Cortical Stimulation of the Epileptogenic Zone for the Treatment of Focal Motor Seizures: An Experimental Study in the Nonhuman Primate

**BACKGROUND:** Cortical stimulation is under investigation in clinical trials of drug-resistant epilepsy. Results are heterogeneous; therefore, more evidence from animal studies is required.

**OBJECTIVE:** To investigate the therapeutic effects of parameters of direct stimulation of the cortical focus in a *Macaca fascicularis* presenting focal motor epilepsy.

**METHODS:** We developed a model of motor seizures after intracortical injection of penicillin G in the primary motor cortex of a *Macaca fascicularis*. We performed electric epidural cortical stimulation at low, medium, and high frequency using continuous or short-term stimulation. Short-term stimulation was triggered on seizure onset, either visually or automatically with a seizure detection algorithm connected to a programmable stimulator.

**RESULTS:** Automated detection could detect 100% of the seizures, but ensuing cortical electric stimulation failed to abort seizures.

**CONCLUSION:** This study demonstrates the inefficacy of the stimulation of the cortical focus to prevent seizures induced by local injection of penicillin G. Because this model may be too severe to allow comparison to human epilepsies, further work is required in other monkey models of focal epilepsy.

**KEY WORDS:** Cortical stimulation, Deep brain stimulation, Drug-resistant epilepsy, Nonhuman primate, Penicillin, Permutation entropy, Seizure detection

**DISCUSSION:**

Drug resistance is a major issue in partial epilepsies because only one-third of the patients are seizure free with medications.\(^1\) Whereas surgical resection is the gold standard in this case, it can be proposed only to the minority of the patients in whom the epileptogenic zone is located apart from eloquent cortex. Thus, central region epilepsies are particularly challenging for the neurosurgeon because postoperative morbidity can reach as high as 52% of the cases, with only 26% of the patients being cured.\(^2\) Therefore, new therapies such as vagal nerve stimulation, deep brain stimulation, or cortical stimulation have been developed during the last 20 years.\(^3,4\) Although cortical stimulation has been used for decades during epilepsy surgery to induce seizures and to map the ictal onset zone,\(^5\) experimental\(^6-8\) or clinical studies have tended to show that electric cortical stimulation could also stop seizures.\(^9-13\) However, the only prospective randomized double-blind trial found a median of only 15% reduction in the frequency of the seizures in mesial temporal lobe epilepsy, and the definite results of the RNS study (Neuropace Inc) have not been published yet.\(^14,15\) Moreover, controversies remain on the stimulation parameters, notably the frequency of stimulation. Whether stimulation should be continuous, to prevent seizure occurrence, or triggered by seizure onset, to abort seizure propagation, is still under investigation.\(^16-19\)

To gather more experimental evidence regarding these important clinical questions, we investigated here the therapeutic effects of parameters of direct stimulation of the cortical focus in a *Macaca fascicularis* presenting focal motor epilepsy. Seizures were induced by the injection of penicillin G into the motor cortex, which induces focal seizures lasting < 48
hours because of the noncompetitive inhibition of the GABAa receptors. We experimented with low-, medium-, and high-frequency electric stimulation on 893 seizures in continuous or short-term conditions. Eventually, we developed a brain computer interface for the automated detection/stimulation of the seizures in monkey on the basis of previous work in rat. Overall, we observed weak, if any, effect of cortical stimulation on seizure activity for any set of parameters.

MATERIALS AND METHODS

Monkey

The study was conducted on a 7-year-old Macaca fascicularis (CRP Port Louis, Mauritius, 8 kg). This Old World monkey is a member of the Cercopithecidae, which is the last phylogenetic bifurcation before the hominoidae. Its motor cortex shares cytoarchitectonic characteristics with humans, and the motor system has a great homology with the human motor system, even if prefrontal cortex is less developed in the macaca.

The project was validated by the regional ethics committee and followed the European norms of November 1986 for the respect and safety of the animals (86/609/EEC).

Surgery

Surgery was performed under general anesthesia (ketamine-xylazine) and aseptic conditions in a stereotaxic frame (David Kopf Instruments, Tujunga, California) with teleradiographic and electrophysiological control. A unilateral 10-mm-diameter hole was made in the skull in the precoronal area, in which 2 epidural quadripolar electrodes (DIXI Microtechniques, Besançon, France) were inserted over the right motor cortex. The location of the motor cortex was determined by direct electric cortical stimulation with an external stimulator. A cannula was screwed epidurally between the electrodes to allow further injections of penicillin G (Figure 1). A head holder was placed on the vertex and secured with dental cement to allow positioning the head of the monkey in a dedicated restrained chair.

Seizure Induction and Analysis

Penicillin G was diluted at 250 IU/µL, and 2000 to 12,000 IU was injected 3 mm deep in the motor cortex at a speed of 4 µL/min with a microneedle and a Hamilton syringe. A total of 17 injections in a period of 5 months were done.

Two acquisition systems were used consecutively. First, a commercial system (Micromed, Treviso, Italy) allowed us to record simultaneously the electrocorticogram, electromyogram, and video, with a sampling rate of 256 Hz and a band-pass filter between 0.53 and 90 Hz. Second, we used the new BioMEA system (Biological, Claux, France) composed of a 64-channel microamplifier operating at a sampling rate of 1.3 or 13 kHz. We interfaced BioMEA with Matlab (Math Works) to allow automated switching between stimulation and electrocorticogram recording on any channel. Data were then analyzed under Spike 2 (Cambridge Electronic Design) or with a homemade Matlab toolbox for intracranial recordings.

Two examiners assessed the number and duration of the seizures visually. Minimal length to define a seizure was 3 seconds. The discharge pattern was studied with time-frequency analysis of the electrocorticogram using Morlet wavelet transform.

Stimulation Paradigm

A WPI A310 Accupulser generator (World Precision Instruments, Sarasota, Florida) delivered monophasic bipolar stimulation through 2 adjacent epidural contacts. We chose the 2 electrode contacts where phase inversion of the spikes was noted underneath and where
stimulation above the motor threshold induced jerks of the arm, closely mimicking the clinical effect produced by a penicillin-induced spike.

For each stimulation frequency, we defined the motor threshold as the minimal intensity that could induce jerk of the contralateral arm in 5 of 10 trials.\textsuperscript{27} Intensity of stimulation was then set at 80% of the motor threshold, and energy delivered by phase was calculated to avoid any tissue injury.\textsuperscript{29} The parameters (frequency, intensity, pulse width) are summarized in the Table and are comparable to other studies on Macaca fascicularis.\textsuperscript{29}

We first tried to abort seizures with short-term stimulation. A delay of 2 seconds between visual detection of the seizure and stimulation was retained to avoid stimulating false seizures. Electric stimulation lasted 5 seconds to minimize any electrically induced neural damage.\textsuperscript{30} We alternated 1 hour without stimulation and 1 hour with stimulation of each seizure, as shown in Figure 2. We then analyzed the effects of continuous stimulation at 1 and 130 Hz, with stimulation starting after the first seizure. Statistical analysis with the nonparametric Friedman test compared the length of the seizures between groups with or without stimulation.

**Automated Seizure Detection/Stimulation**

Seizures were detected online with permutation entropy.\textsuperscript{31} Entropy, a measure of the randomness of the electrocorticogram signal, decreases dramatically during a seizure because of its oscillatory synchronous properties. We used normalized permutation entropy, a variable equal to the maximum theoretical entropy divided by the measured entropy, which therefore had a minimum value of 1 and increased during seizures. Normalized entropy seizure threshold was empirically adjusted to 1.07, and a seizure was assumed to be detected after 3 seconds spent above this threshold to minimize false-positive detection. Once a seizure was detected, stimulation was automatically triggered by the BioMEA system, which in turn piloted the WPI stimulator. Stimulation parameters were as follows: frequency, 100 Hz; intensity, 4 mA; pulse width, 100 microseconds; and duration, 5 seconds. To quantify any effect of stimulation, we chose to stimulate 1 of 2 seizures alternatively, and we compared the length of the seizures in the stimulated and nonstimulated conditions using Wilcoxon nonparametric test.

**RESULTS**

**Seizure Characteristics**

First, we analyzed the pattern of the seizure discharges without any stimulation after 3 consecutive sessions of injection of penicillin performed 4 days apart. Seizures were clinically focal, limited to contralateral rhythmic jerks of the left arm and face, with no secondary generalization. Time-frequency analysis of the electrocorticogram showed 15-Hz discharges followed on the second half of the seizure by a slower 3-Hz discharge (Figure 3). No contralateral diffusion of the seizure was recorded. Interictal symptomatic spikes were noted with a 0.2-Hz periodicity.

Mean duration of the seizures was not significantly different ($P = .2$) in days 1 through 3, with a mean ± SD duration of $53 ± 40.5$, $53.14 ± 23.6$, and $65.4 ± 33.6$ seconds, respectively. However, seizure duration increased with time after the first seizure (Figure 4), yet there was no clear correlation between seizure duration and time (Spearman rank correlation coefficient $r = 0.137$). Number of seizures per hour was statistically different ($P = .03$) between the 3 days with a mean of $17.8 ± 7.7$, $4.6 ± 3.1$, and $2.4 ± 1.9$, respectively.

Penicillin-induced seizures could occur during the 24 hours after intracortical injection. Thus, a minimal delay of 7 days between 2 injections of penicillin was thus incorporated into subsequent experiments to allow the monkey to recover and to avoid a potential decrease in the number of seizures per session.

**Short-term Stimulation**

The effects of 1-, 25-, 130-, and 500-Hz short-term stimulation were analyzed during 3 distinct 6-hour recording sessions after injection of penicillin. For each session, we compared seizure length between the “on” and “off” stimulation periods (Figure 5). Because those periods were alternated every hour, 6 groups of 1 hour were compared for each injection.
We first observed that responsive stimulation did not abort seizures. Second, using the Friedman nonparametric test, we found a significant difference in seizure duration between hours 1 and 6 in each session at 130 Hz ($P = .03$), 25 Hz ($P = .01$), and 500 Hz ($P = .009$). Nevertheless, a posthoc Tukey test showed that the difference in duration was not related to the stimulation but rather to the delay after the first seizure because seizures became longer with time. At 500-Hz stimulation, seizures were longer 4 hours after the first seizures than at 2 hours ($P < .05$). On the other hand, comparison between hours 4 and 5 (500 Hz vs no stimulation) did not reach statistical significance. The same results were observed for the 1-, 25-, and 130-Hz group.

![FIGURE 3. Typical seizures induced by intracortical injection of penicillin. A, time-frequency analysis of a seizure. Spectral power is maximal (red) at 15 Hz at the beginning of the seizures ($t = 0$ seconds) and then at 3 Hz after 25 seconds of evolution. B, electrocorticogram of the same seizure, with a sharp start preceded by 2 spikes. C, electrocorticogram (ECoG; black) and electromyogram of the left upper (EMG1; dark blue) and lower (EMG2; green) limbs of another seizure. Note that preictal spikes are synchronous with jerks of the upper arm.](https://www.neurosurgery.org/content/68/2/482)

![FIGURE 4. Box plots of the duration of seizure under baseline conditions. There was no significant difference in seizure duration between the 3 different injections 4 days apart (left). Seizures were getting longer with time after the first seizures (right), yet the Spearman correlation coefficient was low ($r^2 = 0.137$).](https://www.neurosurgery.org/content/68/2/482)
FIGURE 5. Box plot of the seizure duration during short-term stimulation. Left: Seizure duration according to time after first seizure in hours. Right: Length of seizures according to frequency of stimulation. Grey boxes are the length of seizures without stimulation; horizontal bars represent the median of seizure duration. Length of the seizures was not modified by short-term stimulation.
The dynamic of the seizure duration was thus comparable in the baseline (Figure 4) and stimulation (Figure 5) protocols.

**Long-term Stimulation**

We compared seizure duration during 1 Hz, 130 Hz, and no stimulation after 3 different injections of penicillin (Figure 6). The Friedman test found a significant difference between these groups \( (P < .05) \), but the posthoc Tukey test was not significant (no stimulation vs 1 Hz, \( P = .16 \); no stimulation vs 130 Hz, \( P = .90 \); 130 vs 1 Hz, \( P = .31 \)). During the 6 hours of recording, there were 119 seizures in the no stimulation group, 76 in the 1-Hz group, and 129 in the 130-Hz group.

**Automated Detection/Stimulation**

In 1 session, we performed online automated seizure detection during 3 consecutive hours. The entropy-based algorithm detected 62 seizures (minimal duration, 3 seconds) with 100% sensitivity and 100% specificity. One of 2 seizures was alternatively treated with 100-Hz cortical stimulation. Similar to continuous and manually triggered short-term stimulation protocols, no seizure was abruptly aborted. Median seizure duration was 19 ± 21.4 seconds without stimulation and 17.5 ± 30.3 seconds with 100-Hz short-term stimulation. The difference was not significant \( (P = .99, \text{Wilcoxon test}) \).

**DISCUSSION**

We aimed to assess whether epidural cortical stimulation could stop or prevent seizures induced in the motor cortex after penicillin G injection in a Macaca fascicularis. We also developed in this monkey model an automated seizure detection algorithm that was coupled with a programmable stimulator. After having established the motor threshold as described elsewhere in nonhuman primates, \(^{27,29}\) we delivered electric stimulation on the epileptogenic zone at an intensity set at 80% of the motor threshold intensity.

In the present study, short- or long-term electric stimulation did not abort seizures and did not reduce their length as assessed electrophysiologically or clinically (the clonic of the arm were not stopped by stimulation). This was unexpected in light of previous studies on neocortical seizures. In vitro studies on neocortical slices treated with bicuculline (GABA antagonist) or Mg\(^{2+}\)-free solutions showed that 100-Hz short-term stimulation of the cortex could stop 47% of the seizure-like events.\(^{6}\) Moreover, the remaining seizures were significantly reduced in a frequency-intensity-duration-dependent manner. Such results on neocortical seizures were confirmed by in vivo studies in the rat with ferric chloride-induced seizures.\(^{8}\) The authors found a significant reduction in seizure length after long-term stimulation at 1 or 100 Hz (37% and 39% reduction of seizure length, respectively). This
was further investigated during presurgical evaluation with subdural grids, with low-frequency (0.9 Hz) and medium-frequency (50 Hz) cortical stimulation resulting in a decrease of 24% and 18%, respectively, in the number of the interictal spikes. Moreover, preliminary results in closed-loop systems in humans were also promising. Automated detection and stimulation (100 Hz, 5 mA, 100-microsecond pulse, 1-second duration) in a local closed-loop system led to a mean reduction of 55.5% in the seizure rate for 4 patients in an open study. These results were further confirmed in another preliminary study, with the RNS (NeuroPace Inc) showing that 7 of 8 patients had > 45% reduction in seizure frequency (mean follow-up time, 9 months). In another study, preliminary results of the first 24 of 65 patients showed 43% of responders (> 50% reduction of seizures) in the complex partial seizures group and 35% for total disabling seizures group.

Several factors might explain the negative result of our study. First, the main limitation of our study is the number (n = 1) of the experimental subjects, and we cannot exclude that cortical stimulation could have been efficient for other monkeys (type II error). Nevertheless, because of the high number of seizures (n = 893) analyzed and the obvious lack of efficacy of cortical stimulation, we thought it was not necessary to reproduce the same protocol on other animals. Given that this model is highly reproducible, it is unlikely that significant new benefits will be obtained using the same stimulation paradigm on the same model in a different animal. However, we thought it was important for the scientific community to be aware of our data.

Second, some authors propose that cortical stimulation may have a more predominant effect on the fibers than on the soma of pyramidal cells. Mathematical modeling of the electric field produced by bipolar stimulation over the motor cortex indicates an activation of the fibers parallel to the cortex (short association fibers). This is the basis for regional neuromodulation in pain therapy, which could be inefficient in focal epilepsy. In our experiment, we chose to place the electrodes in the epidural space to lower the risk of cerebrospinal fluid leakage and infectious complications in that we did not hermetically close the skull and skin, and the monkey was implanted for 1 year. If orientation of the electrodes and type of stimulation (monopolar vs bipolar) change the orientation of the electric field, positioning the electrode above or below the dura only changes the current density delivered to the cortex because the dura and cerebrospinal fluid provide a shunting path for the current. Because we determined the minimal intensity that could induce clinical (electromyogram) motor responses, we think we delivered a charge density to the cortex that is comparable to that in human trials in which the electrodes were located in the subdural space.

Third, the timing of stimulation after the onset of the seizures may be relevant. We chose to stimulate 3 seconds after the onset of the seizures during the visual and automated detection protocols because we did not want to stimulate seizures that would have spontaneously stopped within 3 seconds (false-positive results). Nevertheless, it is true that in human protocols of closed-loop systems, the delay between seizure onset and detection/stimulation was shorter, between 0 and 5 seconds, with a minimum duration of 1.84 seconds in a study by Osorio et al. Moreover, it has been reported that late stimulation generally failed when early stimulation succeeded.

Fourth, electric stimulation may have a mostly excitatory effect on the brain. Thus, it has been used for decades (Penfield, Talairach) to induce seizures and to map the epileptogenic zone during invasive presurgical evaluation of focal epilepsy. It has also been shown recently that 5 seconds of 50-Hz stimulation increased the Teager energy \( E = w^2A^2 \), where \( w \) is frequency and \( A \) is amplitude of the signal) as a result of an increase of neural activity in the beta gamma band.

Fifth, our experimental model could explain the lack of efficacy of the stimulation to treat seizures. Seizures were severe in terms of numbers (> 100 in 8 hours) and of the amplitude of the spikes (600 \( \mu \)V with epidural recording). Penicillin G is a non-competitive inhibitor of the GABAa receptors, resulting in “inhibition of the inhibition” with shunting of the GABA interneurons. Whether these interneurons are activated during cortical stimulation is not known and could explain the failure of the therapy in our experiments. Furthermore, we would like to outline the difficulties in obtaining a reliable model of focal epilepsy. The ideal model should have etiology, pathological changes, and electrophysiological changes similar to humans. We chose to perform the experiment on a nonhuman primate because we think that its motor cortex is much closer to the human brain than the rat’s motor cortex. Beyond genetic similarities, the corticomotoneuronal system is more developed in species with more advanced hand function (which is the case in the Cynomolgus monkey). Yet, in terms of nonhuman primate models of epilepsy, to the best of our knowledge, only the Papio papio baboon has spontaneous generalized photosensitive seizures that make it suitable to study sudden unexplained death in epilepsy. If one wants to study focal seizures, then seizures must be induced by a perturbation of the balance between excitation and inhibition. This can be achieved with the application of inhibitory amino acid blockers (penicillin, bicucullin, picrotoxin, strychnine) or with topical application of excitatory agents (acetylecholine agonists like pilocarpine or glutamate agonists like kainite or N-methyl-D-aspartic acid). One can also implant metals (aluminum, cobalt, or zinc) in the cortex. Thus, the alumine model has been used with success in the rhesus monkey, but severe drawbacks have been mentioned. Alumina cream creates cortical lesions (up to 9 mm), and the length of the latency period before electroclinical onset of seizures makes it particularly difficult to use. Another possible agent to induce motor seizure is the tetanus toxin, which blocks synaptobrevin and induces focal seizures lasting for months a couple of days after its injection in the motor cortex. Characteristics of tetanus toxin induced motor seizures are under experimentation in our laboratory.

Sixth, neocortex stimulation might not be the optimal target of therapy in our experimental model. Late synchronization of the internal globus pallidum, preceding the end of the seizures, suggests a role for the basal ganglia in the modulation of the
motor seizures induced by penicillin.49 Therefore, deep brain stimulation of the basal ganglia may be more efficient than direct cortical stimulation in this model (under investigation), with modulation of the excitability of the motor cortex still needing to be demonstrated. Modulation of the epilepticogenic network may also be the mechanism explaining the success of stimulation of the hippocampus for mesial temporal lobe epilepsy. Stimulation of the hippocampus showed significantly better results in patients with normal magnetic resonance images than when there was hippocampal sclerosis on the magnetic resonance image.30 The authors hypothesize that deep brain stimulation could act on the local network and may depend on the amount of residual GABA neurons. This would be reinforced by the anatomic reciprocal loops in the temporal lobe structures between the subiculum, cornu ammonis, and entorhinal cortex. The difference in cortical architecture between allocortical mesial structures and the isocortex of the motor region may also be a cause of the absence of therapeutic efficacy of electric stimulation in our model.

CONCLUSION

Short-term and long-term epidural cortical stimulation could not abort or prevent seizures in our experimental model. Whether this failure can be explained by the blockade of GABA receptor induced by the penicillin injection or by the inefficacy of cortical stimulation in epilepsy may be resolved by future study using a chronic model of focal motor seizure obtained without local GABA inhibition.

Disclosure

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BLAUWBLOMME ET AL


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COMMENT

This article by Blauwblomme et al addresses an important area of active research involving the use of direct cortical stimulation of the epileptogenic zone for the treatment of focal seizures. Several phase 1 and 2 clinical trials have been reported, and the results of a phase 3 clinical trial using responsive stimulation should be reported very soon. Despite the findings of these trials, effective use of this technique will depend on an improved understanding of the cellular mechanisms involved, and this study contributes to the growing knowledge in this area. The findings in this study are negative because stimulation of the cortical focus was ineffective in preventing or aborting seizures produced by the local injection of penicillin G. An important part of this article is the authors’ discussion concerning why cortical stimulation was not effective in this model. The most likely explanation consistent with previously reports relates to the timing of the stimulation. The literature regarding the preclinical and clinical trials consistently supports that for stimulation to be effective in aborting seizures, it must be applied as early as possible. The severity of the seizures may be too great and the delay before stimulation was applied (minimum of 3 seconds of clinical seizure activity in this model) may be too long for the stimulation to be effective.

In summary, this is a well-performed study with negative results that positively contributes to the literature concerning the use of direct stimulation of the epileptogenic zone for the treatment of focal seizures.

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