Vagus Nerve Electroneurogram-Based Detection of Acute Pentylenetetrazol Induced Seizures in Rats

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On-demand stimulation improves the efficacy of vagus nerve stimulation (VNS) in refractory epilepsy. The vagus nerve is the main peripheral parasympathetic connection and seizures are known to exhibit autonomic symptoms. Therefore, we hypothesized that seizure detection is possible through vagus nerve electroneurogram (VENG) recording. We developed a metric able to measure abrupt changes in amplitude and frequency of spontaneous vagus nerve action potentials. A classifier was trained using a "leave-one-out" method on a set of 6 seizures and 3 control recordings to utilize the VENG spike feature-based metric for seizure detection. We were able to detect pentylenetetrazol (PTZ) induced acute seizures in 6/6 animals during different stages of the seizure with no false detection. The classifier detected the seizure during an early stage in 3/6 animals and at the onset of tonic clonic stage of the seizure in 3/6 animals. EMG and motion artefacts often accompany epileptic activity. We showed the “epileptic” neural signal to be independent from EMG and motion artefacts. We confirmed the existence of seizure related signals in the VENG recording and proved their applicability for seizure detection. This detection might be a promising tool to improve efficacy of VNS treatment by developing new responsive stimulation systems.

Keywords: VENG; pentylenetetrazol (PTZ); responsive VNS; seizure detection.

1. Introduction

Vagus nerve stimulation (VNS) was first approved in 1994 as a treatment for refractory epilepsy. Most of the current VNS systems still operate in an open loop setting with the stimulation continuously applied with a predetermined on and off time. Recently, the Aspire SR, an on-demand VNS device, became commercially available. This device relies on the detection of ictal tachycardia to trigger on-demand VNS. One study, in which old devices were replaced by the newer Aspire model, showed an additional clinical benefit although the potential value for patients is debatable since only 64-71% of the seizures are associated with significant changes in

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heart-rate. Moreover, in patients that do present ictal tachycardia, there also exists a high variability of the tachycardia within each patient. The need and importance to develop new on-demand stimulation techniques is highlighted by the fact that several human and animal studies show the importance of stimulating early in the seizure to stop its course. For example, Mc Lachlan et al. demonstrated that the acute abortive effects of VNS in a PTZ model in rats increases whenever the seizure is stimulated earlier. Similarly, data from the E36 study in patients with refractory epilepsy also showed that earlier detection and stimulation, resulted in shorter duration of seizures.

In addition, several studies have shown the beneficial effect of manual triggering stimulation with the use of the magnet at the seizure onset. This on-demand stimulation helped to reduce the intensity and the duration of seizures or even completely terminate the seizure. However, manual on-demand stimulation might not be suitable for all patients, as it requires the ability to swipe the magnet at the beginning of the seizure. Patients without an aura or patients with cognitive impairment may not be able to trigger manually their VNS. To counteract this downside, an automatic on-demand VNS system, based on a reliable seizure detection method, is needed. Other applications like automatic drug delivery systems for the application of rapid epileptic seizure termination treatment or automatic seizure counting to determine treatment outcome could also benefit from a reliable seizure detection method.

Currently, electroencephalography (EEG) is the gold standard for seizure detection in a clinical setting. In the past two decades several methods like wavelet decomposition in combination with support vector machines or convolutional neural network have been employed for automatic seizure detection. Several studies managed to predict seizures in rats and canines preceding the seizure between 6 s and 420 s. As seizures induce autonomic symptoms like cardiac and respiratory changes, seizure detection methods based on the recording of autonomic functions maybe another novel way to detect seizures. As the vagus nerve is being accessed for the implantation of the stimulation electrodes and represents the main parasympathetic output of the nervous system, we believe that vagus nerve recording with the same implanted electrode may contribute to development of a new seizure biomarker.

Several other studies already utilized vagus nerve electromyogram (VENG) signals with the goal to improve therapy of refractory hypertension. One study described VENG-based identification of baroreceptive fibers to develop selective VNS while another study described the extraction of a VENG-based blood pressure biomarker to be used for responsive VNS. In addition, the same group also observed respiration related signals in the VENG, which could be used to distinguish pathological hypertension from nonpathological conditions, such as the increase in blood pressure that occurs during exercise.

Cardiac related energy and spectral pattern of the VENG have been previously suggested as a possible marker for the detection of seizures. Those studies were limited by the lack of independent test and training groups for training of the classifier as well as by the acute, nonimplantable nature of the recording setting. In addition, preliminary results showed an increase of VENG signal power during a seizure in the acute kainic acid (KA) model. Also, the increased vagal activity during KA induced seizures seems to be a possible indicator for increased mortality due to sudden unexpected death in epilepsy (SUDEP). Earlier studies reported extended periods of postictal arrhythmias, which might be explained by an abnormal expression of both sympathetic and parasympathetic neurotransmission further supporting the hypothesis that VENG recording might also be useful for SUDEP detection.

Recently, we established a method to quantify VENG activity in terms of neural spiking detected in the VENG signal. Analysis of spiking activity in the vagus nerve has been the topic of an increasing number of studies with different aims. While only one study investigated the vagus nerve spiking behavior in relation to KA induced seizures, most of the studies described the vagus nerve spiking behavior in relation to nonseizure related topics, such as the inflammatory response, intestinal/gastric extension and hypoxia. In this study, we describe the vagus nerve activity during acute PTZ induced seizures in rats and implement this data into a VENG-based seizure detection algorithm.
2. Materials/Methods

2.1. Animals and surgical procedure

University Health Sciences Sector Laboratory Animal Protection Committee approved the experimental procedures under the reference 2018/UCL/MID/001. Nine male Wistar rats with an average weight of 424.4 ± 31.7 g obtained from the local breeding facility at the UC Louvain have been used. The animals were housed under a 12 h day/night cycle with controlled temperature and humidity and "ad libitum" access to food and water.

All animals were implanted with homemade epidural EEG electrodes made from watchmaker screws (Plastics1, Roanoke, VA, USA), and a microcuff electrode around the left cervical vagus nerve. Epidurals were implanted at the following coordinates: AP 6 mm, ML 0 mm (reference); AP 2 mm, ML 3 mm (left frontal), AP −5 mm, ML 3 mm (left parietal). EEG was recorded differentially between left frontal and parietal electrode at a sampling frequency of 250 Hz. Concomitant video analysis allowed studying the behavior of the animal. ECG needle electrodes were positioned according to Eindhoven Lead II configuration and a venous access was placed in the lateral tail vein.

VENG signals were recorded at a sampling frequency of 80 kHz with a Pt Standard Micro Cuff (Microprobes, Gaithersburg, USA) with 300 μm inner diameter, 100 μm contact width and 2 mm contact spacing.

2.2. Experimental procedure

After the surgery, animals were placed in a Faraday cage and left to stabilize for 15 min. Thereafter, a 60 mg/kg I.P. Ketamine booster injection was applied. Acute seizures were induced in six animals (PTZ1-6) with Pentylenetetrazol (PTZ) and three animals were used as a control (SAL1-3). Electro-physiological and video recording were started within 5 min after the last ketamine injection. Saline infusion (0.5 ml/min/kg) started 5 min after the last ketamine injection and continued for 10 min. These 10 min of saline infusion were used as a baseline interval. In the PTZ group, the IV was switched to 10 mg/ml PTZ solution at 0.5 ml/min/kg and was maintained until the animal reached the tonic clonic phase of the seizure. The electrophysiological monitoring was continued for 10 min after the EEG recovered from the postictal depression. The first occurrence of ictal EEG spikes and the onset of the tonic-clonic stage of the seizure were determined by reviewing manually the video EEG data by an epileptologist.

In the control group, the saline infusion was continued after baseline interval for an additional 10 min and subsequently another 10 min, to allow a similar recording time between PTZ and saline animals. At the end of the experiment, the animal was sacrificed by CO2 asphyxiation.

2.3. Removal of motion/muscle artefacts

Eventual motion/muscle artefacts caused by imperfect sealing of the cuff and/or impedance imbalances have been removed using continuous wavelet transform (CWT) adapted from Ref. 31. To estimate mean and standard deviation of the magnitude of the wavelet coefficients of the whole recording, every 100 s the VENG data was examined by continuous wavelet transformation of a probe window using a Morse mother wavelet. The probe window had a length of 10 s and added overlap to satisfy the cone of interest. The absolute values of the wavelet coefficients (|T|) (Fig. 1(a)) were separated among frequencies and aligned in temporal order. Z-score thresholds for each frequency (Z(f)) were defined in terms of multiples of standard deviation over the mean of the wavelet coefficients magnitude. 11 frequency bands, in ~1 kHz steps (1–7 kHz) and ~250 Hz steps (0.1–1 kHz), have been used to manually select Z-score thresholds, to separate high magnitude components related to motion/muscle artefacts, and low magnitude components related to the VENG signal (Fig. 1(b)). The 11 manually selected Z-scores were fitted to a rational polynomial (numerator degree 2, denominator degree 1) function using Matlab curve fitting tool (the MathWorks, Natick, USA). Goodness of fit was evaluated by the root mean squared error (RMSE: 0.072). The Z-score thresholds at all frequencies (Z(f)) were calculated according to the resulting fitting curve.

Using mean and STD values of the wavelet coefficient magnitude (|T|_{\text{mean}}), derived from the probe run, absolute thresholds (th(f)), for each frequency band was in calculated as in the following
Fig. 1. (Color online) (a) Magnitude scalogram of wavelet transform of a 10 s window of VENG data including EMG/motion artefacts (red ellipses) (b) magnitude of wavelet coefficients for four exemplary frequency bands of (c). Red horizontal lines represent the manually chosen Z-score.

To remove artefacts, the signal was processed in 10 s (800,000 samples) intervals because of high computational costs. To prevent edge effects, an 18.75 ms (1500 samples) overlap was added at the beginning and end of the interval according to the cone of interest, this satisfies signal integrity down to frequencies of 87.9 Hz. The CWT of an 803,000 sample data snippet returned the wavelet coefficients for 167 exponentially distributed frequency bands (0–34730 Hz).

For each wavelet coefficient, the complex magnitude is calculated. Each frequency band was compared to frequency related thresholds calculated by Eq. (1). Contrary to the method in Ref. 31, wavelet coefficients exceeding the threshold are set to 0. In a subsequent filtering step, all wavelet coefficients related to frequency bands higher than the upper border or smaller than the lower border of the pass-band (300–3000 Hz, respectively, 300–6000 Hz) were set to the value 0. We used inverse CWT (ICWT) to obtain the cleaned and filtered signal from the wavelet coefficients. To extract the motion/muscle artefact signal, all coefficients with a magnitude below the frequency specific threshold were set to 0 followed by ICWT.

2.4. VENG spike detection and normalized display

VENG spikes were automatically detected based on previously described methodology. To appreciate evolution of VENG spiking over time, spike frequency and amplitude were pooled and normalized to the amplitude of the standard deviation and aligned in time by aligning the recordings to the mean timestamps of seizure stages/recording intervals and subsequent resampling.

2.5. VENG detection metric

We developed a seizure detection metric based on recording of average VENG spike amplitude and frequency values. To minimize the influence of constant drifts in the signal, two connected sliding windows have been used. The leading window is named foreground (fg) and the following is the background (bg) window. Several window sizes \( s_{fg}, s_{bg} \) have been evaluated with \( s_{bg} > s_{fg} \). The two connected windows were right shifted in 0.5 s steps. The mean spike frequency within the window \( f_{fg}, f_{bg} \) is defined as the number of spikes within the window divided by window length. The mean spike amplitude within the window \( U_{fg}, U_{bg} \) is defined as the average absolute amplitude of all spikes detected within the window. The relative frequency and amplitude slopes \( \Delta f_r, \Delta U_r \) are defined as follows:

\[
\Delta f_r = \frac{f_{fg} - f_{bg}}{s_{fg}} \tag{2}
\]

\[
\Delta U_r = \frac{U_{fg} - U_{bg}}{s_{fg}} \tag{3}
\]
Both relative slopes are normalized by their baseline standard deviation
\[
\Delta f_{\text{norm}} = \frac{\Delta f_{r} - 1}{\text{STD}(\Delta f_{r,\text{baseline}})} \quad (4)
\]
\[
\Delta U_{\text{norm}} = \frac{\Delta U_{r} - 1}{\text{STD}(\Delta U_{r,\text{baseline}})} \quad (5)
\]
The normalized slopes are combined to the detection metric \( M_{\text{det}} \)
\[
M_{\text{det}} = (\Delta U_{\text{norm}} + 1) \cdot (\Delta f_{\text{norm}} + 1) \quad (6)
\]
To avoid edge effects, baseline data was copied, mirrored and added before the baseline.

2.6. Motion/muscle artefact estimator and metric

To estimate the intensity and timestamp of the animal’s motions during the seizure, the 25 fps video recording of the animal was analyzed. The video was recorded using a Canon IXUS 185 digital camera placed on a 20 cm high tripod 20 cm lateral to the animal focusing on head neck and chest area. For each frame, the number of pixels with a change of >20% of the full range (8 bit) in one or more color channels compared to the following frame, were counted. The final motion estimator was then calculated as the standard deviation within a 10s wide sliding window of that signal. The threshold of 20% of the full range of the channel was chosen to avoid detection of noise, fluctuations in the ambient illumination and small motions related to respiration.

Muscle artefacts were estimated based on the VENG recording, bandpass filtered for the typical range of muscle artefacts (0.1–600 Hz). The final muscle artefact estimator was calculated as the standard deviation within a 20s moving window, sliding along the filtered VENG signal.

A motion/muscle artefact-based metric \( M_{\text{art}} \) was calculated in the same fashion as the VENG-based detection metric described in the previous section. Instead of the mean values of VENG spike amplitude and frequency, the standard deviation of the signal extracted from the video and the filtered VENG signal was used.

2.7. Heart rate-based metric

A heart rate-based metric \( M_{\text{HR}} \) was computed in the same fashion as the VENG-based detection metric and motion/muscle artefact-based metric. Instead of the VENG spike frequency and amplitude, this metric was based on the frequency of r-peaks within the fore and background window and was calculated with the normalized r-peak frequency slope \( (\Delta f_{r,\text{peak, norm}}) \) as follows:
\[
M_{\text{HR}} = (\Delta f_{r,\text{peak, norm}} + 1)^2 \quad (7)
\]

2.8. Receiver Operation Characteristic (ROC) curves and algorithm assessment

True positive rate (TPR) and false positive rate (FPR) are defined in Eqs. (8) and (9). Data were pooled for all recordings used for training and ROC curves were calculated.
\[
\begin{align*}
FPR &= \frac{\text{false positives}}{\text{intervals without seizure}} \quad (8) \\
TPR &= \frac{\text{true positives}}{\text{intervals with seizure}} \quad (9)
\end{align*}
\]

For determination of true and false positives, the recordings were separated into three intervals:
- baseline: first 10 min of saline infusion;
- IV: start of PTZ infusion until EEG recovers from post ictal depression plus background window length;
- post IV: end of seizure interval until the end of recording.

In the control group, the post IV interval was defined to start 10 min after start of the saline infusion until the end of saline infusion plus background window length. Seizures detections should only occur during the PTZ IV period, therefore this was the only interval defined as “interval with seizure”, while the baseline and post IV period in the PTZ group, and all intervals in the saline group were defined as “intervals without seizure” Eqs. (8) and (9).

In order to be able to compare thresholds for detections in-between animals, the Z-transformation was used, and the threshold was defined as follows:
\[
\text{th} = \text{mean}_{BL} + Z \cdot \text{STD}_{BL} \quad (10)
\]

By varying the Z-score, different thresholds can be tested. Therefore, the Z-score varied in steps of 0.1 to cover the full detection metric range in all recordings.

For each Z-score, the length of threshold crossings was tested for durations varying between 1 and 80 samples over the threshold (SOT). If several
threshold crossings with a sufficient length occur in a specific interval, they are interpreted as one positive detection. If a positive detection occurs in the PTZ IV interval (“interval with seizure”), this is considered as a true positive detection. If a positive detection occurs during baseline or post IV interval in the PTZ animals or in the saline controls (“interval without seizure”), then the detection is considered to be a false positive detection. TPR and FPR are calculated for each \( Z \)-score and each SOT, pooled over all animals. ROC curves were drawn by plotting FPR against TPR for each SOT. For each ROC curve, the classification quality was calculated by the area under the curve (AUC). The best range of \( Z \)-scores of ROC curves with a perfect classification quality (AUC = 1) was determined based on the \( Z \) values which resulted in FPR = 0% and TPR = 100%. To avoid testing and training of the classifier on the same group of data, a “leave-one-out” method was used. In this method, for each recording (PTZ1-6) to be tested, a training pool (exPTZ1-6) was generated. The training pool consisted out of all recordings except the one to be tested. This way a specific set of classification parameters for each recording (PTZ1-6) was determined.

The classification parameters were then determined by minimizing the weighted distance \( d_w \) Eq. (11) between origin and the minimal SOT related \( Z \)-score \( Z(SOT) \), which satisfies: AUC = 1 & TPR = 1 & FPR = 0;

\[
    d_w = \sqrt{Z(SOT) \cdot w^2 + SOT^2}. \tag{11}
\]

With the weighting factor \( w \) compensating the different scale of \( Z \)-scores and SOT, as well as increasing the weight of lower SOT to promote shorter detection delays rather than low thresholds. In addition, we applied a safety margin of +1 for the SOT and of 0.2 for the \( Z \)-score.

3. Results

3.1. Characterization of the induced seizures

Seizures were recorded in all 6 animals. First motor manifestations of the seizure, namely tonic extension of the whiskers, occurred \( 3.7 \pm 1.8 \) min after start of the PTZ infusion. In 5/6, the PTZ recordings displayed an increased EEG activity around 2Hz at baseline, probably corresponding to the effect of the anesthesia on cerebral function. Short after the start of the PTZ infusion this activity shifts gradually towards higher frequencies.

Fig. 2. (Color online) Spectral behavior of the EEG signal in relation to seizures in the frequency band of 0-6Hz, exemplary for PTZ2 (a) PTZ4 (b) PTZ6 (c) Purple vertical lines indicate start of PTZ infusion, blue vertical lines indicate start of whisker extension, green vertical lines indicate the onset of EEG spikes, vertical red lines indicate start/stop of the tonic-clonic stage of the seizures.
VENG-Based Detection of Acute PTZ

(Δf: 0.7–1.9Hz) (Figs. 2(a) and 2(b)). In 1/6 PTZ-recording, no baseline 2 Hz activity could be observed (Fig. 2(c)). EEG activity in the delta band (<4Hz) is associated with deep sleep. Therefore, missing activity at ∼2Hz could indicate a reduced level of anesthesia. As PTZ1 and PTZ2 show ∼2Hz activity in the EEG and have relatable baseline heart rates (201 BPM/210 BPM versus 203 BPM) altered cardiac activity may reasonably be excluded as a potential cause. However, other possible confounding factors were not monitored during our experiments, such as for example altered level of respiration frequency or different cortisol levels. To counter the problem of the different evolution of EEG spectra, we chose the occurrence of the first ictal EEG spikes as a more robust temporal marker for evolution of cortical activity related to seizures. Mean time of ictal EEG spike onset was 4.0 ± 1.3 min after start of the PTZ infusion. The tonic clonic stage of the seizure was reached at 9.1 ± 1.5 min after start of the PTZ infusion and lasted 0.9 ± 0.3 min. The average delay between the first occurrence of EEG spikes and end of the tonic clonic seizure was 5.9 ± 2.1 min.

3.2. VENG and heart rate during acute PTZ induced seizures

Figure 3 reflects the general behavior of VENG spike frequency (Figs. 3(a) and 3(b)) and amplitude (Figs. 3(c) and 3(d)) during the recording. In the PTZ group VENG spike amplitude and frequency both show a steady increase, and peak during the tonic clonic part of the seizure. After the tonic clonic stage the VENG spike amplitude and frequency drop rapidly towards baseline level and remains relatively constant during the recovery period. In the saline group, we observed a slow increasing drift of the VENG spike amplitude and frequency different from the PTZ animals, indicating an independent effect of the time evolution of the experiment on the VENG recording.

Considering the heart rate during the seizures, we observed three different patterns: 2/6 animals displayed strong ictal tachycardia, a 2/6 displayed minor ictal tachycardia and 2/6 first showed ictal bradycardia followed by tachycardia during the tonic clonic stage of the seizure.

Fig 3. VENG spike frequency (a), (b) and VENG spike amplitude (c), (d), normalized in amplitude by the standard deviation of the full recording, and normalized in time by realignment according to mean timestamps of different seizure stages followed by resampling. Pooled for PTZ group (a), (c) and saline group (c), (d).
In the following, heart rate changes are expressed in % of baseline heart rate. In the animals displaying strong ictal tachycardia, the heart rate increased for 23.3 ± 12.7% during PTZ infusion and for another 15.8 ± 7.0% during the tonic clonic stage, resulting in an overall increase of 39.1 ± 19.7%.

The animals exhibiting minor ictal tachycardia showing an increase of 4.3 ± 1.4% during PTZ infusion and an increase of 3.8 ± 3.1% during the tonic clonic stage, resulting in an overall increase of 8.0 ± 1.8%.

In the animals showing combined ictal brady and tachycardia the heart rate decreased for 15.9 ± 3.5% after start of PTZ infusion, followed by an increase of 15.0 ± 0.6% before the tonic clonic stage and an increase of 61.8 ± 38.0% during the tonic clonic stage, resulting in a maximal increase of 76.8 ± 38.6%.

### 3.3. Metric parameter determination and classifier training

The classification quality in terms of AUC was established for different sets of window sizes. As it can be...
seen in Fig. 3(a), the classification quality decreases with decreasing window sizes. Perfect classification could only be achieved with the window sets: 30 s foreground/90 s background and 10 s foreground/90 s background. When window sizes are decreased to 1 s foreground/3 s background the classification quality is just slightly better than random guessing. The size of the foreground window defines the temporal blur of the detection method. Since the seizure detection method needs to be as precise as possible in time while still maintaining best classification quality, 10 s foreground window and 90 s background window were chosen.

The classification quality indicated by the AUC for each ROC curve for all training pools (exPTZ1-6) are displayed in Fig. 4(b). All training pools except exPTZ6 share the same range of SOT in which perfect classification is possible (29–64 samples), while exPTZ6 exhibits a wider range (23–80 samples). Based on these ranges we can determine the range of Z-scores and SOT which would allow for perfect classification.

As it can be seen in Fig. 4(c), the parameter ranges for perfect classification are mostly identical for training pools exPTZ1-5 while the parameter range for perfect classification in training pool exPTZ6 is larger. Based on the parameter ranges for perfect classification the Z-score and the minimal number of SOT was chosen to satisfy two conditions:

- A small SOT to ensure a short delay to the actual detection.
- A low Z-score to ensure a low threshold to favor false detections rather than missing a seizure.

Based on these conditions, the parameters were chosen as Z-score = 1.6, SOT = 35 for training pools exPTZ1, 2, 4, 5, 6 and: Z-score = 1.4, SOT = 34 for training pools exPTZ3.

Those independently selected classification parameter sets are all located in a very narrow window.

### 3.4. VENG-based seizure detection

For each recorded seizure (PTZ1-6), an individual set of parameters (Z-score, SOT) was established by training our classifier on a pool (exPTZ1-6) consisting of all recordings, except the recording to be tested. From these calculations, we chose the best parameter set which allowed an early and sensitive
detection. The parameter sets were found to lay in a narrow window: \( Z \text{-score} = 1.4 - 1.6, \text{SOT} = 34 - 35 \) (Fig. 4(c), red box). When these parameters were applied in our seizure detection algorithm, several possible candidates for detections were observed (Fig. 4 red asterisks). In recordings with multiple candidate detections, we can observe one or two aggregations of candidates per animal. We define one single aggregation as a group of candidates with a subsequent distance of less than five times the SOT. In addition, only the first candidate in the aggregation is of interest and therefore this candidate will be considered as the single detection hereafter.

We found different patterns of detections in the recorded seizures (PTZ1-6). In 2/6 animals we observed a detection early before the start of the tonic clonic stage (Figs. 5(a) and 5(e), pink arrow), and an additional detection during the tonic clonic stage. (Figs. 5(a) and 5(e), blue arrow). In 3/6, a detection occurred during the tonic clonic stage (Figs. 5(b) - 5(d), blue arrow) and finally in 1/6 a detection was seen at an early stage before the tonic clonic phase (Fig. 5(f), pink arrow), but none in the tonic clonic phase of the seizure.

Detections found during early stages of the seizure are defined as early detections \((n = 3)\), while detections found during the tonic clonic stage of the seizure are defined as late detections \((n = 5)\).

The mean delay between the start of the PTZ infusion and the detections were: 2.57 ± 0.71 min and 9.61 ± 1.34 min for the early detections and late detections, respectively.

![Fig. 6](image_url)

Fig. 6. (Color online) (a)-(c) Superposition VENG based detection metric (purple) and motion/muscle artefact-based metric (green) exemplary shown for (a) PTZ1, (b) PTZ3, (c) PTZ6. (d)-(i) Estimation of EMG artefact amount (green) motion estimator (cyan), for (d) PTZ2, (e) PTZ3, (f) PTZ4, (g) PTZ1, (h) PTZ5, (i) PTZ6. Pink vertical lines indicate the timestamps of early detections, the blue vertical lines indicate the timestamps of late detections. Pink and blue shaded areas indicate the delay between initial threshold crossing and actual detection defined by the SOT.
The mean delay between the start of the first motor manifestation (tonic whisker extension) and the detections were 0.81 ± 1.48 min and 5.58 ± 2.41 min for the early detections and late detections, respectively.

The mean delay between the first occurrence of ictal EEG spikes and the detections was −0.66 ± 0.15 min and 5.43 ± 2.51 min for the early detections and late detections, respectively.

The mean delay between detections and the start of the tonic clonic seizure was −7.11 ± 2.10 min and 0.16 ± 0.09 min for the early detections and late detections, respectively.

3.5. Influence of motion/muscle artefacts

The motion/muscle artefact-based metric and the VENG-based detection metric, as exemplary shown for PTZ1-3 in Figs. 6(a)–6(c), were compared using Pearson’s linear correlation in order to illustrate their independence. For all seizure recordings (PTZ1–6) pooled, we observed a very low mean correlation of $r = −0.018 ± 0.082$ between the motion/muscle artefact-based metric and the VENG detection metric. In other words, the motion/muscle artefact-based metric does not follow the VENG-based metric as shown in Figs. 6(a)–6(c) for PTZ1,3 and 6. Indeed, the early VENG detections appear before any motion or substantial increase in muscle artefacts is visible (Figs. 6(g)–6(i)) and the late detections are only visible after the motion/muscle artefact estimator is decreasing sharply as the seizure evolves to the tonic clonic stage (Figs. 6(d)–6(f)).

3.6. Comparison with Heart rate-based metric

To determine the performance of our VENG-based metric compared to a cardiac-related method, we applied the heart rate-based metric as explained in 2.7. Individual classification parameter sets were determined similarly to the VENG-based detection metric. Compared to the VENG-based metric, the heart rate-based method achieved a lower classification quality, indicated by a maximal AUC of 0.905. The determined individual parameters had a broader window compared to the VENG-based metric: Z-score 0.9-1 and SOT: 33-38 instead of Z-score = 1.4–1.6, SOT = 34–35 for the VENG-based metric.

Only 3/6 seizures were correctly detected (Figs. 7(a)–7(c)), while in 2/6 no detection occurred (Figs. 7(d) and 7(e)) and in 1/6 in addition to valid detections during the seizure a detection

![Fig. 7. (Color online) Heart rate-based metric for PTZ1-6. Green asterisks indicate heart rate-based detection events pink and purple vertical bars indicate early and late VENG-based detections, green arrows indicate first detection of a group, the red arrow indicate false detection, green bars indicate the duration of PTZ infusion, red bars indicate the duration of the tonic clonic stage of the seizure.](2150024-11)
appeared during baseline period (Fig. 1). In the correctly detected seizures, the detection occurred 4.33 ± 4.18 min after start of the PTZ infusion and 0.62 ± 4.82 min after first appearance of EEG spikes. No false detections occurred in 3/3 saline recordings. Application of the grouping as done for the VENG detections resulted in the separation into one to four groups per recording. Due to the varying number of groups, only the latest group (during the tonic-clonic stage) was pooled. The mean delay between this group of detections and the start of the PTZ infusion, the start of first motor manifestations the occurrence of the first ictal EEG spikes and start of the tonic clonic seizure was 9.25 ± 1.18 min, 6.45 ± 2.15 min, −5.68 ± 2.14 min and 0.44 ± 0.44 min, respectively.

4. Discussion
The main goal of this study was to characterize a new seizure detection method based on the recording of spontaneous vagus nerve activity with a fully implantable cuff electrode. Our VENG-based seizure detection algorithm succeeded in detecting 6/6 seizures in six animals either at a very early stage before the first occurrence of ictal EEG spikes ($Δt_s$ = −0.66 ± 0.15 min in 3/6) or after the tonic-clonic stage onset ($Δt_s$ = 0.16 ± 0.09 min in 5/6). No false detections occurred during baseline or recovery period in PTZ animals nor in the saline control animals, indicating the clinical potential of our VENG-based seizure detection method. Previous work has described vagus nerve recordings in other contexts mostly exploring hypertensive. A single previous acute study in rats described VENG signals during induced seizures in rats. Likewise, we see a maximum during the tonic-clonic phase of the seizures. Increased parasympathetic activity in relation to epileptic seizures have been reported on multiple occasions. Brotherstone et al. described elevated cardiac parasympathetic activity through recording of heart rate variability during generalized subclinical seizure. Furthermore, on the basis of remarkably increased parasympathetic activity observed in
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sive differences in the RR intervals (r-MSSD), which is an indirect measure for parasympathetic activity. Therefore, this observation might not reflect an increased parasympathetic activity involving the cardiac branch of the vagus nerve. Instead, the constant infusion of saline may increase total blood volume inducing other parasympathetic activation not specific to the seizure but rather due to the experimental procedure. The parasympathetic pathway may indeed respond to an increased blood volume, leading to higher renal excretion.

4.2. VENG-based seizure detection algorithm

In the prospect of developing a VENG-based detection algorithm, we used several separated training and test data sets to determine the specific classification parameter. We found all parameter sets to lie in a very narrow window (Fig. 1(c), red box), indicating a very uniform underlying cause of the VENG spiking behavior across our animal population. As shown in Fig. 1 we observed early and late detections. Both types of detections showed a much closer relationship to seizure stages defined by epileptiform activity than to the start of motor manifestations or procedures during the recording, indicated by a minimal standard deviation of the delays. The closest relation was seen between the early detection and the start of ictal spikes in the EEG and between the late detections and the start of the tonic clonic phase of the seizure.

In order to appreciate its eventual afferent or efferent character of the recorded increased in VENG activity, we should emphasize that the delay calculated between the early detections and the first ictal spikes on the EEG is probably biased by the fact that the latter does not exactly correspond to the seizure onset. Indeed, in all animals, the baseline EEG was characterized by a 1-4 Hz slow wave activity reflecting the general anesthesia induced by ketamine. Once PTZ was infused, we observed a variable change of the baseline EEG evolving to faster frequencies. However, since we could not observe this activity shift in all recordings, and due to its variable nature, we chose the onset of ictal spikes on the EEG as a marker for the development of the seizure. Santucci et al. described different central manifestations of PTZ-induced seizures in rats, showing that ictal spikes could be preceded by bursts of slow waves and low voltage desynchronized activity. Therefore, ictal spikes may not be the first observable ictal manifestation of PTZ-induced seizures, and its occurrence does not necessarily correspond to the exact onset of the seizure.

Lathers et al. described the so-called “lockstep phenomenon” as the time-locked occurrence of sympathetic/parasympathetic discharges and cortical epileptic activity. In their studies, they found increases in neural discharge rates closely related to the occurrence of cortical epileptic activity. Our early and late detections are based on prominent changes in VENG spike frequency and amplitude. Thus, they represent changes in the amount of recruited nerve fibers and the activity of individual fibers. Due to the close relation of the detections to epileptic activity observed on the EEG, we can relate our results to the lockstep phenomenon. In addition, we have not observed any relation between cardiac behavior and the occurrence of the early/late detections, indicating that the observed VENG signal changes are not caused by cardiac feedback signals.

Late detections were found in 5/6 recordings, while early detections were only observed in 3/6 recordings. The vagus nerve can respond differently to the lockstep phenomenon in function of which fiber type and how many of a specific fiber population is activated. Due to the extra-neural nature of the recording it cannot be excluded that signals from nerve fibers deep in the vagus nerve were missed.
This could explain the higher incidence of late detections compared to early ones. Indeed, as displayed in Fig. 4(c) a higher VENG spike amplitude during the tonic-clonic stage of the seizure indicates a higher percentage of active fibers. Due to the increased number of fibers contributing to the activity change, late detections are less likely to be missed, due to the incomplete recording of the nerve cross section. Nevertheless, in one rat (PTZ6), only an early detection was observed, while the animal did evolve to a tonic clonic seizure. Closer investigation revealed a peak in VENG spike amplitude and frequency during tonic clonic stage of the seizure. However, this peak was less prominent compared to the recordings of PTZ1-5. In addition, PTZ6 showed no prominent baseline delta activity which might indicate a lower anesthesia level. This might partially explain our findings, although further experiments are needed to determine the influence of anesthesia on our VENG detection algorithm.

4.3. Influence of motion and muscle artefacts

Motion and muscle artefacts are usually magnitudes higher in amplitude than the intended nerve signal. To ensure artefact free signals we applied several methods: (1) the raw recording was cleaned using a wavelet technique described in Sec. 2.3. (2) The applied VENG spike detector described in Ref. 27 disregards exceptionally high amplitude spikes which usually associated with muscle artefacts.

In addition, to ensure the independence of our detection method from motor artefacts we created a second test metric based on estimators for motion and muscle artefacts. A mean Pearson’s correlation coefficient of $r = -0.018 \pm 0.082$ between the VENG-based detection metric and the artefact-based test metric indicates that the VENG detection metric and the motion/muscle artefacts-based metric are independent from each other.

When we compare the early detections to the motion and muscle artefact estimators, the early detections clearly precede the sustained motion and muscle artefacts (see Figs. 6(g)–6(i)). They are also preceding the tonic whisker extension, which is the first motor manifestation of the seizure by 0.81 ± 1.48 min. No causal relation between early detection and motion and muscle artefacts could be established, confirming their genuine neural character. In the same line, late detections always occurred after the steep decrease of the motion and muscle artefact estimators (Figs. 6(d) and 6(h)). However, as it can be seen in Figs. 6(d) and 6(i) not all steep downward flanks in the artefact estimator are followed by a late detection. Therefore, we believe the neural signal is independent from motion and muscle artefacts.

In this study, we wanted to isolate neural activity (VENG) from all other signals including muscle and motion artefacts. As significant changes in muscle activity have been shown during tonic-clonic seizures, the muscle and motion artefacts could be utilized as an additional parameter in the future, in order to increase the performance of VENG-based seizure detection.

4.4. Comparison with heart rate-based metric

The only currently commercially available “on-demand” VNS stimulation system (Aspire SR) is based on the detection of ictal tachycardia. Therefore, we compared our VENG-based detection to a heart rate-based method, relatable to the Aspire system. While we could achieve a perfect classification quality using the VENG-based detection, a relatable metric based on the evolution of the mean heart rate, as described in 2.7, performed much poorer. During the classification training on the heart rate-based metric, a maximal AUC of 0.91 ± 0.04 was reached. This indicates a lower classification quality compared to the VENG-based detection. Indeed, the heart rate-based metric only detected 3/6 seizures properly and 2 seizures were not detected at all, and in one recording, a false detection during the baseline interval occurred. In contrast to VENG-based detection, results of the heart rate-based metric showed no close relation to the seizure stages or experimental procedures, as indicated by the small standard derivations of the mean delay. In the two missed detections, the animal did show a minor ictal tachycardia, but the increase heart rate was too low to trigger a detection. One false detection during baseline interval was triggered due to a peak in the heart rate. This peak was not related to the seizure nor the any manipulation during the experiment. No artefacts or ECG abnormalities were observed during this peak. For these reasons, we conclude that VENG-based detection is more reliable than heart rate-based detections, due to two drawbacks of the
heart rate-based method: (1) the lack of a sustained heart rate increase, accompanying the seizure. This is the case in 29-36% of the patients. (2) False positive detections are more likely to occur whenever heart rate increases independently from the seizure. For example, in the E16 study, the Aspire showed a FPR of 6.9/h at the lowest programmed threshold of 20%.

Currently available VNS device utilizing ictal tachycardia detection, triggers stimulation at a heart rate increase of 20–70%. Depending on the window size used to determine the heart rate, the cardiac-based seizure detection of the Aspire would not detect 2 to 4 seizures of our study. Just two animals exhibited high enough heart rate change over a short duration, while two animals exhibited heart rate increases below 20% and two animals exhibited heart rate changes over 20% but due to the very slow nature of those changes, would probably also have been missed.

On the other hand, even though heart rate-based detection seemed more variable, it did detect 3/6 seizures in a time-span shorter than our VENG-based algorithm (2.1 ± 3.59 min earlier). Therefore, it is not excluded that combining both detections algorithms might further improve the seizure detection.

4.5. Limitations
The small dimension of the nerve (about 300 µm in our animals) challenges an appropriate electrode placement with contacts covering the entire circumference of the nerve. This might result in an imperfect representation of the nerve cross section in the recorded signal. To facilitate the electrode nerve contact, we removed the epineurium of the vagus nerve partially, which will not be possible in a human application. However, the human cervical vagus nerves have a 15 times larger diameter compared to rats. Therefore, we assume a higher number of nerve fibers contributing to the VENG signal. In addition, there is more space to separate the electrode contacts. For those reasons we expect to observe higher VENG signals and therefore the removal of the epineurium might not be necessary. On the other hand, as spike amplitude drastically decrease with the distance to the surface signals of fibers deeper in the nerve will drown in the high amplitude signals of superficial fibers. This will result in a limited recording depth and the miss of signals deep in the nerve.

A second limitation of our study is the fact that we acutely recorded seizures in anesthetized animals. There is a debate if and how peripheral nerve signals are affected by different anesthetics. Wilson et al. demonstrated that ketamine may induce a central inhibitory effect on the vagal pathway and have inhibitory effects on vagal nerve signal transmission. However, none of those studies used actual VENG recordings, but only smooth muscle and reflex responses to electric stimulation. In addition, as we only induced seizures acutely, we were not able to evaluate the seizure prediction capability of this method. However, the fact that 3/6 seizures could be recognized in a very early stage, gives us confidence that seizure prediction could be possible. Currently, we are preparing studies using chronic epilepsy models, which will allow us to investigate the seizure detection capability of this method and open eventual future opportunities to work on seizure prediction.

A third limitation is the fact that our seizure detection algorithm has been developed in anesthetized animals, while seizures in awake and freely moving animals are expected to yield higher levels of EMG and motion artefacts that may interfere with the VENG recording. However, encapsulation of the cuff electrode after the implantation promotes electrode-nerve contact and enhances sealing of the cuff. This is expected to reduce these motion artefacts and EMG interferences, due to the true tripolar configuration.

A fourth limitation of this study is the small number of animals. As the scope of this study is to check the feasibility of recording useful seizure related signals in the vagus nerve, no more animals appeared necessary. Of course, higher numbers will be necessary in a later chronic study to evaluate quantitatively the detection method. Note, however, that the small sample number in this study is partly compensated by the leave one out method. This provided us with six training pools of eight animals each and therefore the removal of the epineurium might not be necessary. On the other hand, as spike amplitude drastically decrease with the distance to the surface signals of fibers deeper in the nerve will drown in the high amplitude signals of superficial fibers. This will result in a limited recording depth and the miss of signals deep in the nerve.

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of the foreground window length will affect delay between changes in the vagus nerve signal and detection events as well as the temporal precision of the detection, while changes in the background window length do not further impact the delay or precision. We have shown that we can archive perfect classification with a 10 s foreground window, which matches the foreground window size in the commercially available AspireSR system. This window size can induce a delay up to 10 s. As primary/secondary generalized tonic–clonic seizures show a median duration of more than 1 or 2 min, respectively, we are confident that a delay of 10 s will have a negligible effect on the treatment outcome in a closed loop VNS setting.

A last limitation is the fact, that the spike detection algorithm used in this study is designed for offline use. An adaptation for online use is possible but will induce a delay due to mandatory buffering of the data. Also, the detection algorithm needs to be further optimized to reduce computing time and memory demand.

5. Conclusion

This study suggests that VENG recording might offer a pertinent alternative to heart rate-based seizure detection algorithm. The observed VENG signal changes during seizure are not correlated to motion or EMG artefacts neither related to heart rate fluctuations. Further work is needed to adapt this method for spontaneous seizures in chronic epilepsy models and ultimately in humans.

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Appendix A

In Sec. 2.3 we manually selected thresholds in 11 frequency bands to separate wavelet coefficients related to motion/muscle artifacts from the ones related to neural signals. Those thresholds were based on the magnitude of the wavelet coefficients. In order to provide a more deterministic way to select the thresholds, we developed an automatic method to select thresholds resembling the manually picked ones. As the observed artefact show different footprints in different frequency bands (see Fig. A.3), we grouped frequency band that show similar responses to artifacts: Frequency group 1 (FG1): 103, 253, 506 Hz; Frequency group 2 (FG2): 767, 1013 Hz; Frequency group 3 (FG3): 2025, 3070, 4051, 5345, 6139, 7052 Hz.

To determine the threshold, a probe run of the full recording as in Sec. 2.3 was done by continuous wavelet transformation of 10 s snippets every 100 s. The magnitude of the 11 frequency bands mentioned above were taken, the mean was removed and normalized by the standard deviation. A signal envelope was calculated by the standard deviation of a 1 s (80,000 samples) sliding window. We calculated the threshold (Th) based on the mean of the envelope multiplied by a factor $A$ and its standard deviation multiplied by a factor $B$ as follows:

$$Th = mean(A \cdot envelope) + B \cdot std(A \cdot envelope).$$  \hspace{1cm} (A.1)

The factors $A$ and $B$ have been varied from 1 to 5 in 0.5 steps and from 0 to 7 in 0.5 steps, respectively. According to the lowest mean absolute deviation from the manual selected value, $A$ and $B$ have been chosen for the different frequency groups as: FG1: $A$: 3.5; $B$: 0.0; FG2: $A$: 4.0; $B$: 0.0; FG3: $A$: 4.5; $B$: 1.0.

The resulting thresholds were averaged between the animals for each frequency band and fitted to a rational polynomial (numerator degree 2, denominator degree 1) function using Matlab curve fitting tool (the MathWorks, Natick, USA). Goodness of fit was evaluated by the root mean squared error (RMSE: 0.1643).

As shown in Fig. A.4, the function fitted to automatic selected thresholds resembles the function fitted to the manual selected thresholds very well in the relevant frequency band (mean absolute deviation: 0.58%, max absolute deviation: 2.14%). Deviations outside the relevant frequency band do not affect the signal as all wavelet coefficients not related to the relevant frequency band are set to 0 in order to bandpass filter the signal.

The use of the automatically determined thresholds results in a different processing of less than 0.1% for almost the full relevant frequency band (Fig. A.5).
Fig. A.1. (a) Comparison of fit of manual selected and automatic selected thresholds relevant frequency band (311.7 Hz–5728.3 Hz) for automatic selected thresholds highlighted. (b) Relative deviation between fitted manual thresholds and fitted automatic selected thresholds in % of fitted manual thresholds, relevant frequency band (311.7 Hz–5728.3 Hz) highlighted.

Fig. A.2. (Color online) The red line represents the percentage of differently treated wavelet coefficients during the cleaning step averaged over all recordings, the gray shaded area indicates the standard deviation.

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