Chapter IV

Quantitative EEG analysis: a biomarker for epileptogenesis

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Summary
Purpose EEG recorded during the development of status epilepticus in kainic acid-treated rats is analysed using a method to quantify synchronisation. Moreover, continuous four-week EEG recordings in a kainic acid-treated and a control rat were analysed with this method.

Methods During and after induction of status epilepticus, cortical EEG was continuously recorded for 20 hours from both hemispheres in 8 kainic acid-treated and 7 control rats. In 1 kainic acid-treated and 1 control rat EEG was recorded for four weeks. Event rate (\(ER\)), interhemispheric event synchronisation (\(Q\)), and event interval (\(EI\)) distributions were calculated from the EEG. Events were defined as local maxima in the EEG.

Results In all kainic acid-treated rats, \(ER\) increased from \(ER_{baseline} = 0.37\ \text{s}^{-1}\) to \(ER_{KA} = 3.11\ \text{s}^{-1}\). Events became highly synchronised after minutes to hours (\(Q_{baseline} = 0.24; \ Q_{KA} > 0.90\)). In the 20 hour EEG recordings, 4 phases in the EEG response to status epilepticus-induction were distinguished: Phase I is characterised by low frequency oscillation of periods with high \(ER\) ("bursts of events"). During phase II, \(ER\) increased, and the periods between the bursting shortened. Phase III is characterised by a decreased, constant \(ER\), and a further increase in \(Q\), up to a value of about 1, indicating a very strong interhemispheric coupling. Phase IV is characterised by a strong reduction in \(ER\), with preserved interhemispheric event synchrony. The analysis of the four week EEG-recording in the kainic acid-treated rat showed a remarkable temporal profile. Firstly, kainic acid-induced status epilepticus increased \(ER\) and \(Q\) for about 2 days. Subsequently, both \(ER\) and \(Q\) were very low, after which both parameters were temporarily increased (until day 6), suggesting a silent period of about 4 days. Finally, at day 12 both parameters started to increase slowly, which might be related to progression of epileptogenesis.

Conclusions In the first 20 hours following kainic acid-treatment all rats show a characteristic four-phasic EEG response during the development of status epilepticus, which may parallel phenomena as changes in the receptor dynamics or neuronal cell death. This suggests that a particular timing of interventions in relation to presumed biochemical changes that may occur during induction of status epilepticus is possible. Moreover, the method appears to be very promising for monitoring and quantifying disease progression and automatic seizure detection.
4.1 Introduction

Epilepsy of temporal lobe origin is often progressive in nature and a substantial percentage of patients is unresponsive to treatment with anticonvulsant drugs.\textsuperscript{1} Spontaneous epilepsy established in rats by experimentally induced limbic status epilepticus\textsuperscript{2} shows many of the features seen in human mesial temporal lobe epilepsy, such as neuronal death, gliosis, sprouting and synaptic reorganisation.\textsuperscript{3–7} Status epilepticus-induced epilepsy in rats is therefore considered a good model for mesial temporal lobe epilepsy, which can be used to study the mechanisms underlying epileptogenesis, the development of pharmacoresistance and the development of antiepileptic and disease-modifying drugs.\textsuperscript{8,9}

Limbic status epilepticus can be induced by electrical stimulation of the amygdala or hippocampus or by injection of pilocarpine or kainic acid.\textsuperscript{10–12} Although most animals develop spontaneous seizures in response to status epilepticus, there can be considerable variation in time course, seizure frequency and severity. After hippocampal stimulation seizure frequency progressed up to an average of 10 seizures/day in 47% of the rats.\textsuperscript{10} The other animals in their study showed seizures, but no progression in seizure frequency (24%), or did not develop status epilepticus (29%). Latency to the first seizure ranged from 7–34 days. In the case of treatment with kainic acid a repeated injection schedule has been developed to improve the consistency of status epilepticus-induction.\textsuperscript{13} Nevertheless, in all models certain variability in epileptogenesis is unavoidable. This can be cumbersome if the subject of study requires a homogenous group of epileptic animals. On the other hand, variability in seizure development and associated neuropathological changes can also be advantageous to identify the factors that are critical for epileptogenesis and pharmacoresistance development. In both cases the availability of a parameter that can faithfully predict and monitor the development of spontaneous epilepsy is desirable.

Intracranial EEG can conveniently serve this purpose. Obviously, it can be used to study the time course and properties of seizure activity in brain regions of interest.\textsuperscript{14,15} However, there is more to status epilepticus and epileptogenesis than seizure activity alone. It is a challenging question whether the EEG in the periods preceding and following status epilepticus contains information that can be used to monitor progression and to predict the final outcome of the induced status epilepticus. Various EEG features, such as its spectral properties or synchronisation, can be investigated in relation to the induction of status epilepticus and the ensuing process of epileptogenesis. For instance, Medvedev et al\textsuperscript{14} studied EEG discharges in rats implanted with neocortical and hippocampal electrodes after intravenous infusion of kainic acid, until and including the first convulsive seizure occurring 50–116 min after the infusion. It was found that there is an early increase in gamma activity in the hippocampus from 3–9 minutes after kainic acid administration. Furthermore, in the absence of overt behavioural seizures bilateral spike-wave discharges were observed preferentially in the neocortex, as well as generalised non-convulsive discharges in the neocortex and hippocampus. From the latter type of discharge convulsive seizures could evolve. Development of behavioral seizure activity
correlated strongly and exclusively with an increase in power of frequencies below 10 Hz in the neocortex. Typically, however, these and related studies have been restricted to the first few hours after kainic acid injection.\textsuperscript{14,15} Only a few studies addressed “long-term” electroencephalographic changes (up to 30 hours) during the development of the convulsive status epilepticus. Treiman \textit{et al} describe five stages that were observed during the course of generalised status epilepticus in man. In addition, they observed a similar sequence in untreated generalised convulsive status epilepticus in rats, in which status epilepticus had been induced for example by kainic acid.\textsuperscript{16} Similar stages were observed in soman-intoxicated rats by Koplovitz and Skvorak.\textsuperscript{17} These studies, however, did not include specific quantitative or numerical criteria or definitions as to how each stage starts or ends. A later study by Mikati \textit{et al}\textsuperscript{18} does introduce particular definitions, but still relies on visual interpretation of the single-channel EEG recording.

To our knowledge, a detailed continuous quantitative characterisation by computer analysis of the various phenomena that may be observed during the induction of status epilepticus has not been performed. In this study, we evaluate particular quantitative EEG changes that occur during the kainic acid-mediated induction of status epilepticus in rats, including changes in the synchronisation between the two cerebral hemispheres. To measure synchronisation that is present during seizure activity, different methods have been proposed.\textsuperscript{19} Examples include coherence,\textsuperscript{20} phase synchronisation\textsuperscript{21–23} and synchronisation likelihood.\textsuperscript{24} Recently, Quian Quiroga and co-workers introduced a simple method to measure synchronisation and time-delay patterns between signals, based on well-defined events, such as local maxima or spikes in the EEG.\textsuperscript{25} This method, with some extensions, has been used in this study to quantify the spatiotemporal dynamics of particular events and their synchronisation between the two hemispheres during status epilepticus induced with kainic acid in rats. Furthermore, these events and their interhemispheric synchronisation were quantified in an EEG recording of four weeks following status epilepticus to investigate the feasibility of this method for the analysis of long-term EEG measurements.

\section*{4.2 Methods}

\subsection*{4.2.1 Animals}
The study was approved by the Ethical Committee for Animal Experimentation of Leiden University. Male Sprague-Dawley rats, weighing 200–250 g were obtained from Harlan, Horst, The Netherlands. The animals were housed at a constant temperature of 21 °C.

\subsection*{4.2.2 Implantation of electrodes}
One week before start of the experiments, electrodes were implanted under general anaesthesia with 0.25 mg/kg fentanyl citrate and 8 mg/kg fluanisone (Hypnorm, Janssen Pharmaceutica, Tilburg, The Netherlands) and 18 mg/kg sodiumpentobarbital (Nembutal, Ceva Sante Animale, Maassluis, The Netherlands) intraperitoneally. Two stainless steel electrodes (1.2 mm diameter) were implanted stereotactically over the frontoparietal neocortex at a position 1.0 mm posterior to
bregma and 3.5 mm left and right of the midline. A reference electrode was placed 2.5 mm posterior to lambda. The electrode wires were attached to a connector (MS 363, Plastics One, Roanoke, VA, USA) and the assembly was secured to the skull using dental acrylic cement.

4.2.3 Induction of status epilepticus

The kainic acid-treated rats \((n = 8)\) received intraperitoneal injections with kainic acid once per hour, until the first stage IV/V motor seizure occurred. Typically, 3 injections with kainic acid (first injection 10 mg/kg, all other injections were 5 mg/kg) were needed. Matched controls \((n = 7)\) received intraperitoneal injections with saline.

4.2.4 EEG-recording

In all rats EEG was recorded during 20 hours from the first kainic acid-injection with a sampling frequency of 500 Hz. Baseline EEG was measured for 30 minutes prior to the start of the kainic acid-injections. The EEG of 1 kainic acid-treated and 1 control rat was continuously measured for 4 weeks. Analysis was performed off-line, using Matlab (The Mathworks, Natick, MA) and software developed in our own laboratory. We studied EEG discharges during the first 20 hours using a 2-channel bipolar EEG recording on each hemisphere. Off-line, the data were digitally filtered between 1 and 30 Hz.

4.2.5 Event synchronisation

The EEG was analysed using event synchronisation as reported by Quian Quoroga and coworkers. Here, the method is shortly reviewed. For an extensive treatise the reader is referred to the original paper.\(^{25}\)

Given two simultaneously measured discrete univariate time series \(x_n\) and \(y_n\), with \(n = 1, \ldots, N\). We define particular events, \(E_i^x\) and \(E_j^y\), which occur at event times \(t_i^x\) and \(t_j^y\), with \(i = 1, \ldots, m_x\) and \(j = 1, \ldots, m_y\), with \(m_x\) the number of events in time series \(x_n\) and \(m_y\) the number of events in time series \(y_n\). In the context of the current paper, events will be defined as local maxima in the EEG, which will be further defined. If the signals are synchronised, various events will occur simultaneously. Typically, event synchronisation quantifies the fraction of (nearly) simultaneous events, and also allows quantification of the number of time events from time series \(x_n\) which may lead or lag events from time series \(y_n\).

Events are defined to occur simultaneously if they occur within a (short) time lag \(\pm \tau\). Defining \(c^\tau(x|y)\) as the number of times an event appears in \(x\) after it appears in the time series \(y\), we obtain:

\[
c^\tau(x|y) = \sum_{i=1}^{m_x} \sum_{j=1}^{m_y} J_{ij}^\tau
\]

with:

\[
J_{ij}^\tau = \begin{cases} 
1 & \text{if } 0 < t_i^x - t_j^y \leq \tau \\
0.5 & \text{if } t_i^x = t_j^y \\
0 & \text{else}
\end{cases}
\]

and analogously for \(c^\tau(y|x)\). Subsequently the symmetrical combination is defined by:

\[
Q^\tau = \frac{c^\tau(x|y) + c^\tau(y|x)}{\sqrt{m_x m_y}}
\]
and the anti-symmetrical combination:

$$q_\tau = \frac{c'(y|x) - c'(x|y)}{\sqrt{m_y m_x}}$$  \hspace{1cm} (4.4)

The symmetrical combination ($Q$) measures the synchronisation of the events, and the anti-symmetrical combination ($q$) their delay behavior. Note that $Q$ and $q$ are normalised to $0 < Q < 1$ and $-1 < q < 1$. If and only if the events of the time series $x$ and $y$ are fully synchronised, we obtain $Q=1$. In addition, if the events in $x$ always precede those in $y$ within the time lag $\tau$, $q=1$. In the present study, we set $\tau$ at 3. This value was chosen to achieve sufficient sensitivity to measure synchronisation, but to exclude false positives. The analysis of the continuous recordings shows the validity of this value (see below). Given the sample frequency of $f_s=500$ Hz, a $\tau$ of 3 implies that events occur simultaneously if they occur within 6 ms.

As said above, for the current study, events are defined as local maxima, having an amplitude above a particular threshold level. This level was determined for each EEG channel using the first 500 s of the registration, when the rat was awake. The threshold was set at 4 times the standard deviation of this baseline recording. In addition, this maximum $z$ at $t_i$ should satisfy the condition that $z(t_i) \geq z(t_i + k)$ for $k = -K + 1, \ldots, 0, \ldots, K - 1$ and $z(t_i) > z(t_i + k) + h$ with $K = 3$ and $h = 0.3$. This ensures a sufficient "steepness" towards the local maximum and reduces the likelihood of detection of maxima on a too short local time scale (i.e., within a few samples).

Besides the event synchronisation index $Q$ and the delay behaviour $q$, in the current study the event rate (ER) is defined for each EEG channel as the number of events per unit time, and the event interval (EI) as the period between subsequent events. From the distribution of the $EI$ in overlapping successive time frames, the time course of changes in these $EI$ distributions (bin width = 10 ms) was determined. All parameters were estimated for overlapping windows of 5 min duration. Finally, the cumulative number of events for all rats was calculated by integrating the event rate as a function of time.

### 4.3 Results

#### 4.3.1 EEG during and after induction of status epilepticus

All kainic acid-treated animals underwent a status epilepticus. Figure 4.1 illustrates various phenomena in the EEG of the rats during this status epilepticus or its development. These phenomena were observed in all kainic acid-treated rats, whereas they were absent in the EEG of control rats. Typically, unilateral spikes (figure 4.1A) and bilateral asynchronous spikes (figure 4.1B) were observed when the rats showed staring or wet-dog shakes. During the actual status epilepticus, the EEG showed bilateral synchronous spikes as shown in figure 4.1C. As shown in figure 4.2, these spikes, or events, typically occurred in kainic acid-treated rats, as in these rats the cumulative number of events reached values of $100 \cdot 200 \cdot 10^3$ at the end of the experiment, whereas the cumulative number of events in control rats was only about $10 \cdot 20 \cdot 10^3$.

An example of the temporal behavior of the event rate of both hemispheric recordings is shown in figure 4.3. The duration of these recording is 15 hours. Four distinct phases in the event rate can be observed. In most rats, events first appeared unilaterally; in some rats events were observed bilaterally from the start, but not completely synchronised. This first phase, Phase I, is further characterised by marked oscillations in the event rate, with
Figure 4.1: Three examples of unilateral spikes (panel A), bilateral, asynchronous spikes (panel B) and bilateral synchronous spiking (panel C) as observed in an individual rat at 0.7 hours, 2.9 hours and 8 hours respectively after the first kainic acid injection, which was given at 0.6 hours after start of experiment. Upper trace: recording from the right hemisphere; lower trace: recording from the left hemisphere. For a better visualisation, signals from the left hemisphere are plotted with an offset of -25 μV. The detected events are indicated with a black dot.

ΔT of 8–12 min, where the event rate varied between 10 events per second ("bursts of events") and near-zero values. During these “bursts of events” rats showed staring or wet-dog shakes. At the end of the first phase, the interval between the “bursts of events” shortened and the event rates overall increased signifying the start of the second phase, Phase II. Interestingly, at this time point the rats showed the first class IV/V motor seizure. According to Racine’s scale, a class IV motor seizure is associated with rearing of the animal with concomitant forelimb clonus. A class V seizure is essentially the same, but in addition the animal falls over.13,26

Hereafter, Phase III started, which is characterised by a persistent, intermediate value of the event rate, without the marked oscillations. Behaviourally, the animals experience continuous class IV and/or class V motor seizures. Finally, Phase IV started with a sudden drop in the event rate, which remained low (< 1 s⁻¹), sometimes interspersed with periods with oscillatory high event rates.

Figure 4.2: Time course of the cumulative events in kainic acid-treated rats (black lines) and control rats (grey lines) during status epilepticus. Both the events of the left and the right hemisphere are plotted.
Figure 4.3: Time course of the event rate measured in the right hemisphere (grey line) or in the left hemisphere (black line). For each of the four phases a one hour interval of the event rate is displayed in more detail to show the specific features: in Phase I, the event rate oscillates between high values (10 events per second) and low values near zero, with an interval between two cycles of high event rate, $\Delta T$ of about 8–12 minutes. Phase II shows a gradual increase in the event rate, with a shortening of the interval between the event bursts. Hereafter, Phase III starts, characterised by a persistent, intermediate value of the event rate, without the marked oscillations. Phase IV signifies the end of the stage IV and V seizures, and is characterised by a low event rate, sometimes interspersed with periods with oscillatory high event rates, as indicated.

Figure 4.4: Time courses of the event rate ($ER$) and the event synchronisation ($Q$) for a kainic acid-treated rat (panels A and B) and a control rat (panels C and D). The event rate of the right hemisphere is indicated in grey, of the left hemisphere in black.
Figure 4.5: Signatures of kainic acid induced status epilepticus. In this figure the event interval ($EI$) distributions from the right and left hemispheres are shown as a function of time for all kainic acid-treated rats. The colour bar indicates the mean number of event intervals. At the top of each figure the event rate ($ER$) and the event synchronisation ($Q$) as a function of time are shown. Moreover, the time frames of the four different phases are indicated.
Table 4.1: Individual values of event synchronisation $Q$, event rate $ER$ and delay $q$. Because the start of status epilepticus was not the same for all animals (see figure 4.5), data are calculated using the results of the first 15 hours after start of status epilepticus, rather than from the complete recording of 20 hours.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$Q_{KA}^a$</th>
<th>$Q_{ctrl}^a$</th>
<th>$ER_{KA}$ ($s^{-1}$)$^b$</th>
<th>$ER_{ctrl}$ ($s^{-1}$)$^b$</th>
<th>$q_{KA}$</th>
<th>$q_{ctrl}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.91 (0.59)</td>
<td>0.22 (0.09)</td>
<td>2.88</td>
<td>0.29</td>
<td>-0.018</td>
<td>0.007</td>
</tr>
<tr>
<td>2</td>
<td>0.90 (0.59)</td>
<td>0.18 (0.08)</td>
<td>3.10</td>
<td>0.31</td>
<td>0.018</td>
<td>-0.008</td>
</tr>
<tr>
<td>3</td>
<td>0.95 (0.68)</td>
<td>0.30 (0.10)</td>
<td>3.09</td>
<td>0.40</td>
<td>0.018</td>
<td>-0.012</td>
</tr>
<tr>
<td>4</td>
<td>0.94 (0.75)</td>
<td>0.22 (0.08)</td>
<td>3.11</td>
<td>0.37</td>
<td>0.014</td>
<td>-0.004</td>
</tr>
<tr>
<td>5</td>
<td>0.97 (0.75)</td>
<td>0.22 (0.06)</td>
<td>3.70</td>
<td>0.26</td>
<td>0.024</td>
<td>0.005</td>
</tr>
<tr>
<td>6</td>
<td>0.94 (0.66)</td>
<td>0.25 (0.09)</td>
<td>2.71</td>
<td>0.34</td>
<td>-0.004</td>
<td>0.002</td>
</tr>
<tr>
<td>7</td>
<td>0.89 (0.63)</td>
<td>NA$^c$</td>
<td>2.49</td>
<td>NA$^c$</td>
<td>0.009</td>
<td>NA$^c$</td>
</tr>
<tr>
<td>8</td>
<td>0.89 (0.63)</td>
<td>0.21 (0.08)</td>
<td>3.83</td>
<td>0.21</td>
<td>-0.109</td>
<td>-0.007</td>
</tr>
<tr>
<td>mean</td>
<td>0.92 (0.66)</td>
<td>0.24 (0.09)</td>
<td>3.11</td>
<td>0.37</td>
<td>0.03$^d$</td>
<td>0.006$^d$</td>
</tr>
<tr>
<td>SEM</td>
<td>0.01 (0.02)</td>
<td>0.02 (0.004)</td>
<td>0.16</td>
<td>0.06</td>
<td>0.01$^d$</td>
<td>0.001$^d$</td>
</tr>
</tbody>
</table>

$^a$Maximal value of synchronisation. Between brackets mean of synchronisation.

$^b$Mean for both channels.

$^c$Not available.

$^d$Mean and SEM of the absolute value.

The synchronisation, $Q$, shows a comparable temporal profile. An illustration of the temporal behavior of both the event rate ($ER$) and the concomitant event synchronisation ($Q$) after kainic acid-treatment are shown in figure 4.4 panels A and B respectively. In Phase I, synchronisation values strongly fluctuated between $0.1 < Q < 0.8$. These fluctuations were phase locked to the oscillations in the event rates, indicating that the events occurring during the first phase are synchronised. During Phase II, $Q$ showed a less distinct oscillatory activity, and had a mean value of 0.5. Typically, $Q$ slightly decreased throughout this phase. In the course of Phase III, $Q$ increased to 1, indicating that almost all events occur simultaneously in the right and left hemisphere. This very high synchronisation typically remained present during Phase III. In Phase IV, the event rate
strongly dropped, however, if events occurred, they were strongly synchronised, which is reflected in the high values of $Q$ that were then observed. The results for a control rat are shown in figure 4.4 panel C ($ER$) and panel D ($Q$), showing that the typical temporal profiles for $ER$ and $Q$ as observed in kainic acid-treated rats are not visible in control rats.

Figure 4.5 shows the distribution of the event intervals as a function of time for all kainic acid-rats, with the corresponding event rates and event synchronisation. The colour bar indicates the mean number of event intervals present in each analysis window of 5 seconds, averaged over 5 minutes. Five rats showed very similar time courses of the event interval distribution, whereas the pattern of three other rats was slightly different.

Obviously, the time course of the distribution of the event intervals follows the time course of the event rate. Thus, generally speaking, a higher value of the event rate, was related with a smaller event interval. However, during Phase III some interesting features can be observed. During this phase, most rats showed a very broad and vague band at an event interval of 200–300 ms, and one or two more pronounced and sharply bounded bands at an event interval of 30–80 ms. The occurrence of different bands implicates that complexes consisting of several events are present. The event interval within a cluster was 30–80 ms, whereas the clusters appeared with an interval of 200–300 ms.

Another striking feature of the event interval distribution during Phase III, was that the broad band which starts at 200–300 ms and the sharp band which starts at 30–80 ms came together at the end of Phase III. This suggests that at the end of Phase III single events take place, rather than complexes. These events occur with an interval of about 100 ms.

Figure 4.6: Time course of the event synchronisation ($Q$; upper panel) and the event rate ($ER$; lower panels) in a kainic acid-treated rat and a control rat for about 28 days (650 hours). The time course of $Q$ in the kainic acid-treated rat is displayed in black, and the time course of $Q$ in the control rat in grey (upper panel). For a better visualisation the scale of the event rate is clipped at 1 s$^{-1}$. Data are mean per hour.
No significant differences were found in the delay $q$, indicating that it was not possible to label one of the hemispheres as “driver” and the contralateral hemisphere as “responder”.

In table 4.1, an overview of the event synchronisation, event rate, and delay $q$ for both kainic acid-treated and control rats is presented. These data were calculated from the first 15 hours after the first appearance of events, rather than from the complete recording of 20 hours. This table clearly shows that both the event synchronisation and the event rate were significantly higher in kainic acid-treated rats than in control rats.

### 4.3.2 Time course of event rate and synchronisation during 4 weeks after status epilepticus

As a pilot study, the EEG of a kainic acid-treated and a control rat was continuously recorded for 28 days after induction of status epilepticus. Practical limitations precluded extended observation of the behaviour of the 2 animals and inclusion of more animals in this pilot study, but the results were sufficiently interesting to warrant presentation in this context. The temporal changes of the event rate and the event synchronisation in both rats are shown in figure 4.6. In figure 4.7 the changes during the first 10 days are shown in more detail. These data show a number of interesting features.

During the first day, the kainic acid-treated rat experienced a status epilepticus, that resulted in a pronounced increase in both the event rate and the event synchronisation. Based on observation of the behaviour of the animal the status epilepticus lasted about 10 hours, but interestingly, both event rate and synchronisation returned to baseline only
at 50 hours (2 days) after the start of status epilepticus. Furthermore, at 140 hours (6 days) transient increases in event rate started to appear. Evidently, the events (local maxima) in the EEG were synchronised between both hemispheres, as the event synchronisation was concurrently increased. After 300 hours (12 days) the peaks in event rate were smaller than during the preceding 6 days, but the overall value was constantly increased compared to control. This increase was again correlated with an increased event synchronisation. This temporal profile of event rate suggests that the silent period lasted for 6 days in this rat and that spontaneous epileptic activity started to appear at this point. Examples of EEG recordings in the kainic acid-treated and control rat are presented in figure 4.8.

In the control rat the event rate remained approximately constant during the complete recording. Between 50 and 180 hours after the start of the experiment, i.e. the period corresponding to the silent period in the kainic acid-treated rat, the event rate in both hemispheres was higher than measured in the kainic acid-treated rat. However, these events exhibited a low degree of synchronisation as illustrated by the constant, low, value for $Q$. In contrast, in the kainic acid-treated rat, even a small increase in event rate was always accompanied by a significant increase in event synchronisation (figure 4.7).

### 4.4 Discussion

In this study, the method described by Quian Quiroga et al. was applied to analyse cortical EEG recordings before, during and after induction of status epilepticus with kainic acid. This method is based on the identification of local maxima, or events, in the EEG, including their interhemispheric synchronisation. Interestingly, this showed that during the induction of status epilepticus and status epilepticus itself the EEG can be divided into four phases. Phase I is characterised by low frequency oscillations ($\Delta T = 8–12$ min) of high event rates (“bursts of events”). Phase II marks a period of strong increase and reaching a maximum in the event rate, with shortened interburst
intervals. Subsequently, Phase III is characterised by a small decrease in the event rate, settling at a constant level, and a further increase in the event synchronisation between the two hemispheres, to values near 1, indicating a very strong interhemispheric coupling. A second characteristic of this phase is the presence of two local maxima in the event intervals, the first centred around 30–80 ms and the second initially around 350 ms, but gradually declining. Finally, Phase IV is entered after approximately 8–12 hours, characterised by a strong reduction in event rate, with preserved high interhemispheric event synchrony.

The selectivity and sensitivity of the method to detect epileptic spike discharges, or events, is highly dependent on the values of the parameters defining the minimum amplitude and the steepness of the spike. In the present study, the values for these parameters were experimentally defined. As displayed in figure 4.2, presumably in kainic acid-treated rats events were recorded, whereas only a few events were recorded in the control rats, showing the validity of the used parameter values.

Since the current EEG analysis is based on the quantitative definition of events, including a measure for interhemispheric synchrony, a detailed comparison with previous studies discussing various stages in the sequence of electroencephalographic changes, both in humans and in kainic acid-treated rats, by visual analysis of single-channel ECoG, is not straightforward. Furthermore, these studies did not provide a full temporal profile of the various phases, nor quantitative criteria for transitions between the various phases. We can reliably conclude, however, that our study is not in contradiction to previous observations of the presence of various phases or stages during the course of generalised convulsive status epilepticus.17,18,16

An intriguing question is whether this EEG analysis can be used as a biomarker for epileptogenesis. Important issues to discuss in this respect are the temporal profile of the EEG preceding and during status epilepticus, the strong interhemispheric coupling as represented by the high value of synchronisation, and the analysis of a four week continuous EEG recording in a kainic acid treated and a control rat.

Interestingly, the four phases in the EEG during status epilepticus were related to specific behaviours. During Phase I the animals exhibited periods of staring or wet-dog shakes. Single class IV/V motor seizures were observed during Phase II, and continuous class IV/V motor seizures occurred during Phase III. During Phase IV no specific motor seizures were observed, but the animals were unresponsive to external stimuli. In the present study it was not possible to extensively investigate the behaviour of the rats in a quantitative manner, but measuring the EEG directly in the motor cortex, as was the case in the currently used approach, offers an interesting opportunity to link epileptic behaviour directly to observed EEG responses.

The high synchronisation value, Q, observed in all rats during Phase II and Phase III, indicates the induction of a strong interhemispheric coupling. Several studies have been published about this issue. For instance, Khalilov et al showed in vitro the formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures,
with a delay of about 50 ms. In their study, repeated (10–15 times) brief applications (2–3 min) of kainic acid, inducing repeated ictal episodes, were needed to induce this secondary epileptic focus, which appeared synchronised with the primary focus. Another study by Ono et al., showed in humans interhemispheric delay times of 0 ms. These authors propose a particular facilitation of an epileptogenic susceptible state of the corpus callosum in various forms of epilepsy and suggest there is most likely not a “transfer role”, but a different contribution of the corpus callosum to the interhemispheric recruitment. Whether proper functioning of the corpus callosum, either as a “conductor” or “facilitator”, is a necessary condition for epileptogenicity in the in vivo kainic acid rat model is currently uncertain.

The feasibility of the proposed method to analyse long term EEG recordings was one of the key questions of this study. As a pilot we used a continuous recording studied for four weeks starting with induction of status epilepticus in a rat. Although the study was rather limited the analysis of this recording allows two important conclusions. Firstly, event rate and event synchronisation differ strikingly between the kainic acid-treated and the control rat. Although events were scored in both hemispheres of the control rat, these events were only poorly synchronised as the synchronisation value was very low. In the kainic acid-treated rat however, the events were strongly synchronised, as an increase in event rate was accompanied with an increase in event synchronisation.

Secondly, the event rate and synchronisation show a very interesting temporal pattern in the kainic acid-treated rat, that may be used to characterise the status epilepticus, the silent period and the period of spontaneously occurring seizures. At day 1 both event rate and synchronisation were dramatically increased as result of status epilepticus-induction, and returned to baseline at the end of the second day. Subsequently a silent period was observed until day 6. At that time, the event rate and synchronisation only temporarily increased, and in between returned to baseline. At day 12, both parameters started slowly to increase continuously. In the control rat, however, no remarkable changes in these parameters over time were observed. These results suggest the applicability of the method to plan and measure the effect of interventions.

The promising results of this pilot study offer exciting opportunities to continue these studies in several directions. As stated, in the present study, it was not possible to record the behaviour of the animals continuously during the EEG recording. Therefore, as a next step, the temporal event rate and synchronisation profile in kainic acid-treated rats should be correlated to epileptic behavioural manifestations. This will make it possible to relate EEG events and their synchronisation measure to behavioural motor seizures. As cortical EEG is used in this approach, it is expected that the events in the EEG are very closely correlated to behavioural epileptic manifestations. Therefore, it is hypothesised that the method is useful as a biomarker for monitoring epileptogenesis, and to automatically detect motor seizures.
4.5 Conclusion

In summary, a characteristic four-phasic EEG response during status epilepticus-induction has been demonstrated. The proposed approach allows a quantitative analysis of the effect of interventions, e.g., with seizure modifying drugs or neuroprotective agents. The four-phasic EEG response suggests that a particular timing of these interventions in relation to presumed biochemical changes that may occur during induction of status epilepticus, is possible. Moreover, this approach is also very promising with regard to monitoring disease progression, and automatic detection of epileptic seizures.

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References


