Epileptological investigations in living human brain slices: spontaneously occurring sharp field potentials

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Introduction

It is generally known that epileptic activity is reflected by typical potentials in the conventional EEG. From that the question arises, whether restricted neuronal networks, e.g. in living human brain slices obtained from epilepsy surgery, show bioelectric potentials similar to those found *in situ* and grossly different from those in non-epileptic material. Therefore, electrophysiological investigations on resectates from partial temporal, frontal, parietal and occipital lobectomies were conducted. As the tissue in question originates preferentially from human temporal lobe which has chronically been involved in seizure activity, it is of special interest if the networks of tissue display potentials which occur spontaneously also *in vitro*.

Most studies *in vitro* failed to demonstrate spontaneous epileptiform potentials *in vitro* [1, 2, 4–6, 23]. In only two investigations, field potential activity [3] and spontaneously occurring postsynaptic potentials recorded intracellularly [7] were reported in a few cases.

The fact that spontaneous activity was so rarely observed in living human brain slices from epilepsy surgery could reflect that (1) spontaneous activity appears primarily *in situ* and is eventually lost in *in vitro* preparations or that (2) foci of such activity are very small and thus difficult to detect. In the present study, our group therefore attempted a systematic search for spontaneous activity in human neocortical tissue resected during epilepsy surgery. In fact, spontaneously occurring sharp waves could be detected in field potential recordings. Furthermore, we tried to establish the role of glutamatergic and GABAergic synaptic transmission, as well as the role of voltage-gated calcium channels in the generation of such spontaneous activity.

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Methods

Human tissue obtained was a small portion of that which is normally excised for treatment of pharmacoresistant focal epilepsy mostly originating in the temporal lobe. A flow chart demonstrating the distribution of the human brain material is given in figure 1. Temporal lobe tissue used in this study originated from the anterior portion of the inferior temporal gyrus from standard partial temporal lobectomies. A schematic diagram of the allocation of the tissue probes for different experimental approaches is given in figure 2. The patients, female and male, were aged 1–45 years. In many cases, histopathological analysis of the neocortical samples revealed a mild degree of gliosis or atrophy. In a few cases, cortical dysplasia and a tumour were diagnosed. Hippocampal sclerosis was seen in about 50% of the cases. In 30% of the cases, no pathological abnormalities were found.

The experiments were approved by the local ethics committee. Informed consent was obtained from all patients.

Tissue slices were prepared from 1 cm^3 block of the inferior temporal gyrus (Fig. 2). The techniques for slice preparation were described in detail elsewhere [8–10]. Briefly, neocortical slices of 400–500 µm thickness were cut perpendicular to the pial surface using a vibratome. They were placed in a portable incubation chamber [11] with oxygenated (95% O_2, 5% CO_2) artifical cerebrospinal fluid (ACSF) at a temperature of 28°C and a pH of 7.4. A technical drawing of the transportable incubation chamber is given in figure 3. Slices were allowed to recover for a period of 1–2 h before being transferred into a recording submerged-type chamber. The composition of the ACSF was (in mM): NaCl 124, KCl 4, $CaCl_2$ 2, NaH_2PO_4 1.24, $MgSO_4$ 1.3, $NaHCO_3$ 26 and glucose 10. In the experimental chamber, the tem-

Figure 1 Flow chart demonstrating the distribution of the human brain material.

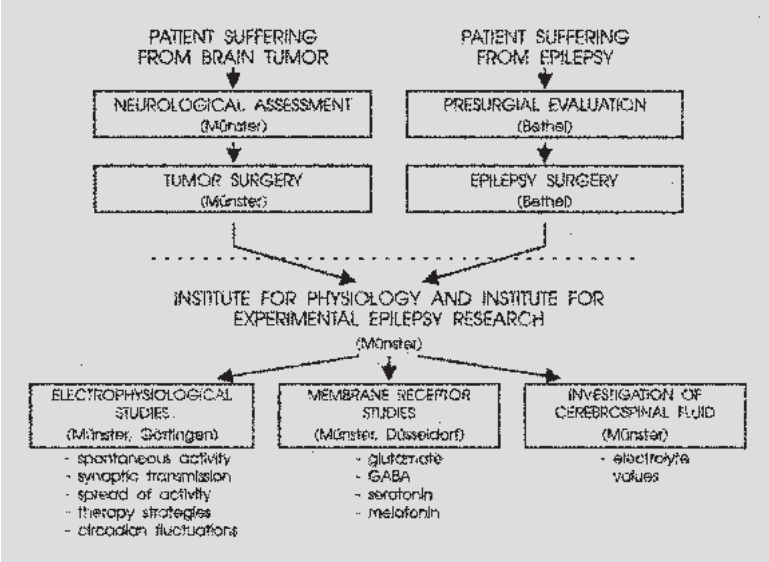
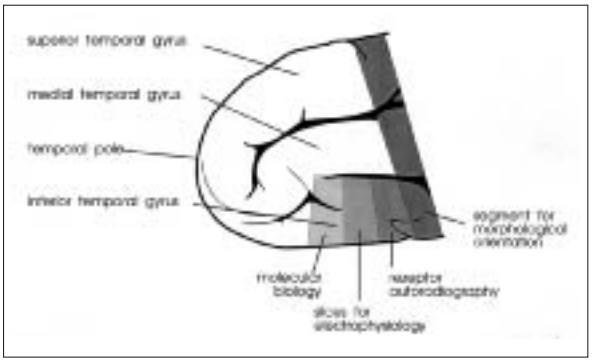


Figure 2 Schematic diagram of the allocation of the tissue probes for different experimental approaches.



perature was raised to 33 °C. In some cases, $MgSO_4$ was omitted from the superfusate. During the experiments, pH, temperature and flow rate (4 ml/min; bath volume 1 ml) were continuously monitored.

All substances used were dissolved in ACSF and added to the superfusate. Drugs applied were: the glutamate subrector antagonists DL-2-amino-5-phosphono-valerate (APV, 100 µM; to block the NMDA receptor) and 6-cyano-7-nitro-quinoxaline-2, 3-dione (CNQX, 5 µM; to block the AMPA receptor); the gamma-aminobutyric acid subreceptor antagonists bicuculline methiodide (5–10 µM; to block the $GABA_A$ receptor) and CGP 55845A (1 µM; to block the $GABA_B$ receptor); the organic calcium channel blocker verapamil (40 µM), and the antiepileptic drugs carbamazepine and phenytoin (25 µM and 100 µM; dissolved in dimethyl-sulfoxide and 1 N NaOH solution diluted to a final concentration of 0.1%, respectively).

Extracellular field potential recordings were performed in layers II–V (as estimated by measuring the position of the electrode 300–2400 µm below pial surface [12] using pipettes (0.5–1.5 MΩ) filled with ACSF. In most trials, recordings were done with two electrodes simultaneously. To search for spontaneous activity, the electrodes were periodically repositioned following an imaginary position grid consisting of squares about 500 µm wide as to cover the whole slice. In addition, field potential recordings were done using a linear array of 8 insulated Karma wire electrode

Figure 3 Technical drawing of the portable incubation chamber for tissue slices (modified after [11]).

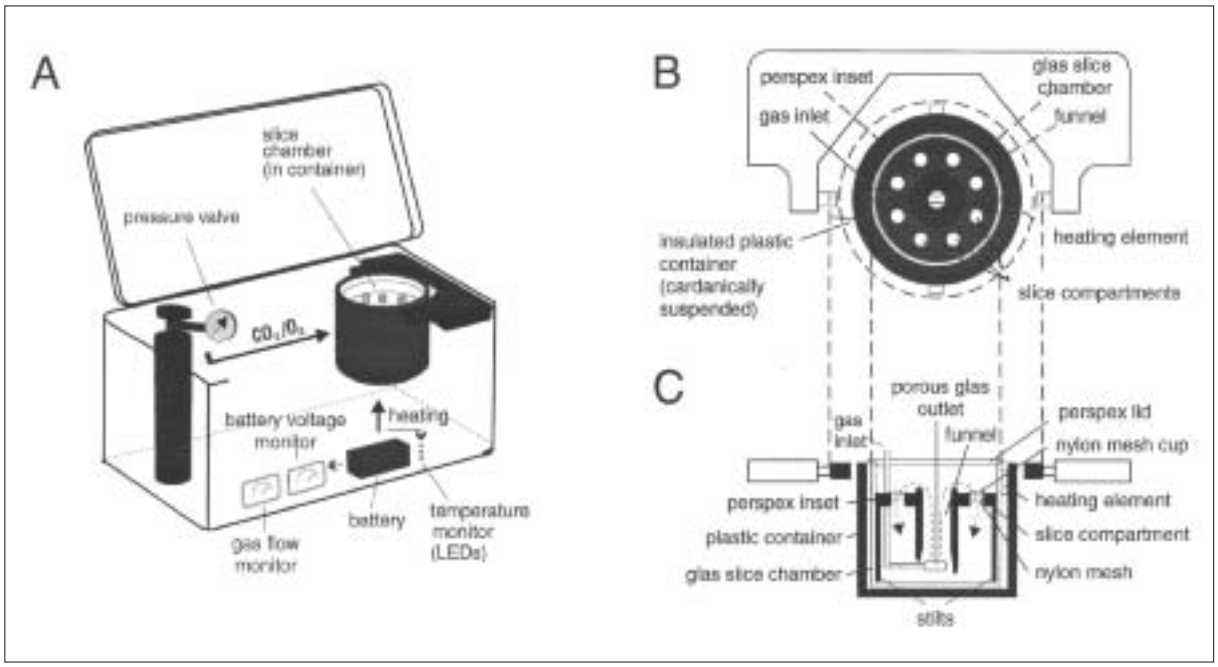


Figure 4 Spontaneous sharp waves (field potentials, FP) recorded from human neocortical slice preparations obtained during epilepsy surgery. Inferior temporal gyrus. GM: grey matter, WM: white matter. A, B: Synchronous (A) and asynchronous (B) appearance of FP at two recording sites. Right part: superimposition of 3 FP with the event FP2 serving as guiding patterns for synchronisation. Inkwriter tracing. Interruptions are indicated. Recordings in A and B stem from different slices. Modified after [22].

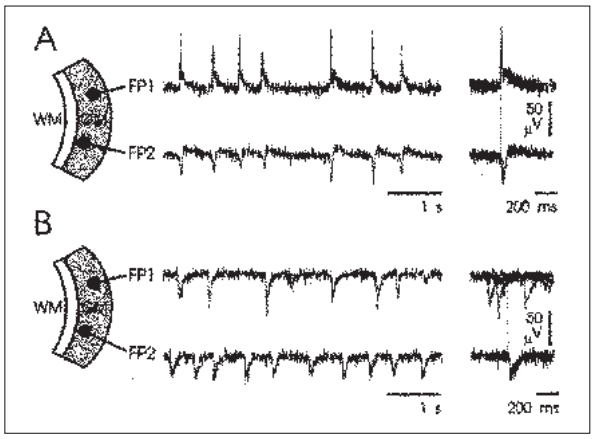
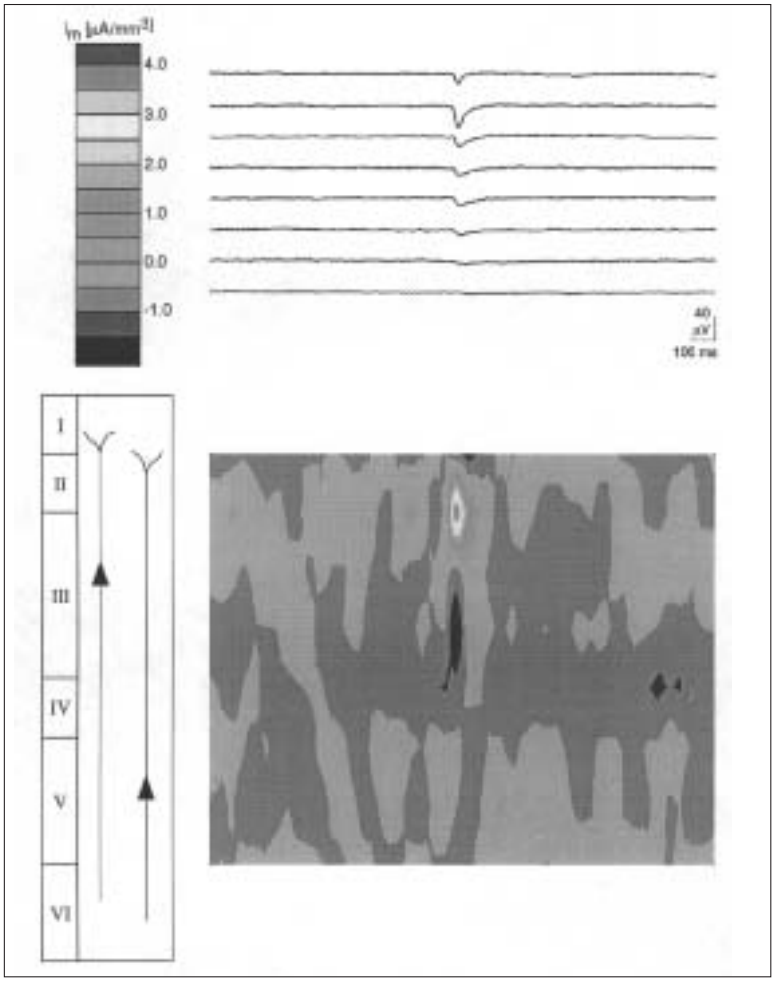


Figure 5 Typical laminar distribution of current sinks with sharp waves occurring spontaneously under control conditions in a human neocortical slice. Slice was obtained during epilepsy surgery; inferior temporal gyrus. Field potentials were picked up by a linear array of 8 electrodes perpendicular to the pial surface (upper panel). From sharp waves, current-source density analyses were performed (lower panel). White: current sinks, black: current sources.



(diameter 33 µm, core diameter 25 µm, California Fine Wire Co., Grover City, CA) with interelectrode distances of 300 µm. To scan a slice for sharp field potentials, the array was placed perpendicular to the pial surface at the midsection of each preparation, and consecutively, at the respective midsections of the resulting halves of the slice. From the potentials recorded with this linear electrode array, current source density (CSD) analyses were performed using conventional algorithms.

Intracellular recordings were performed using sharp micropipettes filled with 2 M potassium methyl sulphate or 2 M KCl (60–180 MΩ) in the immediate vicinity of the field potential electrode and up 800 µm apart. Intracellular data were obtained from cells in layers II to V with a resting membrane potential close to – 60 mV and action potential amplitudes greater than 60 mV.

All signals were recorded on an inkwriter and computer system, as well as a digital oscilloscope.

Results and discussion

Spatial distribution of the field potentials

Sharp field potentials appeared spontaneously in about 60% of temporal neocortical human tissue slices (Fig. 4). In slices not presenting sharp waves, epileptiform activity was induced by application of bicuculline [9, 13] or omission of Mg^{2+} from the bath fluid (0 Mg^{2+} epilepsy) [1, 8] demonstrating viability of the slices.

Activity could be recorded at only one recording site of the slice preparation, or at multiple positions (Fig. 4A, 4B). In the latter experiments, the sharp waves were synchronous (Fig. 4A) or asynchronous (Fig. 4B).

The spatial distribution of the potentials was assessed with two-electrode recordings as described in Materials and Methods. Synchronous activity was recorded with electrode distances from 400 µm to 1000 µm. In some cases, synchronous activity was observed only in a recording field of up to 200 µm as determined by interelectrode distances, and not beyond. Spacing the electrodes at distances of 2000 µm to 4000 µm, activity was seen only rarely in both recording sites, which in these cases was asynchronous.

The spontaneously active networks were possibly not stationary as polarity changes (n = 2) as well as waxing and waning of the potentials sometimes occurred. Such increases and decreases in field potential amplitude could be observed only in irregular intervals which usually lasted more than 30 min.

Figure 6 Population spikes superimposed on spontaneous sharp waves (FP). Three potentials superimposed. Oscilloscope tracings (modified after [22]).

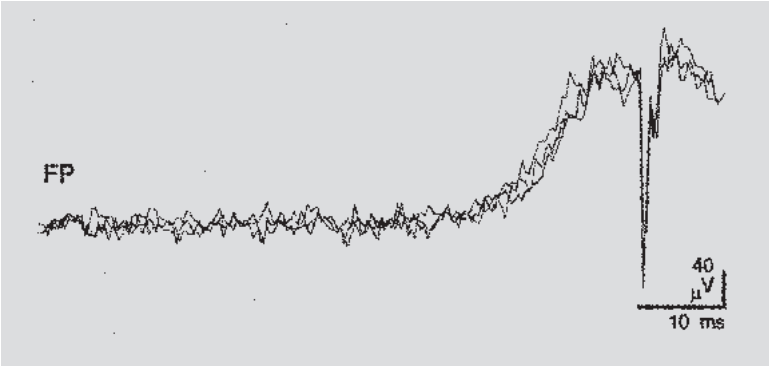
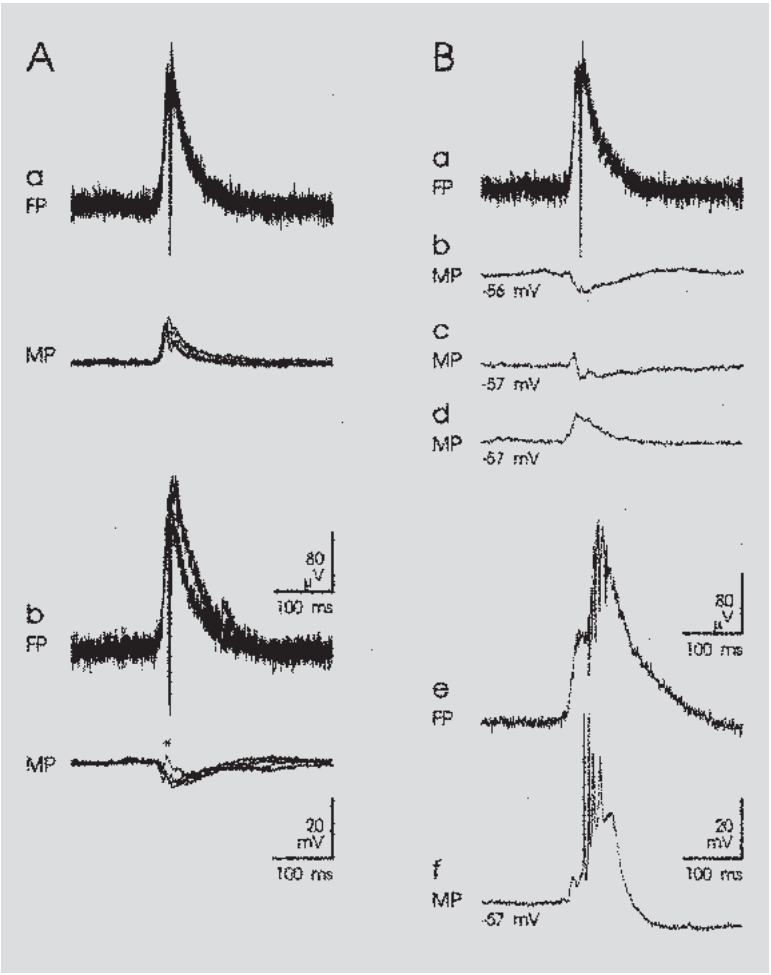


Figure 7 Spontaneous sharp waves and concomitant membrane potential changes (MP). MP recorded from neurons 200 to 800 µm apart from the field potential electrode. Sharp waves (FP) served as guiding patterns for superposition (A, B). A: depolarising (a) or hyperpolarising (b) MP changes. 5 potentials superimposed. Asterisk: note the intermediate depolarisation within the hyperpolarising envelope synchronous to the population spikes riding on the sharp waves. B: FP recordings (a) and, at same MP, corresponding hyperpolarising (b), sequence of de- and hyperpolarising (c) and depolarising potentials (d) in one neuron. e, f: FP (e) and accompanying paroxysmal depolarisation shift (f) after 20 min washout of 5 µM bicuculline (modified after [22]).



Current-source density (CSD) analyses of the spontaneous potentials allowed a more detailed description of the sites of neuronal excitation within the neocortex. A typical example of a CSD analysis is given in figure 5. As in this case, the overwhelming majority of spontaneous potentials were based on current sinks in upper cortical laminae.

Neuronal activity associated with field potentials

The potentials were usually monophasic (Fig. 4–7) an did not represent field potentials brought about by summation of action potentials. On the other hand, clear cut population spikes were found to be superimposed on the potentials in some cases (Fig. 6).

To shed light on the neuronal mechanisms of the generation of the field potentials, intracellular recordings were performed along with field potential recordings. The intracellular electrodes were placed in layers II to V in the direct vicinity of the field potential electrode, or up to 800 µm apart.

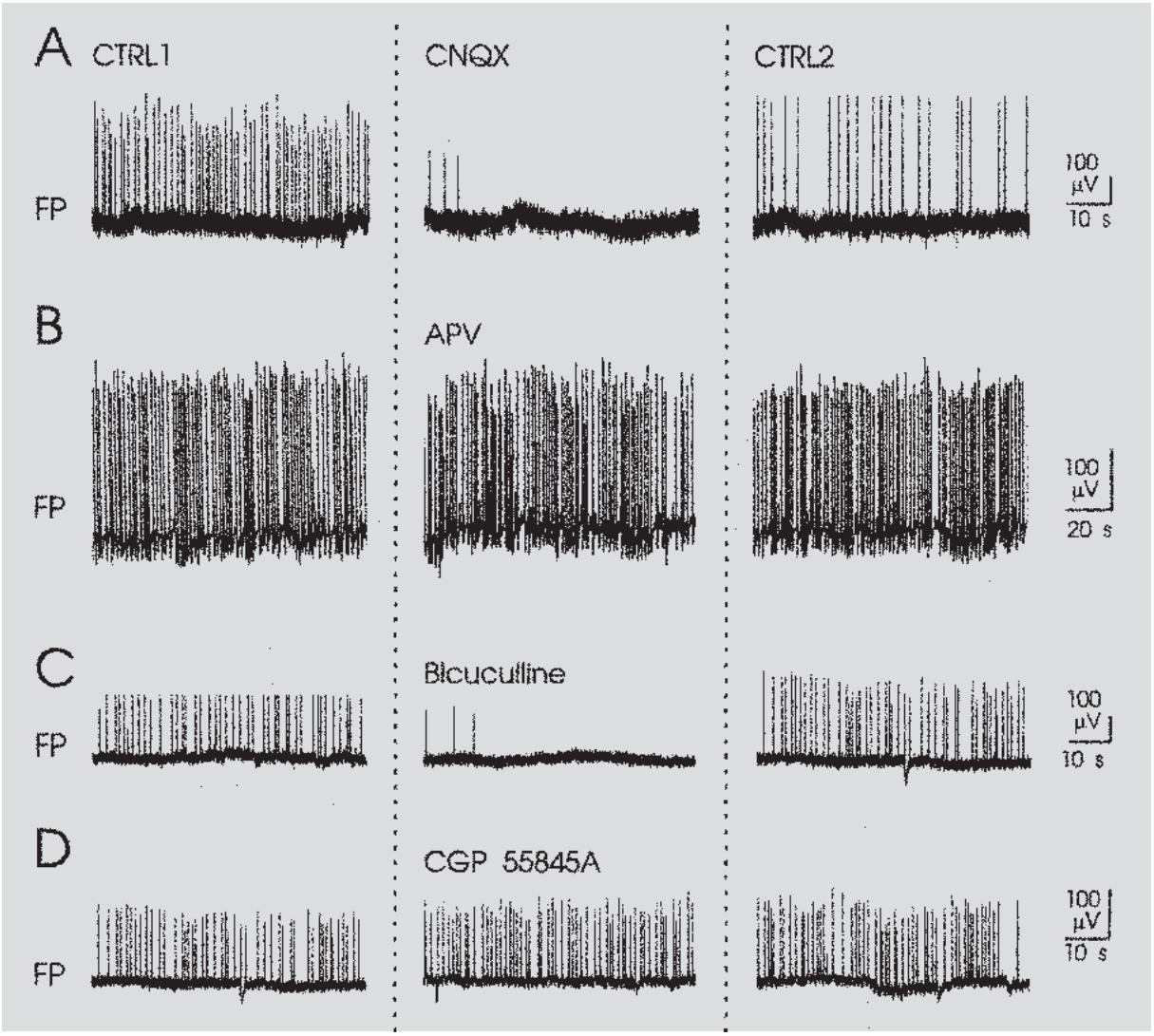
With potassium methyl sulphate filled electrodes, different types of potentials, i. e. depolarising or hyperpolarising or sequences of hyper- and depolarising potentials could be seen in all neurons synchronous to the field potentials (Fig. 7). Never was a paroxysmal depolarisation observed, except after washout of the epileptogenic substance bicuculline. Within a range of membrane potential of –50 to –60 mV, most of the neurons displayed two or all three types of potentials.

To analyse the ionic nature of the membrane potential changes, current was passed over the bridge circuit to alter the resting membrane potential of a given neuron. In experiments in which the neuronal membrane potential was altered, purely hyperpolarising potentials represented monophasic smooth potentials at low membrane potentials which reversed at approximately –68 mV – near the putative E_{GABA} [14] – to a depolarisation of similar shape.

Transmitters involved in the generation of the field potentials

In order to analyse the possible role of excitatory transmission in the generation of the spontaneous potentials, glutamatergic synaptic mechanisms were looked at. To this purpose the non-NMDA receptor antagonist CNQX (5 µM, 30 min) or the NMDA receptor antagonist APV (100 µM, 30–60

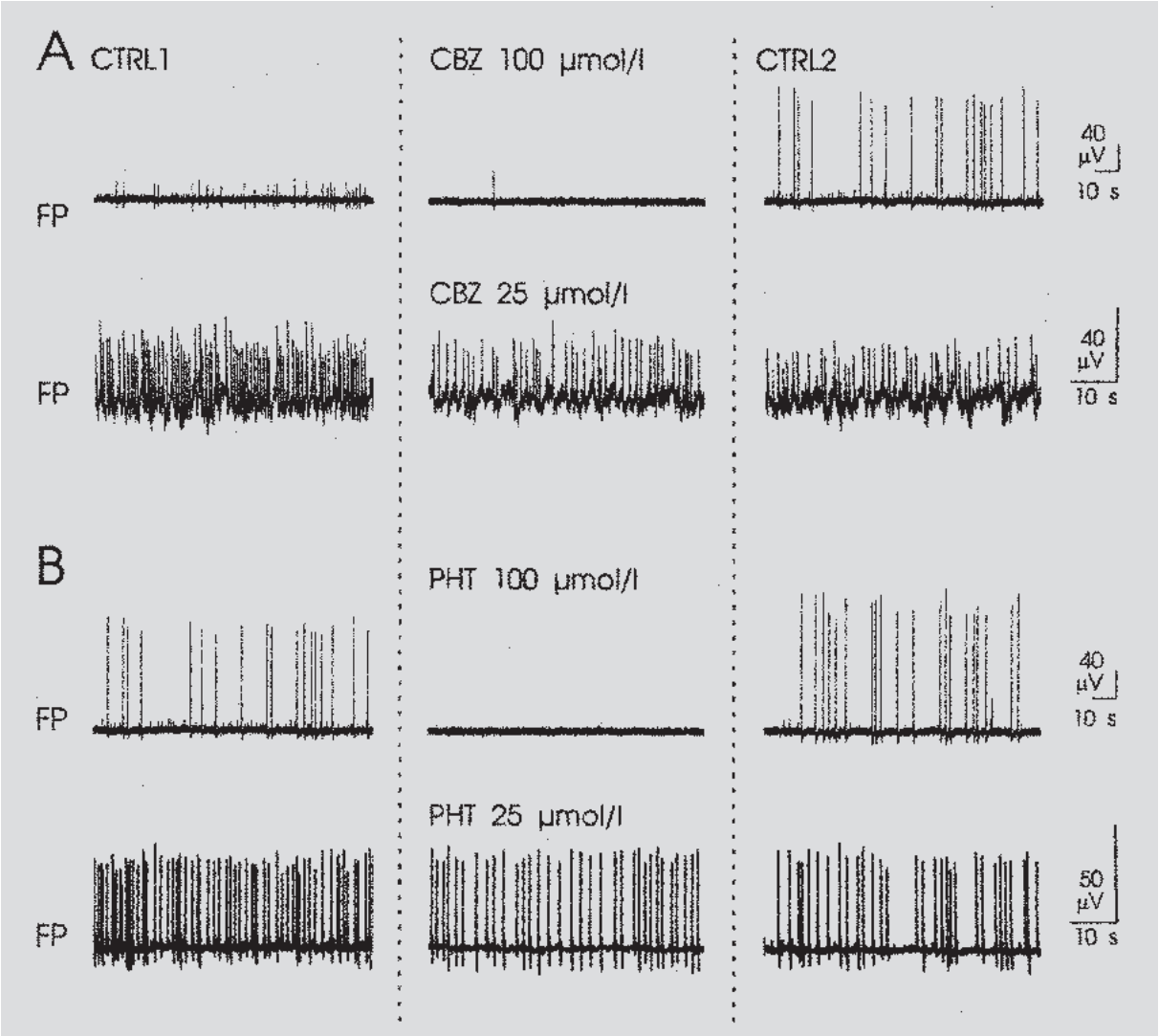
Figure 8 Involvement of excitatory (A, B) and inhibitory (C, D) synaptic mechanisms in the generation of sharp waves (FP). A, B: action of glutamate subreceptor antagonists CNQX (5 µM, A) and APV (100 µM, B). C, D: action of $GABA_A$ receptor antagonist bicuculline (5 µM, C) and $GABA_B$ receptor antagonist CGP 55845A (1 µM, D), CTRL1, CTRL2: recordings before application of substances (CTRL1) an after washout (CTRL2). Inkwriter tracings (modified after [22]).



min) were added to the superfusate. With application of CNQX, the spontaneous sharp waves along with the synchronous postsynaptic potentials were abolished within 15 min in all trials (Fig. 8A). This effect was reversible, as potentials reappeared upon washout of CNQX within 45 min. After washout of the substance, the frequency of occurrence returned to nearly the initial value. The NMDA antagonist APV had no effect on the spontaneous potentials (Fig. 8B).

To test for the functional significance of inhibitory synaptic transmission for the generation of spontaneous activity, the $GABA_A$ receptor antagonists bicuculline (5 µM, 30 min, Fig. 8 C) or picrotoxin (100 µM, 15 min) or the $GABA_B$ receptor antagonist CGP 55845A (1 µM, 30 min, Fig. 8D) were superfused. After application of bicuculline, the field potentials decreased in amplitude and disappeared completely within 10 to 15 min. Upon washout of bicuculline, spontaneous potentials reappeared. Like bicuculline, picrotoxin suppressed the spontaneous field potentials within 5 min, and with washout in both cases grouped activity resembling discharges found with tonic-clonic activity transiently appeared. The $GABA_B$ antagonist CGP 55845 A had no influence on the spontaneous activity, although in one experiment action potentials were generated more frequently than before superfusion of the substance (5 vs. 57 action potentials in 5 min) and the neuron recorded from was slightly depolarised by 2 mV (Fig. 8D).

Figure 9 The conventional antiepileptic drugs carbamazepine (CBZ, A) and phenytoin (PHT, B) fail to suppress spontaneous sharp waves (FP) close to therapeutic concentrations (25 µM), and suppress them in highly supratherapeutical ones (100 µM). CTRL1, CTRL2: recordings before application of the substances (CRTL1) and after washout (CTRL2). Inkwriter tracings (modified after [22]).



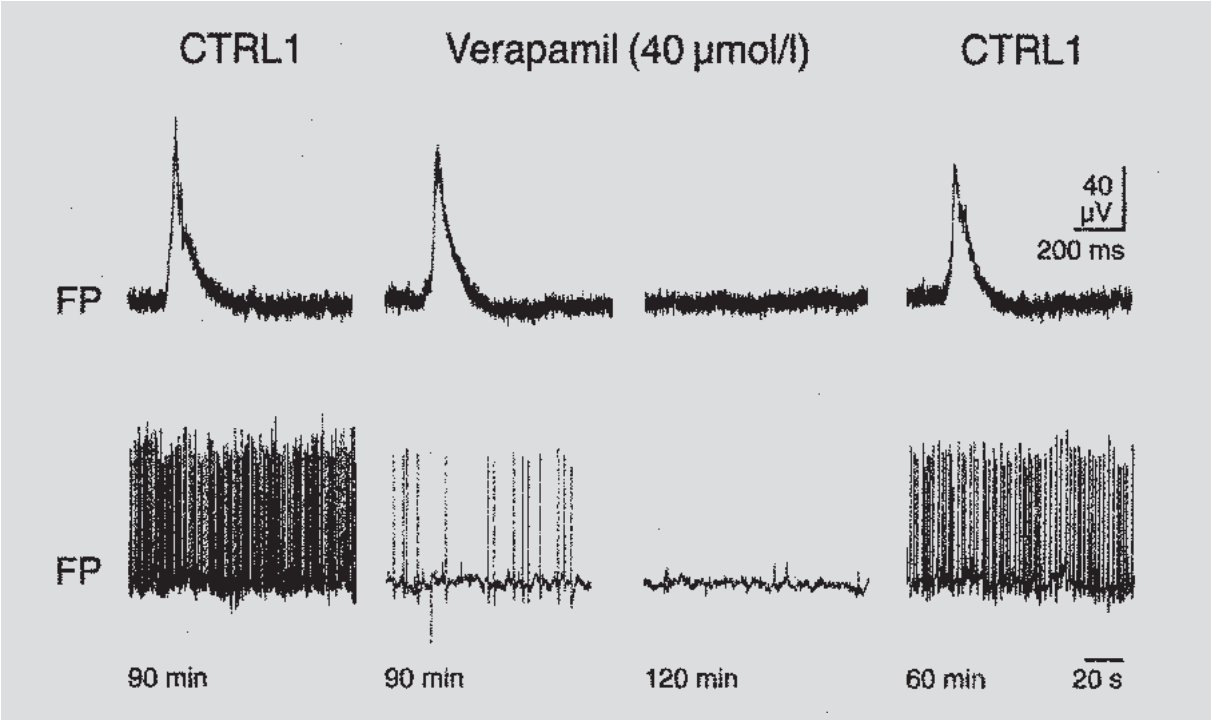
Sensitivity to antiepileptic drugs

Antiepileptic drugs such as carbamazepine (CBZ) and phenytoin (PHT) are given to the patients and are present in their brain tissue up to the operation. These drugs were washed out completely during incubation, and could be responsible for the appearance of spontaneous sharp waves in the slices. Therefore, the effects of the conventional antiepileptic drugs PHT and CBZ on spontaneous activity were tested. PHT and CBZ were given close to or slightly above therapeutic concentrations (25 µM, Fig. 9) [15]. Apart from a slight reduction of the repetition rate to 60–70% of the initial value, the drugs failed to suppress spontaneous activity within 60 min application (Fig. 9). Well beyond therapeutic concentrations (100 µM) both drugs were able to abolish the spontaneous potentials (Fig. 9).

As L-type calcium channels have been shown to be instrumental in experimental epileptogenesis, we also tested the contribution of calcium currents to the spontaneous potentials in question.

In all experiments, the spontaneous potentials were suppressed reversibly by the organic calcium channel blocker verapamil within 60 to 120 min (Fig. 10). Specifically, a suppression by 50% and 90% of the initial repetition rate was reached within half an hour and one hour, respectively. Usually, the potentials did not recover completely when verapamil was washed out and the frequency of occurrence returned to 50% of the value before verapamil superfusion.

Figure 10 Suppression of spontaneous sharp waves (FP) by the organic calcium channel blocker verapamil (40 µM). Inkwriter (lower panels) and oscilloscope tracings at expanded time scale (upper panels). CTRL1, CTRL2: recordings before application of verapamil (CTRL1) and after washout (CTRL2). Traces are given at points in time of the different experimental protocols as indicated (modified after [22]).



The depression of spontaneous activity by the organic calcium channel blocker verapamil corresponds to the antiepileptic action of this drug in many experimental models of epilepsy [16–20] including human tissue [8, 9] and in first clinical trials with other organic calcium channel blockers [21].

Conclusions

As a whole, the spontaneously appearing field potentials in living human brain slices from epilepsy surgery are assumed to reflect a state of increased neuronal synchronisation. A prerequisite for these events are not only the excitatory synaptic transmission via non-NMDA receptors but also the inhibitory synaptic transmission via $GABA_A$ receptors. L-type calcium channels are essentially involved in this synchronised neuronal activity.

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