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Modulation of cortical synchrony by vagus nerve stimulation in adult rats

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***Abstract*—Vagus nerve stimulation (VNS) is a palliative treatment for intractable epilepsy. Therapeutic mechanisms of VNS have not been elucidated. In this study, we measured the local field potential (LFP) with high-spatial resolution using a microelectrode array in adult rats, and analyzed VNS-evoked phase modulation at a local network level. Eight adult Wistar rats (270 – 330 g) were used. Each rat underwent implantation of VNS system (Cyberonics, Houston, TX., USA) under 1.5% isoflurane anesthesia. One week after implantation, right temporal craniotomy was performed under the same as previous anesthesia. Subsequently, a microelectrode array was placed in the temporal lobe cortex, and LFP was recorded with sampling rate of 1000 Hz. Phase-locking value (PLV) between all pairs of electrodes in varied frequency bands was calculated in order to evaluate the effect of VNS in terms of synchrony of neuronal activities. PLV was calculated both in a normal state and in an epileptic state induced by kainic acid. VNS increased PLV in a normal state, particularly in high-γ band. In an epileptic state, VNS increased PLV in high-γ band, and decreased in δ and low-β bands. VNS modulates synchrony in a band-specific and state-dependent manner. VNS might keep cortical synchrony within the optimal state.**

I. Introduction

Vagus nerve stimulation (VNS) is a surgical treatment for intractable epilepsy[1]. An implantable pulse generator continuously and intermittently stimulates the left vagus nerve in the neck, which can alleviate epileptic seizures. Technical details about VNS are described in the Cyberonics's website (http://us.cyberonics.com/en/). Although its clinical efficacy and safety are well established, the underlying mechanisms of action in VNS have not been fully elucidated. Although decrease of paroxysmal spikes in electroencephalography (EEG) appears a year after start of VNS[2], there is no immediate change [3]. However, some kinds of effects to the cerebral cortex are expected to exist because ongoing seizure is aborted by VNS [4]. One possibility is that VNS modulates interaction among cortical neuronal population, or cortical synchrony, thus returning the epileptogenic cortex from an abnormally hyperexcitable state to a stable one.

Furthermore, beneficial effects of VNS include cognitive improvements for memory [5, 6] and decision making [7], modulation of mood [8, 9], and enhanced plasticity [10, 11]. The variable effects other than seizure control suggest that VNS plays more general roles in maintenance of homeostatic property of cortical synchrony. If so, VNS-induced modulation of cortical activity should be state-dependent in order to maintain neuronal synchrony within a normal range.

To examine our hypothesis, we investigate whether and how VNS modulates local synchrony in the cortex in a state dependent manner. We measured the local field potential (LFP) with high-spatial resolution using a microelectrode array in adult rats, and analyzed VNS-induced phase modulation at a local network level both in the normal model and in the epilepsy model induced by kainic acid (KA). Our hypothesis predicts that VNS enhances cortical synchrony in the normal model, whereas suppresses in the epilepsy model, where KA-induced local synchrony is much higher than the normal level.

II. Methods

All procedures were approved by our institutional committee and performed in accordance with "Guiding Principles for the Care and Use of Animals in the Field of Physiological Science" of the Japanese Physiological Society.

A. Animals and surgical procedure

Eight adult male Wister rats (270 – 330 g) underwent implantation surgery of VNS therapy systems (Cyberonics, Houston, TX, USA) under 1.5% isoflurane anesthesia. A stimulation electrode was coiled around the left vagus nerve, and pulse generator was implanted on the back subcutaneously.

One week after implantation surgery, right temporal lobe cortex was exposed under the same as previous anesthesia. Subsequently, a spike microelectrode with a grid of 10 x 10 within 4 mm x 4 mm was inserted at 4th layer in the cortex, and LFP was recorded with sampling rate of 1000 Hz.

We first investigated VNS effects in the spontaneous activities in the normal state. Then, rats received intraperitoneal (i.p.) injections of KA (12mg/kg). Forty five minutes after administration of KA, VNS effects were again investigated in the epileptic state.

B. Stimulation condition and epilepsy induction

The VNS stimulation was given in a series of electrical pulses with a current ranging from 0.25 mA to 2.0 mA, frequency ranging from 10 Hz to 30 Hz, and a constant pulse width of 130 μsec. VNS stimulation per session lasted 30 s in the normal state and 60 s in the epileptic model.

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C. Determination of auditory cortex area

The test area was confined within the auditory cortex (AC), which shared thalamo-cortical inputs from the medial geniculate body, because thalamo-cortical inputs from different regions may result in varied VNS effects. AC and non-auditory cortex (non-AC) were determined using by pure-tone stimulus. Frequency response area (FRA) was estimated using test stimuli of varied frequency and sound pressure. At each recording site of the microelectrode array, characteristic frequency (CF) was defined as the tone frequency that evoked multiple unit activities at the lowest intensity. Recording sites where CF was obtained were defined as AC.

D. Data analysis

We used Phase-locking value (PLV) as the index of the synchrony between electrodes. We applied band-pass filter and Hilbert transform to the obtained LFP, and the instantaneous phase (Φ) was calculated. $\Delta\Phi = (\Phi_{ch1} - \Phi_{ch2})$

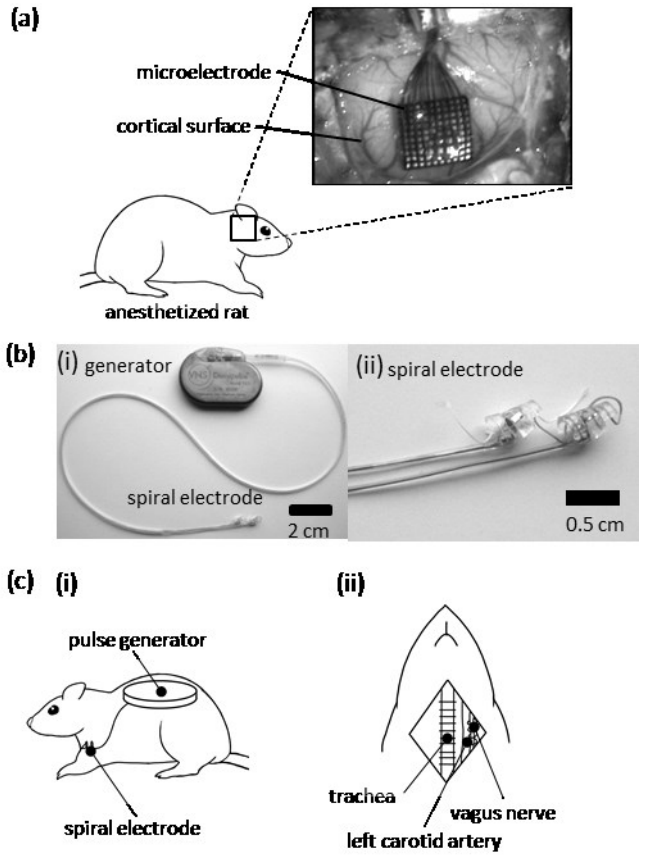

Figure 1 (a) Cortical mapping of neural activities using microelectrode array. (b) Vagus nerve stimulation (VNS) device (Cyberonics). (i) Whole view. The system consists of the generator and spiral electrode. (ii) Magnified view of the spiral electrode. (c) Implantation of VNS device. (i) Whole view. The generator is implanted subcutaneously on the dorsal side of rats. (ii) Anatomical landmarks of vagus nerve in a rat. Spiral electrodes are wrapped around the left vagus nerve.

PLV is defined as the formulae below:

$$PLV(ch1, ch2) = \left| \frac{1}{T} \sum_{t} e^{i(\Phi_{ch1} - \Phi_{ch2})} \right|$$

PLV was calculated in 7 frequency bands as follows: δ: 1 - 4 Hz, θ: 4 – 8 Hz α: 8 – 13 Hz, low-β: 13 – 21 Hz, high-β: 21 – 30 Hz, low-γ: 30 – 45 Hz, high-γ: 55 – 80 Hz. PLV between all pairs of electrodes among auditory cortex in varied frequency bands were calculated to characterize differences between pre-VNS and post-VNS phases. PLV_{mean} is defined as the formulae below:

$$PLV_{mean} = \frac{2}{N(N-1)} \sum_{ch1}^{N} \sum_{ch2}^{N} PLV(ch1, ch2)$$

To examine the effect of VNS in the normal state, PLV was calculated for 25 seconds of VNS-off phase immediately before and after VNS-on phase. To assess PLV changes over time in epileptic state, PLV was calculated every 5 minutes. To examine the effect of VNS in the epileptic state, a stimulation was given 45 minutes after administration of KA, and PLV was calculated for 25 seconds immediately before and after VNS.

III. Results

Figure 2 shows representative VNS modulation of cortical synchrony. In general, VNS increased PLV in the normal state: in Fig. 2 (a), (i), the topography of PLV between electrodes within the auditory cortex demonstrates that, in the normal-state high-γ band, pre-VNS PLV was not generally high, i.e., PLV < 0.55 in half of electrode pairs, while post-VNS PLV in most electrode pairs become higher, i.e., PLV > 0.55. In the epileptic-state low-β band, however, VNS occasionally decreased PLV: in Fig 2. (a), (ii), PLV between most electrode pairs was higher than 0.9 before VNS, while post-VNS PLV in most electrode pairs were less than 0.9. These trends of VNS modulation of PLV were consistently observed across subjects, as summarized in boxplots in Fig. 2 (b): In the normal-state high-γ band, PLV_{mean} tended to increas (0.51 ± 0.03 before VNS vs. 0.56 ± 0.03 after VNS); in the epileptic-state low-β band, PLV_{mean} tended to decreased (0.96 ± 0.05 before VNS vs. 0.91 ± 0.04 after VNS).

Figure 3 shows how the epileptic state involved in terms PLV. PLV gradually increased for 20 min after injection of KA irrespective of frequency bands: in θ band, PLV was 0.73 ± 0.02 at KA administration, subsequently PLV gradually increased up to 0.77 ± 0.04 in 45 minutes; in low-β band, PLV was 0.62 ± 0.02 at KA administration, subsequently PLV gradually increased up to 0.75 ± 0.03 in 45 minutes; in low-γ, PLV was 0.70 ± 0.02 immediately after KA, subsequently 0.77 ± 0.03 in 45 minutes.

Figure 4 demonstrated band-specific VNS modulation of PLV_{mean} in the normal state and in the epileptic state. In the normal state, VNS tended to increase PLV irrespective of bands with ΔPLV_{mean} ranging from 0.0169 ± 0.0282 (low-β band) to 0.0330 ± 0.0223 (high-γ band); in the high-γ band,

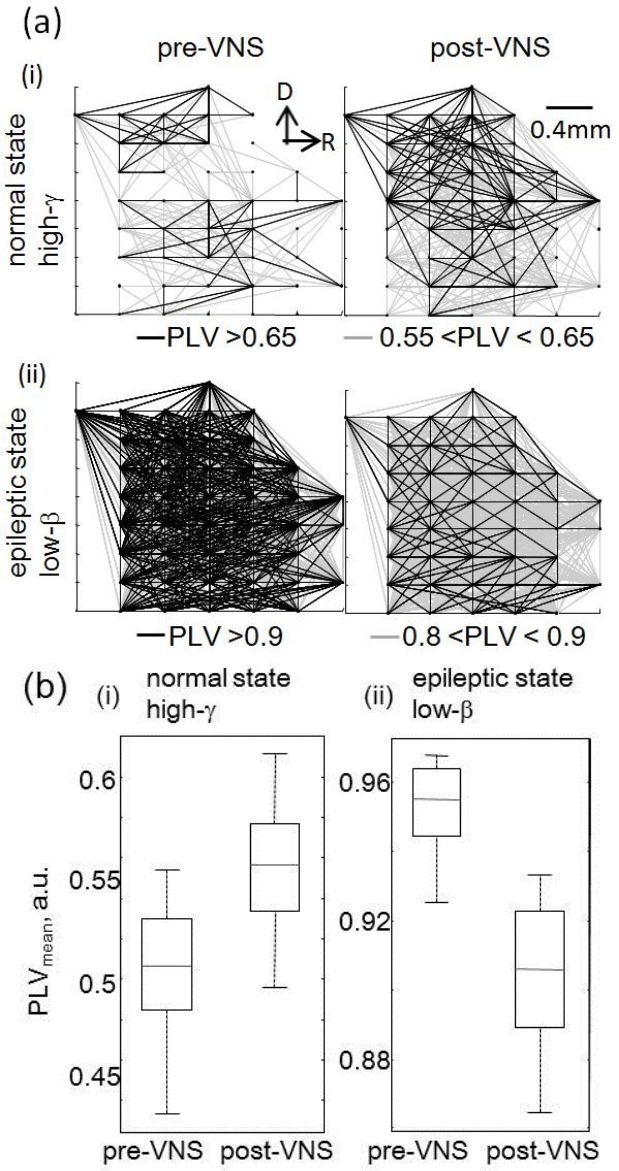

Figure 2 Representative VNS-induced modulation of the cortical synchrony in the auditory cortex. (a) Functional network based on PLV in the normal-state high-γ band (i) and in the epileptic-state low-β band (ii). PLV topographies before and after VNS are shown in the left and right columns, respectively. Black links indicate pairs of recording sites with high synchrony (PLV > 0.65), and gray links, with intermediate synchrony (0.55 < PLV < 0.65). VNS increased the normal-state high-γ band synchrony, but decreased the epileptic-state low-β band synchrony. (b) Boxplots of PLV_{mean} among subjects before and after VNS in the normal state (i) and in the epileptic state (ii). Stimulation conditions in the normal state were current of 0.5 mA, frequency of 10 Hz, and pulse width of 130 μsec, those in the epileptic state were current of 1.0 mA, frequency of 10 Hz, and pulse width of 130 μsec.

VNS-induced ΔPLV_{mean} was statistically significant ($P<0.05$ two-sided t-test). In the epileptic state, on the other hand, ΔPLV_{mean} ranged from -0.0421 ± 0.0270 (δ band) to 0.0164 ± 0.0223 (high-γ band), suggesting that VNS did not always enhance the synchrony. In particular, VNS-induced significant decreases of PLV were observed in the δ and low-β bands ($P<0.05$ two-sided t-test).

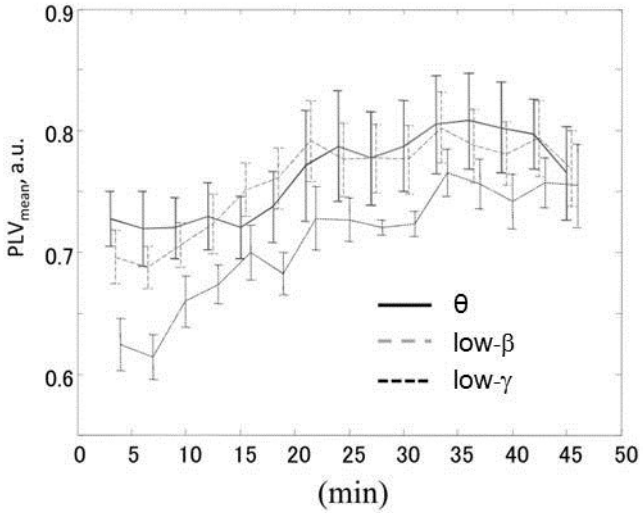

Figure 3 Evolution of epileptic state. Time course of PLV_{mean} in the θ (black solid line), in the low β (gray dotted line) and low γ (black dotted line) bands were shown after KA injection. PLV_{mean} gradually increased after until 20 minutes in both bands.

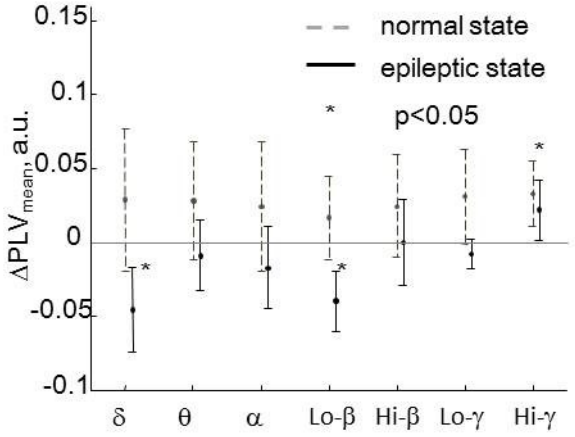

Figure 4 Band specific ΔPLV_{mean} changes by VNS in the normal state (gray dotted line) and epileptic state (black solid line). ΔPLV_{mean} = PLV_{mean} of post-VNS – PLV_{mean} of pre-VNS. In the normal state, ΔPLV_{mean} increased by VNS in all band, and significantly in high-γ band. In the epileptic state, ΔPLV_{mean} significantly decreased by VNS in δ, low-β band. Asterisks indicated that ΔPLV_{mean} was significantly higher or lower than zero (two-sided t-test).

IV. Discussion

In the present study, we demonstrated that VNS modulates local phase synchrony in the cerebral cortex in a state-dependent manner. In the normal state, VNS consistently increased PLV regardless of frequency bands. On the other hand, in the epileptic state, VNS decreased PLV in δ and low-β bands.

The function of synchrony is likely band-specific. The synchrony in low frequency bands may play important roles in global information processing, whereas there exist indication that synchrony in high frequeny bands is a sign of local computation[12].

In particular, the γ-band synchrony is implicated in the activity of GABAergic inhibitory interneurons: excitatory-inhibitory interaction in the low-γ band, and inhibitory-inhibitory interaction in the high-γ band [12].

In the normal state, VNS increased the synchrony in the high-γ band, implying that VNS improves local information processing. Such modulation may account for VNS-induced improvements of cognitive functions [13]. Additionally, the increase of the high-γ band synchrony is a sign of GABAergic enhancements, playing some roles to terminate the seizure [14]. Consistent with this notion, our experiments showed that VNS in the epileptic model increased the γ-band synchrony. Yet, the KA-induced synchronization was much larger than VNS-induced synchronization. The underlying mechanisms of these synchronies are probably different.

In contrast to the high frequency bands, VNS in the epileptic state decreased synchrony in δ and low-β bands. Functionally, γ-band local synchrony is nested in the δ band global synchrony. The β band is possibly related to top-down feedback signaling from the higher-order brain areas. Provided that these bands are crucial to the global information processing, our results suggest that VNS in the epileptic state induces inter-regional desynchronization. Such desynchronization may prevent a seizure from developing further.

Thus, VNS-induced modulation of cortical synchrony is band-specific and state-dependent. Such complicated effects may be caused by multiple mechanisms of neuromodulation. The neurons in the nucleus tractus solitarius send afferents to the locus coeruleus, raphe nuclei, and the basal forebrain [15], which trigger releases of noradrenaline, serotonin and acetylcholine, respectively. These neuromodulations are involved in antidepressant activity and plasticity as well as antiepileptic treatments.

V. Conclusion

In the present study, we investigate whether and how VNS modulates local synchrony in the auditory cortex of rats using a microelectrode array with a grid of 10 x 10 recording sites in 4 x 4 mm area. Our experiments demonstrated that VNS modulates cortical synchrony in a band-specific and state-dependent manner: VNS in the normal state consistently enhanced cortical synchrony irrespective of bands, whereas VNS in the epileptic state suppressed the synchrony in δ and low-β bands. These results suggest that VNS plays a crucial role in maintaining homeostatic properties, by which the cortical synchrony is kept within the optimal state.

Acknowledgment

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