

Dear author,

Please note that changes made in the online proofing system will be added to the article before publication but are not reflected in this PDF.

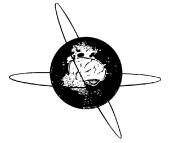
We also ask that this file not be used for submitting corrections.



Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph



We could predict good responders to vagus nerve stimulation: A surrogate marker by slow cortical potential shift

Borgil Bayasgalan^a, Masao Matsuhashi^{b,c,*}, Tomoyuki Fumuro^{b,1}, Haruhiko Nohira^d, Naoki Nakano^e,
Koji Iida^f, Masaya Katagiri^f, Akihiro Shimotake^g, Riki Matsumoto^a, Takayuki Kikuchi^h,
Takeharu Kuniedaⁱ, Amami Kato^e, Ryosuke Takahashi^a, Akio Ikeda^{g,*}

^a Department of Neurology, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan

^b Research and Educational Unit of Leaders for Integrated Medical System, Kyoto University, 54 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan

^c Human Brain Research Center, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan

^d Nihon Kohden Corporation, 1-31-4 Nishiochiai, Shinjuku-ku, Tokyo 161-8560, Japan

^e Department of Neurosurgery, School of Medicine, Kindai University, 377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan

^f Department of Neurosurgery, School of Medicine, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan

^g Department of Epilepsy, Movement Disorders and Physiology, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan

^h Department of Neurosurgery, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan

ⁱ Department of Neurosurgery, Graduate School of Medicine, Ehime University, Shizukawa, Toon City, Ehime 791-0295, Japan

ARTICLE INFO

Article history:

Accepted 31 May 2017

Available online xxxx

Keywords:

EEG

Vagus nerve stimulation (VNS)

Slow cortical potentials (SCPs)

Surrogate marker

Long time constant (TC)

HIGHLIGHTS

- Positive shifts of slow cortical potentials (SCPs) during the vagus nerve stimulation (VNS) period were observed in responders.
- SCP shifts modulated by VNS were more effectively detected by EEGs with long enough time constant.
- Positive polarity of SCP shift on scalp EEG could be an effective marker for response to VNS.

ABSTRACT

Objective: We investigated whether vagus nerve stimulation (VNS) induces a positive shift of slow cortical potentials (SCPs) in patients with >50% seizure reduction (responders) but not in non-responders.

Methods: We analyzed routine clinical electroencephalograms (EEGs) from 24 patients who were undergoing seizure treatment by VNS. The patients were divided into 2 groups by hardware time constant (TC) of EEG: the TC 10-s group (10 patients) and TC 2-s group (14 patients). We compared SCPs at 5 electrodes (Cz and adjacent ones) between the 2 states of VNS: during stimulation and between stimulations. Seizure reduction was independently judged. Correlation between SCP (positivity or not) and seizure reduction (>50% or not) was estimated.

Results: In the TC 10-s group, the correlation between SCP and seizure reduction was significant ($p < 0.05$) (i.e., both good results in 4 and both negative results in 5). In TC 2-s group, the correlation between SCP and seizure reduction was not significant ($p = 0.209$).

Conclusions: A positive shift of SCP recorded by using a TC of 10 s could be a surrogate marker for VNS response.

Significance: SCP could be a biomarker of good responders to VNS.

© 2017 Published by Elsevier Ireland Ltd on behalf of International Federation of Clinical Neurophysiology.

* Corresponding authors at: Research and Educational Unit of Leaders for Integrated Medical System, Kyoto University, 54 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan. Fax: +81 75 751 3202 (M. Matsuhashi). Fax: +81 75 751 3663 (A. Ikeda).

E-mail addresses: matuhasi@kuhp.kyoto-u.ac.jp (M. Matsuhashi), akio@kuhp.kyoto-u.ac.jp (A. Ikeda).

¹ Present address: Department of Medical Technology and Sciences, School of Health Sciences at Fukuoka, International University of Health and Welfare, 137-1, Enokizu, Ohkawa-shi, Fukuoka 831-8501, Japan.

1. Introduction

Vagus nerve stimulation (VNS) is a palliative treatment option for patients with intractable epilepsy who are not good candidates for surgical resection (Connor et al., 2012). VNS uses an electrical stimulator, like those in cardiac pacemakers, which is implanted in the subclavicular area and delivers trains of electrical pulses to

the left vagus nerve via bipolar stimulating electrodes (Terry et al., 1990). The stimulation of the vagus nerve is intermittent, and the usual stimulation condition is set to a signal on-time (VNS ON) of 30 s followed by an off-time (VNS OFF) of 3–5 min (Heck et al., 2002) repeatedly. Positive responses can be obtained within the first 3 months of treatment (Salinsky et al., 1996), and the response has a tendency to increase up to 18 months after surgery (De Taeye et al., 2014). It has been reported that one third of patients have a >50% reduction in seizure frequency, another one third show a 30%–50% seizure reduction, and the remaining one third show no response (Boon et al., 2001).

Neuronal mechanisms of VNS for seizure suppression are not clearly understood yet (Loddenkemper and Alexopoulos, 2008; Fanselow, 2012). In experimental models of epilepsy, it has been reported that VNS produced desynchronization of electroencephalography (EEG) and blocking of spike waves (Zanchetti et al., 1952) as well as an increased seizure threshold (De Herdt et al., 2010). Kralh et al. (1998) demonstrated that the antiepileptic effect of VNS was mediated by locus coeruleus. Chemical activation of parafascicular nuclei, a part of the non-specific thalamocortical projection system, inhibited epileptiform activity (Nail-Boucherie et al., 2005). The thalamocortical projection system has a major role in certain phenomena, such as consciousness, sleep, attention, and idiopathic epilepsy (Hanbery and Jasper, 1954). In humans, a positron-emission tomography (PET) study of VNS response showed activation of the thalamus in patients with seizure reduction (Henry et al., 2004).

Zagon and Kemeny (2000) suggested slow hyperpolarization of cortical pyramidal neurons of the parietal association cortex in anesthetized rats as an underlying mechanism of action of VNS in suppressing seizures. It is clinically useful to extract or record the hyperpolarized state of the cortices in association with VNS in humans by means of scalp EEG. Clinical EEG recorded by using an alternate current (AC) amplifier with very long time constant (TC) can detect slow cortical potential (SCP) or the direct-current (DC) shifts that are generated by either cortical neurons, glias, or their combination, depending on the type of SCP (Ikeda et al., 1996).

Based on previous research on animal studies and the following observations in humans, it can be inferred that depolarization of neurons, seen on EEG as negative SCP shifts, represents activation of excitatory postsynaptic potentials and neuronal excitation. Hyperpolarization of neurons, seen on EEG as positive SCP shifts, represents activation of inhibitory postsynaptic potentials and inhibition of neuronal activity (Prince, 1968; Ayala et al., 1973; Ikeda et al., 1996). Production of scalp-recorded positive SCP by a self-regulation training has been used for suppressing seizures, and some patients have even become seizure free (Kotchoubey et al., 2001; Strehl et al., 2005). On the basis of the above backgrounds, we hypothesized that a positive SCP shift could appear during VNS stimulation in patients with good response to treatment and there could be an absence of positive SCP shift in patients with poor response. Attempts to predict suitable candidates for VNS were reported (De Taeye et al., 2014; Arcos et al., 2014), and showed potential usefulness.

The goal of this retrospective study on data from three different centers was to investigate whether scalp-recorded positive polarity of the SCP shift could be used as a surrogate marker for treatment response to VNS.

2. Methods

2.1. Patient profile

The study was approved by the ethics committees of the Kyoto University Hospital, Kindai University Hospital and Hiroshima University Hospital (IRB#E1736, #25-036 and #Epi1158 respec-

tively). A total of 24 patients (13 women) with intractable epilepsy aged 28.8 ± 17.2 (mean $\pm$ standard deviation) years; ranging from 6 to 66 years, at the time of EEG, who have undergone VNS implantation between November 2010 and August 2014 were studied. 10 patients were diagnosed as having symptomatic generalized epilepsy and 14 as having symptomatic partial epilepsy. Their clinical features and related conditions (VNS stimulation interval, time constant of EEG recording and kind of metal for scalp electrodes) are summarized in Supplementary Table S1.

The mean period between VNS implantation and the latest available EEG recording was 12.2 (range, 4.2–36.7) months. The mean follow-up period or post-implantation seizure assessment at the last visit was 24.1 (range, 11.7–43.0) months, the mean time interval between the EEG and last assessment was 11.9 (range, 0.0–28.8) months (Supplementary Table S2).

2.2. VNS stimulation and seizure frequency assessment

All patients underwent VNS device implantation (Cyberonics, Houston, TX, USA), and the stimulation started within 1–2 weeks after the operation. The stimulation time was from the programmed plateau VNS ON time plus 2 s of ramp-up time and 2 s of ramp-down time (Fig. 1). The output parameters of the pulse generator were individually adjusted, and the adjustment methods were similar to those of other institutions (Labiner and Ahern, 2007). The cycles of stimulation were programmed to use a VNS ON time of 30 s, except in Patient 16 (21 s), and a VNS OFF time of 5, 3, or 1.8 min (Supplementary Table S1).

Seizure frequency was calculated on the basis of the patient's seizure diary when available or the records by the physicians-in-charge at the time of the patient's visit, if available. All types of partial and generalized seizures were singularly counted. Baseline monthly seizure frequency before VNS was calculated on the last visit before VNS implantation, or for the patients with more than one visit, the average of up to 3 monthly visits immediately before the implantation was used.

The monthly seizure frequency was obtained by following 2 methods: (1) if the patient's seizure diary was available, the monthly average of all seizures during the 3 months preceding the EEG recording was assessed; (2) if the seizure number was obtained from the physician's records, the monthly seizure frequency was first calculated, and then the average number of seizures in the 2 consecutive months before and in the month of EEG recording was calculated. When the period between the 2 follow-up visits was >3 months, the average number of seizures at the last 2 clinic visits was used as the number of seizures after the VNS.

If epilepsy surgery, such as corpus callosotomy, was performed after VNS, the seizure frequency after the operation was excluded from the analysis. In 4 patients with multiple types of seizures, an accurate counting of atypical absence seizures was not available: in 3 patients, the frequency of atypical absence seizures only was not calculated, and in the remaining 1 patient (Patient 8), the seizure frequencies for each seizure type were not available. Seizure frequency evaluation after VNS was calculated using the following formula (Labar et al., 1998; Fraschini et al., 2013):

$$\frac{\text{median VNS seizure(per month)} - \text{baseline seizure(per month)}}{\text{baseline seizure(per month)}} \times 100(\%)$$

On the basis of the VNS effect on seizure control, the patients were divided into 2 groups: good responders (>50% reduction in seizure frequency) and non-responders (<50% reduction in seizure frequency). Seizure frequency was analyzed at the time of EEG and at the last visit after VNS.

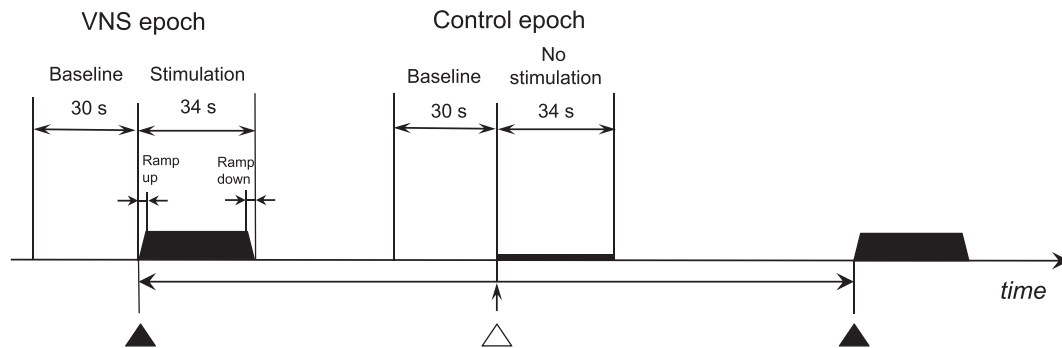


Fig. 1. Schematic diagram of the analysis window of VNS epochs and control epochs. The control epoch for comparison with the VNS epoch is in the middle between 2 VNS epochs. The 34 s of stimulation time consists of a 2-s ramp-up, a 30-s plateau, and a 2-s ramp-down period. Black trapezoid = stimulation, thick line = control period, black triangles = starting point of stimulation, open triangle = starting point of no stimulation.

2.3. EEG recording and analysis

2.3.1. EEG recording conditions

30 min scalp EEGs were recorded by using the EEG1100 or EEG2100 (Nihon Kohden, Tokyo, Japan) with time constants of 10 s in 10 patients (TC 10-s group) and 2 s in 14 patients (TC 2-s group). The signals were digitized with sampling rate of 200 Hz or 500 Hz. The electrodes were placed according to the International 10–20 System (19 scalp electrodes and 2 references of A1 and A2). The types of electrodes used for the EEG recordings were silver electrodes (Nihon Kohden, Tokyo, Japan) at Kindai University and Kyoto University or tin electrodes attached to a special cap (Electro-Cap, Eaton, OH, USA) at Hiroshima University. The electrode impedance was maintained at < 10 k Ω at Kyoto University and Hiroshima University and similar at Kindai University.

2.3.2. EEG analysis

We selected 5 electrodes, C3, C4, Fz, Cz, and Pz, because these electrodes are relatively unsuceptible to electromyographic artifacts. The EEG signal recorded by those electrodes was shown referenced to linked earlobes on a digital display. Data analysis was accomplished in an off-line manner by using Matlab R2012b (MathWorks, Inc., Natick, MA, USA) on a personal computer workstation. For extracting the SCP of the EEG recording, a high-frequency filter of 0.2 Hz was applied to EEG in the C3, C4, Fz, Cz, and Pz electrodes. To identify the stimulation period, a linked earlobe lead or ECG linked to a system reference electrode derivation was employed, and a low-frequency filter (LFF) of 30 Hz was applied only on this channel. The onset points of each train of VNS were marked on the EEG (Figs. 1 and 2A).

As shown in Fig. 1, we averaged the so-called VNS ON time epoch “VNS epoch” (continuous 30 s before VNS onset and the following continuous 34 s of VNS) and the “control epochs,” (continuous 64 s between the 2 consecutive VNS epochs) separately, with an exceptional 55 s in 1 patient (Patient 16, Supplementary Figure S2D, E and F).

Artifacts and inappropriate electrographic signals in EEG were treated by using the following 3 criteria:

- 1) Initial slow negative shifts most likely caused by electrode potentials (Cooper et al., 1969; Epstein, 2011) were observed at the beginning of the recording. This baseline shift almost disappeared within 600 s whenever it was present on EEG. Therefore, we measured the mean potential of the first 60 s in 5 electrodes of analysis, and if it was >150 μ V, then the first 600 s of EEG data were excluded (Fig. 2A). Details of the generator mechanisms of this artifact in the present study are explained in the Supplementary Figure S1.

- 2) EEG epochs with recording reset events, such as pausing in the EEG recording or montage change in certain types of EEG machine, were excluded because the recording reset produced a correction of baseline shifts not infrequently associated with large offset drift (Fig. 2A and Supplementary Figure S2A).
- 3) Epochs containing transient electrographic signal amplitudes >400 μ V (as the peak-to-peak amplitude of the opposite polarity) were excluded from the analysis.

To determine the presence of difference between the above 2 waveforms at 5 electrodes, the Wilcoxon signed rank sum test was calculated (Fig. 2C and Supplementary Figure S2C and F). A positive shift of the SCP was considered to be “present” when it was observed on the 5 electrodes during the stimulation period, and the Wilcoxon test value was <0.05.

We excluded the EEG data of patients with VNS OFF time durations of 1.1 min from analysis, because the consecutive VNS epoch and control epoch overlapped in each analysis window. When the patients had more than one EEG record, the latest one was included for analysis. EEGs were gathered by the coauthors from Kindai University Hospital, Hiroshima University Hospital, and Kyoto University Hospital. All EEGs were analyzed by the authors (B.B., M.M. T.F, Kyoto University). Those processes were completely independent of any EEG acquisition and selection.

2.4. Concordance between the SCP and response to treatment

Concordance was defined as when the patient was a good responder and a positive polarity of the SCP shift was observed or when the patient was a non-responder and a non-positive SCP shift was observed. Any other combinations were judged as non-concordant.

2.5. Statistical analysis

Statistical analysis was performed by using SPSS software (version 15.0, SPSS Inc., Chicago, IL, USA). Fisher's exact test using 2 groups of between-subject factors (responders vs. non-responders, positive SCP shift present vs. positive SCP shift absent) was performed to analyze the relationship between responders and positive shift of SCP. In addition, Fisher's exact test was also employed to compare the two groups (TC 2-s and TC 10-s) regarding the concordance ratio. Two-sided p values of <0.05 were considered as indicating statistical significance.

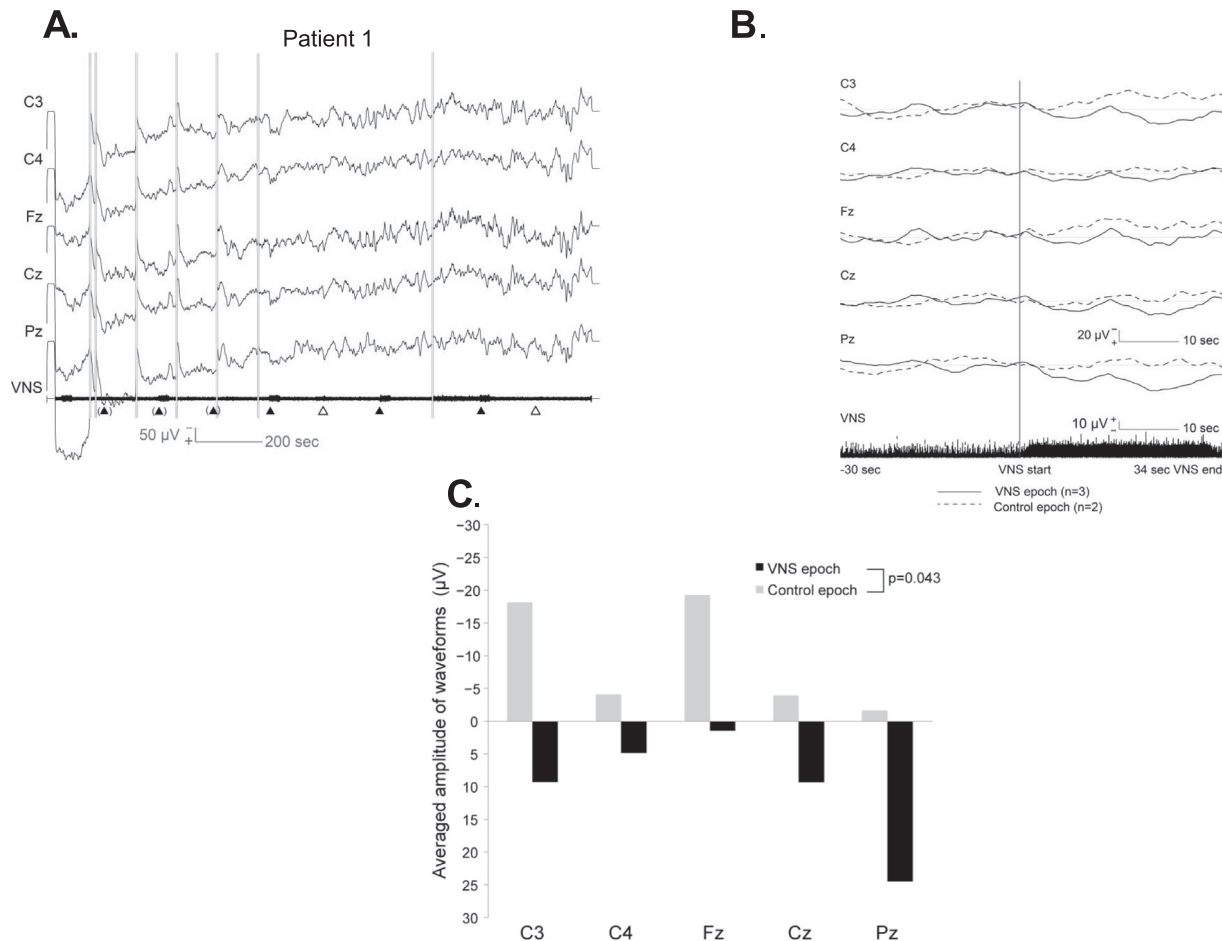


Fig. 2. EEG sample recorded with a TC of 10 s in Patient 1. **A:** EEG waveforms of selected channels. There were 2 recording gaps at 128 s and 1148 s, lasting for 42 s and 33 s, respectively (shown by the first and last vertical lines in the figure). Black triangles enclosed in parentheses represent the excluded epochs because of initial slow drift artifact. Vertical lines = recording breaks, black triangles = the starting point of the stimulation period, open triangles = the starting point of the no stimulation period within the stimulation interval. **B:** Averaged EEG waveforms of the VNS epoch and control epoch. The EEG showed positive polarity of SCP shifts during stimulation. **C:** Quantification of waveforms at 5 electrodes during stimulation of VNS epoch and no stimulation of control epoch.

3. Results

3.1. Effects of VNS on seizure frequency

Among 10 patients in the TC 10-s group, 3 patients (30%) had seizure reductions of >50% at the time of EEG. At the last visit, the number of responders was 4 (40%). Among the 14 patients in the TC 2-s group, the number of responders at the time of EEG was 5 (36%), and this number increased to 8 (57%) at the last visit (Supplementary Table S2). The results of seizure reduction in response to VNS are summarized in Tables 1 and 2. Among all 24 patients, 8 patients had seizure reductions of >50% at the time of EEG, and the number of good responders was 12 (50%) at the last visit. Increase in responder rate over time observed in our study was consistent with previous studies (Morris and Mueller, 1999; Kuba et al., 2009).

3.2. Electrophysiological results

The mean of the period between VNS implantation and the last EEG recording was 12.1 months (range, 4.3–36.6 months).

3.2.1. TC 10-s group (Table 1)

In 10 patients, EEGs were recorded with a 10-s time constant. Recorded VNS epochs were 7.6 ± 3.0 (means $\pm$ standard deviation).

After excluding epochs with artifacts, the final number of available epochs for analysis was 4.1 ± 1.6 per EEG record. Recorded control epochs were 7.5 ± 3.2 . After excluding epochs with artifacts, the final number of available epochs for analysis was 4.4 ± 2.0 (Supplementary Table S3).

Representative waveforms of the whole, 30-min EEG are shown in Fig. 2A. Averaged waveforms (Fig. 2B), amplitudes, and polarities for 5 electrodes (Fig. 2C) are illustrated for each patient with and without a positive shift of SCP (Supplementary Figure S2A, B and C). In the final results, a positive polarity of the SCP shift (Fig. 2C) was observed in 5 patients.

3.2.2. TC 2-s group (Table 2)

In 14 patients, EEGs were recorded with a 2-s time constant. VNS epochs recorded = 10.6 ± 5.7 . After excluding epochs with artifacts, the final number of available epochs for analysis was 7.5 ± 4.6 per EEG record. Control epochs recorded = 10.8 ± 6.0 . After excluding epochs with artifacts, the final number of available epochs for analysis was 8.1 ± 5.3 (Supplementary Table S3).

On some occasions, for instance, Patient 12 had 1 VNS signal obscured by movement artifacts, whereas Patient 15 had 2 VNS signals obscured by the movement artifacts, both during a hyperventilation test on EEG. These 3 VNS signals were not included in the analysis. Representative waveforms of the whole, 30-min EEG are shown in Supplementary Figure S2D, E and F. In the final

Table 1

The degree of positive polarity of slow cortical potential (SCP) shifts and response to VNS treatment in 10 patients in the TC 10-s group.

Patient	Positive polarity of SCP shifts			Response to VNS treatment		Concordance	
	No. of electrodes	P-value ^a	Yes/No	At the time of EEG	At the last follow-up	At the time of EEG ^b	At the last follow-up ^c
1	5	0.043	Yes	NR	R	NC	C
2	5	0.029	Yes	R	R	C	C
3	5	0.001	Yes	NR	NR	NC	NC
4	5	0.043	Yes	R	R	C	C
5	5	0.001	Yes	R	R	C	C
6	2	0.500	No	NR	NR	C	C
7	4	0.080	No	NR	NR	C	C
8	3	0.345	No	NR	NR	C	C
9	4	0.500	No	NR	NR	C	C
10	4	0.138	No	NR	NR	C	C

Yes = positive SCP shift was “present” (as defined in Methods); No = positive SCP shift was not “present”; R = Responder; NR = Non-responder; C = Concordant; NC = Non-concordant.

^a p-value was analyzed by using the Wilcoxon signed rank sum test for 5 electrodes during 2 conditions of both stimulation and no stimulation.

^b p = 0.167.

^c p = 0.048 (Fisher's exact test).

Table 2

The degree of positive polarity of slow cortical potential (SCP) shifts and response to VNS treatment in 14 patients in the TC 2-s group.

Patient	Positive polarity of SCP shifts			Response to VNS treatment		Concordance	
	No. of electrodes	P-value ^a	Yes/No	At the time of EEG	At the last follow-up	At the time of EEG ^b	At the last follow-up ^c
11	4	0.500	No	NR	NR	C	C
12	1	0.138	No	NR	NR	C	C
13	4	0.080	No	NR	NR	C	C
14	5	0.043	Yes	R	R	C	C
15	0	0.043	No	NR	R	C	NC
16	5	0.043	Yes	R	R	C	C
17	1	0.080	No	R	R	NC	NC
18	3	0.500	No	NR	NR	C	C
19	1	0.080	No	NR	NR	C	C
20	3	0.345	No	NR	NR	C	C
21	2	0.225	No	R	R	NC	NC
22	3	0.345	No	R	R	NC	NC
23	5	0.043	Yes	NR	R	NC	C
24	4	0.080	No	NR	R	C	NC

Yes = positive SCP shift was “present”; No = positive SCP shift was not “present”; R = Responder; NR = Non-responder; C = Concordant; NC = Non-concordant.

^a p-value was analyzed by using the Wilcoxon signed rank sum test for 5 electrodes during 2 conditions of both stimulation and no stimulation.

^b p = 0.505.

^c p = 0.209 (Fisher's exact test).

results, a positive polarity of the SCP shift was observed in 3 patients.

3.3. Concordance between SCP analysis and seizure reduction

In the EEGs of the TC 10-s group, 9 of 10 patients showed concordance between the degree of seizure reduction and SCP results at the last visit, and the correlation was statistically significant ($p = 0.048$, Fisher's test). At the time of EEG, the correlation between the 2 was not statistically significant ($p = 0.167$, Fisher's test) (Table 1).

In the EEGs of the TC 2-s group, the correlation between the 2 items was not statistically significant at the last visit ($p = 0.209$, Fisher's test) or at the time of EEG ($p = 0.505$, Fisher's test) (Table 2). However, the difference between the 2 groups was not significant (Fig. 3).

4. Discussion

Our primary goal was to identify a biomarker of good responders to VNS treatment. Based on the reasonable assumption that hyperpolarization or inhibitory properties of patients' brain activity during stimulation is reflected by the positive polarity of SCP,

we compared the SCP between the stimulation (VNS epochs) and control period of the stimulation interval (control epochs). The only outcome of the study protocol was the correlation between the degree of seizure reduction and degree of positive polarity of the SCP shift.

On the basis of the TC value of the EEG recording, we purposely divided the patients into 2 groups: the TC 10-s and TC 2-s groups. Percentages of good responders to treatment were similar between the 2 groups. The EEGs of the good responders at the last visit showed positive shifts of the SCP during stimulation ($p = 0.048$, Fisher's test) in the TC 10-s group, but not in the TC 2-s group ($p = 0.209$) (Tables 1 and 2). These results suggest that a TC of 10 s, but not a TC of 2 s, could extract positive shifts of the SCP appropriately.

Because routine scalp-recorded EEG was used in our analysis, to avoid the external noise from the analysis, we applied 3 kinds of criteria to exclude inappropriate samples (Supplementary Table S3). We deleted almost half of epochs in the TC-10 s group and nearly 30% of epochs in TC-2 s group.

Changes in the conventional EEGs with a LFF of 0.3 Hz (corresponding to a TC of 0.5 s) during VNS have not been observed previously (Salinsky and Burchiel, 1993). However, consistent with the study by Zagon and Kemeny, 2000, a positive polarity of the SCP shifts during VNS was observed in our study.

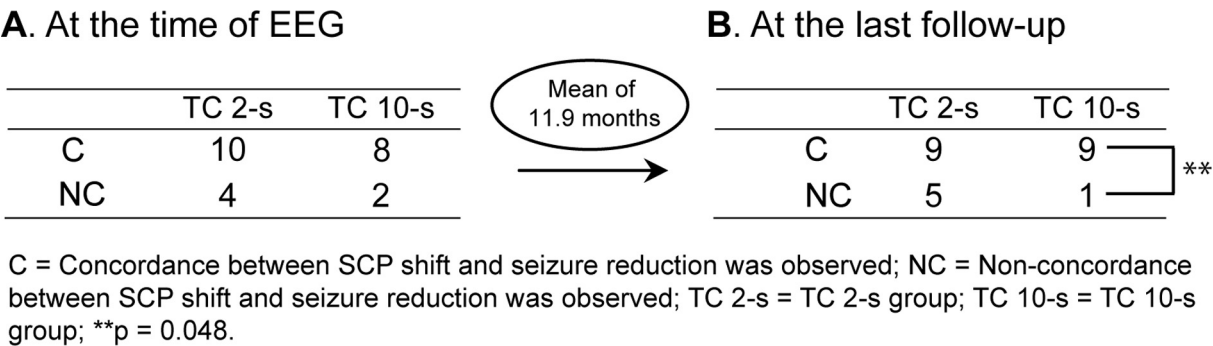


Fig. 3. A summary of Tables 1 and 2: a comparison of concordance and non-concordance in both TC 10-s and TC 2-s groups (number of patients). It showed the number of patients exhibiting concordance and non-concordance between the degree of seizure reduction and SCP results in the TC 10-s and TC 2-s groups. The difference between the TC 10-s and TC 2-s group results did not reach statistical significance: at the time of EEG and at the last follow-up, the p-values were 1.00 and 0.341 respectively.

Although it appeared only as an abstract, a previous study on DC shifts involving DC-coupled amplifiers reported positive DC shifts in 7 of 9 good responders to VNS during and immediately after VNS off-time, whereas all 9 poor responders to VNS did not show any positive shift (Sperner et al., 2005). Very similar to that study, our study found positive polarity of the SCP shifts in good responders during VNS stimulation. The difference between the 2 studies was the occurrence time of positive polarity of the SCP shifts. Specifically, our study showed shorter positive shifts, whereas the other study showed longer positive shifts. These results are simply explained by the usage of DC amplifiers in their study and AC amplifiers in our study.

To interpret the present findings especially regarding sensitivity, the following concern was considered. In the TC 10-s group, the correlation between the seizure reduction and positive polarity shift was not significant when seizure reduction was assessed at the time of EEG recording, but it was significant when seizure reduction was assessed at the last follow-up visit. The time difference between EEG recording and the last follow-up visit was on average 11.9 ± 7.3 months (Supplementary Table S2). Thus, EEG findings regarding the positive polarity of SCP shifts may precede the clinical condition of seizure reduction. Future research could solve whether the delayed effects are common and could assess the underlying mechanism. In addition, a possible limitation of this study is the usage of the Wilcoxon signed rank sum test as an index of positivity of the SCP; although it is usually applied to the values of trials or subjects, we applied the test to 5 electrodes and their average amplitude.

Our findings are consistent with the study by De Taeye et al., 2014, who found more positive P3 waves during VNS condition in the good responder group. The P3 during VNS condition may override on positive SCP observed in our study, resulting in augmenting of P3 amplitude in responders. In comparison with the study by De Taeye, although small in the sample size, in our study it showed statistically significant correlation between SCP and response to VNS.

Recently, noninvasive methods of cranial nerve stimulation, such as transcutaneous VNS (t-VNS), and trigeminal nerve stimulation, such as of the forehead (DeGiorgio et al., 2013), have been reported. The t-VNS is a noninvasive method of VNS, which stimulates the vagus nerve, i.e., tragus, via the auricular branch; its anti-convulsive effect in the first clinical data was equivalent to that of VNS (Stefan et al., 2012). Activation and deactivation of different brain regions by t-VNS were reportedly similar to those produced by an implantable VNS (Frangos et al., 2015). Our findings support the idea that for prediction of seizure reduction by VNS, scalp EEG recorded with a TC of 10 s could be clinically useful if the vagal nerve is properly stimulated noninvasively, such as by t-VNS before VNS surgery. In this regard, use of EEG for prediction is quite

simple and straightforward. The current study findings support further investigation of SCP as a surrogate marker in a larger patient population.

5. Conclusion

This study compared scalp-recorded SCPs during VNS stimulation period with the inter-stimulation interval respectively from 5 electrodes in patients undergoing VNS therapy. The comparison showed that utilization of EEG with a long TC (10 s) revealed positive SCP shifts during VNS in good responders. Positive polarity of SCP shifts could be a possible predictive biomarker of good response to VNS treatment, which may help physicians in selection of parameters and prediction of treatment outcomes before VNS surgery if the vagal nerve can be transiently stimulated. In the future, SCP could be investigated by EEG instruments with long TCs in noninvasive stimulation techniques, such as t-VNS or trigeminal nerve stimulation.

Conflict of interest

Department of Epilepsy, Movement Disorders and Physiology is an endowment department, supported with a grant from GlaxoSmithKline K.K., Nihon Kohden Corporation, Otsuka Pharmaceuticals Co., and UCB Japan Co., Ltd. K. Iida has been receiving honoraria from Nihon Kohden Corporation. Other authors have no conflict of interest to disclose.

Acknowledgements

This work was partly funded by the Japan Ministry of Education, Culture, Sports, Science and Technology (MEXT) KAKENHI Grant Numbers 15H05874, 15H05875, 17H05907 and Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Numbers 26293209, 26282218, 26462223, 25350691 and 23500484.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.clinph.2017.05.019>.

References

Arcos A, Romero L, Gelabert M, Prieto A, Pardo J, Rodriguez Osorio X, et al. Can we predict the response in the treatment of epilepsy with vagus nerve stimulation? *Neurosurg Rev* 2014;37:661–8.

Ayala GF, Dichter M, Gummit RJ, Matsumoto H, Spencer WA. Genesis of epileptic interictal spikes. *Brain Res* 1973;52:1–17.

- Boon P, Vonck K, De Reuck J, Caemaert J. Vagus nerve stimulation for refractory epilepsy. *Seizure* 2001;10:448–55.
- Connor Jr DE, Nixon M, Nanda A, Guthikonda B. Vagus nerve stimulation for the treatment of medically refractory epilepsy: a review of the literature. *Neurosurg Focus* 2012;32:e12. <http://dx.doi.org/10.3171/2011.12.focus11328>.
- Cooper R, Osselson JW, Shaw JC. EEG technology. London: Butterworths & Co. (Publishers) Ltd; 1969. p. 9–25.
- DeGiorgio CM, Soss J, Cook IA, Markovic D, Gombein J, Murray D, et al. Randomized controlled trial of trigeminal nerve stimulation for drug-resistant epilepsy. *Neurology* 2013;80:786–91.
- De Herdt V, De Waele J, Raedt R, Wyckhuys T, El Tahry R, Vonck K, et al. Modulation of seizure threshold by vagus nerve stimulation in an animal model for motor seizures. *Acta Neurol Scand* 2010;121:271–6.
- De Taeye L, Vonck K, van Bochove M, Boon P, Van Roost D, Mollet L, et al. The P3 event-related potential is a biomarker for the efficacy of vagus nerve stimulation in patients with epilepsy. *Neurotherapeutics* 2014;11:612–22.
- Epstein CM. Analog signal recording principles. In: Schomer DL, Lopes da Silva FH, editors. *Niedermeyer's electroencephalography: basic principles, clinical applications, and related fields*. Philadelphia: Lippincott Williams & Wilkins; 2011. p. 111–8.
- Fanselow EE. Central mechanisms of cranial nerve stimulation for epilepsy. *Surg Neurol Int* 2012;3:S247–54.
- Frangos E, Ellrich J, Komisaruk BR. Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: fMRI evidence in humans. *Brain Stimul* 2015;8:624–36.
- Fraschini M, Puligheddu M, Demuru M, Polizzi L, Maleci A, Tamburini G, et al. VNS induced desynchronization in gamma bands correlates with positive clinical outcome in temporal lobe pharmacoresistant epilepsy. *Neurosci Lett* 2013;536:14–8.
- Hanbery J, Jasper H. The non-specific thalamocortical projection system. *J Neurosurg* 1954;11:24–5.
- Heck C, Helmers SL, DeGiorgio CM. Vagus nerve stimulation therapy, epilepsy, and device parameters: scientific basis and recommendations for use. *Neurology* 2002;59(Suppl. 4):31–7.
- Henry TR, Bakay RAE, Pennell PB, Epstein CM, Votaw JR. Brain blood-flow alterations induced by therapeutic vagus nerve stimulation in partial epilepsy: II. Prolonged effects at high and low levels of stimulation. *Epilepsia* 2004;45:1064–70.
- Ikeda A, Terada K, Mikuni N, Burgess RC, Comair Y, Taki W, et al. Subdural recording of ictal DC shifts in neocortical seizures in humans. *Epilepsia* 1996;37:662–74.
- Krahl SE, Clark KB, Smith DS, Browning RA. Locus coeruleus lesions suppress the seizure-attenuating effects of vagus nerve stimulation. *Epilepsia* 1998;39:709–14.
- Kotchoubey B, Strehl U, Uhlmann C, Holzapfel S, König M, Froscher W, et al. Modification of slow cortical potentials in patients with refractory epilepsy: a controlled outcome study. *Epilepsia* 2001;42:406–16.
- Kuba R, Bradzil M, Kalina M, Prochazka T, Hovorka J, Nezadal T, et al. Vagus nerve stimulation: Longitudinal follow-up of patients treated for 5 years. *Seizure* 2009;18:269–74.
- Labar D, Nicolov B, Tarver B, Fraser N. Vagus nerve stimulation for symptomatic generalized epilepsy: a pilot study. *Epilepsia* 1998;39:201–5.
- Labiner DM, Ahern GL. Vagus nerve stimulation therapy in depression and epilepsy: therapeutic parameter settings. *Acta Neurol Scand* 2007;115:23–33.
- Loddenkemper T, Alexopoulos AV. Vagus nerve stimulation: human studies. In: Luders HO, editor. *Textbook of epilepsy surgery*. London: Informa Healthcare; 2008. p. 1188–200.
- Morris 3rd GL, Mueller WM. The Vagus Nerve Stimulation Study Group E01–E05. Long-term treatment with vagus nerve stimulation in patients with refractory epilepsy. *Neurology* 1999;53:1731–5.
- Nail-Boucherie K, Le-Pham BT, Gobaille S, Maitre M, Aunis D, Depaulis A. Evidence for a role of the parafascicular nucleus of the thalamus in the control of epileptic seizures by the superior colliculus. *Epilepsia* 2005;46:141–5.
- Prince DA. Inhibition in “epileptic” neurons. *Exp Neurol* 1968;21:307–21.
- Salinsky MC, Burchiel KJ. Vagus nerve stimulation has no effect on awake EEG rhythms in humans. *Epilepsia* 1993;34:299–304.
- Salinsky MC, Uthman BM, Ristanovic RK, Wernicke JF, Tarver WB. Vagus nerve stimulation study group. Vagus nerve stimulation for the treatment of medically intractable seizures: results of a 1-year open-extension trial. *Arch Neurol* 1996;53:1176–80.
- Sperner JL, Schlegelmilch F, Erdmann L, Schellhorn K. Slow cortical potentials and polygraphic recordings during vagus nerve stimulation: differences between responders and non-responders with pharmacoresistant epilepsy. *Proceedings of American Epilepsy Society: annual meeting of the American Epilepsy Society*. *Epilepsia* 2005;46(Suppl. 8):235.
- Stefan H, Kreiselmeier G, Kerling F, Kurzbuch K, Rauch C, Heers M, et al. Transcutaneous vagus nerve stimulation (t-VNS) in pharmacoresistant epilepsies: a proof of concept trial. *Epilepsia* 2012;53:115–8.
- Strehl U, Kotchoubey B, Trevorrow T, Birbaumer N. Predictors of seizure reduction after self-regulation of slow cortical potentials as a treatment of drug-resistant epilepsy. *Epilepsy Behav* 2005;6:156–66.
- Terry R, Tarver WB, Zabara J. An implantable neurocybernetic prosthesis system. *Epilepsia* 1990;31(Suppl. 2):33–7.
- Zagon A, Kemeny AA. Slow hyperpolarization in cortical neurons: a possible mechanism behind vagus nerve stimulation therapy for refractory epilepsy? *Epilepsia* 2000;41:1382–9.
- Zanchetti A, Wang SC, Moruzzi G. The effect of vagal afferent stimulation on the EEG pattern of the cat. *Electroencephalogr Clin Neurophysiol* 1952;4:357–61.