

# Dynamic metabolic response to multiple spreading depolarizations in patients with acute brain injury: an online microdialysis study

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**Spreading depolarizations (SDs) occur spontaneously with high incidence in patients with acute brain injury. They can be detected by subdural electrocorticographic recordings. We here characterize the dynamic metabolic response to these events. A microdialysis catheter was inserted into perilesional cortical tissue adjacent to a strip for electrocorticography following craniotomy in 10 patients. The microdialysis catheter was connected to an online microdialysis assay measuring glucose and lactate concentrations every 30 to 60 secs. Spontaneously occurring SDs systematically caused a reduction in dialysate glucose by  $-32.0 \mu\text{mol/L}$  (range:  $-92.3$  to  $-18.4 \mu\text{mol/L}$ ,  $n=90$ ) and increase in lactate by  $+23.1 \mu\text{mol/L}$  (range:  $+5.5$  to  $+93.6 \mu\text{mol/L}$ ,  $n=49$ ). The changes were sustained at 20 mins after the SD events and highly significant using an area under the curve analysis ( $P<0.0001$ ). Multiple and frequent SDs led to a progressive stepwise depletion of brain glucose. Hence, SD events cause a massive energy imbalance and their frequent occurrence leads to a local insufficiency of glucose supply. Such a failure would compromise cellular repolarization and hence tissue viability. The findings offer a new mechanism to account for otherwise unexplained instances of depletion of brain microdialysate glucose.**

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## Introduction

Spreading depolarizations (SDs) were first described as cortical spreading depressions by Leao (1944). They have been characterized by large dynamic changes in the distribution of ions between the intracellular and extracellular compartments and dramatic changes in blood flow (Lauritzen *et al*, 1982). There is increasing evidence that SDs occur frequently and spontaneously in the injured human brain: the incidence in patients who have undergone craniotomy lies in the region of 50% to 60% for traumatic brain injury (Strong *et al*, 2002), 72% for

aneurysmal subarachnoid hemorrhage (Dreier *et al*, 2006), and essentially 100% for malignant hemisphere stroke (Dohmen *et al*, 2008). The SDs are waves of transient, abnormal mass depolarizations of neurons and astrocytes that propagate from the core of the injury to the surrounding compromised but potentially salvageable tissue. When occurring adjacent to focal, core lesions in the injured human brain, depolarizations typically occur in temporal clusters of repetitive events (Dreier *et al*, 2006; Fabricius *et al*, 2006). The exact role of SDs in the development of secondary neurologic deterioration in the human brain is still unclear and the relation between SDs and patient outcome is currently under investigation by the COSBID group (Cooperative Study of Brain Injury Depolarizations: <http://www.cosbid.org>).

The occurrence of spontaneous SD-like events in experimental models of stroke (Branston *et al*, 1977) and contusional head injury (Katayama *et al*, 1990) has been well documented. More recent experiments

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have detected complex patterns of transient change in local cerebral blood flow, local tissue oxygen availability, and key energy metabolite pools, using a variety of techniques including laser speckle flowmetry and rapid-sampling microdialysis (rsMD) (Hashemi *et al*, 2009), two-photon microscopy (Takano *et al*, 2007), and oxygen microelectrodes (Piilgaard and Lauritzen, 2009). Recurrent SDs in periinfarct tissue have been shown not only to be associated with the expansion of the damage (Mies *et al*, 1993) but to promote it by progressive infarction of the perilesion tissue (Busch *et al*, 1996; Dijkhuizen *et al*, 1999).

Despite this flurry of findings in experimental models of brain injury, little is known about the effect of SDs on the injured human brain. The reason for that is mostly methodological. The SDs can now be detected reliably using continuous electrocorticography (ECoG) and appropriate frequency filtering (Fabricius *et al*, 2006) over extended periods of time, up to 10 days (Dreier *et al*, 2006). However, it remains difficult to study the metabolic state of the tissue when SDs occur: current sophisticated scanners, including magnetic resonance imaging and positron emission tomography, cannot in practice be used continuously for days, without compromising patient care. The current method of choice for the continuous assessment of metabolism is cerebral microdialysis. It has been used routinely to monitor head injury patients (Glenn *et al*, 2003; Goodman *et al*, 1999; Hutchinson *et al*, 2000) and has enabled the identification of markers for the early detection of ischemia and secondary neuronal deterioration (Sarrafzadeh *et al*, 2003; Vespa *et al*, 2003; Zauner *et al*, 1997). The main pitfall of microdialysis is its limited time resolution, of about 30 to 60 mins in general. This is not sufficient to study the direct and dynamic effects of SDs, as locally the passage of an SD wave may last only 2 to 5 mins. The remedy is to use rsMD, an online microdialysis technique whereby the dialysate, instead of being collected in sample vials, is directly analyzed electrochemically. Typically, microdialysis concentrations of glucose and lactate can be estimated every 30 to 60 secs (Jones *et al*, 2002). This technique has been used successfully in a variety of pathologies, both in the intensive care unit (Parkin *et al*, 2005) and in the operating room (Bhatia *et al*, 2006; Deeba *et al*, 2008).

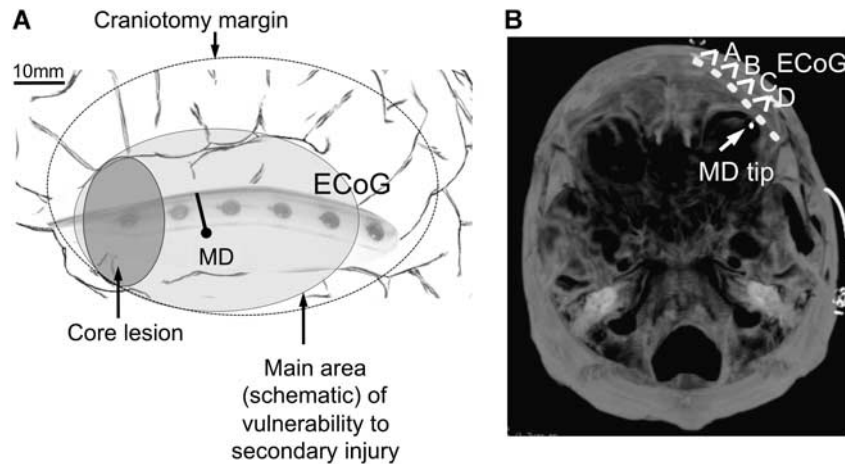
Here, we have sought to determine the dynamic neurometabolic signature of recurrent spontaneous depolarizations in the injured human brain using online rsMD of perilesion tissue.

## Materials and methods

### Patient Recruitment and Monitoring

All human research procedures were approved by the local Research Ethics Committee of King's College Hospital, London, or by Cambridgeshire 4 Research Ethics Committee. Patients requiring emergency craniotomy for traumatic

brain injury ( $n=5$ ), aneurysmal subarachnoid hemorrhage ( $n=2$ ), or intracranial hematoma ( $n=3$ ) were identified and research assent obtained from relatives or the authorized surrogate. Patients under 16 years of age, and/or with a Glasgow Coma Score below 4 at admission and/or with bilateral fixed and dilated pupils were excluded from this study. Towards the completion of surgery, a sterile clinical microdialysis catheter (CMA 70, 10-cm flexible shaft, 10-mm membrane length, 20kDa cutoff, CMA, Stockholm, Sweden) was inserted obliquely into the cortex, so far as possible, to full membrane depth through a minimal pial incision, as described earlier (Parkin *et al*, 2005). Along with the microdialysis catheter, a linear, six-platinum-contact ECoG recording strip (Wyler, 5mm diameter contacts, Ad-Tech Medical, Racine, WI, USA) was placed on the surface of the cortex accessible through the craniotomy, as described earlier (Fabricius *et al*, 2006; Strong *et al*, 2002). The aim was to locate the ECoG strip and microdialysis catheter closely together; it was usually possible to site them on the same gyrus, the microdialysis probe sited in perilesion cortical tissue between contacts 4 and 5 (or 5 and 6) of the ECoG strip that was radiating away from the lesioned area. The intended positioning of the strip and microdialysis catheter are drawn in Figure 1A and can be checked on a postoperative CT, as shown in Figure 1B. We acknowledge that positioning of the MD probe in tissue 'at risk' is crucial for the implications of our findings. Positioning of the microdialysis probe could not rely on physiologically specific imaging that could delineate a clear 'penumbra' (as classically defined in the context of focal cerebral ischemia by marked reductions in cerebral perfusion and availability of oxygen to tissue), as none was available. Instead, pragmatic placement of the probe was guided by our initial practical experience at craniotomy and afterwards. This suggested that after evacuation of a focal TBI hematoma/contusion or an ICH, the region within about 1 to 1.5 cm of the edge of clearly contused tissue risks complete necrosis within some 24 to 36 h or less, perhaps on the basis of new microfoci of intraparenchymal hemorrhage, and can therefore be considered as tissue at 'high risk.' In contrast, there is tissue, apparently more remote from an obvious contusion, where spontaneous depolarizations may occur within 1 to 6 days after trauma (Fabricius *et al*, 2006; Hartings *et al*, 2009). We consider this tissue to be at 'moderate risk,' that is, likely to be more sensitive to secondary insults and/or treatment over a longer period than 24 h, and so more clinically relevant. The aim was thus to place the probe in this tissue at 'moderate risk,' at 1.5 to 2.5 cm from the lesion edge as seen surgically. Insertion of the probe was oblique to the cortical surface, so that the 10-mm membrane was just buried and so putatively largely in cortex. Such placement of the MD probe in the pericontusional zone is well accepted as the best localization for microdialysis measurements of early markers of deterioration of the tissue at risk (Bellander *et al*, 2004). ECoG and microdialysis probes were secured by double suturing onto the skin beside the point of exteriorization. In the experience of surgeons inserting identical strips for assessment of patients with intractable epilepsy, this method of fixation has proved reliable, with negligible movement of probes after placement.



**Figure 1** Probe locations. **(A)** Schematic drawing of the insertion of the probes towards the end of the craniotomy: the ECoG strip is placed near the core lesion, sampling the transition from tissue 'at risk' out towards healthy tissue. The MD probe is inserted in tissue 'at risk' between contacts 4 and 5 on the same gyrus. Both probes are secured by double suturing at the skin exteriorization points. **(B)** Postoperative processed CT volume acquisition scan showing the location of the microdialysis catheter in relation to the ECoG strip. Maximal intensity projection of a selected set of contiguous reformatted slices, orientated to include all six electrodes on subdural strip, and (seen within the subjacent cortex) the tip of the microdialysis catheter, 2 mm below the cortical surface. The ECoG data channels (A to D) are indicated.

After surgery, patients were transferred to intensive or high dependency care. Arterial blood pressure and intracranial pressure were continuously recorded and blood gases, glucose, and electrolytes were documented hourly or 4-hourly. Where required, patients were ventilated, typically to a target partial pressure of arterial carbon dioxide (PaCO<sub>2</sub>) of 30 to 34 torr (4 to 4.5 kPa). Sedation was principally with fentanyl and midazolam, replaced with propofol during weaning. Target cerebral perfusion pressure was 60 mmHg, flexibly applied. Acute intravenous fluid therapy was typically Ringer's lactate or Hartmann's solution, 2 L/24 h, supplemented with colloid for any additional volume requirements. Once tolerated, enteral nutrition replaced this, comprising in general 20 to 25 kcal/kg/day or 60 mL/h, containing ~50% carbohydrate, 30% lipid, and 20% protein, together with electrolytes and trace elements. Glycemic control was targeted to the range 70 to 140 mg/dL (3.9 to 8 mmol/L) using actrapid insulin as a continuous intravenous infusion, typically at 1 to 2 u/h.

### Data Collection

The microdialysis catheter was perfused with sterile artificial cerebrospinal fluid (CMA perfusion fluid CNS: 147 mmol/L NaCl, 2.7 mmol/L KCl, 1.2 mmol/L CaCl<sub>2</sub>, 0.85 mmol/L MgCl<sub>2</sub>) at 2 μL/min using a CMA 100 Micro-injection syringe pump (CMA Microdialysis, Stockholm, Sweden). Perfusion of the MD catheter started immediately after the end of the operation so that the blood-brain barrier was sealed and the initial baseline dialysate levels were steady when the online measurements started in the intensive care unit (typically 1 to 2 h after surgery). The outlet tubing of the probe was adapted to connect to a dual online assay system (Parkin *et al*, 2005). Typically, a 1-m length of low volume connection tubing (Microbiotech, Stockholm, Sweden) was used between the patient and the online assay to facilitate patient movement and nurse care.

The rsMD online assay has been described elsewhere (Jones *et al*, 2002; Parkin *et al*, 2005). Briefly, it couples enzymatic recognition with electrochemical detection to yield two traces of current peaks every 30 to 60 secs. The heights of these peaks are proportional to the dialysate concentrations of glucose and lactate respectively (Figure 2, bottom two traces). The rsMD data, four channels of ECoG (Figure 2), arterial blood pressure, and intracranial pressure (when available) were digitized at 200 Hz using a Powerlab 16/SP analogue/digital converter and Chart-5.5 software (ADInstruments, Oxfordshire, UK) running on an Apple MacBook Pro (A1212, Apple, CA, USA).

A video camera (iSight, Apple, USA) and a customized event marker were used to identify changes due to necessary nursing interventions or to movement artefacts.

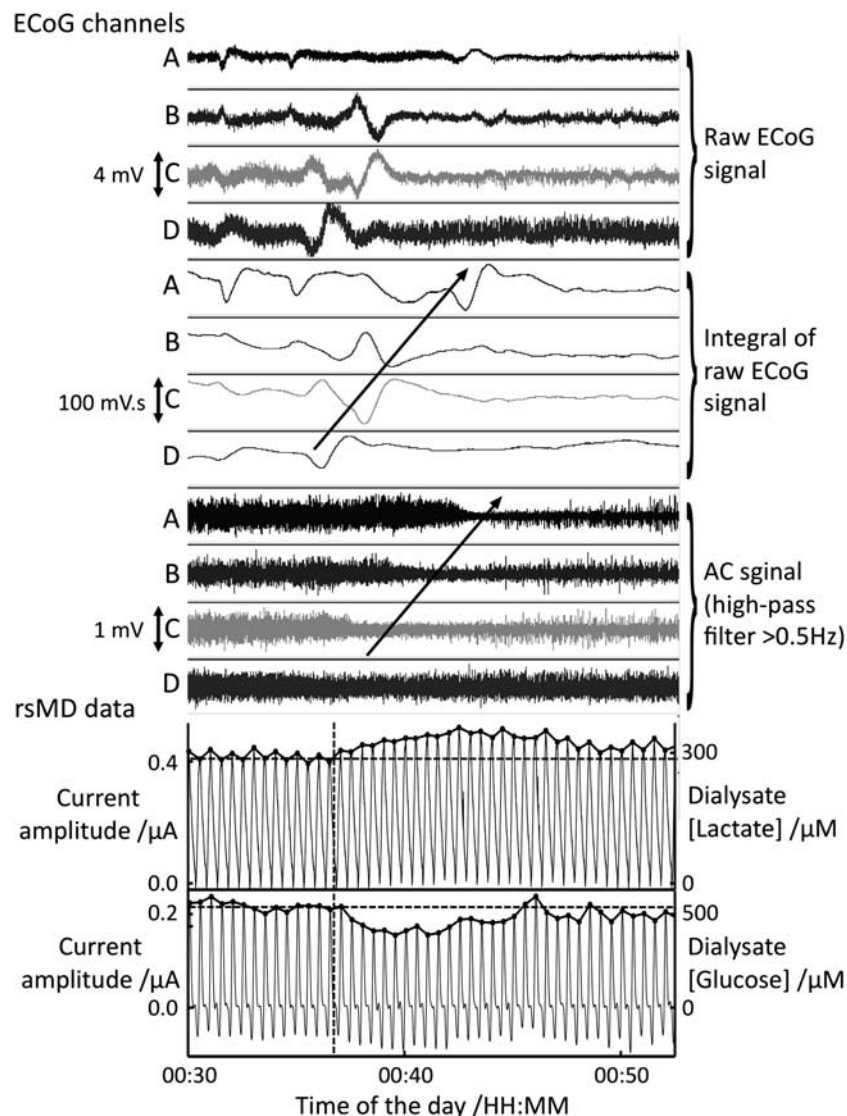
The whole data collection system was placed on a clinically certified trolley (Series 7000, CTL Medical, Essex, UK) situated behind the patient bed in the ICU to cause minimal disruption to clinical care during the study. The data were recorded continuously for a minimum of 24 h and a maximum of 5 days (Table 1).

### Data Analysis

The SDs were identified by their ECoG signature, as described earlier, as the sequential onset in adjacent channels of a propagating, polyphasic slow potential change followed by a rapidly developing reduction in the power of the ECoG amplitude by at least 50% (Fabricius *et al*, 2006) (Figure 2, middle traces).

The rsMD data were analyzed without the knowledge of the ECoG analysis results. The traces were processed into Matlab 7.5 (MathWorks, Natick, MA, USA) according to the following procedure:

1. Noise removal using signal processing techniques newly developed within the group (Feuerstein *et al*, 2009);



**Figure 2** The 25-min recording of four ECoG data channels and rsMD glucose and lactate during an SD in patient 4. The first 12 channels correspond to the bipolar ECoG recording along the six-electrode subdural strip, as described in Fabricius *et al* (2006). The raw traces are shown in the first four channels: all frequencies within the amplifier bandwidth (0.02 to 200 Hz) are recorded. The middle four channels emphasize the slow potential changes: the depolarizations are observed on all channels with a time delay between consecutive contacts, corresponding to a 4 mm/min propagation speed. The lower four channels highlight the depression of the high-frequency activity ( $> 0.5$  Hz) subsequent to the depolarization wave. The arrows indicate a spread of the SD wave from channel D to A, corresponding to contacts 6 to 2 on the subdural strip. The bottom two traces are the rsMD lactate and glucose traces (top and bottom, respectively) time aligned with the ECoG data. The actual current peaks recorded by the assay are shown with the scale on the left axes graduated in  $\mu$ Amperes. The corresponding dialysate concentrations after the extraction of the peak heights (indicated by the dots) are scaled on the right axes. The dotted vertical line indicates the onset of the metabolic response to SD and will define time  $t = 0$  for further analysis. The horizontal dotted lines define the dialysate pre-SD concentrations calculated as the mean concentrations over 5 mins before the SD.

2. Automatic peak detection using a standard local maximum algorithm;
3. Conversion to dialysate concentrations of glucose and lactate based on calibrations performed twice daily at the bedside throughout the monitoring period;
4. Filtering (three-point moving average) to yield dialysate concentration time series.

The dialysate concentration time series were then time-aligned with the ECoG trace corresponding to the closest electrode on the strip to take account of the 9 mins delay

due to the 1-m length low volume connection tubing between the patient and the rsMD assay. Epochs of 5 mins before and 20 mins after ECoG-identified SDs were extracted from the whole glucose and lactate dialysate concentration data for further analysis. All epochs were characterized by their basal levels (defined as the mean of the recorded rsMD values during 5 mins before the SD event), the changes in dialysate concentrations at 10 mins and at 20 mins after SD, and the area under the curve (AUC) for 20 mins after SD. Basal levels were defined over



**Table 1** Summary of patient demographics and recordings

Patient ID	Age	Gender	Diagnosis	Monitoring date (post-ictus)	Duration of monitoring (per hours)	Number of SDs
1	48	F	ICH	Day 0 to day 2	72	5
2	24	M	ICH	Day 1 to day 2	31	10
3	21	M	ICH	Day 2 to day 4	46	30
4	57	F	SAH	Day 11 to day 14	55	28
5	69	F	SAH	Day 2 to day 5	94	22
6	67	M	TBI	Day 0 to day 2	64	17
7	48	M	TBI	Day 1 to day 3	66	7
8	41	M	TBI	Day 1 to day 2	24	4
9 <sup>a</sup>	56	M	TBI	Day 0 to day 5	97	33
10	41	M	TBI	Day 1 to day 5	120	0

Abbreviations: F, female; M, male; ICH, spontaneous intracranial hematoma; SAH, spontaneous subarachnoid hemorrhage; TBI, traumatic brain injury; SDs, spreading depolarizations identified by their electrocorticographic signature.

<sup>a</sup>Patient 9 was diabetic and his initial glycemia was estimated between 325 and 490 mg/dL (18 and 27 mmol/L) at 21:00 on Day 0. The hyperglycemia was subsequently controlled by continuous intravenous insulin infusion together with continuous feeding. The plasma glucose concentrations were maintained between 105 and 230 mg/dL (6 and 12.8 mmol/L) during the rest of the monitoring period.

only 5 mins so as not to include the end of an earlier SD. The choice of 20 mins after SD was determined by the fact that it was generally the minimum interval time between consecutive SDs.

Data that showed transient parallel changes on both the glucose and lactate traces were excluded as they could not be differentiated from non physiological events that could be due to the performance of the assay or the microdialysis recovery, as defined earlier (Parkin *et al*, 2003). Events that were likely due to nurse activities or movements of the patients observed on anonymized video camera recordings were regarded as artefacts and also excluded.

In all patients, 25-min control epochs were selected and characterized at times when the ECoG recordings showed normal background activity (no SD events or epileptiform activity).

All values are given as median dialysate values (interquartile range); results were not corrected for recovery (Parkin *et al*, 2003). Statistical analyses including D'Agostino-Pearson's K2 tests for normality, Pearson product-moment correlations, binomial tests, and two-tailed Wilcoxon signed-rank tests were performed with Matlab 7.5 (MathWorks, Natick, MA, USA) or Igor Pro 6 (WaveMetrics, Portland, OR, USA).

## Results

Ten subjects were studied overall during the first 1 to 5 days of their stay in the intensive care unit following craniotomy. It amounted to a total of 669 h of high-quality ECoG and online microdialysis data. In nine patients, a total of 156 SD events were identified according to their ECoG signatures. The initial baseline microdialysis concentrations (an average of values recorded between 60 and 120 mins after dialysis started) ranged between 300 and 2000  $\mu\text{mol/L}$  for glucose and 400 and 2000  $\mu\text{mol/L}$  for lactate. These are comparable to other microdialysis studies in the injured human brain when accounting for the different recoveries of different probes and perfusion flow rates (Goodman *et al*,

1999; Hutchinson *et al*, 2000; Parkin *et al*, 2005; Sarrafzadeh *et al*, 2003; Vespa *et al*, 2003, 2005; Zauner *et al*, 1997).

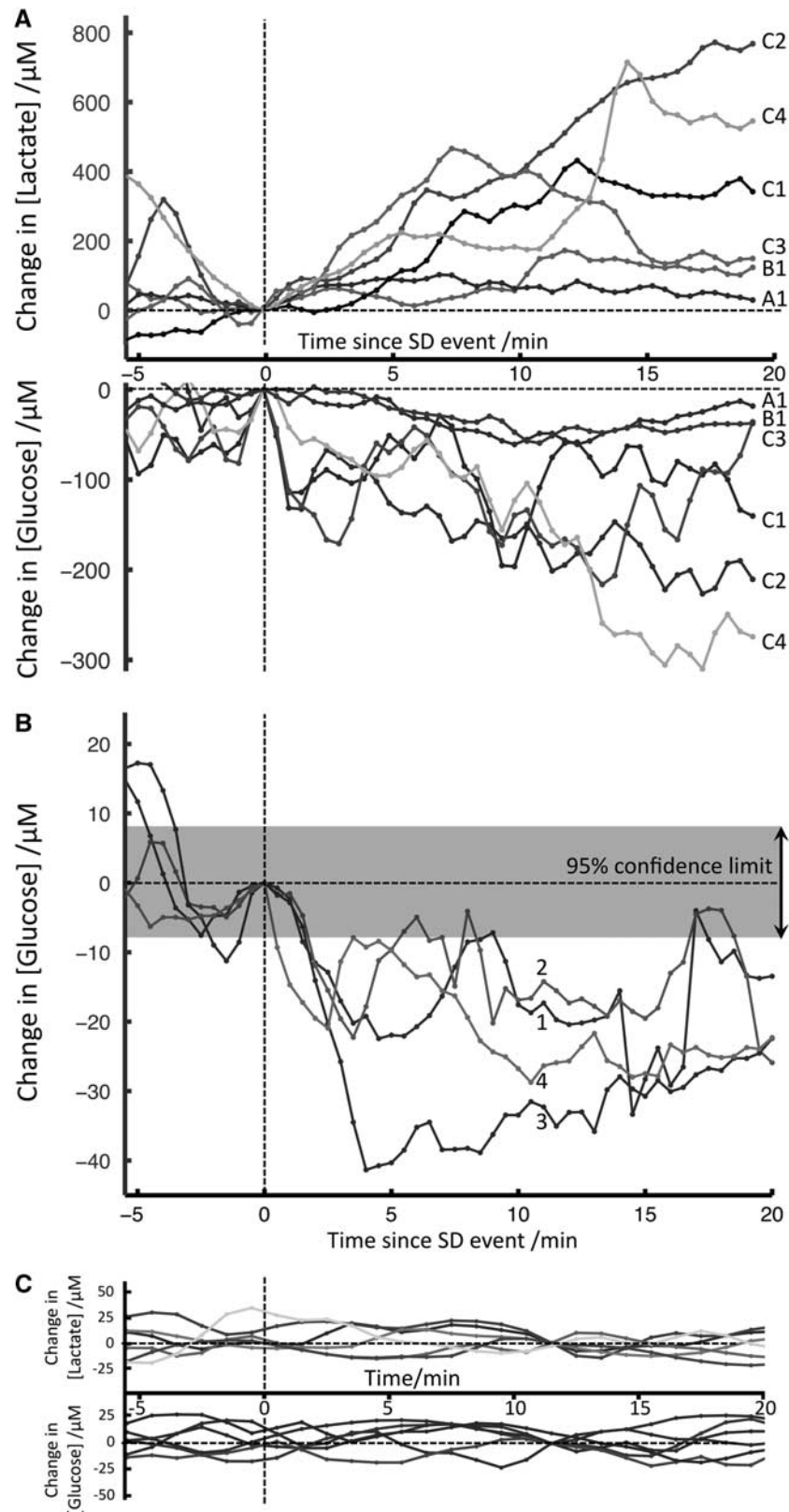
After exclusion of artefacts on the rsMD trace (criteria defined in the Materials and methods section), 90 transients of rsMD glucose and 49 transients of rsMD lactate were associated with the SD events and were fully characterized. The number of lactate events is smaller than that of glucose because the data were either missing or unrecoverable for technical reasons. All 49 lactate events were associated with glucose events, according to the inclusion criteria specified earlier (Parkin *et al*, 2003). An example of 25-min recording of ECoG and rsMD data during a spontaneous SD event in patient 4 is given in Figure 2. The SD wave is followed by a fall in glucose concentrations and an increase in lactate concentrations for >12 mins after SD.

### Metabolic Signature of Spreading Depolarizations

To evaluate the impact of SD events on the microdialysis concentrations of glucose and lactate, 25-min epochs were compared. Ten events are shown in Figure 3A and 3B from two patients. They are all time aligned with the onset of the rsMD response. The general pattern of changes is similar in all cases: each SD is followed by a drop in glucose and a rise in lactate concentrations. In Figure 3A (patient 4), the glucose drops ranged from  $-20$  to  $-96 \mu\text{mol/L}$  and lactate rises from  $+130$  to  $+300 \mu\text{mol/L}$  at 10 mins following the SDs. Some rsMD changes were very small and examples for glucose are given in Figure 3B (patient 3): the glucose changes were uniform, ranging from  $-17$  to  $-33 \mu\text{mol/L}$  at 10 mins following the SDs. Although small, these changes could effectively be resolved and quantified after processing the traces with the denoising filters we have described (Feuerstein *et al*, 2009), as shown by the smallest resolvable change of  $\pm 8 \mu\text{mol/L}$  (given as

the 95% confidence limit) for the assay when used in the ICU environment. The true variability in the baseline measurement due to patient variables is

explicitly shown in Figure 3C for patient 10 who did not show any SD events. These control epochs can be considered as slow fluctuations of the microdialysis



concentrations within an absolute band of 20  $\mu\text{mol/L}$  changes over a stretch of 25 mins. The absolute concentrations in microdialysis glucose and lactate were stable during the whole duration of monitoring in this patient (120 h), and were around 750 to 780  $\mu\text{mol/L}$  for glucose and 660 to 690  $\mu\text{mol/L}$  for lactate.

To integrate both the amplitude and the time course of the rsMD changes, an AUC analysis was performed: for each 20-min period following an SD event, the AUC of dialysis concentration changes was calculated. The distribution of these AUCs is shown in Figure 4. These distributions clearly indicate that the AUCs for glucose are negative, indicating a deficit of dialysate glucose during the 20 mins following SD events, whereas the AUCs for lactate are positive, indicating an excess of dialysate lactate during the 20 mins after SD. This is confirmed by the very high significance of the binomial test, rejecting an equal distribution of positive and negative AUC values, both for glucose and for lactate. The distribution histograms also indicate that this population cannot be described by a normal Gaussian distribution (full details given in the Supplementary Information). As a consequence, a nonparametric statistical analysis was performed on the AUCs for each patient. This is summarized in the box and whisker plot of Figure 5. Using the two-tailed Wilcoxon signed-rank test for each patient, the null hypothesis of a zero-AUC change, i.e., a zero-change of dialysate concentrations during the 20 mins following an SD, was rejected very significantly for each patient, provided there were more than three SD events. Overall, the 90 glucose AUCs and 49 lactate AUCs were very significantly different from zero ( $P < 0.0001$  for both glucose and lactate). In contrast, in patient 10, for whom no SDs were identified on the ECoG recording, we characterized 6 epochs of 25 mins of rsMD data and the 20-min-AUCs were not significantly different from zero (Figure 5). Similarly, when pooling all the control periods across all the 10 patients in this study ( $n = 41$  for glucose and  $n = 29$  for lactate), the AUCs were again not significantly different from zero ( $P = 0.984$

and  $P = 0.308$ , respectively). This confirms that the changes in glucose and lactate dialysis concentrations, though sometimes small, are due to SD events and not to random changes.

### Dynamic and Sustained Changes After the Passage of the Spreading Depolarization Wave

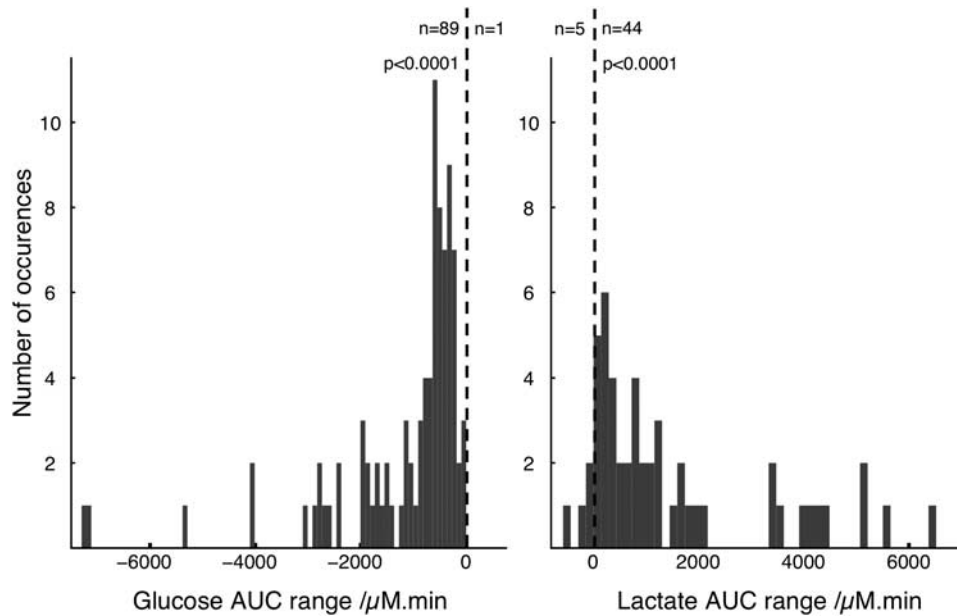
The median and interquartile ranges for dialysate glucose and lactate changes were, respectively,  $-43.4 \mu\text{mol/L}$  ( $-92.2$  to  $-17.8 \mu\text{mol/L}$ ) and  $+41.7 \mu\text{mol/L}$  ( $+8.2$  to  $+93.9 \mu\text{mol/L}$ ) at 10 mins following SDs across all the nine patients included in this study. The relatively wide interquartile range indicates that a variety of rsMD responses were observed. The changes in dialysate glucose at 10-mins showed a significant correlation with the duration of the suppression of ECoG AC signal (Pearson product-moment correlation:  $r = -0.25$ ,  $P = 0.05$ ). The longer the suppression, the greater the fall in dialysate glucose seen.

In most cases, no signs of recovery to the basal values were observed at 20 mins after the SD events: overall, the concentration changes at 20 mins after SDs were of  $-32.0 \mu\text{mol/L}$  ( $-92.3$  to  $-18.4 \mu\text{mol/L}$ ) for glucose and  $+23.1 \mu\text{mol/L}$  ( $+5.5$  to  $+93.6 \mu\text{mol/L}$ ) for lactate. These values were significantly different from zero when using a two-tailed Wilcoxon signed-rank test ( $P < 0.0001$  for both glucose and lactate).

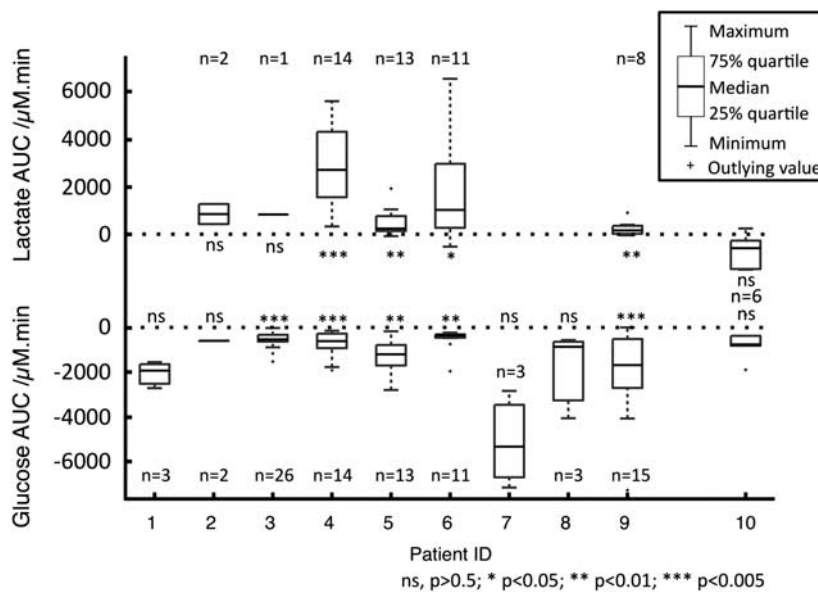
### Cumulative Effects of Recurrent Spreading Depolarizations

The SDs typically occur as clusters of regularly repeating events. We have investigated the effect of frequently occurring SDs on the microdialysis glucose concentrations, and the results are plotted for three patients in Figure 6. Overall, we found that when SD events repeated within  $< 50$ -min interval, the glucose concentrations did not recover fully to their initial pre-SD levels before the onset of the following SD events, thus leading to a progressive stepwise fall in glucose concentrations. Specific

**Figure 3** Changes in dialysate glucose and lactate concentrations following SDs. In this figure, time  $t = 0$  indicates the onset of the rsMD changes, and dialysate change = 0  $\mu\text{mol/L}$  corresponds to the dialysate concentration at time  $t = 0$ . These are indicated by vertical and horizontal dotted lines, respectively. Ten epochs of 5 mins before and 20 mins after SD waves are shown. **(A)** (Patient 4): 6 epochs of rsMD lactate (top) and glucose (bottom) concentration changes following six SD waves. The basal dialysate concentrations ranged from 640 to 930  $\mu\text{mol/L}$  for glucose, and between 1 and 1.5 mmol/L for lactate. The first two SD waves (A1 and B1) were isolated with SDs occurring on Day 12 between 00:30 and 02:30 with a 90-mins interval between the two events. They led to small changes in dialysate glucose and lactate. The other four SDs (C1 to C4) were observed as a temporal cluster of SDs repeating every 35 to 40 mins on Day 12 between 14:00 and 16:00 and they resulted in bigger changes in dialysate concentrations ( $> 100 \mu\text{mol/L}$ ). **(B)** (Patient 3): 4 epochs of small rsMD changes for glucose following four SD waves that occurred on Day 2 at regular intervals of 35 mins between 08:03 and 09:43. The basal dialysate concentrations ranged from 440 to 540  $\mu\text{mol/L}$ . The changes in dialysate glucose concentrations are small ( $< 50 \mu\text{mol/L}$ ) and closely similar within these four events. The band on the top of the graph shows the smallest resolvable change,  $\pm 8 \mu\text{mol/L}$  (95% confidence limit). This is significantly smaller than the changes observed during an SD event. **(C)** (Patient 10): 6 control epochs of rsMD changes for glucose (bottom) and lactate (top). Time  $t = 0$  is arbitrary and the zero on the dialysate change scale was defined as the median value for the 25 mins of data. During the whole period of monitoring, the microdialysis levels were stable, around 750 to 780  $\mu\text{mol/L}$  for glucose and 660 to 690  $\mu\text{mol/L}$  for lactate.



**Figure 4** The AUC distribution for the glucose and the lactate events observed in the rsMD data. The AUC for each rsMD epoch was calculated for 20 mins after SD. The distribution for all the glucose events across the nine patients is shown on the left panel and the distribution for all lactate events on the right. The dotted vertical line specifies the zero-AUC. The number of epochs with a negative AUC is indicated on the left of this line and the number of epochs with positive AUC is indicated on the right for glucose and lactate, respectively. The *P*-value results from a Binomial test with the expectation of a 50% distribution of positive and negative AUCs.



**Figure 5** Per-patient box and whisker plots for the 20-mins-AUCs after SDs. Lactate AUCs are plotted on the top and glucose AUCs on the bottom of the graph. The zero-AUC values are marked by dotted horizontal lines on both parts of the graph. The data were plotted for each patient. For patient 10, who showed no SD events on the ECoG recording, 6 epochs of 20 mins changes in rsMD glucose and lactate were also analyzed for their AUCs. A two-tailed Wilcoxon signed-rank test was performed for each patient, and the results are indicated on the figure with the number of events (*n*) for each patient.

narratives for the three patients in Figure 6 are given in the Supplementary Information.

## Discussion

There are four principal findings from this study. First, SD waves in the perilesion zone in the *human*

brain lead to a fall in extracellular glucose concentrations and a rise in extracellular lactate concentrations. Second, the effect of SDs on glucose and lactate could be resolved dynamically using rsMD in the ICU and showed a wide range of patterns of changes. Third, the metabolic response to the passage of the SD wave is sustained, with no sign



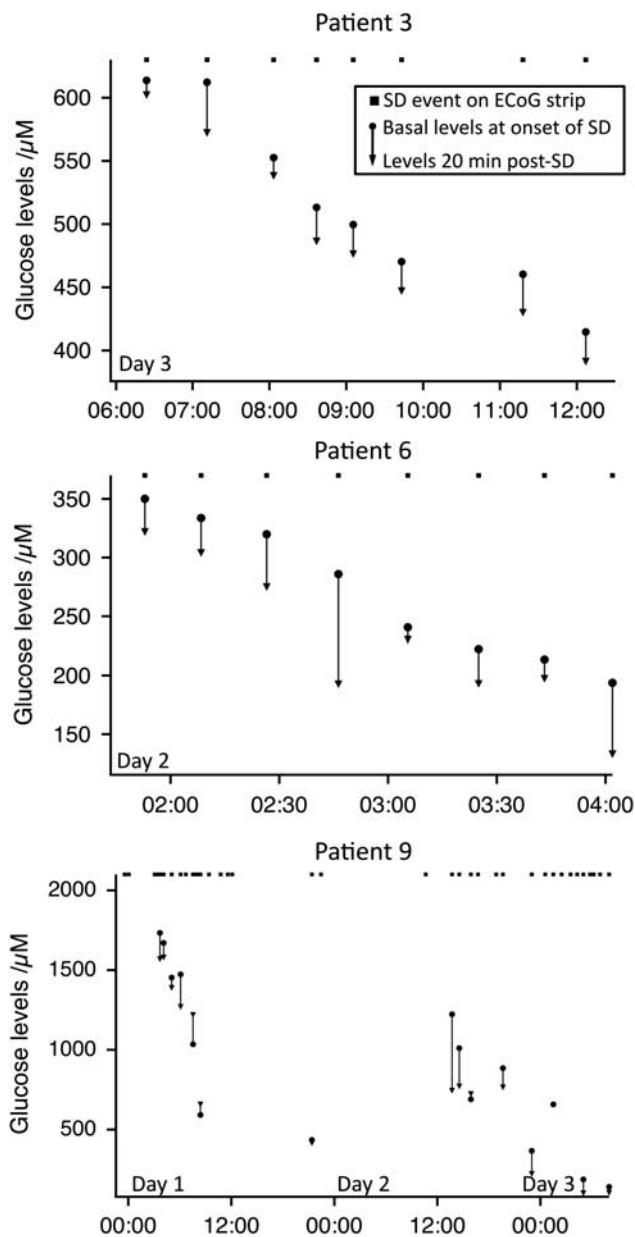
of recovery at 20 mins after SD. Finally, spontaneous SDs occurring frequently in perilesion zones in the injured human brain lead to a stepwise progressive depletion of the extracellular glucose pool.

### Fall in Glucose and Increase in Lactate Following Spreading Depolarization Waves

We have described here a characteristic metabolic response to SD waves in the *human* brain: a fall in dialysate glucose and an increase in dialysate lactate concentrations. Such changes in extracellular glucose and lactate levels have been observed in experimental models of stroke with induced or spontaneous SD waves. Mies *et al* (1993) found a decline in glucose in brain slices following the

passage of a single SD wave, and a microdialysis study showed a marked increase in lactate during the repolarization phase in the rat striatum (Taylor *et al*, 1994). Selman *et al* (2004) observed a marked decrease in extracellular glucose concentration and increase in lactate concentration at the SD wavefront and after the passage of the SD in rats using a freeze trapping technique. More recently, the metabolic response to induced SD was quantified in cats using the same rsMD assay, with results (Hashemi *et al*, 2009) very similar to our values now described for the human brain. This same study showed that the fall in microdialysate glucose follows rather than causes depolarization.

Clinically, Parkin *et al* (2005) previously demonstrated a clear correlation between the total number of SD events and a reduction of dialysate glucose at 1 to 2 h after the last SD compared with initial values. However, although the association between low glucose concentrations and SD events was observed, the causation could not be demonstrated, for example, from a typical time course of metabolic changes linked to single depolarization events. Here, we provide the first evidence that SD waves in the



**Figure 6** Cumulative effect on glucose concentrations following frequently occurring SDs in three patients. For each patient, the square indicates an SD event detected on the ECoG subdural strip. For each SD event associated with an rsMD change in glucose, the basal levels and 20 mins levels are plotted. *Patient 3*: Eight SD events on Day 3. The first two events were separated by 50 mins, with an initial 613  $\mu\text{mol/L}$  dialysate glucose down to 574  $\mu\text{mol/L}$ . The next four SD events repeated at a regular interval of 35 mins (thus constituting a cluster) and the glucose levels fell from 553 to 448  $\mu\text{mol/L}$ , with no recovery to basal concentrations between subsequent SD events. Finally, after a gap of 1 h and 30 mins, by which time the glucose levels nearly recovered the basal levels of the preceding SD, two SDs occurred with 50-min interval and the glucose levels were just below 400  $\mu\text{mol/L}$ . The glucose levels did not fall further and patient 3 recovered well (extended Glasgow Outcome score of 6). *Patient 6*: During this cluster of eight SD events repeating every 15 to 20 mins at the end of the monitoring period, the glucose dialysate concentrations fell from 334 to 134  $\mu\text{mol/L}$ . The falls in glucose concentrations varied between 15 and 92  $\mu\text{mol/L}$  for all the eight SDs. The glucose levels remained below 150  $\mu\text{mol/L}$  for the rest of the monitoring period and patient 6 died on Day 4. Note that in the case when the SDs were observed at < 20-min intervals, the 20-min value was defined as the basal levels just preceding the next SD. *Patient 9 (diabetic)*: three initial temporal clusters of SD events until 12:00 on Day 1 repeated every 20 to 35 mins and drove the glucose concentrations down from 2.0 mmol/L to 591  $\mu\text{mol/L}$ , with no recovery in between the events. After a gap of 9 h and an apparent stabilization of the glucose levels, two SDs at 21:30 on Day 1, repeating at an hour interval, led to a small  $-14 \mu\text{mol/L}$  change in glucose concentrations. During 12 h with no SD events, the glucose concentrations recovered to a high level of 1.2 mmol/L. Another cluster of six SDs repeating every 50 to 60 mins between 13:44 and 16:44 on Day 2 resulted in a drop of glucose down to 768  $\mu\text{mol/L}$ . Two isolated SDs followed a few hours later, and a final cluster of 13 SDs early on Day 3 led to a fall of glucose down to 106  $\mu\text{mol/L}$ . Patient 9 never recovered and deceased on Day 6.

injured human brain do indeed lead to declines in extracellular glucose and increases in extracellular lactate concentrations. The increase in lactate represents an increase in local production (possibly due to a local lack of oxygen). The levels of dialysate glucose represent a balance between supply from the blood and utilization by the brain. Hence, a fall could either be due to increased utilization with an invariant supply or a falling supply for a given utilization.

### Dynamic Changes in the Metabolic Response

We observed a wide variety of glucose and lactate responses to SD in terms of amplitude and time course of the changes as exemplified in Figure 3A (e.g., trace A1 compared with traces C2 and C4). The variability of these responses is much greater than the random noise from the rsMD assay following denoising techniques (Feuerstein *et al*, 2009), and illustrated in Figure 3B. We conclude that the observed variety in the responses is physiological and likely to reflect different responses of the injured tissue to SD waves.

Recent findings by the COSBID group indicate that SDs in SAH patients can be associated with different patterns of hemodynamic response: either physiological or inverse (Dreier *et al*, 2009). The former corresponds to a hyperemic response that delivers enough oxygen and energy metabolites to repolarize the tissue and hence sustain recurrent SDs. This could correspond to small changes in the rsMD data both in amplitude and in duration. The inverse response is seen as a transient (but sometimes sustained) oligemia that spreads across the tissue in a similar pattern as the SD waves (and has been termed '*cortical spreading ischemia*' (Dreier *et al*, 2009)). This vascular change, reasonably designated as microvascular spasm (of varying severities and durations), has been shown to *follow* rather than *lead* to the depolarization event (Dreier *et al*, 2000). This inverse coupling results in tissue hypoxia and could account for the larger prolonged responses observed in the rsMD data. Another factor that could account for the variability of the rsMD responses is the duration of depolarizations. In support of this, we found a positive correlation between the duration of the depression of the background ECoG activity and the amplitude of the glucose drop at 10 mins after SD. These findings suggest that the amplitude and duration of the glucose and lactate changes could serve as measures of the insult severity associated with SD waves in cortical perilesion boundary zones in human brain injury.

### Sustained Changes After the Passage of the Spreading Depolarization Wave

Despite variability in the dynamic responses, the observed metabolic changes were on average still

present 20 mins after SD, with no sign of recovery. Increased utilization of glucose in cortical tissue during the depolarization phase of the SD wave has been demonstrated (Shinohara *et al*, 1979). It has been attributed to the activation of the ATP-dependent ionic pumps to restore ionic homeostasis following the depolarization. However, this alone cannot explain the sustained decrease in glucose at 20 mins since at this time, cells are no longer depolarized, but are hyperpolarized. Alternatively, glucose may be used at this stage to replenish the astrocytic glycogen. Hertz *et al* (2007) suggested that glycogenolysis offers a higher anaerobic yield of ATP than fresh glucose and is thus likely to dominate ATP supply when there is acute energy demand. A significant reduction of glycogen levels after the passage of an SD wave has indeed been observed (Selman *et al*, 2004) and glycogen resynthesis is therefore likely to be required between 10 and 20 mins after SD. Glucose utilization by activated microglia may constitute a further drain on the glucose pool: whether microglial glucose utilization increases with the same time course as glucose microdialysis declines with an individual SD is unknown, but upregulated background microglial (and/or astrocytic) utilization would likely render the brain glucose pool more labile in the face of an abrupt increase in utilization linked to an SD.

The concomitant sustained increase in lactate concentrations suggests a possible lack of oxygen locally and/or a resort to nonoxidative metabolism to sustain the additional metabolic workload following SDs in perilesion tissue. Tissue hypoxia has been observed to coincide with the passage of SD waves (Piilgaard and Lauritzen, 2009; Takano *et al*, 2007; Tsacopoulos and Lehmenkühler, 1977). It has also been suggested that the early increased aerobic metabolism in response to massive depolarization (Rosenthal and Somjen, 1973) could switch to glycolysis at a later stage (Hashimoto *et al*, 2000). Hyperglycolysis was also observed in other microdialysis studies in head injury patients (Glenn *et al*, 2003; Goodman *et al*, 1999) and ultimately leads to increased lactate concentrations, as observed here. Such increases in lactate were recently found to be the most sensitive biochemical marker for predicting spontaneous episodes of intracranial hypertension in TBI patients (Adamides *et al*, 2009).

### Cumulative Stepwise Fall of Glucose with Recurrent Spreading Depolarizations

We show that the sustained drop in glucose concentrations after depolarizations, combined with their capacity to recur in clusters at intervals of <50 mins leads in the injured brain to a progressive depletion of the cerebral extracellular glucose pool. We estimated from our data that changes in the levels of plasma glucose could influence the microdialysis glucose concentrations with a sensitivity of

$40.8 \pm 10.7 \mu\text{mol/L}$  per  $\text{mmol/L}$  plasma glucose change (mean  $\pm$  s.e. of the mean,  $n=8$ ) (please see Supplementary Information for details). This suggests that large changes in blood glucose are a confounding variable. However, plasma glucose changes alone are not sufficient to explain the changes reported here.

A stepwise decrease in glucose concentrations similar to those reported here was observed linked to spontaneous periinfarct SDs following experimental middle cerebral artery occlusion (Hopwood *et al*, 2005). Similarly, Takeda *et al* (1993) described a depletion of tissue glucose and production of lactate with recurrent SDs in rats.

There is also evidence from *in vivo* experiments for a reverse chain of causality, in which a higher incidence of spontaneous PIDs is associated with relatively mild reductions in plasma glucose, which was interpreted as the independent variable here (Hopwood *et al*, 2005; Strong *et al*, 2000). This combination of findings led to the suggestion of a vicious circle (Hopwood *et al*, 2005) in which recurrent SDs (or, more certainly, peri-infarct SDs) drive brain glucose down, in turn increasing the frequency of SDs as proposed earlier (Nedergaard and Astrup, 1986), and in turn further lowering tissue glucose concentrations. The interaction of frequency of recurrence of depolarizations with delayed restoration of tissue glucose that we have demonstrated here suggests that this concept of a vicious circle, strengthened by mild hypoglycemia, can indeed be applied to patients. To this threatening combination of factors (perhaps present to some degree in injured tissue even without depolarizations) must now be added the impact of microvascular spasm promoted by depolarizations in ischemic brain, as first characterized experimentally as 'cortical spreading ischemia' (Dreier *et al*, 2000) and recently demonstrated in patients with SAH (Dreier *et al*, 2009). However, low tissue/plasma glucose is by no means the only factor likely to influence depolarization frequency, with clear evidence for an effect of lower cerebral perfusion pressure (Hartings *et al*, 2009), with suspicion falling also on pyrexia, seizures, choice of sedation,  $\text{PaO}_2$ , and ICU nursing procedures.

Several clinical studies have demonstrated a correlation between low brain glucose concentrations and poor patient outcomes. An initial study reported 'undetectable' (lower than  $50 \mu\text{mol/L}$ ) dialysate glucose in most patients who died after severe head injury (Zauner *et al*, 1997). More recently, a strong relationship between periods of low brain glucose concentrations (lower than  $200 \mu\text{mol/L}$  for 2 h) and poor outcome was demonstrated in TBI patients (Vespa *et al*, 2003) and in aneurysmal SAH patients (Schlenk *et al*, 2008). In all these studies, clinical factors (e.g., seizures, hypoglycemia, activated macrophages or neutrophils, or elevated glutamate concentrations) either did not have a time course that corresponded well to the observed increased glucose utilization (Zazulia *et al*, 2009) or simply accounted for only a minority of the observed prolonged periods of low glucose values

(Vespa *et al*, 2003). We suggest that clusters of frequent SDs are an important mechanism leading to the low brain glucose concentrations measured in these studies, augmenting the likely effect of mitochondrial failure (Verweij *et al*, 2000).

Given the small number of patients ( $n=9$ ) and the diversity of their primary pathologies (TBI, SAH, and ICH), we cannot presently conclude that repetitive SDs are *the dominant* cause for low brain glucose. A prospective study would be required to address this question, together with, for example (1) whether there is a difference in the responses to SDs when they occur at early versus late days following injury, (2) whether there is a discernible pattern in the metabolic response to SDs depending on the primary pathology and its location (requiring interval imaging, preferably with MR-diffusion-weighted imaging), and (3) whether the magnitudes of the glucose drops are correlated with the frequency of SD events.

## Conclusions

For the first time, it has been possible to resolve the dynamic metabolic response to SDs in the injured human brain using rapid sampling of cerebral microdialysate for glucose and lactate, together with noise-reduction postprocessing. We here show reductions of tissue glucose in association with depolarization events. Our findings suggest that clusters of frequent SDs are one mechanism that leads to the low brain glucose concentrations that are often observed. The resulting accumulated failure of glucose supply to meet the energetic demand for the restoration and maintenance of cell membrane polarization could compromise the viability of the tissue and hence lead to further expansion of the lesion and poor neurologic outcome.

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## Conflict of interest

The authors declare no conflict of interest.

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