

Electrical Cortical Stimulations Modulate Spike and Post-Spike Slow-Related High-Frequency Activities in Human Epileptic Foci

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Abstract

Objective: Using interictal epileptiform discharges (IEDs), consisting of spikes and post-spike slow waves (PSSs), and IED-related high-frequency activities (HFAs), we elucidated inhibitory effects of electrical cortical stimulation (ECS) on human epileptic foci.

Methods: We recruited 8 patients with intractable focal epilepsy, and 50-Hz ECS was applied to the seizure-onset zone (SOZ) and non-SOZ. Before (5-min) and after (20-min) ECS, we evaluated the number of IED, the amplitudes of spikes and PSSs, spike-related HFA power, and PSS-related low gamma (30-50 Hz) activities.

Results: SOZ stimulation significantly decreased the number of IEDs and amplitude of spikes.

Spike-related HFA power values in fast ripple (200-300 Hz) and ripple (80-150 Hz) bands were significantly suppressed only by SOZ stimulation in 4 and 3 patients, respectively.

Among 4 patients with discrete PSSs, the amplitude ratio of spike/PSS decreased and the PSS-related low gamma activity power increased significantly in 2 patients and marginally in 1 patient.

Conclusions: ECS potentially modulates cortical excitability by reducing excitation and increasing inhibition, and monitoring IED-related HFAs may help achieve the optimal effects of ECS.

Significance: IED and IED-related HFAs are dynamic, potential surrogate markers for epileptic excitability during the interictal period.

Highlights

1. The number and amplitude of spikes decreased after 50-Hz stimulation of the seizure onset zone (SOZ).
2. Spike-related high-frequency activities (HFAs) also decreased and post-spike slow wave-related HFAs increased.
3. 50-Hz stimulation of the SOZ modulated cortical excitability toward less excitation and more inhibition.

Keywords: Electrical Cortical Stimulation, Neuromodulation, Intractable Focal Epilepsy, Interictal Epileptiform Discharges, High Frequency Oscillation/Activity.

Abbreviations: IED = interictal epileptic discharge; PSS = post-spike slow wave; HFA = high-frequency activity; ECS = electrical cortical stimulation; SOZ = seizure-onset zone.

1. Introduction

Epilepsy, a neurological disorder, is caused by abnormal electrical discharge due to excessive neuronal activity. Interictal epileptiform discharges (IEDs) are used to diagnose epilepsy and identify the localization of the seizure onset zone (SOZ) (de Curtis and Avanzini, 2001). Each IED usually consists of spikes (excitation) and post-spike slow waves (PSSs) (inhibition) (Blumenfeld, 2005, Serafini and Loeb, 2015). Wide-band electrocorticogram (ECoG) has enabled us to record high-frequency oscillations or activities (HFOs/HFAs). Epileptic HFOs, usually divided into ripple (R: 80-200 Hz) and fast ripple (FR: above 200 or 250 Hz) according to their frequencies, have been recently suggested as possible surrogate markers for epileptogenicity (Crepon et al., 2010, Jefferys et al., 2012). Interictal HFOs are more reliable markers for the SOZ compared with epileptic spikes (Jacobs et al., 2012, Jirsch et al., 2006) and are significantly associated with favorable seizure outcomes (Cho et al., 2014, Jacobs et al., 2010, Kerber et al., 2014). For instance, one study demonstrated significant spatial and temporal associations between IEDs and HFOs (Engel et al., 2009), and another study showed that IEDs with abnormal HFOs, particularly fast ripples (FRs, 250–500 Hz), had greater sensitivity in identifying the epileptogenic region than those without concomitant abnormal HFOs (Jacobs et al., 2008). Thus, HFOs have been extensively examined due to their specific significance for the SOZ. One study demonstrated that both visible HFOs and those with sharp spikes (HFAs) are strongly associated with the epileptogenic zone and are good surrogate markers for favorable surgical outcomes (Burnos et al., 2016). Notably, our

previous study found that the HFA power of early cortico-cortical evoked potentials (CCEP) is significantly higher in the SOZ than in the non-SOZ (Kobayashi et al., 2017). These findings suggest that HFAs related to the sharp components reflect the epileptogenicity-related hyperexcitability.

Brain stimulation techniques, such as transcranial magnetic stimulation (TMS) (Kinoshita et al., 2005a, Tergau et al., 1999), transcranial direct/alternating current stimulation (tDCS/tACS) (Herrmann et al., 2013, Reato et al., 2013), and responsive neurostimulation system (RNS) (Ben-Menachem and Krauss, 2014, Morrell, 2011), have recently been employed to treat various neurological and psychiatric disorders. In addition, electrical cortical stimulation (ECS) is considered an adjunctive therapy for patients with contraindications for resection surgery. Accumulating studies demonstrated that ECS produces inhibitory effects, especially on the acquisition of epileptogenicity due to kindling in rodents (Lopez-Meraz et al., 2004), and that high frequency (50 Hz) ECS suppresses IEDs and beta band activities (10–32 Hz) (Kinoshita et al., 2005b), and low-frequency ECS inhibits IEDs and seizure frequency in humans (Koubeissi et al., 2013, Lim et al., 2016). However, the mechanisms by which ECS controls cortical excitability remain unclear.

We hypothesized that ECS inhibits interictal epileptic activities, as does the RNS, which can help decrease the frequency of debilitating ictal seizures (Bergey et al., 2015). Using IEDs, which are known as pathological expressions of the synchronization of neuronal assemblies, and spike-related HFAs as dynamic measures of abnormal cortical excitability,

we investigated the effect of ECS on the epileptic focus in patients with intractable epilepsy.

2. Methods

2.1. Patients

A total of 9 consecutive patients with intractable focal epilepsy who had undergone invasive presurgical evaluation were recruited in this study. One patient was excluded owing to excessive motion artifacts on ECoG. Four (Patients 1–4) of the included 8 patients were partly described in a previous paper (Kinoshita et al., 2005b). Among the 8 patients, 5 exhibited neocortical epilepsy (NE), and 3 had mesial temporal lobe epilepsy (MTLE). Detailed patient information is summarized in Table 1.

The study was approved by the Ethical Committee of Kyoto University Graduate School of Medicine (C235/580), and written informed consent was obtained from all patients before the examination. All the work described was carried out in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

2.2. ECoG recording

ECoG was recorded with EEG 1000, 1100, or 1200 (AC amplifier with input impedance of 200 M Ω , which is capable of long-term video-EEG monitoring; Nihon Kohden, Tokyo, Japan), with a sampling rate of 1,000 Hz and bandpass filter of 0.08–300 Hz in Patients 1–4, and a sampling rate of 2,000 Hz and bandpass filter of 1.6–600 Hz in Patients 5–8, 1–2 weeks after the implantation surgery while the patients were taking the ordinary dose of anti-

epileptic drugs. The subdural electrodes, which were disks (3 mm in diameter) with a 2.3-mm (diameter) recording surface and were made of platinum (Ad-Tech, Racine, WI, U.S.A), were placed at a center-to-center distance of 1 cm. The reference electrodes, contralateral to the subdural electrodes, were placed on the skin over the mastoid process. Throughout the study, patients were instructed to lie on a bed and remain awake to maintain consistent awareness. We recorded ECoG for 5 min before and 20 min after the ECS. The SOZ was identified using the earliest ictal ECoG changes in each patient. The electrodes that were most distant from the SOZ within the same lobe were defined as the electrodes of the non-SOZ. In Patient 3, the right frontal lobe in which the SOZ was located was too irritative due to frequent IEDs; hence, as an exception, we defined the most distant implanted electrodes as non-SOZ. The electrode configuration for each patient is shown in Fig. 1.

2.3. Electrical cortical stimulation

During functional mapping for clinical purposes, we performed high frequency (50 Hz) ECS using the implanted electrodes. We used the same conditions (50 Hz, bipolar, alternating square pulse of 0.3-ms duration, 1–15 mA, 1–5 s) to stimulate the SOZ and non-SOZ (Fig. 2). The 50-Hz stimulation was first applied at 1 mA for 1 s, and we then increased the stimulus intensity and duration until after-discharges emerged, clinical symptoms occurred, or the maximum intensity of 15 mA was applied for 5 s. We recorded maximum charge density, stimulus duration, and total delivery coulomb in SOZ and non-SOZ stimulation conditions independently (Table 2). The total stimulus duration and delivery coulomb were maintained

within safety limits (Gordon et al., 1990). SOZ stimulation in Patient 4 and non-SOZ stimulation in Patient 5 could not be conducted due to shortage of time for clinical examination. Thus, we obtained 7 sets of data for SOZ, as well as for non-SOZ stimulation.

2.4. IED analysis

We semi-automatically detected IEDs at one core electrode of the SOZ after applying a time constant of 0.03 s (1st order IIR filter) to the ECoG using a custom-made script (written by M.M.) (The MathWorks, Natick, MA, U.S.A). IEDs with larger spike amplitudes than the independently settled threshold in each patient (150–250 μV) were counted during each of the 5-min periods before (1 period: Pre [pre-stimulus 5 min]) and after stimulation (4 periods: Post 1 [post-stimulus 0–5 min], Post 2 [post-stimulus 5–10 min], Post 3 [post-stimulus 10–15 min], and Post 4 [post-stimulus 15–20 min]). We then visually confirmed the actual IEDs in each 5-minute period by removing spurious activity due to artifacts. The number of IEDs was compared between the 1 Pre-stimulus and 4 Post-stimulus periods.

We also calculated the amplitudes of spikes and PSSs for each IED from the trough to the peak, as shown in Fig. 3A. We measured the amplitudes of PSSs only in 4 patients who presented well-defined PSSs following the spikes. We compared the average amplitudes of spikes and PSSs before and after ECS among all patients in both the SOZ and non-SOZ.

2.5. Analysis of spike- and PSS-related HFAs

We analyzed the power spectrum of spike-related HFAs, which was defined as a square of instantaneous amplitude using Hilbert transformation, for FR (200–300 Hz), R (80–150 Hz),

low gamma (30–50 Hz), and beta (15–30 Hz) bands and calculated the maximum power values during spike components (Fig. 3B) in reference to the baseline activities (the mean power from 200 to 100 ms before spike peak). We used IIR (Butterworth) filter, as well as zero phase shift by applying 1st order filter bidirectionally, to obtain each range of activities. The time-frequency analyses for the IEDs were performed using the short-time Fourier transform, as previously reported (Kobayashi et al., 2017), and showed a dramatic decrease in the low gamma band power during PSS. Therefore, we also assessed the power of PSS-related low gamma activities in the ascending phase of PSS after the spike component and calculated the power of low gamma activities during 100 ms of the PSS ascending phase from its onset (Fig. 3C). However, in Patient 7, because the PSS ended within 100 ms, this power was calculated during 50 ms of the PSS ascending phase from its onset.

In order to exclude the effects of background power changes induced by ECS (Kinoshita et al., 2005b), the power of background activities for each band was calculated after excluding the 1,000-ms epoch from 300 ms before to 700 ms after the spike peak (Fig. 3D). We meticulously adjusted the ranges of spike- and PSS-related HFA power by considering the power change in background activities. After these adjustments, we compared the power of spike- and PSS-related HFAs in each patient.

2.6. Statistical analysis

We evaluated the following factors for the SOZ and non-SOZ: 1) The number of IEDs, 2) The spike component amplitude, 3) The spike/PSS amplitude ratio, 4) The power spectrum of

spike-related HFAs for each frequency band (FR, R, low gamma, and beta), and 5) The power spectrum of PSS-related low gamma activities. We first compared these 5 factors between the periods before (Pre) and immediately after the ECS (Post 1) and then investigated the post-stimulus effects 20 min after the stimulation (Post 2–4). We used the Wilcoxon signed-rank test for non-parametric comparisons. All statistical values were corrected based on the false discovery rate (FDR, $p < 0.05$) for multiple comparisons. Significance was set at $p < 0.05$ for all analyses.

3. Results

Table 3 summarizes the results for the five evaluation factors. The result for each factor is as follows.

3.1 IED number

The time-dependent average IEDs in a representative patient (Patient 1) are shown in Fig. 4A and B, and the number of IEDs in the SOZ (after SOZ and non-SOZ stimulation) in each period is shown in Fig. 4C and D. In all patients, the number of IEDs was significantly smaller after (during Post 1) than before SOZ stimulation (during Pre). We also observed a significant decrease in the number of IEDs throughout Post 2–4. On SOZ stimulation, the most prominent inhibitory effect on the number of IEDs was observed in Post 1. In contrast, no significant change in the number of IEDs was found after stimulating the non-SOZ.

3.2 Spike and PSS amplitudes

On stimulating the SOZ, spike amplitudes significantly decreased in all patients, as did the number of IEDs (Fig. 4E). The suppressive effect of the SOZ stimulation seemed to last throughout the 20-minute period after stimulation. In contrast, no significant change in spike amplitudes was found on stimulating the non-SOZ (Fig. 4F).

In contrast, the PSS amplitude after SOZ stimulation was the highest in Post 1 and gradually reduced in Patient 1 (Fig. 5A and B). Thus, the morphology of IEDs changed after SOZ stimulation: Spike components (excitatory components in IED) decreased, and PSS (inhibitory components in IED) increased, mainly in Post 1. Spike and PSS amplitudes gradually returned to the pre-stimulus period values. Based on these amplitude changes in both spikes and PSSs, we compared the amplitude ratio of spike/PSS in the 4 patients who showed overt PSSs. We found a significant decrease in the amplitude ratio in 2 patients. A similar tendency was observed in another patient (Fig. 5C), although the changes were not statistically significant. However, this decrease was not observed after non-SOZ stimulation (Fig. 5D). There was a significant interaction between the spike/PSS amplitude ratio and the stimulus site (SOZ vs. non-SOZ; Fig. 5E).

3.3. Spike-related HFA power changes

Representative spike-related HFAs of the FR band after SOZ stimulation (Patient 1) are shown in Fig. 6A and B. Compared with those in the pre-stimulus period, the spike-related HFAs in Post 1 decreased greatly and then gradually increased to reach the pre-stimulation values. Spike-related HFAs of the FR band were significantly lower in Post 1 than in the pre-

stimulus period (Fig. 6C). Similarly, the power of spike-related HFAs of the R band significantly decreased after SOZ stimulation (Fig. 6D). Significant decreases were also observed in spike-related low gamma and beta bands throughout the 20-min period after stimulation (Fig. 6E and F). The power changes in each band (FR, R, low gamma, and beta) were substantially higher than those of background activities in each period after SOZ stimulation.

The degrees of spike-related power changes in each frequency band differed among patients. Individual analysis of the patients showed significant decreases in the FR and R bands in 4 and 3 patients, respectively, after SOZ stimulation. In contrast, non-SOZ stimulation did not result in any consistent effects on spike-related HFA power. These results are shown in Table 4.

3.4. PSS-related low gamma activity power changes

Representative results (Patient 1) of PSS-related low gamma activities after SOZ stimulation are shown in Fig. 3C. We observed a decrease in low gamma activity power, i.e., post-IED depression, from the onset of the PSS to 100 ms after the onset (corresponding to the PSS ascending phase) (Fig. 3C). After SOZ stimulation, the power of PSS-related low gamma activities increased in Post 1 and gradually returned to pre-stimulation values (Fig. 7A). A significant increase was noted in 2 out of 4 patients with discrete PSSs. In 1 patient, the significant difference was observed in the individual analysis but disappeared after the FDR correction ($p = 0.0508$), probably owing to the small number of IEDs during Post 1 (Fig. 7B).

These changes were detected in the same patients who showed decreased spike/PSS ratios.

None of these changes were found after non-SOZ stimulation.

4. Discussion

We examined the suppressive effects of 50-Hz ECS on IEDs. We found the following: 1) frequencies of IEDs and amplitudes of their spike components significantly decreased after SOZ stimulation; 2) the spike-related HFA power in FR and R bands significantly decreased in 4 and 3 patients, respectively; 3) the spike/PSS amplitude ratio decreased significantly in 2 and marginally in 1 out of 4 patients with clear PSSs; and 4) PSS-related low gamma activities increased in 3 patients. To the best of our knowledge, this study is the first to demonstrate IED morphologic changes and spike- and PSS-related HFA power changes induced by clinical ECS.

4.1. Inhibitory effects of ECS on IEDs

Stimulation methods, such as TMS (Akamatsu et al., 2001, Fregni et al., 2006a), DBS (Hamani et al., 2008), tDCS (Fregni et al., 2006b), tACS (Reato et al., 2013), and VNS (Schachter and Saper, 1998) show suppressive effects on cortical excitability by reducing the IED rate or seizure occurrence. Our previous studies demonstrated the suppressive effects of both 50-Hz (Kinoshita et al., 2005b) and 0.9-Hz (Yamamoto et al., 2002) SOZ ECS on IEDs. Several case reports showed that ECS suppressed cortical excitability in patients with epilepsy, as indicated by reductions in the seizure frequency, IED rate, or severity of epilepsy

(Lundstrom et al., 2016, Velasco et al., 2007). Studies with experimental animal models found that high- (Yu et al., 2016) and low-frequency (Velisek et al., 2002) ECS could inhibit the development of after-discharges. Because the morphology of IEDs has not been well documented, this present study investigated the suppressive effects of ECS specifically on SOZ in terms of each component of IEDs and IED-related HFAs. Our results on the suppressive effects of ECS on IEDs are consistent with those in those previous studies mentioned above. However, the effects of ECS on HFOs/HFAs remain unclear. We found that spike-related HFA power values in FR (200-300 Hz) and R (80-150 Hz) bands were significantly suppressed only by SOZ stimulation. However, Jacobs et al. (Jacobs et al., 2014) showed that 50-Hz ECS reduced the FR (200–500 Hz) and R (80–200 Hz) rates not only in the SOZ but also in the neighboring regions, as well as outside the SOZ. Therefore, further studies are needed to clarify the effects of ECS on HFOs/HFAs.

Several mechanisms can explain the inhibitory effects of ECS on IEDs. One possible mechanism is changed synchrony. Synchronization and desynchronization are most relevant to these morphological IED changes since an increased synchronization in synaptic membrane potentials of cortical neurons is necessary for the generation of spike components (Annegers, 1998). Another possibility involves changes in both GABAergic and glutamatergic synaptic conductances (Hiller et al., 2007). Since IEDs are blocked by glutamate or GABA synaptic transmission antagonists (Cohen et al., 2002, Huberfeld et al., 2007), both circuits play pivotal roles in neuronal synchronization and generation of

population spikes. Other conceivable reasons for IED changes include the mutual effects of potassium currents and synaptic disfacilitation (Steriade and Amzica, 1999), intra/extracellular pH changes (de Curtis et al., 1998), chloride changes (Khazipov, 2016), and decoupling of gap junctions (Spray et al., 1981).

4.2. Changes in spike-related HFAs

Although our sample was small, we observed significant decreases in the spike-related HFA power in FR and R bands after applying 50-Hz ECS to the SOZ (Fig. 6C and D). Because R band superimposition in IEDs, as well as neocortical FR, is a specific marker for SOZ, (Wang et al., 2013), our findings might support the suppressive effect of ECS on SOZ excitability.

Additionally, low gamma and beta power overriding IEDs also significantly decreased. Since spikes included bands in the low gamma to beta range, the contribution ratio between spike amplitudes and low gamma or beta bands showed a strong correlation, whereas that between spike amplitudes and FR or R bands had a minor correlation (see Supplementary Material), suggesting that decrements in FR and R activities reflect a direct ECS influence on HFA.

None of these factors changed after stimulating the non-SOZ, except for Patient 8. We address the possible explanations in the limitations section.

This study did not focus on the HFOs, which represent oscillatory activities, but on the HFAs, which reflect power changes in high-frequency bands. We found that the maximum spike-related HFA power occurred in the trough or the ascending phase of the spike component. The findings are consistent with those in previous studies examining the

relationship between IEDs and HFOs (Li et al., 2018, van Klink et al., 2016). Our findings suggest that spike-related HFAs are possible surrogate markers for cortical excitability and less likely to be merely caused by filtering.

The observed decrease in the spike-related HFA power can be partly explained by mechanisms similar to those underlying IED changes. Recently, mathematical simulation studies have shown that IEDs and FRs partially share common mechanisms, such as GABA reversal potential and altered synaptic potential (Demont-Guignard et al., 2012), and the factors separating HFOs from spikes might be differences in pyramidal cell synchronization and the jitter of feed-forward activation from cortical interneuron connections onto pyramidal cells. In addition, unlike IEDs, decoupling gap junctions may play a key role in inhibiting the spike-related HFAs because HFOs are sustained even in the presence of glutamatergic and GABAergic receptor antagonists but cease after the application of drugs known to decouple gap junctions, such as carbenoxolone (Roopun et al., 2010).

4.3. Changes in PSS components

A small study population (4 patients with discrete PSSs) was included in the PSS-component analyses of IEDs. We found a significant decrease in the amplitude ratio of spike/PSS components in 2 patients and a marginally significant decrease in 1 patient. The spike component is thought to be generated by the synchronous firing of pyramidal neurons, i.e., paroxysmal depolarization, and this abnormal paroxysmal depolarization is followed by temporary neuronal hyperpolarization during PSS. An increased epileptiform discharge

threshold after the spike component has been demonstrated by stimulation studies in experimental animal models (de Curtis and Avanzini, 2001) and in epilepsy patients (de Curtis et al., 2005). The decrement in neuronal excitability during PSSs is presumably caused by the recurrent inhibitory networks activated by adjacent neuronal firing (Bragin et al., 2007, Csercsa et al., 2010). These findings confirm the original concept: spike is an excitatory component of IED, whereas PSS is an inhibitory component of IED. Our present finding (PSS amplitudes, but not spike components, increase after SOZ stimulation) provides direct evidence for the activation of inhibitory components in the brain.

4.4. Changes in PSS-related low gamma activities

Notably, we found that the amplitude ratio of spike/PSS decreased and the PSS-related low gamma activity power increased significantly in 2 patients and marginally in 1 patient. This finding seems paradoxical because PSSs reflect post-spike power depression. We assume that the increase in the power of low gamma bands after ECS is induced by the synchronized local neuronal firing of inhibitory interneurons, thereby resulting in increased PSS amplitudes, since GABA-mediated inhibition is preserved in the SOZ to maintain interictal homeostasis (Engel, 1995, Prince and Jacobs, 1998). Similar findings were demonstrated in clinical studies by Lega et al. (Lega et al., 2015) using single-pulse ECS; they investigated the post-stimulus power change 100–800 ms after 1-Hz ECS and found that low (40–70 Hz) but not high gamma activities were evoked more significantly frequently in the early seizure propagation network than in the late seizure propagation network in the SOZ. They hypothesized that this

finding was caused by a relative decrease in local inhibitory input in the SOZ. The difference in stimulus frequencies (50 Hz versus 1 Hz) might explain the discrepancy between our results and theirs. In addition, the activation of GABA-mediated inhibition by high-frequency ECS was observed in the caudate nucleus (Li et al., 2004), the nucleus accumbens (Xie et al., 2014) of rats, the primary visual cortex of monkeys (Logothetis et al., 2010), and human cortical tissue specimens (Argiti et al., 2016). Consistently, the importance of low gamma activities during the PSS was demonstrated in the human epileptic focus (Urrestarazu et al., 2006), and a significant increase in sub to low gamma activities (0–40 Hz) was found during the PSS at and around the foci, especially in MTLE patients.

Moreover, the findings in recent animal studies focusing on interneuron activities support our hypothesis about the low gamma band change after ECS. Interneurons are believed to play a key role in GABA-mediated inhibition and are activated during interictal discharges (Cohen et al., 2002, Domann et al., 1991). Studies have shown that low gamma activities (20–80 Hz) specifically reflect interneuron activity, especially of parvalbumin (PV) interneurons (Cardin et al., 2009, Chen et al., 2017, Cobb et al., 1995). Additionally, both inhibiting and driving PV neurons have been specifically demonstrated to modulate gamma frequency rhythmicity in vivo using optogenetics (Sohal et al., 2009). Furthermore, in a rat model, ictal seizures could be terminated optogenetically not only by inhibiting excitatory principal neurons but also by activating PV neurons (Krook-Magnuson et al., 2013).

Taken together, we propose that the low gamma increase is caused by the activation

of the recurrent inhibitory network, as demonstrated previously (Ayala et al., 1973, Logothetis et al., 2010). Further studies are needed to bridge the gap between basic and clinical studies and to confirm whether the augmentation of the PSS component with increased low gamma activities reflects ECS-induced GABA-mediated inhibition.

4.5. Limitations

This study has some limitations. First, we could not observe whether the inhibitory effect continued 20 min after the stimulation. Although it is challenging to explain the cause of long-lasting modifications, the effects might arise from the long-term depression of synaptic plasticity, as previously demonstrated in stimulation experiments (Hess and Donoghue, 1996, Kirkwood et al., 1993). Second, as the sample size was small, we could not show pathology-dependent differences in stimulation effects. Third, in Patient 8, the decrease in the spike-related HFA power was observed after stimulating the non-SOZ, but not the SOZ. One possible explanation is that the stimulated areas in the non-SOZ were still irritative despite being far away from the SOZ in the same temporal lobe. Another possibility is that the electrical charge delivered to the SOZ (0.15 mC) was lower than that applied to the non-SOZ (7 mC) in this particular patient. Fourth, in this study, we divided IEDs into two parts (spike and PSS) to discuss the mechanism underlying the inhibitory effects of ECS. However, it has been argued that IEDs are not simple paroxysms of hypersynchronous excitatory activity (Matsumoto, 1964), but rather representatives of the interplay between multiple distinct neuronal types within neuronal networks (Le Van Quyen et al., 2008). Although we found

that ECS could decrease the excitatory component (spike) and increase the inhibitory component (PSS) in IEDs, caution is required when ascribing the IED modulation simply to the change of the excitation/inhibition balance. Additional analyses other than power spectrum analysis are needed to clarify the modulation effects on the synchronization of neuronal assembly during IEDs (Avoli and de Curtis, 2011). Moreover, we cannot entirely rule out the possibility that the IED modulation is derived from the interaction with the subcortical structures, such as the thalamus, by antidromic or orthodromic activation. Further investigations using animal models should focus on individual pyramidal and inhibitory neurons to provide additional evidence to endorse our extrapolation of the mechanism underlying IED morphological changes.

4.6. Future perspectives

Our findings shed light on the clinical applications of ECS as a neuromodulation therapy for epilepsy. However, in some conditions, especially during stereo-electroencephalography use, ECS has been applied to induce epileptic seizures (Bartolomei et al., 2017, Chauvel and McGonigal, 2014). Because increased or excessive interneuron activity and synchronous GABA-mediated inhibitory postsynaptic potentials have been shown to be paradoxically associated with seizure generation (Neumann et al., 2017, Uva et al., 2015), the degree of GABA-mediated modulation may help explain the mechanisms underlying reciprocal effects of ECS. Thus, IED-related HFAs are presumably excellent markers for well-controlled suppressive effects.

A recent report showed that IEDs are associated with favorable surgical outcomes (Cuello-Oderiz et al., 2018). Hence, we speculate that the factors examined in this study (IED occurrence, spike/PSS amplitude ratio, spike-related HFA power, and PSS-related low gamma activity power) can be used to assess the effect of the treatment. The reduction in IED activity may contribute to the preservation of higher brain functions (Glennon et al., 2016, Ung et al., 2017) at and around the focus. RNS, which has already been used to terminate seizures, is considered a safe and effective adjunctive treatment for intractable focal-onset epilepsy. The adjustment of stimulation parameters and monitoring of the effects on IEDs are required to further optimize the utility of “interictal” neuromodulation therapy. Our findings provide additional evidence regarding the suppressive effect of ECS and can help develop practical treatment strategies to modulate the excitability of the epileptic focus, even during interictal periods.

5. Conclusions

This study demonstrated the inhibitory effects of the 50-Hz stimulation at the SOZ. This stimulation reduced the number of IEDs, the amplitude of spike, and the power of spike-related HFAs and augmented the amplitude of PSS and PSS-related low gamma power. This study suggests that ECS potentially modulates cortical excitability, thereby reducing excitation and increasing inhibition, and the findings could help identify the optimal parameters for stimulation therapy in patients with epilepsy.

Disclosure of Conflicts of Interest

None of the authors have any conflicts of interest or potential financial interests to disclose.

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Figure Legends

Fig. 1. Electrode configurations during electrocorticograms (ECoGs) for each patient.

Each figure shows the location of implanted and stimulation electrodes. Circles with solid and dotted lines indicate the stimulation electrodes of the SOZ and non-SOZ, respectively. SOZ was defined by other examination findings, such as ictal or interictal ECoG results. Electrodes most distant from the SOZ within the same lobe were classified as non-SOZ, except for Patient 3 who exhibited very frequent interictal epileptiform discharges in the frontal lobe. FLE: frontal lobe epilepsy, MTLE: Mesial temporal lobe epilepsy, TLE: temporal lobe epilepsy, CS: central sulcus; SOZ: seizure onset zone.

Fig. 2. Stimulation protocol.

During cortical stimulation mapping, we used burst electrical stimulation to the SOZ and non-SOZ. Each stimulation consisted of 50-Hz, bipolar, alternating square pulses (0.3 ms duration, 1–15 mA) within 5 s with a total charge of 0.3–23.0 mC. The 50-Hz stimulation was applied at 1 mA for 1 s to 15 mA (maximum intensity) for 5 s. For each stimulus condition, we divided ECoG data before and after stimulation into 5-minute blocks and then compared the values among these periods from 5 min before stimulation to 20 min after stimulation.

SOZ: seizure onset zone; ECoG: electrocorticogram.

Fig. 3. Evaluation methods of interictal epileptiform discharges (IEDs) and spike-related high-frequency activities (HFAs).

A. Representative average IED (Patient 1) obtained in each 5-min block within a time-window of 0.3 s before to 0.7 s after the spike peak. After detection of the spikes, we calculated the amplitude of the spike and post-spike slow wave (PSS) from peak to trough for each IED in each patient.

B. Representative average spike-related HFA power (Patient 1). After Hilbert transformation, for the power of spike-related HFAs, we calculated the power of the square absolute value of each range activity by considering the maximum power and mean power from 200 to 100 ms before the spike peak as the baseline.

C. IED power spectrum obtained using short-time Fourier transform. The power suppression of the low gamma band was observed during the PSS compared with the baseline from 200 to 100 ms before the spike peak. The power of PSS-related low gamma activities was calculated with a focus on those in the ascending phase of PSS during 100 ms after the PSS trough (red dot line).

D. Representative evaluation methods of background activity (Patient 1). To remove the effects of background power changes induced by electrical cortical stimulation (ECS), we excluded the 1,000-ms epoch from 300 ms before to 700 ms after the spike peak (red dot line) to calculate the power of background activities for each band.

Fig. 4. Changes in the spike component of IED, the IED number, and the spike amplitude after stimulating the SOZ and non-SOZ.

A. Representative average IEDs (Patient 1) after SOZ stimulation. Average IED in each period is shown by a different color line (black for Pre-stimulus, red for Post 1, green for Post 2, dark blue for Post 3, and light blue for Post 4 periods) according to the time-series.

B. Enlargement of the spike component indicated by a black dot in (A)

C, D. Changes in the IED number after stimulating the SOZ (C) and non-SOZ (D). Each patient is shown in a different color. Among all patients, the IED number significantly decreased, especially in Post 1 (Wilcoxon signed-rank test, FDR-corrected, * $p < 0.05$). No significant effect on the IED number was observed in patients after stimulating the non-SOZ.

E, F. IED amplitude changes after stimulating the SOZ (E) and non-SOZ (F). Patients are indicated by the same colors as in (C) and (D). The spike amplitude significantly decreased throughout the 20 min after SOZ stimulation in all patients (Wilcoxon signed-rank test, FDR-corrected, * $p < 0.05$). No significant change was found after stimulating the non-SOZ.

IED: interictal epileptiform discharge; SOZ: seizure onset zone; FDR: false discovery rate.

Fig. 5. Changes in the PSS component of IED and the amplitude ratio of spike/PSS after stimulating the SOZ and non-SOZ.

A, B. Panel (A) is the same as Fig. 4A. (B) shows the enlargement of the PSS component of IED as indicated by a black dot in (A).

C, D. Changes in the amplitude ratio of spike/PSS after stimulating SOZ (C) and non-SOZ (D). Patients are indicated by different colors. Among the 4 patients with overt PSSs, the amplitude ratio significantly decreased in 2 patients (Wilcoxon signed-rank test, FDR-corrected, $** p < 0.03$). No significant effect was observed after non-SOZ stimulation.

E. Interaction between periods of SOZ and non-SOZ stimulation. Average spike/PSS amplitude ratios among 4 patients after stimulating SOZ (red line) and non-SOZ (blue line) are shown. Different stimulus sites significantly affected the changes in the amplitude ratio among patients with overt PSSs (two-way ANOVA test, $* p < 0.05$).

IED: interictal epileptiform discharge; SOZ: seizure onset zone; FDR: false discovery rate; PSS: post-spike slow wave.

Fig. 6. Changes in spike-related HFAs after stimulating SOZ.

Spike-related HFA power values were obtained by Hilbert transformation of raw ECoG in each frequency range of HFAs [fast ripple (FR, 200–300 Hz), ripple (R, 80–150 Hz), low gamma (30–50 Hz), and beta (15–30 Hz)].

A. Representative spike-related HFAs in the FR band after SOZ stimulation (Patient 1)

Line colors indicate different periods, as shown in Figure 4. The power of spike-related FR

band HFAs decreased mostly in Post 1 and gradually increased toward values observed before stimulation.

B. Enlargement of the absolute power of spike-related HFAs in the FR band as indicated by a black dot in (A)

C, D, E, and F. Changes in spike-related HFAs in each frequency range (C, FR; D, R; E, low gamma; F, beta). Power values in the FR band were significantly lower in Post 1, 2, and 4 than in the Pre-stimulus period (* $p < 0.05$, Wilcoxon signed-rank test, FDR-corrected).

Similarly, the R band power significantly decreased after stimulation (Post 1: ** $p < 0.01$, Post 2: ** $p < 0.01$, Post 3: * $p < 0.05$, Post 4: ** $p < 0.01$). Significant decreases in spike-related activities in low gamma and beta bands were also observed throughout the 20 min after stimulation.

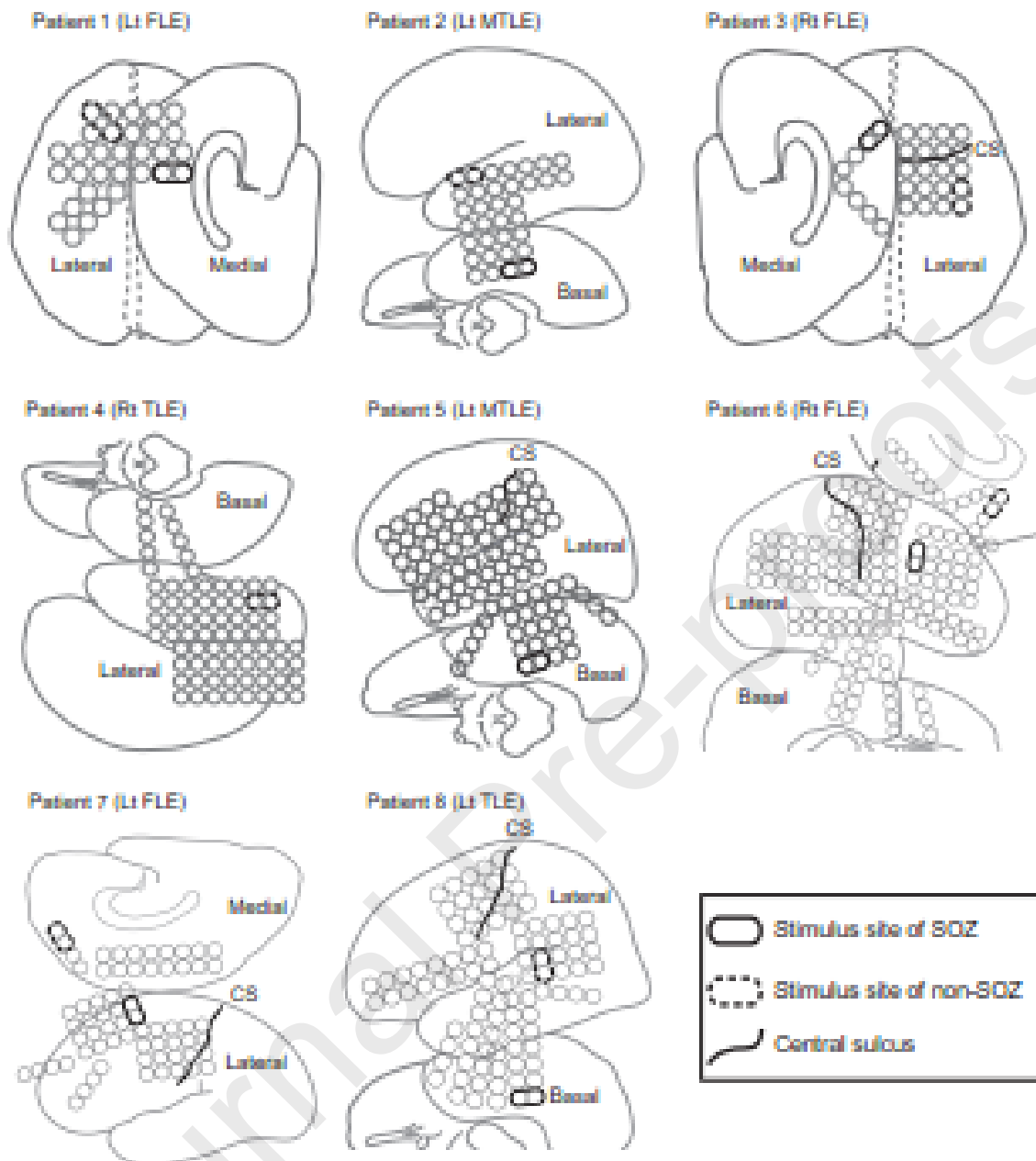
HFA: high-frequency activity; SOZ: seizure onset zone; ECoG: electrocorticogram; FDR: false discovery rate.

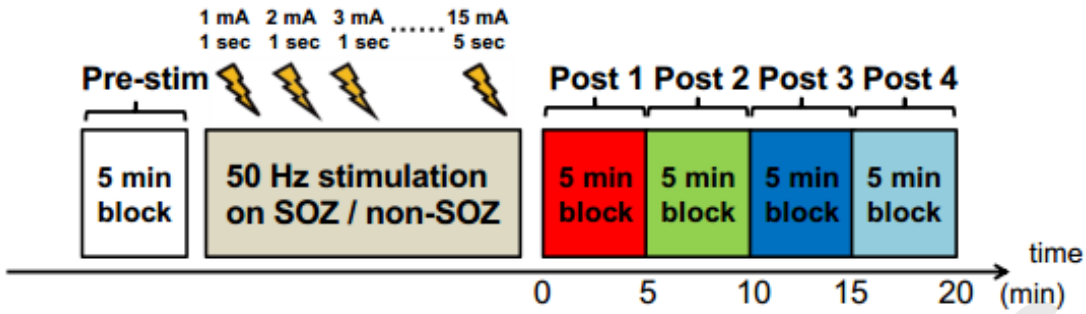
Fig. 7. Change in PSS-related low gamma activities.

A. The absolute power of PSS-related low gamma activities was calculated using the Hilbert transformation. The red dotted square shows the analyzed bins for frequency and time window, corresponding to the PSS ascending slope. The power significantly increased in Post 1 and Post 2 (** $p < 0.01$, Wilcoxon signed-rank test, FDR-corrected).

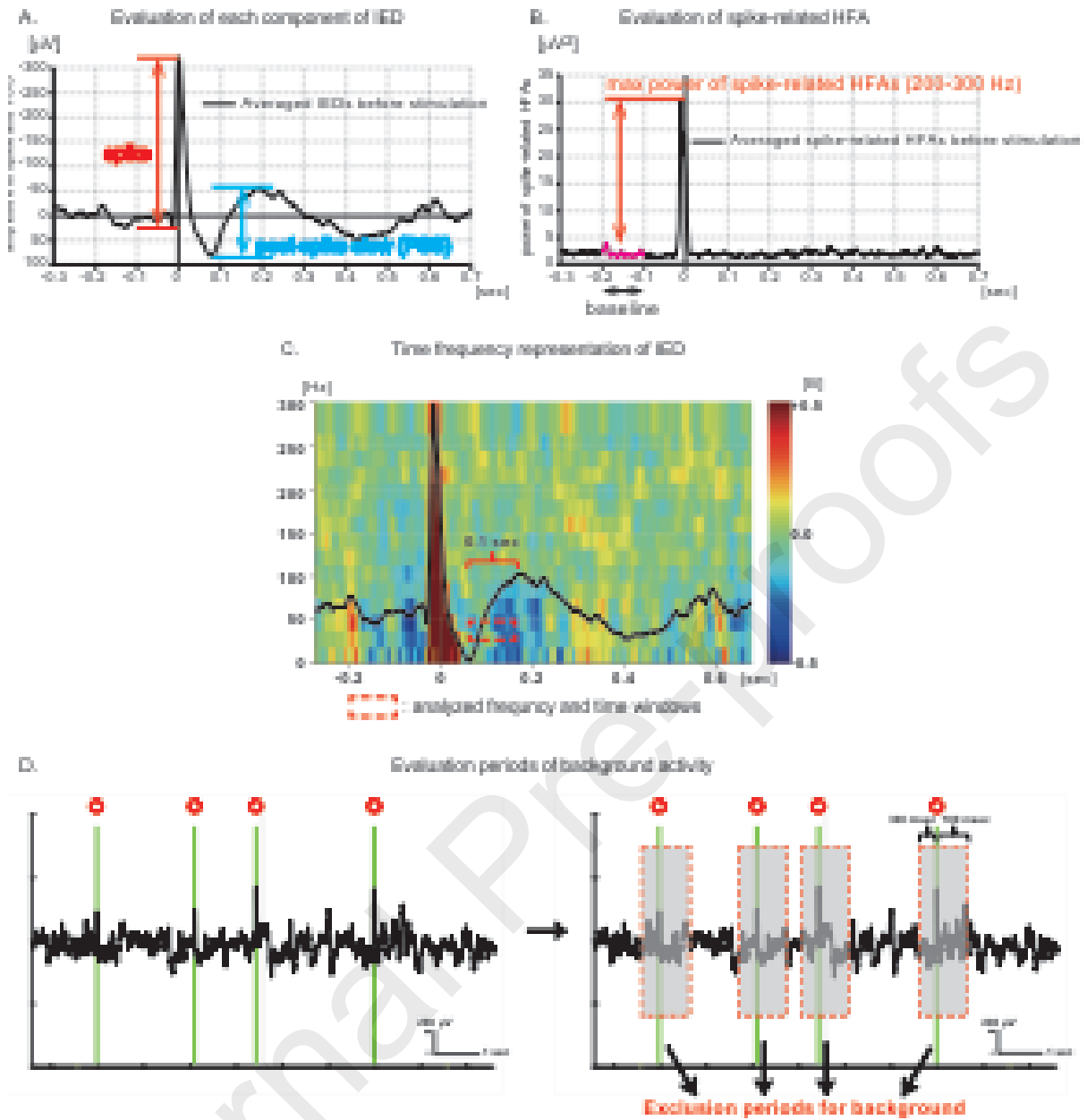
B. Changes in PSS-related low gamma activities in patients with overt PSSs are shown by the boxplot and contour line. The power values significantly increased compared with those in Pre-stimulus (Patient 1: ** $p < 0.01$ in Post 1 and 2; Patient 2: * $p < 0.05$ in Post 2, 3, and 4, Wilcoxon signed-rank test, FDR-corrected; Patient 7: (*) $p = 0.0127$ in Post 1, Wilcoxon signed-rank test, not significant after FDR correction for multiple comparisons).

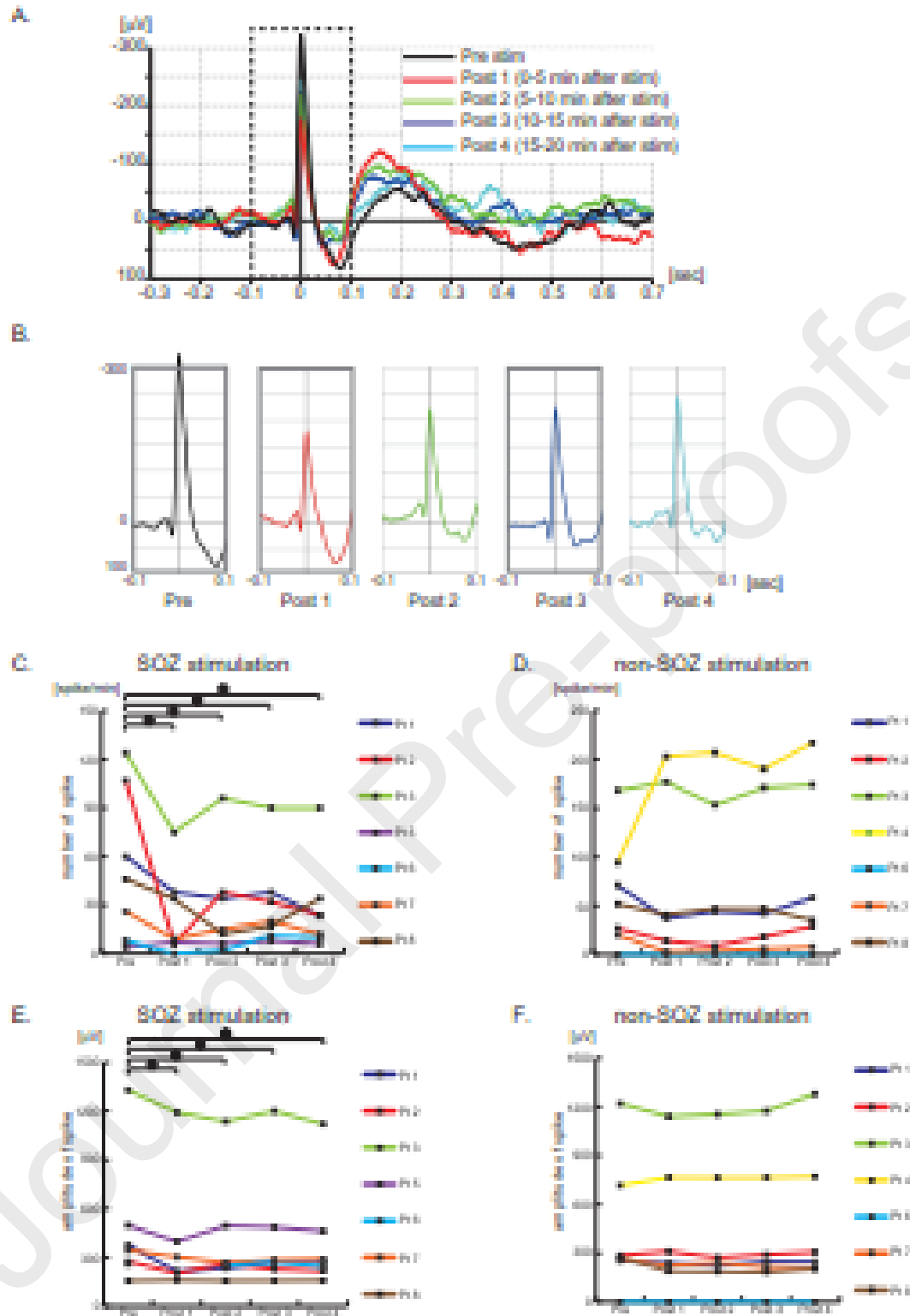
PSS: post-spike slow wave; FDR: false discovery rate.

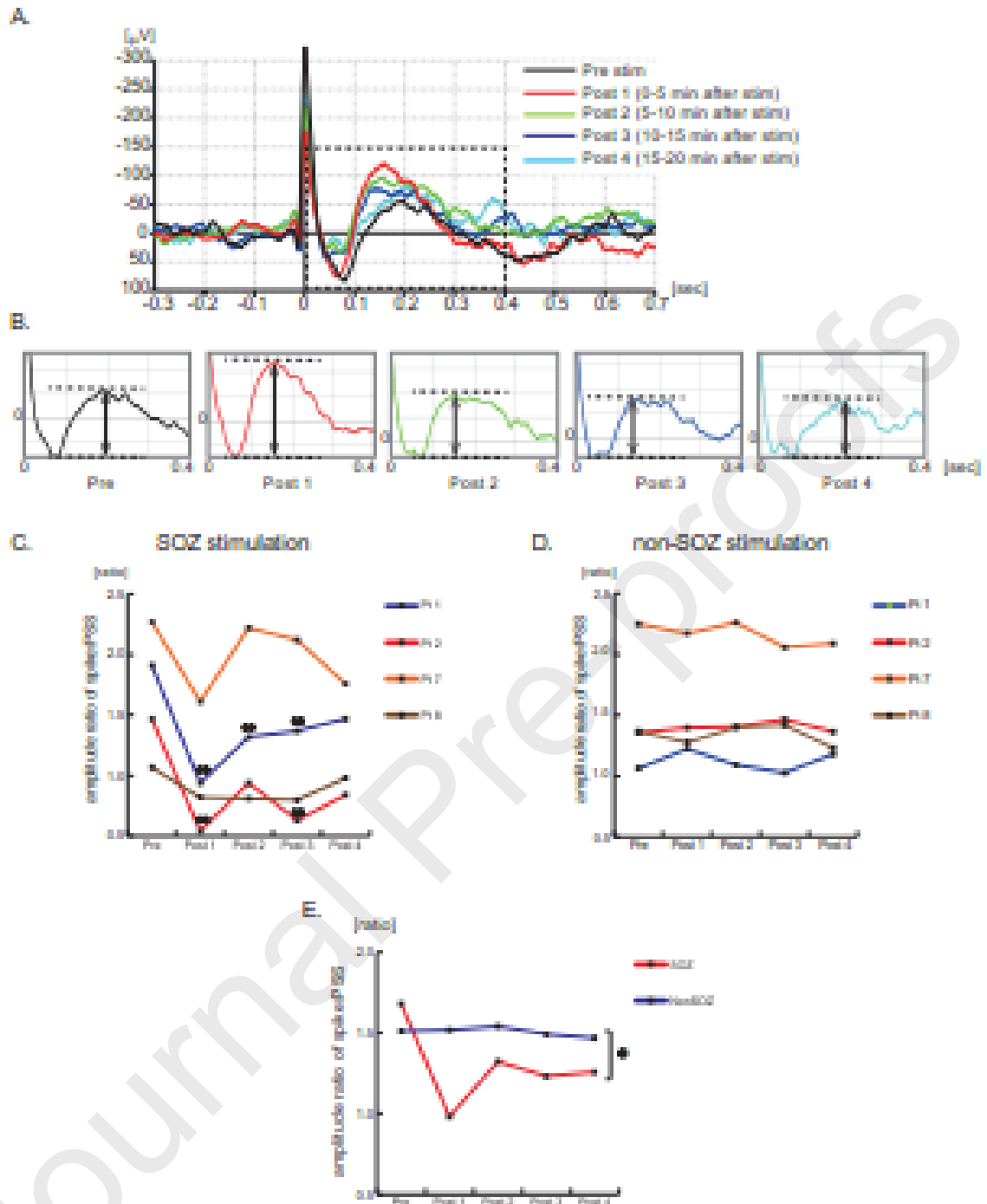


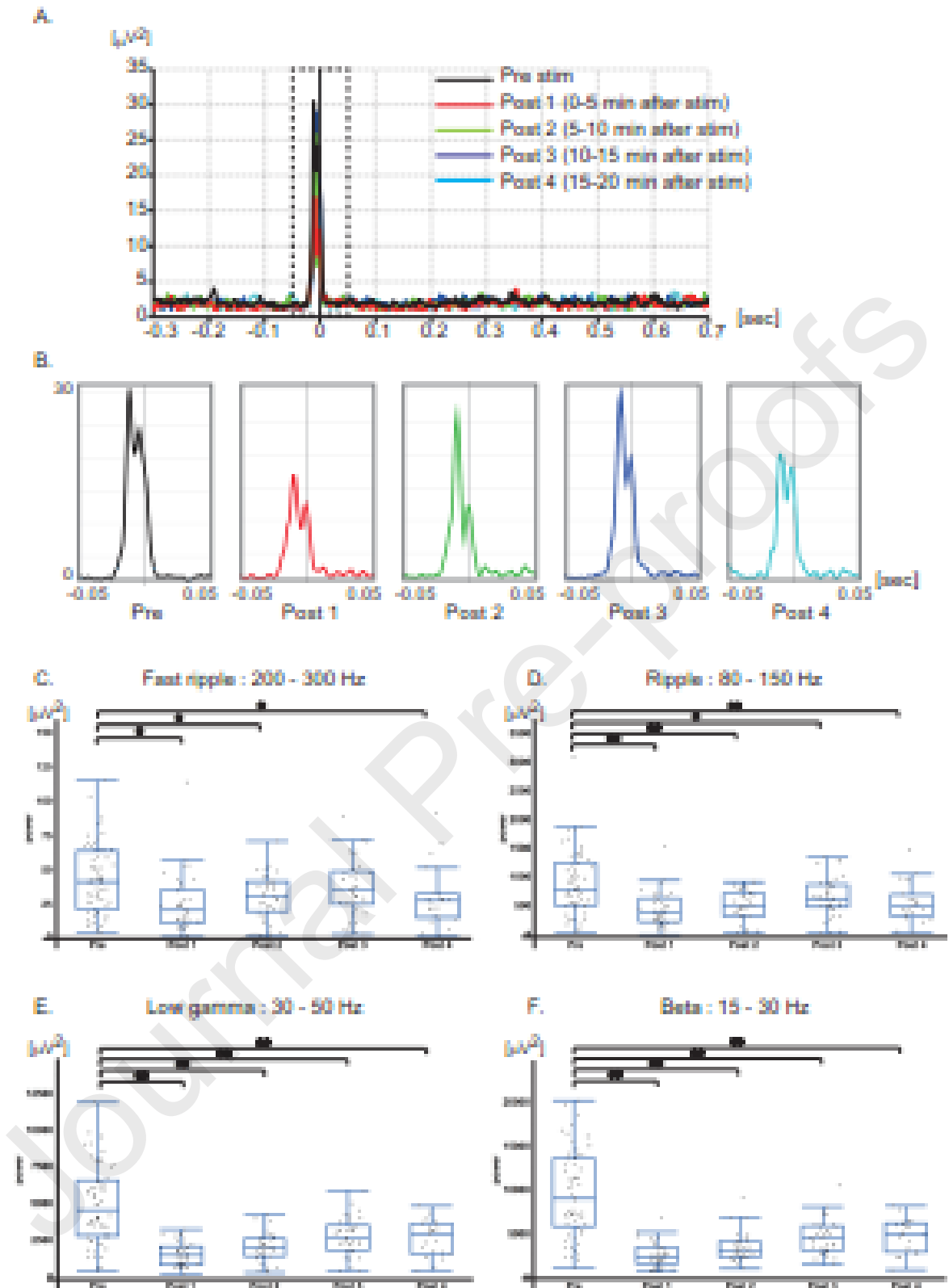


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<Table 1> Patient profile

Patient No.	Age	Sex	Diagnosis	Pathology	Outcome (Engel)
1	19	F	Lt FLE	FCD IIa	class Ic
2	25	F	Lt MTLE	HS	class Ia
3	31	M	Rt FLE	FCD type IIb	class Ib
4	25	M	Rt TLE	FCD type IC	class Ia
5	41	M	Lt MTLE	FCD type IC	class Ia
6	17	M	Rt FLE	FCD type IC	class IVb
7	39	F	Lt FLE	FCD type IIb	class Ia
8	34	M	Lt TLE	FCD type IA	class Ia

FLE: Frontal lobe epilepsy, MTLE: Mesial temporal lobe epilepsy,

FCD: Focal cortical dysplasia, HS: Hippocampal sclerosis

*: there were less than two years after the surgery to evaluate the outcome in Pt.8

<Table 2> Stimulation parameters and delivery Coulomb

Patient No.	condition	Maximum charge density /phase ($\mu\text{C}/\text{cm}^2/\text{phase}$)	Total stimulus time/site (sec)	Total delivery Coulomb (mC)
1	(1)	29.7	79.0	8.1
	(2)	29.7	49.0	4.8
2	(1)	29.7	224.0	23.0
	(2)	29.7	118.0	11.6
3	(1)	8.7	11.0	0.3
	(2)	59.2	12.0	1.2
4	(1)	N.A.	N.A.	N.A.
	(2)	63.7	76.5	12.0
5	(1)	34.0	34.0	2.9
	(2)	N.A.	N.A.	N.A.
6	(1)	63.7	69.0	9.2
	(2)	63.7	69.0	9.2
7	(1)	34.0	34.0	2.9
	(2)	55.2	59.0	7.0
8	(1)	16.9	4.0	0.15
	(2)	55.2	59.0	7.0

Condition (1): 50-Hz stimulation of seizure onset zone (SOZ),

Condition (2): 50-Hz stimulation of non-SOZ

N.A.: not available

All stimulation studies were conducted within safety limits of stimulus duration and delivery coulombs.

<Table 3> Summary of the results in SOZ and non-SOZ stimulation

		SOZ stimulation	non-SOZ stimulation
Number of IED		↓ 8 out of 8 patients	→ 8 out of 8 patients
Spike amplitude		↓ 8 out of 8 patients	→ 8 out of 8 patients
Spike/PSS amplitude ratio		↓ 3 out of 4 patients (1; tendency)	→ 8 out of 8 patients
Power of	FR	↓ 4 out of 8 patients	→ 8 out of 8 patients
spike-related HFAs	R	↓ 3 out of 8 patients	→ 7 out of 8 patients
Power of		↑ 3 out of 4 patients	→ 8 out of 8 patients
PSS-related low gamma		(1; marginally)	

SOZ: seizure onset zone, non-SOZ: non seizure onset zone

IED: interictal epileptic discharge, PSS: post-spike slow

HFA: high frequency activities, FR: fast ripple, R: ripple

The majority of evaluation factors were modulated after SOZ stimulation.

<Table 4> The change of the power of spike related activities among all patients.**(i) spike-related HFAs (FR: 200-300 Hz)**

	After stimulating SOZ							After stimulating non-SOZ						
	Pt. 1	Pt. 2	Pt. 3	Pt. 5	Pt. 6	Pt. 7	Pt. 8	Pt. 1	Pt. 2	Pt. 3	Pt. 4	Pt. 6	Pt. 7	Pt. 8
Post 1	↓*	n.s.	n.s.	n.s.	no spike	↓*	n.s.	n.s.	n.s.	n.s.	n.s.	no spike	n.s.	n.s.
Post 2	↓*	↓**	n.s.	n.s.	↓*	↓*	n.s.	n.s.	n.s.	n.s.	n.s.	no spike	n.s.	↓*
Post 3	n.s.	↓**	n.s.	n.s.	↓*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	no spike	n.s.	↓*
Post 4	↓*	n.s.	n.s.	n.s.	↓*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	no spike	n.s.	↓*

(ii) spike-related HFAs (R: 80-150 Hz)

	After stimulating SOZ							After stimulating non-SOZ						
	Pt. 1	Pt. 2	Pt. 3	Pt. 5	Pt. 6	Pt. 7	Pt. 8	Pt. 1	Pt. 2	Pt. 3	Pt. 4	Pt. 6	Pt. 7	Pt. 8
Post 1	↓**	n.s.	n.s.	n.s.	no spike	↓*	n.s.	n.s.	n.s.	n.s.	n.s.	no spike	n.s.	n.s.
Post 2	↓**	↓**	n.s.	n.s.	n.s.	↓**	n.s.	n.s.	n.s.	n.s.	n.s.	no spike	n.s.	↓*
Post 3	↓*	↓**	n.s.	n.s.	n.s.	↓*	n.s.	n.s.	n.s.	n.s.	n.s.	no spike	n.s.	n.s.
Post 4	↓**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	no spike	n.s.	n.s.

(iii) spike-related low gamma activities (30-50 Hz)

	After stimulating SOZ							After stimulating non-SOZ						
	Pt. 1	Pt. 2	Pt. 3	Pt. 5	Pt. 6	Pt. 7	Pt. 8	Pt. 1	Pt. 2	Pt. 3	Pt. 4	Pt. 6	Pt. 7	Pt. 8
Post 1	↓**	↓**	n.s.	n.s.	no spike	no spike	n.s.	n.s.	n.s.	n.s.	↑*	no spike	n.s.	n.s.
Post 2	↓**	↓**	n.s.	n.s.	n.s.	↓**	n.s.	n.s.	n.s.	↓*	n.s.	no spike	n.s.	↓*
Post 3	↓**	↓**	n.s.	n.s.	n.s.	↓**	n.s.	n.s.	n.s.	n.s.	↑*	no spike	n.s.	↓*
Post 4	↓**	↓**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	↓*	↑*	no spike	n.s.	n.s.

(iv) spike-related beta activities (15-30 Hz)

	After stimulating SOZ							After stimulating non-SOZ						
	Pt. 1	Pt. 2	Pt. 3	Pt. 5	Pt. 6	Pt. 7	Pt. 8	Pt. 1	Pt. 2	Pt. 3	Pt. 4	Pt. 6	Pt. 7	Pt. 8
Post 1	↓**	↓**	n.s.	n.s.	no spike	no spike	n.s.	n.s.	n.s.	↓*	↑**	no spike	n.s.	n.s.
Post 2	↓**	↓**	n.s.	n.s.	n.s.	↓**	n.s.	n.s.	n.s.	n.s.	↑**	no spike	n.s.	↓**
Post 3	↓**	↓**	n.s.	n.s.	n.s.	↓**	n.s.	n.s.	n.s.	n.s.	↑**	no spike	n.s.	↓**

Post 4 ↓** ↓** n.s. n.s. n.s. n.s. n.s. n.s. n.s. ↓* ↑** no spike n.s. ↓*

FR: fast ripple, R: ripple, n.s.: not significant

↑: increase, ↓: decrease (** p<0.01, * p<0.05)

The mode of modulation (i.e., power decrease), if present, was consistent among patients for SOZ stimulation, while it was not consistent for non-SOZ stimulation.

Journal Pre-proofs