Cellular Mechanisms Underlying Antiepileptic Effects of Low- and High-Frequency Electrical Stimulation in Acute Epilepsy in Neocortical Brain Slices In Vitro

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Submitted 13 May 2006; accepted in final form 29 November 2006

Schiller Y, Bankirer Y. Cellular mechanisms underlying antiepileptic effects of low- and high-frequency electrical stimulation in acute epilepsy in neocortical brain slices in vitro. J Neurophysiol 97: 1887–1902, 2007. First published December 6, 2006; doi:10.1152/jn.00514.2006. Approximately 30% of epilepsy patients suffer from drug-resistant epilepsy. Direct electrical stimulation of the epileptogenic zone is a potential new treatment modality for this devastating disease. In this study, we investigated the effect of two electrical stimulation paradigms, sustained low-frequency stimulation and short trains of high-frequency stimulation, on epileptiform discharges in neocortical brain slices treated with either bicuculline or magnesium-free extracellular solution. Sustained low-frequency stimulation (5–30 min of 0.1- to 5-Hz stimulation) prevented both interictal-like discharges and seizure-like events in an intensity-, frequency-, and distance-dependent manner. Short trains of high-frequency stimulation (1–5 s of 25- to 200-Hz stimulation) prematurely terminated seizure-like events in a frequency-, intensity-, and duration-dependent manner. Roughly one half of the seizures terminated within the 100-Hz stimulation train (P < 0.01 compared with control), whereas the remaining seizures were significantly shortened by 53 ± 21% (P < 0.01). Regarding the cellular mechanisms underlying the antiepileptic effects of electrical stimulation, both low- and high-frequency stimulation markedly depressed excitatory postsynaptic potentials (EPSPs). The EPSP amplitude decreased by 75 ± 3% after 10-min, 1-Hz stimulation and by 86 ± 6% after 1-s, 100-Hz stimulation. Moreover, partial pharmacological blockade of ionotropic glutamate receptors was sufficient to suppress epileptiform discharges and enhance the antiepileptic effects of stimulation. In conclusion, this study showed that both low- and high-frequency electrical stimulation possessed antiepileptic effects in the neocortex in vitro, established the parameters determining the antiepileptic efficacy of both stimulation paradigms, and suggested that the antiepileptic effects of stimulation were mediated mostly by short-term synaptic depression of excitatory neurotransmission.

INTRODUCTION

Epilepsy is a common disease effecting nearly 1% of the population (Browne and Holmes 2001; Forsgren et al. 2005). Clinically, epilepsy manifests as recurrent unprovoked seizures, which in turn vary widely in their frequency, clinical manifestations, and severity (Browne and Holmes 2001; Duncan et al. 2006). Antiepileptic drugs (AEDs) render most patients with epilepsy seizure free. However, ~30% of all patients with epilepsy suffer from drug-resistant epilepsy and continue to experience seizures despite adequate AED treatment. Because of the ongoing recurrence of seizures, these patients suffer from a devastating disease with severe implications on the longevity and quality of life (Brodie and French 2001; Cockerell et al. 1997; Duncan et al. 2006; Kwan and Sander 2004; Schmidt and Loscher 2005; Sperling 2004). One emerging new treatment modality for drug-resistant epilepsy is direct electrical stimulation of the epileptogenic zone (for review, see Cohen-Gadol et al. 2003; Durand and Bikson 2001; Loscher and Schmidt 2004; Theodore and Fisher 2004). Cortical electrical stimulation for treating epilepsy can potentially be performed with either open- or closed-loop stimulation paradigms. In open-loop stimulation, the cortex is stimulated using predetermined sequences, unaffected by the underlying cortical activity. In contrast, closed-loop devices on-line detect impending or ongoing seizures and administer a short stimulation protocol to terminate the emerging seizure (for review, see Cohen-Gadol et al. 2003; Durand and Bikson 2001; Loscher and Schmidt 2004; Theodore and Fisher 2004).

Previous studies in hippocampal brain slices in vitro and in human patients with temporal lobe epilepsy have shown that cortical electrical stimulation can eliminate epileptiform discharges, including epileptic seizures. Most previous studies have used open-loop stimulation paradigms (Barbarosie and Avoli 1997; Bikson et al. 2001; Cuellar-Herrera et al. 2004; Khosravani et al. 2003; Kinoshita et al. 2004; Krauss and Gordon 1999; Lian et al. 2003; Theodore and Fisher 2004; Velasco et al. 2000, 2001; Vonck et al. 2002). However, recently, few preliminary studies tested the ability of closed-loop stimulation to prematurely terminate seizures in epilepsy patients (Fountas et al. 2005; Kossoff et al. 2004; Osorio et al. 2005). In addition, Lesser et al. (1997) have reported neocortical electrical stimulation eliminated evoked afterdischarges in patients with intractable extratemporal neocortical epilepsy.

Despite growing evidence regarding the antiepileptic effect of electrical stimulation, the use of electrical stimulation for treating intractable epilepsy is only in its early stages of development. The studies performed thus far tested a small number of patients in a nonblinded manner and used mostly acute short-term rather than chronic long-term stimulation protocols. Moreover, the efficacy of stimulation reported thus far in the literature was probably not sufficient for clinical use (Cuellar-Herrera et al. 2004; Vonck et al. 2002), possibly because of the fact that the stimulation parameters were not optimally determined.
optimized in these studies. In addition, despite growing interest in the potential use of electrical stimulation for treating various neurological diseases including intractable epilepsy, the mechanisms underlying the antiepileptic effect of stimulation remain unclear. Three major candidate mechanisms have been proposed: reduction in the excitability of neurons, increased inhibitory neurotransmission, and depression of excitatory neurotransmission (for review, see Durand and Bikson 2001; McIntyre et al. 2004b).

Neocortical brain slices treated with bicuculline (BCC) or magnesium-free solutions serve as acute models of neocortical epilepsy in vitro, because they produce both interictal-like depolarizing paroxysmal shift (PDS) discharges and seizure-like events (Avoli et al. 1991; Hablitz 1987; Schiller 2002, 2004; Valenzuela and Benardo 1995). In this study, we used extracellular field potential measurements and whole cell intracellular voltage recordings from neocortical brain slices treated with either BCC or magnesium-free solution to investigate the antiepileptic effects of two stimulation paradigms: sustained low-frequency stimulation (0.1–5 Hz for 5 min or longer), which is best suited for prolonged open-loop stimulation, and short trains of high-frequency stimulation (25–200 Hz for 1–5 s), which is best suited for closed-loop stimulation. We will concentrate on three main questions. First, can neocortical epileptiform discharges be eliminated by two stimulation paradigms sustained low-frequency and short trains of high-frequency electrical stimulation? Second, what are the optimal stimulation parameters for obtaining maximal antiepileptic effects with the stimulation paradigms? Third, what are the cellular mechanisms underlying the antiepileptic effects of cortical electrical stimulation?

**Methods**

**Slice preparation and electrophysiological recordings**

Parasagittal neocortical brain slices (300–400 μm in thickness) were prepared from 13- to 35-day-old Wistar rats, as previously described (Schiller 2004). The slice and single neurons were visualized using infrared illumination and differential interference contrast optics (IR-DIC) video microscopy. The microscope used was a fixed stage BX-51WI (Olympus). Extracellular field potential recordings and whole cell voltage recordings from the soma of single neurons were obtained using the Multi-clamp 700A amplifier (Axon Instruments, Foster City, CA). Extracellular recordings, which were performed with glass pipettes (0.1–0.5 MOhm) filled with the extracellular solution, were amplified 1,000-fold and filtered with a low-pass filter of 3 KHz and high-pass filter of 1 Hz. Intracellular whole cell recordings were performed from the soma of layer 5 and layer 2/3 pyramidal neurons as previously described (Schiller 2002; Schiller et al. 2000). The slice was bathed in artificial cerebrospinal fluid (ACSF) that contained (in mM) 125 NaCl, 25 NaHCO₃, 25 glucose, 4 KCl, 1.25 NaH₂PO₄, 1.5 CaCl₂, and 1 MgCl₂, 0.1 BCC, or 0 MgCl₂; pH 7.4. Somatic (3- to 6-MOJM resistance) whole cell recording pipettes were filled with (in mM) 115 K-gluconate, 20 KCl, 2 Mg-ATP, 2 Na₂-ATP, 10 Na₂-phosphocreatine, 0.3 GTP, 10 HEPES, pH 7.2, and either 0.1 calcium green-1 or 0.1 1,2-bis-(2-aminophenox)-ethane-N,N,N,N-tetraacetic acid tetrakis (BAPTA). The experiments were performed at 35 ± 0.5°C. All chemicals were purchased from Sigma except for calcium green-1 (Molecular Probes, Portland, OR), 2-aminophosphonopentanoic acid (APV), 6-cyano-nitroquinoxaline-2,3-dione (CNQX), and BCC (Tocris, Bristol, UK).

In all intracellular experiments, we continuously monitored the resting membrane potential and repeatedly tested the series resistance and the amplitude and shape of action potentials evoked by depolarizing somatic current injections. The recording was discontinued when the resting membrane potential changed by >3 mV, when the series resistance was >25 MOhm or changed by >30%, or when the amplitude or shape of action potentials evoked by somatic current injection changed. In addition, the position of the intra- and extracellular electrodes was monitored visually using the IR-DIC optics.

In this study, brain slices treated with either BCC or magnesium-free extracellular solution produced both interictal-like discharges and seizure-like events. Interictal-like discharges were defined as events of intense firing (usually consisting of 1–2 PDS discharges), lasting <0.5 s. Seizure-like events were defined as events of intense firing lasting >2 s. Events lasting 0.5–2 s were left undefined.

**Electrical stimulation**

Electrical stimulation was administered using a Master-8 stimulator and Iso-flex isolators (AMPI, Jerusalem, Israel). We used two different types of stimulating electrodes: double barrel theta patch pipettes containing two silver wires, one in each barrel and filled with ACSF (composition of ACSF described above) (Polsky et al. 2004; Schiller et al. 2000), and platinum-iridium metal microelectrodes with impedance of 0.2–0.5 MOhm (Microprobe, Gaithersburg, MD). In both cases, a negative current was administered against a ground electrode. In the case of the double barrel theta pipettes, one wire served as active electrode while the other wire served as the ground electrode, whereas in the case of metal microelectrodes, a separate chlorinated silver wire was inserted into the bath and served as the ground electrode. The results obtained with the two types of stimulating electrodes were analyzed together, because no differences between them were observed. In all experiments, we stimulated the slice with depolarizing square pulses lasting 0.2–0.4 ms. In all experiments, we first characterized the threshold for initiation of the PDS response in the slice, and the stimulus intensity used for stimulation was 2.5- to 3-fold the PDS threshold unless specifically stated. In all experiments, we applied only a single sustained low-frequency stimulation train per slice aside from experiments in which multiple data points were compared in the same slice. These included experiments examining the effects of stimulus frequency, stimulus intensity, and stimulation site, where no more than five consecutive stimulation sessions were applied per slice. In these experiments, we waited ≥30 min between stimulation sessions, and the order of stimulation sessions was randomly chosen. In experiments where two stimulating electrodes were used, the interelectrode distance between the two stimulating electrodes was measured separately in the horizontal and vertical directions.

All experiments were performed after slices were prebathed in the magnesium-free solution or the BCC-containing solution for ≥1.5 h, and spontaneous epileptiform discharges were recorded at a minimal rate of 1.5 discharges/min. Slices that produced spontaneous discharges at lower rates (<1.5 discharges/min) were excluded. For experiments that examined the effect of stimulation on excitatory postsynaptic potentials (EPSPs), we chose slices with a relatively low frequency of spontaneous epileptiform discharges (1.5–2.5 epileptiform discharges/min).

**Data analysis**

The data were analyzed using Igor (WaveMetrics, Lake Oswego, OR) and Excel software and presented in the form of average and SD values. To measure the amplitude of epileptiform discharges or EPSPs with over-riding action potentials, the waveform was filtered with a low-pass filter of 300 Hz to filter out the over-riding fast action potentials. Statistical analysis was performed using the Student’s t-test and ANOVA statistical tests using Excel and SPSS software.
RESULTS

Prevention of epileptiform discharges by sustained open-loop low-frequency electrical stimulation

To study the effect of sustained low-frequency electrical stimulation on epileptiform discharges, we stimulated BCC-treated neocortical brain slices at 0.1–5 Hz for 5–45 min. Figures 1 and 2 show two typical examples where the slice was stimulated at 1 Hz. During the control prestimulation period, the slice spontaneously produced both interictal-like discharges and seizure-like events (Hablitz 1987; Schiller 2002, 2004). At the onset of the 1-Hz electrical stimulation, PDS discharges were evoked. However, as stimulation progressed, the epileptiform discharges gradually attenuated, and with time, epileptiform discharges disappeared altogether (Figs. 1 and 2). The effect of stimulation was reversible. On discontinuation of stimulation, evoked and spontaneous epileptiform discharges gradually recovered (Fig. 1C; data not shown for spontaneous events).

Similar experiments to those presented in Figs. 1 and 2 were performed in 36 slices with the same general results (24 slices using extracellular field potential recordings and 12 slices using intracellular whole cell recordings). In all these experiments, epileptiform discharges were evoked at the onset of stimulation. In 15 of 36 slices (42%), a seizure-like event was evoked at the onset of stimulation, whereas in the remaining 21 slices, isolated interictal-like PDS discharges were evoked at the onset of stimulation. As stimulation progressed, epileptiform discharges gradually attenuated. In all slices, seizure-like events disappeared altogether within the first minute of stimulation, and in all but two slices (94%), epileptiform discharges disappeared altogether within the initial 5 min of the 1-Hz stimulation. Suppression of epileptiform discharges was maintained throughout the duration of stimulation (≤45 min) in all recorded slices. In all recorded slices, evoked epileptiform discharges gradually recovered within the first 1–2 min after discontinuation of the 1-Hz stimulation, whereas spontaneous PDS discharges and seizure-like events reappeared within 10 min after discontinuation of sustained stimulation in 32 of the 36 slices examined (89%).

We next characterized the stimulation parameters influencing the antiepileptic effect of sustained low-frequency electri-
We next examined the spatial constraints of the antiepileptic effects of sustained electrical stimulation. To do so, we used a new experimental paradigm, where we placed two stimulating electrodes: one to administer the sustained electrical stimulation and the other to administer infrequent test pulses (once every 20–30 s). The location of the recording and infrequently stimulating test electrodes remained unchanged, whereas the sustained stimulating electrode was moved between different locations. In this way, we could test how stimulation at one location affects the ability to generate epileptiform discharges at other locations in the slice. Figure 3B presents the results of one such experiment. Sustained 1-Hz electrical stimulation at one site suppressed epileptiform discharges evoked by the second electrode in a distance-dependent manner. At an interelectrode distance of 100 μm, the responses were completely suppressed, whereas when the electrodes were placed 750 μm apart, only partial suppression of the response was observed (Fig. 3B). Similar results were obtained in all nine slices examined (5 for horizontal and 4 for vertical distances between the 2 stimulating electrodes). At interelectrode distances of 50–100 μm, complete suppression occurred. Partial suppression was evoked at interelectrode distances of 250–1,000 μm, whereas an average horizontal interelectrode distance of 1,160 ± 305 μm (n = 5) did not significantly affect the epileptiform discharges evoked by the second stimulating electrode (Fig. 3C). Our results also indicated that the antiepileptic efficacy of stimulation was greater in the vertical direction (same cortical column) compared with the horizontal direction (Fig. 3C).

It is interesting to note that, despite the spatial constraints of electrical stimulation, sustained 1-Hz stimulation at a single site was sufficient to prevent spontaneous epileptiform discharges in the vast majority of slices. Hence initiation of spontaneous epileptiform discharges probably involved a larger network of neurons, and the antiepileptic effect induced by stimulation in part of this network was sufficient to prevent spontaneous PDS discharges and seizure-like events.

As pointed out earlier, seizure-like events were evoked at the onset of sustained low-frequency stimulation in 42% of cases. We attempted to overcome this potential drawback by gradually increasing the stimulus intensity at the onset of stimulation. In these experiments, slices were stimulated initially at intensities equal to one half the threshold for PDS initiation. Later the stimulus intensity was gradually increased by 30% every 1 min until it reached 2.5-fold the PDS threshold. Under these conditions, no seizure-like events were observed at the onset of stimulation. Instead, only interictal-like PDS discharges were evoked during the initial stages of stimulation. These findings were observed in eight slices. It is important to stress that, in human patients, interictal discharges usually are of no clinical significance, and the main concern is from initiation of seizures by stimulation.

We further studied the antiepileptic effects of sustained electrical stimulation in a second model of acute epilepsy,
significant attenuation of epileptiform discharge was observed. Sustained stimulation was frequency dependent. At 0.05 Hz, no antiepileptic effects of sustained low-frequency stimulation. Similar to BCC-treated slices, the antiepileptic effects of sustained stimulation. If the distance between the two stimulating electrodes was decreased, the antiepileptic effects gradually decreased, and at an interelectrode distance of 1,500 μm (left trace) horizontally from test stimulating electrode (S1). D: peak amplitude (mean ± SD) of responses evoked by 2nd test stimulating electrode (S1) is plotted as a function of horizontal (filled circles) and vertical (stars) distances between test (S1) and sustained stimulating electrode (S2). Plotted data were averaged from 5 experiments. All measurements in C and D were obtained after 5 min of 1-Hz sustained stimulation. Statistical analysis with ANOVA test revealed P < 0.01. We compared individual values using the t-test, we found P < 0.01 for comparison of control responses and responses obtained at all interelectrode distance of 1,500 μm where P > 0.2. ANOVA test revealed a P < 0.01 for comparison of vertical and horizontal interelectrode distance of 1,000 μm. All experiments were performed in BCC-treated neocortical slices.

neocortical brain slices bathed in a magnesium-free extracellular solution. Similar to BCC-treated slices, sustained 1-Hz stimulation suppressed both evoked and spontaneous epileptiform discharges in magnesium-free-treated neocortical slices. Figure 4, A and B, shows one such typical experiment. At the onset of stimulation, an epileptiform discharge was evoked. However, as the stimulation progressed, responses gradually attenuated until only EPSs were evoked. Concomitantly spontaneous epileptiform discharges, which were abundant before the sustain stimulation, disappeared altogether after 280 s of stimulation. Similar experiments were performed in 10 additional neurons. In all these 11 experiments, epileptiform discharges were evoked at the onset of stimulation and were gradually replaced by either sub- or suprathreshold EPSs. Concomitantly spontaneous epileptiform discharges disappeared altogether in 6 of 11 slices, and in the remaining 5 slices occurred infrequently throughout the 15-min sustained stimulation. On discontinuation of the 1-Hz stimulation, epileptiform discharges rapidly recovered (Fig. 4B).

We next examined the effect of stimulation frequency on the antiepileptic effects of sustained low-frequency stimulation. Similar to BCC-treated slices, the antiepileptic effects of sustained stimulation was frequency dependent. At 0.05 Hz, no significant attenuation of epileptiform discharge was observed (n = 5). Sustained stimulation at 0.1 Hz (3 of 5 neurons) or 0.2 Hz (4 of 4 neurons) already resulted in gradual suppression of epileptiform discharges, whereas further increasing the stimulation frequency enhanced the rate of suppression of epileptiform discharges during stimulation (7 of 7 neurons, data not shown). The antiepileptic effects of sustained stimulation in magnesium-free-treated slices were location dependent. In these experiments, we used two stimulating electrodes. One electrode stimulated the slice continuously at 1 Hz, and the other electrode was used to stimulate the slice infrequently (2 stimuli/min). To study the spatial efficacy of sustained stimulation, the distance between the two stimulating electrodes was changed during the experiments (for more details, see description of this paradigm in BCC-treated slices). As shown in Fig. 4C, the efficacy of stimulation decreased as the distance between the two stimulating electrodes increased. Sustained stimulation effectively eliminated epileptiform discharges at interelectrode distances of ≤100 μm. At larger interelectrode distances, the antiepileptic effects gradually decreased, and at interelectrode distances of 400–600 μm or greater, sustained 1-Hz stimulation with one electrode did not have noticeable antiepileptic effects on epileptiform discharges evoked by the second, infrequently stimulating electrode (Fig. 4C). It is interesting to note that the spatial efficacy of stimulation in magnesium-free–treated slices was smaller than that observed.
in BCC-treated slices (1,160 ± 305 µm for BCC, n = 5, compared with 490 ± 74 µm for magnesium free, n = 6).

Termination of seizure-like events by short closed-loop high-frequency electrical stimulation

In the previous section, we showed that sustained open-loop low-frequency electrical stimulation (0.33–5 Hz for 5–45 min) prevented interictal-like discharges and seizure-like events. A second potential stimulation paradigm for treating epilepsy is a closed-loop stimulation. Ideally, these devices impending seizures will be detected and the appropriate stimulation will be applied to prevent initiation of full-blown clinical seizures. In our experiments, we were unable to detect impending seizures. Hence, we wanted to examine whether we are able to terminate ongoing seizures with short trains of high-frequency electrical stimulation (25–200 Hz for 1–5 s). We used high-frequency stimulation trains in these experiments for two main reasons. First, in contrast to low-frequency stimulation, with its slow onset time and initial excitatory effect, high-frequency stimulation seems more likely to be more appropriate for closed-loop stimulation. Second, high-frequency stimulation paradigms have been used in previous and ongoing human stimulation trials (Fountas et al. 2005; Kossoff et al. 2004; Lesser et al. 1997; Osorio et al. 2005). We limited the duration of high-frequency stimulation to 5 s, because longer stimulation hampered our ability to electrophysiologically monitor the duration of seizures. To study the antiepileptic effects of short trains of high-frequency stimulation, we used two different experimental designs. In the first, we evoked seizure-like events with one electrode, and 1–2 s later we stimulated the slice at 100 Hz for 1–3 s with a second electrode (Fig. 5A). In the second experimental design, we waited for initiation of spontaneous seizure-like events. Once such an event was visually identified, we manually applied the 1- to 3-s 100-Hz stimulation train (Fig. 5B). To ensure that stimulation was applied during seizure-like events rather than interictal-like PDS discharges, we only included cases in which stimulation was applied 0.7–3 s after seizure initiation.

Stimulating the slice at 100 Hz terminated 47% of seizures-like events during the 1- to 3-s stimulation period (Fig. 5, A and B; 57 of 121 seizure-like events in 11 slices). In these cases, no residual seizure-like activity was observed after the 100-Hz stimulation train ended. In comparison, only 12% of control seizures lasted <3 s (n = 43, P < 0.001). The results in spontaneous and evoked seizures were averaged together, because no significant differences were observed between them (P = 0.2). The remaining 53% of seizures that persisted beyond the duration of stimulation were significantly shortened by the 1- to 3-s, 100-Hz stimulation train (Fig. 5, A and B, bottom traces). The average duration of seizure-like events that persisted beyond stimulation decreased from 14.3 ± 4.6 s under control conditions (n = 38) to 6.7 ± 2.3 s after stimulation (n = 64, P < 0.001). Again, no significant differences were observed between evoked and spontaneous seizures (P = 0.1), and hence their data were combined.

We next wanted to study how different stimulation parameters influenced the ability of high-frequency stimulation to prematurely terminate seizure-like events. In our experiments, we studied four different stimulation parameters: intensity, duration, frequency, and location of stimulation. Figure 6A

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**FIG. 4.** Antiepileptic effects of sustained low-frequency stimulation in magnesium free–treated neocortical brain slices. A: spontaneous epileptiform discharges recorded intracellularly from a layer 5 pyramidal neuron in a magnesium free–treated slice under control conditions before stimulation. Note appearance of spontaneous epileptiform discharges in this trace. B: whole cell voltage recording from a layer 5 pyramidal neuron in a magnesium free–treated slice during sustained 1-Hz electrical stimulation. Traces are shown for beginning of stimulation, 10, 60, 240, and 300 s into 1-Hz stimulation, and 120 s after sustained 1-Hz stimulation ended. C: spatial efficacy of sustained stimulation in magnesium free–treated slices. Two stimulating electrodes were placed in neocortical layer 2/3. Sustained 1-Hz stimulation was administered with 1st electrode (sustained stimulating electrode, S2), whereas the 2nd electrode (test electrodes, S1) was used to administer infrequent test stimuli. Single traces recorded during stimulation with the 2nd test electrode in isolation (S1, left trace) and during sustained 1-Hz stimulation with the sustained stimulating electrode (S2) are shown. S2 electrode was located 200 µm horizontally from test stimulating electrode (S1). Bottom left: schematic presentation of experimental scheme. A photograph of a parasagittal slice with a single layer 5 pyramidal neuron filled with biocytin (not from this experiment). Location of intracellular recording pipette (gray) and 2 stimulating electrodes (S1 and S2) are schematically shown. Movement of sustained stimulating electrode (S2) is shown in arrows. Bottom right: peak amplitude (mean ± SD) of responses evoked by 2nd test stimulating electrode (S1) is plotted as a function of horizontal interelectrode distance from S1 and S2 electrodes. Results were obtained by averaging results from 6 experiments. All measurements were obtained after 5 min of sustained 1-Hz stimulation. To measure amplitude of responses in these experiments, voltage responses were filtered with a high-pass filter of 100 Hz to filter out fast over-riding action potentials. Statistical analysis revealed P < 0.05 with ANOVA test. In addition, P < 0.01 for comparison of control responses and responses obtained at interelectrode distance of ≤300 µm. P < 0.05 for interelectrode distances of 400–500 µm. P was not significant for distances of 600 and 1,000 µm.
shows the average percent of seizures terminated during a 2-s, 100-Hz stimulation train as a function of the stimulus intensity. To average the results of different slices, stimulus intensities were normalized to the PDS discharge threshold. Termination of seizure-like events was dependent on the stimulus intensity. Increasing the stimulus intensity 2.5-fold of the PDS threshold gradually increased the fraction of seizures terminated during the stimulation period. However, additional increase of the stimulus intensity from 2.5- to 7-fold of the PDS threshold did not further enhance termination of seizure-like events.

The duration of stimulation also influenced the antiepileptic efficacy of high-frequency stimulation. Extending the duration of stimulation from 0.25 to 3 s gradually increased the fraction of seizures terminated during stimulation. Further prolonging stimulation from 3 to 5 s did not significantly effect seizure termination (Fig. 6B).

We next examined the effect of frequency on the antiepileptic effect of high-frequency stimulation. Four different stimulation frequencies were tested ranging from 25 to 200 Hz. Increasing the frequency from 25 to 50 Hz markedly increased the fraction of seizures that terminated during stimulation. However, further increasing the frequency from 50 to 100–200 Hz did not further increase the fraction of seizures terminated during stimulation (Fig. 6C).
In contrast to the intensity, frequency, and duration of stimulation, termination of seizure-like events was insensitive to the site of high-frequency stimulation. We observed no significant differences in the percent of seizures terminated by a 2-s, 100-Hz stimulation when we changed location of the stimulating electrode between neocortical layer 2/3, layer 5, and subcortical white matter (20 seizures in layer 2/3 and 18 seizures in layer 5 in 6 slices; data not shown). Moreover, changing the interelectrode distance between the electrode that evoked seizure-like events and the electrode that applied the 2-s, 100-Hz stimulation (≤1,500 μm horizontally) did not significantly change the percent of seizures terminated (Fig. 6D). The fact that seizure termination was unaffected by the interelectrode distance probably reflected the fact that, once seizure-like events initiated, they involved the entire neuronal network of the slice rather than the site of initiation.

To further study the antiepileptic effects of high-frequency electrical stimulation, we repeated our experiments in magnesium free–treated neocortical brain slices. Magnesium free–treated neocortical brain slices only infrequently produced discharges lasting ≥3 s. Thus to study the antiepileptic effects of short trains of high-frequency stimulation, we evoked epileptiform discharges with one electrode and 200 ms later applied a 0.6-s, 100-Hz stimulation train with a second electrode. The distance between the two stimulating electrodes was 50–100 μm in the horizontal direction. When we compared the duration of epileptiform discharges with and without the 100-Hz stimulation train, we found that epileptiform discharges were significantly shorter when the 0.6-s, 100-Hz stimulation train was administered. In these experiments, the average duration of epileptiform discharges decreased from 1.49 ± 0.72 s under control conditions (n = 48) to 1.08 ± 0.46 s (n = 42) during stimulation (experiments were performed in 10 slices, P < 0.01). Moreover, 22 of 42 epileptiform discharges terminated during the 0.6-s stimulation, in contrast to only 14 of 48 discharges that were shorter than 0.8 s under control conditions. Hence, our findings indicated that, similar to BCC–treated slices, short high-frequency stimulation trains prematurely terminated epileptiform discharges in magnesium free–treated neocortical brain slices.

**Cellular mechanisms underlying the antiepileptic effect of low- and high-frequency electrical stimulation**

In the previous sections, we showed that seizure-like events can be prevented by sustained low-frequency stimulation on the one hand and prematurely terminated by short trains of high-frequency stimulation on the other hand. We next studied the cellular mechanisms underlying the antiepileptic effect of electrical stimulation. In our experiments, we considered two potential mechanisms: reduction of neuronal excitability and depression of excitatory neurotransmission. In our experimental model, a third potential antiepileptic mechanism of enhanced inhibition was not relevant because GABA_A receptors were pharmacologically blocked.

**Ability of axons to sustain firing during low- and high-frequency electrical stimulation**

In these experiments, we performed whole cell recordings from a neocortical pyramidal neuron and visually identifying the axon of the recorded neuron using fluorescent and DIC imaging. To directly stimulate the axon, we placed the stimulating electrode in close proximity to the identified axon (Fig. 7A). To eliminate ionotropic synaptic neurotransmission, we added CNQX (20 μM), APV (100 μM), and BCC (10 μM) to the bath solution and confirmed that no EPSPs or inhibitory postsynaptic potentials (IPSPs) were evoked in response to extracelluar synaptic stimulation.

The results of these experiments showed that the neuron reliably generated action potentials at 1 Hz throughout the 15- to 30-min, 1-Hz stimulation period (n = 16, 9 layer 5 and 7 layer 2/3 neurons). In contrast neurons could not reliably sustain high frequency firing. More specifically, when axons were stimulated at 25 Hz for ≤45 s, neurons reliably generated axonal action potentials after each stimulus. However, when the stimulation rate was increased to 100–200 Hz (and in some neurons, 50 Hz as well), a fraction of action potentials failed to initiate within the first second of stimulation, as determined by somatic recordings. On average, during the third second of 100-Hz axonal stimulation, neurons generated only 45 ± 5 action potentials (n = 13, 7 layer 5 neurons and 6 layer 2/3 neurons). Further raising the stimulus intensity transiently increased the firing frequency, but it rapidly decreased again to the baseline value (data not shown). It is interesting to note that a recent modeling study raised the possibility that, during high-frequency axonal stimulation, action potentials failed to propagate to the soma while successfully propagating along the axonal arborization (McIntyre et al. 2004a).

**Effect of low- and high-frequency electrical stimulation on neuronal excitability**

One mechanism by which electrical stimulation can exert its antiepileptic effect is by reducing the excitability of neurons or axons. Neurons in the stimulated neocortical slice can be divided into two main groups with respect to the effect of stimulation. The first group consists of neurons directly stimulated by the stimulating electrode, whereas the remaining neurons are indirectly activated by electrical stimulation through mono- or polysynaptic connections. The antiepileptic effects of stimulation can be mediated by decreased excitability of one or of both subgroups of directly stimulated and synaptically activated neurons. To study the effects of stimulation on excitability of the different neurons, we used four different experimental paradigms. First, we examined the ability of axons to sustain firing during stimulation. Second, we examined the ability of directly stimulated neurons (by sustained axonal stimulation) to fire action potentials in response to somatic depolarizing waveforms (input–output response curves). Third, we studied the ability of indirectly (synaptically) activated neurons to fire action potentials in response to somatic depolarizing waveforms. Fourth, we studied the antiepileptic effect of partial pharmacological blockade of voltage-gated sodium channel that in turn artificially decreased the excitability of neurons.

**Ability to generate action potentials in response to depolarizing waveforms in directly stimulated neurons**

When the axon of the neuron is directly stimulated, sustained action potential firing can reduce the neuron’s ability to
generate additional action potentials in response to incoming synaptic potentials. To study this possibility, we directly stimulated the visually identified axon in a similar manner to that described in the previous experiment and examined the response of the neurons to depolarizing somatic current injections before, during, and after the axonal stimulation. When we compared the number of action potentials evoked by 100-ms somatic depolarizing current injections of different amplitudes (input–output response curve) before and during the 1-Hz axonal stimulation, we found a small, yet insignificant, reduction in the number action potentials generated in response to the depolarizing current injection (Fig. 7, A and C; n = 16, 9 layer 5 and 7 layer 2/3 pyramidal neurons). All these small effects were reversible on discontinuation of electrical stimulation (data not shown). Hence sustained action potential firing at low frequencies did not significantly affect the ability of neurons to generate action potentials in response to incoming depolarizing waveforms.

In addition, we examined the effect of 100-Hz stimulation on the ability to generate action potentials in response to depolarizing somatic current injections. To test this question, 100-ms somatic depolarizing current injections were applied either during or immediately after a short train of 100-Hz stimulation. When the 100-ms depolarizing current injection was applied 2 s after the initiation of a 4-s, 100-Hz stimulation train, it was very difficult to evoke additional action potentials by somatic depolarizing current injections. Even when we injected large 100-ms somatic depolarizing currents, only one to two additional action potentials were produced (n = 15, 8 layer 5 and 7 layer 2/3 neurons). When the 100-ms depolarizing current injection was applied immediately after a 2-s, 100-Hz stimulation, the number of action potentials generated was markedly decreased (for 220 pA, the average number of action potentials decreased from 2.5 ± 0.9 before the 100-Hz stimulation to 1.7 ± 0.6 immediately after the 2-s, 100-Hz stimulation, P < 0.01).

Taken together, our findings indicated that, in directly stimulated neurons, sustained low-frequency stimulation did not significantly affect excitability of neurons, whereas short high-frequency stimulation trains impaired the ability of neurons to further respond to depolarizing waveforms.

Effects of sustained synaptic stimulation on the ability to generate action potentials in response to depolarizing waveforms

We next examined whether sustained 1-Hz extracellular synaptic stimulation impaired the ability of neurons to generate action potentials in response to depolarizing current injections. In these experiments, we examined the input–output response curve evoked by 100-ms depolarizing current injections of different amplitudes under control conditions and 1 and 10 min into sustained 1-Hz extracellular synaptic stimulation. After 1 min of sustained 1-Hz stimulation, one to two action potentials usually accompanied the synaptic response, whereas 10 minutes into sustained 1-Hz stimulation, only subthreshold EPSPs remained (e.g., Figs. 2 and 4). Our experiments indicated that sustained 1-Hz stimulation had no effect on the number of action potentials evoked by the depolarizing current injections (input–output curve) either after 1 (data not shown) or 10 min...
of sustained 1-Hz stimulation (Fig. 7, D and E; 9 layer 5 neurons and 7 layer 2/3 neurons).

We next examined the effect of short trains of 100-Hz synaptic stimulation on generation of action potentials. We compared the number of action potentials generated by a 100-ms depolarizing current injection under control conditions 2 s after initiation of a 4-s, 100-Hz synaptic stimulation and immediately after a 2-s, 100-Hz stimulation train. We found that the 100-Hz stimulation slightly (but insignificantly) increased the number of action potentials generated, whereas immediately after the 2-s, 100-Hz stimulation train, no significant difference was observed in the number of action potentials generated compared with prestimulated control values. The 100-ms, 100- and 220-pA depolarizing current injections evoked 1.4 ± 0.5 and 2.8 ± 0.4 action potentials under control conditions, 1.6 ± 0.5 and 3.2 ± 0.8 action potentials during the 100-Hz stimulation (the 100-ms, 100- and 220-pA depolarizing current injections were applied 2 s after initiation of a 4-s, 100-Hz stimulation), and 1.3 ± 0.6 and 2.9 ± 1.0 immediately after the 100-Hz stimulation ended, respectively (n = 6, 4 layer 5 neurons and 2 layer 2/3 neurons; P > 0.15 for all 3 cases).

Taken together, our findings indicated that the excitability of neurons indirectly activated by synaptic inputs are unaffected by both sustained low-frequency stimulation and short trains of high-frequency stimulation.

Antiepileptic effects of partial pharmacological blockade of voltage-gated sodium channels

To study the potential antiepileptic effects of increasing the threshold for action potential initiation, we pharmacologically reduced the excitability of neurons by adding low concentrations (0.1–0.2 μM) of extracellular TTX to the bath solution. In these experiments, we first measured the rate of spontaneous epileptiform discharges and the threshold for axo-somatic action potentials initiation under control conditions. The threshold for axo-somatic action potential initiation in the recorded neuron was measured during 100-ms depolarizing current injections. Next we added 0.1 μM TTX to the bath solution and again measured the threshold for action potential initiation in the recorded neuron. If the threshold was increased by <2.5 mV, we further increased the concentration of TTX to 0.2 μM. Later we again measured the frequency of spontaneous epileptiform discharges and the response to a 5-min, 1-Hz sustained stimulation in the presence of TTX. The average frequency of spontaneous epileptiform discharges was 3.7 ± 0.7 under control conditions and 3.4 ± 0.9 after the addition of 0.1–0.2 μM TTX (Fig. 7F; n = 5, P > 0.25). In addition, suppression of epileptiform discharges during the 5-min, 1-Hz stimulation was not affected by 1–2 μM TTX (data not shown, n = 3). In these experiments, the average threshold for action potential initiation in the recorded neuron was increased by 3.9 ± 1.5 mV (n = 5). On further increasing the concentration of TTX to 1 μM to eliminate action potentials altogether, spontaneous and evoked epileptiform discharges disappeared altogether (Fig. 7F; n = 3). Hence mild reduction in excitability, as manifested by increased initiation threshold of axo-somatic action potentials, had no significant antiepileptic effects.

Taken together, the findings of our experiments in directly stimulated neurons, indirectly synaptically activated neurons, and with low concentration of TTX indicated that the antiepileptic effects of electrical stimulation were unlikely to be caused by a reduction in the excitability of neurons.

Effect of low- and high-frequency electrical stimulation on excitatory synaptic transmission

We next examined the effect of electrical stimulation on excitatory neurotransmission. In these experiments, we decreased the stimulus intensity below the threshold for epileptiform discharge initiation, such that either EPSPs or action potentials were evoked, and measured the effect of electrical stimulation on the amplitude of EPSPs or the probability to fire action potentials. Under these stimulation parameters, spontaneous epileptiform activity persisted. Thus we chose for these experiments slices with relative low rates of spontaneous epileptiform discharges (below 2 discharges/min) and performed measurements ≥20 s after epileptiform discharges ended. Figure 8 shows a typical experiment that examined the effect of sustained low-frequency stimulation on excitatory synaptic transmission. In this experiment, the slice was stimulated at 1 Hz with two different stimulus intensities: one evoked EPSPs (Fig. 8A) and the other evoked action potentials (Fig. 8B). Sustained low-frequency stimulation significantly attenuated excitatory synaptic transmission. The amplitude of EPSPs gradually decreased with stimulation (Fig. 8A), and at the higher stimulus intensity, above the threshold for action potential initiation, the 1-Hz sustained stimulation resulted in gradual failure of action potential firing with only infrequent action potentials observed as the stimulation progressed (Fig. 8B). After stimulation ended, the EPSP amplitude partially recovered and reached 93% of the prestimulus control value 30 min after stimulation was discontinued (P < 0.01 compared with the prestimulus control value). The fact that EPSPs only partially recovered after stimulation probably resulted from the induction of long-term depression (LTD) by the 15-min, 1-Hz stimulation (Froc et al. 2000; Perrett et al. 2001).

Similar results were obtained in all 16 additional neurons examined (7 layer 2/3 neurons and 9 layer 5 neurons). On average, the amplitude of EPSPs decreased by 61 ± 10% after 10 min of sustained 1-Hz stimulation (n = 16, P < 0.01, no significant differences between layer 2/3 and layer 5 neurons). Moreover, similar to the results shown in Fig. 8A, EPSPs did not fully recover after stimulation probably resulted from the induction of LTD (n = 8, P < 0.01, indicating the induction of LTD).

When the stimulus intensity was increased above the threshold for action potential initiation, only 2 ± 4% of stimuli evoked action potentials after 10 min of 1-Hz sustained stimulation compared with 100% during the prestimulus control period (n = 9).

To study the possibility that depression of EPSPs by sustained low-frequency stimulation was related to BCC, we performed similar experiments in brain slices bathed in ACSF without BCC. Similar to BCC-treated brain slices, 5 min of 1-Hz stimulation decreased the amplitude of EPSPs by 53 ± 14% (n = 4).

Depression of EPSPs by sustained low-frequency stimulation was dependent on the stimulation frequency. The higher the stimulation frequency (examined between 0.5 and 5 Hz),
the greater the depression of EPSP amplitude. On average, after 5 min of stimulation at 0.5, 1, 2, and 5 Hz, the amplitude of EPSPs decreased by 32 ± 8 (n = 6), 61 ± 10 (n = 16), 67 ± 8 (n = 5), and 82 ± 12% (n = 5; P < 0.01 for comparison of 0.5 Hz with all other frequencies and comparison of 1 and 5 Hz; P < 0.05 for comparison of 2 and 5 Hz).

Similar to BCC-treated slices, in magnesium free–treated brain slices, sustained low-frequency stimulation also markedly depressed the amplitude of EPSPs. At 1-Hz stimulation, the average amplitude of EPSPs decreased by 59 ± 19 and 67 ± 16% after 5 and 10 min of stimulation (n = 5). Depression of the EPSP amplitude by sustained stimulation was frequency dependent. Very little depression of the EPSP amplitude was observed at 0.1-Hz stimulation, whereas at stimulation frequencies of 0.5–5 Hz, the amplitude of the EPSP was significantly depressed. Moreover, the higher the stimulation frequency, the bigger the depression of the average EPSP amplitude we observed (Fig. 9A).

To further study the antiepileptic effects of suppressing EPSPs, we partially blocked ionotropic glutamate receptors by low concentrations of CNQX (2 μM) and APV (10 μM) and examined the frequency of spontaneous epileptiform discharges and the antiepileptic effects of 1-Hz sustained stimulation. Under these conditions, the average amplitude of suprathreshold EPSPs in the recorded neurons was decreased by 35 ± 16% (n = 6). Partial blockade of glutaminergic neurotransmission by 2 μM CNQX and 10 μM APV significantly decreased the rate of spontaneous epileptiform discharges in both BCC- and magnesium-free treated brain slices (Fig. 9B).

On average, the frequency of spontaneous epileptiform discharges decreased by 69 ± 17% in magnesium free–treated slices (n = 6, P < 0.01) and by 63 ± 21% in BCC-treated slices (n = 5, P < 0.01). In addition, we examined the effect of a 5-min, 1-Hz stimulation before and after the addition of 2 μM CNQX and 10 μM APV. For these experiments, we chose cells that produced PDS discharges for at least the initial 10 consecutive stimuli (e.g., Figs. 2 and 9C). In all five neurons that qualified for this condition (4 BCC-treated and 1 magnesium free–treated slices) after adding 2 μM CNQX and 10 μM APV, the initial stimulus evoked a PDS discharge, although with a smaller amplitude and duration than under control conditions (e.g., compare the initial PDS responses in Fig. 9C). Under these conditions, suppression of epileptiform discharges was markedly enhanced, and the responses rapidly transformed into EPSPs within the initial two to five stimuli (Fig. 9C). Similar results were obtained in all five neurons examined.
We next examined the effect of higher concentrations of CNQX (10 μM) and APV (50 μM). In these experiments, the pharmacological blockers were washed in gradually, and thus glutamate receptors were progressively blocked over a period of 15–20 min. In all eight slices examined, addition of 10 μM CNQX and 50 μM APV to the bath solution gradually eliminated spontaneous epileptiform discharges, and at a later stage, evoked epileptiform discharges as well. In three slices where whole cell recordings were performed from layer 5 pyramidal neurons, the average EPSP amplitude decreased by 46 ± 15% when spontaneous epileptiform discharges disappeared and by 58 ± 18% when evoked spontaneous discharges disappeared. Taken together, our findings indicated that depression of EPSPs was sufficient to eliminate epileptiform discharges.

We next characterized the effect of high-frequency stimulation trains on excitatory neurotransmission. Figure 10 shows the results of one such experiment. The stimulus intensity was decreased such that EPSPs rather than epileptiform discharges were evoked. During the 100-Hz stimulation, the EPSP amplitude rapidly and markedly decreased. Already after the fifth stimulus (40 ms of stimulation), the EPSP amplitude decreased by 77% compared with the prestimulus control EPSP, whereas after 2 s of 100-Hz stimulation, it decreased by 89 ± 2% (average of 10 consecutive EPSPs). The effect of the 100-Hz stimulation on EPSP amplitude was reversible, as shown in Fig. 10B. Fifteen minutes after stimulation ended, the amplitude of EPSPs returned to 96 ± 8% that of the prestimulus control EPSP (P = 0.3). Similar results to those shown in Fig. 10, A and B, were observed in all seven additional experiments performed. The average amplitude of EPSPs decreased by 86 ± 6 and 89 ± 4% after 1 and 2 s of 100-Hz stimulation, respectively (n = 8, 5 layer 5 and 3 layer 2/3 pyramidal neurons). Similar results were obtained in two additional experiments performed on brain slices bathed in ACSF without BCC. In addition, we repeated these experiments in magnesium free–treated brain slices with similar results. On average, after 1 s of 100-Hz stimulation, the amplitude of EPSPs decreased by 81 ± 12% (n = 3) in magnesium free–treated slices. We did not examine the effect of high-frequency stimulation in the presence of 2 μM CNQX and 10 μM APV, because the rate of spontaneous and evoked seizure-like events markedly decreased. Hence these experiments were virtually impossible to perform.

It is interesting to note that, in our experiments, we did not observe induction of long-term synaptic potentiation after the high-frequency stimulation trains. Fifteen minutes after the 1- to 3-s, 1-Hz stimulation trains, the average EPSP amplitude was 103 ± 9% of the prestimulus control EPSP amplitude (n = 6, P = 0.4) (see also Teyler 1989).

FIG. 9. Antiepileptic effects of partial pharmacological blockade of glutamatergic synapses. A: synaptic depression of excitatory neurotransmission in magnesium-free–treated neocortical brain slices. Left: average EPSP (average of 5 consecutive EPSPs) under control conditions and 5 min into a 1-Hz (top) and 2-Hz (bottom) sustained stimulation. Experiments were performed in magnesium-free–treated brain slices, and synaptic stimulus intensity was reduced such that subthreshold EPSPs were evoked. Control EPSPs were evoked at 0.05 Hz. Right: average normalized EPSP amplitudes (mean ± SD), presented as percent of control EPSP, are plotted as a function of frequency of sustained stimulation. All measurements were performed 5 min into sustained stimulation. An average of 5 consecutive EPSPs was obtained for each experiment, and values of different experiments were further averaged. Data for 0.1-, 0.5-, 2-, and 5-Hz were averaged from 4 experiments each and for 1-Hz from 5 experiments. P < 0.01 for comparison of 0.1 Hz with all other frequencies and 0.5 and 2 Hz. 0.5 ± 0.05 Hz and 5 and 0.5 Hz with 2 Hz. All these experiments were performed in magnesium-free–treated brain slices. Recordings were performed from 4 layer 5 pyramidal neurons, and stimulation electrode was located in layer 2/3 400–600 μm above (vertically) and 50–100 μm lateral (horizontal) from the recording pipette. B: suppression of epileptiform discharges by partial pharmacological blockade of AMPA and N-methyl-D-aspartate (NMDA) receptors. Left: traces recorded in a magnesium-free–treated slice before (top trace) and 30 min after (bottom trace) addition of 2 μM CNQX and 10 μM APV. Note reduction in frequency of spontaneous epileptiform discharges after partial blockade of glutamate receptors. Right: average (mean ± SD) frequency of spontaneous epileptiform discharges under control conditions, after partial (2 μM CNQX and 10 μM APV) and complete (10 μM CNQX and 50 μM APV) blockade of AMPA and NMDA glutamate receptors in magnesium-free–treated brain slices. Partial blockade of AMPA and NMDA receptors significantly decreased frequency of spontaneous epileptiform discharges (P < 0.01 for both magnesium-free–treated and BCC–treated), whereas complete blockade of ionotropic glutamate receptors eliminated epileptiform discharges altogether. C: left: traces recorded during a 1-Hz electrical stimulation under control conditions and after addition of 2 μM CNQX and 10 μM APV to the bath solution. This recording was performed from a layer 5 pyramidal neuron, and stimulating pipette was located 300 μm above and 50 μm lateral to recording pipette. Right: graphs of peak amplitude of response during a sustained 1-Hz stimulation under control conditions and after addition of 2 μM CNQX and 10 μM APV to bath solution. Top graph: results from a single experiment (same experiment as right panel). Bottom graph: average results from 6 slices treated with BCC. Control value was averaged from 5 consecutive responses evoked once every 10 s. Note that low doses of glutamate receptors blockers decreased amplitude and duration of initial PDS and rapidly enhanced antiepileptic effects of stimulation. Amplitude of responses with over-riding action potentials was measured after filtering with a 300-Hz low-pass filter.
DISCUSSION

In this study, we investigated the antiepileptic effect of two different stimulation paradigms, sustained low-frequency (5–45 min of 0.1–5 Hz) and short trains of high-frequency (1–5 s of 25–200 Hz) electrical stimulation, in two fundamentally different acute models of epilepsy: BCC-treated slices in which GABA<sub>A</sub> receptors are blocked, and magnesium free–treated slices, where N-methyl-D-aspartate (NMDA) currents are enhanced. We chose both these stimulation paradigms because the first is better fitted for continuous open-loop stimulation, whereas the second is better suited for closed-loop stimulation in response to an impending or ongoing seizure. Our study yielded three main results.

1) Sustained open-loop low-frequency electrical stimulation prevented interictal-like discharges and seizure-like events. The antiepileptic effect of sustained low-frequency stimulation was dependent on the frequency and the relative distance of the stimulating electrode from the onset site of seizure-like events, but was independent of the cortical layer stimulated. Effective elimination of seizure-like events was achieved at stimulation frequencies >0.5 Hz and when the stimulating electrode was located within 1 mm of the “epileptic focus.”

2) Short (1–5 s) trains of high-frequency stimulation (50 Hz and above) prematurely terminated a fraction of seizure-like events. On average, 47% of seizures terminated during the high-frequency stimulation trains, and the remaining seizures, which persisted beyond the stimulation train, were shortened by an average of 53 ± 21%. Both these values showed a statistically significant antiepileptic effect of stimulation. The antiepileptic effect of high-frequency stimulation was dependent on the intensity, duration, and frequency of stimulation, but was independent of the cortical layer stimulated and the relative distance of the stimulating electrode from the site of seizure onset. The dependence on stimulation duration was observed up to, but not beyond, 3 s, and the dependence on stimulation frequency was observed up to, but not beyond, 50 Hz. It is important to stress that this study was the first to examine the ability of high-frequency trains to prematurely terminate seizure-like events in vitro.

3) We studied the cellular mechanisms underlying the antiepileptic effects of stimulation. We concentrated on two main potential candidate mechanisms: depression of excitatory neurotransmission and reduced excitability of the neurons. We found that both sustained low-frequency and short high-frequency electrical stimulation markedly depressed EPSPs. Moreover, we showed that partial blockade of EPSPs by postsynaptic pharmacological blockers indeed suppressed epileptiform discharges. Hence depression of EPSPs (synaptic depression) can account for the antiepileptic effects of electrical stimulation. To the best of our knowledge, this is the first...
time the antiepileptic effects of stimulation have been linked to depression of EPSPs.

We also examined the effects of stimulation on several aspects of excitability, including the ability of directly stimulated axons to sustain action potential firing, the ability to generate action potentials in response to depolarizing waveforms in directly and synaptically activated neurons, and the effects of pharmacologically compromising excitability by low doses of TTX on epileptiform discharges. We found that sustained low-frequency stimulation had no significant effects on excitability, whereas high-frequency stimulation only reduced the ability to generate high-frequency firing in directly stimulated neurons (not in synaptically activated neurons). Additional experiments with low doses of TTX showed that a mild reduction in the ability to generate axo-somatic action potentials had no significant antiepileptic effects, and generation of epileptiform discharges was compromised only when firing of axo-somatic action potentials was markedly suppressed. Taken together, our findings suggested that the antiepileptic effects of electrical stimulation were mediated by depression of excitatory neurotransmission, whereas reduction of excitability may somewhat contribute in high-frequency stimulation.

It is interesting to note that our findings are different from those reported for sinusoidal high-frequency electrical field stimulation in hippocampal brain slices where the antiepileptic effects were attributed to potassium efflux, depolarization of the membrane potential, and in turn, depolarization block of action potential firing (Bikson et al. 2001). In our experiments, we found no evidence for depolarization of the membrane potential during low-frequency stimulation.

The antiepileptic effect of sustained open-loop low-frequency stimulation paradigms has been examined in the past in hippocampal brain slices in vitro and in human patients with mesial temporal and neocortical epilepsy. Application of both constant DC and sinusoidal electric fields has been shown to suppress epileptiform discharges in the hippocampus in vitro (Bikson et al. 2001; Gluckman et al. 1996; Lian et al. 2003; Warren and Durand 1998). In addition, low-frequency stimulation applied either continuously or intermittently also prevented seizure-like events in hippocampal brain slices in vitro (Albensi et al. 2004; Barbarosie and Avoli 1997; Jerger and Schiff 1995; Khorosravani et al. 2003).

In patients with epilepsy, brief trains of high-frequency stimulation have been shown to terminate stimulus evoked afterdischarges in the neocortex (Lesser et al. 1999). In addition, continuous high-frequency stimulation administered to the hippocampus for 2–3 wk (Cuellar-Herrera et al. 2004; Velasco et al. 2000, 2001) or 5 mo (Vonck et al. 2002) decreased the number of seizures in a small number of patients suffering from intractable temporal lobe epilepsy.

Our study adds important additional information to that reported previously in the literature. The main new findings of this study are as follows. First, to the best of our knowledge, all previous in vitro studies were performed on hippocampal and entorhinal brain slices, and this is the first study that investigated the antiepileptic effect of electrical stimulation in extratemporal neocortical brain slices in vitro. Second, to the best of our knowledge, our study is the first to investigate the antiepileptic effects of closed-loop high-frequency stimulation in vitro. Third, in this study, we carefully characterized the parameters affecting the antiepileptic effects of stimulation. Hence our findings can be used to optimize the stimulation parameters. Fourth, and most importantly, our study shows for the first time that the antiepileptic effects of stimulation are mediated most likely by synaptic depression of glutaminergic synapses.

One of the main goals of this study was to define the stimulation parameters most effective in seizure prevention. We found that efficient antiepileptic low-frequency stimulation required stimulation frequencies of 0.5 Hz and above and placement of the stimulating electrodes in close proximity to the site of seizure onset. Similarly, Lian et al. (2003) also reported that the antiepileptic effect of monopolar electrical stimulation in hippocampal slices was limited to a region surrounding the stimulation electrode. The limited spatial efficacy of sustained low-frequency stimulation in seizure prevention may present a significant problem for clinical use. Possible ways to overcome this problem are to increase the stimulus intensity or use multiple stimulating contacts. In our experiments, the duration of sustained low-frequency stimulation was limited to 45 min. Although under our experimental condition in vitro, the effect stabilized after 5–10 min of low-frequency stimulation, further experiments are needed in vivo to examine the effect of open-loop sustained low-frequency stimulation lasting days. It is important to stress that other open-loop stimulation paradigms can be used, for example, intermittent high-frequency trains as used in vagal nerve stimulation. This study was limited to continuous low-frequency stimulation paradigms. Additional studies are needed to address the antiepileptic efficacy of other potential open-loop stimulation paradigms.

This study was carried out in two acute models of epilepsy: BCC- and magnesium free–treated neocortical brain slices in vitro. There are several apparent differences between our models and human epilepsy. Seizure frequency in our models was much higher than human epilepsy or chronic animal models of epilepsy. Neocortical slices contain only a fraction of the cortical network and lack all subcortical and brain stem structures that can either amplify or attenuate seizures. Synaptic transmission was different in our model either because inhibition was compromised (BCC) or NMDA currents were enhanced (magnesium free). Moreover, the different physical environment in vitro and in vivo can affect the efficacy of stimulation. For all these reasons, further studies are needed to verify our results in chronic models of epilepsy in intact rats in vivo.

In addition to open-loop sustained low-frequency stimulation, a second more elegant approach to treat intractable epilepsy is with computer-controlled closed-loop devices. Such devices will automatically detect impending or ongoing seizures, and in response, generate the appropriate short stimulation trains to terminate emerging seizures. In contrast to open-loop stimulators, closed-loop devices will only generate short stimulation trains, while most of the time, the cortex will remain uninterrupted.

In our study, we were unable to detect the prodromal phase of seizures. Thus we were limited to examining the efficacy of various closed-loop stimulation protocols in prematurely terminating ongoing seizures. Our findings indicated that short high-frequency stimulation protocols had a clear antiepileptic effect. However, they only succeeded in terminating ~50% of
seizures during the 1- to 3-s, 100-Hz stimulation trains and shortening the remaining one half of the seizures by ~50%. It is likely that if and when we have the capability to on-line identify impending seizures and apply stimulation during the prodromal phase of seizures, the antiepileptic efficacy of stimulation will increase.

In this study, we examined the underlying mechanisms responsible for the antiepileptic effect of electrical stimulation. We found that electrical stimulation marked depressed EPSPs and somewhat attenuated the excitability of neurons. Synaptic depression is one form of activity-dependent short-term synaptic plasticity (Von Gersdorff and Borst 2001; Zucker and Regehr 2002). Synaptic depression has previously been described in various peripheral and central synapses including excitatory synapses innervating layer 2/3 and layer 5 neocortical pyramidal neurons, the synapses examined in this study (Markram and Tsodyks 1996; Thomson et al. 1993; Varela et al. 1997). The main mechanism responsible for synaptic depression is probably depletion of vesicles from the readily available pool of synaptic vesicles (Zucker and Regehr 2002). However, other pre- and postsynaptic mechanisms probably also contribute to synaptic depression including desensitization of AMPA receptors, saturation of glutamate receptors, inactivation of presynaptic voltage-gated calcium channels, and activation of presynaptic metabotropic glutamate receptors (Schneggenburger et al. 2002; Von Gersdorff and Borst 2001; Zucker and Regehr 2002).

In addition to short-term synaptic depression, sustained low-frequency stimulation also induced long-term synaptic depression. This finding is consistent with findings of previous studies that described long-term synaptic depression in the neocortex (Bear 1999; Froc et al. 2000; Perrett et al. 2001). The magnitude long-term synaptic depression is much smaller than short-term synaptic depression. However, it may have a long-standing antiepileptic effect (Albensi et al. 2004). It is interesting to note that, despite the induction of LTD in our preparation, we observed no significant long-term effects of sustained low-frequency stimulation on the magnitude of PDS discharges or on the frequency of spontaneous seizure-like events. This was possibly caused by the relative small magnitude of LTD.

In addition to synaptic depression, electrical stimulation also reduced the excitability of neurons during high-frequency stimulation. The reduction of neuronal excitability during high-frequency stimulation possibly resulted from inactivation of voltage-gated sodium channels (Goldin 2003).

In conclusion, this study, we showed that both low- and high-frequency cortical electrical stimulation can eliminate seizure-like events in BCC-treated neocortical brain slices. Moreover, we identified the relevant parameters influencing the antiepileptic effect of cortical electrical stimulation and showed that synaptic depression was the main mechanism responsible for the antiepileptic effect of electrical stimulation. In the future, studies are needed to further investigate the antiepileptic effect and safety profile of open- and closed-loop cortical stimulation in chronic animal models of epilepsy in vivo.

GRANTS

This study was supported by the Israeli Science Foundation and the Rapapport Foundation.

REFERENCES


Cockerell OC, Johnson AL, Sander JW, Sharvon SD. Diagnosis of epilepsy: a review and further analysis of the first nine years of the British National General Practice Study of Epilepsy, a prospective population-based study. Epilepsia 38: 31–46, 1997.


