

1 **Left Cervical Vagal Nerve Stimulation Reduces Skin Sympathetic Nerve Activity in**
2 **Patients with Drug Resistant Epilepsy**

3 **Short title: VNS Reduces Sympathetic Nerve Activity**

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1 **Abstract**

2 **Background:** We recently reported that the skin sympathetic nerve activity (SKNA) can
3 be used to estimate the sympathetic tone in humans. In animal models, vagal nerve
4 stimulation (VNS) can damage the stellate ganglion, reduce stellate ganglion nerve
5 activity and suppress cardiac arrhythmia. Whether or not VNS can suppress
6 sympathetic tone in humans remain unclear.

7 **Objective:** To test the hypothesis that VNS suppresses SKNA in patients with drug
8 resistant epilepsy.

9 **Methods:** We used electrocardiogram (ECG) patch electrodes to continuously record
10 SKNA in 26 patients with drug resistant epilepsy who were admitted for video
11 electroencephalographic monitoring. Among them, 6 (2 men, age 40 ± 11 years) were
12 previously treated with VNS and 20 (7 men, age 37 ± 8 years) did not. The signals from
13 ECG leads I and II were filtered to detect SKNA.

14 **Results:** The VNS had an ON-time of 30 s and OFF-time of 158 ± 72 s, with output of
15 1.92 ± 0.42 mA at 24.17 ± 2.01 Hz. The average SKNA (aSKNA) during VNS OFF-time
16 was $1.06\ \mu\text{V}$ [95% confidence interval, CI, 0.93 to 1.18] in lead I and $1.13\ \mu\text{V}$ [CI, 0.99
17 to 1.27] in lead II, which was significantly lower than $1.38\ \mu\text{V}$ [CI, 1.01 to 1.75, $p=0.036$]
18 and $1.38\ \mu\text{V}$ [CI, 0.98 to 1.78, $p=0.035$] of the control Group, respectively. The heart
19 rate was 65 bpm [CI, 59 to 71] in VNS group, significantly lower than 77 bpm [95% CI,
20 71 to 83] in control group.

21 **Conclusion:** Patients with VNS had significantly lower SKNA than patients without VNS.
22

1 **Key words:** Autonomic nervous system; neuromodulation; sudden unexpected death in

2 epilepsy

3

4

ACCEPTED MANUSCRIPT

Introduction

1
2 Vagal nerve stimulation (VNS) is commonly used to manage patients with drug resistant
3 epilepsy and has been reported to reduce the incidence of sudden unexpected death in
4 epilepsy (SUDEP).^{1, 2} Recent studies showed that there is significant interictal cardiac
5 electrical instability (elevated T wave alternans) in patients with drug-resistant epilepsy,
6 which was effectively suppressed by VNS.³ Because T wave alternans is enhanced by
7 sympathetic activation,⁴ these findings suggest that VNS might be associated with
8 reduced sympathetic tone. Consistent with that hypothesis, Clancy et al⁵ reported that
9 non-invasive stimulation of the auricular branch of the vagal nerve suppressed muscle
10 sympathetic nerve activity in normal human volunteers. However, as far as we know,
11 there was no study that demonstrated reduced sympathetic nerve activity in patients
12 with chronically implanted vagal nerve stimulators. The cervical and thoracic vagal
13 nerves contain significant sympathetic components.^{6, 7} In ambulatory dogs, left cervical
14 VNS could be associated with high rate of activation of the left stellate ganglion.⁸
15 Repeated high rate of excitation might cause neurotoxicity and promote neuronal cell
16 death.⁹ Consistent with the neurotoxicity hypothesis, we recently reported that there
17 was significant damage to the left stellate ganglion in dogs receiving chronic VNS.¹⁰ The
18 stellate ganglion damage was associated with a significant reduction of the stellate
19 ganglion nerve activity and ventricular rate in atrial fibrillation (AF). However, it is difficult
20 to record the stellate ganglion nerve activity in humans. We recently developed a new
21 method (neuECG) to simultaneously record the skin sympathetic nerve activity (SKNA)
22 and electrocardiogram (ECG) in ambulatory dogs.^{11, 12, 13} and in humans.¹⁴ The purpose
23 of the present study was to record SKNA from patients with drug resistant epilepsy to

1 test the hypothesis that the SKNA is lower in patients with VNS than in patients without
2 VNS.

3

4 **Methods:**

5 This research protocol was approved by the Institutional Review Boards of the Indiana
6 University (IU) School of Medicine. We prospectively enrolled 26 patients with drug
7 resistant epilepsy to participate in the study. All patients were recruited after they were
8 admitted to the Epilepsy Monitoring Unit of the IU-Methodist Hospital to record video
9 electroencephalogram. As part of the standard care, anti-epileptic medications were
10 completely or partially withdrawn while the patients were confined to bed with bathroom
11 privileges. The recordings were made continuously except when the patients went to
12 the bathroom. The neuECG was recorded using standard ECG patch electrodes
13 connected to a modified ME6000 (Biomation, Almonte, Ontario) recorder with a wide
14 bandwidth (2 KHz) and high sampling rate (10 KHz). All 26 patients had at least 24-hr of
15 recording (Table 1). Among them, six had previously underwent VNS implantation, while
16 20 did not receive it. The standard VNS protocol was 30 s on and 3-5 min off.¹⁵
17 Because this is an observational study, the off-time was variable based on the clinical
18 needs. The baseline SKNA of 19 patients in the latter group was included in a previous
19 report.¹⁴ A total of 8 patients had seizure during the recording period. We excluded the
20 data recorded 40-min before to 40-min after the seizure from analyses.

21

22 **Data Analysis**

1 Because VNS causes large artifacts on the neuECG recording, we only analyzed data
2 during the VNS-off time. The data were analyzed with a custom written software which
3 selected the R waves and calculated the RR intervals and heart rate automatically. All
4 selections were then confirmed by manual examination to exclude artifacts. The
5 average SKNA (aSKNA) and heart rate were determined for 2 min at the beginning, 20-
6 min past and 40-min past the hour for 24 hours, resulting in 72 data points per patient. If
7 the patient went to the bathroom during the analyses window, we used the data
8 acquired after the resumption of recording for analyses. We bandpass filtered the
9 recorded neuECG signals between 200 Hz to 1000 Hz and also between 500 Hz to
10 1000 Hz to display SKNA. As shown in our previous report,¹⁴ the sensitivity of detecting
11 SKNA is higher with the former filter setting but the specificity is higher with the latter
12 filter setting. The same signals were bandpass filtered between 0.5 Hz and 150 Hz to
13 display the surface ECG. For quantitative analyses, we integrated all digitized SKNA
14 signals over each 1-min window and divided the results (in μV) by the number of
15 samples in that window (10,000 X 60, or 60,000 samples) to obtain the aSKNA. The
16 quantitative analyses were done for both filter settings. The aSKNA included both the
17 signal and noise. The noise level of the recording device (recorded when the two poles
18 were shorted) is 1.17 μV (bandpass 200-1000 Hz) and 0.74 μV (bandpass 500-1000
19 Hz). Because vagal nerve stimulation is unlikely to change the noise level, the
20 differences between VNS group and control group are due to the differences of nerve
21 activity. Heart rate was determined in the same 2-min windows, resulting in 72 data
22 points per patient. The average heart rate of these 72 data points were used as the

1 heart rate for that patient.

2

3 **Statistical Analysis**

4 Linear regression was used to study the relationship between heart rate and SKNA
5 activity for each patient. Linear mixed-effects models were used to compare SKNA
6 activity between control and VNS stimulation groups where patients were treated as
7 random effects. Generalized additive mixed-effects models were used to characterize
8 the circadian pattern. T test was used to compare average SKNA activities and heart
9 rate (over 24-hour period), and the correlation coefficients between SKNA and heart
10 rate. Two-sided p values ≤ 0.05 were considered statistically significant.

11

12 **Results:**

13 The VNS group included 6 patients (2 men, age 40 ± 11 years). The control group
14 included 20 patients (7 men, age 37 ± 8 years) (Table 1). None of the patients had
15 cardiovascular diseases. The admission diagnoses were generalized tonic-clonic
16 seizure (N=1), complex partial seizure with secondarily generalized tonic-clonic seizure
17 (N=2) and complex partial seizure (N=3) in VNS group; generalized tonic-clonic seizure
18 (N=6), complex partial seizure with secondarily generalized tonic-clonic seizure (N=6)
19 and complex partial seizure (N=8) in control group. Patients in the VNS group had
20 received VNS treatment for an average of 81 ± 39 months (range 20 to 121 months).
21 VNS was programmed to an ON-time of 30 s and OFF-time of 158 ± 72 s. The output
22 was 1.92 ± 0.42 mA with a frequency of 24.17 ± 2.01 Hz.

23

1 *Patterns of SKNA in control group*

2 **Figure 1** shows a typical example of SKNA episodes recorded in a patient without VNS.
3 The SKNA can be either prolonged or episodic. Panel A shows a prolonged nerve
4 discharge episode associated with heart rate acceleration. The data from the red
5 rectangles are enlarged and shown in Panel B. Panel C shows short bursts of SKNA
6 which were also associated with heart rate elevation. The data from the red rectangles
7 were enlarged and shown in Panel D. These SKNA episodes were not pulse
8 synchronous and were morphologically similar to that recorded from the superficial
9 branches of radial nerves with microneurography techniques.¹⁶ The artifacts on surface
10 ECG (red dots) suggest that some of the SKNA episodes occurred coincidentally with
11 the physical activity.

12
13 *Patterns of SKNA in VNS group*

14 As compared with patients in control group, the VNS group had reduced SKNA
15 episodes. **Figure 2A** shows SKNA and heart rate in control patient who had an active
16 SKNA and corresponding heart rate elevation. Similar episodes were rare in VNS
17 patients. When present, the SKNA were also associated with heart rate changes. The
18 VNS episodes typically interrupts the SKNA episode and reduces the heart rate
19 variations (Figure 2B). In most patients with VNS, the SKNA episodes were infrequently
20 observed. **Supplement Figure 1** shows a longer term (5-min) recording from a VNS
21 patient (Panel A) and a control patient (Panel B). In VNS Patient, there were very low
22 amplitudes of SKNA during the VNS ON and OFF times, with only little heart rate

1 variations. In comparison, a control patient had high SKNA activity and robust heart
2 responses to SKNA.

3

4 *Correlation between heart rate and SKNA*

5 There was a positive correlation between heart rate and SKNA in all patients studied.
6 We tested the correlation using two different filter settings for SKNA detection. For all
7 patients studied, the average Pearson's correlation coefficients between aSKNA (200
8 Hz-1000 Hz bandpass filter) and heart rate was 0.52 [CI, 0.39 to 0.65] in VNS group,
9 and 0.72 [CI, 0.65 to 0.79, $p=0.024$] in control group. The correlation coefficients were
10 0.45 [CI, 0.35 to 0.56] in VNS group, and 0.60 [CI, 0.43 to 0.66, $p=0.019$] in control
11 group after 500 Hz-1000 Hz bandpass filtering. **Supplement Figure 2** shows the typical
12 examples of the correlation between heart rate and aSKNA in both groups of patients. A
13 total of 144 dots were in each graph. Panel A and B were obtained a after bandpass
14 filtering between 200 Hz and 1000 Hz. Panel C and D were obtained a after bandpass
15 filtering between 500 Hz and 1000 Hz. Each dot represents the average with a 1-min
16 window.

17

18 *Circadian variation of SKNA*

19 **Figure 3** summarizes hourly averaged aSKNA over a 24-hour period from lead I
20 (Panels A and B) and lead II (Panels C and D) in all patients studied. With either filter
21 setting, the aSKNA of patients in the VNS group for both filter settings was significantly
22 ($p<0.00001$) lower than the aSKNA in patients of the control group. In both VNS and
23 control groups, aSKNA showed significant ($p<0.00001$) circadian variations.

1

2 *Comparison of aSKNA between control group and VNS group*

3 **Figure 4** shows the range of aSKNA in Lead I, Lead II and heart rate of all patients
4 studied. The control group had significantly higher aSKNA and heart rate than the VNS
5 group. When bandpass filtered between 200 Hz and 1000 Hz, the aSKNA during VNS
6 OFF-time was 2.44 μV [95% confidence interval, CI, 2.13 to 2.74] in lead I and 2.49 μV
7 [CI, 2.05 to 2.94] in lead II in the VNS group. These aSKNA values were significantly
8 lower than 3.72 μV [CI, 2.58 to 4.87, $p=0.012$] and 3.26 μV [CI, 2.14 to 4.39, $p=0.014$],
9 respectively, of the control Group. When filtered between 500 Hz and 1000 Hz, the
10 aSKNA during VNS OFF-time was 1.06 μV [CI, 0.93 to 1.18] in lead I and 1.13 μV [CI,
11 0.99 to 1.27] in lead II, which were significantly lower than 1.38 μV [CI, 1.01 to 1.75,
12 $p=0.036$] and 1.38 μV [CI, 0.98 to 1.78, $p=0.035$], respectively, of the control Group. The
13 average heart rate was 65 bpm [CI, 59 to 71] in VNS group and 77 bpm [CI, 72 to 83] in
14 the control group ($p=0.013$). The average systolic blood pressure of VNS patients and
15 non-VNS patients were 116 ± 10 mmHg and 127 ± 16 mmHg, respectively ($p=0.1369$).
16 The average diastolic blood pressure of VNS patients and non-VNS patients were 69 ± 6
17 mmHg and 75 ± 10 mmHg, respectively ($p=0.1807$).

18 Because patients with hypertension may have heightened sympathetic tone,¹⁷ we
19 removed patients with hypertension and repeated the analyses of the remaining
20 patients (5 with VNS and 14 without VNS). Using 200-1000 Hz filter setting, the aSKNA
21 in Lead I was 2.41 μV (CI 2.01 to 2.80) in VNS group and 3.81 μV (CI 3.36 to 4.27) in
22 non-VNS group ($p=0.023$). The aSKNA in Lead II was 2.37 μV (CI 1.96 to 2.78) in VNS
23 group and 3.64 μV (CI 2.79 to 4.49) in non-VNS group ($p=0.001$). Using 500-1000 Hz

1 filter setting, the aSKNA in Lead I was 1.02 μV (CI 0.92 to 1.12) in VNS group and 1.69
2 μV (CI 1.29 to 2.09) in non-VNS group ($p=0.049$). The aSKNA in Lead II was 1.08 μV
3 (CI 1.04 to 1.12) in VNS group and 1.57 μV (CI 1.34 to 1.81) in non-VNS group
4 ($p=0.015$). Therefore, excluding hypertensive patients did not change the conclusion of
5 the study.

6

7 **Discussion:**

8 The results of the present study show that patients with left VNS had lower heart rate
9 and aSKNA than patients without VNS. These findings suggest that chronic intermittent
10 VNS can reduce sympathetic tone in humans.

11

12 *Vagal nerve stimulation and cardiovascular diseases*

13 VNS is a well-established therapeutic option for patients with drug resistant epilepsy^{18, 19}
14 but also had significant effects on cardiovascular function. Studies in animal models
15 showed that VNS might be helpful in managing acute ischemia and in prolonging
16 survival in heart failure,²⁰⁻²³ although recent clinical trials failed to confirm those
17 findings.²⁴ Another potential application of VNS in heart diseases is in the rate and
18 rhythm control of AF.^{10, 25, 26} VNS has been reported to reduce the incidence of sudden
19 unexpected death in epilepsy (SUDEP),^{1, 2} but others failed to confirm that finding.²⁷

20

21 *Mechanisms of VNS*

22 The vagal nerve is a complex anatomical structure. It is generally accepted that
23 parasympathetic activation is responsible for some of the therapeutic effects of VNS.

1 However, if parasympathetic activation is responsible for heart rate control, then the
2 therapeutic effects of the VNS should be limited only to the time when VNS is turned on.
3 When VNS is turned off, ventricular conduction should accelerate, leading to a loss of
4 therapeutic benefit. Recordings in ambulatory dogs showed that the ventricular
5 responses during AF was reduced even when VNS was off.¹⁰ One possibility for this
6 carry over effect is that stimulation of the sympathetic component in the vagal nerve
7 may activate the ganglion cells in the SG at high rates,⁸ which may cause neurotoxicity,⁹
8 damages stellate ganglion to reduce the stellate ganglion nerve activity. In addition to
9 interacting with the stellate ganglion, VNS also targets convergent local circuit neurons
10 of the intrinsic cardiac nervous system to mitigate the potential for neurally induced
11 AF.²⁸

13 *Skin sympathetic nerve recording*

14 The skin is well innervated by sympathetic nerve fibers.^{29, 30} The somata of the nerves in
15 the upper extremities and thorax resides in the cervical and stellate ganglia.³¹ Our
16 recent studies showed that SKNA could be detected in humans using conventional
17 patch electrodes using a recording system with high sampling rate and wide frequency
18 bandwidth.^{32, 33} High pass filtering could eliminate the ECG signals and retain high
19 frequency signals that included both muscle and nerve activities. Like most diagnostic
20 tests, specificity could be increased only at the cost of reduction in sensitivity and vice
21 versa. A wider bandpass filter setting (200 Hz to 1000 Hz) allowed us to increase the
22 sensitivity of detecting SKNA but reduced specificity because the frequency of muscle
23 activities could reach 400 Hz.³⁴ A narrow bandpass filter setting (500 Hz to 1000 Hz)

1 was more specific but might be less sensitive in detecting the SKNA. We have therefore
2 used both filter settings to detect nerve activity in the present study. With either filter
3 setting, we were able to document robust SKNA in control patients and reduced SKNA
4 in patients with VNS. These findings suggest that, similar to VNS in ambulatory dogs,¹⁰
5 VNS in humans also suppresses sympathetic tone and maybe potentially useful in
6 cardiac arrhythmia control.

7

8 *SKNA and heart rate*

9 The heart rate is controlled by both sympathetic and parasympathetic nerve activities.
10 While the SKNA is a useful measure of sympathetic tone, it does not adequately
11 measure all nerve activities that control the sinus node. Therefore, the average
12 correlation coefficient between aSKNA and heart rate was around 0.45-0.72 in different
13 groups and using different filter settings. Similar correlation coefficients were also
14 observed between sympathetic nerve activities and heart rate in ambulatory dogs.¹¹ It
15 is unclear why VNS patients had reduced heart rate responses to SKNA. However,
16 because VNS may damage the stellate ganglion and reduce sympathetic outflow,¹⁰ the
17 damaged stellate ganglion might not be able to generate enough sympathetic tone to
18 overcome the parasympathetic influence of the sinus node.

19

20 *Limitations of the study*

21 We do not have the histological sections or direct recordings of the stellate ganglion in
22 patients with VNS. Therefore, it is not possible to determine if VNS has caused stellate
23 ganglion damage or a reduction of stellate ganglion nerve activity. None of the patients

1 died suddenly or had significant arrhythmias during the recording. Therefore, the
2 relationship between SKNA and SUDEP remains unclear. The electrode impedance
3 was not measured in the present study. However, we have previously reported that the
4 average impedance of the electrodes as the one used in the present study was 37 ± 8
5 k Ω (range 28–52 k Ω).³²

6

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17

18

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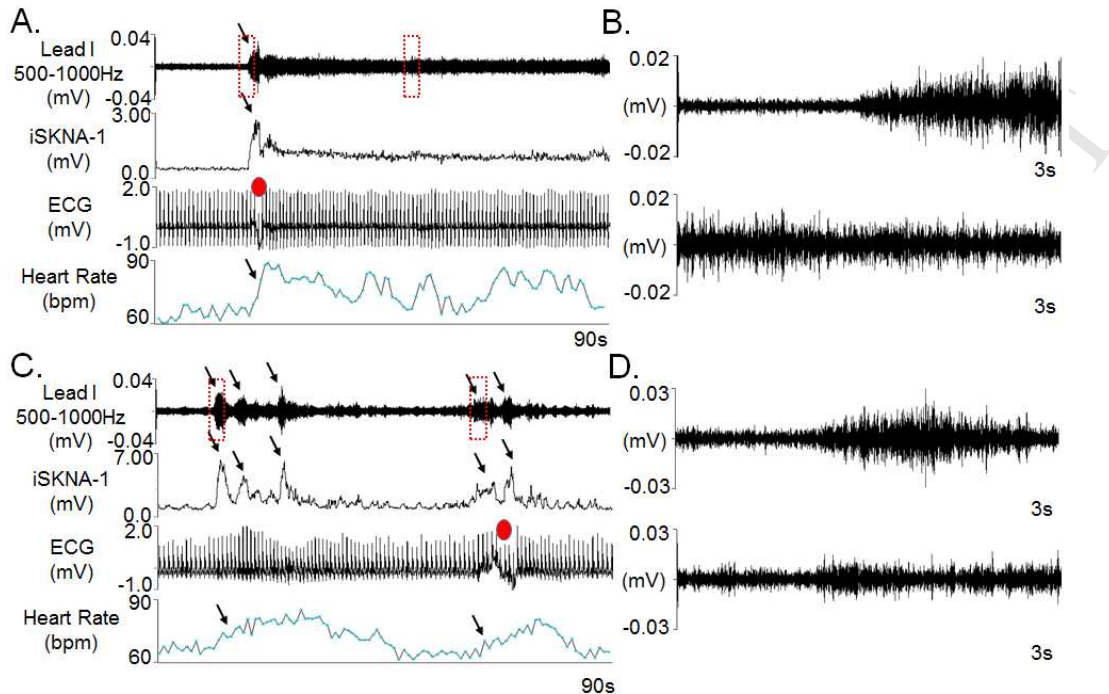
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1 **Table 1:** Clinical characteristics of the study population.

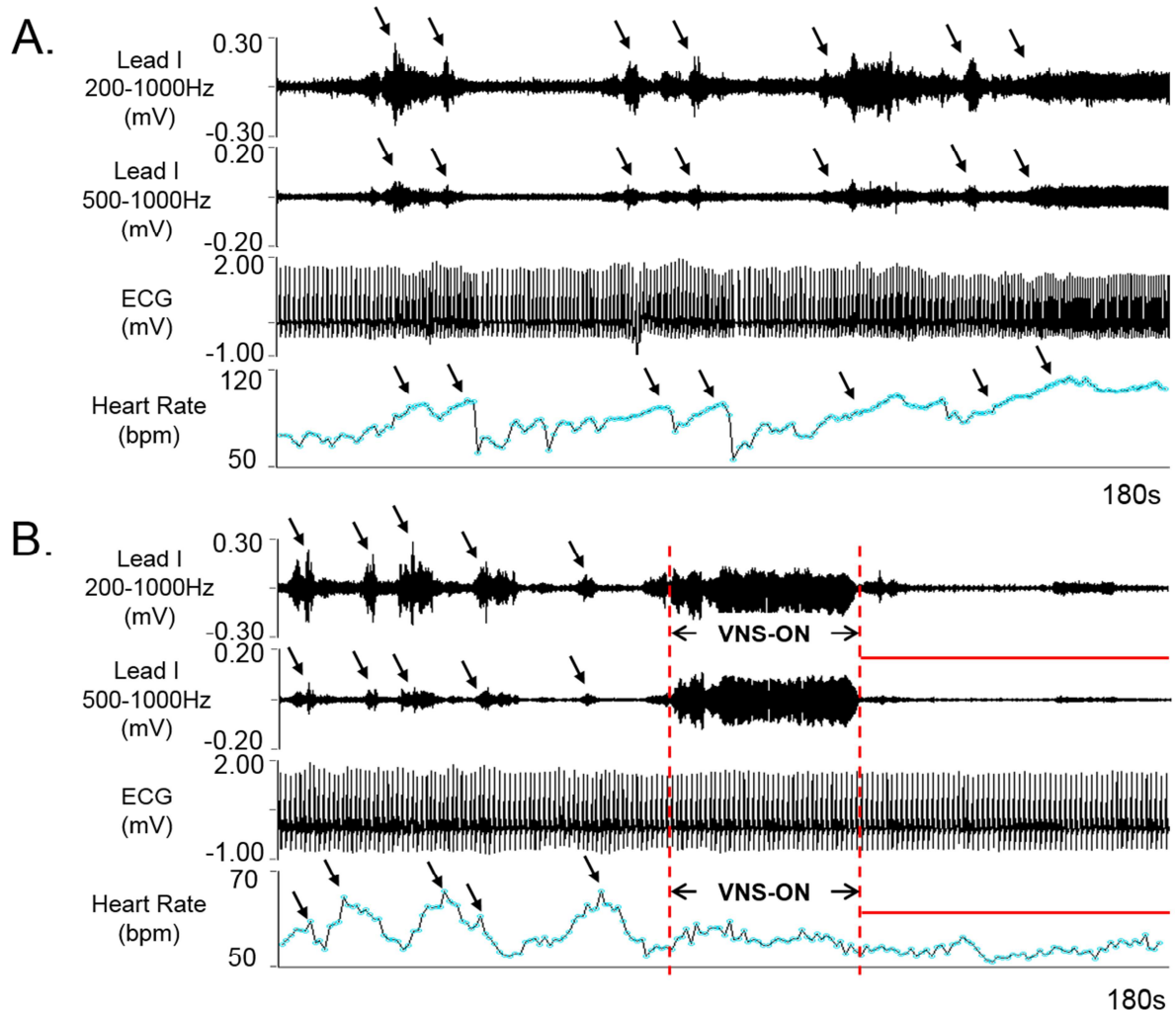
Pt No.	Age (yrs)	Gender	Group	Epilepsy History (yrs)	Seizure type	Hypertension	Diabetes	Organic Heart disease
1	25	M	VNS	21	Generalized	No	No	No
2	40	F	Control	24	Complex Partial	No	No	No
3	47	F	Control	27	Complex Partial with secondary generalization	No	No	No
4	53	M	VNS	41	Complex Partial with secondary generalization	Yes	No	No
5	44	M	VNS	30	Complex Partial with secondary generalization	No	No	No
6	30	F	Control	17	Generalized	No	No	No
7	41	F	Control	2	Complex Partial	Yes	No	No
8	36	F	VNS	12	Complex Partial	No	No	No
9	24	F	Control	1	Complex Partial	No	No	No
10	37	F	Control	5	Complex Partial	No	No	No
11	26	F	Control	2	Generalized	No	Yes	No
12	68	M	Control	48	Complex Partial with secondary generalization	No	No	No
13	40	F	Control	1	Complex Partial	Yes	Yes	No
14	18	M	Control	15	Generalized	No	No	No
15	38	F	Control	18	Complex Partial with secondary generalization	No	No	No
16	34	F	Control	7	Complex Partial	No	No	No
17	29	M	Control	26	Generalized	Yes	No	No
18	27	M	Control	21	Complex Partial with secondary generalization	No	No	No
19	36	M	Control	6	Complex Partial with secondary generalization	Yes	No	No
20	25	M	Control	10	Generalized	No	No	No
21	29	F	VNS	16	Complex Partial	No	No	No
22	44	F	Control	1	Complex Partial	Yes	No	No
23	46	F	Control	27	Complex Partial with secondary generalization	No	No	No
24	50	F	VNS	36	Complex Partial	No	No	No
25	53	F	Control	33	Complex Partial	Yes	Yes	No
26	38	M	Control	37	Generalized	No	No	No

- 1 The patient (pt) numbers correspond to the sequence of recruitment. F, female; M, male;
 2 sec, secondary; VNS, vagal nerve stimulation; Yrs, years



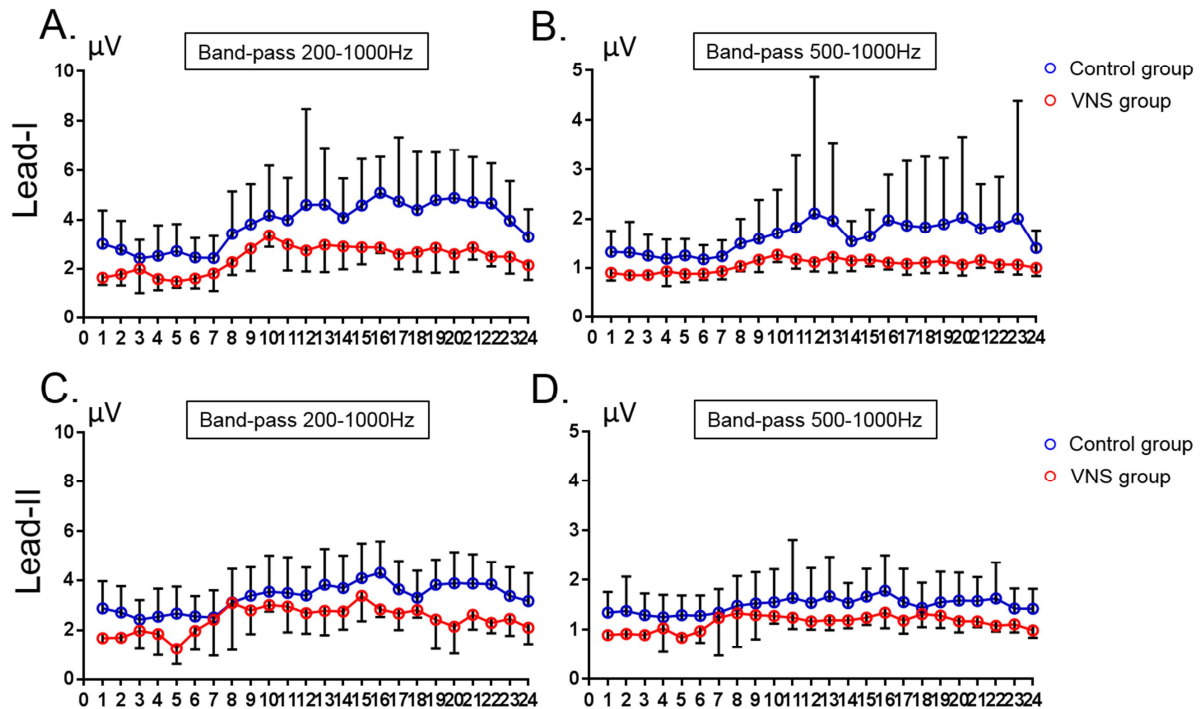
- 3
 4 **Figure 1:** Patterns of baseline SKNA. All data were obtained from ECG Lead I. **A** shows
 5 persistent SKNA associated with heart rate acceleration. The digitized signals were
 6 bandpass between 500-1000 Hz to display the high frequency nerve activities. The
 7 integrated SKNA of Lead I (iSKNA-I) display the sum of SKNA over each 100 ms
 8 window, which is a common method to display nerve activities in microneurography
 9 studies.³⁵ The same signals from ECG Lead I were bandpass filtered from 0.5 to 150 Hz
 10 to display surface ECG. Note the artifact on ECG (red dot) suggests physical activity at
 11 the beginning of the episode. **B** shows the data in the two red boxes of **A** in greater
 12 detail. **C** shows short bursts of SKNA associated with corresponding heart rate elevation
 13 (arrows). **D** shows the data in the two red boxes of **C** in greater detail. (SKNA=skin

- 1 sympathetic nerve activity, iSKNA= integrated skin sympathetic nerve activity, ECG=
 2 electrocardiogram.)



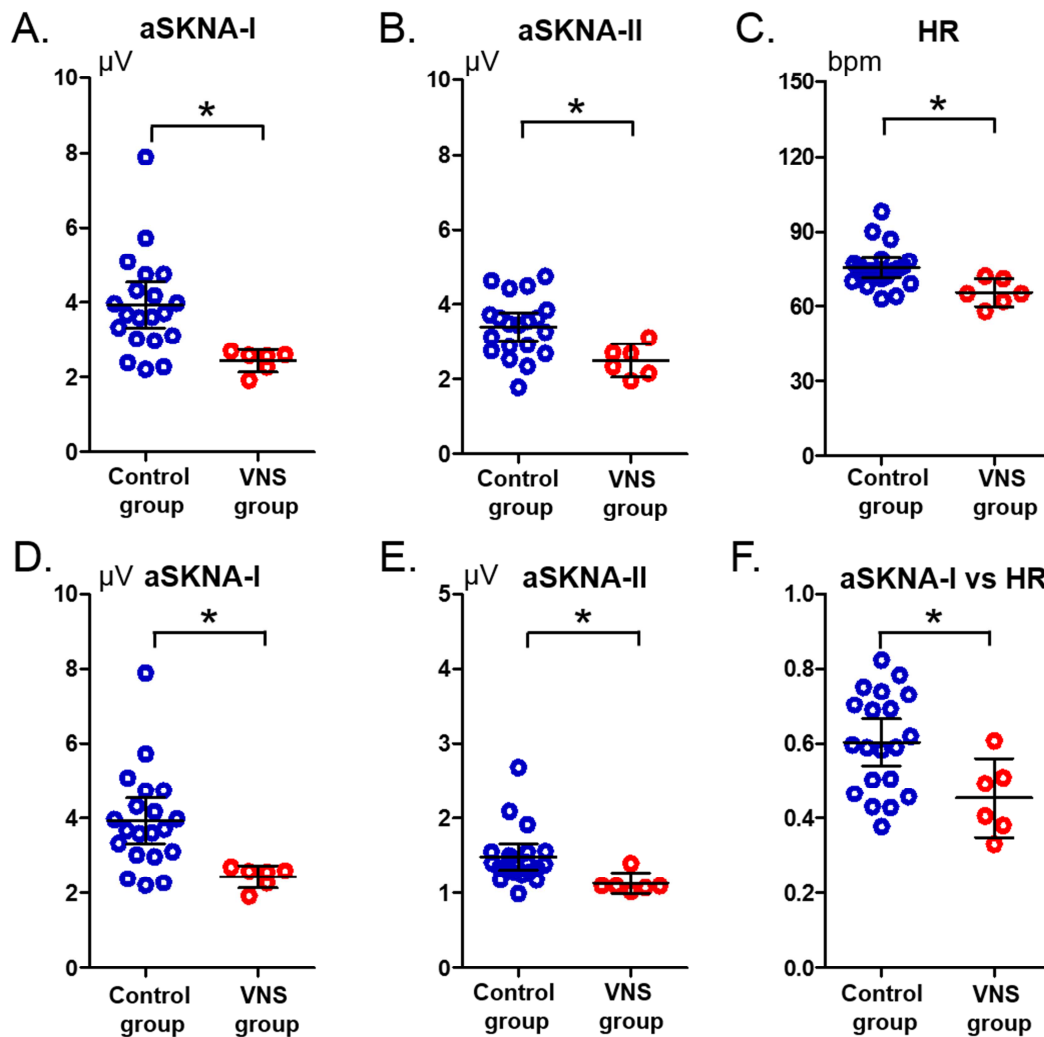
- 3
- 4 **Figure 2:** Effects of VNS on nerve activities. **A** shows recording made on ECG Lead I in
 5 a control patient. There were nerve discharges associated with episodic heart rate
 6 elevation (arrows). Persistent SKNA occurred towards the end of the recording. **B**
 7 shows a recording from VNS patient. Note that there were intermittent episodic SKNA
 8 prior to VNS, but no SKNA after VNS. These intermittent heart rate elevations were
 9 noted prior to VNS, corresponding to the SKNA episodes. Little heart rate variations

- 1 were noted after VNS (red bar). (SKNA=skin sympathetic nerve activity, iSKNA=
 2 integrated skin sympathetic nerve activity, ECG= electrocardiogram.)



- 3
 4 **Figure 3:** Hourly averaged nerve activities from Lead I and II in all patients studied. **A**
 5 **and B** show SKNA from Lead I while **C and D** show SKNA from Lead II. The filter
 6 settings are labeled in each panel. With either filter setting, the aSKNA was higher in the
 7 control group than in the VNS group. A significant circadian variation was noted in each
 8 graph. (Lead I: average SKNA at ECG Lead I. Lead II: average SKNA at ECG Lead II).

9



1

2 **Figure 4:** Average skin sympathetic nerve activities (aSKNA) and heart rate recorded of
 3 all patients studied. Each dot represents the aSKNA of a patient over a 24-hr period. **A**
 4 and **B:** The SKNA was bandpass filtered between 200 Hz to 1000 Hz. **D** and **E:** The
 5 SKNA was bandpass filtered between 500 Hz to 1000 Hz. **C:** Heart rate of both groups.
 6 **F:** Correlation coefficients of the Pearson's correlation test of both groups. Horizontal
 7 bars represent mean and 95% confidence interval. *: p<0.05. (aSKNA-I: average skin
 8 sympathetic nerve activity at ECG Lead I. aSKNA-II: average skin sympathetic nerve
 9 activity at ECG Lead II)