standard error of the mean and $P < 0.05$ was considered statistically significant.

**Limitations of the technique**

The manufacturer of the parenchymal thermal diffusion probe (Hemedex, Inc.) suggests recording during implantation of the device to ensure placement away from thermally significant vessels with substantial pulsatility (probe placement assistant, PPA). If the probe tip is near a thermally significant vessel ($K > 6.5$) that produces significant pulsatility (PPA > 5), the monitor will produce an error message and prevent regional CBF measures. However, we did not follow this recommendation in the operating room; therefore, data collection was limited in some cases because of placement in tissue with unstable thermal conductivity ($K > 6$) and/or near a pulsatile vessel (PPA > 5). The probe, which was tunneled into position, could not be adjusted in the intensive care unit. Data collection was also limited in patients with fever; for patient safety, the thermal diffusion probe would shut off if the patient’s temperature reached 39.5°C, thus ensuring tissue was not heated above 41°C. The device, which recalibrated every 2 h for ~5–15 min, also interrupted continuous data collection.

**Assessment of clinical outcomes**

Clinical outcome was assessed during telephone interview or clinical visit using the extended Glasgow Outcome Scale; scores were rated as good (moderate disability or good recovery; 5–8) or poor (dead, vegetative state or severe disability; 1–4).

**Results**

**Summary of patients’ clinical and neuromonitoring data**

Patients’ clinical and neuromonitoring data are presented in Tables 1 and 2. A total of 876 CSDs were recorded in 17 of 24 patients in 106 days of electrophysiological monitoring. Regional CBF data collection was limited to 39% of this duration for several reasons (Table 2). First, probe placement in an instable thermal conductive field ($K > 6.5$) limited data collection in five patients and placement in close proximity to a thermally significant vessel ($PPA > 5$) limited data collection in nine patients. Second, data collection was impeded for six patients whose temperatures reached or exceeded 39.5°C. Third, haemodynamic responses of Patient 15 were excluded when CSD-related temperature waves were detected at the proximal temperature sensor, likely due to shallow placement of the thermal diffusion probe in the cerebral cortex. For these reasons, regional CBF data were only available for 196 of 876 CSDs detected. For 42% of the CSDs (82/196) stereotyped haemodynamic responses were observed in a time-locked fashion relative to the CSD. During these CSDs, average intracranial pressure and mean arterial pressure values were maintained in their normal physiological range ($<20$ mmHg and $>70$ mmHg, respectively) and temporary deviations outside this range did not affect relevant electrophysiological or blood flow measures. The haemodynamic responses, which occurred in five of the 17 patients with CSD, were characterized by either physiological neurovascular coupling (hyperaemia, $n = 20$ CSDs) or pathological inverse coupling (hypoperfusion, $n = 62$ CSDs), further described below. For the remaining 58% of the CSDs (114/196), no consistent changes in regional CBF were observed.

**Physiological neurovascular coupling to CSD during intact cerebrovascular autoregulation**

Patient 13 exhibited only physiological neurovascular coupling to seven CSDs that occurred over 28–34 h after injury (Fig. 2A). Baseline perfusion was similar at the start (25.3 ml/100 g/min) and end of this period (24.7 ml/100 g/min), but varied before each CSD (range: 23.1–45.5 ml/100 g/min). The magnitude and duration of the hyperaemic responses to CSD were consistent, with a maximum increase of 794 ± 43% above baseline and a $T_{50}$ of 499 ± 48 s ($n = 7$). Regional CBF increases started 112 ± 32 s after detection of the CSD, which depression durations of 569 ± 83 s ($n = 7$). Cerebrovascular autoregulation was intact (average $rCBFx$ 0.05 ± 0.03) during the period of hyperaemic responses (Fig. 2B), and post-monitoring brain scans suggest electrodes were placed on cortex that remained viable (Fig. 2C).

**Inverse neurovascular coupling to CSD during impaired cerebrovascular autoregulation**

All haemodynamic responses to CSDs were the pathological inverse type in three patients. Patient 14 showed inverse neurovascular coupling for 10 CSDs that occurred 16–29 h after injury (Fig. 2D). Baseline perfusion at the start (15.6 ml/100 g/min) was substantially lower than at the end of this period (44.2 ml/100 g/min) and varied prior to each CSD (range: 15.6–73.3 ml/100 g/min). Magnitude and duration of the transient hypoperfusions were consistent and reproducible, with a maximum decrease –24 ± 6% below baseline and a $T_{50}$ of 227 ± 17 s ($n = 10$). Regional CBF decreases started 55 ± 3 s after detection of CSDs, which had depression durations of 633 ± 44 s ($n = 10$). Cerebrovascular autoregulation became progressively impaired during the inverse haemodynamic responses, with an average $rCBFx$ 0.30 ± 0.04 during this period (Fig. 2E). Brain scans show electrodes were placed directly adjacent to an intracerebral haematoma (Fig. 2F).
Table 1 Patient and recording characteristics

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MVA = motor vehicle accident; MVA-P = pedestrian involved in motor vehicle accident; MC = motorcycle accident; ADM GCS = Admission Glasgow Coma Scale; F = frontal; T = temporal; L = left; R = right; Bi = bilateral; Cont = contusion; SDH = subdural haematoma; EDH = epidural haematoma; ICH = intracerebral haemorrhage; SAH = sub-arachnoid haemorrhage; ECoG = electrocorticography.

A similar pattern of consistent inverse neurovascular coupling in response to nine CSDs was also observed in Patient 1 (data not shown) at 144–151 h after injury. Baseline perfusion was similar at the start (38.8 ml/100 g/min) and end of this period (35.5 ml/100 g/min), but varied prior to each CSD (range: 24.0–56.3 ml/100 g/min). The hypoperfusions, which started 206±13 s after detection of CSDs, had a maximum decrease of −40±4% below baseline and a T50 of 238±20 s (n = 9). Depression durations for these CSDs were 740±130 s (n = 6); however, the last three events occurred on a background of isoelectricity induced by prior CSDs. No post-monitoring imaging studies were performed and cerebrovascular autoregulation could not be analysed because blood pressure was not recorded.

In contrast with the consistency of the inverse neurovascular coupling observed in Patients 1 and 14, Patient 12 had progressive worsening of the pathological inverse response to CSDs over time [ANOVA: F(2,27) = 11.52, P < 0.001] that coincided with a progressive loss of cerebrovascular autoregulation [ANOVA: F(2,35) = 12.8, P < 0.001] (Fig. 3A–C). Haemodynamic responses began 303±12 s before detection of CSDs. Early in the recording, 23–42 h after injury, seven CSDs elicited hypoperfusions with an average maximum decrease of −14±3%, and a T50 of 60±1 s (n = 7). For the next eight CSDs, at 43–49 h after injury, the hypoperfusions had a maximum decrease of −29±4%, T50 49±3 s (n = 8), that was significantly worse than the prior period (P < 0.05 Tukey’s post hoc). Finally, at 50–66 h after injury, hypoperfusions had a maximum decrease of −79±3%, T50 51±3 s (n = 15, P < 0.05 Tukey’s post hoc). During these three periods, cerebrovascular autoregulatory status progressed from a rCBF of 0.04 (±0.03) to 0.14 (±0.02) to 0.28 (±0.04) (P < 0.05 Tukey’s post hoc compared to initial period). Baseline perfusion was similar between the early (average: 5.2 range: 2.8–7.0 ml/100 g/min) and middle periods (average: 5.9 range: 3.7–14.2 ml/100 g/min), but was significantly higher later (average: 31.8 range: 10.1–68.4 ml/100 g/min) [ANOVA: F(2,27) = 15.52, P < 0.0001]. Cerebral perfusion pressures at CSD onsets were constant from first to last period (75±1, 72±1, 76±2 mm Hg, respectively) [ANOVA: F(2,54) = 1.86, P = 0.17], and there were no significant differences in depression durations (775±40, 800±28, 703±26 s) [ANOVA: F(2,25) = 3.18, P = 0.058]. As indicated in serial brain scans, electrodes were placed on cortex undergoing lesion expansion (Fig. 3D).

Transformation from physiological to inverse neurovascular coupling coincides with impairments in cerebrovascular autoregulation

Comparisons across the previous patients suggested an association between physiological neurovascular coupling and intact autoregulation on one hand, and inverse neurovascular coupling with
impaired autoregulation on the other. This association was additionally observed as a transformation of both variables through time in Patient 5 (Fig. 4A–C). In this patient, haemodynamic responses started 389/C631 s after detection of CSDs. In the initial period 25–32 h after injury, cerebrovascular autoregulation was intact (rCBFx = 0.19/C60.04) and haemodynamic responses to 13 CSDs were all hyperaemic (maximum increase 44/C66%, T = 50/C6150/C68s, n = 13). During the subsequent period at 33–45 h, however, autoregulation became impaired (rCBFx = 0.32/C60.04) and haemodynamic responses transformed suddenly, rather than progressively, to hypoperfusions (maximum decrease 30/C62%, T = 50/C6244/C628 s, n = 13). Changes in both regional CBF response [t(24) = 12.2, P < 0.001] and rCBFx [t(19) = 2.2, P = 0.0398] were statistically significant between the two periods. Baseline perfusion between the two periods was similar (32.0 versus 36.0 ml/100 g/min) (P = 0.05), but varied before each CSD (range: 17.1–60.1 ml/100 g/min). Cerebral perfusion pressures measured at CSD onsets were also similar (90 ± 3 versus 84 ± 3 mm Hg) (P > 0.05). There was no difference in the depression durations for these CSDs (639 ± 47 versus 563 ± 24 s) [t(24) = 1.41, P = 0.18]. Post-monitoring CT scan shows a large hypodense region throughout the right frontal lobe that included the area of neuromonitoring (Fig. 4D).

Transformation from a physiological hyperoxic response to an inverse hypoxic response to CSD

To confirm haemodynamic responses to CSD with a second technique, PbtO2 probes were placed intraoperatively alongside electrode strips in three patients. In two patients, probes became dislodged and no measurements were obtained. In Patient 10, however, transient PbtO2 changes were observed consistently beginning 71/C646 s after 26 CSDs. As in Patient 5, PbtO2 responses to CSD transformed from physiological transient increases (hyperoxic response) to inverse transient decreases (hypoxic response) over time (Fig. 5A). At 40–53 h after injury, the PbtO2 responses to 14 CSDs were hyperoxic (maximum increase 20/C61%, T = 50/C6307/C614 s, n = 14) during intact cerebrovascular autoregulation (rCBFx = 0.03/C60.02). At 54–68 h, PbtO2 changes inverted and became hypoxic (maximum decrease 14/C62%, T = 50/C6244/C628 s, n = 12). Depression durations of CSDs with hypoxic responses were significantly longer than those with hyperoxic responses (1229/C6140 versus 847/C643 s) [t(18) = 3.4, P = 0.003]; 6 of the 12 hypoxic CSDs were isoelectric. Cerebral perfusion pressures fell significantly from the earlier period of hyperoxia to the later period of hypoxia but remained in normal range (110 ± 2 versus 94 ± 2 mmHg) (P < 0.001). Cerebrovascular autoregulation could not be determined during the later hypoxic changes because no

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**Table 2 Neurovascular coupling to CSD**

<table>
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<th>Patient</th>
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<th>Neurovascular Coupling to CSD</th>
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<th>Average rCBF (ml/100 g/min)</th>
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PPA = probe placement assistant; K = thermal conductivity; Temp = patient temperature; GOS-E = Extended Glasgow Outcome Score; rCBF = regional CBF.

Brain tissue oxygen responses denoted by brackets.

*Analysis excluded due to temperature wave on proximal thermistor producing regional CBF artefact.
regional CBF data were collected during this period. Based on brain scans, electrodes were placed on cortex bordering a large area of oedema with ongoing lesion expansion (Fig. 5B). No consistent haemodynamic changes were observed in association with CSD during the 79 h of regional CBF measurement.

**Role of baseline blood flow**

Baseline levels of regional CBF were analysed to determine the association of ischaemia (<18 ml/100 g/min) with CSDs. CSDs occurred over a wide range of perfusion levels, with only 27% occurring at ischaemic levels (Fig. 6A). During ischaemia, a greater proportion (32% of CSDs, 20/62) of pathological inverse responses occurred and thus exacerbated perfusion deficits, compared with only 10% of CSDs (2/20) with physiological haemodynamic responses (Fisher’s exact test $P = 0.08$). However, there was no significant difference in baseline perfusion for CSDs with physiological responses (30.1 ± 2.6 ml/100 g/min) compared with inverse responses (28.2 ± 2.3 ml/100 g/min) [$t(80) = 0.45, P = 0.65$]. The average depression time was significantly longer for CSDs with the inverse haemodynamic response (772.6 ± 33.5 s: 11 isoelectric CSDs) than CSDs with the physiological response (666.6 ± 33.0 s: two isoelectric CSDs) [$t(93) = 2.22, P = 0.03$]. No differences in perfusion during the entire recording period occurred between patients with and without CSDs (36.3 ± 4.4 versus 39.0 ± 6.1 ml/100 g/min) [$t(22) = 0.35, P = 0.73$].

**Figure 2** Physiological neurovascular coupling to CSDs during intact cerebrovascular autoregulation and inverse neurovascular coupling to CSDs during impaired cerebrovascular autoregulation. (A) Bipolar electrocorticographic (ECoG) recordings from corresponding electrodes, CSD (black trace, 0.01 Hz high pass filter) spreading depression (blue trace, 0.5–50 Hz bandpass filter), regional CBF (rCBF, red trace), and blood pressure (BP, gold trace). Lapse in regional CBF data was caused by calibration of the device. (B) Intact autoregulation (rCBF$_x$) during period of physiological coupling (red box). (C) Brain images show placement of electrode (Day 1 CT, blue arrow) on cortex with normal intensity on the post-monitoring scan (Day 11 MRI). (D) Recordings same configuration as A. (E) Impaired autoregulation during period of inverse coupling (blue box). (F) Brain images show placement of electrode (Day 1 CT, blue arrow) directly adjacent to an intracerebral haematoma where cortical lesions developed (Day 11 MRI). ICP = intracranial pressure.
Average regional CBF was significantly higher in patients with good 6-month outcomes (5–8, 46.8 ± 6.5 ml/100 g/min) than those with poor outcomes (1–4, 32.2 ± 3.7 ml/100 g/min) \( t(22) = 2.08, P = 0.0487 \) (Fig. 6B). Similarly, patients with poor outcomes trended strongly toward longer total times of ischaemia compared to those with good outcomes (23.2 ± 6.5 versus 5.3 ± 2.4 h) \( t(22) = 1.90, P = 0.071 \). Analysis of daily perfusion values did not reveal a specific time frame responsible for these differences. At least one ischaemic period (hourly average <18 ml/100 g/min) occurred in 19 of 24 patients (79%, range 1–78 h).

**Figure 3** Progressive worsening of inverse neurovascular coupling to CSD coincides with impairment of cerebrovascular autoregulation. (A) Recordings similar configuration as Fig. 2. (B) Average hypoperfusion during each period depicts the progressive worsening of inverse neurovascular coupling (average in blue, SEM in black). (C) Progressive impairment in autoregulation during this period. (D) Brain images show placement of electrode (Day 2 CT, blue arrow) on a developing cortical lesion. ECoG = electrocorticography; rCBF = regional CBF.
Lastly, there was no significant difference in patient outcomes between those with and without CSDs \( t(22) = 0.53, P = 0.59 \).

**Discussion**

Here, we characterized the haemodynamic response to CSD in surgical patients with TBI using a parenchymal thermal diffusion blood flow probe placed intraoperatively adjacent to a subdural electrocorticography strip. With continuous multimodal monitoring during intensive care, we detected pathological inverse coupling to CSD (transient hypoperfusion) which, in this patient series, was more prevalent than physiological coupling (hyperaemia). Specifically, inverse coupling was observed for 74 CSDs in five patients while physiological coupling was observed for 34 CSDs in three patients. Roughly one-third of CSDs that induced hypoperfusions occurred in tissue with baseline regional CBF \(<18 \text{ ml/100 g/min}\) and thus exacerbated existing ischaemic conditions. We found that physiological haemodynamic responses occurred only when cerebrovascular autoregulation was intact and that pathological inverse responses were associated with progressive autoregulatory impairments. In three patients, a pathological evolution of neurovascular coupling was observed, marked by a switch from hyper- to hypoperfusion responses to CSD, or by increasing severity of hypoperfusions over time. Furthermore, pathological inverse coupling was only observed with electrodes placed in or adjacent to evolving lesions and was associated with significantly longer depression periods. Physiological coupling to CSDs was observed throughout the recording period in only one patient whose electrodes were located on tissue that remained viable.

These results establish inverse neurovascular coupling to CSD as a novel mechanism of secondary injury in TBI. Furthermore, these results suggest that CSD, the neurovascular response, cerebrovascular autoregulation, and ischaemia are critical processes to monitor and target therapeutically in the management of TBI.

**Significance of the inverse haemodynamic response**

In focal cerebral ischaemia models, the haemodynamic response to CSD conveys the status of underlying cortex; that is, cortical regions remote from the injury exhibit the physiological response, the ischaemic core exhibits the inverse, and the penumbra exhibits a mixture of the two, as well as biphasic signals (Luckl *et al.*, 2009; Nakamura *et al.*, 2010; Offenhauser *et al.*, 2011). In our study, one patient exhibited physiological haemodynamic responses to all CSDs, and five patients had only inverse responses or exhibited a transformation from physiological to pathological coupling. The location of monitoring, which was remote from damaged tissue in the former patient and located perilesional in the latter patients, was consistent with the spatial mapping of regional CBF responses to CSD in animal models of focal cerebral ischaemia. However, our finding showing the transformation from physiological increases to pathological decreases in perfusion through time in two patients has not previously been described in animals. Rather, this temporal evolution has only been reported previously in a single case report of a patient with severe TBI who eventually died (Mayevsky *et al.*, 1996). Similar to our results, the initial CSDs detected with an invasive multiparametric probe
induced hyperaemia whereas later events were coupled to the pathological inverse response. Although hypotension is capable of inducing a transformation from hyperaemia to hypoperfusion (Sukhotinsky et al., 2008), no such changes in blood pressure occurred in our patients.

A likely cause of inverse coupling to CSD is intracerebral or subarachnoid haemorrhage, because haemoglobin scavenges the vasodilator, nitric oxide, and unmasks the vasoconstrictive effects of elevated extracellular [K⁺] during CSD (Hablit and Heinemann, 1989; Dreier et al., 2000). There is clinical support for this mechanism as the inverse haemodynamic response to CSD is observed in patients with aneurysmal subarachnoid haemorrhage (Dreier et al., 2009). However, inverse coupling also occurs experimentally after focal ischaemia (Luckl et al., 2009; Nakamura et al., 2010; Offenhauser et al., 2011) and clinically after malignant ischaemic stroke (Woltz et al., 2013) when haemorrhage is not present. In TBI, several pathophysiological factors may contribute. Here, all patients with TBI with inverse

Figure 5  Transformation from physiological hyperoxic to inverse hypoxic responses to CSD. (A) Recordings similar configuration as Fig. 2 with the addition of brain tissue oxygenation (PbtO₂, green trace). (B) Brain images show electrode (Day 5 CT, blue arrow) on cortex bordering a large area of oedema and lesion expansion. MAP = mean arterial pressure.
coupling had Fisher Grade 1 or 2 in addition to subdural or intracerebral haemorrhage and cerebral contusion. Furthermore, our data suggest that impairments in cerebrovascular autoregulation play an important role in inverse neurovascular coupling.

Cerebrovascular autoregulation

Cerebrovascular autoregulation describes the ability of pial resistance vessels to modulate their tone in response to changes in cerebral perfusion pressure in order to maintain constant levels of blood flow to neural tissue. The association between impaired cerebrovascular autoregulation and inverse neurovascular coupling to CSD observed in our small patient series suggests that the two phenomena may result from common underlying perturbations. In particular, the metabolic hypothesis of autoregulation asserts that energy supply-demand mismatch causes release of chemical factors in neural tissue that effect corresponding changes in vascular tone. In this regard, it is possible that CSD itself contributes to both inverse neurovascular coupling and impaired autoregulation.

The repeated recurrences of CSDs in subarachnoid haemorrhage patients at close intervals were associated with progressively increasing hypoxic responses; Bosche et al. (2010) suggested that impairment of neurovascular coupling resulted from the earthing and intracranial pressure, is the most common method for the measurement of haemodynamic responses to CSD in patients with subarachnoid haemorrhage (Dreier et al., 2008; Dreier et al., 2009). With laser Doppler probes integrated into a subdural electrode strip, hyperaemic coupling to CSD measured a 257% increase in regional CBF with duration of 689 s. Hypoperfusions induced decreases of −59% with a shorter duration 118 s. Similarly, our hyperaemic responses measured with thermal diffusion probes were of larger amplitude (419% increase) and longer duration (T50 325 s) than hypoperfusion responses (−36% decrease, T50 183 s). The broad spectrum of regional CBF changes observed within and between patients strongly supports the validity of these measurements as reflecting regional CBF changes induced by CSD.

A substantial 58% of CSDs that occurred during valid perfusion recordings exhibited no haemodynamic response, which was similar to the lack of responses detected in 40–70% of CSDs in patients with subarachnoid haemorrhage (Dreier et al., 2009; Bosche et al., 2010). These may in part represent true negatives since absent perfusion responses are observed along the continuum from hyperaemia to hypoperfusion. However, many recordings

Figure 6 Perfusion role in CSD and patient outcomes. (A) Distribution of baseline regional CBF at the time of CSD suggests occurrence of CSD was independent of baseline perfusion. (B) Patients with good outcomes (extended Glasgow Outcome Scale 5–8) had significantly higher regional CBF than those with poor outcomes (1–4). GOS-E = extended Glasgow Outcome Scale.

Further work is needed to assess the similarity between these measures.

Methodological considerations

Here, we lost a substantial amount of data by placing the blood flow probe near thermally significant vessels or in tissues with unstable thermal conductivity. The absolute values of perfusion may not be accurate in this study due to the relatively high K value (5.9 ± 0.1), the wide variability in baseline perfusion observed, and measurement of values outside the physiological range. The aforementioned complications would have been largely avoided by verifying the readings in the operating room when adjustment was still possible. Nonetheless, the measurements obtained answered a major question and confirmed that vascular responses to a cortical phenomenon could be detected at a 2.0–2.5 cm depth in the white matter. For at least 42% of CSDs, the vasodilation or constriction of penetrating pial vessels accompanying CSD were sufficient to alter the perfusion of white matter at this depth. Furthermore, the characteristics of these changes are in general agreement with previous studies of cortical surface measurement of haemodynamic responses to CSD in patients with subarachnoid haemorrhage (Dreier et al., 2009).
were likely false negatives caused by deep or poor probe placement. Patient 10 confirmed this possibility as 26 CSDs induced \( P_{tO2} \) responses with no observed changes in regional CBF. Another source of false negatives could arise from CSDs with varying propagation patterns and velocities that would be excluded from analysis due to an inconsistent temporal relationship.

### Role of ischaemia

Focal cerebral ischaemia experiments have suggested cerebral blood flow <18 ml/100 g/min as an infarction threshold, which if maintained over some hours, causes irreversible tissue damage \( (\text{Jones et al., 1981}) \). This ischaemic threshold has been widely adopted in experimental and clinical studies, regardless of the techniques used. The incidence of ischaemia in patients with severe TBI detected with \( ^{133}\text{Xe} \) with xenon CT is high (30–45% of patients) and increased perfusion is associated with better outcomes \( (\text{Obrist et al., 1984; Marion and Bouma, 1991; Marion et al., 1991; Bouma et al., 1992; Muizelaar and Schroder, 1994; Schroder et al., 1996}) \). Similar to these studies, we detected significantly higher perfusion in patients with good outcomes compared with patients with poor outcomes. Our high (79%) incidence of ischaemia detected was likely attributable to the use of continuous, rather than intermittent, perfusion monitoring, and to the increased severity of injury affecting our patients, all of whom underwent surgical intervention. Only 27% of the CSDs occurred during ischaemic conditions. However, these results apply only to the brain regions monitored and do not rule out a significant role of focal ischaemia in regions where CSD is initiated. Still, the 32% of CSDs with the pathological inverse response that occurred during baseline ischaemic condition further exacerbate perfusion deficits and likely increased the probability of tissue damage.

### Conclusion

In this multicentre study, our findings confirm the pathological inverse haemodynamic response to CSD as a secondary injury mechanism in severe TBI that is associated with loss of cerebrovascular autoregulation. Furthermore, neurovascular responses to CSD may aid in determining the relative status of the underlying tissue as healthy (spreading hyperaemia), impaired (inverse coupling), or deteriorating (switch from hyperaemic to inverse coupling). Our findings provide groundwork for advanced multimodal monitoring to assess a patient’s autoregulatory status, identify optimal target ranges for cerebral perfusion, detect secondary injury mechanisms in real-time, and determine the efficacy of interventions targeted against these processes.

### Funding

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### Supplementary material

Supplementary material is available at Brain online.

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