Aus dem Zentrum für Schlaganfallforschung
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Cortical spreading depolarization in patients with aneurysmal subarachnoid hemorrhage

zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)

vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

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Datum der Promotion: 24. 02. 2012

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1 Zusammenfassung

In Deutschland erleiden jedes Jahr etwa 6000 Patienten eine aneurysmatische Subarachnoidalblutung (aSAB). Die Mortalität dieser unmittelbar lebensbedrohlichen Erkrankung beträgt trotz intensivmedizinischer Versorgung etwa 30%. Weitere 30% der Patienten behalten schwere Behinderungen.
Die wichtigste und folgenreichste Komplikation nach der aSAB ist die verzögerte zerebrale Ischämie, sie tritt bei etwa einem Drittel aller Patienten auf und führt in etwa 10% der Fälle zu computertomografisch nachweisbaren Hirninfarkten.

Lange Zeit wurden angiografisch nachweisbare Spasmen der proximalen Hirnarterien als Hauptursache für die Entwicklung der verzögerten zerebralen Ischämie erachtet. Neuere Erkenntnisse deuten jedoch auch auf einen Zusammenhang zwischen verzögerter zerebraler Ischämie und Spreading Depolarization hin.
Der Begriff Spreading Depolarization beschreibt ein elektrophysiologisches Phänomen, bei dem sich eine anhaltende neuronal-astrogliale Zellentladung wellenartig über den Kortex ausbreitet. Die damit einhergehenden Ionenkonzentrationsveränderungen zwischen Intra- und Extrazellulärraum führen durch Wassereinstrom in die Zellen zu einem zytotoxischen Ödem. Gleichzeitig sistiert die elektrokortikografische Aktivität (Spreading Depression). Die invasive Elektrokortikografie bietet gegenwärtig die einzige Möglichkeit, Spreading Depolarization und Spreading Depression bei Patienten mit aSAB nachzuweisen. Die Implantation des subduralen Elektrodenstreifens erfolgt durch den Neurochirurgen nach der operativen Versorgung des Aneurysmas.

Für die vorliegende Dissertation wurden die elektrokortikografischen Monitoringdaten von 23 Patienten mit aSAB analysiert. 13 dieser 23 Patienten waren mit nicardipine prolonged-release implants (NPRI) behandelt worden. NPRI werden vom Neurochirurgen in die basalen Zisternen eingebracht und sollen durch die verzögerte Freisetzung ihres Wirkstoffs eine Prophylaxe des proximalen Vasospasmus bewirken. In der Elektrokortikografie waren Spreading Depolarizations als langsame Potentialveränderungen im niedrigen Frequenzspektrum, und die begleitende elektrokortikografische Depression als Amplitudenreduktion im hohen Frequenzspektrum erkennbar. Während einer 10-tägigen Monitoringphase wurden insgesamt 664 Spreading Depolarizations bei 18 der 23 Patienten (78%) beobachtet. Die Depressi-

onsdauer betrug insgesamt 104,2 Stunden. Die Depolarisationen traten entweder vereinzelt, oder gruppiert in sogenannten Clustern mit wiederkehrenden Depolarisationen und zunehmenden, mitunter andauernden Depressionsdauern auf.

Trotz einer deutlichen Reduktion von Vasospasmen in der NPRI-Gruppe konnte kein signifikanter Unterschied zwischen dem Auftreten von Spreading Depolarization oder Spreading Depression in beiden Studiengruppen nachgewiesen werden. Diese Tatsache deutet darauf hin, dass Auftreten und Schweregrad von Vasospasmen nicht mit dem Auftreten von Spreading Depolarizations korrelieren. Vielmehr deuten die Ergebnisse darauf hin dass Spreading Depolarization ein eigenständiger Mechanismus in der Entwicklung von verzögerten zerebralen Ischämien nach aSAB ist.

Nur die invasive Elektrokortikografie ermöglicht derzeit einen verlässlichen Nachweis von Spreading Depolarization. Wir erhoffen uns für die Zukunft, dass das Monitoring von Spreading Depolarizations eine Vorhersage der verzögerten zerebralen Ischämie gestattet. Auf diese Weise könnte es zukünftig zur Therapiestratifizierung verwendet werden und Patienten könnten in Abhängigkeit ihres individuellen Risikos für verzögerte Ischämien behandelt werden. Außerdem kann das Monitoring zur Entwicklung neuer Therapiekonzepte beitragen, die z. B. auf die Blockierung von Spreading Depolarization ausgerichtet sind.

2 Summary

Aneurysmal subarachnoid hemorrhage (aSAH) is a life-threatening disease. In Germany, approximately 6000 patients suffer from aSAH every year. Mortality and disability rates of patients reaching medical care are 30% and 30%, respectively. Delayed cerebral ischemia (DCI) is the most important in-hospital complication after aSAH and occurs in about one third of patients. Approximately 10% of patients with aSAH develop computed tomography (CT)-proven delayed brain infarcts.

It has been hypothesized that proximal vasospasm is the prime mechanism of DCI. Digital subtraction angiography (DSA) is the gold standard for diagnosing proximal vasospasm. More recently, it has been found that DCI is also associated with clusters of spreading depolarizations in the cortex. The term spreading depolarization describes electrophysiologically a near-complete sustained mass depolarization of neurons associated with near-complete breakdown of the ion concentration gradients across neuronal membranes and loss of electrical activity (spreading depression) as well as, biochemically and morphologically, a cytotoxic edema with distortion of dendritic spines. Invasive electrocorticography (ECoG) using a subdural strip is a reliable tool for detecting spreading depolarization. A subdural electrode strip can be implanted after surgical clipping of the ruptured aneurysm.

In my thesis, I analyzed the ECoG monitoring data of 23 patients with aSAH. 13 of the 23 patients had received nicardipine prolonged-release implants (NPRI) to prevent proximal vasospasm. While spreading depolarization was observed as a slow potential change, the accompanying spreading depression of synaptic activity was seen as an arrest of brain electrical activity. During a monitoring period of 10 days, 664 spreading depolarizations occurred in 18 of 23 patients (78%). The total ECoG depression period was 104.2 hours. Spreading depolarizations occurred either as isolated events or as parts of a cluster of recurrent depolarizations. During a cluster, a persistent depression of ECoG activity was possible between the recurrent events.

Despite a successful treatment of proximal vasospasm with NPRI, there was no significant difference in the occurrence of spreading depolarizations or depression of brain electrical activity in both study groups. This indicates that there is no correlation

between the occurrence, or the degree of proximal vasospasm and the occurrence of spreading depolarization. These findings rather imply that both proximal vasospasm and spreading depolarization act as independent factors in the pathophysiological spectrum of DCI.

Currently, only ECoG monitoring reliably detects spreading depolarization, one of the presumed key players in the pathogenesis of DCI. In the future, monitoring of spreading depolarization could become an important tool for the treatment stratification in aSAH patients and make an important contribution to the development of novel therapeutic strategies, reducing the rates of impairment, morbidity and mortality after aSAH.

3 Abbreviations

(a)SAH	(aneurysmal) subarachnoid hemorrhage
ATP	adenosine triphosphate
Ca^{2+}	calcium
CBF	cerebral blood flow
CSI	cortical spreading ischemia
CT	computed tomography
DC	direct current
DCI	delayed cerebral ischemia
DIND	delayed ischemic neurological deficit
DSA	digital subtraction angiography
ECoG	electrocorticography/electrocorticogram
GCS	Glasgow Coma Scale
K^+	potassium
MRI	magnetic resonance imaging
Na^+	sodium
NO	nitric oxide
NPRI	nicardipine prolonged-release implants
SPC	slow potential change
TCD	transcranial Doppler sonography
WFNS	World Federation of Neurological Surgeons

4 Introduction

4.1 Aneurysmal subarachnoid hemorrhage (aSAH)

The incidence of spontaneous intracranial hemorrhage after aneurysm rupture remains stable at around 6 cases per 100 000 per year (van Gijn and Rinkel, 2001). Most patients are less than 60 years old. Case fatality is approximately 50%. About one third of surviving patients will remain dependent and suffer consequently from a significant reduction in quality of life.

The severity of the initial ictus and delay in referral of patients with ruptured aneurysms to neurosurgical and intensive care accounts for a high level of mortality and morbidity (Kassell et al., 1985). Over the past years, some improvement in the timing of aneurysm surgery has been accomplished. Patients undergoing surgery within the first three days after the initial bleeding (regardless of their clinical condition on admission) are more likely to achieve a better recovery and overall outcome compared to patients with delayed surgery due to unstable clinical condition on admission (Haley et al., 1992). However, despite successful treatment of the aneurysm by clipping or coiling, complications after aneurysmal subarachnoid hemorrhage (aSAH) have remained frequent.

4.2 Complications after aSAH

Frequent in-hospital complications include rebleeding, hydrocephalus, periprocedural ischemia, and delayed cerebral ischemia (DCI) (Macdonald et al., 2007).
DCI occurs in 33-38% of all patients with aneurysmal SAH and approximately 10 % of patients eventually develop computed tomography (CT)-proven delayed brain infarcts (Woitzik et al., 2011 in press).
DCI typically develops between days 4 and 14 after aSAH. The clinical symptoms of DCI are often referred to as delayed ischemic neurological deficits (DIND). Symptoms include worsening headache, nuchal rigidity, increasing body temperature, a decline in level of consciousness and focal neurological deficits (Frontera et al., 2006; Vergouwen et al., 2008). DCI often develops gradually over hours and days in contrast to thromboembolic stroke where symptoms appear abruptly (Kassell et al.,

1985). To diagnose DCI, other causes of neurological worsening such as hydrocephalus should be excluded. For this purpose, a CT scan should be performed.

Vasospasm has been regarded the prime mechanism of DCI for many years. However, more recently, it has been increasingly questioned whether angiographic vasospasm is the only key player. Many different factors could contribute to DCI after aSAH as well, including microthrombosis, impaired large and small vessel reactivity (Vollmer et al., 1992; Ohkuma et al., 2000), systemic volume contraction (Wijdicks et al., 1985), impaired autoregulation (Jaeger et al., 2007; Neil-Dwyer et al., 1994), spreading ischemia (Dreier et al., 1998), chemical factors such as local potassium (K^+) or hemoglobin release from the blood clot (Dreier et al., 1998), oxygen free radicals (Mori et al., 2001), bilirubin oxidation products (Clark et al., 2002), or hyponatremia (Wijdicks et al., 1985).

4.3 DCI and cerebral vasospasm

Cerebral vasospasm is a result of prolonged smooth muscle cell contraction mediated by various factors, including proteins with vasoactive potential such as endothelin-1, an endogenous vasoconstrictor. Elevated levels of endothelin-1 can be found in patient plasma and cerebrospinal fluid after aSAH. Breakdown products of erythrocytes, such as oxyhemoglobin, adenosine triphosphate (ATP) and K^+ accumulate in the subarachnoid space after aneurysm rupture and also act as vasoactive agents. Fatty acids and other lipid derivates and superoxide free radicals, responsible for inactivation of nitric oxide (NO), may play a role in the pathology as well (Macdonald et al., 2007; Clark and Pyne-Geithman, 2005).

70% of patients experience angiographic vasospasm after aSAH. Of those, about one third develops DCI (Kassell et al., 1985). However, not all patients with DCI have vasospasm. Inversely, not all patients with vasospasm develop clinical symptoms and signs of DCI (Rabinstein et al., 2004; Vergouwen et al., 2008). The medical prevention of proximal vasospasm alone has not yet contributed to either a significant reduction of the appearance of DCI, or an improvement in clinical outcome (Pluta et al., 2009).

4.4 DCI and microthrombosis

It has become evident that microthrombosis, as a result of various processes such as activation of the coagulation cascade and impaired fibrinolytic activity, correlates with the development of DCI and cerebral infarction after aSAH (Vergouwen et al., 2008). Studies showing increased levels of serological coagulation markers, as well as evidence of microthrombi in the vicinity of clinically ischemic lesions in autopsy studies (Stein et al., 2006) indicate that microthrombosis may be yet another pathway in the pathogenesis of DCI. It may not be entirely independent of proximal vasospasm but may rather play a complementary role in the pathogenesis of DCI. It remains to be investigated whether microthrombosis after SAH is also associated with the occurrence of spreading depolarization.

4.5 DCI and spreading depolarization

DCI after aSAH co-occurs with spreading depolarization in electrocorticographic (ECoG) recordings (Dreier et al., 2006).
Spreading depolarization describes a wave in the cortex characterized by near-complete sustained depolarization of neurons, breakdown of ion gradients, and near-complete loss of membrane resistance. It occurs when passive cation influx across the cellular membranes exceeds ATP-depending sodium (Na^+) and calcium (Ca^{2+}) pump activity. Neuronal swelling with distortion of dendritic spines and a cessation of neuronal function with a loss of brain electrical activity follow (Takano et al., 2007).

Spreading depolarization was first discovered by Leão in rabbit brain in 1944 via the accompanying spreading depression of brain electrical activity. The term spreading depression describes a silence of brain electrical activity as an epiphenomenon of spreading depolarization since the sustained neuronal depolarization is above the inactivation threshold of the action potential generating channels. Thus, neurons loose the ability to fire signals during spreading depolarization.

Spreading depression is recorded as a cessation of activity in the higher frequency spectrum of the ECoG. This higher frequency spectrum of the ECoG is mainly due to summation of postsynaptic neuronal activity in the cortex. In contrast to spreading depression of activity, spreading depolarization is observed as a large propagating

negative slow potential change (SPC). The SPC is explained by the synchronous sustained depolarization of millions of cortical neurons. In a single neuron, a depolarization changes along the neuronal main axis, i.e. perpendicular to the surface. This creates a slowly changing intracellular electrical potential difference that is in turn associated with a slow extracellular field potential shift. Thus, the SPC that is visible in the ECoG is a summary measure for spreading depolarization in the tissue (Canals et al., 2005).

The ignition of spreading depolarization occurs passively, driven by direct electrical and diffusion forces. Energy consumption however, paradoxically increases since the correction of the intracellular Na^+ and Ca^{2+} surge requires an immediate activation of ATP-dependent Na^+ and Ca^{2+} pumps. In normal healthy brain, the intense neuronal and astrocytic depolarization acts as a potent stimulus to increase cerebral blood flow (CBF) in the cortex (spreading hyperemia) in order to meet the increased neuronal energy demand (Lauritzen, 1994). In other words, spreading depolarization does not cause a cellular energy shortage when CBF is regulated normally and oxygen and glucose supply is sufficient (Somjen, 2004).

Under certain conditions, however, spreading depolarization induces severe hypoperfusion, rather than hyperemia. These conditions include hypoxia or partially ischemic conditions, as well as the presence of high K^+ and/or low glucose levels in combination with decreased NO availability after aSAH (Dreier et al., 1998; Petzold et al., 2008). Here, the coupling of neuronal activity and CBF can invert. Thus, spreading depolarization induces severe microarterial spasm instead of vasodilatation which leads to spreading ischemia (Dreier et al., 1998).

This spreading perfusion deficit then in turn creates a vicious circle since it produces a mismatch between neuronal energy demand and supply (Strong et al., 2007). As a consequence of the energy deficit, the neurons cannot repolarize and continue to release vasoconstrictors. Through the sustained release of vasoconstrictors from neurons and astrocytes, the ischemic condition persists and prevents the repolarization of the neurons. Consistently, severe spreading ischemia can lead to wide-spread cortical necrosis (Dreier et al., 2000).

4.6 Treatment of DCI

The treatment of DCI has been the subject of a variety of clinical studies. In 2001, Treggiari-Venzi et al. collected the most relevant literature on clinical trials investigating prophylactic therapies for cerebral vasospasm in a topic review. These included 1) hypervolemic/hypertenisive/hemodilution therapy (triple-H therapy), 2) calcium antagonists such as nimodipine, nicardipine, and fasudil hydrochloride, 3) endovascular treatment such as balloon angioplasty or intra-arterial injection of vasodilators, and 4) other proposed treatments such as intracisternal fibrinolysis, antioxidants and free radical scavengers, immunosuppression, serine protease inhibitors, and thromboxane-A_2 synthetase inhibitors.

Many of these studies follow the traditional concept of assuming that proximal vasospasm acts as the prime mechanism in the pathogenesis of DCI and clinical trials focus on prevention of vasospasm with the aim to improve clinical outcome. However, successful treatment of proximal vasospasm does not translate into an improvement of patient outcome (Macdonald et al., 2007), nor can a better patient outcome in return be associated to reduced vasospasm.

In many of these clinical trials calcium antagonists were used for the prevention and treatment of DCI. Both nimodipine and nicardipine inhibit slowly inactivating, voltage-sensitive Ca^{2+} channels (L-type channels) and block Ca^{2+} entry from the extracellular space into smooth muscle which leads to cerebral vasodilatation and an increase in cerebral perfusion (Krischek et al., 2007). Initially, the rationale for the anti-ischemic mechanism of calcium antagonists was based on the assumption that the blockade of excitotoxic Ca^{2+} entry protects neurons directly. However, nimodipine failed to be consistently effective in several animal studies of focal cerebral ischemia, and thus the cytoprotective effect on neurons in ischemic stroke unrelated to aSAH has to be questioned. The clinical effect could not be explained by a reduction of angiographic vasospasm (Dreier et al., 2002).

Side effects of the systemical administration of calcium antagonists such as hypotension, pulmonary edema, and anemia are common (Treggiari-Venzi et al., 2001) and these complications are independently associated with poor outcome. It remains to be investigated whether the dissociation between vasospasm and clinical outcome is

due to the detrimental side effects that counterbalance any benefits, or because mechanisms other than vasospasm contribute to the development of DCI.

In order to reduce the side effects of a systemical administration of nicardipine, more recent clinical trials concentrate on a topical administration by implanting prolonged-release pellets (Omeis et al., 2008; Barth et al. 2007). Nicardipine prolonged-release implants (NPRI) are rod-shaped 2x10 mm polymers, containing 4 mg of nicardipine. Release kinetics and pattern of distribution of nicardipine have been described by Kasuya et al. (2002). Pellets implanted into the basal cisterns in close contact to the proximal cerebrovascular system at the time of aneurysm surgery release the drug over 14 days, covering the peak-incidence of cerebral vasospasm and spreading depolarizations while avoiding systemic side effects. They are believed to provide a better treatment option since proximal vasospasm is prevented more effectively and the cerebrospinal fluid circulation might transport the drug over the hemispheric surface where microvascular spasm and CSI are targeted. NPRI are tolerated well and no drug-related adverse events have yet been noticed (Bart et al., 2007).

4.7 Goals

The purpose of this work was to find a clinically useful approach to the ECoG monitoring data of patients with aSAH and further to establish an analytical framework that enables a standardized and reliable detection of pathological patterns. First, the duration of the total recording time, the quality of recording, spontaneous baseline activity, ictal epileptiform activity, as well as any kind of spreading depolarization events with or without corresponding depression of brain electrical activity had to be assessed. Since it is well established that events of spreading depolarization and spreading depression of brain electrical activity do occur in patients with aSAH, I tested the applicability of the analytical procedure for clinical studies by comparing the occurrence of spreading depolarization and spreading depolarization-induced spreading depression between patients treated with NPRI and those not treated.

5 Material and methods

5.1 Patient recruitment and clinical care

23 patients with major aSAH were recruited in the department of Neurosurgery, Universitätsmedizin Mannheim, Germany. Inclusion criteria for patients were

1) age > 18,
2) spontaneous aSAH,
3) necessity for surgical intervention, and
4) written consent.

CT and digital subtraction angiography (DSA) were performed on the day of admission. During surgical aneurysm clipping basal cisterns were opened and blood clots washed out. 13 of the 23 patients had NPRI placed into the basal cisterns in direct contact to the exposed vessel walls, as described by Barth et al. (2009). The remaining 10 patients without NPRI served as control group.

After surgery, all patients were transferred to the intensive care unit and bipolar ECoG recordings were acquired continuously in four active channels (A-D) from the 6-electrode (linear array) subdural strip implanted during aneurysm surgery (Strong et al., 2002; Fabricius et al., 2006). It had been aimed to place each of the electrode strips on a single gyrus, parallel to the assumed path of spreading depolarizations. Electrode 1 served as ground, electrodes 2-6 were connected in sequential bipolar fashion to an amplifier (as described by Dreier et al., 2006). Data were sampled at 200 Hz, recorded and reviewed with the use of Powerlab 16/sp analog/digital converter and Chart-6 software. Figure 1 shows a schematic drawing of the electrode strip and its connection to the amplifier.

Serial CT scans were performed post-operatively, selectively at the time of clinical deterioration, and after the monitoring period to screen for delayed infarcts. This was complemented by magnetic resonance imaging (MRI) in selected cases. Transcranial Doppler sonography (TCD) and a daily thorough neurological examination were performed at bedside on each monitoring day. Monitoring periods lasted 8-10 days for patients without clinical symptoms and 12-15 days in the case of DCI. Afterwards, the electrode strip was removed.

Data are given as median (1^{st}, 3^{rd} quartile). Statistical analysis was performed using Mann-Whitney rank sum test, two-tailed Fisher exact test, and Wilcoxon signed rank test in order to compare the number of spreading depolarizations and total duration of the ECoG depression periods in both study groups. A probability value of $P < 0.05$ was considered statistically significant.

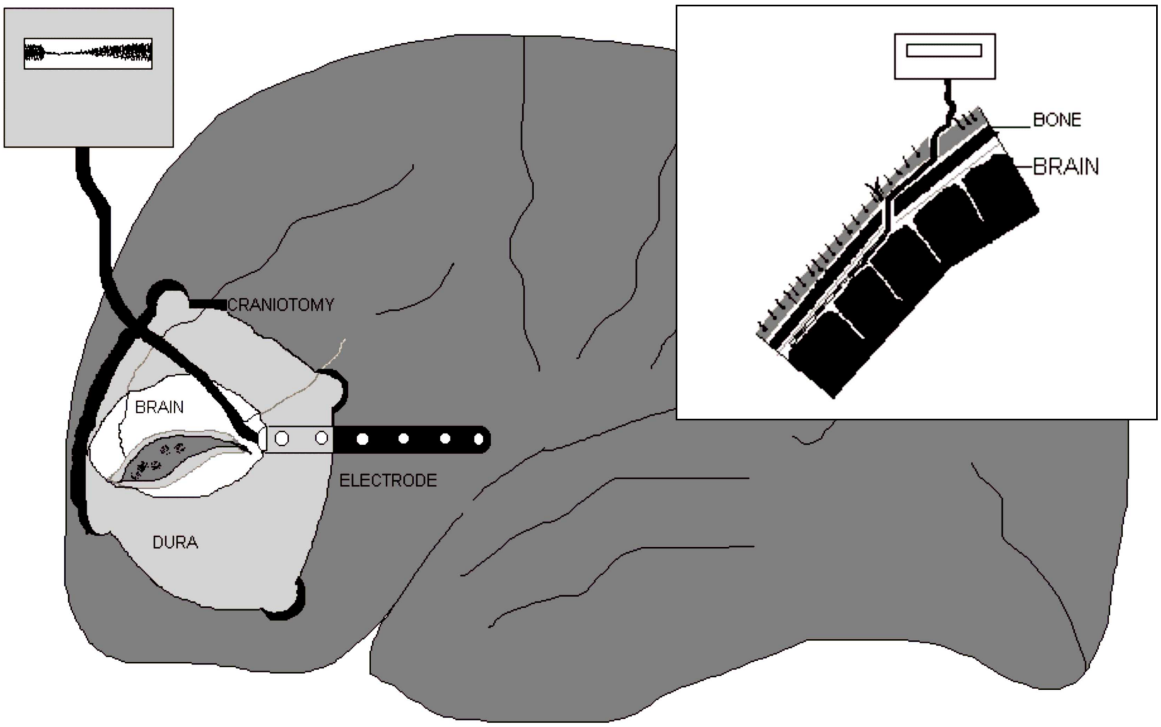

Figure 1: Electrode strip on the cortex accessible from the craniotomy. The boxed image shows the lead cable from the strip tunneled under the skin and externalized through a stab wound which allows removal of the strip at the end of the monitoring period without re-opening the wound.

6 Results

The clinical and demographic characteristics of the 23 patients, as well as monitoring data are summarized in Table 1.

No.	Age (years), sex	Duration of ECoG monitoring (h)	Number of SDs	Total duration of ECoG depression period (min)	Ictal epileptiform activity	NPRI
1	46, f	116	45	234.4		yes
2	34, f	188	136	570		yes
3	51, f	191	44	767.9		yes
4	69, m	228	31	278.6		yes
5	47, f	214	4	16.7		yes
6	43, f	139	116	1319.6		yes
7	63, m	218	22	140.9		yes
8	49, m	137	31	336.3		yes
9	71, f	172	42	359.6		Yes
10	67, f	166	0	0		yes
11	50, f	160	0	0		yes
12	54, m	159	0	0	yes	yes
13	62, f	224	51	528.1	yes	yes
14	55, f	213	10	88.6		
15	51, f	107	10	37.9		
16	61, f	193	10	82		
17	46, f	147	4	10.3		
18	68, f	126	13	85.8		
19	56, f	177	12	69.8		
20	41, f	105	51	632.9		
21	41, f	246	32	659.7		
22	50, f	20	0	0		
23	63, f	136	0	0		

Table 1: Summary of demographic, clinical and monitoring data f = female; m = male; ECoG = electrocorticography; SD = spreading depolarization; NPRI = nicardipine prolonged-release implants. For statistical evaluation, events of SiD and isoelectric depolarization were both counted as spreading depolarization events. Patients No. 1-13 formed the NPRI-group, patients No. 14-23 served as a control.

6.1 Data analysis: baseline activity and ictal epileptiform activity

Curves were scanned for quality, baseline activity and ictal epileptiform activity.
Noise signals which lasted for more than 30 minutes were neither included in further evaluations nor did they contribute to the total duration of recording time. Good quality in only one channel (with three noise channels) was accepted for up to 8 hours.
For baseline activity, the high-frequency ECoG was split into different frequency bands roughly following classically defined electroencephalographic conventions: delta (1-4Hz), theta (5-8 Hz), and alpha (9-14). To evaluate the baseline activity, an interval of approximately 1 minute was assessed every four hours, around 1 AM, 5 AM, 9 AM, 1 PM, 5 PM, and 9 PM. Baseline activity was classified as

a) flat signal,
b) burst suppression pattern,
c) burst suppression pattern in some channels, delta in others,
d) delta,
e) delta/theta, or
f) theta/alpha activity.

Ictal epileptiform activity was roughly classified as

a) spike or spike-and-wave seizures,
b) ictal train of rhythmic delta or theta activity, or
c) ictal train of rhythmic alpha or beta activity.

6.2 Data analysis: spreading depolarization and ECoG depression

Finally, curves were scanned for events of cortical spreading depolarization. Spreading depolarization was defined by the sequential onset in adjacent channels of a propagating SPC as originally described by Fabricius et al., 2006. The propagation velocity was estimated from the delay between first and second SPC of a given spreading depolarization recorded at two neighboring electrodes, and assuming a linear spread along the recording strip.

The parallel high-frequency ECoG depression was defined by a rapidly developing reduction of power of the ECoG amplitude, best visualized in the traces representing the power of the bandpass filtered ECoG (described in detail in figure 2, see below).

To determine the duration of the ECoG depression period (i.e. the interval between depression onset and onset of restoration of activity), the integral of power of the bandpass filtered activity (time constant decay, 60 s) was used, as described by Dreier et al., 2006.

The duration periods were determined separately for all channels involved in the depression. For subsequent statistical analysis, only the longest depression period was selected, since it is assumed that especially the prolonged depression periods reflect energy depletion in brain tissue (Fabricius et al., 2006; Dreier et al., 2006).

Episodes of SPC that occurred in only one channel (i.e. lacking propagation to another channel) were scored as single electrode depolarization (SiD) and counted as event of depolarization. The duration of the accompanying depression period was marked in a similar fashion to that of spreading depolarization affecting more than one electrode (described above).

The data base eventually included an account of each patient's total duration of ECoG recording, the various types of baseline activity (evaluated every 4 hours), the spreading depolarizations and the durations of ECoG depression. In case of ictal epileptiform activity, onset, duration, and number of seizures were stated. All values were correlated to the time of the initial bleeding.

6.3 Illustrative case 1: isolated spreading depolarizations, clusters of spreading depolarization, and the corresponding depression of ECoG activity.

This 62 year-old female patient suffered from World Federation of Neurological Surgeons (WFNS) Grade 5, Fisher Grade 3 aSAH and was admitted to hospital with a Glasgow Coma Scale (GCS) of 6. A clinical decision was made that surgery was required and the aneurysm was successfully clipped. NPRI and the 6-electrode subdural strip were implanted. ECoG monitoring started on the day of the bleeding. Total recording time was 224.2 hours. Delta baseline activity was observed until repeated spreading depolarizations started two and a half hours after the onset of recording. Until day 5 after the bleeding, a total of 50 spreading depolarizations occurred (with a peak incidence of 16 on day 1) resulting in a total depression time of 3.3 hours. On day 2, baseline activity changed from delta activity to a burst suppres-

sion or burst suppression/delta pattern indicating sedation. Figure 2 shows an early, isolated spreading depolarization and a relatively short interval of corresponding depression of ECoG activity.

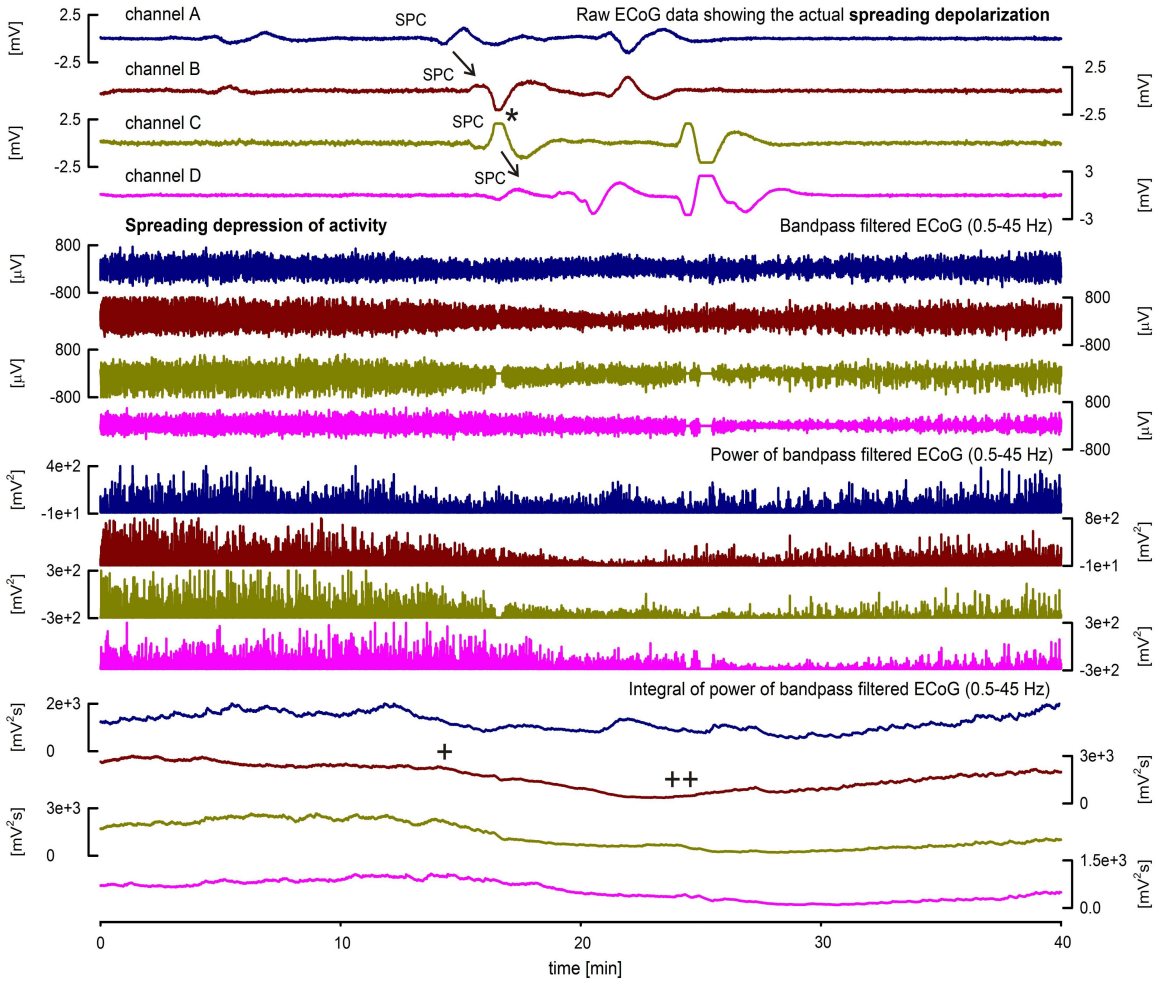

Figure 2: The subdural ECoG over a time period of 40 minutes shows an isolated spreading depolarization in the 62 year-old female patient described in illustrative case 1.

Channel A is a differential signal between electrodes 2 and 3, Channel B between 3 and 4, etc. The upper four traces represent the raw ECoG signal. The next four traces demonstrate the bandpass filtered signal in the frequency range from 0.5-45 Hz. A bandpass filter is a device that allows frequencies to pass within a certain range ("pass-band"), and attenuates frequencies outside that range. Here, the pass band is between 0.5 and 45 Hz.

Traces 9 to 12 and 13 to 16 display the power of the bandpass filtered ECoG and the integral of power of the bandpass filtered ECoG, respectively. Squaring the high-frequency ECoG signals helps to visualize the loss of the signal amplitude during spreading depression of activity. The integral of power helps to determine the duration of the depression period.

In conclusion: The upper four traces identify the spreading depolarization, and the lower 12 traces identify the subsequent, corresponding spreading depression of ECoG activity.

In this case, the spreading depolarization propagates from channel A (representing electrodes 2 and 3) to channel D (electrodes 5 and 6). Channel C shows the phase reversal (*) of the SPC in B. We see a phase reversal whenever a localized change of the SPC occurs at the electrode common to two channels. Phase reversal means that the SPC deviates in opposite directions in the two channels.
The amplitude of the spreading depression is seen best in the traces representing the power of the bandpass-filtered ECoG signal. Note that the propagation of the spreading depolarization (highlighted by arrows in the upper four trace) resembles the propagation of spreading depression, both starting in channel A and propagating along the electrode strip towards channel D.
The traces of the integral of power help us to determine the duration of the depression periods. In this example, the depression in channel B starts at the timepoint indicated by "+", and the recovery of activity indicated by "++".

In contrast to an isolated spreading depolarization with fast restoration of brain electrical activity, a cluster of spreading depolarizations is a status of recurrent depolarizations without recovery of the high-frequency ECoG activity in at least one channel between the events. Clusters consist of spreading depolarizations that occur at high frequency (a median frequency of 2.6 events/h in 5 patients with clusters of spreading depolarizations after aSAH was reported by Dreier et al., 2009) and thus, the intervals between depolarizations can be as short as 20 minutes. During clusters, spreading depolarizations co-occur with progressively increasing periods of electrocorticographic silence, and/or eventually complete silence of brain electrical activity at the site of recording. The evolution of ischemic stroke after SAH is associated with these clusters of spreading depolarizations and increasingly prolonged depression periods (Dreier et al., 2006). In this illustrative case, the patient showed severe clusters of repeated spreading depolarizations during the later days of the monitoring period. By the time of explantation of the electrode strip (day 15) the patient had developed a tetraparesis, global aphasia, and was ventilated via tracheostoma (5 points on the Modified Rankin Scale).

Figure 3 shows a cluster of spreading depolarizations and the corresponding depression of brain electrical activity.

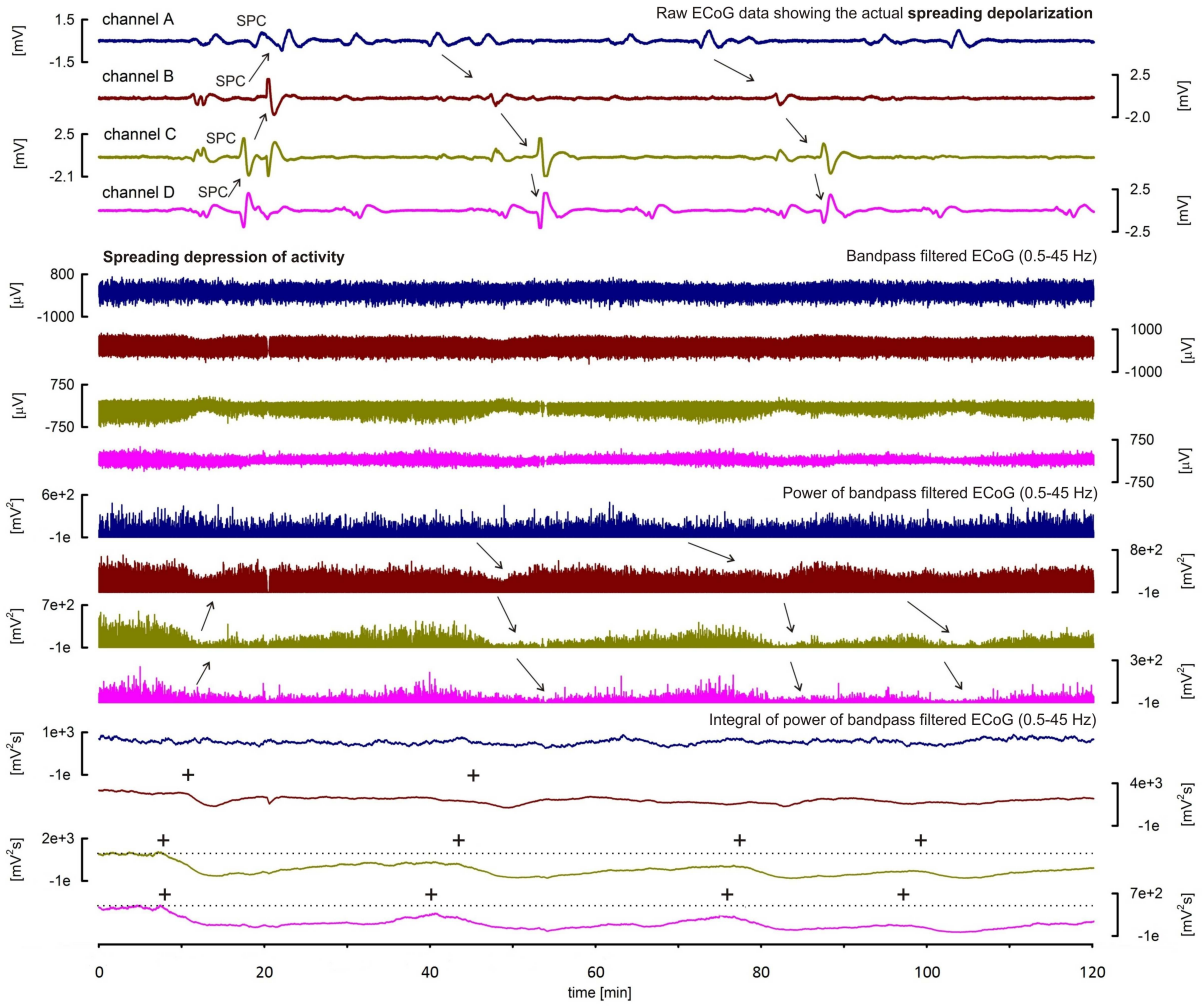

Figure 3: Cluster of spreading depolarizations in the 62 year-old female patient described in illustrative case 1.

Traces 1 to 4 show the first SPC that propagates along the electrode strip from channel D through channels C and B to A. Arrows highlight the direction of the SPC. Note that the next and the susbsequent SPCs originate from the opposite direction.

The arrows in traces 9 to 12 (showing the power of the bandpass filtered ECoG) indicate that the propagation of the corresponding depression of ECoG activity roughly resembles the direction of the SPC propagation. Decreases of brain electrical activity in the traces showing the integral of power (13 to 16) are indicated by "+". The dotted lines in traces 15 and 16 help to visualize a decline of the initial amplitude of the integral of power. This supports the hypothesis that spreading depolarizations recurring at short intervals (i.e. clusters) lead to a progressive decrease of brain electrical activity and the evolution of ischemic stroke (Dreier et al., 2006).

6.4 Illustrative case 2: ictal epileptiform activity.

This 54 year-old male patient presented with WFNS Grade 3 and Fisher Grade 3 SAH and was admitted to hospital where he underwent aneurysm surgery and NPRI implantation. GCS on admission was 13. ECoG recordings started later that same day. During a total recording time of almost 159 hours, no spreading depolarizations were observed and baseline activity was mostly flat. On the second day of recording, we observed prolonged ictal epileptiform activity (158 events of ictal train of rhythmic delta or theta activity) which lasted until day 3 when baseline activity returned to a continuous flat signal. Decrease of GCS level by 4 points necessitated reintubation. Upon removal of the electrode strip (day 15), the patient had a newly developed hemiparesis and aphasia (5 points on the Modified Rankin Scale).

In comparison to spreading depolarization and corresponding ECoG depression, ictal epileptiform activity is not associated with large propagating SPCs.

Figure 4 shows an episode of ictal epileptiform activity in this patient. Here, we see only minor SPCs compared to the SPCs in the preceding examples of isolated spreading depolarization or clusters of spreading depolarization, and they do not propagate. The most distinctive feature, however, is a marked increase of brain electrical activity, in contrast to the decrease described above. This marked increase is usually visualized best in traces showing the integral of power of the bandpass filtered ECoG. In this case, spontaneous baseline activity was already flat, so that the marked increases of brain electrical activity are also clearly visible in traces representing the bandpass filtered ECoG and the power of the bandpass filtered ECoG. A higher temporal resolution of the bandpass filtered ECoG shows rhythmic discharges with spikes or sharp waves, or a sharp, pointed variation of a preceding kind of spontaneous activity (e.g. rhythmic delta activity). That latter, however, was not observed in this illustrative case.

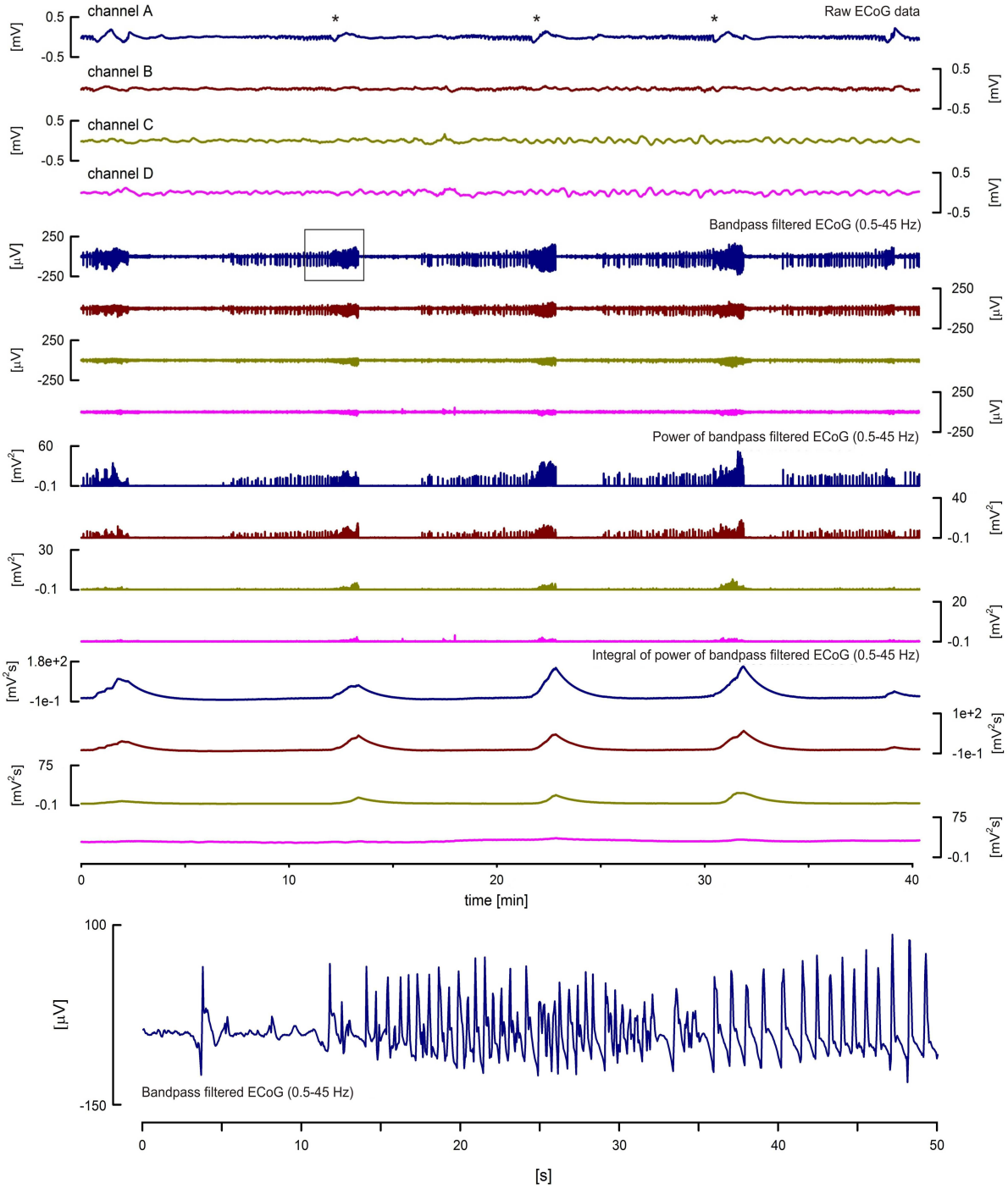

Figure 4: Repetitive bursts of ictal epileptiform activity in the 54 year-old patient described in illustrative case 2.

The upper four traces show the raw ECoG signal. Asterisks (*) highlight negative slow potential shifts that cause an increase in brain electrical activity (traces 5 to 16). Note that the SPCs have considerably lower amplitudes than the SPCs in the preceding illustrative cases of spreading depolarization. Here, they also do not propagate. The most striking distinctive feature, however, is the marked increase of brain electrical activity in contrast to depression of activity. The bandpass filtered ECoG signal displays fusiform patterns indicating the increasing ictal epileptiform activity while the spontane-

ous high-frequency activity is already depressed. The integral of power of the bandpass filtered ECoG signal (traces 13 to 16) shows the increase of activity as large peaks visible in channels A to C.
The bottom trace of the illustration shows a higher temporal resolution of the boxed section of channel A in the bandpass filtered ECoG signal. The preceding burst-suppression pattern changes to an ictal epileptiform activity that is characterized by recurrent synchronized discharges.

6.5 Summary of results

During a total recording time of 3831.6 hours, 664 spreading depolarizations were observed in 18 of 23 patients, resulting in a total depression time of 104.2 hours. Of the 13 patients with NPRI, only three did not show any spreading depolarizations. Similarly, only two of the ten patients without NPRI showed no spreading depolarizations.
In most patients, initial ECoG baseline activity was continuous or discontinuous delta activity, in some cases mixed with a burst suppression pattern.

Spreading depolarizations in individual patients are mostly stereotypical. In the majority of cases we encountered isolated spreading depolarizations accompanied by depression of brain electrical activity with either fast or prolonged recovery. In fewer cases, clusters of spreading depolarizations occurred. Some of these clusters were characterized by isoelectric spreading depolarizations, i.e. spreading depolarizations that occurred while the brain electrical activity is still depressed. Two patients from the NPRI group showed multiple events of ictal epileptiform activity. In both cases, seizure activity was not associated with spreading depolarization or spreading depression but with marked local increases in brain electrical activity.

6.6 The occurrence of spreading depolarizations in both study groups

To compare the occurrence of spreading depolarizations per day recording time in both study groups (i.e. patients with NPRI, and those without), Mann-Whitney rank sum tests were performed. In the nicardipine group the highest incidence of spreading depolarizations was observed on day 1 (median 11.9). The highest incidence for the occurrence of spreading depolarizations per day recording time in the control group was on day 4 (median 1.5).

There was no significant difference in the occurrence of spreading depolarizations when each day of recording was compared separately between the two study groups. In a cumulative analysis which examined the occurrence of spreading depolarization during the early period of recording (days 0 to 4), and the late time period (days 6 to 10), statistical trend was found that more patients with NPRI showed three or more spreading depolarizations per day recording time in the early period than patients from the control group (P = 0.08 in the two-tailed Fisher exact test, n = 13 for NPRI group, n = 9 for controls). In the early time period, the median number of spreading depolarizations per day recording time was 7.7 (0.9, 13) in the NPRI group, and 1.7 (0.4, 2.5) in the control group.

In the late recording period, the median number of spreading depolarizations per day recording time was only 0.6 (0.0, 1.0) in the NPRI group, and 0.3 (0.0, 2.1) in the control group. For the NPRI group, this frequency was significantly lower than that in the early time period (Wilcoxon Signed Rank Test, P = 0.006) whereas no such statistical difference was found for the number of spreading depolarizations per day recording time between early and late time period for the control group.

The box plots diagram in Figure 6 shows a day-by-day analysis of the number of spreading depolarizations per day recording time for both groups.

6.7 The total duration of ECoG depression in both study groups

In a day-by-day analysis of the total duration of ECoG depression per day recording time, values for both study groups did not differ significantly (Mann-Whitney rank sum test). There was a statistical trend that in comparison with the control group there is a longer duration of ECoG depression on day 1 for patients treated with NPRI (P = 0.056). Similarly, a statistical trend was observed that more patients treated with NPRI experienced depression periods of more than 25 minutes per day recording time between days 0 to 4 (P = 0,099 in the two-tailed Fisher exact test, n = 13 for NPRI group, n = 9 for controls) than patients left untreated.

The median duration of ECoG depression per day recording time for the NPRI group was significantly longer in the early time period (38.6 (4.7, 139.4)min) than in the late period (5.9 (0, 13.3) min) (Wilcoxon signed ranked test, P = 0.006, n = 13), while we observed no significant difference in the duration of ECoG depression between early

and late time period in the control group (median depression per day recording time during days 0 to 4: 7.8 (3.5, 24.6)min , and 0 (0, 13.2) min from day 6 onward).
The box plots diagram in Figure 5 summarizes the results of the day-by-day analysis of the total depression period per day recording time.

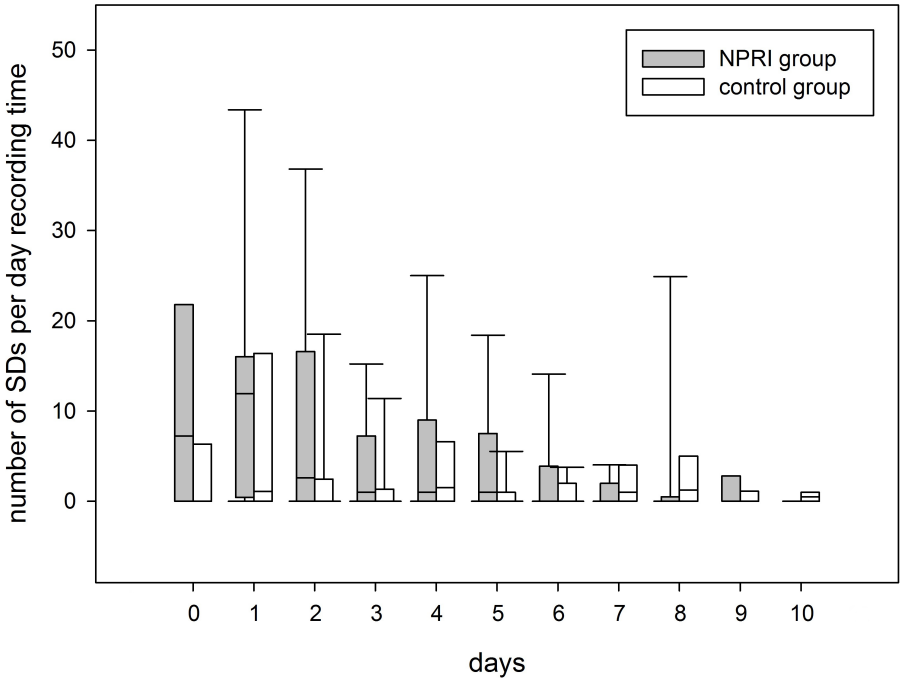

Figure 6: Number of spreading depolarizations per day recording time.
Comparison performed using Mann-Whitney rank sum tests.

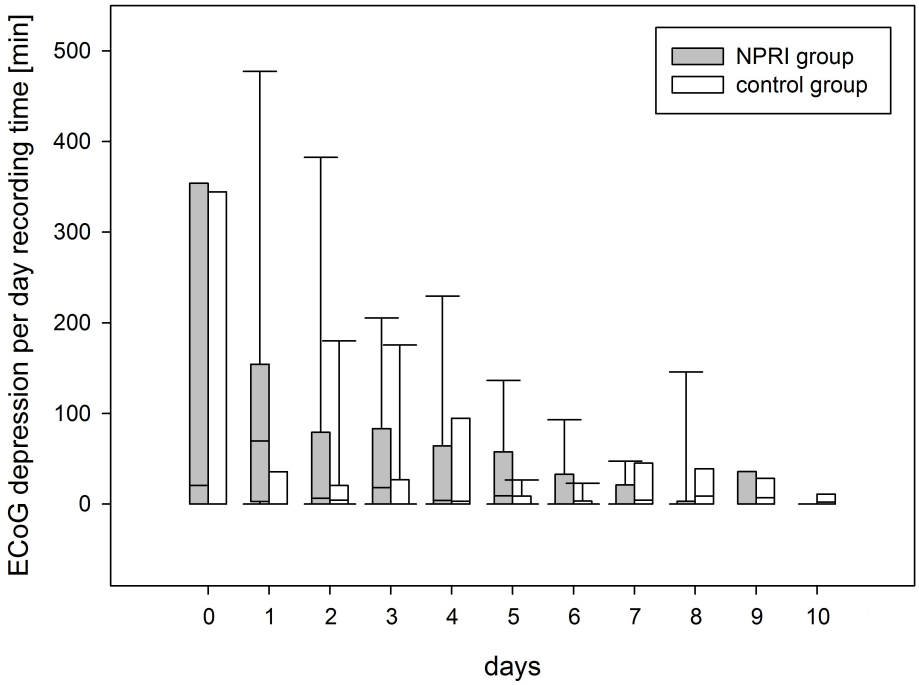

Figure 5: ECoG depression per day recording time.
Comparison between patients treated with NPRI and control group using Mann-Whitney rank sum test.

7 Discussion

7.1 Prerequisites for ECoG monitoring of patients with aSAH

The poor clinical outcome of patients with aSAH is determined mainly by brain damage induced by the initial hemorrhage and by DCI, the most serious in-hospital complication after aSAH (Hijdra et al., 1988; Ferguson and Macdonald, 2007). Currently, the definition of the term DCI encompasses symptomatic neurological deterioration and/or radiographic evidence of ischemia or infarction (Frontera et al., 2009). The diagnosis of DCI is limited, however, because it is typically made retrospectively once infarct development is already on its way or even completed. Hence, a reliable tool for detecting *ongoing* ischemia is needed in order to selectively start neuroprotective therapy at the earliest point.

It has been increasingly questioned whether vasospasm of major cerebral arteries alone determines DCI. Chronic vasospasm of distal arteries and arterioles, microthrombosis, spreading depolarization, and spreading ischemia are likely to be complementary factors in the pathogenesis of DCI. Clinical assessment of neurointensive care patients with aSAH is often difficult because of reduced consciousness (e.g. analgo-sedation). In many cases, the sometimes subtle early symptoms of DCI escape attention and delayed infarcts develop under the eye of the treating intensivist. While cerebral vasospasm is typically diagnosed by TCD and/or DSA, these diagnostic tools are unsuitable to detect spreading depolarization and spreading ischemia. Since transient depolarizations can also occur in the absence of angiographic vasospasm, a complementation of the conventional diagnostic procedure is required.

In routine electroencephalography (EEG), depolarizations and spreading depression are typically hidden by the contamination with undepressed activity from neighboring cortical areas, and the volume of the cortical surface involved in the depolarization wave is too small to have a significant effect on the conventional EEG. Anatomical structures such as the dura and the skull act as high-pass filters and the SPC created by the synchronous depolarization of millions of neurons is removed from the signal. Currently, only invasive ECoG reliably detects spreading depolarizations and spread-

ing depolarization-induced spreading depression. The invasive nature of the methodology certainly represents a limitation to the indication for further, broader studies on spreading depolarizations and their effects on patient outcome. However, most patients that are admitted to hospital with severe aSAH do require surgical intervention for aneurysm treatment, evacuation of the hematoma, or decompressive surgery, so that placement of the subdural electrode strip for the recording and monitoring of brain electrical activity is available.
The information provided by invasive ECoG experiences may enable a translation and application to less invasive methods in the future.

7.2 ECoG reliably detects pathological changes in brain electrical activity, such as spreading depolarizations, spreading depression, and ictal epileptiform activity

It has been shown that DCI occurs time-locked to recurrent spreading depolarizations in patients with aSAH (Dreier et al., 2006) and prolonged ECoG depression periods may indicate the development of delayed ischemic stroke in the recording area (Woitzik et al., 2011 in press). During clusters, isoelectric depolarizations, (i.e. depolarizations that evolve in an area where brain electrical activity is already or still depressed from previous depolarization events) are presumably associated with worse tissue outcome since they increase the mismatch between energy demand and supply (Fabricius et al., 2006).

In invasive ECoG recordings, spreading depolarizations can be detected as propagating waves of marked polyphasic SPCs in adjacent channels. The accompanying depression or silence of neuronal activity is defined by a rapidly developing reduction of the power of the ECoG amplitude. An SPC of large (>1 mV) amplitude and duration of 1-2 minutes spreading synchronously with the ECoG depression is regarded as the gold standard for identifying spreading depolarization (Fabricius et al., 2008).

As described in the methods section, we gained an even better visualization of the loss of ECoG amplitude during spreading depression by calculating the power of the bandpass-filtered ECoG signal. Here, the amplitude reduction appears more pronounced. A simple mathematical integration of the power of the bandpass-filtered

ECoG activity allows a determination of the duration of ECoG depression since starting and endpoints of the depression period can be clearly identified.

Ictal epileptiform activity is another pathological phenomenon that follows primary brain injury and has a potential to exacerbate tissue damage. It occurs either independently, or co-occurs with spreading depolarization in humans after aSAH (Fabricius et al., 2008). Ictal epileptiform activity is described by the development of a spike pattern which results from the melting of paroxysmal depolarization shifts (PDS) in the neurons. As in spreading depolarization, seizure activity is also associated with a sustained depolarization of the neurons but this is less pronounced than that of the near-complete depolarization of spreading depolarization. It is also below the inactivation threshold for the action potential generating channels which allows for highly frequent firing of action potentials. The corresponding extracellular field potential shift creates a steep potential change visible as the spike pattern.

Like spreading depolarization, the majority of acute seizures occur without overt manifestation while the patient is in neurointensive care. Therefore, it is important to obtain reliable records of seizure activity in these patients and treat them accordingly, once a distinction from spreading depolarization has been made. In general, the stimuli that induce spreading depolarization can also provoke seizure discharge, and there are no simple rules by which to predict which one of the two will occur (Somjen, 2004). In 2008, Fabricius et al. performed ECoG analysis of patients with severely injured brain and showed that ictal epileptiform activity either precedes or is interrupted (and possibly blocked) by spreading depolarization events. In other cases spreading depolarizations seem to be triggered by ictal events or they are entirely independent of seizure activity. ECoG recordings allow unambiguous distinction between ictal epileptiform activity and spreading depolarization, as described in the results section. In the second illustrative case depicted above, no events of spreading depolarization were observed at the site of the electrode strip throughout the entire recording period, yet the patient developed severe disabilities which may have been caused by prolonged ictal epileptiform activity.

7.3 Limitations

Placement of only one single subdural electrode strip on the cortex accessible from the craniotomy renders it likely that spreading depolarizations in other regions of the brain escape detection. Hence, a patient's clinical deterioration in the presumable absence of spreading depression of ECoG activity may be misjudged (Dreier et al., 2006). In the majority of cases, however, delayed ischemia after aSAH involves the aneurysm bearing vascular territory where the electrode strip is placed. Moreover, the propagating nature of spreading depolarization implies that even if the recording strip is placed remote from the actual ischemic zone, depolarizations from the ischemic zone should spread to the recording strip.

It could be argued that the implantation of the electrode strip increases the individual's risk of infection and rebleeding after aneurysm surgery. Lee et al. (2000) and Espinosa et al. (1994) assessed a risk of 0 - 2% for epidural and delayed subdural hematoma after electrode implantation, and a risk for infection of 2.6 -3.9%. In 2002, Strong et al. reported no significant increase in this incidence of rebleeding or infection but one must certainly not forget that any additional invasive intervention bares its additional risks.

Because spreading depolarizations also occur as a consequence of brain contusion (Strong et al., 2002, Fabricius et al., 2006), it could be argued that the placement of the electrode strip on cortex previously retracted and damaged during aneurysm surgery produces a confounding bias. Cortical tissue is indeed highly sensitive to mechanical disturbance and spreading depolarizations are easily induced (Leão, 1944). However, the fact that we see patient data without any signs of neuronal depolarization despite neurosurgical intervention speaks against this argument.

Another prevalent assumption is that the electrode strip acts as a conductor and facilitates the spreading of depolarization (Strong et al., 1996). However, the fact that spreading depolarizations do not move immediately from gyrus to gyrus, but rather alongside gyri implies that the pia-arachnoid forms a barrier and in this fashion also prevents short-circuits between surface electrodes and cortical grey matter.

ECoG recording and monitoring remains very sensitive to patient movement (Dreier et al., 2009). Nursing procedures required in intensive care necessarily involve patient movement, as do patients' transports to clinical examinations outside the intensive care unit. Prior to our analysis large segments had to be cut out due to artifacts and noise so that they would not interfere with data from good quality recording. Other segments were already missing because monitoring had been interrupted during clinical examinations such as DSA or CT. In these cases, spreading depolarizations occurring during those periods were not detected.

7.4 The use of ECoG as an analytical tool in clinical studies

After the establishment of an analytical framework that allows a standardized and reliable detection of pathological changes in the ECoG activity, I tested its applicability for clinical studies in a prospective study of 23 patients. 13 of these 23 patients had received NPRI during aneurysm surgery, the remaining 10 patients served as the control group.

Spreading depolarizations and the corresponding depression of brain electrical activity were observed in both study groups. Moreover, it was found that there was neither a significant reduction in the number of spreading depolarizations per day recording time, nor in the total duration of ECoG depression per day recording time in patients with NPRI when data was compared in a day-by-day analysis. The cumulative analysis of the number of spreading depolarizations per day recording time during the early period of recording (days 0 to 4) revealed a statistical trend that patients treated with NPRI showed even more events of spreading depolarizations than patients left untreated. Similarly, patients from the NPRI group experienced significantly longer periods of ECoG depression in the early recording period.

A simple interpretation of these results would support the hypothesis that spreading depolarizations occur abundantly and independently despite a reduction of proximal vasospasm. This in turn could be evidence for the occurrence of DCI in the absence of proximal vasospasm. In this case, NPRI would fail to act as a sufficient neuroprotective therapy and once again, the limited association of angiographic vasospasm

with DCI would suggest the existence of another pathogenic component. However, the small size of the study group limits such a conclusion.

A possible explanation for the lack of clear evidence of a reduction of spreading depolarizations in patients treated with NPRI is that implants themselves induce spreading depolarization. We know that depolarization waves and spreading depression of brain electrical activity can be elicited by mechanical stimulation (Leão, 1944). Foreign body reactions or inflammatory responses to nicardipine implants have not yet been reported, but cannot be ruled out as possible triggers of spreading depolarizations.

In addition to this, it has been shown in experimental studies by Pluta et al. (2009) that intrathecal drug distribution after aneurysmal SAH is hampered by clots around proximal arteries. Even new drug packaging techniques such as NPRI probably fail in releasing their components into the vicinity of distal arteries (that are at the high risk of delayed cerebral vasospasm) because the released nicardipine is sequestrated within the local cistern by the surrounding blood clots. In this case, of course it would be impossible to conclude that spreading depolarization is indeed a factor that operates independently from proximal vasospasm since the latter might not have been sufficiently blocked by NPRI.

The number of patients included in this study, however, was only very small. A comparison of the occurrence of spreading depolarizations and ECoG depression per day recording time failed to reach statistical significance in the day-by-day analysis. To confirm (or refute) the hypotheses that spreading depolarizations occur less or more frequently in patients treated with NPRI, more patients would have to be recruited in future studies.

7.5 Outlook

As previously described in the introduction to this work, there have been several clinical studies examining the treatment of delayed neurological deficits. Most of these studies measured success or failure of the therapeutic regimes by evaluating clinical outcome or results of imaging diagnostics. However, there is growing evi-

dence that the pathogenesis of DCI is multifactorial (Pluta et al., 2009), and that it occurs in the absence of angiographic vasospasm. Whatever new pharmacological inventions will give grounds for clinical studies in the future, ECoG recording and monitoring should be included in patient surveillance (together with thorough and standardized serial neurological examinations) in order to correlate clinical deterioration with their possible causal agent and to further elucidate the pathogenesis of DCI.

8 Conclusion

Cortical spreading depolarizations and the depression of ECoG activity are independent mechanisms in the pathophysiological spectrum of delayed neurological deterioration and delayed ischemia after SAH, and there is evidence that they do occur in the absence of angiographic vasospasm.

The clinical diagnosis of symptomatic delayed ischemia remains challenging, especially in ventilated and sedated patients. A reliable neurological assessment is often impossible and appropriate timing of therapeutic measures that are not risk-free, such as aggressive triple-H therapy, cannot be made. Serial MRI or CT/CT perfusion imaging that would detect infarction in its development is time-consuming, expensive, and not always accessible.

Proximal vasospasm, one of the assumed key players in the development of DCI, is diagnosed by TCD and/or DSA. Here, cortical spreading depolarizations escape detection. To date, ECoG is the only reliable tool for detecting of spreading depolarizations at the bedside (Hartings et al., 2006). Stable and sensitive recordings and their online interpretation using the analytical framework described above are an efficient way to detect pathological changes in brain electrical activity after aSAH, and help to examine in future studies whether particular patterns have adverse, benign or protective consequences. This is of major interest, because one day it might become possible to stratify the population of SAH patients into one group of patients with high risk of developing delayed ischemia and another group with lower or without such risk.

Routine ECoG monitoring after aSAH may complement in the diagnosis of delayed ischemic neurological deficits, facilitate the treatment, or even help to prevent infarct development in the future.

9 Appendix

Glasgow Coma Scale -- Scale for recording the conscious state of a patient

Eye Opening	Verbal Response	Motor Response
4=Spontaneous	5=Normal conversation	6=Normal
3=To voice	4=Disoriented conversation	5=Localizes to the Pain
2=To pain	3=Words, but not coherent	4=Withdraws to pain
1=None	2=No words, only sounds	3=Decorticate posture
	1=None	2=Decerebrate
		1=None

Minimum = 3
Maximum =15

(Teasdale GM, Jennett B. Assessment of coma and impaired consciousness. A practical scale. Lancet No. 2 81-84 (1974))

WFNS SAH grading scale

Grade 1	GCS 15, without motor deficit
Grade 2	GCS 14-13, without motor deficit
Grade 3	GCS 14-13, with motor deficit
Grade 4	GCS 12-7, with/without motor deficit
Grade 5	GCS 6-3, with/without motor deficit

(Teasdale GM, Drake CG, Hunt W, Kassell N, Sano K, Pertuiset B, De Villiers JC. A universal subarachnoid hemorrhage scale: report of a committee of the World Federation of Neurosurgical Societies. Journal of Neurology, Neurosurgery & Psychiatry Vol. 51(11) 1457 (1988))

Fisher Grade -- classifies the appearance of SAH on CT scan

1 No hemorrhage evident**.**

2 SAH less than 1mm thick.

3 SAH more than 1mm thick.

4 SAH of any thickness with intra-ventricular hemorrhage or parenchymal extension.

(Fisher C, Kistler J, Davis J (1980). "Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning". Neurosurgery 6 (1): 1–9.)

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11 Statutory declaration

Eidesstattliche Versicherung gemäß der Promotionsordnung der Charité

Hiermit erkläre ich, dass

1. keine staatsanwaltschaftlichen Ermittlungsverfahren gegen mich anhängig sind,
2. weder früher, noch gleichzeitig ein Promotionsverfahren durchgeführt oder angemeldet wurde,
3. die vorgelegte Promotion ohne fremde Hilfe verfasst,
4. die beschriebenen Ergebnisse selbst gewonnen wurden, sowie die verwendeten Hilfsmittel,
5. die Zusammenarbeit mit anderen Wissenschaftlerinnen oder Wissenschaftlern und technischen Hilfskräften und die Literatur vollständig angegeben sind,
6. dem Bewerber die geltende Promotionsordnung bekannt ist.

Berlin, den 24. 02. 2012

12 Curriculum vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

13 List of publications

Woitzik J, Dreier JP, Hecht N, Fiss I, Sandow N, Major S, Winkler M, Dahlem YA, Manville J, Diepers M, Muench E, Kasuya H, Schmiedek P, Vajkoczy P; for the COSBID study group. **Delayed cerebral ischemia and spreading depolarization in absence of angiographic vasospasm after subarachnoid hemorrhage: a small case series study.** (J Cereb Blood Flow Metab, *in press*)

Dreier JP, Major S, Pannek HW, Woitzik J, Scheel M, Wiesenthal D, Winkler M, Hartings JA, Fabricius M, Speckmann EJ, Gorji A; for the COSBID study group. **Spreading convulsions, spreading depolarizations, and epileptogenesis in human cerebral cortex**. (Brain, *in press*)

Drenckhahn C, Winkler M, Major S, Scheel S, Kang EJ, Grozea C, Hartings JA, Woitzik J, Dreier JP. **Correlates of Spreading Depolarization in Human Scalp Electroencephalography** (Brain, *in press*)

Oliveira-Ferreira AI, Winkler M, Reiffurth C, Milakara D, Woitzik J, Dreier JP. **Spreading depolarization, a pathopysiological mechanism of stroke and migraine aura**. (Future Neurology, *in press*)

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14 Acknowledgements

Danksagung

Mein Dank gilt in erster Linie Herrn Prof. Dr. Jens P. Dreier, der mich an dieses spannende Thema herangeführt hat und bei der Betreuung außerordentliche Geduld und Motivation gezeigt hat. Vielen Dank für das andauernde Vertrauen!

Bei meinen Kolleginnen und Kollegen der AG Dreier, insbesondere Ana I. Oliveira-Ferreira und Sebastian Major, sowie Dr. Yuliya A. Dahlem, bedanke ich mich für die unermüdliche Hilfsbereitschaft und freundschaftliche Zusammenarbeit im Rahmen dieses Projekts!

Meiner wunderbaren Familie danke ich für ihre liebevolle Unterstützung und immer-währenden Rückhalt in allen Lebenslagen!