Seizure 20 (2011) 513–519

Contents lists available at ScienceDirect

Seizure

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A characteristic time sequence of epileptic activity in EEG during dynamic penicillin-induced focal epilepsy—A preliminary study

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ARTICLE INFO

Article history:
Received 16 September 2010
Received in revised form 23 February 2011
Accepted 18 March 2011

Keywords:
Epilepsy
Electroencephalography
Penicillin
Experimental model
Pig

ABSTRACT

Penicillin-induced focal epilepsy is a well-known model in experimental epilepsy. However, the dynamic evolution of waveforms, DC-level changes, spectral content and coherence are rarely reported. Stimulated by earlier fMRI findings, we also seek for the early signs preceding spiking activity from frequency domain of EEG signal. In this study, EEG data is taken from previous EEG/fMRI series (six pigs, 20–24 kg) of an experimental focal epilepsy model, which includes dynamic induction of epileptic activity with penicillin (6000IU) injection into the somatosensory cortex during deep isoflurane anaesthesia. No ictal discharges were recorded with this dose. Spike waveforms, DC-level, time–frequency content and coherence of EEG were analysed. Development of penicillin induced focal epileptic activity was not preceded with specific spectral changes. The beginning of interictal spiking was related to power increase in the frequencies below 6 Hz or 20 Hz, and continued to a widespread spectral increase. DC-level and coherence changes were clear in one animal. Morphological evolution of epileptic activity was a collection of the low-amplitude monophasic, bipolar, triple or double spike-wave forms, with an increase in amplitude, up to large monophasic spiking. In conclusion, in the time sequence of induced epileptic activity, immediate shifts in DC-level EEG are plausible, followed by the spike activity-related widespread increase in spectral content. Morphological evolution does not appear to follow a clear continuum; rather, intermingled and variable spike or multispike waveforms generally lead to stabilised activity of high-amplitude monophasic spikes.

1. Introduction

In acute experimental focal epilepsy models, $GABA_A$ receptors are blocked with a local cortical application of a blocking agent (e.g. picrotoxin, bicuculline or penicillin G), which prevents the GABA-mediated inhibitory control of the main population of pyramidal neurons.[1,2] Layer IV of the cerebral cortex is the most sensitive to penicillin epileptogenesis.[3]

During the first minutes of development of an epileptic focus, the earliest sign is the increased number and frequency of spiking as a neuron's response to (e.g. visual) stimuli, i.e., an enhanced physiologic response. Then next sign is the intracellular paroxysmal depolarisation shift (PDS), coupled to interictal activity in ECoG. Response inhibition occurs in neurons surrounding the initial focus (the injection site), creating an "inhibitory surround" as a projection to the neighbouring interictal activity.[4] Each interictal spike is followed by an immediate transient inhibition that terminates the massive paroxysmal burst of action potentials.[5] Inhibition is shown to be partly GABA-mediated via both $GABA_A$ and $GABA_B$ receptors in penicillin-induced foci[6] and causes a profound postsynaptic inhibitory potential of 1–2 s duration.[7]

With a sufficient penicillin dose, interictal spikes appear in ten seconds to minutes, increase their frequency in several minutes, then develop short seizure-like "bursts" in around twenty minutes, up to ictal discharge, i.e., seizure activity in electrocorticography (ECoG).[8] With a higher penicillin dose, severe seizure activity appears in ten minutes.[9] Also, slow DC-shifts in EEG may occur, as

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doi:10.1016/j.seizure.2011.03.006

consistently related to ictal epileptic activity in humans[10] with a time scale of seconds. Slow DC-shifts may be generated by glial cell-originated spatial potassium buffering. Another DC-potential, PDS, mentioned above has time scale of 250–300 ms[5] compared to the dynamics of slow DC-shift. Each extracellular interictal spike is generated by synchronized population of intracellular PDSs.[5] In our study, the recording is performed with EEG, corresponding to extracellular population-level measurement.

The characteristic time sequence of epileptic activity in EEG, i.e., DC-level changes, dynamic evolution of waveforms, spectral content and coherence are rarely reported in detail. Recently, the study by Canan et al.[11] suggested changes in latent and epileptiform periods compared to basal activity in their time–frequency analysis of rat ECoG with an intracerebroventricular application of penicillin in urethane anaesthesia. With a different experimental model, our aim was to further investigate plausible DC-level shifts and the morphology of waveform development as well as to revisit spectral analysis. Especially, the previous fMRI findings in[12–14] indicate a search for the early signs of spectral content changes preceding spiking activity in EEG. In this study, we utilized the EEG data from the previous experimental EEG/fMRI series of a focal epilepsy model,[12] which included a dynamic induction of epileptic activity and simultaneous EEG/fMRI in isoflurane anaesthesia. We compared this EEG-analysis with our previously published fMRI findings reported in [12].

2. Materials and methods

2.1. The animal model and experimental procedure

The full procedure of development of the model with dynamic focal epilepsy induction and application of EEG/MRI experiment has been documented in detail elsewhere.[12] The results reported here include EEG data from six female piglets (2–3 months, 20–24 kg). During simultaneous fMRI and EEG recording, a bolus of penicillin (6000IU) was manually injected into the somatosensory cortex[15] at a target depth of 5 mm below the dura mater, through a cranial hole on the left side anteriorly to the coronal suture. The catheter was fixed securely with tissue glue. Somatosensory area was selected as a target area known to be sensitive to epileptogenic agents. We did not have co-ordinates, but the location is specified in [15]. The opening in the skull was a drill hole of diameter approximately 3 mm, allowing enough space for the plastic catheter insertion. Plastic epidural catheter (Portex® epidural minipack, 19-gauge) for penicillin injection was carefully prefilled to avoid air bubbles with 0.9% NaCl (tip) and benzylpenicillin sodium (Geepenil®) in 0.9% NaCl. The injected volume was 0.11 ml due to the catheter volume. The baseline before the injection was either 1 min ($N = 3$) or 3 min ($N = 3$) long, respectively. Due to technical limitations in fMRI, there was an interrupted time continuity, i.e., about a 20 s technical delay at 8 min 33 s from the start (a dotted line in the figures below) during the monitored period of 17 min.

Animals were fasted 12 h prior to the induction of the anaesthesia. Premedication consisted of i.m. midazolam (Dormicum®, 1.5 mg/kg) and ketamine (Ketalar®, 15 mg/kg). A venous cannula was inserted into the ear of a pig. Intubation was facilitated with i.v. administration of thiopental (Pentothal®, 25 mg/ml). The animals were normoventilated (7–8 L/min, 18 rpm) with 40% oxygen in air. Anaesthesia was maintained with isoflurane at end-tidal concentrations of 1.4–1.8%, the EEG burst-suppression pattern as an end-point. Muscular paralysis was obtained with repeated doses of pancuronium bromide (Pavulon®, 4 mg/h). Relaxation was not induced before the preferred anaesthesia level was obtained. Prior to the preparation required for catheter insertion, local anaesthesia was applied (lidocaine–adrenaline, Xylocain® adrenalin, 10 mg/ml + 5 μg/ml) together with fentanyl boluses 50 μg i.v. Anaesthesia throughout the study period was supervised by a senior anesthesiologist (E.S.).

At the end of the experiment, the pigs were euthanized with an overdose of pentobarbital. The protocol of these experiments had the approval of the local Research Animal Care and Use Committee.

2.2. EEG recording and analysis

Two EEG electrodes were attached with tissue glue onto the surface of the pig skull, contra- and ipsilaterally to lesion, posteriorly to the coronal suture. The distance between ipsilateral electrode and injection site was about 2 cm, as they were in the opposite sides of coronal suture. Contralateral electrode was about 2 cm from the ipsilateral electrode, about 28 mm from the catheter insertion site. The reference and ground electrodes were attached around the frontal sinuses. Digital EEG (Scan®, SynAmps®, NeuroScan®, MagLink®, NeuroScan, El Paso, TX) was continuously recorded (amplifier set-up: DC-recording, bandwidth 0–200 Hz, gain 150, sampling frequency 1000 Hz, range 37 mV, accuracy 0.559 mV) throughout the MRI session. Imaging resulted in EEG artefact, making the signal readable only during short time periods between fMRI acquisitions. Therefore, 1500 ms signal segments between the fMRI artifacts were extracted from the original EEG recording for further analysis. First, the DC-level change in function of time was determined by calculating the median value per each segment. For time–frequency analysis, signal segments were linearly detrended before the calculation of power spectral density (PSD) estimate (Welch's method, 1000 sample time window, 900 sample overlap, Hamming window). To better illustrate the activity changes in all frequencies, the PSDs were amplitude normalized by dividing the values in a specific frequency by its mean value during one minute period prior to the penicillin injection. Normalized time–frequency representation of the signals is not dominated by the low frequency activity, and therefore illustrates better the variation in high frequency activity. The magnitude squared coherence estimate of the contra- and ipsilateral signals was obtained using the Welch's averaged periodogram method (the same parameters as in the PSD estimation). The epileptic spikes were detected by using thresholding and visual review. All the signal processing was performed with the Matlab technical computing language (The MathWorks Inc., Natick, MA).

3. Results

After penicillin-injection, the appearance of spike activity occurred within minutes (Table 2 in [12]) ipsilaterally with blunt, wide, small-amplitude spikes (in four out of six animals). This shape was followed by multispike complexes (polyspikes) of several kinds (triplets or bursts) and double spikes until a constant large spike-activity saturated (Figs. 1 and 2). There was not a clear continuum of waveforms but an intermingled collection of polyspikes, which at first had a distinctive positive dip (a 'bipolar' waveform) in the beginning (B in Fig. 1) and later the vice versa. In three animals, this bipolar waveform remained during the whole period. A single sharp wave could first arise early intermingled with other forms and further began to dominate as high-amplitude monophasic spikes. In the contralateral side, however, development was not identical to the ipsilateral. These consistent forms and the epileptic activity evolution had variations in all animals (Fig. 1, Table 1). An example of the summary of waveforms, spectral content and time comparison is shown in Fig. 3.

Beginning straight after the penicillin injection, ipsilateral DC-level had a negative shift followed by a positive evolution, which shouldered and restabilized to a slow negative shift with a later drop clearly visible in contralaterally but also ipsilaterally. In contralateral side, there was not shouldering but a slow positive

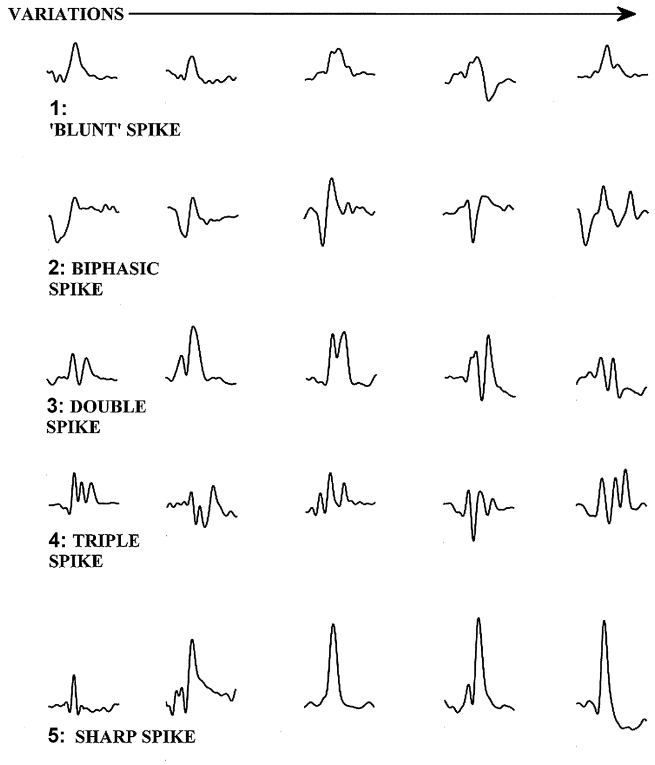

Fig. 1. Development of interictal spike activity after the penicillin injection (6000IU). Collection and comparison of waveform morphologies ipsi- and contralaterally in different animals. Variability in different morphologies exists within and between these distinctive forms.

evolution until the drop. The drop was not instantaneous like an amplifier DC-artefact, however. This occurred clearly in one animal (Fig. 2, DC-level) and to a lesser extent, but with a similar negative shift, in the beginning, in two other cases.

Table 1
Epileptic evolution: waveforms 1–5, see Fig. 1.

Pig	Channel	Morphology – time evolution				
A	Ipsi	1→	3/5→	4→	2/5	
	Contra	3/5/4→	2/5			
B	Ipsi	1/3→	2→	5/2		
	Contra	1→	2			
C	Ipsi	4→	2→	3→	5	
	Contra	2→	4/1/5/3→	3/5		
D	Ipsi	2/5→	3/2/4/5			
	Contra	1,1/3→	5/3/1			
E	Ipsi	4/5→	1/4/5→	3/4/5		
	Contra	1→	3/5/1			
F	Ipsi	1→	2/1→	3/2/5→	5/3/4→	3/5
	Contra	1→	1/3/5→	3/4/5→	3/4→	5

In the normalized time–frequency plots (Fig. 4), before the first spike, no evident changes in any frequencies were found here, when scaling was done to suppress baseline activity before the penicillin injection as a background. At the first spike (arrowheads), power increases varied from less than 6 Hz frequencies up to 20 Hz. In two animals, it was difficult to see any change (A, E). Then, a simultaneous wide-band frequency (below 20 or 50 Hz) power increase followed during the evolution of the constant epileptiform activity.

Coherence analysis between ipsi-and contralateral electrode did not show marked changes in five animals. In one pig (Fig. 5), coherence did show a slight wide-band reduction at the onset of the spiking, and also a disappearance of dominant alpha coherence, after theta coherence was lost. When the stabilised monophasic spike activity was reached, an increase in beta and delta frequencies was apparent.

4. Discussion

In this study, we sought for any characteristic time sequences of interictal epileptic activity in the EEG data from the experimental

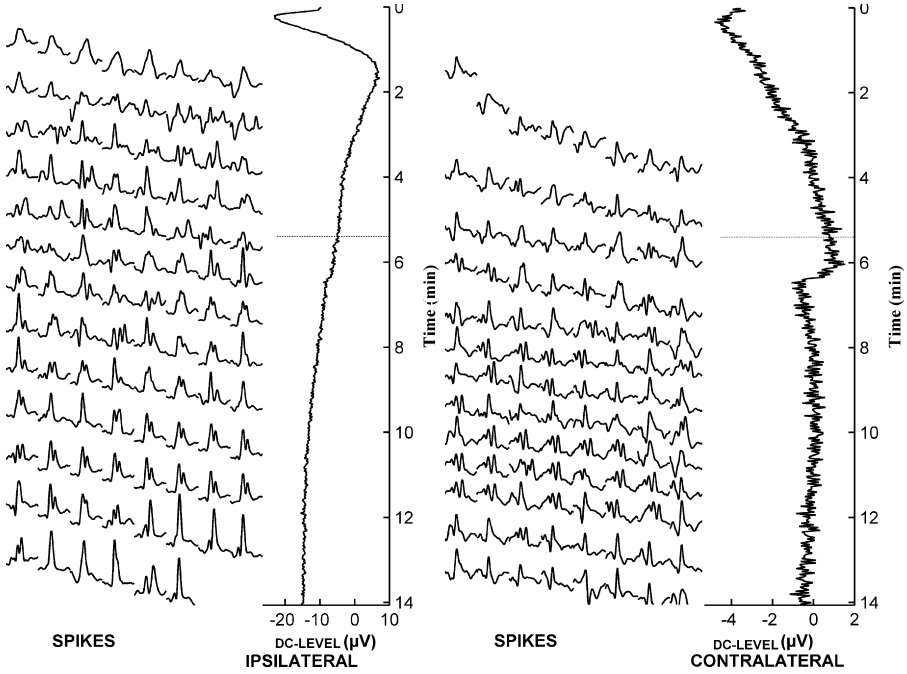

Fig. 2. An example of the development of interictal spike waveforms ipsi- and contralaterally to penicillin-induced (6000IU) epileptic focus (pig F). In four of six animals, a 'blunt' spike was the first sign of epileptic activity. Either the first or second change, a 'bipolar' waveform evolves generally with positive–negative peaks and later the vice versa. Also, a triplet and/or a double waveform follows or they are intermingled. A single sharp wave-like morphology, with some variations, can first arise, early intermingled with other forms, and further began to dominate the stabilised activity of high-amplitude monophasic spikes. Dotted line represents the interrupted time continuity (approximately a 20 s pause), see Section 2 for details.

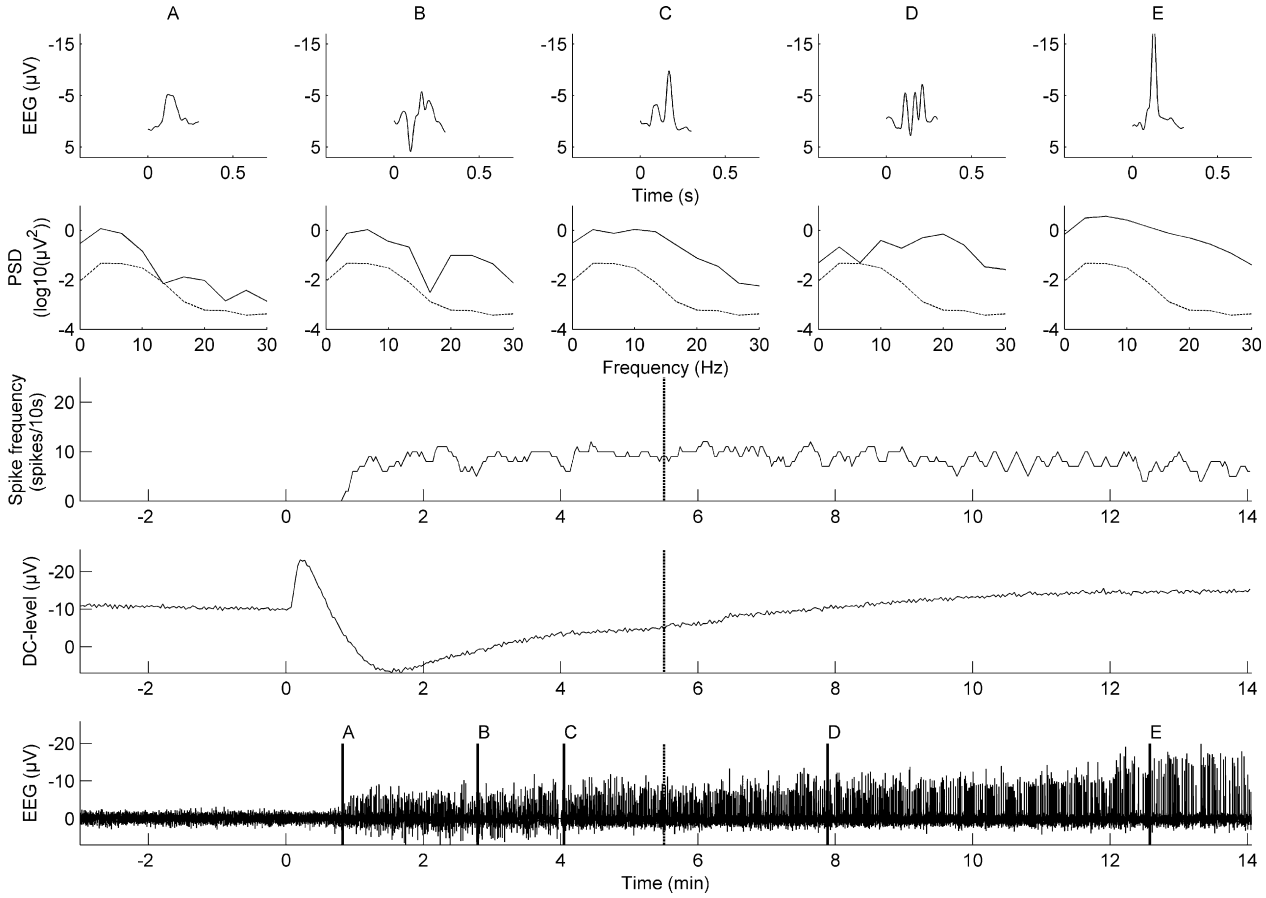

Fig. 3. Summary of the characteristic time sequence of epileptic activity in EEG during a dynamic penicillin-induced focal epilepsy. Dominant waveform and their power spectral densities, respectively in two upper rows. Respective time points are marked in the EEG plot. Also spike frequency and DC-level changes are shown. Dotted line represents the interrupted time continuity (approximately a 20 s pause), see Section 2 for details.

EEG/fMRI series of a focal epilepsy model.[12] This model included dynamic induction of epileptic activity and simultaneous EEG/fMRI in isoflurane anaesthesia. It provided a repeatable finding[13,14] that an initial blood–oxygen-level-dependent (BOLD) fMRI signal increase can occur consistently prior to the appearance of the epileptic spiking in scalp EEG. This is contrary to the conventional view of a delayed hemodynamic response following the spike activity. However, we did not find any consistent changes in the time–frequency analysis of EEG prior to the beginning of interictal spiking. Our results nevertheless suggest that an immediate DC-level shift pattern might exist despite the fact that the finding was not consistent, followed by evolution of interictal spike waveforms and related spectral changes ranging from frequencies under 6 Hz and above.

In humans, either negative DC-shifts or positive ones followed by a negative DC-shift have been associated consistently with seizures in temporal lobe epilepsy.[10] In our study, we found a negative–positive DC-shift ($N = 3$), but not in all cases. It is difficult to say why this finding was limited to only a few animals despite consistent fMRI findings reported in[12] following the penicillin-injection. In cats, positive DC-shift on scalp has been associated with deepening of isoflurane anaesthesia,[16] with a possible origin of blood–brain-barrier opening, first in thalamus. In our study, isoflurane in concentrations of 1.4–1.8 ET%, was used in burst-suppression level. Therefore, a positively shifted DC-level can be present at the moment of penicillin-injection. Isoflurane is also a gap-junction blocker of astrocytes *in vitro* with $10(-3)$ M concentration.[17] The role of gap-junctions in epilepsy is still controversial, but they may contribute to the high-frequency oscillations preceding the ictal activity and inhibit the spread of synchronized neuronal activity. In our study, no continuous epileptic seizure activity was induced, however, not even with a repeated penicillin-injection.

Isoflurane anaesthesia may also limit the spread of epileptic activity via another mechanism: the enhancement of $GABA_A$ergic inhibition, and a blockade of thalamocortical information transfer *in vivo*.[18] This may even enable the localization of the primary epileptogenic zone better than in the awake condition. In humans, isoflurane at anaesthesia levels lower than the burst-suppression level does not suppress epileptic spikes.[19] In hippocampal slice preparations, a dose-dependent reduction of population spikes in epileptiform bursts and their frequency have been found in [20]; however, a typical epileptiform character was retained.

Contrary to isoflurane, penicillin G is a known $GABA_A$ receptor antagonist, impairing the function of GABA-mediated inhibitory neurotransmission (see [1]). Since the inhibition is impaired, recurrent excitatory post-synaptic potentials and intrinsic bursting of a subpopulation of pyramidal cells (in the hippocampus or in neocortical layer V) lead to an excessive cell firing in interconnected cortical neurons, and to a highly synchronized activity of the neuronal population.[1,2]

With the selected penicillin dose (6000IU), the first distinctive epileptic spikes visible in EEG appeared in 49 s – 2 min 44 s after the penicillin injection.[12] The underlying baseline burst-suppression pattern due to isoflurane anaesthesia developed either towards more epileptiform appearance or disappeared.[12] Waveform evolution included variability within and between different morphologies. However, a collection of distinctive forms were

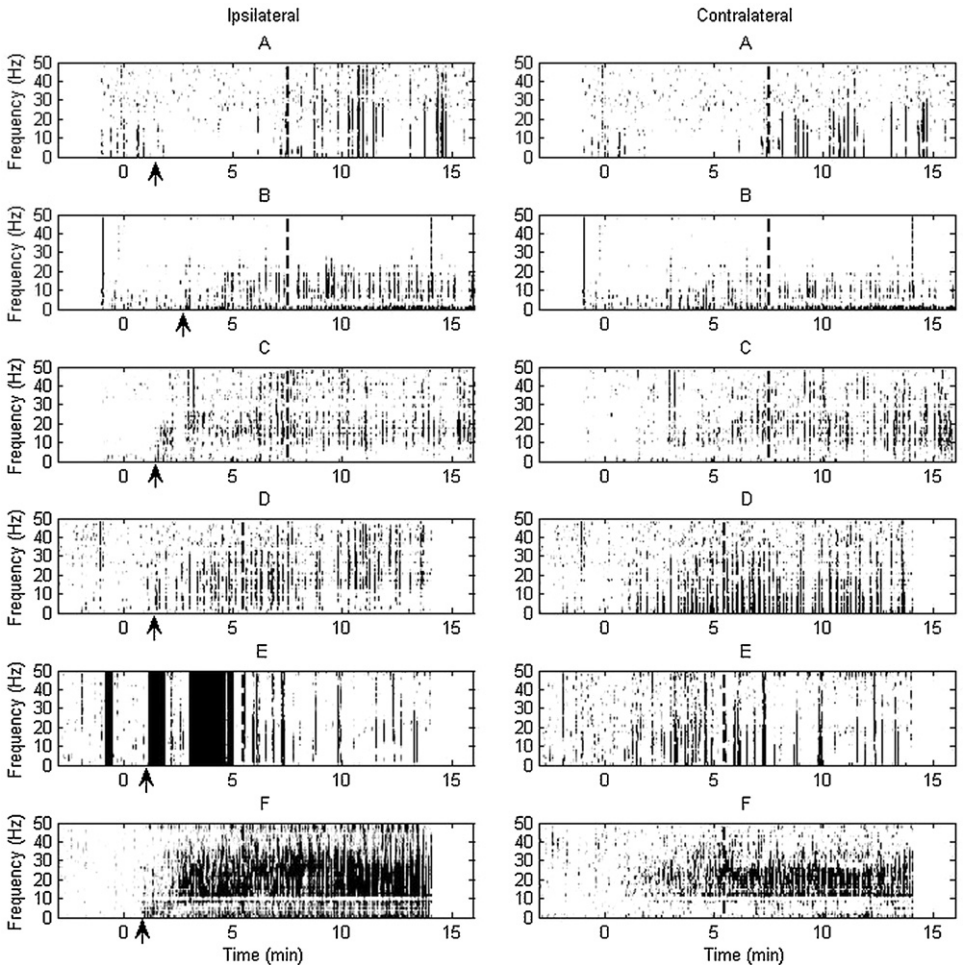

Fig. 4. The normalized time–frequency plots. No evident changes in any frequencies were seen here before the first spike (normal to baseline activity before the penicillin injection). After the first spike (arrowheads), power increases varied from less than 6 Hz frequencies up to 20 Hz. During the evolution of the constant epileptiform activity, a simultaneous wide-band frequency (below 20 or 50 Hz) power increase occurs, as assumed. Dotted line represents the interrupted time continuity (approximately a 20 s pause), see Section 2 for details.

dominating, including a 'blunt' spike or wave; a 'bipolar' waveform (positive–negative peaks or the vice versa), a double spike with varying amplitude relationships (uneven, even), polyspike complexes (double or triplet) and a single sharp wave (or spike-and-wave complex). Most often, the blunt spike, with an appearance (not duration) resembling the 'broad sharp waves' of Bauer et al.,[21] was the first epileptiform wave detected, but not always. In some cases, a bipolar waveform or triplet complexes (sometimes intermingled with single sharp waves or double complexes) began the epileptic activity. Typically, polyspike complexes occurred between blunt and sharp single spikes; however, no definite sequence was found and the different dominating waveforms could be present intermittently despite an emphasis of one form. Similarity to different waveforms of periodic epileptiform discharges (PED) is also striking. Whether PEDs are best regarded as an ictal or a terminal phase of status epilepticus; or post- or interictal phenomenon, is controversial.[22] Ipsilateral and contralateral spike evolution did always differ (Table 1), in accordance with the fact that these two spiking phenomena are underlain by different mechanisms. However, the morphologies were not strikingly different between ipsi- and contralateral sites (Fig. 2).

In the literature, the closest comparison of theoretical models of epilepsy can be found in the results of the related works of Marten et al., Rodrigues et al. and Breakspear et al.[23–25] In their cortico-thalamic model, the key parameters were an effective delay (mean delay of $GABA_B$ coupling between reticular nucleus and thalamic relay centres) and coupling strength of cortical excitatory neurons to thalamic relay nuclei. Adjusting these parameters, different spike-and-wave morphologies arise containing blunt, double and triple spikes (in addition to waves)[23] or single and double spikes as in [24]. Notably, positive–negative (or the vice versa) biphasic (like 'bipolar' stimulus) waveforms were not produced by their model.

In our study, we did not find any consistent changes in the time–frequency analysis of EEG prior to the beginning of interictal spiking, despite the simultaneous 2.5–13.5% average BOLD signal increase of focus region[12] and the known coupling between neuronal activity and BOLD response (see, e.g. [26]). Otherwise, it is known that after penicillin administration the first enhanced physiologic response occurs, then, the paroxysmal depolarisation shifts coupled to interictal activity in electrocorticogram.[4] These changes may precede spiking visible on the skull EEG. Neuronal changes can occur in a smaller cortical area than required for appearance of an EEG "wave".[27] It is surprising that no hint of a consistent change in 'latent' period spectral content was detected, especially in the light of the recent work of Canan et al.[11] In their model of rat ECoG with intracerebroventricular application of penicillin in urethane anaesthesia, during the latent period, the spectral power in all frequencies was decreased compared to basal activity. After epileptiform activity began, spectral power in 3–4 Hz frequencies decreased and it increased in all other frequencies. Later, spectral power showed a marked decrease in 1–2 Hz and a prominent increase in 4–10 Hz. In our study, the beginning of

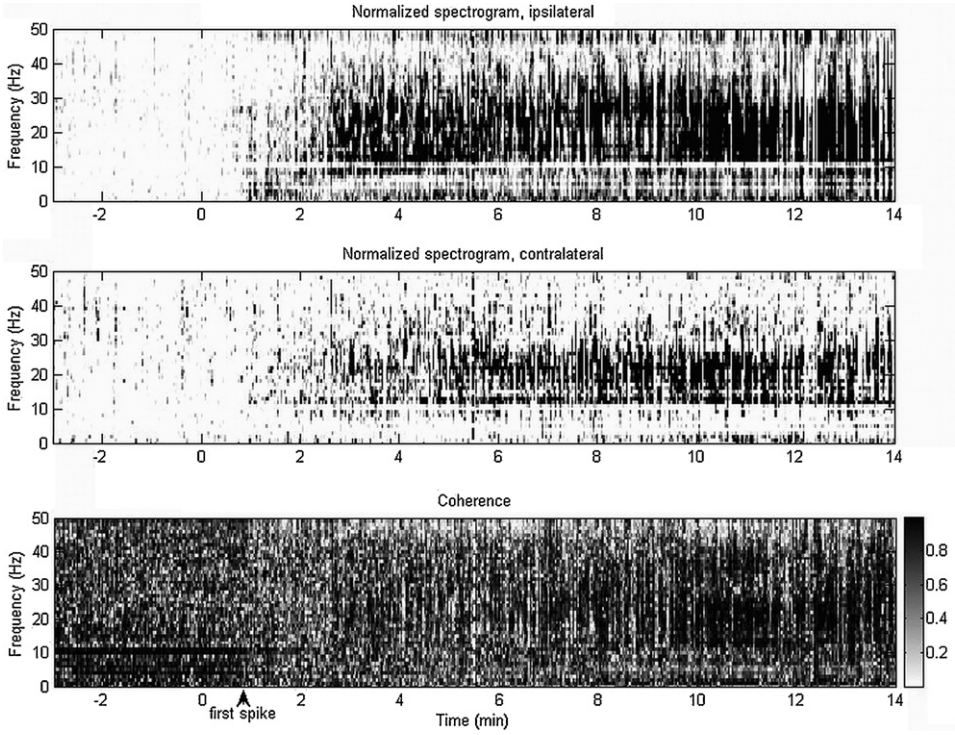

Fig. 5. Coherence in function of time for the pig F (arrowhead = first spike) compared to spectrograms. Note theta and alpha coherence changes and a slight but apparent decrease in widespread frequencies after first spike. See Section 3 for details. Dotted line represents the interrupted time continuity (approximately a 20 s pause), see Section 2 for details.

interictal spiking was related to a power increase in the frequencies below 6 Hz or 20 Hz, and continued to a widespread spectral increase over frequencies with fully developed spikes, as assumed. High frequency oscillations have been associated in intracerebral EEG to the beginning of seizure activity and to localization of the seizure onset zone.[28] However, power in the high frequencies preceding the first spike did not change in our study. Nor coherence between ipsi- and contralateral electrodes, respectively, changed except in one animal (pig F), where a striking reduction of theta coherence followed by alpha frequencies at the onset of spiking occurred, and a weak wide-band reduction as well. It should be noted, however, that alpha power did not change in the spectral analysis during the experiment in this animal. As the isoflurane both blocks gap-junctions and has a counter-effect on penicillin, thalamic generation of alpha may be disrupted. This could suggest a topic for further studies in similar experimental models.

Limitations of the present study have been discussed in Mäkiranta et al.[12] The absolute spike frequency is underestimated here, due to the size of the analysis window that was limited between imaging artifacts. Similarly, the use of this time window leads to uncertainties in the estimation of the power spectral density in the low frequencies. However, the time window was kept the same throughout the analysis for relative comparison. Burst-suppression level anaesthesia may also have a confounding effect. Also, for future development of the model, it is notable that most prominent changes occurred only in one animal, pig F. The reason for that may be the location of the tip of the catheter. In other pigs, the tip was deeper than in pig F. As the insertion of the tip was manually controlled, the exact location was approximate and *a posterior* measurement was made from MRI images.[12] The sensitivity of the cortical layers to induced epilepsy is maximum in layer IV.[3] For further study, it is recommended to use a very shallow and controlled insertion of the catheter. Control injections (solvent/saline) are further suggested in order to exclude any doubt about the nature of EEG spikes: spikes might either be mediated by GABA-mechanism or induced by a mechanic irritation of cortical tissue.

In conclusion, the characteristic time sequence of epileptic activity in EEG may be the following in respect to DC-level changes, the dynamic evolution of waveforms, and spectral content and coherence. First, immediate negative–positive slow shifts in DC-level EEG are plausible. Then, spike activity appears. Spike activity evolution does not follow a clear continuum, however, but variable waveform morphologies are intermingled. Finally, a stabilised activity of high-amplitude monophasic spikes evolves in the time frame of 14–16 min. Spiking-induced widespread increase in power spectral density arises first under 6–20 Hz.

For further EEG/fMRI experiments, investigation of the DC-EEG changes could provide an interesting view of prior-spiking events in EEG. In future studies, a quantitative analysis of spectrograms (e.g. extract dominant frequency, etc.) might help to understand dynamic changes in frequency domain. Also, the findings here gain significance by illuminating a dynamic evolution of epileptic activity for purposes of the on-going modelling work of epilepsy and automatic classification of spikes.[29]

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