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Laryngeal Muscle-Evoked Potential Recording as an Indicator of Vagal Nerve Fiber Activation

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ABSTRACT

Background: Vagus nerve stimulation (VNS) is an adjunctive therapy for drug-resistant epilepsy. Noninvasive evoked potential recordings in laryngeal muscles (LMEPs) innervated by vagal branches may provide a marker to assess effective vagal nerve fiber activation. We investigated VNS-induced LMEPs in patients with epilepsy in acute and chronic settings.

Materials and Methods: A total of 17 of 25 patients underwent LMEP recordings at initiation of therapy (acute group); 15 of 25 patients after one year of VNS (chronic group); and 7 of 25 patients were tested at both time points (acute + chronic group). VNS-induced LMEPs were recorded following different pulse widths and output currents using six surface laryngeal EMG electrodes to calculate input/output curves and estimate LMEP latency, threshold current for minimal ($I_{threshold}$), half-maximal (I_{50}), and 95% of maximal (I_{95}) response induction and amplitude of maximal response (V_{max}). These were compared with the acute + chronic group and between responders and nonresponders in the acute and chronic group.

Results: VNS-induced LMEPs were present in all patients. $I_{threshold}$ and I_{95} values ranged from 0.25 to 1.00 mA and from 0.42 to 1.77 mA, respectively. Estimated mean LMEP latencies were 10 ± 0.1 milliseconds. No significant differences between responders and nonresponders were observed. In the acute + chronic group, $I_{threshold}$ values remained stable over time. However, at the individual level in this group, V_{max} was lower in all patients after one year compared with baseline.

Conclusions: Noninvasive VNS-induced LMEP recording is feasible both at initiation of VNS therapy and after one year. Low output currents (0.25–1.00 mA) may be sufficient to activate vagal Aα-motor fibers. Maximal LMEP amplitudes seemed to decrease after chronic VNS therapy in patients.

Keywords: Biomarkers, epilepsy, laryngeal muscle-evoked potentials, response, vagus nerve stimulation

Conflict of Interest: Jean Delbeke and Frank Dewaele are consultants for Synergia Medical. Frank Dewaele has been a consultant for LivaNova. Ann Mertens has received registration grants from MagVenture to participate in conferences on Transcranial Magnetic Stimulation. Robrecht Raedt, Kristl Vonck, and Paul Boon have received free devices for research studies in normal volunteers from Cerbomed. Kristl Vonck and Paul Boon have received consultancy fees from LivaNova and Synergia Medical. Kristl Vonck and Paul Boon have participated in advisory board meetings for LivaNova and Synergia Medical. Kristl Vonck also has received consultancy fees from All Man Foundation. The remaining authors reported no conflict of interest.

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INTRODUCTION

Vagus nerve stimulation (VNS) is an efficacious treatment for patients with drug-resistant epilepsy.[1] Intermittent electrical pulses are delivered through a subclavicular implanted pulse generator to the left vagus nerve in the low-cervical region, inducing action potentials that travel toward the nucleus tractus solitarius and to various intracranial brain regions.[2] One third of patients do not benefit from the therapy,[3] and the optimal stimulation parameters required to effectively activate the vagus nerve and evoke its therapeutic effects are still unknown. Therefore, a technique to assess whether the vagal fibers of VNS-treated patients are effectively stimulated during VNS is needed in clinical practice.

We have previously demonstrated that it is feasible to record VNS-induced laryngeal muscle-evoked potentials (LMEPs) in rats that were chronically implanted using a noninvasive electromyography (EMG) technique.[4] The LMEPs result from contractions of the laryngeal muscles induced by coactivation of the Aα-motor fibers in the recurrent laryngeal nerve which branches off from the vagus nerve at the level of the aortic arch. Therefore, LMEPs are reflective of the efficient induction of action potentials in part of the vagal fibers during VNS therapy. In humans, VNS-induced LMEPs also have been recorded using acute intraoperative vocal cord EMG, endotracheal tubes with electrodes, and laryngoscopy.[5–7] The intraoperative measurement of LMEPs is invasive, prolongs the implantation procedure, and is unfeasible in a more chronic VNS treatment setting for therapy follow-up. We previously investigated the feasibility of noninvasive recording of LMEPs in patients who were chronically implanted and focused on feasibility and methodology for surface recording of human VNS-induced LMEPs.[8] The aim of this study was to investigate these measurements in a routine clinical setting and to confirm the feasibility to reliably record VNS-induced LMEPs in patients who were implanted awake using a noninvasive, easy-to-use EMG tool. Furthermore, the stability of LMEP characteristics over time and a correlation between LMEP characteristics and response were investigated.

MATERIALS AND METHODS

Patients

A total of 25 patients (15 men, 10 women, 40 ± 14 years) with drug-resistant epilepsy and implanted with a VNS device (VNS Therapy AspireSR Model 106 Generator or VNS Therapy Demipulse Model 103 Generator; LivaNova, Inc, Houston, TX) participated in the study: 17 of the 25 patients at the initiation of VNS therapy (acute group) and 15 of the 25 patients after one year of VNS therapy (chronic group), of whom 7 of the 25 patients at both time points (acute + chronic group). The study was conducted at Ghent University Hospital, Ghent, Belgium and approved by the ethics committee of Ghent University Hospital and conformed to the ethical standards of the Declaration of Helsinki. After a full description of the procedure was provided and explained, all patients gave written informed consent.

VNS-Induced LMEP Recordings

LMEP recordings were performed at the time of a 24-hour video-EEG monitoring at the initiation of VNS therapy or after one year. Six Ag/AgCl [1a, 1b, 2a, 2b, 3a, 3b, Kendall H92SG ECG electrodes (Kendall Healthcare products, Minneapolis, MN), Ø 35 mm] recording electrodes were placed in the neck according to the three perpendicular axes around the larynx (Fig. 1). A ground electrode (G1) was placed on the forehead. A common reference electrode (rb) was placed at the sternum. The EMG was recorded using Micromed System Plus (Micromed, Mogliano, Italy). Before all study measurements, VNS electrode impedance was normal.

For the duration of the experiment, VNS frequency and duty cycle were set at 30 Hz and seven seconds ON/18 seconds OFF for all patients to assure reproducibility and to limit the time of the procedure, ie, the LMEP recordings. A frequency of 30 Hz was chosen because no overlap of stimulation artifacts and electrophysiological responses was observed and because this frequency is most often used in clinical practice. The recordings were repeated three times using different pulse widths of 130, 250, and 500 μs. For each pulse width, the output current was ramped-up in

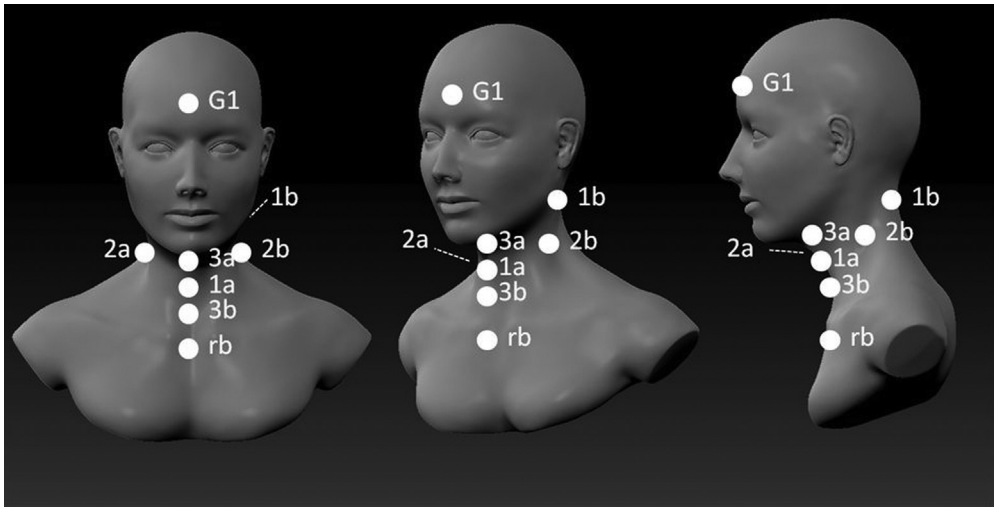

Figure 1. Schematic representation of the electrode placement for LMEP recording. Six EMG surface electrodes were placed in the neck according to the three perpendicular axes around the larynx: electrode pair 1a–1b was placed according to the sagittal axis, 2a–2b according to the horizontal axis, and 3a–3b according to the vertical axis. G1, ground; rb, common reference.

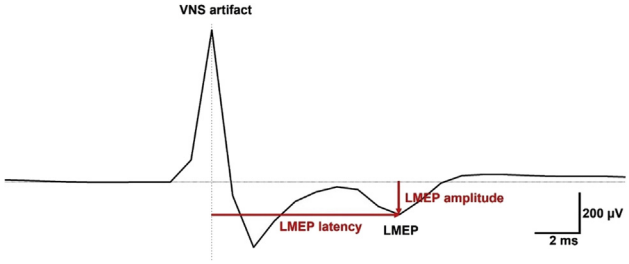

Figure 2. Visual representation of a typical LMEP signal. This figure shows an example of an LMEP recorded in patient 1 after one year of VNS therapy at a VNS output current of 2.5 mA and a pulse width of 500 µs. The VNS artifact and LMEP are marked. Moreover, determination of LMEP latency and LMEP amplitude are indicated in the figure. x-axis: latency in milliseconds (ms); y-axis: LMEP amplitude in microvolts (µV). [Color figure can be viewed at www.neuromodulationjournal.org]

steps of 0.125 mA (for patients implanted with a VNS Therapy AspireSR Model 106 Generator) or 0.25 mA (for patients implanted with a VNS Therapy Demipulse Model 103 Generator) until the individual tolerable threshold was reached for newly implanted patients or until the chronic stimulation output current of the patient was reached for patients tested after one year of VNS therapy. Four trains of stimulation were recorded at each output current. The signals were digitized online with a sampling frequency rate of 1024 Hz, an antialiasing filter of 250 Hz, a gain of 50 dB, and a resolution of 16 bits on a 6400-µV range (97.6 nV/digit).

Afterward, the LMEPs were computed offline in Brain Vision Analyzer 2.0 (Brain Products, Gilching, Germany)si using a specific analysis sequence (Fig. 2). First, difference waves were calculated by comparing the channels (1a–1b, 2a–2b, and 3a–3b). Next, data were filtered using a high-pass filter with a cutoff frequency between 100 and 200 Hz and various slopes. VNS artifact peaks were marked on the filtered data using a level trigger and these markers were imported in the nonfiltered data. Next, a segmentation was performed based on the imported markers and based on a time interval from 10 milliseconds before the VNS artifact marker to 20 milliseconds after the VNS artifact marker. After performing a zero-baseline correction, between 6 milliseconds and 2 milliseconds before the VNS artifact marker, the average of the full range was calculated.

After the experiment, the pulse generator was programmed to the chronic stimulation parameters of the patients.

Outcome Parameters

An LMEP was defined as the initial peak that was reproducibly recorded after each observable VNS artifact. LMEPs were identified after each VNS pulse of all four seven-second trains. Per individual patient, the best electrode pair was selected based on maximal LMEP amplitudes. The threshold for LMEP induction ($I_{threshold}$) was defined as the lowest output current (mA) at which an LMEP could be reproducibly identified in the recorded EMG signals. The LMEP latency (Fig. 2) was defined as the time (milliseconds) between the peak of the VNS artifact and the peak of the LMEP. The LMEP amplitude (Fig. 2) was defined as the amplitude of the LMEP peak relative to baseline (µV).

Input/Output Curves

For each patient and pulse duration (130, 250, and 500 µs), input/output (I/O) curves were made by plotting LMEP amplitudes (µV) against VNS output currents (mA) using Sigmaplot 13 (Systat Software, San Jose, CA). As no LMEPs could be measured when the VNS output current was put at 0 mA, a zero point was added for every measurement. I/O curves with a plateau at maximum LMEP values, and at least two values along the slope of the sigmoid curve were fitted to a Boltzmann function using the least squares method (Fig. 3):

$$V = \frac{Vmax}{1+e^{-\left(\frac{I-I50}{b}\right)}}$$

with V = LMEP amplitude (in µV); V_{max} = maximum LMEP amplitude (in µV); I = VNS output current used (in mA); I_{50} = VNS output current resulting in an LMEP of half-maximal amplitude (in mA); and b = slope (in µV/mA).

From these Boltzmann functions, the I_{50} and I_{95} were calculated as the VNS output current (mA) required to evoke an LMEP of half-maximal and 95% of maximal amplitude (V_{max}), respectively.

I/O curves that fitted to Boltzmann functions with R^2 values greater than 0.9 were normalized by dividing the LMEP amplitudes by the maximal LMEP amplitude. The Boltzmann fits to these normalized I/O curves were then averaged across patients to determine an average I/O function per VNS pulse duration.

Patient Groups

Patients (n = 25) were classified into two groups depending on the reduction in mean monthly seizure frequency they experienced after VNS therapy: responders (≥50% reduction) and non-responders (<50% reduction). The mean monthly seizure frequency was defined as the mean seizure frequency during the

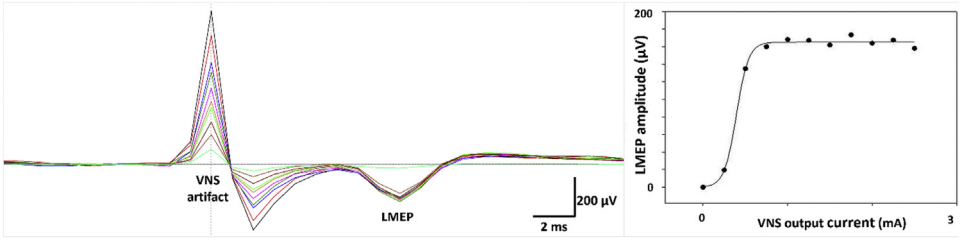

Figure 3. Visual representation of LMEP signals and the resulting I/O curve. This figure illustrates how the I/O curves were created. On the left, an overlay of the recorded LMEP signals for every recorded VNS output current (0.25–2.5 mA in steps of 0.25 mA) in patient 1 at a pulse width of 500 µs is shown. x-axis: latency in milliseconds (ms); y-axis: LMEP amplitude in microvolts (µV). On the right, the resulting I/O curve is shown. x-axis: VNS output currents in milliamperes (mA); y-axis: LMEP amplitudes in microvolts (µV). The following values for a Boltzmann function gave the best fit to the individual dots: V_{max} = 165.24 µV; I_{50} = 0.39 mA; and b = 0.07 µV/mA. [Color figure can be viewed at www.neuromodulationjournal.org]

three consecutive months before implantation (pre-VNS) or before the LMEP measurements after one year of VNS (post-VNS). Reduction in mean monthly seizure frequency was calculated as follows: Seizure reduction (%) = ([seizure frequency pre-VNS − seizure frequency post-VNS]/seizure frequency pre-VNS) × 100.

The acute group (n = 17/25) includes all patients tested at the initiation of VNS therapy, two weeks after implantation of the VNS device. The chronic group (n = 15/25) includes all patients tested after one year of VNS therapy. Some of these patients were tested both at the start and after one year of VNS therapy and were included in the acute + chronic group (n = 7/25).

Statistical Analyses

All statistical analyses were performed in SPSS version 26 (SPSS, Chicago, IL) with the level of statistical significance set at 0.05. Unless otherwise stated, data are expressed as median and range values. Nonparametric tests were used because of the low sampling rate and limited number of patients for comparisons. A logarithmic transformation of V_{max} values (log[V_{max}]) was performed for statistical analyses because amplitude values are known to have a normal distribution in the logarithmic space. Wilcoxon signed rank test was used to assess the stability of log(V_{max}), $I_{threshold}$ and I_{50} values over time in the acute + chronic group. Moreover, a linear mixed model analysis of I_{50} values was used to investigate the effect of pulse width.

RESULTS

Patient Characteristics

Patient characteristics are summarized in Table 1. A total of 16 of the 17 patients from the acute group, 8 of the 15 patients from the chronic group, and six patients from the acute + chronic group were implanted with a VNS Therapy AspireSR Model 106 Generator. The remaining patients had a VNS Therapy Demipulse Model 103 Generator implanted. A total of 9 of the 17 patients from the acute group, 8 of the 15 patients from the chronic group, and four patients from the acute + chronic group were classified as responders, whereas the remaining patients were nonresponders.

Qualitative Analysis

LMEPs were reproducibly recorded in all 25 patients. The rough LMEP traces (Fig. 2) and fitted I/O curves (Fig. 3) were easily identifiable.

Latency and Estimated Conduction Velocity

The estimated mean latency (±SEM) was 10 ± 0.1 milliseconds. To assess the origin of the LMEP signal, ie, the activated fibers involved, the mean conduction velocity needed to be estimated from our estimated LMEP latency. It is important to note that this could only be estimated very roughly with the available data. First, the mean onset latency was estimated by subtracting 3 milliseconds from our average peak latency, based on knowledge from previous experiments (Fig. 2 in the study of Vespa et al[8]), resulting in a roughly estimated mean LMEP onset latency of 7 milliseconds. Next, the mean conduction velocity was estimated on the basis of the mean length of the left recurrent laryngeal nerve in humans of 13.7 cm,[9] knowing that the VNS electrode is implanted approximately 8 cm above the clavicle and that the recurrent laryngeal nerve branches off from the vagus nerve approximately 5 cm under the clavicle,[10] leading to a total distance of 26.7 cm from the VNS electrode to the laryngeal muscles. A 1 ms neuromuscular junction delay was taken into account.[11] A roughly estimated mean conduction velocity of about 45 m/s was obtained.

Thresholds for LMEP Induction

$I_{threshold}$ values in the acute and chronic groups for different pulse widths are provided in Table 2. No significant differences in $I_{threshold}$ over time were observed in the acute + chronic group. When looking at individual patients in this group, the thresholds for LMEP induction remained relatively stable over time, with a maximal difference of no more than one increment (ie, 0.125 mA) as shown in Figure 4. The chronic output parameters were higher than the $I_{threshold}$ values in all patients.

V_{max} and I_{95}

V_{max} is reached when there is a maximum plateau phase in the I/O curves (Materials and Methods section, Input/Output Curves). V_{max} was reached in 13 of the 15 patients of the chronic group with I_{95} values between 0.5 and 1.8 mA, whereas the amplitude of the VNS artifact (Fig. 3) kept on increasing with the VNS output current. V_{max} was not reached in one responder and one nonresponder of this group. In the acute group, V_{max} was only reached in seven of the 17 patients. These patients did not tolerate high stimulation currents at that time (I_{95} values ranging from 0.4 to 0.9 mA). The estimated V_{max} ranged from 23.1 to 482.4 μV in all patients. V_{max} values were lower after one year of VNS therapy than at the start in three of three individual patients (3/3 at 250 μs, 2/2 at 500 μs).

VNS Output Current Needed to Evoke an LMEP of Half-Maximal Amplitude

I_{50} values of the calculated I/O curves, together with other fit parameters, can be found in Table 3. Linear mixed model analysis of I_{50} values in the chronic group revealed a significant effect of pulse width ($F = 59.370$; $p < 0.001$) with lower I_{50} values for longer pulse widths: mean (± SEM) I_{50} values were 0.8 ± 0.1 mA, 0.6 ± 0.1 mA, and 0.5 ± 0.0 mA for pulse widths of 130, 250, and 500 μs, respectively (Fig. 5). The influence of pulse duration on I_{50} values also is illustrated in Figure 6, where averaged I/O curves in the chronic group are plotted. Good I/O curves, allowing for the comparison of I_{50} values between the start and after one year in patients, were only observed at both time points in three patients of the acute + chronic group (3/3 at 250 μs, 2/3 at 500 μs). In these three patients, I_{50} values were higher after one year than at the start in three of three cases at 250 μs and in one of two cases at 500 μs.

DISCUSSION

The results of this study show that LMEPs can be reproducibly recorded in both patients who were acutely and chronically VNS implanted and treated using a noninvasive EMG approach. We previously investigated the use of VNS-induced LMEPs as a possible marker of effective nerve activation in patients with chronically treated VNS (between eight months and 14 years),[8] but LMEPs were never investigated in both acute and chronic settings before. The focus of this study was to assess the feasibility of recording LMEPs in a larger group of patients at fixed time points following therapy initiation.

The recorded traces and derived I/O curves visually resembled typical signals found in previous VNS-induced LMEP responses. A similar sigmoid shape of dose-response curves for VNS-induced LMEPs was found in preclinical experiments of our group with

Table 1. Main Clinical Characteristics and Habitual VNS Parameters of Patients.

Patient ID	Sex (M/F)	Age (y)	Model VNS	Start epilepsy	Response at 1 y	Time point for LMEP recording (start/1 y)	VNS parameters				
							Output current (mA)	Frequency (Hz)	Pulse width (μs)	ON time (s)	OFF time (min)
Patient 1	F	28	103	15–16 y	Nonresponder	Start	0.5	30	500	30	5
		29				1 y	2.75	30	500	7	3
Patient 2	F	34	106	Since childhood	Nonresponder	Start	0.25	30	500	30	5
Patient 3	M	39	106	1 y	Responder	Start	0.25	30	500	30	5
		40				1 y	2	30	250	30	5
Patient 4	F	59	106	16 y	Nonresponder	Start	0.25	30	500	30	5
Patient 5	M	20	106	18 mo	Responder	Start	0.25	30	500	30	5
		21				1 y	2.25	20	500	30	5
Patient 6	F	36	106	22–24 y	Responder	Start	0.5	30	500	30	5
Patient 7	M	54	106	9 y	Responder	Start	0.5	30	500	30	5
		55				1 y	1.375	30	500	30	5
Patient 8	M	40	106	21–22 y	Responder	Start	0.5	30	500	30	5
Patient 9	F	53	106	13 y	Nonresponder	Start	0.25	30	500	30	5
Patient 10	M	45	106	7 y	Nonresponder	Start	0.5	30	500	30	5
		46				1 y	2	30	500	30	5
Patient 11	M	30	106	15 y	Responder	Start	0.25	30	500	30	5
Patient 12	F	23	106	6 y	Nonresponder	Start	0.5	30	500	30	5
Patient 13	M	36	103	29 y	Nonresponder	1 y	2.75	30	500	30	5
Patient 14	M	69	103	11 y	Responder	1 y	2	20	250	30	5
Patient 15	M	17	103	9 mo	Nonresponder	1 y	2.25	30	500	30	5
Patient 16	M	40	103	8 mo	Responder	1 y	2.5	25	500	30	3
Patient 17	M	23	106	10 y	Nonresponder	1 y	2	30	500	30	5
Patient 18	F	38	103	9–10 y	Nonresponder	1 y	2.25	30	500	30	5
Patient 19	M	20	103	5–6 y	Responder	1 y	1.5	20	500	30	5
Patient 20	F	59	106	30 y	Responder	1 y	1.5	20	250	30	5
Patient 21	M	41	106	35 y	Responder	Start	0.375	30	500	30	5
		42				1 y	1.75	15	250	30	5
Patient 22	F	35	106	4 y	Nonresponder	Start	0.25	30	500	30	5
		36				1 y	1.75	20	250	30	5
Patient 23	M	45	106	6 y	Responder	Start	0.5	30	250	30	5
Patient 24	M	55	106	6 y	Nonresponder	Start	0.5	30	500	30	5
Patient 25	F	58	106	6 mo	Responder	Start	0.5	30	500	30	5

In this table, the sex of the patients and the duration of epilepsy are given, as well as the implanted VNS device, the age, and the therapeutic VNS parameters at the moment the measurements are included. Moreover, a distinction between responders (≥50% seizure reduction) and nonresponders (<50% seizure reduction) was made after 1 year of VNS therapy.

Table 2. $I_{threshold}$ Values.

Threshold for LMEP induction	130 µs			250 µs			500 µs		
	Minimum (mA)	Maximum (mA)	Median (mA)	Minimum (mA)	Maximum (mA)	Median (mA)	Minimum (mA)	Maximum (mA)	Median (mA)
Acute group (n = 17)	0.375	1	0.5	0.25	0.625	0.375	0.25	0.625	0.375
Chronic group (n = 15)	0.25	1	0.563	0.25	0.75	0.5	0.25	0.5	0.375

This table summarizes the minimal, maximal, and median $I_{threshold}$ values in patients of the acute and chronic groups for different pulse widths.

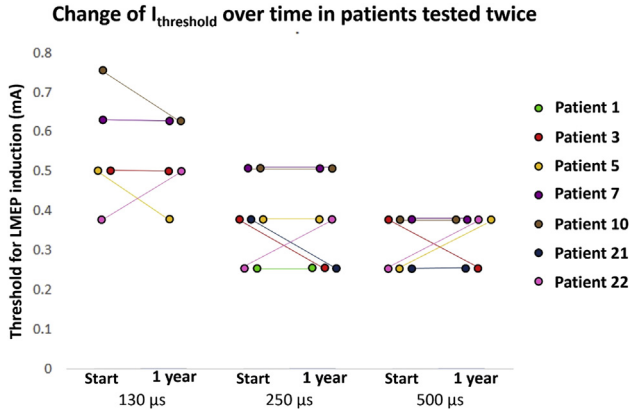

Figure 4. Change of $I_{threshold}$ over time in patients tested twice. This figure summarizes the change in $I_{threshold}$ values (in mA) between the start and after one year in the acute + chronic group (n = 7). Individual $I_{threshold}$ values are depicted as dots with each color representing one patient. Lines connecting the dots indicate the change in $I_{threshold}$ values over time. [Color figure can be viewed at www.neuromodulationjournal.org]

VNS-treated rats[12,13] as wells as in a previous clinical study with patients who were chronically VNS implanted.[8] Similar dose-response curves also were found by other research groups doing experiments in mice,[14] rats,[15] and pigs.[16] The estimated mean latency of the LMEP peak was 10 ± 0.1 milliseconds. LMEP latencies could not be exactly determined because of the low sampling rate. This implicated that artifact timings are not very precise because VNS stimuli have very high frequencies that could not be determined with the physiological equipment used and resulted in little predictable interactions with the device. Moreover, the indicated LMEP peaks were most likely not the real LMEP peaks because these probably lay in between two sampled data points in most cases. Therefore, the mentioned LMEP latencies are only estimations based on the estimated timings of VNS artifacts and LMEP peaks. To compare this with other experiments, estimation of onset latencies and conduction velocities is required. The estimated mean onset latency of 7 milliseconds and an estimated conduction velocity of 45 m/s found in this study are in line with previous studies. The estimated conduction velocity in our previous study with patients who were chronically VNS implanted[8] was identical to the conduction velocity estimated in this study, ie, 45 m/s. Invasive LMEP recordings upon direct stimulation of the recurrent laryngeal nerve in 42 patients[17] resulted in a conduction velocity of approximately 30 m/s. In a preclinical rat study in our laboratory,[4] the conduction velocity was estimated to be 28 m/s. Taking into account the difficulty in estimating the recurrent length, the error in stimulus timing because of filtration distortion, the effect of temperature in different studies, and the limited sampling rate in this study, we can conclude that our findings are similar and support the hypothesis that the signals recorded are of the same origin, ie, the activation of the Aα-motor fibers of the recurrent laryngeal nerve and subsequent contraction of the laryngeal muscles.

In all patients included in this study, LMEPs were already recorded at low VNS output currents, ie, in the range of 0.25 to 1 mA, 0.25 to 0.75 mA, or 0.25 to 0.625 mA for pulse widths of 130 µs, 250 µs, or 500 µs, respectively. This is in line with the results of our previous clinical study where thresholds for LMEP induction upon VNS stimulation at a pulse width of 250 µs and a frequency of 20 Hz

Table 3. Boltzmann Fit Parameters of Selected I/O Curves.

Patient ID	Time (st/1 y)	Electrode pair	Pulse duration (μs)	$I_{threshold}$ (mA)	V_{max} (μV)	Slope (μV/mA)	I_{50} (mA)	I_{95} (mA)	R^2
1	st	3a–3b	250	0.25	258.4	0.08	0.40	0.64	0.9987
	1 y		130	0.25	182.7	0.20	0.68	1.26	0.9785
			250	0.25	181.8	0.15	0.49	0.92	0.9613
			500	0.25	165.2	0.07	0.39	0.60	0.9957
3	st	1a–1b	130	0.50	73.5	0.04	0.59	0.70	1.0000
	1 y		500	0.25	307.4	0.06	0.48	0.67	0.9763
5	1 y	2a–2b	130	0.63	48.3	0.10	0.70	0.99	0.9726
			500	0.38	43.2	0.11	0.52	0.85	0.9743
7	st	3a–3b	250	0.50	49.4	0.02	0.47	0.52	0.9919
			500	0.38	42.4	0.03	0.37	0.45	0.9658
	1 y		130	0.63	23.3	0.05	0.71	0.86	0.9980
			250	0.50	29.1	0.06	0.53	0.71	0.9919
			500	0.38	27.2	0.05	0.40	0.56	0.9966
8	st	2a–2b	250	0.38	52.3	0.02	0.35	0.42	0.9907
			500	0.25	51.7	0.07	0.30	0.51	0.9853
9	st	2a–2b	130	0.63	76.1	0.02	0.60	0.66	0.9979
			250	0.38	74.0	0.04	0.39	0.50	0.9974
			500	0.25	58.0	0.04	0.30	0.42	0.9968
10	st	2a–2b	250	0.50	47.3	0.08	0.65	0.89	0.9886
			500	0.38	44.8	0.10	0.54	0.82	0.9945
	1 y		130	0.63	23.5	0.14	0.88	1.28	0.9830
			250	0.50	24.9	0.13	0.66	1.04	0.9832
			500	0.38	23.1	0.09	0.51	0.77	0.9789
12	st	3a–3b	130	0.50	47.4	0.05	0.52	0.66	0.9974
			500	0.38	60.3	0.07	0.41	0.61	0.9908
13	1 y	2a–2b	250	0.50	8.5	0.13	0.52	0.91	0.9699
14	1 y	3a–3b	130	1.00	71.8	0.15	1.18	1.61	0.9891
			250	0.75	76.2	0.14	0.85	1.27	0.9898
			500	0.50	67.0	0.18	0.67	1.20	0.9704
15	1 y	1a–1b	130	0.50	93.0	0.07	0.91	1.11	0.9887
			250	0.50	83.4	0.08	0.67	0.89	0.9940
			500	0.50	65.9	0.12	0.56	0.90	0.9861
16	1 y	2a–2b	130	0.75	52.5	0.21	1.14	1.77	0.9783
			250	0.50	48.5	0.15	0.76	1.22	0.9744
			500	0.50	55.1	0.13	0.67	1.06	0.9904
18	1 y	2a–2b	500	0.50	83.0	0.11	0.60	0.93	0.9850
19	1 y	3a–3b	130	0.50	34.9	0.08	0.73	0.96	0.9916
			500	0.50	30.6	0.06	0.48	0.65	0.9969
21	1 y	1a–1b	130	0.38	190.0	0.07	0.56	0.77	0.9989
			250	0.25	192.4	0.07	0.43	0.63	0.9953
			500	0.25	179.5	0.05	0.34	0.48	0.9918
22	1 y	1a–1b	130	0.50	38.8	0.09	0.63	0.89	0.9933
			250	0.38	38.6	0.10	0.51	0.81	0.9804
			500	0.38	33.0	0.08	0.47	0.71	0.9762

This table summarizes the fit parameters that describe the I/O curves fitting Boltzmann functions per pulse duration. Additional parameters indicated in the table are the selected electrode pair per individual patient, $I_{threshold}$ and I_{95}.
st, start.

ranged from 0.25 to 1 mA (median 0.50 mA).[8] These results are consistent with the findings from the invasive study of Ardesch et al,[7] in which the effect of VNS on the vocal folds was already present at VNS output currents of 0.25 to 0.50 mA, without necessarily causing VNS-induced hoarseness.

A shift in activation curves over time could not be demonstrated within patients. Because I_{50} values could only be compared between the start and after one year in three patients at a pulse width of 250 μs and in two patients at a pulse width of 500 μs, this has to be confirmed in a larger group of patients. We experienced difficulties in obtaining good I/O curves in patients tested at the initiation of the VNS therapy, as ramp-up in these patients was limited because of tolerance. Therefore, it is suggested to include an additional intermediate testing point after six months of VNS therapy in future studies. In future studies, it also would be interesting to study the relationship between tolerance for VNS-related side effects and VNS intensity.

Individual thresholds for the activation of vagal A and B fibers will vary as a result of anatomical differences, ie, the relative position of fibers within the nerve, electrode contact position, and local tissue gliosis. It was shown by Helmers et al[2] that the presence of 110 μm of fibrotic tissue can decrease fiber activation by 50%. Furthermore, LMEP recording is expected to be slightly lower than the VNS output current required to achieve therapeutic efficacy

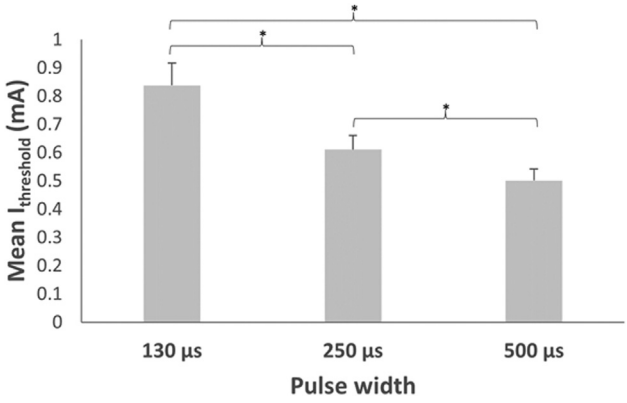

Figure 5. Mean I_{50} values for different pulse widths. This figure illustrates the effect of pulse width on I_{50} values. Longer pulse widths result in lower I_{50} values, with significant differences in I_{50} values between all tested pulse widths. *$p < 0.001$.

because it records the activity of the thicker and therefore low threshold Aα-motor fibers, whereas the therapeutic effects of VNS are believed to be mediated by the slightly thinner and higher threshold A and B fibers.[18–20] The exact threshold ratio between Aα-motor fibers and slightly higher threshold A and B fibers still has to be demonstrated. Given that the variation in threshold intensity with fiber diameter is rather limited in the large diameter range,[21] we expect to find a ratio value close to unity between the $I_{threshold}$ and efficient therapeutic VNS parameters. Therefore, we suggest that the presented noninvasive LMEP recording technique can be used to guide individual titration of the stimulation parameters.

It may be expected that the vagus nerve of some nonresponders is not adequately activated because of various reasons such as poor nerve-electrode contact, local gliotic scar, or vagus nerve damage such as a diabetic or other neuropathy or traumatic surgery for example.[22–25] However, in this study, LMEPs with latencies within the expected range were recorded in all patients including

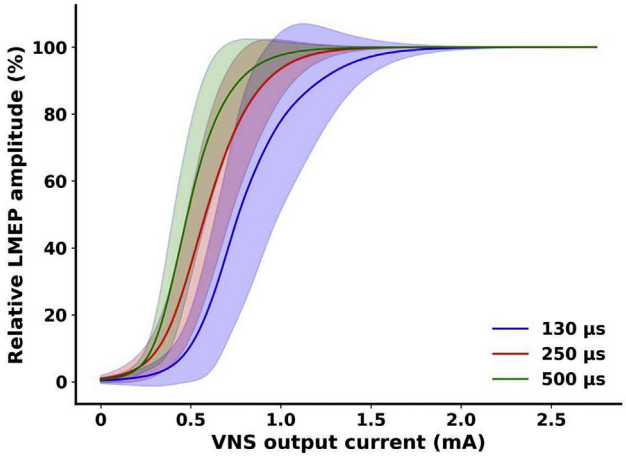

Figure 6. Averaged I/O curves for the different pulse durations in the chronic group. This plot shows the average Boltzmann fits (±SEM) to LMEP I/O curves for the different pulse durations (130 µs, 250 µs, and 500 µs represented in blue, red, and green, respectively). The average Boltzmann fits were described by the following parameters: I_{50} = 0.8 mA, 0.6 mA, and 0.5 mA; b = 0.16 µV/mA, 0.14 µV/mA, and 0.11 µV/mA for 130 µs, 250 µs, and 500 µs, respectively. [Color figure can be viewed at www.neuromodulationjournal.org]

nonresponders, and no significant differences in LMEP characteristics between responders and nonresponders were found. Of course, nonresponse also could result from the heterogeneity of the underlying pathophysiological mechanisms or the variability in characteristics of more central structures in the neural pathway involved in VNS. Therefore, the LMEP is not a biomarker for the antiepileptic efficacy of VNS but rather an indication that the nerve is effectively stimulated, which is an essential step to achieve therapeutic stimulation. An LMEP is the result of efferent stimulation of the vagus nerve, thereby reflecting the effective activation of efferent Aα motor fibers, whereas stimulation of the higher threshold afferent Aβ-fibers is believed to be required for the antiepileptic action of VNS.[26,27] Therefore, LMEPs only serve as a surrogate marker for effective transfer of activating current from the VNS electrode to the vagus nerve. However, LMEP recruitment curves as shown in Figure 6 are most likely an underestimation of the stimulation needed to achieve a therapeutic VNS effect. All patients were stimulated at chronic VNS output currents higher than $I_{threshold}$ values. In all patients of the chronic group, except in one responder and one nonresponder, V_{max} was reached. In the nonresponder, no ramp-up was performed until the chronic stimulation parameters after one year because of technical issues. Ineffective stimulation, reflected by the absence of an LMEP at reasonable stimulation currents or a prolonged latency, could not explain nonresponse in this study.

We observed a large interindividual variation of the estimated maximal LMEP amplitudes, ranging from 23.1 to 482.4 µV. LMEP amplitudes are merely estimations as with the sampling frequency of 1024 Hz; data points were only sampled every 1 millisecond, causing real LMEP peaks to be missed in most cases. This also was seen in our previous clinical study.[8] Variation in LMEP amplitude between patients and at different time points might be explained by differences or changes in tissue composition (thickness of subcutaneous tissue and body fat), underlying pathology, positioning of electrode pairs, and VNS electrode impedance. Because of the low sampling rate of 1024 Hz, our amplitude values may have been somewhat underestimated. A decrease in maximal LMEP amplitudes over time was observed in three of three patients of the acute + chronic group. Higher maximal LMEP amplitudes at the initiation of the VNS therapy may be related to acute effects of surgery as LMEPs were recorded only two weeks after the implantation of the VNS device. Therefore, it would be of interest to compare maximal LMEP amplitudes between additional time points, for example, between initiation, after six months, one year, and two years of VNS therapy.

As we aimed to implement the LMEP measurements in patients during their routine examinations in the hospital in a normal video-EEG monitoring setting using the available equipment, a significantly lower-than-optimal sampling rate (and low-pass filter) was used. However, LMEPs could be identified very reproducibly, proving that the method can easily be implemented in the frame of the routine follow-up of patients who were VNS implanted. Because of the easy implementation and the possibility to analyze LMEPs recorded from different electrode pairs simultaneously, we would suggest recording LMEPs on all three described axes and choose the best axis per individual patient at the end of the analyses. For more detailed analyses of LMEP characteristics and their stability over time, a higher sampling frequency and more standardized placement of electrode pairs as described before[8] may be recommended. However, implementation of LMEP measurements in routine clinical practice as described in this study using a

sampling frequency of 1024 Hz (and 250 Hz low-pass cutoff) may already be sufficient to guide titration of VNS parameters in a more objective and individualized way.

CONCLUSION

Our results show that VNS-induced LMEPs can be recorded very reproducibly in both patients who were acutely and chronically VNS-treated using noninvasive skin electrodes. This study shows that VNS-induced LMEP recordings can be easily implemented in routine hospital examinations using available equipment. LMEPs can be used for checking correct transfer of current from the electrode to the vagus nerve and to give an indication of system integrity, a prerequisite to achieve clinical efficacy, and may help guide proper titration of stimulation parameters.

Authorship Statements

Charlotte Bouckaert was responsible for the conceptualization, methodology, software, formal analysis, investigation, data curation, writing—original draft preparation, and project administration. Robrecht Raedt was responsible for the conceptualization, methodology, software, validation, data curation, writing—original draft preparation, supervision, and funding acquisition. Lars Emil Larsen was responsible for the data curation and writing—review and editing. Riëm El Tahry, Evelien Carrette, Veerle De Herdt, Ann Mertens, and Alfred Meurs were responsible for writing—review and editing. Stefanie Gadeyne and Frank Dewaele were responsible for resources and writing—review and editing. Silke Proesmans was responsible for the formal analysis, investigation, and writing—review and editing. Jean Delbeke was responsible for the data curation and writing—review and editing. Paul Boon was responsible for the writing—review and editing and funding acquisition. Kristl Vonck was responsible for the conceptualization, methodology, validation, resources, writing—review and editing, data curation, writing—original draft preparation, supervision, and funding acquisition. All authors approved the final manuscript.

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COMMENTS

Bouckaert and colleagues investigated the use of noninvasive evoked potential recordings in laryngeal muscles as a potential noninvasive biomarker to assess effective vagal nerve fiber

activation. They found the concept to be feasible both at the initiation of therapy and at a year of VNS therapy. Unfortunately, no differences between responders to the VNS therapy and nonresponders could be found. Be that as it may, the technique was shown to at least determine that the vagus nerve was being effectively stimulated. This feature may offer greater value in assessing patients with "late failure" of their devices, and it should be investigated as a technique to allow for a noninvasive method to potentially differentiate lead fracture, device failure, perineural fibrosis, neuropathy or other reasons for patients presenting with a worsening response to previous effective VNS therapy.

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