Epilepsia, 49(9):1586–1593, 2008
doi: 10.1111/j.1528-1167.2008.01592.x

FULL-LENGTH ORIGINAL RESEARCH

Effects of high-frequency stimulation on epileptiform activity in vitro: ON/OFF control paradigm

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SUMMARY

Purpose: To determine the effects of high-frequency electrical stimulation on electrographic seizure activity during and after stimulation (ON-effect and OFF-effect).

Methods: The modulation and suppression of epileptiform activity during (ON-effect) and after (OFF-effect) high-frequency electrical stimulation was investigated using the high-K^+ and picrotoxin hippocampal slice epilepsy models. Uniform sinusoidal fields (50 Hz) were applied with various intensity levels for 1 min across brain slices. Extracellular and intracellular activity were monitored during and after stimulation.

Results: The ON-effects of high-frequency stimulation were highly variable across individual slices and models; ON-effects included modulation of activity, pacing, partial suppression, or activity resembling spreading-depression. On average, epileptic activity, measured as power in the extracellular fields, increased significantly during stimulation. Following the termination of electrical stimulation, a robust poststimulation suppression period was observed. This OFF suppression was observed even at relatively moderate stimulation intensities. The duration of OFF suppression increased with stimulation intensity, independent of ON-effects. Antagonism of $GABA_A$ function did not directly effect OFF suppression duration.

Conclusions: The present results suggest that "rational" seizure control protocols using intermittent high-frequency electrical stimulation should control for both ON and OFF effects.

KEY WORDS: Electrical stimulation, Epilepsy, Hippocampus, Electric fields, CA1, CA3, Ictal.

Technologies applying electrical stimulation to control pharmacologically intractable epileptic seizures are being actively explored (Velasco et al., 2000c; Cohen-Gadol et al., 2003; Goodman, 2004; Polkey, 2004; Murphy & Patil, 2005; Morrell, 2006; Albensi et al., 2007; Li & Mogul, 2007). A variety of stimulation paradigms including DC or slow-adaptive electric fields (Gluckman et al., 1996; Ghai et al., 2000; Gluckman et al., 2001; Lian et al., 2003), high-frequency stimulation (Bikson et al., 2001; Lian et al., 2003; Feddersen et al. 2007; Jensen & Durand, 2007), and low-frequency pulsed stimulation (Albensi et al., 2004) have been developed. A range of potential suppression mechanisms have been proposed with the specific mechanisms depending on the precise waveform applied (Durand & Bikson, 2001; McIntyre et al., 2004c; Li & Mogul, 2007) and the underlying seizure dynamics (e.g., clinical focus, animal model).

Significant unknowns remain about the mechanisms of electrical-stimulation control of seizures. "Rational" protocols based on quantitative predictive control for optimizing stimulation waveform are lacking (Bikson et al., 2006). Moreover, empirical clinical optimization is limited by the relative infrequency of seizures and safety concerns (Theodore & Fisher, 2004). In vitro epilepsy models provide a preliminary tool to prescreen, characterize, and optimize stimulation paradigms and waveforms (Durand & Bikson, 2001). In this report, we investigated the effects of high-frequency stimulation on high-K^+ and picrotoxin hippocampal slice models of epilepsy. We considered effects during stimulation (ON-effects) and poststimulation modulation of activity (OFF-effects). We discuss whether stimulation approaches that balance ON/OFF effects may provide a more robust method for seizure control.

Accepted February 26, 2008; Early View publication April 4, 2008.

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METHODS

Recordings were obtained from the CA1 or CA3 pyramidal cell regions of hippocampal brain slices (0.35–0.40 mm) prepared from male Sprague–Dawley rats (125–175 g; CCNY-IACUC protocol 0406). A total of 29 animals were used in this study. Slices were superfused in an interface recording chamber at 36°C oxygenated (with 95% O_2, 5% CO_2) "normal" artificial cerebrospinal fluid (ACSF) consisting of (in mM) 125 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1.6 $CaCl_2$, 1.5 $MgSO_4$, 26 $NaHCO_3$, and 10 dextrose.

"High-potassium" (high-K^+) ACSF consisted of (in mM): 125 NaCl, 8.0 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 1.5 $MgSO_4$, 26 $NaHCO_3$, and 10 dextrose. "Picrotoxin ACSF" was made by adding 100 μM picrotoxin ($GABA_A$-receptor antagonist) to normal ACSF. "High-potassium plus picrotoxin" ACSF was made by adding 100 μM picrotoxin to high-potassium ACSF. Perfusion with these solutions resulted in spontaneous epileptiform activity (electrographic seizures) in the CA1 or CA3 regions of the hippocampus; epileptiform activity was characterized by spontaneous bursts of population-spike trains. Slices in which spreading depression-like activity was observed in the absence of stimulation were excluded. Individual slices were superfused with only a single epileptiform solution. All chemicals were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.).

Recordings of extracellular field potentials were obtained using glass micropipettes (10–15 MΩ, pulled on a P-97; Sutter Instruments, Novato, CA, U.S.A.) filled with 125–150 mM NaCl. One recording electrode was positioned in the somatic layer of the CA1 or CA3 region. A second electrode was positioned at an isopotential site in the bath (Ghai et al., 2000; Durand & Bikson, 2001). For intracellular recording, the potential from a field electrode positioned next to the intracellular electrode (50–100 MΩ, filled with 3 mM KCl) was subtracted (Bikson et al., 2004; Radman et al., 2007).

Uniform 50 Hz sinusoidal electric fields were generated across individual slices by passing current (A-M Systems stimulus isolator Model 2200, Carlsborg, WA, U.S.A.) between two parallel AgCl-coated silver wires placed on the surface of the ACSF in the interface chamber (Bikson et al., 2004). Stimulation was applied for 1 min. Stimulation intensity (mV/mm) ranged from 70 to 414 mV/mm.

Suppression during ON or OFF periods was defined as reduction in population spike activity to 20% of prestimulation values. ON and OFF power ratios (dB) were quantified by comparing field power during stimulation (1 min) or immediately poststimulation (first 1 min) relative to the power of the 1-min field base-line before stimulation (all 100 Hz high-passed). "Spreading depression-like" events were defined extracellularly as slow shifts in the extracellular field potential ≤ -10 mV for >10 s (Haglund & Schwartzkroin, 1990; Tong & Chesler, 2000; Bikson et al., 2003) that were followed by a refractory period (absence of spontaneous or evoked population spikes). "Spreading depression-like" events were defined intracellulary as shifts in membrane potential to ≥ 15 mV for >10 s (Haglund & Schwartzkroin, 1990). In the analysis, we excluded cases in which spreading depression-like events were induced by stimulation.

Signals were amplified and filtered with an Axoclamp-2B (Axon Instruments, Union City, CA, U.S.A.) and FLA-01 amplifiers (Cygnus Technology, Delaware Water Gap, PA, U.S.A.); then digitized and processed using a Power 1401 and Signal software (CED, Cambridge Electronic Design, Cambridge, U.K.). Additional filtering (including 50 Hz band-stop), statistical analysis, and figure generation were implemented using MATLAB R14 (Mathworks, Inc., Natick, MA, U.S.A.). Results are reported as mean ± standard error. After combining data from all slices for each epilepsy model, Pearson's correlation coefficient was used to determine the total correlation (r_t) between electric field intensity, OFF suppression period, and ON power ratio. Significance of correlation (p-value) was calculated using Student's *t*-test on the combined data; $p < 0.05$ reported as significant. In addition, we calculated correlation coefficients for each slice and averaged across slices to obtain a pooled within-slice covariance (r_w).

RESULTS

The effects of high-intensity 50 Hz sinusoidal electric fields on epileptiform activity were evaluated in the high-K^+ and picrotoxin epilepsy models. Modulation during stimulation (ON-effects) and suppression after stimulation (OFF-effects) were quantified. For all three models, ON-effects were classified as: (1) "modulation/pacing": epileptiform activity remained and population spikes could occur in phase with stimulation; (2) "partial suppression": epileptiform activity was suppressed for more than 10 s, but less than the 1-min duration of stimulation; (3) "spreading depression-like event": during stimulation a spreading depression-like event (see Methods) is triggered.

For determining "maximal" on stimulation effects, stimulation intensity was increased until either: (1) partial suppression was observed; (2) a spreading depression-like event was induced (at intensities below spreading depression-like events, modulation/pacing was observed); or (3) the maximal field amplitude tested (320–414 mV/mm) continued to induce modulation/pacing. Unless othwerise stated, the high-intensity ON results reported below refer to these "maximal" stimulation effects.

ON-effects of stimulation during high-K^+ epileptifom activity

The effects of stimulation on high-K^+ electrographic field activity was evaluated in 24 slices. Low-intensity stimulation resulted in modulation/pacing of activity in all

slices tested. High-intensity stimulation (between 200 and 414 mV/mm) resulted in modulation/pacing in 16 slices (Fig. 1A; 16.8 ± 1.0 dB ON power ratio), partial suppression of activity for a portion of the stimulation period in three slices (average field threshold 223 mV/mm; 14.5 ± 1.5 dB ON power ratio; Fig. 1B,C), and a spreading depression-like event in five slices (Fig. 1D). Partial suppresion could be associated with a characteristic slow-field shift; this slow-field shift (Fig. 1C) was distinct from spreading depression-like events as it was relativly short and activity (pacing or spontaneous epileptiform activity) resumed immediately after return to baseline (see Methods).

Intracellular recording confirmed observations with field electrodes (Fig. 2). Intracellularly high-intensity fields resulted in cell modulation/pacing (n = 1; Fig. 2A), transient suppresion (n = 2; Fig. 2B), or a spreading depression-like event (n = 2; Fig. 2C).

ON-effects of stimulation during picrotoxin epileptifom activity

High-intensity stimulation (160–400 mV/mm) of picrotoxin induced activity (Fig. 3) resulted in partial suppression of activity (Bikson et al., 2001) in four of eight slices tested (average field threshold 235 mV/mm; 4.2 ± 0.7 dB ON power ratio) and modulation/pacing in three slices (8.3 ± 2.0 dB ON power ratio), with one slice showing a spreading depression-like event. Lower-intensity stimulation (80–100 mV/mm) resulted in activity modulation/pacing in a total of 12 slices tested.

ON-effects of stimulation during high-K^+ plus pictroxin epileptifom activity

In the high-K^+ model, inhibitory synaptic function is intact (Jensen & Yaari, 1997). We tested the role of GABAergic function during high-frequency stimulation of high-K^+ by adding picrotoxin (0.1 mM) during high-K^+ bursting. Stimulation (75–414 mV/mm) of high-K^+ plus picrotoxin activity resulted in modulation/pacing in five of six slices tested (Fig. 4; 15.6 ± 0.8 dB ON power ratio) and a spreading depression-like event in the remaining slice.

OFF-effects of stimulation

Succesful OFF suppression was defined as a poststimulation suppression period greater than twice the baseline (prestimulation) interelectrographic seizure period. High-intensity stimulation (160–414 mV/mm) resulted in successful OFF suppression in all 19 slices tested in the high-K^+ model, six of seven slices tested in the picrotoxin model, and all five slices tested in the high-K^+ plus picrotoxin model. The minimum field amplitudes required for OFF suppression were on average 147, 134, and 125 mV/mm in the high-K^+, picrotoxin, and high-K^+ plus picrotoxin models, respectively. The average poststimulation OFF power ratios, at the minimum stimulation intensities proceeding succesful OFF suppression, were −4.6 ± 0.9 dB, −0.6 ± 0.3 dB, and −0.9 ± 0.5 dB in the high-K^+,

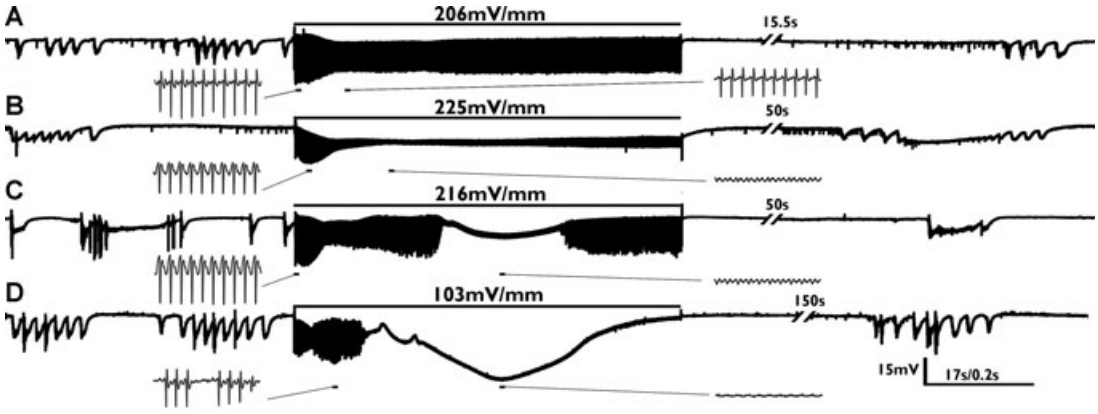

Figure 1.
Effects of high-intensity sinusoidal (50 Hz) stimulation on high-K^+-induced epileptiform extracellular field activity. Typical modulation/pacing of activity (**A**) and examples of partial suppression during stimulation (**B, C**), and a stimulation induced spreading depression-like event (**D**); traces from different slices, see text for classification scheme. All signals were 50 Hz band-stop filtered, removing the stimulation artifact, but leaving spontaneous and paced activity. Note that in all cases, particularly at the initiation of stimulation, episodes of population spike pacing were observed; the interspike interval was generally (a multiple of) the stimulation period (20 ms = 1/50 Hz). Episodes of suppression (including during spreading depression-like events) were characterized by the absence of synchronized population activity. In all cases, a poststimulation OFF suppression period was observed.

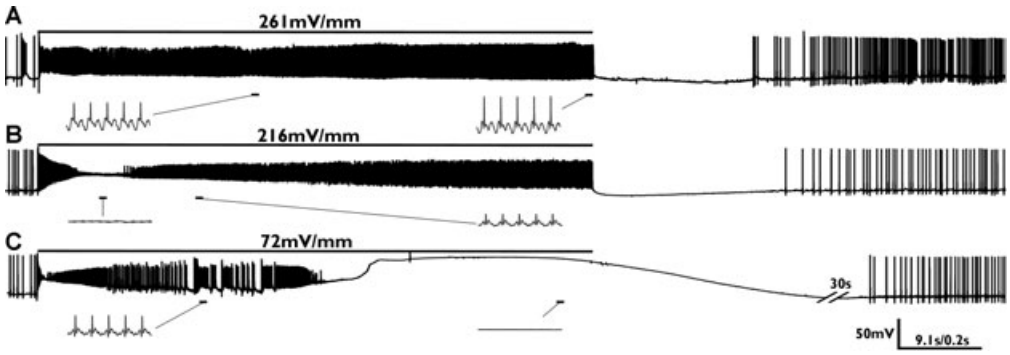

Figure 2.
Effects of high-intensity sinusoidal (50 Hz) stimulation on high-K^+-induced epileptiform intracellular activity. Typical modulation/pacing of activity (**A**) and example of partial suppression (**B**) and spreading depression-like event (**C**) during stimulation. All signals were 50 Hz band-stop filtered, removing the stimulation artifact, but leaving spontaneous and paced action potentials. During stimulation pacing of action potentials generally occurred at the frequency of stimulation (50 Hz) or subharmonic. During stimulation episodes of action potential suppression or action potential attenuation could clearly be observed. Note that in all cases, a poststimulation OFF suppression period was observed.
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picrotoxin, and high-K^+ plus picrotoxin models, respectively.

In each model, the duration of poststimulation OFF suppression increased with stimulation intensity (Fig. 5, left; significant correlation in all three models with $p < 0.01$). For the high-K^+ and picrotoxin models, no correlation between ON power ratio and poststimulation OFF suppression duration was observed (Fig. 5, right top and center). However, the high-K^+ plus pictroxin model did show a correlation (Fig. 5, right bottom). This correlation remained even when a linear effect of stimulus intensity on each of these variables was subtracted (residuals remain correlated with $r = 0.57$, $p < 0.02$).

Discussion

ON/OFF electrographic seizure control

Fundamental differences exist between electrographic seizure genesis in vivo and the in vitro models studied here; for example, in vivo seizures occur at a comparatively low

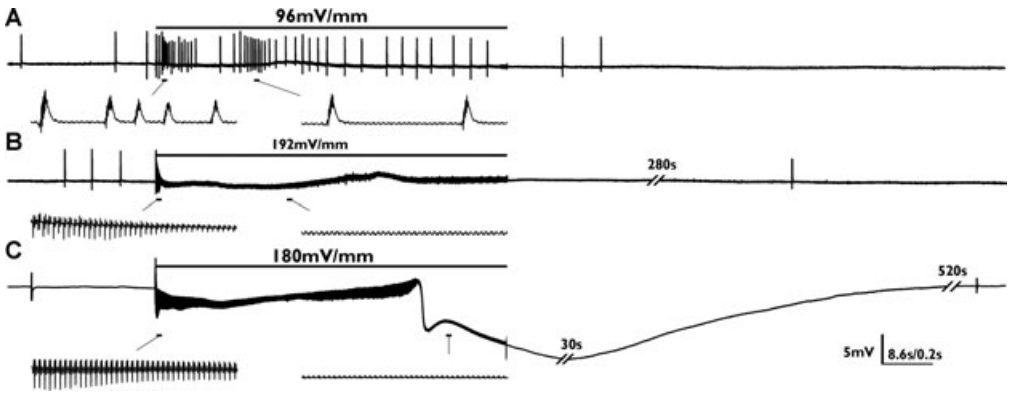

Figure 3.
Effects of high-intensity sinusoidal (50 Hz) stimulation on picrotoxin-induced epileptiform extracellular field activity. Lower intensity stimulation resulted in modulation/pacing (**A**) while higher intensity stimulation resulted in partial suppression of activity (**B**) or spreading depression-like event (**C**). All signals were 50 Hz band-stop filtered, removing the stimulation artifact, but leaving spontaneous and paced activity. Note that lower-intensity stimulation could modulate/aggravate activity. In all cases, a poststimulation OFF suppression period was observed.
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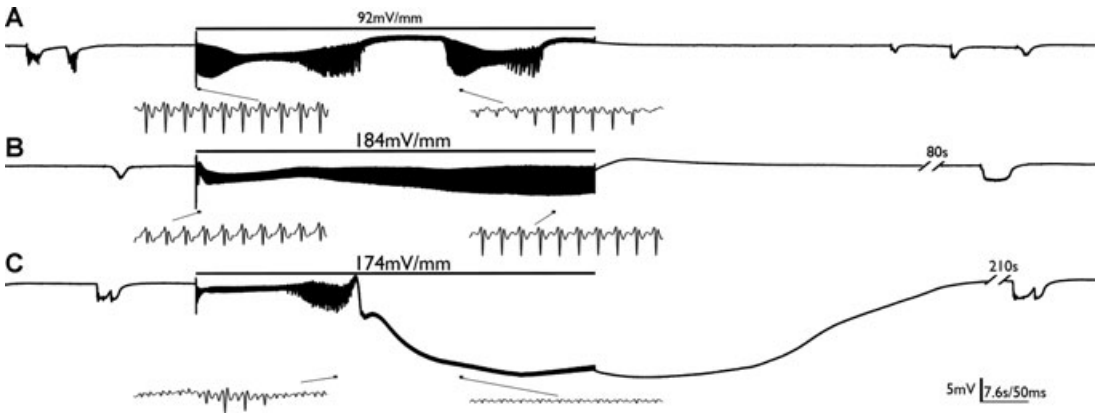

Figure 4.
Effects of high-intensity sinusoidal (50 Hz) stimulation on high-K^+ plus picrotoxin-induced epileptiform extracellular field activity. Both lower intensity and higher intensity stimulation resulted in modulation/pacing (**A, B**) or could trigger a spreading depression-like event (**C**). All signals were 50 Hz band-stop filtered, removing the stimulation artifact, but leaving spontaneous and paced activity. Note that in all cases, particularly at the initiation of stimulation, episodes of population spike pacing were observed; the interspike interval was generally (a multiple of) the stimulation period (20 ms = 1/50 Hz). In all cases, a poststimulation OFF suppression period was observed.

rate. In addition, the clinical manifestations of electrical-stimulation induced pacing or spreading depression-like activity are unclear. Previous experimental reports have considered low-frequency "pacing" as antiepileptic (Schiller & Bankirer, 2007) or targeted only the low-frequency (<10 Hz) field component of electrographic seizures (Bikson et al., 2001; Lian et al., 2003). In this report, we distinguished between pacing and (partial) suppression of population spikes (see Methods). However, even in cases of partial suppression, periods of pacing were observed during high-frequency stimulation. Moreover, our results and previous studies (Bikson et al., 2001; Lian et al., 2003) suggest that continuous high-frequency stimulation eventually fails to suppress electrographic seizures after >3 min.

All the ON-effect classification schemes we evaluated were problematic due to the shifting effects during the course of the 1 min stimulation (e.g., between pacing and suppression). The quantitative ON power ratio depended on stimulus intensity for the high-K^+ but not the picrotoxin model (Fig. 5). The incidence of partial suppression during stimulation did not depend on stimulus intensity. As stimulation intensity increased the quantitative ON power ratio metric could either increase, reflecting enhanced pacing of activity, or decrease, reflecting robust suppression of activity. In addition, for two of the three models tested, the duration of post stimulation OFF suppression did not correlate with ON power ratio. Thus broadly speaking, ON stimulation effects were variable, unpredictable, and are of unclear clinical relevance (average pacing and partial-suppression ON power ratio, across models, 12.4 ± 0.6 dB), and may be poor predictors of OFF suppression efficacy.

In contrast to the variable ON stimulation effects, in all models tested here, high-frequency (50 Hz) sinusoidal stimulation resulted in consistent poststimulation OFF suppression of activity. This OFF suppression was observed at relatively low intensities (compared to those necessary to induce ON partial suppression) and was characterized by a robust inhibition of synchronized activity (at the minimum stimulation intensity producing successful OFF suppression the average OFF power ratio, across models, was −3.4 ± 0.4 dB). Furthermore, poststimulation OFF suppression period increased reliably with stimulation intensity. This effect was robust across models and did not depend on the specifics of the ON-effects. OFF suppression of epileptiform activity has previously been observed after sustained high-frequency stimulation in vitro (Bikson et al., 2001; Lian et al., 2003; Schiller & Bankirer, 2007).

We propose that "rational" stimulation protocols, which intelligently balance ON and OFF effects may provide a robust approach to seizure control.

Cellular mechanisms of ON/OFF suppression

The mechanisms of ON modulation/suppression by high-frequency electric fields have been previously addressed (Jensen & Yaari, 1997; Bikson et al., 2001; Lian et al., 2003; Schiller & Bankirer, 2007). The cellular mechanisms underlying the OFF-effects of electrical stimulation are less characterized, though poststimulation inhibition has been observed in multiple systems (McIntyre et al., 2004a; Shin et al., 2007). Just as high-frequency

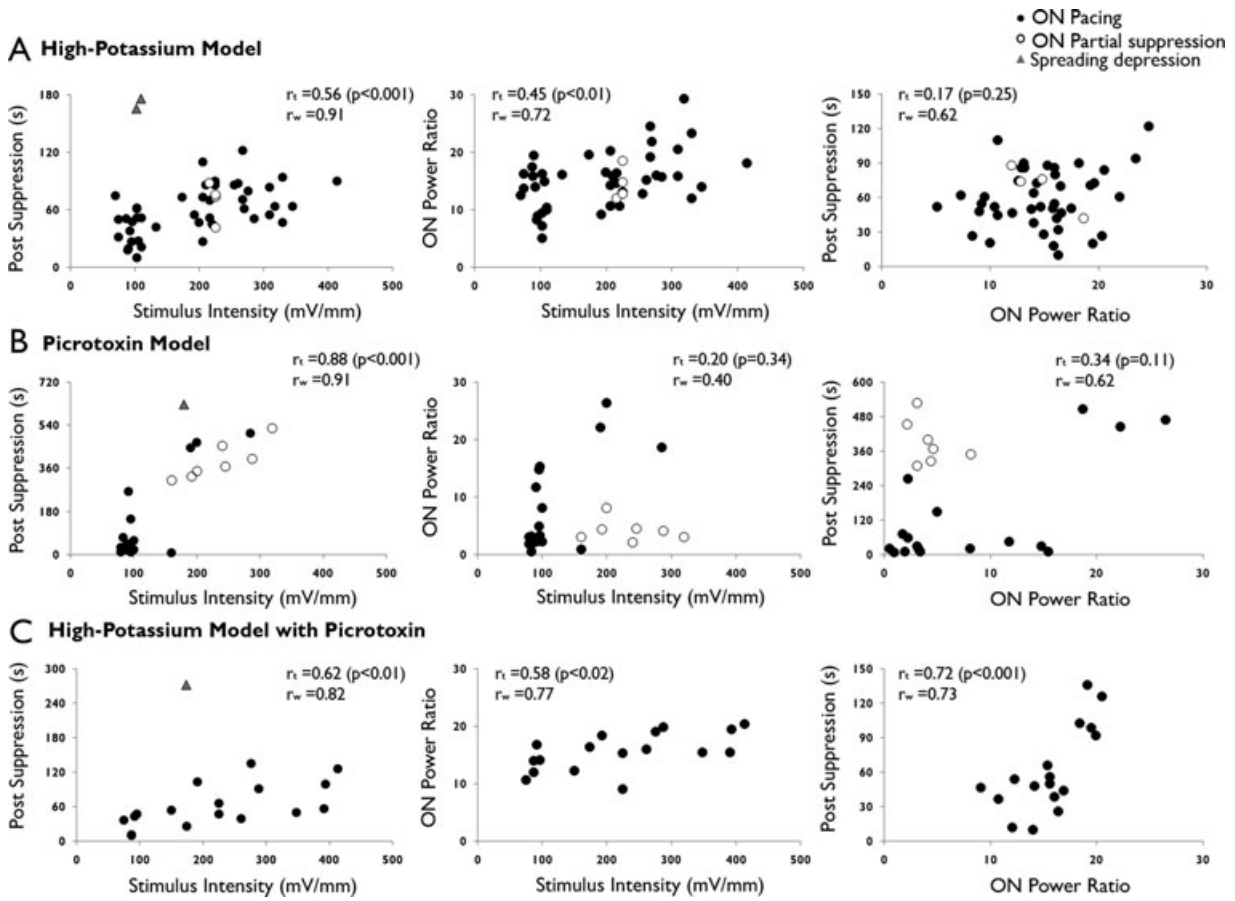

Figure 5.

Summary of ON and OFF stimulation effects for each epilepsy model. The relationship between pairs of three metrics were compared (postsuppression duration, stimulation intensity, and ON power ratio) separately for each model (high-K^+, picrotoxin, high-K^+ plus picrotoxin). Each point represents a field application (with multiple field amplitudes tested in specific slices). r_t is the correlation coefficient of combined data; p-values indicate significance of this total correlation. r_w is the within-slice correlation coefficient averaged across slices (see Methods), and reflects predictability for a given slice. Symbols indicate pacing (filled circle), partial suppression (open circle), or spreading depression-like events (filled triangle) resulted from given stimulations. Spreading depression-like events were excluded from calculation of correlation coefficients. Note that poststimulation duration increased consistently with stimulation intensity. ON power ratio did not correlate consistently with stimulation intensity or with postsuppression duration (see text).

supra-threshold brain stimulation results in constellation of ON-effects (e.g., excitability, ion concentration, and synaptic modulation), the OFF-effects of stimulation presumably reflect the recovery of multiple processes. From the perspective of seizure-control, interest should focus on parameters with a slow-recovery rate in the OFF period. One such candidate is extracellular potassium concentration, which decreases below baseline after stimulation termination and gradually recovers to baseline (approximately 5 min) concurrently with electrographic seizure recovery (Bikson et al., 2001; Lian et al., 2003; Shin et al., 2007).

We observed marked differences in postsuppression period between the high-K^+ and picrotoxin epilepsy models. One hypothesis is that this difference reflects antagonism of $GABA_A$ function in the picrotoxin model. However, we found that antagonism of GABA-function in the high-K^+ model did not significantly effect OFF-suppression duration. Alternatively, differences between models may reflect that extracellular potassium concentration transient dynamics, as baseline extracellular potassium concentration was different. Similarly, extracellular Ca^{2+} baseline (and thus transient) levels may modulate poststimulation duration.

Clinical application: safety and efficacy

Variable clinical efficacy reinforces the need to develop stimulation protocols that are robust across epilepsy cases. Across models, we found ON suppression partially successful in 8 of 32 slices (average threshold 231 mV/mm) while OFF suppression was evidently successful in 31 of 32 slices (average threshold 135 mV/mm). Even the highest stimulation intensities tested in the present report (414 mV/mm) are within peak electric field amplitudes generated near Food and Drug Administration (FDA) approved deep brain stimulation (DBS) devices (McIntyre et al., 2004b; Elwassif et al., 2006). OFF suppression requires still less peak current than ON suppression and inherently low-duty cycle (ON/OFF) stimulation. Reduction of stimulation intensity will increase safety by minimizing electrochemical (Merrill et al., 2005) and heating damage (Elwassif et al., 2006), reducing side effects by increased spatial focality (McIntyre et al., 2004b), as well as improved implanted device performance (e.g., battery life).

The ON/OFF paradigm introduced here, places emphasis on both the ON and poststimulation OFF effects of nervous system activation. Therefore, protocols optimized for only ON suppression (e.g., short pulse trains) may not successfully prevent electrographic seizures in the OFF period (Boex et al., 2007; Feddersen et al., 2007). A rational extension of ON/OFF suppression paradigms includes stimulation protocols incorporating periodic application of high-frequency trains (ON) at a sufficiently short intertrain duration (OFF period) such that seizures are never generated (i.e., the system is tonically maintained in an OFF suppression refractory state). Indeed, successful clinical seizure control designs (Group VNSS, 1995; Handforth et al., 1998; Velasco et al., 2000a, 2000b; Vonck et al., 2002; Boon et al., 2007) have used intermittent (e.g., 1 min on/5 min off) stimulation protocols.

Spreading depression-like events occurred with increasing stimulus intensities. Clinically, spreading depression has been associated with as relatively mild phenomena as migraine (Calabresi et al., 2007) and as severe effects as necrosis (Balestrino & Somjen, 1986; Pomper et al., 2006). Lower-intensity ON/OFF suppression approaches may be used to avoid any potential complications of spreading depression induction. Alternatively, the pronounced antiepileptic effects of spreading depression (e.g., triggered on seizure prediction) may be clinically favored to severe seizure symptoms. Any necrosis resulting from spreading depression could result in secondary aggravation or amelioration of the epileptic network.

Continued refinements in stimulation protocols, including the use of feedback, will improve successful seizure control (Gluckman et al., 2001; Fountas & Smith, 2007); indeed monitoring of activity in the OFF period will be stimulation-artifact free, allowing accurate monitoring of brain state. An implanted system would further need to consider the spatial distribution of induced fields. Field generated with implantable electrodes are generally nonuniform; however, our experience with high-frequency stimulation (as opposed to DC stimulation) suggests suppression is not orientation specific (Bikson et al., 2001; Lian et al., 2003). In summary, our results support the consideration of "rational" stimulation paradigms designed around both ON and OFF stimulation effects.

ACKNOWLEDGMENTS

The authors acknowledge technical support from Dolores Miranda and Je Hi An from City College of New York. This research was supported in part by PSC-CUNY, The Andy Grove Foundation, and NIH.

Conflict of interest: We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. The authors report no conflicts of interest.

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