Is burst activity in cortical slices a representative model for epilepsy?

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Abstract

Neocortical slices including sensory cortex of mouse were used to study cellular and network activity during bicuculline evoked seizure-like activity. The relationship between the activities of single cells and the network was quantified using entropy measures of spike trains. The network shows a large increase in synchronous burst activity during the seizure-like phase. Surprisingly, the individual cells do not seem to follow this pattern of increased synchrony. It is hypothesized that the recruitment of silent units during the seizure onset may explain this paradoxical finding. Our data agrees with recent findings in experimental seizures and intra-operative recordings in humans.

Introduction

Epilepsy is the most common serious pathological condition in neurology [2]. From the standpoint of electrophysiology, seizures are frequently associated with synchronized hyperactivity in neural networks and high levels of cellular depolarization.

Synchronization in the nervous system is not always pathological. The normal sleep state is characterized by a slow and highly coordinated activation of millions of neurons [6], while the awake state is characterized by faster and less coordinated activity of neurons.

Since synchronization, high levels of activity and membrane depolarization are found in both epileptic and normal bursts, quantification of various aspects of burst activity might help to detect different mechanisms between normal and pathologic activity. To study burst activity at the tissue level, we performed simultaneous extra- and intracellular electrophysiological recordings on neocortical slices. This enabled us to quantify to what extend the cells are synchronized with a network. We evoked seizure-like bursts by application of bicuculline, an antagonist of the $GABA_A$ receptor [1].

Methods

Neonatal (P8-13) male or female CD-1 mice were deeply anesthetized with ether, decapitated at the C3/C4 spinal level and the cortex isolated in ice-cold artificial cerebrospinal fluid (ACSF). Neocortical slices (500 μm thick), encompassing the somatosensory cortex, were sectioned and transferred into a recording chamber and submerged under a stream of ACSF (temperature, 30^{o}C; flow rate, 10 ml/min) containing (mM/l): 118 NaCl, 3 KCl, 1.5 $CaCl_2$, 1 $MgSO_4$, 25 $NaHCO_3$, 1 NaH_2PO_4 and 30 D-glucose equilibrated with carbogen (95% O_2 and 5% CO_2). After 30 min the potassium concentration was raised from 3 to 5 mM/l to obtain spontaneous rhythmic activity.

An overview of the experimental setup is shown in Fig 1. Population activity was recorded extracellularly with suction electrodes positioned onto the surface of the cortical layers II/III. The signals were amplified 2000 times, filtered between 0.25 and 1.5 kHz,

rectified and integrated using an electronic filter (time constant of 30-50 ms). With these settings, the filter output was a measure for extracellular spike activity. Intracellular patch-clamp recordings were obtained from cortical neurons with the blind-patch technique. Patch electrodes were filled with a solution containing (mM/l): 140 K-gluconic acid, 1 $CaCl_2*6H_2O$, 10 EGTA, 2 $MgCl_2*6H_2O$, 4 Na_2ATP, 10 HEPES. In several recording sessions biocytin (4.5 mg/ml) was added to the solution. The recordings were low-pass filtered (0-2 kHz, Bessel 4-pole filter, -3dB). An example of simultaneous intracellular recording and population activity is depicted in Fig. 1. The cells described in this study had membrane potentials ranging from –57 mV to –68 mV and action potentials with an overshoot.

At concentrations between 3-5 mM K^+ in the ACSF, the slice preparation generated rhythmic synchronized population activity. This activity was used as the baseline (BLN). During the BLN phase, the intrinsic firing properties of the neuron were determined by injecting depolarizing currents. After the blockade of GABA-ergic synaptic transmission with bicuculline (10μM/l), seizure-like activity (SLA) bursts of population activity were generated, followed by a steady state (STD) of ongoing activity. An examples of an extracellular recording including the three phases: BLN, SLA, and STD can be seen in Fig. 1B. In one of six recordings, the network didn't transition to STD. This recording was not included in the quantitative analysis.

Signal processing was applied to describe the entropy and information content in the signals during different bursting patterns. Bursts were detected as they exceeded a visually determined threshold (Fig. 1C). The population activity and cellular signals were averaged and aligned on the basis of the activity bursts in the population (burst related

window, Fig. 1C). Spike activity in each of the trials in the average was determined. An example of a series of burst-aligned trials is shown in the raster plot in Fig. 2. To study information content in the cellular activity, entropy was calculated using Shannon's formula for entropy:

$$H = -\sum_{i=0}^{\infty} p(i) \cdot \log_2(p(i))$$

where H is the entropy in bits and p(i) the probability of observing the i^{th} activity pattern. The method we applied is similar to the one described by Reich and coworkers [5]. In short, the spike trains in all trials were aligned according to the population burst and subdivided into time bins that varied in size from 5-200 ms. The probabilities to observe i spikes was determined from the spike counts in the bins. The total entropy was determined by taking into account all bins, and represents the variability across time (H_t, Fig. 2). The noise entropy was determined by taking into account all bins across trials at a fixed time (H_n, Fig. 2). We used the mean value of all noise estimates as the noise estimate for further analysis. The bin size that generates the highest noise entropy rate was selected. The values were calculated in bits and were normalized by dividing by the bin size (bits/s). Burst-related information was calculated as the difference between the total and noise entropy:

$$H_b = H_t - \text{mean}[H_n]$$

Each of the values (H_t, H_n, H_b) was determined for each phase (BLN, SLA, STD) and for each cell. The significance of the changes of the measures between the phases was determined with a Wilcoxon test.

Results

The average network burst frequency during the BLN and STD is around 0.1 Hz and increases to and average of 0.4 Hz during the SLA. These differences in burst rate were significant ($p < 0.05$). The amplitude of the individual network bursts didn't change significantly between states.

Five of the neurons were (according to their intrinsic properties) regular spiking neurons, one was an intrinsically bursting neuron. During the 'spontaneous' activity, the intrinsic burster could not be distinguished from the other neurons. Two regular spiking neurons we patched were labeled with biocytin, and found to be layer II/III pyramidal neurons. The relationship between the cellular activity and the population was recorded in each of the phases. A typical example of cellular BLN activity at the population burst (Fig. 3A) shows an excitation followed by inhibition. This is reflected in the time-course of the average membrane potential and the spike activity (firing rate and superimposed spike trains). During the SLA (Fig. 3B) the inhibition is earlier in the burst, and the excitation pattern becomes irregular. The STD (Fig. 3C) shows a strong synchrony between cell and population.

The total entropy per second (Fig. 3D) is highly correlated with the spike rate ($r = 0.99$, $p< 0.001$). The largest part of the total entropy is a noise component, i.e. not-burst-related (Fig. 3E). The difference between total entropy and noise entropy is the burst-related component, which is a measure for the similarity between the action potential activities of the cell and the network. The ratio between signal and noise (S/N) is minimal during the SLA and maximal during the STD (Fig. 3F). The difference between these maximum and minimum values is significant ($p < 0.05$).

Discussion

We demonstrated that the relationship between cellular and network activity can be quantified by entropy values of the spike trains, and that the S/N parameter changes from the SLA to the STD (Fig. 3F). It is remarkable that there is no significant increase in the burst-related entropy or S/N parameter during the SLA as compared to the BLN and STD (Fig. 3D, E, F). This indicates that the activity of single cells do not become massively synchronized with the network at the onset of the SLA. Both visual inspection of the average activity (Fig. 3A, B, C) and the significant change in S/N (Fig. 3F) suggest that increased synchrony between unit and network occurs later during the STD.

The network burst rate is significantly increased during the SLA ($p < 0.05$). The amount of synchronous behavior of the network can be assumed proportional to the product of burst rate and average amplitude of the burst. Considering the extracellular activity of the network during the SLA, one can therefore conclude that the synchronous network activity per unit time increases significantly during SLA ($p < 0.05$). Since this phenomenon cannot be explained from the activity of the individual units, there must be another factor playing a role. We think that the most likely explanation is an increased number of active units during seizure onset. Indeed an increase of evoked population activity after administration of bicuculline was shown in an intrinsic optical imaging study by Kohn et al [3]. Due to recruitment of previously silent neurons, the network shows increased bursting during the SLA. Under this assumption, the SLA is characterized by a large number of active neurons, and each may show a low synchrony with the network bursting activity. Low synchrony between individual neurons during

seizure like discharges was described by Netoff and Schiff [4]. Although in a general sense, our findings are similar to the results of this study, a detailed comparison is difficult because these authors used a different definition of seizure-like activity, and focused on the relationship between the membrane potential of cells in which the action potential generation mechanism was disabled.

Our results are in agreement with intra-operatively recorded seizure-related neuronal activity in humans, where low synchrony was found to be present at seizure onset [7]. It is compelling that recordings in slices demonstrated an important similarity between SLA and seizures in the intact brain. This similarity is an indication that (for some aspects) SLA may be a representative model for epilepsy, although at this stage it would be premature to answer the question in our title.

Acknowledgement

This work was supported in part by a grant from the Falk Center for Advanced Study and Care of Pediatric Epilepsy.

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Legends

Fig. 1: Overview of the experimental setup and recording.

A. Intra- and extracellular electrodes were placed in a neocortical slice.

B. This setup allowed us to record an integrated measure of extracellular bursting (upper trace) and cellular activity (lower trace). After adding bicuculline, the activity transitioned from baseline (BLN) to a seizure-like activity (SLA). In five out of six cases, SLA spontaneously transitioned into a steady state (STD) of activity.

C. Details from B show the population burst and the cellular activity in analysis windows around the network burst

Fig. 2: Raster plot of subsequent spike trains aligned with the network burst. A value for the total entropy (H_t) is determined across time. A noise entropy value (H_n) is estimated

across trials for each bin. Burst related entropy (H_b) is determined by subtracting the mean noise entropy from the total entropy.

Fig. 3: Average results during the baseline (BLN, A), the seizure-like activity (SLA, B) and steady state (STD, C). Each graph shows the average network burst waveform (NB), the average membrane potential (MP), and the average firing rate (FR). The lower trace in each panel are the superimposed spike trains (ST).

The total entropy (D), the noise related entropy (E), and the signal-to-noise ratio (F). The arrow indicates a significant difference ($p < 0.05$).

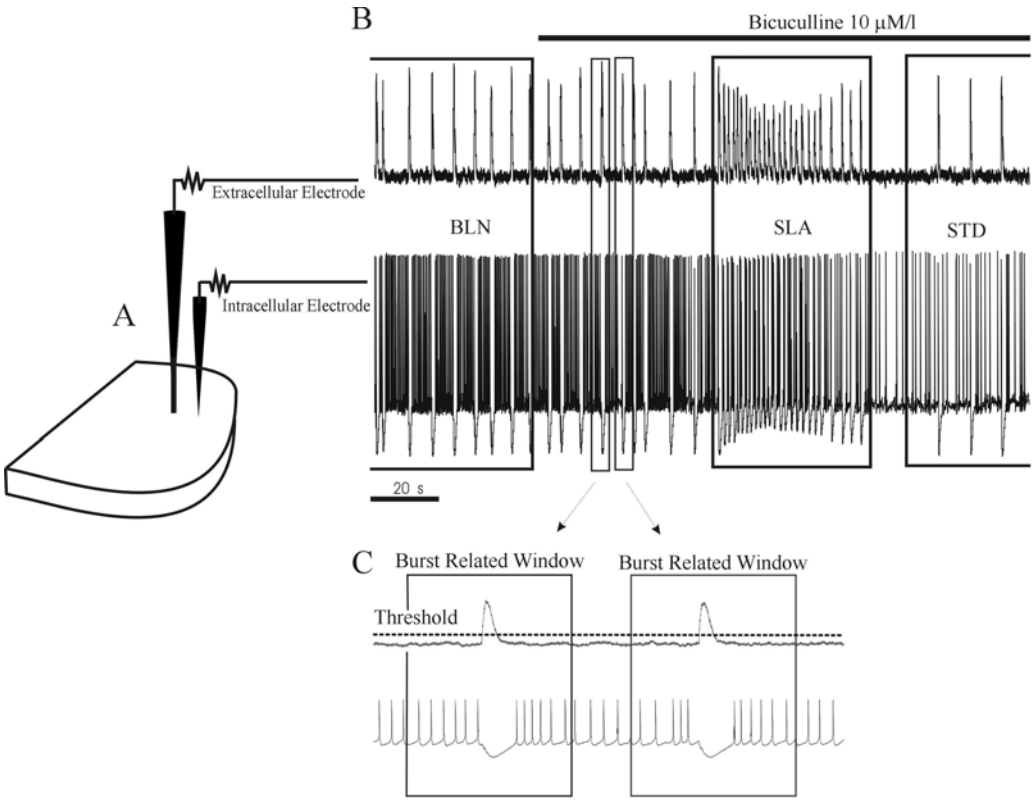

Fig. 1

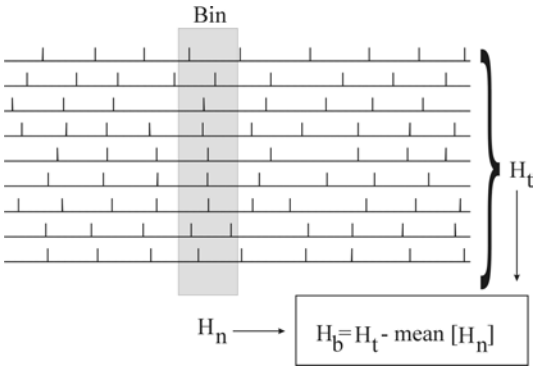
Bin
H_t
H_n
$H_b = H_t$ - mean [H_n]

Fig. 2

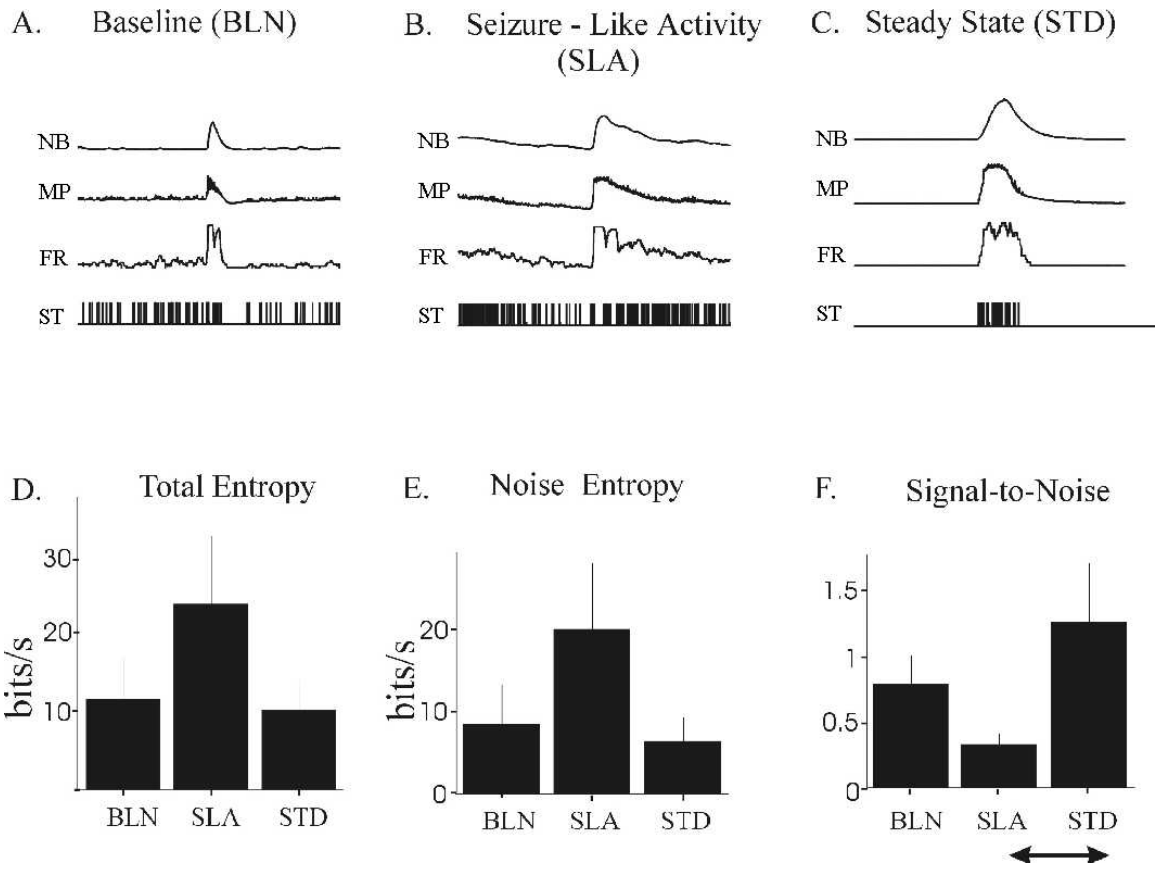
A. Baseline (BLN)
B. Seizure - Like Activity (SLA)
C. Steady State (STD)
NB
MP
FR
ST
D. Total Entropy
E. Noise Entropy
F. Signal-to-Noise
bits/s
30
20
10
0
1.5
1
0.5
BLN
SLA
STD

Fig. 3