

# Delayed ischaemic neurological deficits after subarachnoid haemorrhage are associated with clusters of spreading depolarizations

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**Progressive ischaemic damage in animals is associated with spreading mass depolarizations of neurons and astrocytes, detected as spreading negative slow voltage variations. Speculation on whether spreading depolarizations occur in human ischaemic stroke has continued for the past 60 years. Therefore, we performed a prospective multicentre study assessing incidence and timing of spreading depolarizations and delayed ischaemic neurological deficit (DIND) in patients with major subarachnoid haemorrhage (SAH) requiring aneurysm surgery. Spreading depolarizations were recorded by electrocorticography with a subdural electrode strip placed on cerebral cortex for up to 10 days. A total of 2110 h recording time was analysed. The clinical state was monitored every 6 h. Delayed infarcts after SAH were verified by serial CT scans and/or MRI. Electrocorticography revealed 298 spreading depolarizations in 13 of the 18 patients (72%). A clinical DIND was observed in seven patients 7.8 days (7.3, 8.2) after SAH. DIND was time-locked to a sequence of recurrent spreading depolarizations in every single case (positive and negative predictive values: 86 and 100%, respectively). In four patients delayed infarcts developed in the recording area. As in the ischaemic penumbra of animals, delayed infarction was preceded by progressive prolongation of the electrocorticographic depression periods associated with spreading depolarizations to >60 min in each case. This study demonstrates that spreading depolarizations have a high incidence in major SAH and occur in ischaemic stroke. Repeated spreading depolarizations with prolonged depression periods are an early indicator of delayed ischaemic brain damage after SAH. In view of experimental evidence and the present clinical results, we suggest that spreading depolarizations with prolonged depressions are a promising target for treatment development in SAH and ischaemic stroke.**

**Keywords:** cortical spreading depression; electrocorticography; ischaemic stroke; spreading ischaemia; subarachnoid haemorrhage

**Abbreviations:** AD = anoxic depolarization; DIND = delayed ischaemic neurological deficit; DSA = digital subtraction angiography; ICP = intracranial pressure; MCA = middle cerebral artery; SAH = subarachnoid haemorrhage; SD = spreading depression; TCD = transcranial Doppler sonography

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## Introduction

In 1944, the Brazilian physiologist Leão first described the spreading depression (SD) of electrocorticographic activity in rabbit cerebral cortex, and he subsequently reported the large spreading negative slow voltage variation of the extracellular space that accompanies this depression (Leão, 1947). Furthermore, he showed that a similar spreading negative slow voltage variation occurs in the cortex in response to ischaemia induced by bilateral carotid artery occlusion (Leão, 1947), concluding that 'in the SD of activity, a change of the same nature as one resulting from prolonged interruption of the circulation, occurs in the cerebral cortex' (authors' emphasis).

The cellular correlate of Leão's spreading negative slow voltage variation is a spreading mass depolarization of neurons and astrocytes (Martins-Ferreira *et al.*, 2000; Somjen, 2001). Under conditions of anoxia and severe ischaemia, spreading depolarization and the corresponding electrocorticographic depression are persistent and referred to as anoxic depolarization (AD), whereas under normal conditions they are transient, and are referred to as SD. AD and SD are similar in their rate of propagation across the cortex, as demonstrated by recordings of the intrinsic optical signal (Jarvis *et al.*, 2001), and also in their associated dramatic changes of intra/extracellular ion concentrations (Kraig and Nicholson, 1978; Hansen and Zeuthen, 1981). The restoration of ion homeostasis, and hence electrocorticographic activity, after a spreading depolarization is energy dependent (Somjen, 2001). During SD, regional cerebral blood flow increases in order to meet this demand, thus preventing neural damage (Lauritzen, 1994). In contrast, when AD is superimposed on preexisting ischaemia, a further decrease of cerebral blood flow occurs (Sonn and Mayevsky, 2000; Shin *et al.*, 2005).

Focal ischaemia is a special condition since there are gradients of perfusion, oxygen, and glucose between the core ischaemic region and the normal tissue where blood flow and energy substrates are unrestricted. Accordingly, direct evidence from microelectrode studies and indirect evidence with laser speckle imaging indicates that the spreading negative slow voltage variation starts in the ischaemic core as persistent AD, becomes a transient depolarization as it spreads through the metabolically compromised penumbra, and traverses the surrounding healthy tissue as SD (Nedergaard and Hansen, 1993; Koroleva and Bures, 1996; Nallet *et al.*, 1999; Shin *et al.*, 2005). Often referred to as peri-infarct or transient ischaemic depolarizations, these intermediate forms of spreading depolarizations in the penumbra typically occur in a temporal cluster of repetitive events (Nedergaard and Hansen, 1993; Koroleva and Bures, 1996; Nallet *et al.*, 1999; Hartings *et al.*, 2003). Peri-infarct depolarizations cause neuronal damage since, like AD, they increase the mismatch between energy demand and supply (Busch *et al.*, 1996; Takano *et al.*, 1996; Strong *et al.*, 1996) and exacerbate ischaemia in the penumbra (Shin *et al.*, 2005). As a consequence, the period of electrocorticographic

depression becomes increasingly prolonged with repeated transient depolarizations, evolving to a state of persistent depression with loss of function but preserved ion homeostasis (Back *et al.*, 1994; Ohta *et al.*, 2001; Hartings *et al.*, 2006). Under these conditions, subsequent spreading depolarizations are reflected only in slow negative voltage variations without the opportunity for further depression of electrocorticographic activity.

Spreading depolarization appears to be a highly conserved trait in the phylogeny of vertebrates (Kraig and Nicholson, 1978; Hansen and Zeuthen, 1981). However, it has been a matter of speculation for the last 60 years whether spreading depolarizations occur in ischaemic stroke in man. Were this to be shown, AD and the intermediate forms of spreading depolarization could be used as a real-time and comprehensive indicator of progressive ischaemic damage, marking metabolic challenge, glutamate release, and toxic calcium entry into neurons (Koroleva and Bures, 1996; Ohta *et al.*, 2001). Moreover, therapy might then target the intermediate forms of spreading depolarizations so as to protect the penumbra against recruitment into the infarct core.

Delayed ischaemic neurological deficit (DIND) after subarachnoid haemorrhage (SAH) is a distinctive syndrome of cerebral ischaemia. Increased headache, meningism and body temperature are typically followed by a fluctuating decline in consciousness and appearance of focal neurological symptoms (Hijdra *et al.*, 1986). The recent multicentre trials of tirilazad mesilate recorded a rate of 33–38% for DIND after SAH and of 10–13% for CT-proven delayed infarcts in the vehicle groups, which comprised a total of over 1000 patients (Haley *et al.*, 1997; Lanzino and Kassell, 1999; Lanzino *et al.*, 1999).

The risk of developing DIND correlates with the amount of blood observed in the initial CT scan (Kistler *et al.*, 1983; Brouwers *et al.*, 1993), and its onset coincides with the time of peak subarachnoid haemolysis in a primate model of SAH (Pluta *et al.*, 1998). This led to the hypothesis that breakdown products of erythrocytes in the subarachnoid space induce DINDs (MacDonald and Weir, 1991). The assumed prime mechanism of DIND is the induction of vasospasm. Proximal vasospasm is visualized with digital subtraction angiography (DSA) or measured with transcranial Doppler sonography (TCD) (Kistler *et al.*, 1983; Vora *et al.*, 1999; Unterberg *et al.*, 2001). However, the positive predictive values of TCD and DSA for the development of DIND are only between 30 and 50% (Vora *et al.*, 1999; Unterberg *et al.*, 2001). Using PET, Minhas *et al.* (2003) showed that after SAH, TCD in particular was unable to distinguish a state of tissue ischaemia from hyperaemia. These PET findings, similar to those of a study with single-photon emission CT, called for research aimed at the microcirculatory mechanisms underlying DIND (Ohkuma *et al.*, 2000; Minhas *et al.*, 2003). Notably, in animals, spreading depolarization, in the presence of breakdown products of erythrocytes, is the most

potent inducer of microarterial spasm currently known, leading to spread across the cortex of virtual disappearance of microcirculation for periods of minutes or even hours. This stimulated the question whether spreading depolarization is involved as both consequence of proximal and cause of distal arterial spasm in the complex sequence of events underlying DIND (Dreier *et al.*, 1998, 2000).

Early treatment of ruptured intracranial aneurysms is performed to reduce the risk of rebleeding. Aneurysms are either treated with endovascular detachable coils or craniotomy and clipping. If craniotomy is indicated for aneurysm occlusion or for surgical evacuation of an intracerebral haematoma, implantation of a subdural electrode strip for electrocorticography is possible. This allows electrocorticographic monitoring for the whole period of ischaemic stroke development, since DINDs have a delayed peak incidence at Day 7 after SAH.

Here, we studied prospectively whether a cluster of spreading depolarizations, with delayed onset following SAH, is associated with DIND. For the first time we show that (i) spreading depolarizations occur in patients with SAH, (ii) a cluster of spreading depolarizations accompanies DIND, and (iii) the evolution of delayed infarcts on CT/MRI is associated with prolonged electrocorticographic depression periods similar to features of spreading depolarizations in the ischaemic penumbra or in the presence of breakdown products of erythrocytes (animal experiments).

## Material and methods

### Patient recruitment and clinical care

Patients with major SAH were consecutively recruited by four centres in the Co-Operative Study on Brain Injury Depolarizations (COSBID, see [www.cosbid.org](http://www.cosbid.org)). The research protocol was approved by the local ethics committees. Clinical and research consents were obtained after a clinical decision had been taken to offer surgical treatment. SAH was diagnosed by assessment of CT scans. Haemorrhage was graded according to the Fisher scale (Kistler *et al.*, 1983), and clinical presentation according to the World Federation of Neurological Surgeons (WFNS) scale (Drake *et al.*, 1988). The aneurysm was assessed using four-vessel DSA, or a more restricted study when indicated. The aneurysm was clipped ( $n = 16$ ), coiled ( $n = 1$ ) or wrapped ( $n = 1$ ). A single, linear, 6-contact (platinum) electrocorticography recording strip (Wyler, 5 mm diameter; Ad-Tech Medical, Racine, WI, USA) was placed on cortex accessible through the craniotomy, as previously described (Strong *et al.*, 2002; Fabricius *et al.*, 2006). After surgery, patients were transferred to the intensive care unit. Intracranial pressure (ICP) was monitored via ventricular drainage catheter or ICP transducer (Codman or Camino systems). Glasgow Coma Scale (GCS), blood gases, glucose and electrolytes were documented every 6 h. A thorough neurological examination was performed at least daily. A clinical DIND was defined by either a delayed decrease of consciousness by at least two GCS levels and/or a new focal neurological deficit. Serial CT scans were performed post-operatively, at the time of clinical deterioration and

after the monitoring period to screen for delayed infarcts. This was complemented by MRI in selected cases. Vasospasm was defined using DSA as  $>30\%$  narrowing of the arterial luminal diameter in one of the following arterial segments: A1, A2, M1, M2, and C1–C2 (Unterberg *et al.*, 2001). Magnification errors were corrected by comparing extradural segments of the internal carotid artery (C4–C5). Using TCD, significant vasospasm was defined by a mean velocity  $>200$  cm/s in at least one middle cerebral artery (MCA) and vasospasm was excluded if the MCA mean velocities remained below 120 cm/s throughout the observation period (Vora *et al.*, 1999). Patients with DIND were treated with triple-H therapy (hypertension, hypervolaemia and haemodilution). Triple-H-therapy aimed at a mean arterial pressure of 100–110 mmHg, a haematocrit around 30% and a central venous pressure  $>10$  mmHg. For this purpose hydroxyethyl starch (10%) 250 ml was given four times daily and crystalloids were infused at a rate of  $\sim 120$  ml/h. Catecholamines, mostly noradrenaline, were administered if fluid therapy alone failed to secure adequate mean arterial pressure (Unterberg *et al.*, 2001; van Gijn and Rinkel, 2001). Oral nimodipine was given prophylactically in 11 patients (i.e. in 3 of 4 centres) (Pickard *et al.*, 1989). The use of a single, narrow linear electrode strip allowed withdrawal at the bedside. No local haemorrhagic or infectious complications of the subdural strip were encountered.

### Electrocorticography

Electrocorticographic recordings were acquired continuously in four active channels (A–D) from the 6-electrode (linear array) subdural strips. Electrode 1 served as ground while Electrodes 2–6 (interelectrode distance 1 cm) were connected in sequential bipolar fashion to one GT205 or two Dual Bioamp amplifiers (0.01–100 Hz) (ADIInstruments, New South Wales, Australia). Data were sampled at 200 Hz and recorded and reviewed with the use of a Powerlab 16/SP analogue/digital converter and Chart-5 software (ADIInstruments, New South Wales, Australia).

### Data analysis

Spreading depolarization was defined by the sequential onset in adjacent channels of a propagating, polyphasic slow potential change (Fabricius *et al.*, 2006) that corresponds to the negative slow voltage variation described by Leão (1947) and Hartings *et al.* (2006). The parallel electrocorticographic depression was defined by a rapidly developing reduction of the power of the electrocorticographic amplitude by at least 50% (Strong *et al.*, 2002; Fabricius *et al.*, 2006). The duration of the depression period of electrocorticographic activity ( $>0.5$  Hz) was used as an indirect indicator of tissue energy supply since restoration of this activity after spreading depolarization is energy dependent (Nedergaard and Hansen, 1993; Back *et al.*, 1994). This duration, used here to assess the spatial and temporal relation between compromised energy supply and the development of brain infarcts, was measured as the interval between depression onset and onset of restoration of activity using the integral of power of the high-pass filtered activity (lower frequency limit, 0.5 Hz; time constant decay, 60 s).

Data are given as median (1st, 3rd quartile). Statistical analysis was performed using Wilcoxon signed rank or Mann–Whitney rank sum test.  $P < 0.05$  was considered statistically significant.

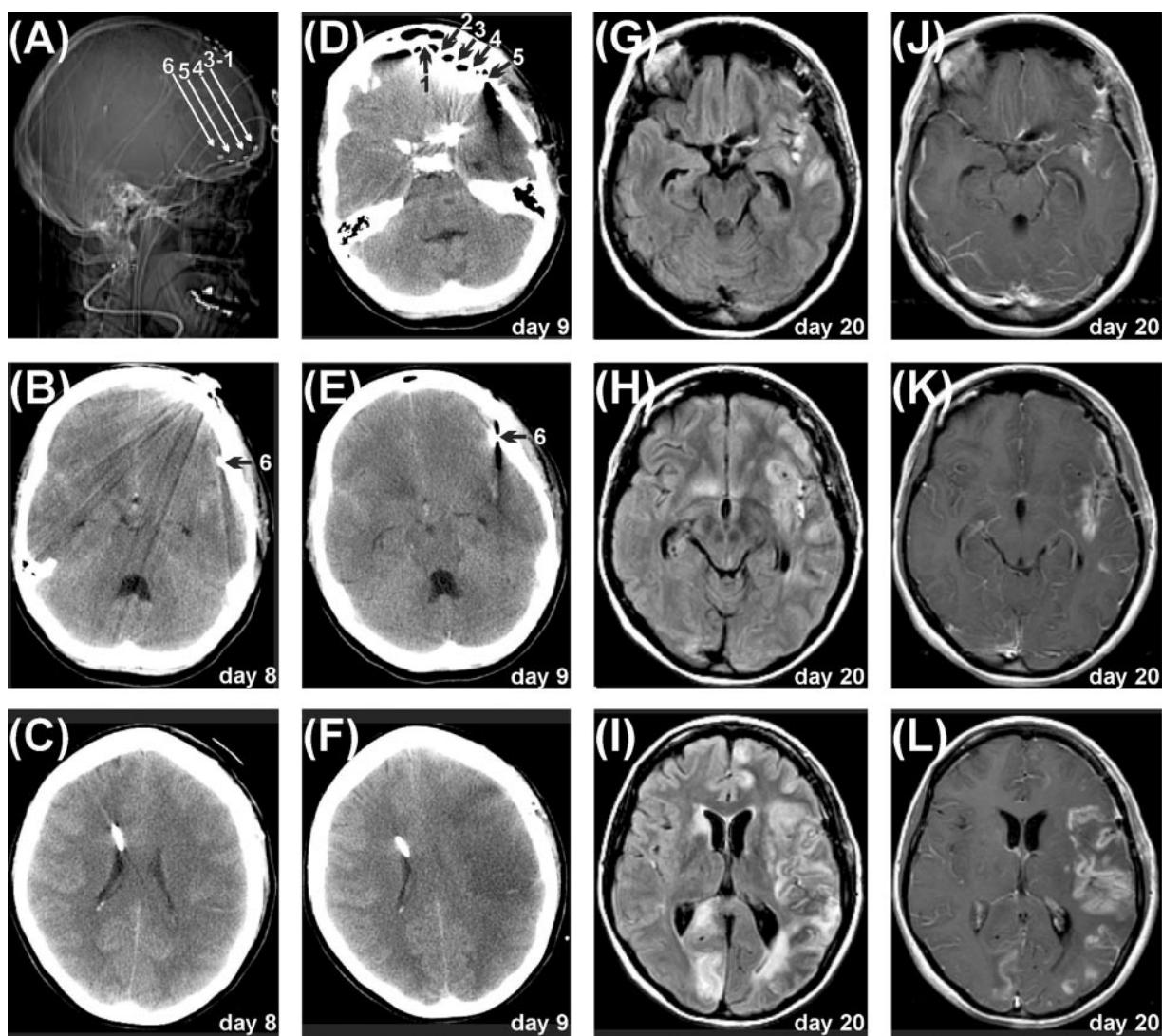
## Results

### Illustrative cases

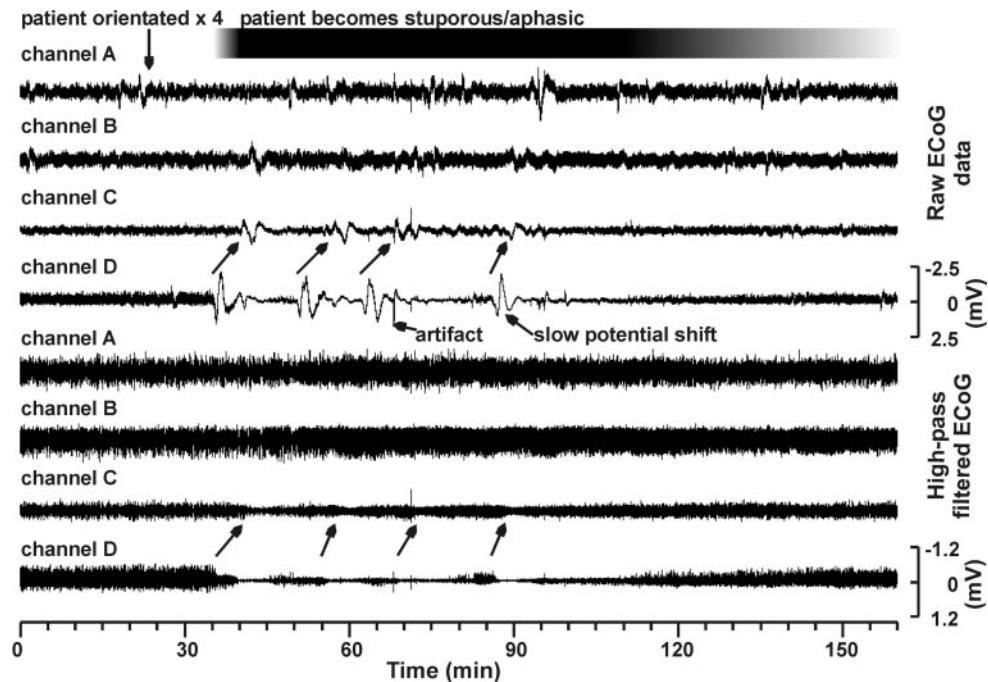
#### Patient 1

A 44-year-old female with WFNS Grade 2 and Fisher Grade 3 SAH is presented. On Day 1 after SAH, an anterior communicating artery aneurysm was clipped, an external ventricular drain was established, and the electrode strip was placed at the left frontolateral base (Fig. 1A, B, D and E). The patient showed good recovery on Days 2 and 3. On Day 4, some confusion coincided with the occurrence of nine spreading depolarizations with short-lasting depression

periods. On Day 5 10:30 h, the patient was again fully orientated, without signs of focal neurological deficits. Around 10:45 h the GCS level decreased from 15 to 7, coinciding with a series of four spreading depolarizations (Fig. 2). TCD detected a significant mean velocity of  $>200$  cm/s in the left MCA suggesting vasospasm. Triple-H therapy was started. A CT scan was normal. Consciousness improved transiently, followed rapidly by a decline during the next series of four spreading depolarizations. Level of consciousness fluctuated over the following 2 days in correlation with repeated spreading depolarizations. The



**Fig. 1** CT and MR images for the spatial and temporal correlation between occurrence of spreading depolarizations and delayed infarct evolution in Patient 1. In the area of Electrode 6 (channel D), the figure shows a predominantly cortical infarct, which evolved between Days 8 and 9 after several prolonged spreading depolarizations were recorded. (A) CT-topogram showing the left subdural electrode strip; (B and C) CT on Day 8; (D–F) CT on Day 9; (G–I) MRI using fluid-attenuated inversion recovery (FLAIR) pulse sequence, Day 20; (J–L) Gadolinium-enhanced  $T_1$ -weighted MRI, Day 20. No major hypodensity is observed in the left lateral frontal and perisylvian region on Day 8 (B and C) (note Electrode 6 in B and E and Electrodes 1–5 in D). The CT of Day 9 (F) demonstrates a large new hypodensity in the left lateral frontal and perisylvian region, which evolved between the scans of Days 8 and 9. The FLAIR and contrast-enhanced MR images in (G–L) of Day 20 demonstrate the full extent of the lesions, with band-like signal changes in the left lateral frontal cortex (Electrode 6 location), the perisylvian cortex (corresponding with the global aphasia of the patient), the left posterior MCA territory, and the right mesial occipital region.



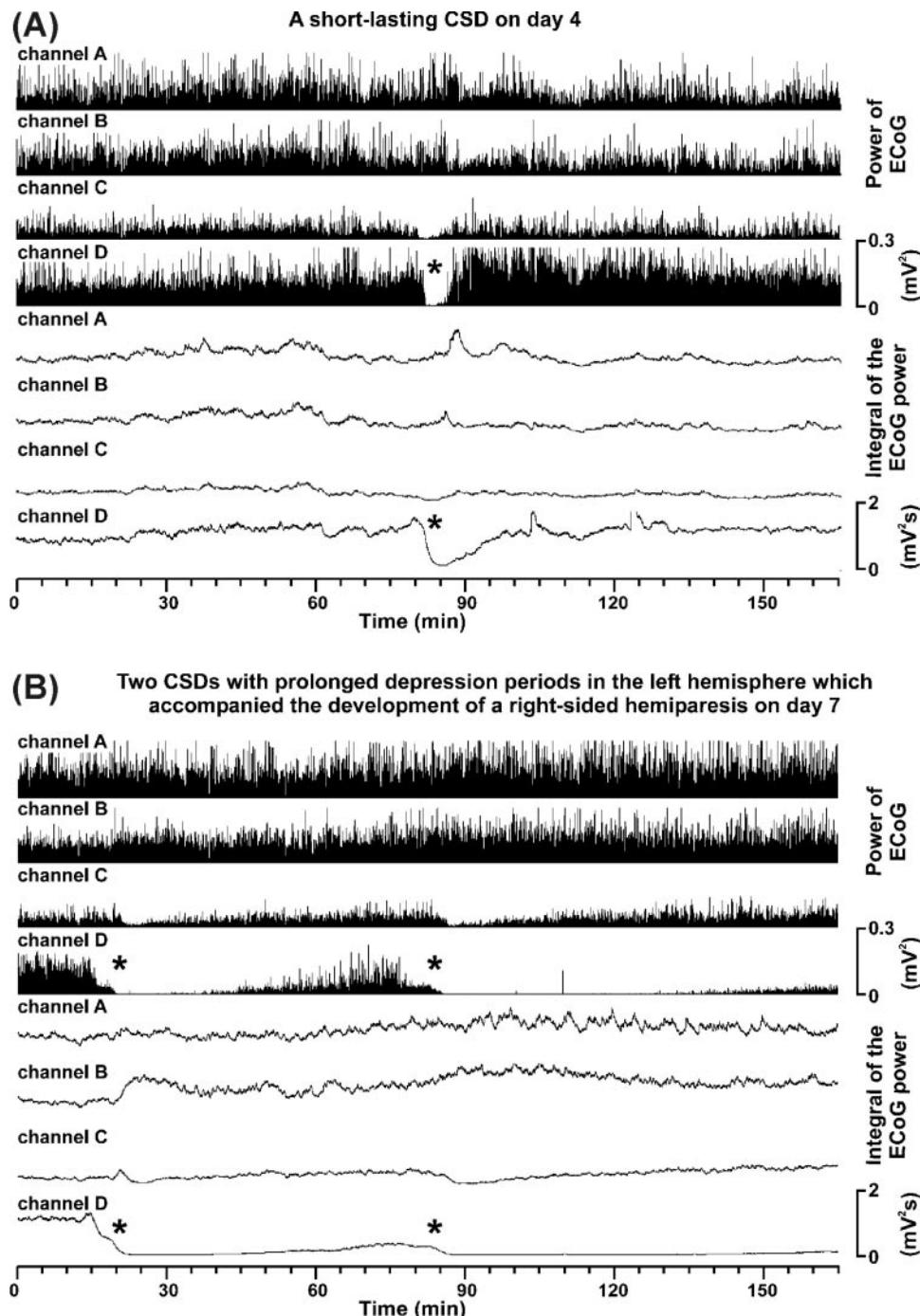
**Fig. 2** A close temporal correlation between a series of 4 spreading depolarizations (arrows) and the development of a transient stuporous/aphasic state in Patient 1 around 10:45 h on Day 5 after SAH. In a thorough neurological exam around 10:30 h, the patient had been orientated to date, space, person and situation (patient orientated  $\times$  4) and no focal neurological deficit had been found. Then, she spontaneously developed a state in which she did not respond to her name and hardly moved her arms and legs in response to painful stimuli. The figure demonstrates bipolar recordings between Electrodes 2 and 3 (channel A), 3 and 4 (channel B), 4 and 5 (channel C) and 5 and 6 (channel D) during the change of her mental status. Compare the spatial arrangement of each electrode in relation to the cortical regions using the CTs of Fig. 1A, B, D and E. The upper four traces show the raw electrocorticographic (ECoG) data which contain the slow potential shifts of the spreading depolarizations (lower frequency limit of the GT205 amplifier, 0.01 Hz). The lower four traces give the high-pass filtered electrocorticographic activity (lower frequency limit, 0.5 Hz) in which the spread of the electrocorticographic depression is observed. The 4 spreading depolarizations propagated from channel D to C (arrows). The calibration bars for channel D also apply to channels A–C.

development of a right hemiparesis on Day 7 correlated with two spreading depolarizations characterized by very prolonged electrocorticographic depression periods in channel D (= Electrodes 5–6, duration: 37 and 66 min, Fig. 3). On Day 8, the propagation pattern of the spreading depolarizations changed; spreading depolarizations now propagated along the whole distance from channel D to A (representing Electrodes 6–2, Fig. 4). A CT scan showed sulcal effacement of the left hemisphere (data not shown). On Day 9, channel D showed spreading depolarization without restoration of electrocorticographic activity. While the electrocorticogram was depressed, two more episodes with slow potential changes occurred in channel D. A CT scan on Day 9 showed a large new hypodensity of the left lateral frontal and perisylvian region [compare the CT scan of Day 9 (Fig. 1F) with the scan of Day 8 (Fig. 1C)]. Later, a rapid increase of ICP to 50 mmHg necessitated sedation, intubation and mannitol i.v. Electrical silence in channel D lasted until the end of the recording period on Day 11. In contrast, there was a burst-suppression pattern interrupted by some persisting short-lasting spreading depolarizations at channels A and B (Electrodes 2–4). On Day 20, MRI was performed.

Note the impressive MR signal abnormalities in the left lateral frontal and perisylvian cortex in Fig. 1G–L. Such MR-signal abnormalities were previously shown to correspond with the characteristic histological pattern of cortical necrosis in patients after SAH (Birse and Tom, 1960; Neil-Dwyer *et al.*, 1994; Dreier *et al.*, 2002). Thus, electrophysiological changes at Electrode 6, where the presumed cortical necrosis evolved, consisted of repeated spreading depolarizations displaying progressively increasing electrocorticographic depression periods (compare Fig. 3A and B), whereas fewer spreading depolarizations with only short electrocorticographic depression periods were recorded at Electrodes 2–4, where cortex remained normal. The patient was transferred to a rehabilitation unit with global aphasia and severe right-sided hemiparesis on Day 22.

### Patient 2

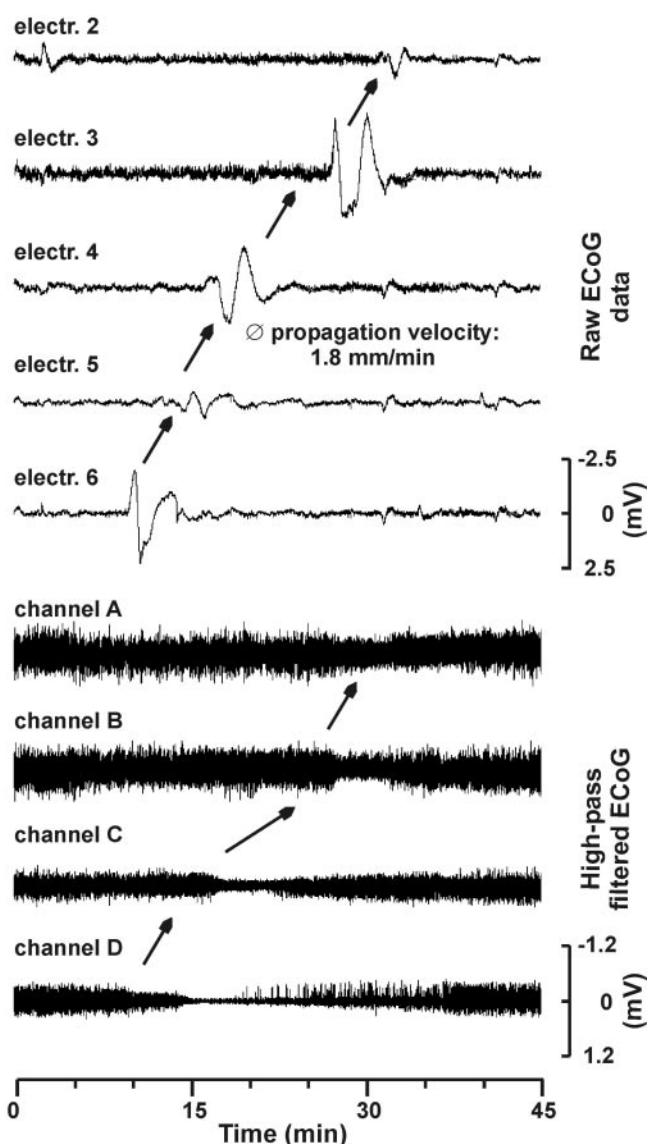
This 50-year-old female with WFNS Grade 5 and Fisher Grade 3 SAH (Fig. 5A) had a right-sided ophthalmic artery aneurysm which was coiled. A frontal haematoma was



**Fig. 3** Comparison between a short-lasting spreading depolarization in Patient 1 on Day 4 (A) and two long-lasting spreading depolarizations on Day 7 (B). The two long-lasting spreading depolarizations accompanied the development of a new right-sided hemiparesis. Bipolar recordings are demonstrated between Electrodes 2 and 3 (channel A), 3 and 4 (channel B), 4 and 5 (channel C) and 5 and 6 (channel D). Compare the spatial arrangement of each electrode in relation to the cortical regions using the CTs of Fig. 1A, B, D, E. In A and B, the upper four traces show the power of the high-pass filtered electrocorticographic (ECoG) activity (lower frequency limit, 0.5 Hz). The lower four traces show the integral of the power (lower frequency limit, 0.5 Hz; time constant decay, 60s). The integral of the power was used to calculate the duration of the depression period (\* = depression periods). (B) Note the long-lasting depression periods in channel D (Electrode 5–6), where the new infarct is evolving (compare images of Fig. 1). The calibration bars for channel D also apply to channels A–C.

surgically evacuated, extraventricular drainage was established and a subdural electrode strip was placed at the right frontolateral base. Electrodes 4 and 5 of the strip are shown in Fig. 5C and E. Until Day 8 after SAH (20:23 h), a total of

37 spreading depolarizations were observed in this intubated and sedated patient. They appeared to originate from the site of the intracerebral haematoma (Fig. 5A and C) since they all propagated in the direction of Electrode 6–4 (Fig. 5B and D).



**Fig. 4** On Day 8, the propagation pattern of spreading depolarizations changed in Patient 1; the spreading depolarizations now propagated along the whole distance from channel D to A (representing Electrodes 6–2). The upper five traces are unipolar recordings from individual electrodes, each referenced to one ipsilateral subgaleal platinum electrode [raw electrocorticographic (ECoG) signals]. The slow potential change is thus visualized for each electrode independently of the others, clearly showing the propagation (arrows) of the slow potential change from Electrode 6 along the strip to Electrode 2 (distance: 4 cm). The average propagation velocity was 1.8 mm/min in this example. Note that these slow potential changes do not represent true direct current (DC) shifts but are high-pass filtered by the GT205 with a lower frequency limit of 0.01 Hz. Compare the spatial arrangement of each electrode in relation to the cortical regions using Fig. 1A, B, D and E. The lower four traces show the high-pass filtered electrocorticographic data with a lower frequency limit of 0.5 Hz of the simultaneous bipolar recordings which allow the visualization of the spreading electrocorticographic depression (arrows). The calibration bar in trace 5, Electrode 6, also applies to traces 1–4 and the calibration bar in trace 9, channel D, also applies to traces 6–8.

Initially, these spreading depolarizations were all associated with short-lasting depression periods (Fig. 5B). Starting on Day 5, the depression periods progressively increased (Fig. 5D), coinciding with the advent of vasospasm in the ipsilateral MCA. On Day 8 (20:23 h), for the first time, a spreading depolarization propagated in the opposite direction from Electrode 4–6. At 1:54 h, another spreading depolarization spread in the new direction; depression period was now 93 min. The next spreading depolarization occurred on Day 9 at 4:25 h and was followed by several spreading depolarizations in a row without restoration of electrocorticographic activity for 455 min (Fig. 5F and H). On Day 9 at 15:41 h, CT showed large new infarcts in the ipsilateral anterior cerebral artery (Fig. 5E) and MCA territories, thus providing an explanation for both the change in direction of propagation of the spreading depolarizations (i.e. new anterior source of initiation) and prolongation of the electrocorticographic depression period. The next night, increase of ICP necessitated decompressive hemicraniectomy. The recording area showed gyral swelling but remained relatively well preserved on the MRI on Day 13, consistent with a penumbral region (Fig. 5G). At discharge from the intensive care unit on Day 25 after SAH, the patient had deviation of gaze to the right, left-sided hemiplegia and right leg paresis.

### Incidence of spreading depolarizations in patients with SAH

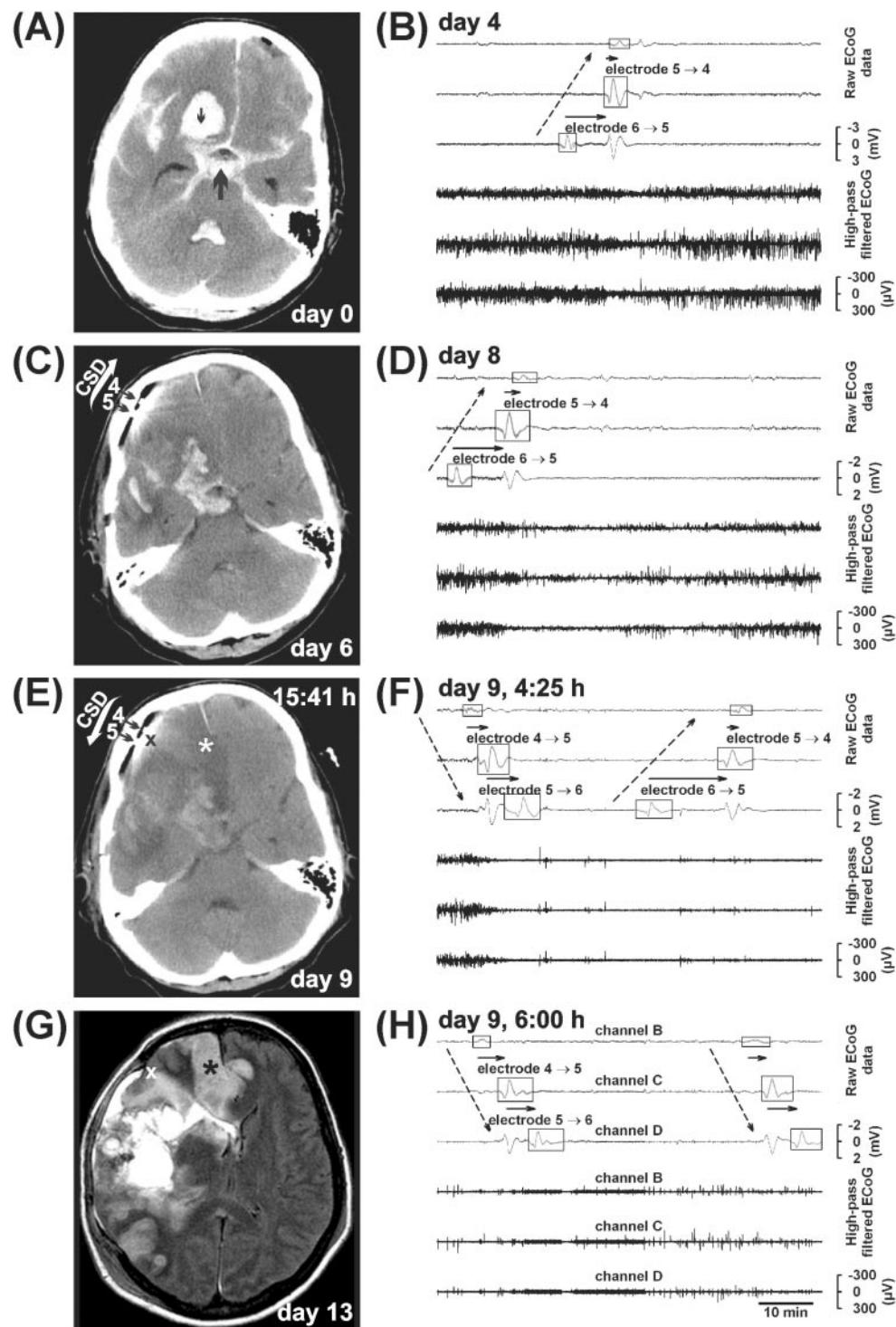
Patient characteristics are given in the Table 1. In a total electrocorticographic recording time of 2110 h, 298 spreading depolarizations [peak-to-peak amplitude: 1.9 (1.6, 2.6) mV, propagation velocity: 2.0 (1.5, 2.4) mm/min] occurred in 13 of 18 patients (72%). Figure 6 gives the duration of electrocorticographic depression in logarithmic scale for every single spreading depolarization in each patient. This variable is important as patients who developed depression periods lasting >10 min (Patients 1–11) had worse outcome on discharge from the acute unit to the rehabilitation unit than the other patients (Patients 12–18) (modified Rankin scale: 5 (3.5, 5) versus 2 (1.5, 2.5),  $P = 0.008$ , Mann–Whitney rank sum test).

### Clusters of spreading depolarizations accompany DIND in SAH

The grey-shaded areas in Fig. 6 indicate the periods of DIND defined by GCS, constituting a delayed decrease from 14 (12, 15) to 9 (8, 11) (Patients 1 and 3–7,  $P = 0.031$ , Wilcoxon signed rank test) and/or the finding of a delayed brain infarct (dark grey-shaded areas, Patients 1–4). Clinical DIND began 7.8 days (7.3, 8.2) after SAH and was always time-locked to a new cluster of spreading depolarizations. This was reflected in the statistical analysis: the number of spreading depolarizations per day was significantly higher in patients with DIND and/or delayed

brain infarct at Days 7–9 after SAH [5.6 (3.8, 8.4),  $n = 7$  versus 0.0 (0.0, 0.0),  $n = 4$ ,  $P = 0.006$ , Mann–Whitney rank sum test]. The positive predictive value of a new cluster of spreading depolarizations was 86% for a DIND while the negative predictive value if no cluster occurred was 100%. On the day of DIND, none of the core variables changed significantly from the values 1 day before, but ICP [20 (19, 24) mmHg], maximal body temperature in 24 h

[38.7 (38.2, 38.7) °C], serum glucose [136 (131, 138) mg/dl], leucocyte count [13.9 (8.8, 15.1)/nl] and C-reactive protein [7.4 (4.0, 8.2) mg/dl] were above the normal range. Haematocrit was slightly diminished [33.8 (30.0; 34.7)%]. Normal values were recorded for cerebral perfusion pressure, arterial oxygen saturation, arterial pH and serum sodium. Epileptic electrocorticographic activity, hydrocephalus, metabolic and pharmacological causes



for delayed neurological deterioration were excluded in all cases.

Dense clusters of spreading depolarizations occurred shortly after operation in Patients 8 and 9 and were associated with an acute focal and global neurological deficit (Fig. 6) (Unterberg *et al.*, 2001). This acute deficit was related to a large intracerebral haematoma as a sequel of the aneurysmal haemorrhage in Patient 8 and to the operation in Patient 9. Both patients showed a protracted post-operative recovery.

### Spreading depolarizations with prolonged electrocorticographic depression precede delayed cortical infarcts at the recording site

In Patients 1–4, imaging demonstrated delayed brain infarcts in the recording area (compare illustrative cases). While 53% of all spreading depolarizations showed depression periods shorter than ten minutes in all channels, spreading depolarizations with depression periods beyond 60 min in at least one channel were (i) only observed in Patients 1–4 ( $n = 9$  spreading depolarizations), (ii) occurred at 7.8 days (7.2, 9.1) after SAH, and (iii) were always spatially confined to electrocorticography channels of cortical areas showing delayed infarcts on CT/MRI. This was reflected in the statistical analysis: the electrocorticographic depression per day was significantly higher in patients with delayed infarcts at Days 7–9 after SAH [307 (212, 426) min,  $n = 4$  versus 26 (0, 30) min,  $n = 7$ ,  $P = 0.006$ , Mann–Whitney rank sum test; Fig. 7].

All 11 patients who were electrocorticographically monitored at Days 7–9 had a Fisher Grade 3 haemorrhage,

which is typically associated with very high rates of significant vasospasm and DIND (Kistler *et al.*, 1983). DSA was carried out to assess vasospasm in 8 and daily TCD in 9 of these patients. Only one patient had neither DSA nor TCD. The percentage of significant vasospasm was 90%.

### Discussion

Aneurysmal SAH has a 30 day mortality rate of ~45% (Broderick *et al.*, 1993) and represents one quarter of haemorrhagic stroke (Nilsson *et al.*, 2000). DIND is a prominent complication of SAH, is a major cause of disability and mortality, is familiar to specialists in neurosurgery, neurology, radiology, anaesthesiology and internal medicine, and is difficult to treat (van Gijn and Rinkel, 2001). One reason for this difficulty is incomplete understanding of the pathophysiology. This paper documents for the first time the occurrence of spreading depolarizations in patients with SAH, similar to previous findings in animals (Hubschmann and Kornhauser, 1980; Busch *et al.*, 1998). In a total recording time of 2110 h, 298 spreading depolarizations were observed in 13 of 18 patients with SAH. It would appear that spreading depolarizations are very frequent after major SAH.

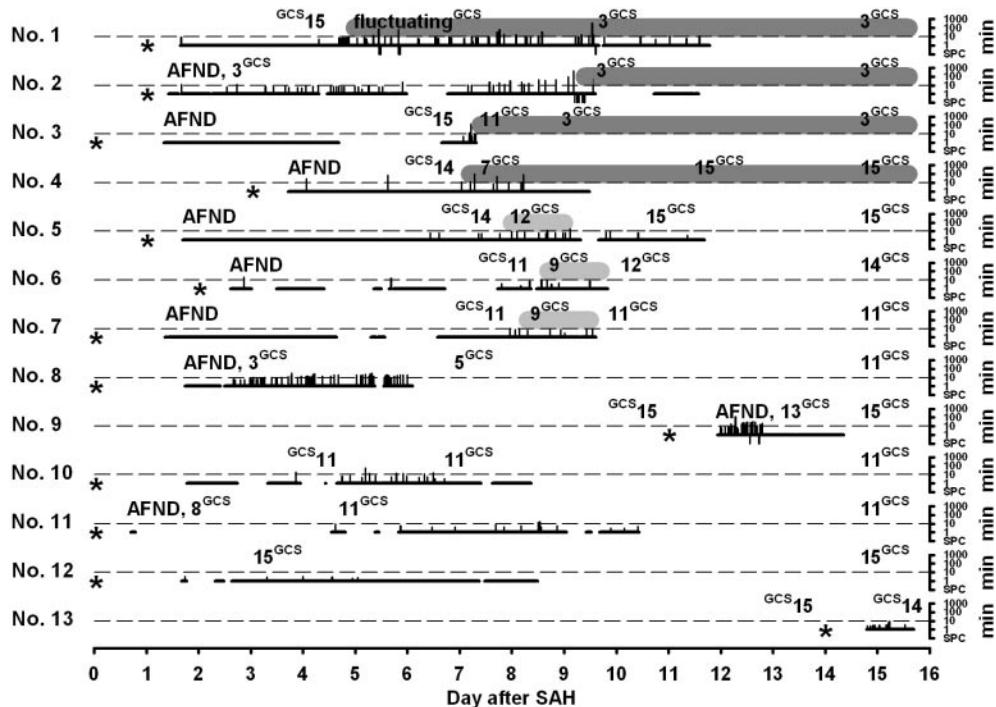
Consistent with animal studies, the evolution of ischaemic stroke, as studied in these patients, was associated with a cluster of spreading depolarizations and increasingly prolonged periods of electrocorticographic depression (Nedergaard and Hansen, 1993; Back *et al.*, 1994; Ohta *et al.*, 2001; Hartings *et al.*, 2006). The progressive prolongation of the depression period of spreading depolarization was locally restricted to areas of new infarct evolution as demonstrated on serial CTs and MRI. Within

**Fig. 5** Temporal relationship between spreading depolarizations and the evolution of an infarct in the anterior cerebral artery territory in Patient 2. **(A)** (Day 0) shows the initial CT scan with massive SAH (large arrow) and right frontobasal haematoma (small arrow). **(B)** (Day 0) gives bipolar recordings between Electrodes 3 and 4 (channel B), 4 and 5 (channel C) and 5 and 6 (channel D) (names of the channels and time scale are found in **H**). In **B** the raw electrocorticographic (ECoG) activity which contains the slow potential changes is shown in the upper three traces whereas the high-pass filtered electrocorticographic activity demonstrating the electrocorticographic depression is shown in the lower three traces (lower frequency limit, 0.5 Hz). The horizontal black arrows and squares in the upper three traces indicate the propagation from one electrode to the next in detail. Note that in this bipolar recording, a simultaneous slow potential change with phase reversal in two neighbouring channels originates at the electrode common to the two channels. This is best seen for Electrode 5 in channels D and C. The calibration bar in the third trace also applies to traces 1 and 2; the calibration bar in trace 6 also to traces 4 and 5. **(B)** A spreading depolarization with short-lasting electrocorticographic depression (lower three traces) as recorded during the first days after SAH. The dashed arrow illustrates the direction of propagation of the spreading depolarization from Electrode 6 to 4. **(C)** CT at 6 days after SAH and 5 days after surgical haematoma evacuation. Note Electrodes 5 and 4 in the CT. The white arrow in **(C)** illustrates the direction of propagation of spreading depolarization from Electrode 6 to 4 along the subdural strip which was observed in the first 37 spreading depolarizations in this patient [compare the spreading depolarizations in **B** and **D** (dashed arrows)]. **(D)** A spreading depolarization on Day 8 after SAH. Note that the depression period (lower three traces) is considerably longer compared with that of the spreading depolarization in **B**, which is typical of this later time period and suggests some energy compromise (compare Patient 2 in Fig. 6). Late on Day 8, the direction of propagation of the spreading depolarization suddenly changed and the spreading depolarization now started mostly from the more anterior electrodes as shown in **F** (Day 9, 4:25 h) and **H** (Day 9, 6:00 h) (in 3 of 4 spreading depolarizations dashed arrows now point from Electrode 4 to 6). Also, electrocorticographic activity remained depressed between subsequent spreading depolarizations on Day 9, typical of spreading depolarizations in the ischaemic penumbra in animals. These changes correlated with the evolution of a new infarct in the territory of the right anterior cerebral artery as shown in **E** (Day 9, 15:41 h; asterisk) and the territory of the right MCA (data not shown). The white arrow in the CT of **E** illustrates the new direction of propagation of spreading depolarization in this period from Electrode 4 to 6 [corresponding with the first spreading depolarization in **F** and both spreading depolarizations in **H** (dashed arrows)]. **(G)** An MRI of Day 13 with FLAIR sequence demonstrating the lesions. Asterisk = delayed brain infarct in the anterior cerebral artery territory; cross = previous recording area. Note that increase of ICP had necessitated right-sided decompressive hemicraniectomy on Day 10.

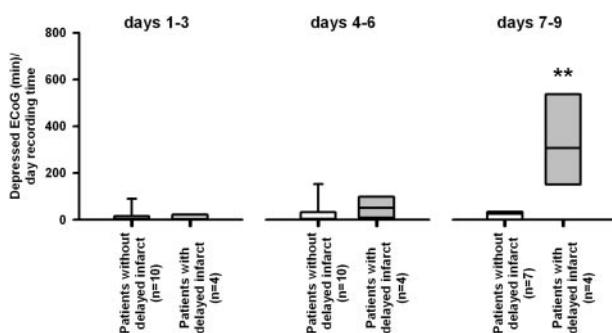
**Table I** Summary of demographic and treatment-related data

No.	Age (years), sex	WFNS grade	Fisher GCS/ MRS on dis-charge	Location of aneurysm	Location of electrode strip (vascular territory)	Day of operation after SAH	Ipsilateral hemispheric gyral swelling	Clinical DIND	Delayed CT-/MRI-proven infarct	Significant proximal vaso-spasm	Day of control DSA	*daily TCD (h)	Duration of ECoG monitoring	Total number of spreading depolarizations	Total duration of ECoG depression periods (min)
1	44, f	2	3	10/5	1,2	ACoA	ACA + MCA	—	Y	Y	*	*	240.3	67	1081
2	50, f	5	3	10/5	3T	Ophth	ACA + MCA	—	Y	Y	*	*	191.7	47	1167
3	34, f	3	3	3/5	3T	ACoP <sup>1</sup>	MCA	0	Y	Y	7*	—	96.3	6	180
4	59, f	1	3	15/3	2	MCA	MCA	3	Y	Y	—	—	138.5	10	406
5	63, m	5	3	15/3	2	MCA	ACA + MCA	—	Y	Y	7*	7*	207.7	19	156
6	55, f	2	3	14/4	0	ACoA + MCA	ACA + MCA	2	Y	Y	7*	7*	107.8	10	90
7	61, f	5	3	11/5	2	ACoP	ACA + MCA	0	Y	Y	6*	6*	157.3	9	117
8	41, f	4	3	11/5	2T	MCA	MCA	0	Y	Y	8*	8*	125.5	51	729
9	57, f	1	3	15/3	1	MCA	MCA	—	Y	Y	11	11	57.7	31	564
10	68, f	5	3	11/5	0T	ICA	MCA	0	Y	Y	7*	7*	122.9	17	209
11	56, f	5	3	11/5	2T	MCA	MCA	0	Y	Y	9*	9*	106.9	14	96
12	46, f	1	3	15/2	0	ACoA + ICA	ACA + MCA	0	Y	Y	7*	7*	145.0	6	17
13	42, f	—	3	15/1	0	ACoP + ICA	MCA	14	Y	Y	14	14	21.6	11	46
14	31, m	—	3	15/0	0	MCA	MCA	7	Y	Y	7	7	53.1	0	0
15	49, f	1	3	15/2	0	ICA	MCA	2	Y	Y	7	7	64.3	0	0
16	45, m	4	4	11/4	1,2	ACoA	ACA	0	Y	Y	—	—	127.6	0	0
17	39, f	1	3	15/1	0	MCA	MCA	0	Y	Y	—	—	22.7	0	0
18	51, f	4	3	15/3	0	MCA	MCA	0	Y	Y	6*	6*	123.3	0	0

WFNS = World Federation of Neurological Surgeons scale; GCS = Glasgow Coma Scale; MRS = modified Rankin Scale; focal deficit on discharge: 0 = no deficit, 1 = aphasia, 2 = hemiparesis, 3 = tetraparesis, T = tracheotomized; location of aneurysm: ACoA = anterior cerebral artery; ACoP = posterior communicating artery, ACA = anterior cerebral artery, MCA = middle cerebral artery, Ophth = ophthalmic artery, ICA = internal carotid artery, ACA = anterior cerebral artery, MCA = middle cerebral artery territory, MCA = middle cerebral artery territory; DIN = delayed ischaemic neurological deficit (sedated = sedated during most of the monitoring period so that neurological assessment was limited); ECoG = electrocorticography.



**Fig. 6** Time correlation of clinical scores and incidence of spreading depolarizations in patients with and without DIND. The figure gives an overview of 13 of 18 patients who developed spreading depolarization after SAH. No. 1 = Patient 1 etc. (corresponding with table, text and other figures); asterisk = day of surgery; horizontal black line = recording period; vertical lines = cortical spreading depolarizations (the length of the vertical lines gives the duration of the depression period in logarithmic scale); light grey-shaded area = clinical DIND (delayed decrease of consciousness by at least two levels of GCS and/or new focal neurological deficit) without delayed infarct; dark grey-shaded area = DIND associated with delayed brain infarct; AFND = acute focal neurological deficit due to acute SAH or surgery; GCS = Glasgow Coma Scale; SPC = pure slow potential change which started within a period of electrocorticographic depression. Patient 2 was sedated throughout the monitoring period so that clinical assessment was limited. The finding of a new infarct on CT on Day 9 is taken as the onset of the DIND (compare illustrative case 2).



**Fig. 7** Delayed brain infarcts are associated with prolonged electrocorticographic depression. Patients with delayed CT-/MRI-proven infarcts showed significantly prolonged electrocorticographic (ECoG) depression periods during infarct development at Days 7–9 after SAH ( $**P = 0.006$ ), whereas early after SAH, no significant difference was found between patients who later developed a delayed infarct and those who did not. Prolongation of the recovery phase of spreading depolarization is one of the electrocorticographic hallmarks of penumbral spreading depolarizations in animals.

the limits of a small study, it is suggested that this pattern is an early, sensitive indicator for impending tissue necrosis, as in animals. In addition, only patients with the clinical syndrome of DIND showed a statistically significant delayed

increase in the number of spreading depolarizations per day. The presence and absence of a delayed cluster of spreading depolarizations had high positive (86%) and negative predictive values (100%), respectively, for a DIND.

However, our study has certain limitations.

(i) Similar to previous studies (Kistler *et al.*, 1983), we observed a very high rate of significant proximal vasospasm in this patient group. Although this suggests that similar electrocorticographic patterns occur in the pathology of ischaemic stroke, it is possible that our results are more or less specific for DIND after SAH. Strictly speaking, our results apply only to patients with Fisher Grade 3 SAH and a large amount of blood in the subarachnoid space. Only patients with this Fisher grade carry a high risk of developing a DIND, which was a principal inclusion criterion for the study (Kistler *et al.*, 1983).

Consistent with this reservation, it remains possible that the lysing blood, which covers in abundance wide areas of cortex after SAH, has important direct effects on neurons and astrocytes with relevance for spreading depolarization. Indeed, in animals, recurrent spreading depolarizations with prolonged electrocorticographic depressions are found not only in the ischaemic penumbra after MCA occlusion but also when breakdown products of erythrocytes are applied to

the subarachnoid space (Dreier *et al.*, 1998, 2000; Windmüller *et al.*, 2005). Whether these different conditions converge on very similar cellular and molecular mechanisms is unresolved (Dreier *et al.*, 1998, 2000; Sonn and Mayevsky, 2000; Shin *et al.*, 2005; Windmüller *et al.*, 2005).

(ii) The use of AC-coupled amplifiers with a 0.01 Hz high-pass cut-off enabled detection of transient voltage negativities associated with SD, but on the other hand precluded recording of sustained negative potentials associated with persistent depolarizations such as AD. Hence, there is potential ambiguity in instances of spreading depolarizations with prolonged depression periods: does tissue remain depolarised throughout the depression, as in AD, or is there rapid repolarization followed by an induced penumbral state of continued electrical silence and preserved ion homeostasis? In many instances in our recordings, spreading negative slow voltage variations recurred while the spontaneous electrocorticographic activity still exhibited a prolonged depression from a previous event (e.g. Case 2). This same phenomenon has been previously reported in focal ischaemia in rats and cats (Back *et al.*, 1994; Ohta *et al.*, 2001; Hartings *et al.*, 2006). This demonstrates that in such instances spreading depolarizations with prolonged depression are in fact transient depolarizations with delayed recovery, since tissue experiencing sustained depolarization during AD cannot generate subsequent negative slow voltage variations.

Other characteristics of AD and SD shed additional light on this ambiguity. First, it is one of the hallmarks of AD in animal experiments that the negative slow voltage variation is preceded by a period of a few minutes with non-spreading cessation of the electrocorticographic activity (Leão, 1947) accompanied by gradual acidification and a slow rise of the extracellular potassium concentration (Obrenovitch *et al.*, 1990; Nedergaard and Hansen, 1993). This non-spreading cessation of spiking activity within seconds of anoxia or severe ischaemia is explained by the high sensitivity of the underlying synaptic processes to energy compromise (Fleidervish *et al.*, 2001). Characteristically, the spreading depolarizations in our human recordings did not show this electrocorticographic pattern typical of AD. Rather, consistent with SD, the depression of electrocorticographic activity began simultaneously with the spreading negative slow voltage variation. Second, in AD, the lag time of the negative slow voltage variation between neighbouring electrodes is often shorter than in SD since AD often initiates at multiple cortical sites (Nedergaard and Hansen, 1993; Jarvis *et al.*, 2001). In our human recordings, in contrast with AD, the lag times of the negative slow voltage variations were not significantly different between spreading depolarizations with short- and long-lasting depressions.

We observed a continuous spectrum of events with depression periods ranging from <10 min to several hours. Rather than two strictly separate types of spreading depolarizations such SD and AD, we suggest that many of these events represent intermediate forms of spreading

depolarizations, referred to as peri-infarct or transient ischaemic depolarizations (Nedergaard and Hansen, 1993; Koroleva and Bures, 1996). As mentioned, peri-infarct depolarizations have prolonged depression periods and are associated with expansion of neuronal damage in animal experiments, and these characteristics were observed in the present study. Although the above points argue against the possibility that prolonged depressions reflect AD phenomena, it remains possible that some events with prolonged depression had transient depolarizations that endure longer than typical SD and can occur as a characteristic of peri-infarct depolarization (Nedergaard and Hansen, 1993; Koroleva and Bures, 1996).

Although our study is too small to provide precise numerical values to allow discrimination between SD and intermediate forms of spreading depolarization, it indicates in principle that a cluster of spreading depolarizations with a dynamic change of progressively increasing depression periods carries a high risk for the development of a delayed brain infarct. We believe that our data are sufficient to justify the use of this electrocorticographic pattern as a reliable indication for triple-H therapy (in addition to clinical status, TCD and DSA). Electrocorticography appears to have particular value for sedated and ventilated patients in whom clinical assessment is very limited and the positive predictive values of TCD and DSA alone are insufficient to diagnose delayed ischaemia at an early stage (Vora *et al.*, 1999; Unterberg *et al.*, 2001; Minhas *et al.*, 2003; Rabinstein *et al.*, 2005). This implies that patients with higher WFNS grade will derive greatest benefit from electrocorticographic monitoring.

(iii) The present study is based only on electrocorticographic, clinical and imaging data. Neuroimaging with CT and MRI is the gold standard in the clinics to demonstrate ischaemic lesions and a good correlation exists between neuroimaging and histopathological findings of delayed ischaemia (Birse and Tom, 1960; Neil-Dwyer *et al.*, 1994; Dreier *et al.*, 2002; Rabinstein *et al.*, 2005). However, in future studies, the combination of electrocorticography with microdialysis to monitor metabolism and methods to monitor the tissue oxygen level and cerebral blood flow will further increase our understanding of the precise relations between spreading depolarizations and delayed ischaemic damage in the human brain (Hopwood *et al.*, 2005; Parkin *et al.*, 2005).

(iv) Based on animal studies, it was previously hypothesized that microvascular spasm and spreading ischaemia in response to spreading depolarization causes the characteristic widespread focal laminar cortical infarcts in SAH as seen in Patient 1 (Dreier *et al.*, 1998, 2000, 2002). We are unable to prove or disprove this hypothesis since we did not measure local cerebral blood flow in conjunction with the electrocorticogram. However, the occurrence of abundant spreading depolarizations during the evolution of these distinctive lesions warrants future study of this issue.

(v) Because electrocorticography was performed with a single electrode strip, it is possible that spreading depolarizations in other regions of the brain escaped detection. With limited spatial sampling, the incidence of spreading depolarizations is likely underestimated and the statistical results influenced accordingly.

(vi) It could be argued that the electrode strip facilitated the spread of depolarizations by acting as a conductor. However, in several studies in which the spread of spreading depolarization has been imaged continuously in the gyrencephalic brain (Strong *et al.*, 1996, 2000; Bowyer *et al.*, 1999), events have never been seen to move immediately from one gyrus to another. Rather, a delay is invariably seen with timing appropriate for propagation around the depth of the sulcus. Thus the pia-arachnoid forms a barrier to the depolarization current, and would prevent short-circuiting by surface electrodes.

(vii) Part of the electrode strip might have been placed on cortex previously retracted during surgery. As recently shown, spreading depolarization can occur as a consequence of brain contusion (Strong *et al.*, 2002; Fabricius *et al.*, 2006) and this was possibly a confounding factor. Experimental experience is that penumbral tissue is highly sensitive to even gentle mechanical disturbance, manifest as easy, inadvertent induction of peri-infarct depolarizations, and neurosurgeons recognise well the risks of ill-timed surgery in SAH. The present findings offer a possible explanation for this clinical observation.

(viii) Based on the fluctuating clinical course, it has been previously suggested that recurrent spreading depolarizations could be the pathophysiological basis of the syndrome of fever, coma and focal neurological deficits in patients with familial hemiplegic migraine (Dreier *et al.*, 2005). In the present study, a correlation of a cluster of spreading depolarizations with decreased level of consciousness has been demonstrated directly for the first time. However, the exact pathophysiological mechanism remains uncertain and needs to be investigated in future studies.

In conclusion, we found overall that spreading depolarizations with electrocorticographic depression of >10 min duration indicated a significantly worse outcome when the patient was discharged from the acute unit. Additionally, clusters of spreading depolarizations, with onset delayed for some days after SAH, correlated with DIND, and the electrocorticographic recovery phases after spreading depolarizations were progressively prolonged during infarct evolution. These data support the notion that in SAH spreading depolarizations with prolonged depression are indicators of the impending and progressive neuronal damage in the human brain.

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