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# Spreading convulsions, spreading depolarization and epileptogenesis in human cerebral cortex

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**Spreading depolarization of cells in cerebral grey matter is characterized by massive ion translocation, neuronal swelling and large changes in direct current-coupled voltage recording. The near-complete sustained depolarization above the inactivation threshold for action potential generating channels initiates spreading depression of brain activity. In contrast, epileptic seizures show modest ion translocation and sustained depolarization below the inactivation threshold for action potential generating channels. Such modest sustained depolarization allows synchronous, highly frequent neuronal firing; ictal epileptic field potentials being its electrocorticographic and epileptic seizure its clinical correlate. Nevertheless, Leão in 1944 and Van Harreveld and Stamm in 1953 described in animals that silencing of brain activity induced by spreading depolarization changed during minimal electrical stimulations. Eventually, epileptic field potentials were recorded during the period that had originally seen spreading depression of activity. Such spreading convulsions are characterized by epileptic field potentials on the final shoulder of the large slow potential change of spreading depolarization. We here report on such spreading convulsions in monopolar subdural recordings in 2 of 25 consecutive aneurismal subarachnoid haemorrhage patients *in vivo* and neocortical slices from 12 patients with intractable temporal lobe epilepsy *in vitro*. The *in vitro* results suggest that $\gamma$-aminobutyric acid-mediated inhibition protects from spreading convulsions. Moreover, we describe arterial pulse artefacts mimicking epileptic field potentials in three patients with subarachnoid haemorrhage that ride on the slow potential peak. Twenty-one of the 25 subarachnoid haemorrhage patients (84%) had 656 spreading depolarizations in contrast to only three patients (12%) with 55 ictal epileptic events isolated from spreading depolarizations. Spreading depolarization frequency and depression periods per 24 h recording episodes showed an early and a delayed peak on Day 7. Patients surviving subarachnoid haemorrhage with poor outcome at**

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**6 months showed significantly higher total and peak numbers of spreading depolarizations and significantly longer total and peak depression periods during the electrocorticographic monitoring than patients with good outcome. In a semi-structured telephone interview 3 years after the initial haemorrhage, 44% of the subarachnoid haemorrhage survivors had developed late post-haemorrhagic seizures requiring anti-convulsant medication. In those patients, peak spreading depolarization number had been significantly higher [15.1 (11.4–30.8) versus 7.0 (0.8–11.2) events per day, $P = 0.045$]. In summary, monopolar recordings here provided unequivocal evidence of spreading convulsions in patients. Hence, practically all major pathological cortical network events in animals have now been observed in people. Early spreading depolarizations may indicate a risk for late post-haemorrhagic seizures.**

**Keywords:** epilepsy; subarachnoid haemorrhage; spreading depression; spreading depolarization; delayed cerebral ischaemia
**Abbreviations:** GABA = $\gamma$-aminobutyric acid; SAH = subarachnoid haemorrhage

# Introduction

Spreading depolarization is characterized as a wave in the grey matter of the CNS with near-complete sustained depolarization of neurons. It consists of massive ion translocation between intra- and extracellular space, neuronal swelling, distortion of dendritic spines, glial depolarization, large change of the slow electrical potential and silencing of brain bioelectrical activity (spreading depression) (Kraig and Nicholson, 1978; Somjen, 2001; Dreier, 2011). The slow electrical potential change is a direct extracellular summary measure for the spreading depolarization of the neurons at the recording electrode (Canals *et al.*, 2005). The spreading depression (silencing) of bioelectrical activity is an epiphenomenon as it is initiated by the sustained depolarization of the neurons above the inactivation threshold for the action potential generating channels (Kager *et al.*, 2002). Spreading depression does not follow spreading depolarization if brain electrical activity has already ceased for other reasons. The latter is observed in focal or global ischaemia, for example, where non-spreading depression of spontaneous activity precedes the eruption of spreading depolarization by 1–5 min (Leão, 1947; Farkas *et al.*, 2010). Such non-spreading depression of activity is due to mechanisms unrelated to spreading depolarization, such as disturbed vesicular transmitter release or release of adenosine by astrocytes in response to energy depletion (Fleidervish *et al.*, 2001; Canals *et al.*, 2008). While neurons are depolarized during spreading depression of activity, they are hyperpolarized during non-spreading depression of activity (Somjen, 2001).

These two fundamental depression patterns of neuronal activity have important clinical implications as they seem to translate into two different temporal patterns characterizing neurological deficits in the patient's cognition, perception and/or behaviour. It is assumed that non-spreading depression of activity is the pathophysiological correlate of the sudden and simultaneous neurological deficits of transitory ischaemic attacks, non-migrainous stroke and cardiac arrest, whereas spreading depression of brain electrical activity is the correlate of the creeping neurological deficits of migraine aura and migrainous stroke. The depression pattern thus determines the clinical symptoms but it does not determine whether neurons will survive or die. It is assumed that the countdown to neuronal death is initiated by the spreading depolarization process and its toxic effects including intracellular calcium surge, mitochondrial depolarization and massive release of excitatory amino acids (Somjen, 2001; Dreier, 2011). Whether or not the tissue recovers from spreading depolarization depends on proper function of the energy-dependent sodium pump (LaManna and Rosenthal, 1975). Even under healthy resting conditions, this enzyme consumes ~50% of the brain energy. During spreading depolarization, energy consumption by the sodium pump is markedly increased, resulting in a 50% decrease in tissue ATP, even if energy supply is normal (Mies and Paschen, 1984). In energy-depleted tissue, ATP for fuelling the sodium pump is lacking, which results in prolonged spreading depolarizations with increasingly harmful consequences up to the death of the neurons. For a more comprehensive account of these signals in relation to the clinic, we refer the reader to a former review (Dreier, 2011).

Nevertheless, in animals, spreading depolarization is not always accompanied by depression of neuronal activity. Thus, Leão as well as Van Harreveld and Stamm found that the effects of spreading depolarization on spontaneous activity changed during a series of minimal electrical cortical stimulations (Leão, 1944, 1972; Van Harreveld and Stamm, 1953). After inducing several typical spreading depolarizations with depression of spontaneous activity, a few isolated spikes appeared during the depression. Upon further repetition of the stimulus, short runs of high-amplitude electrocorticographic spikes developed. These became longer in duration in consequent stimulations, until eventually a continuous high potential activity of a 'clearly convulsive nature' (author's emphasis) was often recorded during the period which had originally shown the spreading depression. Van Harreveld and Stamm (1953) coined the term 'spreading convulsion' for this phenomenon to distinguish it from spreading depression of activity.

In the present study, we provide unequivocal evidence that spreading convulsions occur in the human brain both in neocortical slices from patients with intractable epilepsy and *in vivo* in patients with aneurismal subarachnoid haemorrhage (SAH). We demonstrate *in vitro* that $\gamma$-aminobutyric acid (GABA)-mediated inhibition protects from spreading convulsions, and analyse *in vivo* whether type, frequency or severity of spreading depolarizations are associated with a higher proportion of late post-haemorrhagic seizures.

# Materials and methods

## Clinical study on ictal epileptic field potentials and spreading depolarizations in patients with aneurismal subarachnoid haemorrhage

Patients with major aneurismal SAH were consecutively recruited by one centre of the Co-Operative Studies on Brain Injury Depolarizations (COSBID, see www.cosbid.org) within 72 h after aneurysm rupture. Patient characteristics are given in Table 1. The research protocol was approved by the local ethics committee of the Charité University Medicine Berlin. Clinical and research consent was obtained after a clinical decision had been taken to offer surgical treatment after aneurismal SAH. Aneurismal SAH was diagnosed by assessment of CT scans. Haemorrhage was graded according to the Fisher scale, and clinical presentation according to the World Federation of Neurological Surgeons scale. A study neurologist or neurosurgeon performed a neurological and general medical evaluation on admission. Baseline demographic data, social history including tobacco and alcohol use, medical history including history of epilepsy, migraine and cardiovascular risk factors and clinical signs and symptoms of the initial haemorrhage were recorded. The aneurysm was assessed using four-vessel digital subtraction angiography, or a more restricted study when indicated. The craniotomy allowed placement of a single, linear, 6-contact (platinum) recording strip for electrocorticography recordings (Wyler, 5-mm diameter; Ad-Tech Medical) as described previously (Dreier *et al.*, 2006). After surgery, the patient was transferred to the intensive care unit. Intracranial pressure was monitored via ventricular drainage catheter or intracranial pressure transducer (Codman or Camino systems). Glasgow Coma Scale, blood gases, glucose and electrolytes were documented at least every 6 h. A thorough neurological examination was performed at least daily. A delayed ischaemic neurological deficit was defined as the occurrence of focal neurological impairment (such as hemiparesis, aphasia, apraxia, hemianopia or neglect), or a decrease of at least 2 points on the Glasgow Coma Scale [either on the total score or on one of its individual components (eye, motor on either side, verbal)]. Moreover, the diagnosis of a delayed ischaemic neurological deficit required that the neurological deficit was not present immediately after aneurysm occlusion, that it lasted for at least 1 h and could not be attributed to other causes such as hydrocephalus or re-bleeding by means of clinical assessment, CT or MRI of the brain and appropriate laboratory studies (Vergouwen *et al.*, 2010). Serial CT scans were performed postoperatively, at the time of clinical deterioration and after the monitoring period to screen for delayed infarcts. This was complemented by MRI in selected cases. Admission and follow-up neuroimages were independently evaluated by a study neuroradiologist, blinded to the electrocorticography data, for the presence of focal or global cerebral oedema, the Fisher grade, the presence and degree of hydrocephalus, the presence of infarction, intracerebral or subdural haematoma. Using transcranial Doppler-sonography, significant vasospasm was defined by a mean velocity $>200$ cm/s in at least one middle cerebral artery. Vasospasm was considered possible in the range between 120 and 200 cm/s and excluded if the middle cerebral artery mean velocities remained $<120$ cm/s throughout the observation period (Vora *et al.*, 1999). Patients with delayed ischaemic neurological deficit were treated with haemodynamic therapy (hypertension, hypervolaemia (van Gijn and Rinkel, 2001). Oral nimodipine was given prophylactically. The use of a single linear electrode strip allowed withdrawal at the bedside. No local haemorrhagic or infectious complications of the subdural strip were encountered. Management and treatment of epileptic seizures was decided by the intensive care specialists in charge during the neuromonitoring period and by community neurologists after discharge from the hospital. Clinical outcome was assessed at 6 months according to the extended Glasgow Outcome Scale.

## Diagnosis of late post-haemorrhagic seizures

The patients were administered a semi-structured telephone interview by a neurologist in training. If the patient was unable to give a history, relatives or care givers were interviewed. Medical notes were taken into account when possible. The interview started with a screening questionnaire according to Placencia *et al.* (1992). If patients fulfilled the screen-positive criteria, a detailed history of the seizures was taken including the timing, seizure type, concurrent medical problems and anti-convulsant medication. The definition of a seizure followed the current guidelines of the International League Against Epilepsy (1989). For the purpose of the present study, onset seizures were defined as occurring within 12 h of the initial haemorrhage. In-hospital seizures were observed in the subacute phase while the patient was in hospital. Late post-haemorrhagic seizures were defined as occurring after discharge from hospital. In all cases, discharge from hospital was later than 1 week after the initial haemorrhage.

## Electrocorticography and data analysis in the *in vivo* study

Five monopolar electrocorticography recordings were acquired continuously from Electrodes 2–6 (interelectrode distance 1 cm) of the 6-electrode (linear array) subdural strip against a subgaleal reference electrode using a GT205 amplifier (0.01–45 Hz; ADInstruments). Electrode 1 served as ground. In a subgroup of patients, the direct current electrocorticogram was additionally recorded using a BrainAmp amplifier (0–45 Hz; BrainProducts GmbH) as reported previously (Dreier *et al.*, 2009; Oliveira-Ferreira *et al.*, 2010). The here-applied platinum electrodes have much better low-frequency recording properties than stainless steel electrodes but are polarizable and thus inferior to silver chloride. Silver chloride electrodes are nevertheless precluded from invasive recordings in patients because of their toxicity (Tallgren *et al.*, 2005). Data were sampled at 200 Hz and recorded and reviewed with the use of a Powerlab 16/SP analog/digital converter and Chart-7 software (ADInstruments). The electrocorticography analysis was performed by two neurologists blinded to the neuroimaging analysis and patient outcome.

Spreading depolarization was defined by the sequential onset in adjacent electrodes of a propagating, polyphasic slow potential change (Figs 1A and 2A) (Dreier *et al.*, 2006; Fabricius *et al.*, 2006). The parallel electrocorticography depression was defined by a rapidly developing reduction of the power of the high-frequency electrocorticography amplitude (bandpass: 0.5–45 Hz). The electrocorticography depression period ($>0.5$ Hz) was assessed using the integral of power of the high-pass filtered activity (lower frequency limit, 0.5 Hz; time constant decay, 60 s) as described previously (Dreier *et al.*, 2006).

For the purpose of this study, ictal epileptic field potentials were defined as rhythmic discharges with spikes, polyspikes or sharp waves or characteristic rhythmic delta or theta activity of at least 10 s duration (Figs 1–3). Episodes with ictal epileptic field potentials

**Table 1 Summary of demographic, spreading depolarization- and ictal epileptic activity-related data (aneurismal SAH)**

| No. | Age (years), sex | WFNS grade | Fisher grade | Location of aneurysm | Intervention | Late epilepsy | Anti-convulsive drug treatment of late post-haemorrhagic seizures | Start of ECoG monitoring (day after insult) | Recording time (h) | Total number of spreading depolarizations | Total number of spreading depressions | Total number of isoelectric[a] spreading depolarizations | Total number of spreading depolarizations with pulse artefacts | Total number of spreading convulsions | Total depression period (min) | Number of isolated ictal epileptic events | Total duration of isolated ictal epileptic events (min) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 38, f | 5 | 3 | ACoA | Clipping, EVD | No | | 2 | 35.6 | 0 | 0 | 0 | 0 | 0 | 0.0 | 0 | 0.0 |
| 2 | 50, f | 5 | 3 | ICA | Coiling, EVD | CPS | *LTG* | 0 | 191.3 | 48 | 41 | 7 | 0 | 0 | 878.4 | 0 | 0.0 |
| 3 | 35, f | 3 | 2 | ACoP | Clipping | Death | | 1 | 153.3 | 6 | 3 | 3 | 0 | 0 | 70.6 | 0 | 0.0 |
| 4 | 62, m | 5 | 3 | MCA | Clipping, EVD | CPS | *CBZ* | 0 | 227.6 | 17 | 17 | 0 | 0 | 0 | 112.4 | 0 | 0.0 |
| 5 | 44, f | 2 | 2 | ACoA | Clipping, EVD | SGS | LVT | 0 | 239.9 | 65 | 62 | 3 | 0 | 0 | 3618.0 | 0 | 0.0 |
| 6 | 46, m | 5 | 3 | MCA | Clipping, EVD | SGS | *CBZ* | 1 | 150.3 | 11 | 7 | 1 | 0 | 3 | 177.8 | 7 | 29.4 |
| 7 | 44, m | 5 | 4 | ACA | Coiling, EVD | No | | 3 | 47.7 | 9 | 0 | 9 | 0 | 0 | 648.7 | 0 | 0.0 |
| 8 | 61, m | 1 | 3 | MCA | Clipping, EVD | No | | 1 | 214.4 | 0 | 0 | 0 | 0 | 0 | 0.0 | 0 | 0.0 |
| 9 | 65, f | 4 | 1 | MCA | Clipping, EVD | No | | 1 | 221.4 | 15 | 15 | 0 | 0 | 0 | 143.9 | 0 | 0.0 |
| 10 | 46, m | 5 | 4 | MCA | Clipping, EVD | Death | | 1 | 212.6 | 3 | 3 | 0 | 0 | 0 | 9.5 | 0 | 0.0 |
| 11 | 70, f | 1 | 3 | PericA | Clipping, EVD | No | | 0 | 60.3 | 3 | 3 | 0 | 0 | 0 | 25.1 | 0 | 0.0 |
| 12 | 41, f | 2 | 2 | MCA | Clipping, EVD | No 3 year follow-up | | 1 | 217.1 | 87 | 87 | 0 | 0 | 0 | 660.0 | 1 | 3.1 |
| 13 | 50, f | 4 | 3 | MCA | Clipping, EVD | No | | 0 | 229.5 | 29 | 28 | 0 | 1 | 0 | 221.5 | 0 | 0.0 |
| 14 | 49, f | 1 | 3 | MCA | Clipping, EVD | No 3 year follow-up | | 1 | 206.1 | 5 | 5 | 0 | 0 | 0 | 25.6 | 0 | 0.0 |
| 15 | 56, f | 4 | 3 | MCA | Clipping, EVD | Death | | 1 | 186.9 | 2 | 2 | 0 | 0 | 0 | 11.9 | 0 | 0.0 |
| 16 | 57, m | 1 | 3 | MCA | Clipping, EVD | Death | | 1 | 208.4 | 27 | 15 | 8 | 4[b] | 0 | 639.5 | 0 | 0.0 |
| 17 | 50, f | 5 | 3 | ACoA | Coiling, EVD | Death | | 1 | 116.9 | 0 | 0 | 0 | 0 | 0 | 0.0 | 0 | 0.0 |
| 18 | 71, m | 5 | 4 | ACoA | Clipping, EVD | SGS | *LVT, LTG* | 1 | 248.4 | 118 | 62 | 54 | 0 | 2 | 2987.0 | 47 | 421.7 |
| 19 | 52, f | 2 | 4 | ICA | Clipping | No | | 0 | 16.7 | 0 | 0 | 0 | 0 | 0 | 0.0 | 0 | 0.0 |
| 20 | 38, m | 5 | 3 | ACoA | Clipping, EVD | SGS | *CBZ* | 0 | 153.3 | 15 | 15 | 0 | 0 | 0 | 728.3 | 0 | 0.0 |
| 21 | 53, m | 5 | 3 | ACA | Coiling, EVD | No | | 1 | 237.2 | 54 | 54 | 0 | 0 | 0 | 309.4 | 0 | 0.0 |
| 22 | 47, m | 4 | 3 | MCA | Clipping, EVD | SGS | *CBZ, LVT* | 1 | 237.7 | 31 | 31 | 0 | 0 | 0 | 395.8 | 0 | 0.0 |
| 23 | 47, f | 4 | 3 | MCA | Clipping, EVD | No | | 1 | 132.3 | 70 | 48 | 22 | 0 | 0 | 1970.1 | 0 | 0.0 |
| 24 | 47, m | 4 | 3 | ACoA | Clipping, EVD | No | | 1 | 281.4 | 51 | 50 | 1 | 0 | 0 | 745.4 | 0 | 0.0 |
| 25 | 51, f | 4 | 3 | MCA | Clipping | SGS | *LVT* | 0 | 230.4 | 24 | 14 | 8 | 2 | 0 | 694.2 | 0 | 0.0 |

ACA = anterior cerebral artery; ACoA = anterior communicating artery; ACoP = posterior communicating artery; CBZ = carbamazepine; CPS = complex partial seizure; EVD = extraventricular drainage; ICA = internal carotid artery; LTG = lamotrigine; LVT = levetiracetam; MCA = middle cerebral artery; PericA = pericallosal artery; SGS = secondarily generalized seizure; WFNS = World Federation of Neurological Surgeons Scale.

a In isoelectric spreading depolarizations brain electrical activity has already ceased before the onset of the spreading depolarization (Dreier *et al.*, 2006; Fabricius *et al.*, 2006; Hartings *et al.*, 2011).

b Isoelectric spreading depolarization with superimposed arterial pulse artefacts.

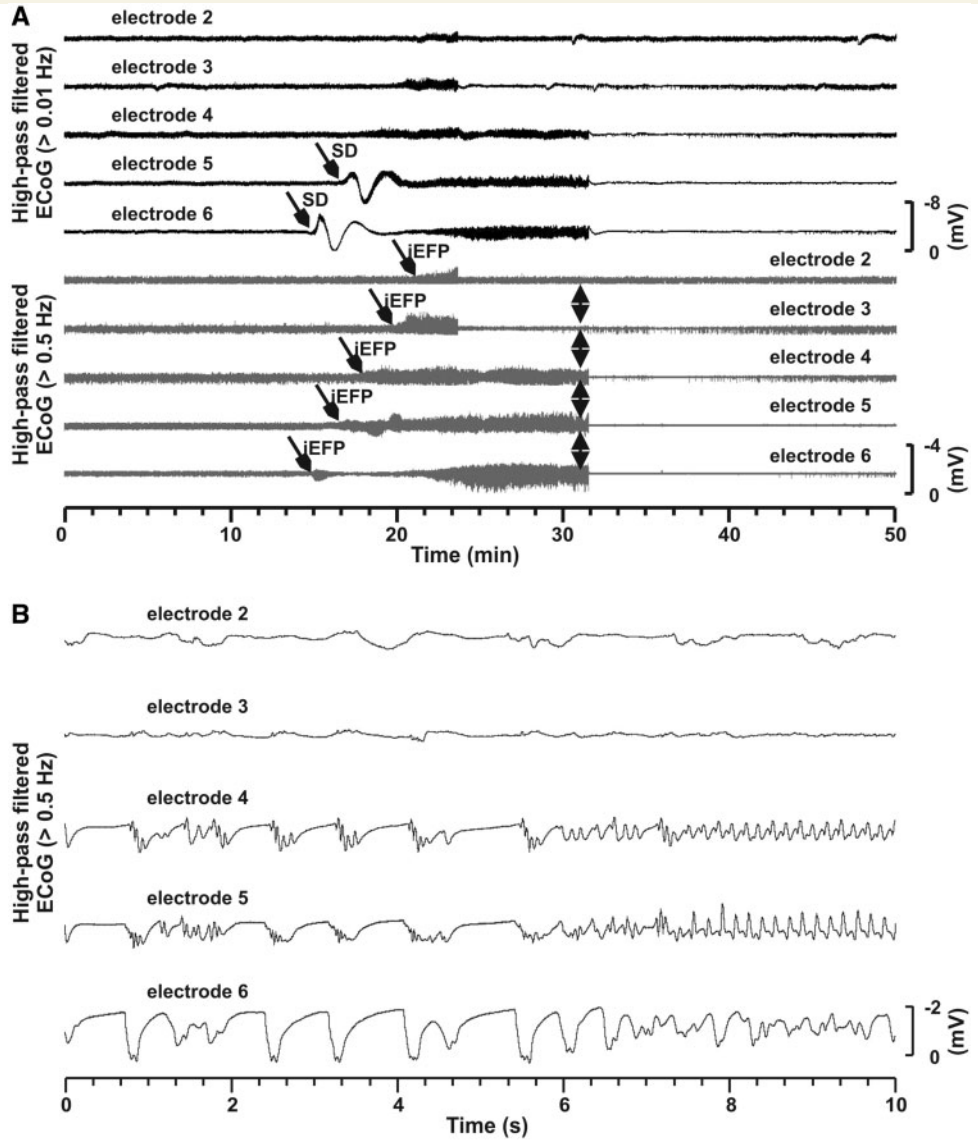


**Figure 1** (**A**) Electrocorticography recording of spreading convulsion. The spreading depolarization started at Electrode 6 55 h after aneurismal SAH and 15 h after discontinuation of the sedation with propofol. It only spread to Electrode 5. The propagation velocity was 5.5 mm/min assuming a wavefront oriented perpendicular to the electrode strip. The peak-to-peak amplitudes were 5.7 and 4.4 mV in Electrodes 6 and 5, respectively. The slow potential change started at Electrode 6 superimposed with ictal epileptic field potentials which propagated from Electrode 6 along the strip until Electrode 2 at an average velocity of 6.6 mm/min. At Electrode 6, two periods with ictal epileptic field potentials were interrupted by a period of silence in brain electrical activity for 3.5 min. The ictal epileptic field potentials showed a maximal amplitude of 2.3 mV and abruptly ceased first in Electrodes 2 and 3 while continuing in Electrodes 4–6 where they simultaneously ceased only 7 min later. The whole ictal epileptic event lasted for 14.5 min. It was followed by complete and long-lasting depression of the brain electrical activity. Recovery of the brain electrical activity started after about 7 min. A clinical seizure was not documented on the intensive care unit. (**B**) Shows synchronous polyspike wave activity in Electrodes 4–6 at the end of the ictal epileptic event as indicated by the arrows in (**A**). Note that in this and subsequent figures negative potential deflection is plotted upward.

isolated from spreading depolarizations were quantified as the total number of epileptic seizure periods (Fabricius *et al.*, 2008).

## Brain slice preparation and *in vitro* experiments

All *in vitro* experiments were approved by the local ethics committee (Ethikkommission der Ärztekammer Westfalen-Lippe und der Medizinischen Fakultät der Westfälischen Wilhelms-Universität Münster). Informed consent was obtained from all patients.

The cortical tissue was taken from a small portion of that excised for treatment of pharmacoresistant focal epilepsy ($n = 12$). Detailed data of patients are given in Table 2.

Slices were prepared from a 1 $cm^3$ tissue block within 5 min after resection. The techniques for slice preparation have been described previously (Gorji and Speckmann, 2004). Briefly, neocortical slices of

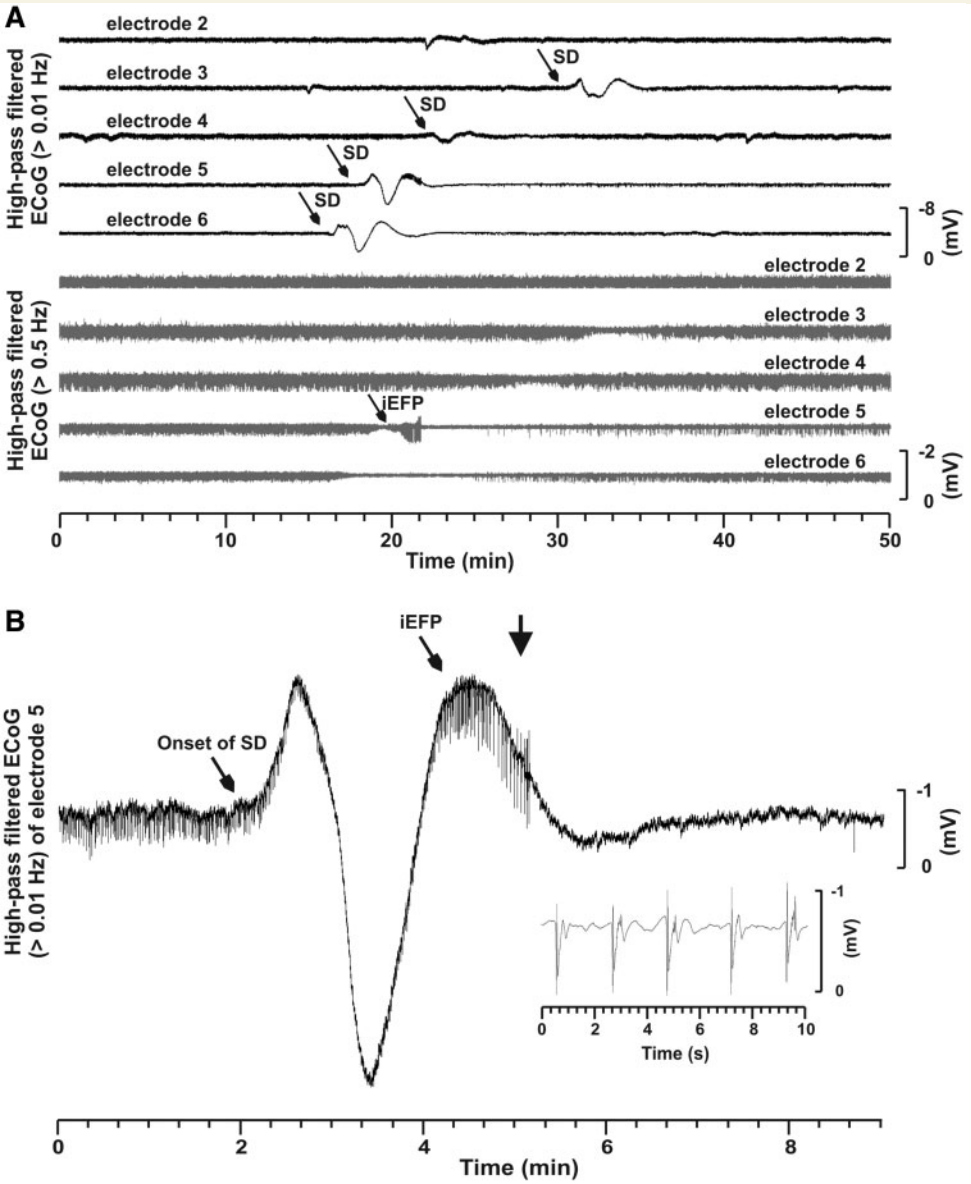


**Figure 2** (**A**) Electrocorticography recording of spreading convulsion. Two and four hours after the spreading convulsion in Fig. 1, the patient developed two more spreading convulsions but the ictal epileptic field potentials (iEFP) were only observed at Electrode 5 and the spreading depolarization (SD) propagated not only from Electrode 6 to 5 but from 6 to 3 at an average velocity of 2.4 mm/min. (**B**) Shows in more detail that the ictal epileptic field potential in Electrode 5 started at the end of the slow potential change. The inset demonstrates the polyspike pattern of the ictal epileptic field potentials. Thereafter, the patient had another seven spreading depolarizations propagating from Electrodes 6 to 3 not associated with ictal epileptic field potentials and four ictal epileptic events restricted to Electrode 5, which did not show a close temporal relationship with spreading depolarization. Spreading depolarization and the ictal epileptic activities cleared after Day 4 without anti-convulsant medication. Nevertheless, the patient developed late epilepsy.

500-μm thickness were cut in frontal plane perpendicular to the pial surface using a vibratome. They were placed in a portable incubation chamber with oxygenated (95% $O_2$ and 5% $CO_2$) artificial CSF consisting of (in mM): NaCl 124, KCl 4, $CaCl_2$ 2, $NaH_2PO_4$ 1.24, $MgSO_4$ 1.3, $NaHCO_3$ 26 and glucose 10 at a temperature of 28°C and a pH of 7.4. Slices were allowed to recover from transport for a period of 1–2 h before being transferred into an interphase-type recording chamber. In the experimental chamber, the temperature was raised to 32°C. During the experiments, the pH, the bath temperature and the flow rate (1.5–2.0 ml/min) remained constant.

Extracellular field potentials were recorded with glass microelectrodes (150 mM NaCl; 2–10 MΩ) in the third and fifth layer of the neocortical slices, judged by visual placement of the recording pipette, connected to the amplifier by an Ag/AgCl–KCl bridge. Field potentials were traced by an ink writer and recorded by a digital oscilloscope.

To search for spontaneous activity (Table 2), the electrodes were periodically repositioned following an imaginary grid consisting of 500 × 500 μm squares covering the whole slice (Kohling *et al.*, 1998). To evaluate slices, subcortical white matter was stimulated electrically by means of a bipolar platinum electrode placed at the border

**Table 2 Summary of demographic data of the *in vitro* study in slices from patients with intractable epilepsy**

| No. | Age (years), sex | Seizure type | Seizure frequency per month | Seizures for *n* years | Anti-convulsive drug treatment | MRI | Pathology | Ammon's horn sclerosis | Tissue for slices | Slice activity |
|---|---|---|---|---|---|---|---|---|---|---|
| 26 | 49, f | CPS, GS, SGS, SPS | 12 | 39 | CBZ, VPA | Sclerosis | Focal dysplasia, gliosis (fibre) | Yes | TL l | Sp |
| 27 | 34, m | CPS, GS, SGS, SPS | 3 | 28 | CBZ, GPT, LTG, MSX, OXCZ, PHB, PHT, TOP, VGB | Sclerosis | | Yes | TL r | Sp |
| 28 | 39, f | CPS, GS, SGS, SPS | 3 | 14 | | | Astrocytosis, gliosis (fibre) | | TL r | Nsp |
| 29 | 44, f | CPS, GS, SGS, SPS | 300 | 22 | GPT, OXCZ, VPA | Sclerosis | Astrocytosis, atrophy, gliosis (fibre) | Yes | TL l | Nsp |
| 30 | 47, f | CPS, SPS | 2 | 30 | PHT, LTG | Sclerosis | Astrocytosis, gliosis (fibre) | Yes | TL r | Nsp |
| 31 | 15, m | GS, SPS | | 1 | OXCZ | Tumor | Ganglioglioma | | TL r | Sp |
| 32 | 33, m | CPS, GS, SGS, SPS | 4 | 29 | CBZ, PHT, VPA | Lesion, sclerosis | Astrocytosis, gliosis (fibre) | Yes | TL r | Nsp |
| 33 | 18, m | CPS, SPS | 2 | 3 | CBZ, OXCZ, VPA | Mesial atrophy, dysplasia, lesion, sclerosis | Astrocytosis, focal dysplasia | Yes | TL r | Nsp |
| 34 | 14, f | CPS, SPS | 20 | 12 | CBZ, ESX, LTG, OXCZ, STM | Lesion, sclerosis | | Yes | TL l | Sp |
| 35 | 36, m | CPS | 4 | 30 | CBZ, LTG, OXCZ, TGB, TOP, VPA | Lesion, sclerosis | Astrocytosis, focal dysplasia, gliosis (fibre), laminar disorder | Yes | TL r | Sp |
| 36 | 46, m | CPS, GS, SGS, SPS | 8 | 39 | CBZ, ESX, LTG, PHB, PHT, STM, VPA | Mesial atrophy | Astrocytosis, focal dysplasia | Yes | TL r | Sp |
| 37 | 20, m | CPS, GS, SGS, SPS | 7 | 12 | OXCZ, TOP, VPA | | Astrocytosis, focal dysplasia | Yes | TL r | Sp |

CBZ = carbamazepine; CPS = complex partial seizure; ESX = ethosuximide; GPT = gabapentin; GS = primarily generalized seizure; HP = hippocampus; l = left; LTG = lamotrigine; MSX = metosuximide; Nsp = no spontaneous; OXCZ = oxcarbazepine; PHB = phenobarbitale; PHT = phenytoin; r = right; SGS = secondarily generalized seizure; Sp = spontaneous; SPS = simple partial seizure; STM = sulthiame; TGB = tiagabine; TL = temporal lobe (neocortex); VGB = vigabatrin; VPA = valproic acid.

of white and grey matter adjacent to the recording electrodes and evoked field potentials were tested across the whole slice (Berger *et al.*, 2008).

Spreading depolarization was elicited by KCl injection. A glass electrode filled with 2 M KCl was fixed in a special holder connected with a plastic tube to a pressure injector and the tip inserted into the sixth layer of the neocortex. A high-pressure pulse of KCl, sufficient to induce spreading depolarization, was injected into the tissue (tip diameter: 2 μm; injection pressure 1.0–2.0 bar applied for 200–500 ms, up to two separate injections, 3–5 nl). In some experiments, spreading depolarization was evoked in the presence of the $GABA_A$ receptor and small-conductance calcium-activated potassium (SK) channel antagonist bicuculline (5 μM) or *N*-methyl-D-aspartate (10 μM) that activates the ionotropic *N*-methyl-D-aspartate receptor of glutamate.

Data are given as median (1st and 3rd quartile). Statistical analysis was performed using two-sided Fisher's exact or Mann–Whitney rank sum tests as indicated in the text. $P < 0.05$ was considered statistically significant.

# Results

## Spreading convulsions in patients with aneurismal subarachnoid haemorrhage

Demographic details of the 25 consecutive patients are given in Table 1. During the monitoring period of 4456.5 h (median: 208.4 h per patient), electrocorticography recordings revealed five spreading convulsions and 55 ictal epileptic events isolated from spreading depolarizations. Following the original definition of Van Harreveld and Stamm (1953), a spreading convulsion was defined as a spreading depolarization with ictal epileptic field potentials riding on the final shoulder of the slow potential change where, normally, depression of spontaneous activity is observed. Ictal epileptic field potentials could either start before the spreading depolarization, transiently interrupted by the slow potential change and reappearing on its final shoulder, as shown in Fig. 1, or they could only start on its final shoulder as shown in Fig. 2 from the same patient. Figure 2 also illustrates that spread of the ictal epileptic field potentials was not invariably observed between electrodes but the ictal epileptic field potentials could remain locally restricted to one electrode. The ictal epileptic field potentials were characterized by spike and polyspike wave activity in one patient (Figs 1 and 2) or rhythmic sharp waves in the other (Fig. 3). Ictal epileptic field potentials were highly synchronized between neighbouring recording sites (Fig. 1). Both patients with spreading convulsions also had ictal epileptic events in the electrocorticography isolated from spreading depolarizations ($n = 54$ events) (Fig. 3). One of the patients displayed repeated spells with breath holding and tonic posturing in conjunction with the isolated ictal epileptic events in the electrocorticography. The patient was treated with levetiracetam abolishing both epileptic symptoms and electrocorticography events. In the other patient, the ictal epileptic activities occurred while the patient was unattended and resolved spontaneously. Nevertheless, both patients were readmitted to hospital for a generalized status epilepticus after 3 and 12 months, respectively (Table 1). Another patient had no spreading convulsions during the monitoring period but

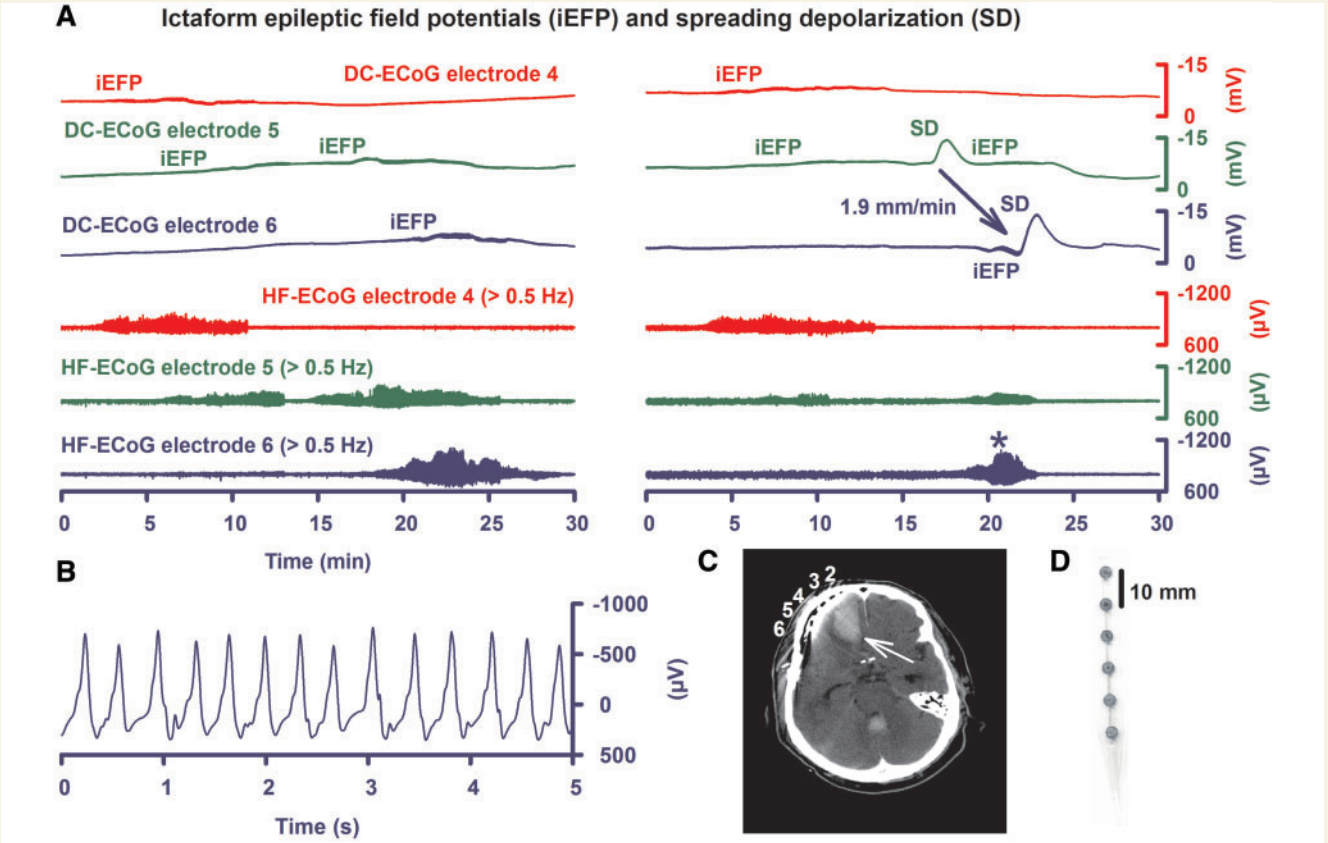


**Figure 3** Electrocorticography recording showing the cooccurrence of ictal epileptic field potentials with spreading depolarization at the vicinity of a 6 × 2 cm right frontal intracerebral haematoma. (**A**) On the left, isolated ictal epileptic field potentials are shown spreading from Electrodes 4–6. Such events were clinically associated with short epileptic spells of breath holding and tonic posturing in this unconscious patient. Epileptic ictal activity and seizures disappeared after treatment was started with levetiracetam. On the right, another time period is demonstrated in which ictal epileptic field potentials at Electrode 5 precede and follow the spreading depolarization (spreading convulsion). In the upper three traces the full frequency band from 0 to 45 Hz is given including the direct current component in contrast to Figs 1 and 2 where the lower frequency limit was 0.01 Hz. Full band recordings allow assessment of the slow potential change (indicated by spreading depolarization in the figure) without signal distortion. It is obvious that the slow potential change of spreading depolarization (up to 10.7 mV in the figure) is much larger than that associated with the ictal epileptic field potentials (up to 1.8 mV in the figure). In the lower three traces, a lower frequency limit of 0.5 Hz caused the lower frequency components of the electrocorticography to disappear which eases the assessment of the high-frequency band $>0.5$ Hz containing the ictal epileptic field potentials. (**B**) Three per second sharp waves superimposed on shallower delta activity at Electrode 6. In (**A**), this episode is indicated by the star in the lowest recording trace. (**C**) CT showing the intracerebral haematoma (white arrow) in the right frontal lobe of the patient and Electrodes 2–6 of the subdural recording strip. Note that both ictal epileptic field potentials and spreading depolarization seem to spread away from the haematoma site. (**D**) Subdural 6-contact (platinum) electrocorticography recording strip.

one isolated ictal epileptic event in the electrocorticography characterized by a 3 /s spike-and-wave pattern and associated with oral automatisms.

## An arterial pulse artefact mimicking spreading convulsions in patients with aneurismal subarachnoid haemorrhage

We observed an interesting arterial pulse artefact in seven spreading depolarizations of three patients where the spindle-shaped, arterial pulse-synchronous electrocorticography changes occurred superimposed on the peak of the slow potential change in a single electrode (Fig. 4 and Table 1). This arterial pulse artefact should not be mistaken for ictal epileptic field potentials riding on the slow potential change.

## *In vitro* study of spreading convulsions

Microinjection of KCl into neocortical slices (layer VI, $n = 119$ spreading depolarizations in 38 slices from Patients 26–37) elicited displacements of the direct current potential consisting of initially negative shifts, followed by positive waves (Fig. 5A). The amplitude and duration of negative direct current deflections were $-11.6 \pm 0.7$ mV and $93 \pm 12.4$ s, respectively. The triggered wave propagated against the flow of the superfusate and reached first the nearby electrode, and a few seconds later the other electrode located closer to the inlet of superfusate in the chamber. The

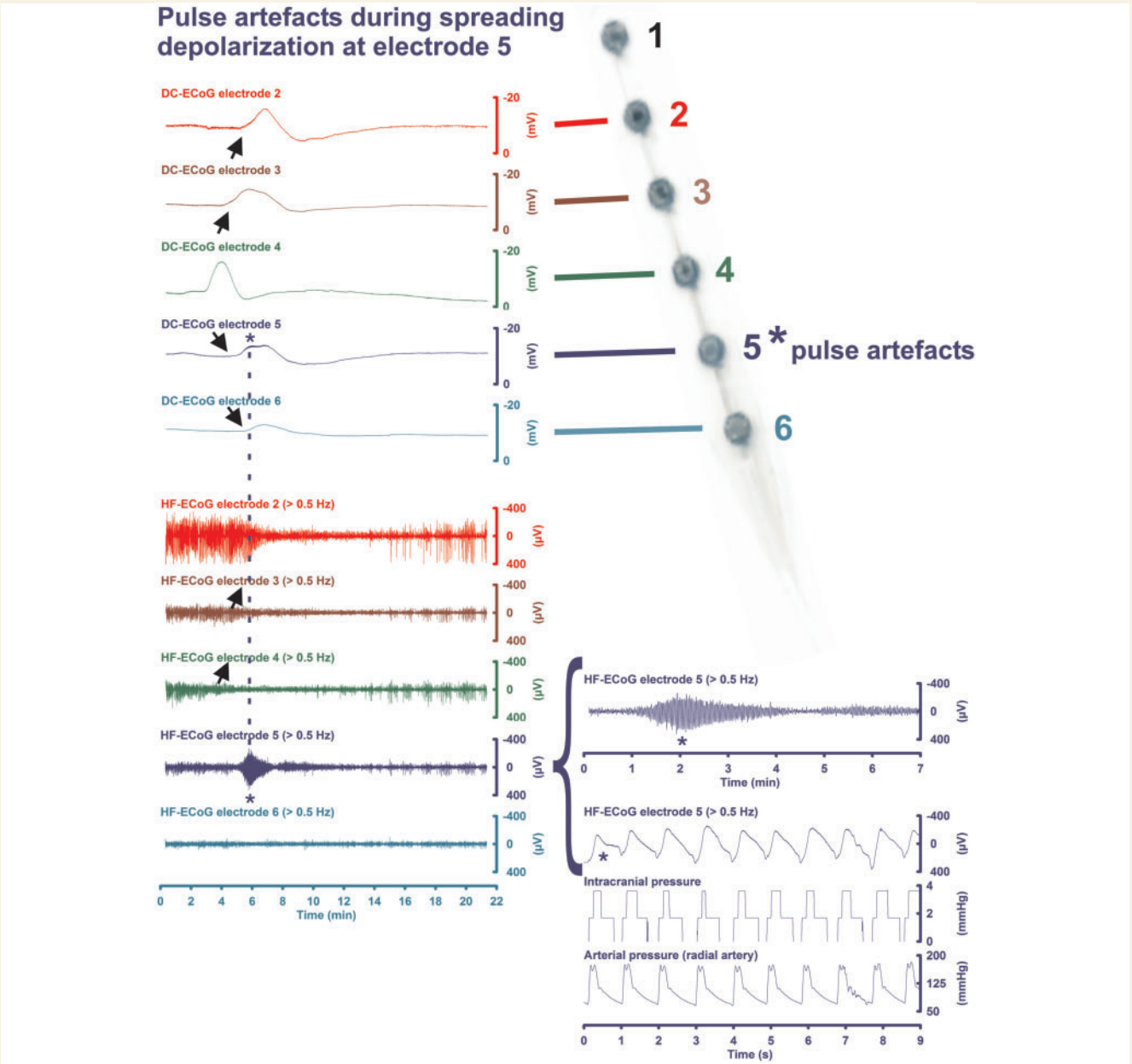


**Figure 4** Electrocorticography recording of arterial pulse artefacts on the peak of the slow potential change of spreading depolarization at only one electrode (Electrode 5). The upper five traces show the full band recordings from 0 to 45 Hz including the direct current component and thus containing the slow potential change. The lower five traces simultaneously demonstrate the high-frequency (HF) recordings excluding the direct current (DC) component but easing the assessment of the arterial pulse-synchronous electrocorticography changes at Electrode 5 and the spreading depression of activity at Electrodes 2–5. At Electrode 6, the brain electrical activity had been already depressed before the spreading depolarization started. Hence, the spreading depolarization, as indicated by the slow potential change, did not lead to spreading depression of activity here.

propagation velocity of the spreading depolarizations was determined by dividing the distance between two microelectrodes by the interval between the direct current potential shift appearances. The velocity of vertical propagation of the negative direct current shift was $3.3 \pm 0.1$ mm/min.

In slices obtained from three patients (Patients 26, 32 and 37), induction of spreading depolarization ($n = 35$ events in 10 slices; interval between elicitations: 45 min) spontaneously and repeatedly triggered ictal epileptic field potentials within 10–20 s (Fig. 5A). The ictal epileptic field potential first appeared in layer V (amplitude of $1.9 \pm 0.8$ mV and duration of $98 \pm 12$ s) propagated opposite to the direction of the flow of the superfusate to layer III (amplitude of $0.7 \pm 0.7$ mV and duration of $35 \pm 9$ s) at a velocity of $5.7 \pm 0.2$ mm/min (Fig. 5A). All slices obtained from these three patients showed such spreading convulsions.

In nine patients (Patients 27–31 and 33–36), induction of spreading depolarization in neocortical slices ($n = 18$ slices) did

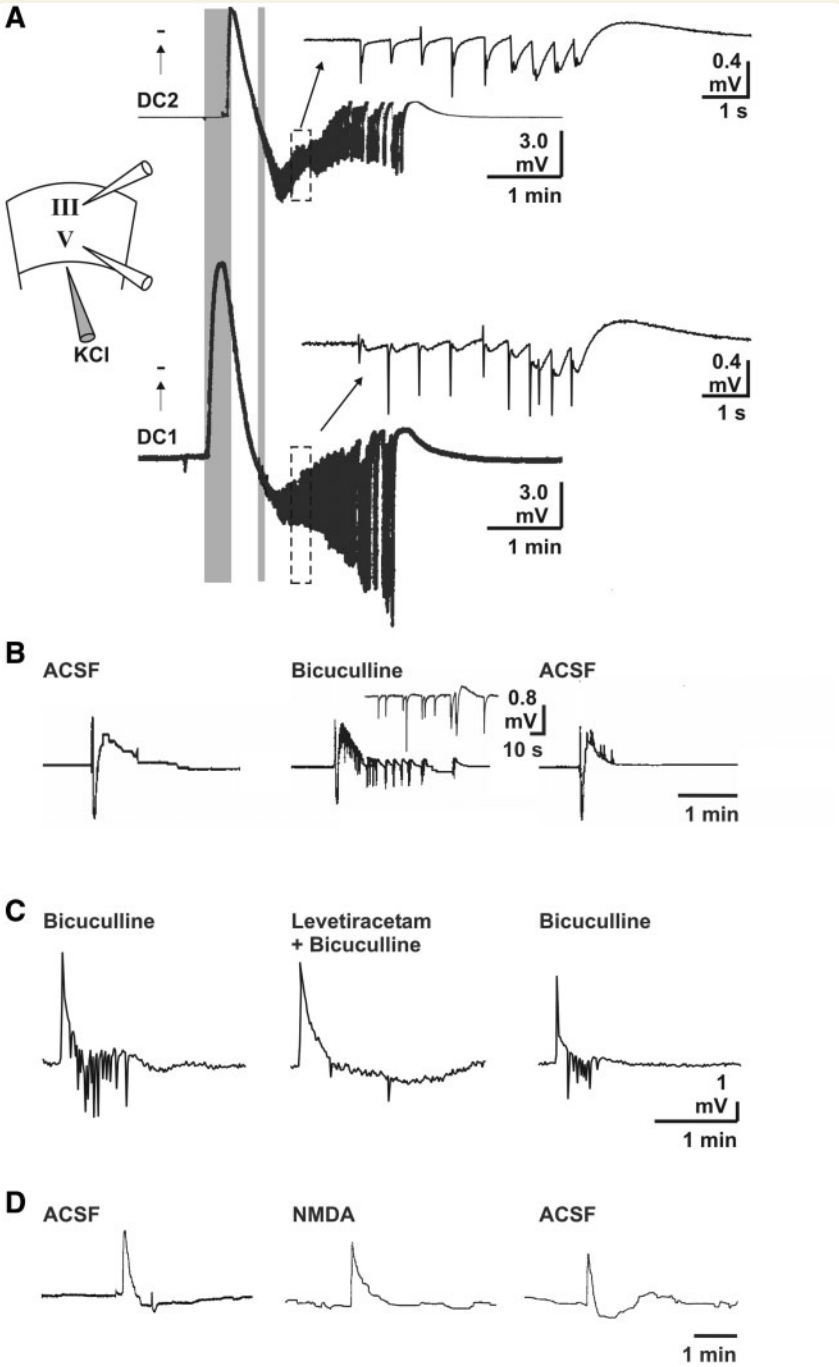


**Figure 5** Spreading convulsions in human temporal neocortex slices obtained during epilepsy surgery. Spreading depolarizations were induced in these experiments by KCl (2 *M*) microinjection in layer six. (**A**) Propagation of the negative direct current (DC) (slow potential) shift characteristic of spreading depolarization from layer five to three followed by ictal epileptic field potentials. In this case, the spreading depolarization triggered the ictal epileptic field potentials spontaneously, i.e. without addition of bicuculline. Note the slow propagation of the spreading depolarization from layer five to three, in contrast to the synchronization of the later ictal burst discharges between the two layers. Lower rows traced by ink writer and upper rows recorded by a digital oscilloscope. (**B**) The $GABA_A$ antagonist bicuculline caused the spreading depolarization to trigger ictal epileptic field potentials in those cases where it did not trigger the ictal epileptic field potentials spontaneously. From *left* to *right*: spreading depolarization before, 45 min after bicuculline (5 μM) application and 45 min after washout of bicuculline (lower trace in the middle: digital oscilloscope; upper trace: ink writer). (**C**) Addition of levetiractem (500 μM) to the bath solution inhibited the induction of ictal epileptic field potentials by the spreading depolarization under continuous application of bicuculline. This effect was reversible. (**D**) *N*-methyl-D-aspartate did not cause spreading depolarizations to trigger ictal epileptic field potentials. Spreading depolarization before 45 min, after application of *N*-methyl-D-aspartate (10 μM) and 45 min after washout.

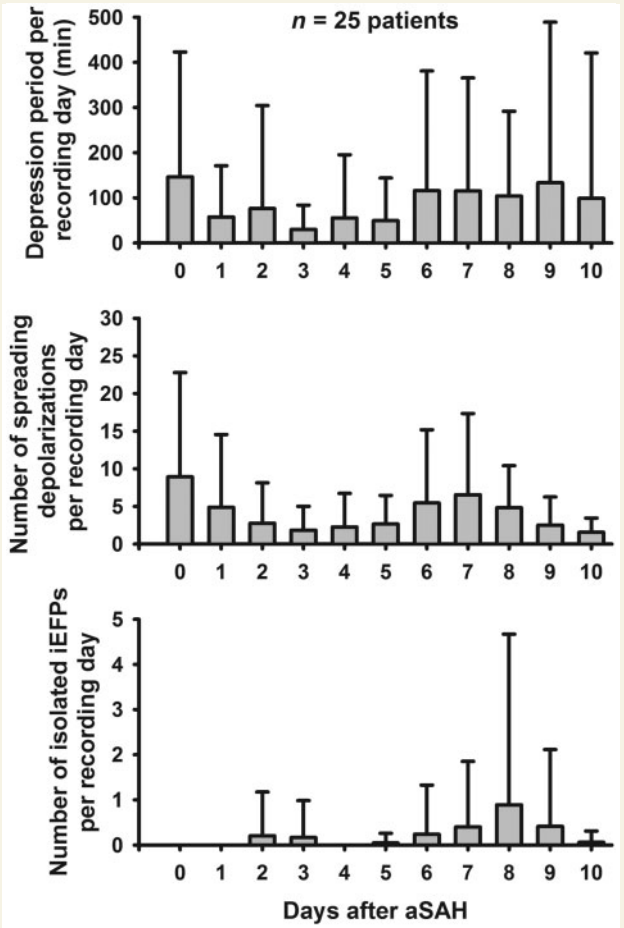


**Figure 6** Temporal distribution of the average depression periods, spreading depolarizations and isolated ictal epileptic events (iEFPs) in the 25 patients over the first 11 days after aneurismal SAH. Whereas the depression periods and spreading depolarizations showed both an early peak on the day of the haemorrhage and a delayed peak on Day 7 after aneurismal SAH, the isolated ictal epileptic events only showed a delayed peak on Day 8. Spreading depolarizations were significantly more frequent than isolated ictal epileptic events on every single day.

not trigger ictal epileptic field potentials. In order to study the possible role of reduced inhibition for the development of spreading convulsions, a low concentration of bicuculline was applied (Fig. 5B). After addition of bicuculline (5 μM) to the superfusate for 45 min, induction of spreading depolarization triggered ictal epileptic field potentials in all tested slices (second spreading depolarization in Fig. 5B; $n = 16$). After washout of the drug for 45 min, induction of spreading depolarizations was not followed by ictal epileptic field potentials anymore in 14 of 16 slices (third spreading depolarization in Fig. 5B). In control experiments in which no spreading depolarization was triggered, application of low-dose bicuculline upon human neocortical slices for at least 120 min did not elicit any ictal epileptic field potentials (data not shown). Addition of levetiracetam (500 μM) to the bath solution in six slices (Patients 34–36) inhibited the induction of ictal epileptic field potentials by the spreading depolarization under continuous application of bicuculline (second spreading depolarization in Fig. 5C). In 12 slices obtained from five patients (Patients 31 and 33–36), addition of *N*-methyl-D-aspartate (10 μM) to the bath solution for 30–45 min did not trigger any ictal epileptic field potentials (second spreading depolarization in Fig. 5D). Induction of spreading depolarization after washout of *N*-methyl-D-aspartate for 45 min did neither evoke any ictal epileptic field potentials (Fig. 5D).

## Frequency and temporal distribution of spreading depolarizations and isolated ictal epileptic events after aneurismal subarachnoid haemorrhage

Twenty-one of the 25 aneurismal SAH patients (84%) displayed 656 spreading depolarizations, and 3 of 25 patients (12%) 55 isolated ictal epileptic events. The slow potential change of the spreading depolarizations showed an amplitude of −2.6 (−1.4, −2.7) mV as assessed in the bandpass-filtered recordings of the GT205 amplifier (bandpass: 0.01–45 Hz). The propagation velocity of the spreading depolarizations was 2.5 (1.9–3.6) mm/min assuming an ideal linear spread along the electrode strip. The peak-to-peak amplitude of the isolated ictal epileptic events was 1046 (952–1858) μV and their propagation velocity 4.2 (2.9–20.9) mm/min (range from 1.5 to 37.5 mm/min) (bandpass: 0.01–45 Hz). Figure 6 shows the temporal distributions of the average depression periods, spreading depolarizations and isolated ictal epileptic events per recording day over the first 11 days after aneurismal SAH (Day 0 started with the time point of the initial haemorrhage). Whereas the spreading depolarizations and associated depression periods both showed an early peak on the day of the initial haemorrhage and a delayed peak on Day 7 after aneurismal SAH, the isolated ictal epileptic events only showed a delayed peak on Day 8. Spreading depolarizations were more frequent than isolated ictal epileptic events on every single day ($P = 0.011$, Mann–Whitney rank sum test, Day 0 was not tested because recordings were performed in only 8 of the 25 patients).

## Clinical and radiological characterization of the aneurismal subarachnoid haemorrhage study population

Clinical assessment during the first 10 days after aneurismal SAH was limited in this patient population with mostly severe aneurismal SAH. Only 7 of the 25 patients showed days with a Glasgow Coma Scale score above 13 which allowed neurologic assessment to some degree. Six of those patients had spreading depolarizations leading to spreading depression of activity. Interestingly, none of those reported on the creeping symptoms typical of migraine aura. One patient with up to 40.1 spreading depolarizations per recording day and 442.8 min of electrocorticography depression on Day 6 developed an obsessive–compulsive behaviour with frequent and intense tooth brushing that she had never displayed

before. On Day 7, a transient right arm paresis was noted. Two of the seven patients developed marked falls in consciousness (from a Glasgow Coma Scale score of 14 and 15, respectively, to 3), focal neurological deficits and delayed infarcts in temporal relationship with clusters of spreading depolarizations associated with permanent depression of brain electrical activity. Sixteen of the 25 patients showed evidence of structural brain damage in the ipsilateral hemisphere in the early peri-operative time window (Days 0–4). This consisted of either an early ischaemic infarct ($n = 3$) or an ipsilateral intracerebral haematoma ($n = 4$) or both ($n = 9$). Ten patients developed a CT proven delayed ischaemic infarct. One patient showed a delayed ischaemic neurological deficit (transient paresis of the right arm) without CT-proven infarct. Delayed cerebral ischaemia thus occurred in 11 of the 25 patients (44%) in this population with high World Federation of Neurological Surgeons and Fisher grades. According to transcranial Doppler sonography criteria, 11 of the 25 patients (44%) had significant, 44% insignificant and 3 of 25 patients (12%) had no evidence of proximal vasospasm. No significant relationship between delayed cerebral ischaemia and proximal vasospasm was observed.

## Onset and late post-haemorrhagic epileptic seizures

Ten of the 25 patients were reported to have onset seizures (40%). Five patients died within the first 6 months. Two patients were unavailable for the semi-structured telephone interview 3 years after the initial haemorrhage. Eight of the remaining 18 patients (44.4%) had developed late post-haemorrhagic seizures (Table 1). One patient had only one complex partial seizure but was treated with lamotrigine by her neurologist based on the frequent seizure recurrence in this patient population (Claassen *et al.*, 2003). No further seizure was observed under this medication. The patient fulfilled the criterion for a post-haemorrhagic seizure but failed the criteria for epilepsy, which includes the occurrence of two or more seizures (1989). The other patients had two or more seizures. One patient developed complex partial and the remaining six secondarily generalized seizures. The proportion of patients developing late post-haemorrhagic seizures was similar in patients who presented with and without onset seizures (42.9% versus 45.5%).

## Relationship of spreading depolarizations with clinical and radiological parameters

During Days 1–4 after aneurismal SAH, the peak depression period of the 24 h recording episodes showed a statistical trend towards a longer duration in patients with early structural brain damage [53.0 (18.4–377.9) versus 0.0 (0.0–129.0) min/24 h, $P = 0.095$, $n = 24$, Mann–Whitney rank sum test]; the difference in the peak number of spreading depolarizations was insignificant [8.0 (3.4–18.5) versus 0.0 (0.0–7.0) events per 24 h]. During Days 5–10 after aneurismal SAH, the peak depression period was significantly longer [201.4 (102.4–676.8) versus 43.5 (0.0–117.9) min, $P = 0.029$, $n = 21$, Mann–Whitney rank sum test], and the peak number of spreading depolarizations showed a statistical trend towards a higher incidence in patients with delayed cerebral ischaemia [12.0 (3.6–13.1) versus 4.0 (0.0–7.9) events per day, $P = 0.093$, Mann–Whitney rank sum test]. Among the 20 survivors at 6 months, patients with poor outcome (extended Glasgow Outcome Scale scores 1–4) showed a longer total depression period per recording day than patients with good outcome (extended Glasgow Outcome Scale scores 5–7). This difference was highly significant [72.3 (31.3–288.6) versus 7.4 (0.0–20.1) min per recording day, $P = 0.007$, $n = 20$, Mann–Whitney rank sum test]. Moreover, the total number of spreading depolarizations per recording day was significantly higher in patients with poor versus good outcome [3.1 (2.3–6.0) versus 1.2 (0.0–1.8) events per recording day, $P = 0.017$, $n = 20$, Mann–Whitney rank sum test]. Peak depression periods and peak numbers of spreading depolarizations were significantly different as well [307.5 (146.2–583.3) versus 37.5 (0.0–89.2) min, $P = 0.014$, and 13.0 (11.0–30.4) versus 6.9 (0.0–8.7) events, $P = 0.039$, respectively, $n = 20$, Mann–Whitney rank sum test]. Patients with either early or late structural brain damage showed a higher proportion of late epilepsy (8 of 14, 57.1%) than patients without structural brain damage (0 of 4, 0.0%) but this only showed a statistical trend ($P = 0.092$, $n = 18$, Fisher's exact test). In patients developing late post-haemorrhagic seizures, the peak number of spreading depolarizations was significantly higher [15.1 (11.4–30.8) versus 7.0 (0.8–11.2) events per day, $P = 0.045$, $n = 18$, Mann–Whitney rank sum test]. The peak depression period showed a statistical trend towards a longer duration in patients who developed late post-haemorrhagic seizures [365.6 (161.6–688.4) versus 69.3 (6.3–149.1) min, $P = 0.056$, $n = 18$, Mann–Whitney rank sum test].

# Discussion

Until the turn of the millennium, electrophysiological evidence of the major pathological cortical network events had been restricted to the spectrum of epileptic activities in the clinic. The last decade has, however, seen a remarkable progress in the translation of technologies from bench to bedside that has allowed the identification of the other important spectrum, the spectrum of spreading depolarizations. Only electrocorticography with subdural electrodes has thus provided unequivocal evidence that spreading depolarizations take place in abundance in individuals with structural brain damage (Strong *et al.*, 2002; Dreier *et al.*, 2006, 2011; Fabricius *et al.*, 2006; Dohmen *et al.*, 2008; Hartings *et al.*, 2011). Refinement of recording technology and analysis made it possible to identify different depression patterns associated with spreading depolarization (Dreier *et al.*, 2006, 2011; Fabricius *et al.*, 2006; Hartings *et al.*, 2011). Direct current amplifiers for direct current electrocorticography and laser Doppler flowmetry for regional cerebral blood flow recordings enabled description of the two fundamental types of haemodynamic responses to spreading depolarization, the normal and the inverse response (Dreier *et al.*, 2009). Moreover, very prolonged negative direct current shifts were found, typical of spreading depolarization in the core of ischaemia; often referred to as anoxic depolarization

(Oliveira-Ferreira *et al.*, 2010). The present article provides unequivocal electrophysiological evidence that spreading convulsions occur in patients. Hence, practically all major pathological cortical network events in animals have now been observed in people. Identification of spreading convulsions was possible through the use of monopolar recordings where the signal of one electrode is not contaminated by that of another electrode in contrast to bipolar recordings (Fabricius *et al.*, 2008). Monopolar recordings thus allow for the detailed analysis of the temporal relationship between the large slow potential change and ictal epileptic field potentials at one recording site. Direct current recordings are particularly helpful since the slow potential change carrying the ictal epileptic field potentials are not distorted (Hartings *et al.*, 2009).

## Basic features of epileptic activities in comparison with spreading depolarization

At the cellular level, epileptic activity is characterized by the so-called paroxysmal depolarization shift. A paroxysmal depolarization shift lasts for 80–200 ms and is the correlate of a synchronous network event resulting from a giant excitatory postsynaptic potential. The giant excitatory postsynaptic potential is presumably the consequence of synchronous activation of recurrent excitatory paths (Ayala *et al.*, 1973). The interictal spike is the correlate of the paroxysmal depolarization shift in the electrocorticography trace. Ictal epileptic activity is assumed to result from longer cellular depolarizations, which are probably the consequence of a melting of paroxysmal depolarizations shifts; ictal epileptic field potentials are its correlate in the electrocorticography trace; their clinical correlate is an epileptic seizure. Of note, the sustained depolarization underlying ictal epileptic field potentials remains below the inactivation threshold for the action potential-generating channels. This allows for continuous, synchronous, highly frequent firing of the neurons that occurs superimposed on the moderate sustained depolarization. In contrast, spreading depolarization is characterized by near-complete sustained depolarization above the inactivation threshold for the action potential generating channels and in consequence initiates electrical silencing (depression) (Kager *et al.*, 2002). The depression period nevertheless outlasts the near-complete sustained depolarization period indicating additional mechanisms underlying the blockade of action potential generation such as the delayed recovery of calcium homoeostasis (Somjen, 2001).

Interictal spikes and ictal epileptic field potentials spread between neurons. The propagation rate is usually higher than that of spreading depolarization, implicating different mechanisms of spread (Weissinger *et al.*, 2005). In the present study, however, we not only observed a fast spread of isolated ictal epileptic events of almost 40 mm/min but also a slow spread similar to that of spreading depolarization. This is particularly impressive in Fig. 1 where the spreading depolarization developed into an isolated ictal epileptic event at more distal recording sites. This event continued to propagate in the tissue at the same slow rate as the spreading depolarization. We can only speculate whether such a scenario may underlie a Jacksonian march as previously suggested by Leão since the patient was unattended during this moment and his Glasgow Coma Scale score was only 6 (Leão, 1972).

## The relationship between epileptic activities and spreading depolarization is complex

There are several common pathways for the induction of epileptiform burst discharges and spreading depolarization in acute experimental epilepsy models such as decline in extracellular magnesium (Mody *et al.*, 1987; Avoli *et al.*, 1991), increase in extracellular potassium (Somjen, 2001; Gabriel *et al.*, 2004), blockade of the sodium pump (Balestrino *et al.*, 1999; Vaillend *et al.*, 2002) and inhibition of GABA receptors (Hablitz and Heinemann, 1989; Köhling *et al.*, 2003). Moreover, spreading depolarization can be initiated in a susceptible area by a single discharge of an epileptic focus termed spike-triggered spreading depolarization (Koroleva and Bures, 1983). Repeated spreading depolarizations may enhance epileptic activities (Gorji and Speckmann, 2004) by selective suppression of GABAergic function (Kruger *et al.*, 1996). Nevertheless, the relationship between epileptic activity and spreading depolarization is complex since the susceptibility to spreading depolarization is increased in acute epilepsy models but it seems to be strongly decreased in chronic epilepsy (Koroleva *et al.*, 1993; Tomkins *et al.*, 2007). In addition to differences in cytoarchitecture and tissue electrical resistivity between rodent and primate cortex (Van Harreveld *et al.*, 1956), this was possibly a major reason why McLachlan and Girvin failed to trigger spreading depolarizations in the exposed cortex of patients with epilepsy using electrode configurations and current intensities similar to those that consistently provoked spreading depolarizations in normal rats (McLachlan and Girvin, 1994). Correspondingly with this notion, it was recently found that the potassium threshold for spreading depolarization was markedly elevated in brain slices from chronically epileptic rats compared with age-matched controls, and this threshold of epileptic rodent tissue was similarly high as that of brain slices from patients with intractable epilepsy (Maslarova *et al.*, 2011). This finding does not preclude the occurrence of epileptic spike-triggered spreading depolarizations in chronically epileptic patients (Koroleva and Bures, 1983) but it suggests the development of intrinsic protective mechanisms in epileptic tissue against them. Recurrent spike-triggered spreading depolarizations presumably explain the 2.4 times higher likelihood of patients with epilepsy to suffer from migraine, mostly migraine with aura, than their relatives without epilepsy (Marks and Ehrenberg, 1993; Ottman and Lipton, 1994). Speculatively, the frequency of migraine aura would be substantially higher without such intrinsic protective mechanisms against spreading depolarization in chronically epileptic tissues.

In contrast to chronic epilepsy where the neuronal network disturbance thus favours the occurrence of ictal epileptic events over that of spreading depolarization, it was shown previously in patients that acute brain injury favours the occurrence of spreading depolarization over that of ictal epileptic events

(Fabricius *et al.*, 2008). This notion was strongly supported in the present clinical study on aneurismal SAH.

## Spreading convulsion, a hybrid of spreading depolarization and ictal epileptic field potentials

Another degree of complexity comes into play when the intersection between ictal epileptic activities and spreading depolarization is considered. The correlate of spreading depolarization in electrocorticography traces is the large slow potential change propagating at a rate of ~2–8 mm/min (Canals *et al.*, 2005). Seconds ahead of this slow potential change, a subthreshold pacemaker field oscillation was recorded in rat hippocampus (Herreras *et al.*, 1994). This entailed a synchronization of the initial firing among nearby neurons that presented as a high-frequency burst of population spikes lasting for ~1–5 s. This synchronization is presumably carried by intercellular calcium communication between neurons via gap junctions (Herreras *et al.*, 1994; Kunkler and Kraig, 1998). Of note, such 'epileptoid' activity occurs in healthy tissue ahead of spreading depolarization and does not represent full-blown ictal epileptic field potentials. Nevertheless, ictal epileptic field potentials can precede spreading depolarization in acute epilepsy models in human and rodent brain slices, induced by electrical stimulation or low magnesium, for example (Mody *et al.*, 1987; Avoli *et al.*, 1991; Bragin *et al.*, 1997). In a similar fashion, ictal epileptic field potentials were also demonstrated in the front of spreading depolarizations in brain-injured patients (Fabricius *et al.*, 2008). Under such conditions, ictal epileptic field potentials and spreading depolarization usually occur in an alternating fashion and, after recovery from spreading depolarization, ictal epileptic field potentials are still blocked for periods of up to 10 min (Mody *et al.*, 1987; Avoli *et al.*, 1991; Kreisman and Smith, 1993; Bragin *et al.*, 1997). Spreading convulsions are characterized by the opposite behaviour as ictal epileptic field potentials occur superimposed on the final shoulder of the slow potential change where, normally, depression of activity is observed (Van Harreveld and Stamm, 1953). In our *in vivo* recordings, the ictal epileptic field potentials of the spreading convulsions could outlast the large slow potential change by >10 min.

## Mechanisms of spreading convulsion

Following spreading depolarization in healthy rodent cortex, the restoration of inhibitory interneurons seems to be delayed compared with that of excitatory neurons (Piilgaard and Lauritzen, 2009). This might contribute to the mechanism of spreading convulsions but their actual occurrence is restricted to more or less unphysiological conditions. Thus, Van Harreveld and Stamm (1953) found spreading convulsions under hypercapnia as well as cholinergic drugs (pilocarpine, acetylcholine). Later, spreading convulsions were observed in juvenile hippocampal slices induced by hyperthermia or bath-applied 4-aminopyridine (Psarropoulou and Avoli, 1993; Wu and Fisher, 2000). In juvenile hippocampal slice cultures, spreading convulsions were found both under low magnesium and in response to electrical stimulation under either replacement of NaCl with sodium acetate in the bathing medium or reduction of oxygen to a low normal level (Kunkler and Kraig, 1998; Pomper *et al.*, 2004). The present *in vitro* study on neocortex slices from patients with intractable epilepsy revealed that a reduction of inhibitory tone plays a critical role for spreading convulsions. The proportion of spreading convulsions thus increased significantly when we applied a $GABA_A$ and small-conductance calcium-activated potassium channel antagonist. Impairment of GABA-mediated inhibition is believed to increase the susceptibility of neuronal tissues to both ictal epileptic field potentials and spreading depolarization. Application of GABA blocks the propagation of spreading depolarization (Ochs and Hunt, 1960), and GABA receptor blockade elicits spreading depolarization in neocortical tissues (Hablitz and Heinemann, 1989; Köhling *et al.*, 2003). Vice versa, spreading depolarization in rat neocortex modulates $GABA_A$ binding sites in several subcortical regions (Haghir *et al.*, 2009). Disequilibrium of the chloride distribution was suggested in selected patients with intractable epilepsy (Gorji *et al.*, 2006). Perturbation of chloride homeostasis and GABAergic signalling in downstream regions are thought to facilitate induction of epileptic discharges (Hochman *et al.*, 1999) and spreading depolarization (Lenz *et al.*, 1997). Evidence from animal models and human studies suggests that altered expression of GABA receptors may contribute to the pathogenesis of medically intractable epilepsy (Loup *et al.*, 2000). Whether such mechanisms play a role in epileptogenesis after aneurismal SAH is unknown. It is nevertheless interesting in this respect that two patients with aneurismal SAH who were later readmitted to hospital for status epilepticus had shown spreading convulsions during the monitoring period although this finding is not sufficient to suggest any statistical association.

The arterial pulse artefacts riding on the peak of the slow potential change in some of the spreading depolarizations should not be mistaken for ictal epileptic field potentials. They are possibly the consequence of an increased arterial pulse amplitude that was recently described in response to the spreading depolarization induced spreading hyperaemia using laser Doppler flowmetry (Dreier *et al.*, 2009). Whether they are always associated with hyperaemia deserves further study however.

## Potential clinical implications of spreading depolarizations for epileptogenesis

The proportion of patients with late post-haemorrhagic seizures was higher in our study than in previous surveys (Claassen *et al.*, 2003; Buczacki *et al.*, 2004; Gilmore *et al.*, 2010). This was presumably due to the severity of the initial haemorrhage, a high rate of intracerebral haemorrhage, the high proportion of delayed cerebral ischaemia, the prospective design of the study and the fact that the indication for neurosurgical intervention was an inclusion criterion (Gilmore *et al.*, 2010). The only statistically significant finding associated with the development of late post-haemorrhagic seizures was a higher peak number of spreading depolarizations in the electrocorticography monitored,

subacute phase after aneurismal SAH. Within the limitations of a small study, this is an interesting observation but the peak number of spreading depolarizations may not be an independent risk factor since there also seems a relationship between spreading depolarizations and the early and delayed development of brain damage after aneurismal SAH (Dreier *et al.*, 2006, 2009; Bosche *et al.*, 2010). This was statistically significant for the development of delayed cerebral ischaemia and the peak depression period during Days 5–10 after aneurismal SAH in the present study. Moreover, patients surviving aneurismal SAH with poor outcome showed significantly longer total and peak depression periods as well as significantly higher total and peak numbers of spreading depolarizations than patients with good outcome. These issues have to be investigated in a larger trial. Such a trial should also consider the blood–brain barrier disruption after aneurismal SAH (Doczi *et al.*, 1984), which can be assessed in serial magnetic resonance images. Blood–brain barrier disruption is currently under intense investigation as a causative factor for post-traumatic epilepsy (Shlosberg *et al.*, 2010). As spreading depolarizations induce blood–brain barrier disruption in animals (Gursoy-Ozdemir *et al.*, 2004), there could be a complex cycle by which spreading depolarizations, brain damage and blood–brain barrier disruption contribute to epileptogenesis after aneurismal SAH. Targeting spreading depolarizations early in the course of the disease could potentially interrupt this cycle and in consequence lower the incidence of late post-haemorrhagic seizures. This could be more promising than the prophylactic anti-convulsant treatment of early seizures, which has consistently failed to reduce the risk for late epilepsy after traumatic brain injury for example (Schierhout and Roberts, 1998). Candidate drugs should be selected with great care in preclinical studies. Some anti-convulsants also lower the susceptibility to spreading depolarization (Ayata *et al.*, 2006), but it is likely that their effects are too weak in patients with aneurismal SAH as spreading depolarizations are increasingly pharmaco-resistant in energy-depleted tissue (Somjen, 2001; Dreier, 2011).

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