Recording of spontaneous vagus nerve activity during Pentylenetetrazol-induced seizures in rats

Lars Stumpp\textsuperscript{a},* Hugo Smets\textsuperscript{b}, Simone Vespa\textsuperscript{a}, Joaquin Cury\textsuperscript{b}, Pascal Doguet\textsuperscript{c}, Jean Delbeke\textsuperscript{e}, Emmanuel Hermans\textsuperscript{a}, Christian Sevcencu\textsuperscript{d}, Thomas N. Nielsen\textsuperscript{e}, Antoine Nonclercq\textsuperscript{b}, Riem El Tahry\textsuperscript{a,\textoline{f}}

\textsuperscript{a} Institute of Neuroscience, Université Catholique de Louvain, Belgium
\textsuperscript{b} BEAMS Department, Université libre de Bruxelles, Belgium
\textsuperscript{c} Synergia Medical SA, Mont-Saint-Guibert, Belgium
\textsuperscript{d} National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj-Napoca, Romania
\textsuperscript{e} Department of Health Science and Technology, Aalborg University, Denmark
\textsuperscript{f} Cliniques Universitaires Saint Luc, Center for Refractory Epilepsy, Belgium

\textbf{ARTICLE INFO}

Keywords: Vagus nerve Seizure detection Vagus nerve electroneurogram PTZ

\textbf{ABSTRACT}

\textbf{Background:} Vagus nerve stimulation is a treatment for refractory epilepsy. The vagus nerve carries parasympathetic information and innervates multiple organs. As seizures are commonly associated with autonomic manifestations, we believe that biomarkers for diseases affecting autonomic functions such as epilepsy can be found in vagus nerve signals.

\textbf{New method:} We present a method to record vagus nerve electroneurogram (VENG) and detect in the VENG single unit activity in anesthetized rats during Pentylenetetrazol induced seizures using a true tripolar cuff electrode.

\textbf{Results:} The VENG consisted of high amplitude bursts and lower amplitude bursts synchronous to respiration and heartbeat respectively. The average spikes exhibited a triphasic shape with duration below 1.5ms and root mean square amplitude varied between 5.5 +/- 0.2 \(\mu\)V and 11.4 +/- 3.1 \(\mu\)V depending on the type of recording. An increase of the contact distance resulted in a signal amplitude increase. Application of Lidocaine led to a total disappearance of the recorded spontaneous spiking of the nerve.

\textbf{Comparison with existing methods:} True tripolar cuff electrodes exhibited a better performance in terms of artefact rejection, stability and reproducibility of the signal compared to commonly used hook electrodes which is of special interest in seizures where important motion and EMG artifacts are expected.

\textbf{Conclusion:} We present a new method to record single unit activity of the vagus nerve during acute chemically induced seizures in rats and verified the neural origin of the recorded signals. This recording method might be a powerful tool to develop seizure biomarkers based on VENG.

1. Introduction

Epilepsy is a serious neurologic condition which affects about 50 million people worldwide (Fiest et al., 2017). It is associated with stigma, psychiatric co-morbidity, and high economic costs (Allers et al., 2015). One third of the patients suffer a ‘refractory’ form of the disease meaning that they do not respond to antiepileptic drugs. In this group, epilepsy surgery is considered to be the treatment with the best outcome. However only a fraction (5 %-50 %) (Ryvlin and Rheims, 2008) of these patients are suitable candidates for surgery. For the remaining patients, vagus nerve stimulation (VNS) may be an alternative.

Recently, a new model of VNS has been commercialized (AspireSR), which was designed to exploit ictal tachycardia using a patented cardiac-based seizure-detecting algorithm. The device triggers VNS on the basis of tachycardia. The performance of this automated seizure detection was assessed in a prospective observational multi-site study (Boon et al., 2015). Despite the rather accurate system, the expected additional or potential benefit for patients is still a matter of debate. A possible explanation is that a substantial number of patients do not have ictal tachycardia while this is a basic requirement for this “closed loop” system. However, the abortive effect of on demand VNS, prior or soon after seizure onset is being confirmed by several human and
animal studies (Boon et al., 2001; McLachlan, 1993; Zabara, 1992).

To this day, seizure detection is mainly based on scalp or intracranial electroencephalography (EEG). However, seizures also induce autonomic symptoms such as pupil dilation, skin blushing, cardiac and respiratory changes (Wannamaker, 1985; Sevcencu and Struijk, 2010). Within this context, the vagus nerve is a key bidirectional information pathway between the brain and different visceral organs. For this reason, exploiting the vagal neural traffic related to seizures might offer a novel method for early detection of seizures as needed to control an on demand therapeutic stimulation of the same nerve.

Several reports in the literature support the idea of extracting different physiological signals from the vagus nerve. For example, it was previously shown that vagus nerve signals can reflect blood pressure fluctuations (Sevcencu et al., 2014) and respiration cycles in healthy pigs (Sevcencu et al., 2018). The authors suggested the use of the vagus nerve electrophysiology (VENG) in order to extract a blood pressure biomarker as a feedback signal to detect hypertension (Sevcencu et al., 2014), and the use of respiratory neural profiles also extracted from VENG as biomarkers to distinguish pathological from non-pathological blood pressure increase related to physical exercise (Sevcencu et al., 2018) in closed loop VNS therapy of hypertension. Furthermore, Plachta et al. used VENG signals recorded with multi-contact cuff electrodes to identify baroreceptive fibers in the vagus nerve in order to increase efficacy and decrease side effects in selective VNS for the treatment of refractory hypertension (Plachta et al., 2012). Moreover, Zanos et al. were able to record single spike activity in the vagus nerve in mice and described cytokine-specific sensory neural signals based on vagal nerve recordings (Zanos et al., 2018).

In addition, some early studies have focused on VENG recordings during seizures in rats, but these studies were performed with non-implantable hook electrodes (Harreby et al., 2011b, 2011). Cardiac activity related features of the neural signal (energy or spectral pattern) were suggested as a possible marker for seizure activity. However, these results were not further exploited for the development of possible seizure detection systems.

Analyzing single unit activity recorded from a fully implantable setting is a novel approach to investigate ictal vagus nerve activity. In this study, we compare different technical options to record spontaneous VENG during acute Pentylenetetrazol (PTZ) induced seizures in rats and describe a new method to record and identify single unit activity and sort out the detected spikes into specific amplitude groups.

2. Materials/methods

2.1. Animals and surgical procedure

The experimental procedures have been approved by the University Health Sciences Sector Laboratory Animal Protection Committee (2018/UCL/MD/001). Fourteen male Wistar rats with an average weight of 368 ± 46 g obtained from the local breeding facility at the Université Catholique de 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.

The animals were initially anesthetized with 6% Sevoflurane in pure O₂ at a 3 L/min flow rate. As the initial anesthesia takes effect, the Sevoflurane anesthesia was stopped, and the animals were injected with 60 mg/kg Ketamine and 7 mg/kg Xylazine intraperitoneally (i.p.). Depth of anesthesia was controlled by the withdrawal reflex upon noxious paw stimulation and maintained by additional i.p. injections of 60 mg/kg Ketamine, approximately every 30 min. The body temperature was controlled via a rectal probe and maintained at 37.5 °C by a heating pad.

The electrocardiogram (ECG) was recorded via subcutaneous tungsten needle electrodes, placed on the limbs according to Eindhoven’s lead II.

Rats were secured in a stereotactic frame (David Kopf Instruments, Tujunga, USA), the skull was exposed, and epidural electrodes were screwed into previously drilled holes as described by Harreby et al. (2011). A two channels EEG was recorded bipolarly as illustrated in Fig. 1a.

For recording the VENG, the left vagus nerve was exposed. The epineurium was desheathed over a length of 10 – 15 mm and the exposed nerve was placed either on an elevated tripolar hook electrode or into a tripolar cuff electrode described below (Fig. 1b, c). The nerve and the surrounding tissue were covered with silicone oil (Merck KGaA, Darmstadt, Germany) to prevent dehydration.

The lateral tail vein was cannulated with a 26 G catheter to provide a venous access for the administration of PTZ or saline.

2.2. Data acquisition

2.2.1. Electrode types

Two types of electrodes were used. 1) hook electrodes made of three 70 mm long, 200 μm thick Ag-wire (>99.99 % Ag) sandwiched between two silicone sheets either at 1 mm (Hook_1) or 2 mm (Hook_2) inter-contact distance. The contacts were submerged into bleach for 1 h to form a silver chloride layer (Silverman et al., 2018). Before the recording started, the electrodes were briefly cleaned electrochemically. To vary the anode-cathode distance (AC-distance) different contact settings, presented in Table 1 were used for Hook_1 and Hook_2.

2) Pt Standard Micro Cuff (Microprobes, Gaithersburg, USA) with 300 μm inner diameter, 100 μm contact width and 2 mm contact spacing. The ground consisted of a 20 mm long 200 μm thick Ag/AgCl wire, placed between skin and sternomastoid muscle (Fig. 1c).

A set of custom-made amplifiers were used. This set consisted out of two VENG, one ECG and two EEG amplifiers. All signals were filtered...
2.2.2. VENG spike detection

The spike detection method was adapted from (Nonclercq et al., 2012) for VENG signals. In the following description and in the supplemental MethodsX article, the word ‘peak’ refers to a maximum above the threshold in the VENG signal convoluted with a spike template (i.e. template matching) which indicates the possible location of a spike, while the word ‘spike’ refers to a detected and confirmed monophasic single axonal action potential. The algorithm works in five steps. 1) Template matching was performed with a generic spike template. Peaks were detected using parameters suitable for highly sensitive detection. 2) Detected peaks were separated into clusters. 3) The number of clusters was automatically adjusted. 4) Centroids were used as templates for more specific spike detections, therefore adapting to the types of spike morphology. 5) Final sorting was used to separate positive and negative spikes and sort spikes into two amplitude groups for each polarity (Positive: P1.2, Negative: N1.2). Spikes with extremely high or small absolute amplitudes are likely to correspond to electromyogram (EMG) artifacts (Falcone et al., 2018) (too high amplitude) or to background noise (too low amplitude) and considered as outliers. The main changes compared to the method in (Nonclercq et al., 2012) are 1) adaption to the VENG specific spikes by the use of a generic template mimicking the shape of spikes in the VENG recording. 2) The use of adapted thresholds and non-normalized cross-correlation instead of fixed thresholds and normalized cross-correlation to reduce the number of false positives. 3) The use of a correlation-based metric to determine the number of specific templates based on spike-shapes instead of fixed thresholds and normalized cross-correlation to reduce the number of false positives. 3) The use of a correlation-based metric to determine the number of specific templates based on spike-shapes instead of fixed thresholds and normalized cross-correlation to reduce the number of false positives. 4) The ability to detect and distinguish nearby spikes instead of rejecting spikes below a certain inter spike interval to also detect activity of different coincidentally firing fibers. 5) Final sorting was used to separate positive and negative spikes and sort spikes into two amplitude groups for each polarity (Positive: P1.2, Negative: N1.2). Spikes with extremely high or small absolute amplitudes are likely to correspond to electromyogram (EMG) artifacts (Falcone et al., 2018) (too high amplitude) or to background noise (too low amplitude) and considered as outliers. The main changes compared to the method in (Nonclercq et al., 2012) are 1) adaption to the VENG specific spikes by the use of a generic template mimicking the shape of spikes in the VENG recording. 2) The use of adapted thresholds and non-normalized cross-correlation instead of fixed thresholds and normalized cross-correlation to reduce the number of false positives. 3) The use of a correlation-based metric to determine the number of specific templates based on spike-shapes instead of fixed thresholds and normalized cross-correlation to reduce the number of false positives. 4) The ability to detect and distinguish nearby spikes instead of rejecting spikes below a certain inter spike interval to also detect activity of different coincidentally firing fibers. For a detailed description including all parameters see supplemental MethodsX article.

2.2.3. Respiration estimator

The respiration estimator was calculated by the cubic spine interpolation of the r-wave amplitude at the r-peak timestamp extracted from the ECG, followed by zero phase shift high pass filtration at 1 Hz, to obtain a differentiable estimated respiration trace.
1) The most distinctive bursts were high in amplitude and synchronous to the negative peaks in the respiration estimator (Fig. 2a, yellow shaded).
2) Lower amplitude bursts were identified in the VENG at a frequency corresponding to the heart rate (Fig. 2a, dotted lines). At higher heart rates these heartbeat related bursts overlapped and became less obvious.

Within the recorded VENG signal, spikes were detected. Fig. 2b displays the mean shape for positive and negative spikes, pooled together for 3 recordings, using a hook electrode with 4 mm AC-distance. The average spikes exhibited a triphasic shape with duration below 1.5 ms. Table 2 displays VENG spike features, effective signal amplitudes represented by the root mean square (RMS) as well as the number of animals in which spikes could be detected in baseline recordings for different electrode types.

Vagotomy caudal to the recording electrode reduced the signal amplitude, with vanished respiratory burst activity in some animals (Fig. 2c), while those burst activity became more prominent in others (Fig. 2d).

Alternatively, an increase in the AC-contact distance of the hook electrode resulted in a signal amplitude increase (Fig. 2e–g). When a 1 mm AC-distance was used, the burst-modulation in the VENG signal was hidden in the background noise and often not, or just barely visible (Fig. 2e). By increasing, the AC-distance to 2 mm or more the burst modulation of the VENG signal became clearly visible (Fig. 2f–g).

The mean signal amplitude, measured by RMS, changed according to the electrode distance as shown in Table 3. Note that, starting with the value at 1 mm inter-contact distance, the RMS amplitude is roughly squared for each doubling of the distance (Indicated as estimated values).

### 3.2. Silencing of the vagus nerve

Topical application of Lidocaine to the nerve resulted within 30 s in

![Fig. 2. VENG recording and features during baseline, after vagotomy and with different electrode setups a) top: Baseline VENG signal of RAT1 at low respiration rate. Broken vertical lines indicate timestamps of R peaks in the ECG. Bottom: ECG derived respiration estimator of RAT1, synchronous to the VENG signal. Negative slopes indicate inspiration (yellow shaded intervals). Timestamps of r-peaks are marked with red diamonds. b) average positive(top) and negative(bottom) spikes extracted from 3 recordings using a hook electrode with 4 mm AC-distance, shaded area indicates ± STD. c,d) VENG signal before and after vagus nerve ligature caudal to the recording electrode in RAT2 (c) and RAT3 (d). e-g) VENG signals recorded with different AC-distances: 1 mm in RAT4 (e), 2 mm in RATS (f), 4 mm in RAT6 (g).](image-url)
a total disappearance of the bursts as well as the arrest of spontaneous spiking activity of the nerve (Fig. 3).

3.3. Comparison with ENS

The RMS amplitude of the VENG signal was much larger (by the factor 27.7 for INT1, 8.8 for INT2) than the corresponding amplitude of the ENS signal (Fig. 3c). The ENS also exhibited respiration related bursts, but no heartbeat related bursts were identified (Fig. 3c). The VENG and ENS signal envelope was calculated using a sliding standard deviation with a 125 ms window. A lag between the VENG and ENS signals was found by the cross-correlation of the signal envelopes. In the case of the ENS, the burst preceded the VENG by an average 54.6 ± 10.8 ms (for INT, INT2). The Pearson’s correlation coefficient between the VENG signal envelope and the ENS envelope corrected for the lag were 0.816 for INT1 and 0.4625 for INT2.

3.4. Cuff vs. hook electrodes

Stability of the recording was estimated by the fraction of artifacts in the recording signal. Artifact percentage was defined as the percentage of sample points of a moving standard deviation with a 100 ms window that were exceeding the range of 0.3–3 times the standard deviation of the baseline interval. Hook electrodes yielded a relatively large average artifact percentage of 30.06 ± 13.98 %. Cuff electrodes offered a higher stability during the recording as indicated by an average artifact percentage of 0.12 ± 0.15 %.

The raw VENG signals recorded via a micro-cuff electrode in a true tripolar setting show the same characteristics as recordings using a hook electrode, namely the respiration-related, the heartbeat-related bursts and the average spike shape.

3.5. VENG during PTZ induced seizures

Generalized tonic clonic seizures were induced in 2 animals using 66.5 ± 6.6 mg/Kg PTZ. (PTZ1 and 2). In both cases the VENG recording was performed using a cuff electrode. The onset of seizures in both
animals, based on concomitant EEG recording was 115 ± 64 s after PTZ injection. Total duration of the seizures was 754 ± 129 s. In addition, seizures were induced in 3 other animals (RAT1,7,8), using a hook electrode with 4 mm AC-distance. Due to a loss of contact between nerve and electrode caused by motions during the seizure, the recordings were not usable. They will not be further discussed in this section.

Fig. 5 shows the evolution over time of the spiking activity of amplitude bins P1, P2, N1 and N2 as well as the vital parameters of rats infused with PTZ and saline (control).

In one animal, PTZ1, the global spiking activity of the VENG decreased 117 s after start of the seizure (Fig. 5a, arrow). The decrease was gradual for amplitude groups P1 and N2 and very abrupt for group N1. Later in the seizure (432 s after seizure onset in the EEG), VENG amplitude groups increased gradually and peaked during the tonic-clonic stage. The combined average firing rate for all spike groups (P1,2 and N1,2) was 119.6 Hz. Rat PTZ1, presented ictal bradycardia, with a decrease in heart rate of 13 % until 543 s after seizure onset, where after heart rate increased gradually again to peak during tonic-clonic seizure (Fig. 5d). The respiration rate was much more variable. At seizure onset there was an initial increase with peaks short after the initial body jerks and onset of the tonic-clonic seizure. During the tonic-clonic seizure, the respiration rate decreased and stabilized later (Fig. 5g).

In contrast to rat PTZ1, in the second animal (PTZ2), all amplitude groups increased gradually but showed, as in PTZ1, a maximal peak when the tonic-clonic stage was reached (Fig. 5b). The combined average firing rate for all groups (P1,2 and N1,2) was 137.3 Hz. Rat PTZ2 showed ictal tachycardia, with a gradual increase of heart rate of 13 % from the start of the PTZ infusion until the tonic-clonic stage and

animals, based on concomitant EEG recording was 115 ± 64 s after PTZ injection. Total duration of the seizures was 754 ± 129 s. In addition, seizures were induced in 3 other animals (RAT1,7,8), using a hook electrode with 4 mm AC-distance. Due to a loss of contact between nerve and electrode caused by motions during the seizure, the recordings were not usable. They will not be further discussed in this section.

Fig. 5 shows the evolution over time of the spiking activity of amplitude bins P1, P2, N1 and N2 as well as the vital parameters of rats infused with PTZ and saline (control).

In one animal, PTZ1, the global spiking activity of the VENG decreased 117 s after start of the seizure (Fig. 5a, arrow). The decrease was gradual for amplitude groups P1 and N2 and very abrupt for group N1. Later in the seizure (432 s after seizure onset in the EEG), VENG amplitude groups increased gradually and peaked during the tonic-clonic stage. The combined average firing rate for all spike groups (P1,2 and N1,2) was 119.6 Hz. Rat PTZ1, presented ictal bradycardia, with a decrease in heart rate of 13 % until 543 s after seizure onset, where after heart rate increased gradually again to peak during tonic-clonic seizure (Fig. 5d). The respiration rate was much more variable. At seizure onset there was an initial increase with peaks short after the initial body jerks and onset of the tonic-clonic seizure. During the tonic-clonic seizure, the respiration rate decreased and stabilized later (Fig. 5g).

In contrast to rat PTZ1, in the second animal (PTZ2), all amplitude groups increased gradually but showed, as in PTZ1, a maximal peak when the tonic-clonic stage was reached (Fig. 5b). The combined average firing rate for all groups (P1,2 and N1,2) was 137.3 Hz. Rat PTZ2 showed ictal tachycardia, with a gradual increase of heart rate of 13 % from the start of the PTZ infusion until the tonic-clonic stage and
an abrupt increase of 25% during the tonic-clonic stage of the seizure (Fig. 5e). Similar to rat PTZ1, the respiration trace was more variable, with an increase towards the tonic-clonic stage and a steep decrease at the onset of the tonic-clonic stage (Fig. 5h).

The control rat (CTRL) showed relatively stable activity of all amplitude groups (Fig. 5c) and vital functions. The average combined firing frequency for P1,2 and N1,2 was 119.7 Hz. Only a brief step-like increase in VENG spiking activity could be noted. This followed a brief step-wise increase in heart rate of 7% just after the start of the saline infusion, which then gradually returned to the baseline (Fig. 5f).

4. Discussion

The aim of our study was to analyze spontaneous vagus nerve activity during acute PTZ-induced seizures in anesthetized rats. This manuscript describes a method to record VENG during seizures and reports different technical aspects of it. In addition, several experiments were performed to verify the genuine origin of the recorded signals. Two burst modulations were observed in the VENG signal, one of high amplitude synchronous with the respiration and one of lower amplitude and synchronous with the cardiac activity. Our results are compatible with previous studies, in which the respiratory modulation was shown to be the dominant component of the VENG signal in anesthetized rats and mice (Falcone et al., 2018; Silverman et al., 2018; Zanos et al., 2018).

Within the VENG signal, we detected spikes with a duration of less than 2 ms (Fig. 2b), which is similar to those reported by Falcone et al. (2018). However, Falcone et al. detected mostly biphasic spikes Falcone et al. (2018), while the majority of the detected spikes in this study had a triphasic shape and smaller amplitude. Falcone et al. (2018). This can be explained by the different recording configuration. Falcone et al. used monopolar recording Falcone et al. (2018), while our study sorted to a true tripolar setting. In contrast with the study performed by Silverman et al. in mice Silverman et al., 2018, our recordings showed a much higher firing frequency. This can be explained by several factors such as species difference, different anesthetics used and the feeding status of the animals. The rat vagus nerve has a 50% larger diameter compared to mice Falcone et al. (2018). This might be reflected in a higher number of nerve fibers and thus a larger total number of action potentials contributing to the signal. Silverman et al. used Isoflurane as an anesthetic, which is known to suppress peripheral nerve activity depending on the concentration used Silverman et al., 2018; Falcone et al. (2018). This is not the case for ketamine used in our study Silverman et al., 2018. Furthermore, it is known that the vagus nerve spiking activity is higher in well-fed mice as compared to starved animals Silverman et al., 2018. The animal used in this study had ‘ad libitum’ access to food and water, which was not the case in the mentioned previous studies Silverman et al., 2018.

Our spike sorting method was able to detect VENG spikes of positive and negative polarity and different amplitudes. In contrast to our initial expectations, the obtained amplitude distribution did not point to specific clusters but only segregates positive and negative spikes (see supplementary MethodsX article). Zanos et al. (2018) isolated specific spike clusters in the VENG encoding cytokine-specific information. They used t-distributed stochastic neighbor embedding (t-SNE) for dimensionality reduction and density-based spatial clustering of applications with noise (DBSCAN). In our study, the distribution of the spike features after dimensionality reduction did not show a high enough variation in density to employ DBSCAN. Therefore, the recorded VENG spikes were only sorted arbitrarily into amplitude groups. In future studies the use of different spike features may allow us to identify distinct clusters.

In order to ensure that our recordings consisted of genuine neural signals, we performed experiments in which we selectively silenced the nerve by topical application of 2% Lidocaine solution during VENG recording. When lidocaine was applied, all bursts and spike activity of the VENG vanished, while cardiac and respiratory activity were still present. We also performed experiments where we compared signals recorded with an identical hook electrode positioned in near proximity of the VENG electrode, but not on the nerve (i.e. ENS signals) and VENG signals recorded with the electrode placed on the vagus nerve. We found a very clear difference in signal amplitude between the ENS and the VENG signals. This indicated that the signal recorded from the vagus nerve was not likely to be generated outside the nerve. Indeed, the control hook in contact with the surrounding tissue should have shown the same or higher amplitude, if the source of the interference was non-neural and located in the surrounding tissue. Although the extra-neural signal was much lower in amplitude, it still contained small bursts similar to those in the VENG signal but with a considerable time lag. The Pearson’s correlation coefficient between VENG and ENS envelope, the latter corrected for the lag, of 0.816 (INT1) and 0.463 (INT2) indicate a certain similarity in general shape of the envelopes. The reduced coefficient in INT2 can be explained by less prominent respiratory related bursts due to a lower signal quality. In hook electrode recordings, the signal quality can vary due to the inferior electrode-nerve contact, compared to cuff-electrodes, and the inability to reject noise and artifacts resulting in less visible respiratory related bursts. This indicates that the dominant component in both signals is related to respiration. However, the lag between VENG and ENG envelope, the difference in amplitude as well and a clear divergence of the correlation coefficient from 1 indicates a different source or transmission for both signals.

We hypothesized that the origin of respiration related burst in the VENG may lie in feedback signals from slow adapting stretch receptors transmitting lung information to respiratory centers from the brain stem via vagal afferents. (Sevcencu et al., 2018; Schelegle and Green, 2001). However, our results (Fig. 2c, d) showed that respiratory related burst activity was not entirely of afferent nature because this activity was not completely suppressed by vagotomy caudal to the recording electrode. This indicated that burst activity synchronized with the respiratory cycle also comprised some efferent component. Those signals could originate from α-motor neurons innervating the larynx, which were also detected after vagotomy in previous vagus nerve studies Sevcencu et al., 2018. The variability of the response to the vagotomy can be caused by different physiological and technical factors.

Beside the motor neurons innervating the larynx, the vagus nerve is known to comprise efferent fibers innervating the heart lungs, airways and gastrointestinal tract Câmara and Griessenauer, 2015; Berthoud et al., 1991; McGovern and Mazzone, 2010. The signals of fibers that are not innervating lungs or airways are unlikely to be in phase with the respiration. They might be tonic in nature with respect to respiratory cycles and appear as background activity. The recruitment of laryngeal motor fibers is probably modulated by respiration rate and/or depth of respiration, which affects signal shape and amplitude. In addition, vagal efferents control the function of sinoatrial and atrioventricular node Rinkema et al., 1982 as well as secretion of gastric acid Berthoud et al., 1991. As part of a complex biological regulatory system, efferent VENG signals might depend heavily on the current state of that system, which can be influenced by factors like respiration, cardiac activity or feeding status. The difference in respiration rate (RAT2 ∼ 133/min, RAT3 ∼ 41/min) clearly indicates an altered physiological state, which can be caused e.g. by different levels of anesthesia. It might be argued that in Rat2 (Fig. 2c) phasic respiratory related signal are present after vagotomy but are submerged in background activity, while in Rat3 (Fig. 2d) background activity is sufficiently low to allow phasic respiratory related signals to stand out of the background. On the technical side, the nature of the recording can also play a role. Hook electrodes allow the recording of superficial signals at the point of contact between the electrode and the nerve. These signals could easily be missed if the motor neurons transmitting it ran in a segment of the nerve, located opposite to the electrode contacts Plachta et al., 2013. Those hypotheses can be proven by experiments utilizing tightly
controlled food deprivation, closely monitored continuous intravenous anesthesia including neuromuscular block, mechanical ventilation, and individual capturing of each signal component, e.g. by the use of sieve electrodes. However, this would be out of the methodological scope of this manuscript.

In addition to the respiration-related bursts, the recording of bursts synchronous with the heartbeat (Fig. 2a) is also compatible with previous studies reporting the presence of blood pressure profiles in VENG recordings, (Sevcencu et al., 2014; Plachta et al., 2020). Plachta et al. and Sevcencu et al. suggested the aortic baroreceptors (ABR) to represent the origin of those VENG components. Indeed, the ABR fibers transmit blood pressure-related signals via the aortic depressor nerve, which merges with the vagus nerve caudal to our recording site (Plachta et al., 2013; Sevcencu et al., 2017).

It is known that increased AC-distances result in higher signal amplitude. Former studies have shown that the signal amplitude increases with the square of the AC distance for the range (1 – 4 mm) used in this study (Stein et al., 1975; Sahin and Durand, 1998). Taking into account a certain degree of inaccuracy of the contact distances due to the flexible electrode material, our results are compatible with those findings.

Although typical components of the VENG signal were observed in both hook electrode and cuff electrode recordings, our results illustrate that cuff electrodes offer a more stable VENG recording with a greater artefact rejection compared to hooks (Fig. 4). This is of major importance, especially if we intend to record VENG during seizures where a lot of different artefacts (motion/EMG, etc.) can be expected. Those differences in artefact rejection were illustrated by averaging signal VENG snippets on the cardiac activity (Fig. 4). The coincidence with the R-peak in the ECG of the first component, which was visible in all recordings, indicated that this extra-neural component originated from the QRS complex of the ECG. The absence of the late component in the extra-neural electrode and the decreased amplitude in the silenced nerve suggested a mixed origin of that late component, which may correspond to afferent signals from the aortic baroreceptors (Plachta et al., 2013) mixed with motion artefacts induced by the pulsations of the nearby carotid artery. Ideally, the true tripolar configuration should reject all artifacts (Demosthenous et al., 2004) and exhibit twice the signal amplitude seen in bipolar recordings with the same AC-contact distance (Pachnis et al., 2007). The lower amplitude and the non-ideal artefact rejection might be explained by the imperfect seal of our split cylinder cuff (Andreasen et al., 2000) and the impedance imbalances within the cuff electrode (Pachnis et al., 2007). In future work, these shortcomings could be compensated by sealing the cuff electrode using silicone adhesive and the use of an impedance matching algorithm based on artifact comparisons between the two branches of the true tripolar setting (Levy et al., 2019).

Our method allowed to record VENG during acute PTZ induced seizures. This is a first step towards our main goal to develop VENG seizure detection methods. Up till today, only Harreby et al. (2011b) reported on work in that direction, showing an increased VENG power during PTZ induced seizures suggesting an increased parasympathetic activity as indicated by an increase in the RMS of successive R-R interval differences (r-MSSD). In two animals, we found that seizures affected the vagus nerve activity and vital functions in two different ways. One animal exhibited ictal bradycardia, and ictal tachycardia was observed in the second one. Ictal tachycardia is most commonly but not always seen in different localized focal seizures in humans (Sevcencu and Struijk, 2010). In both cases, VENG activity mostly mirrored the heart rate and not respiration. In the animal showing ictal bradycardia (PTZ1), we could observe an abrupt change in firing frequencies 1.9 min after onset of the first EEG spikes and shortly after the first ictal motor manifestations. Those changes were very prominent in small negative spikes and present but less prominent in other amplitude groups. Due to the small number of animals we cannot rule out the possibility that this is caused by artifacts. However, neither ECG nor EEG nor video recordings provided any indication in that sense. Further recordings need to be done to evaluate this finding.

The infusion of saline in the CTRL recording did not impact VENG activity or vital functions, although we did observe a short transient increase of VENG and heart rate after onset of saline infusion (see Fig. 5c,f). This transient increase may be attributed to the Bainbridge reflex (Hakumäki, 1987) which was also observed in another study in pigs (Sevcencu et al., 2016). Interestingly this reflex was either not present in the rats treated with PTZ or masked by the cardiovascular manifestations of the PTZ-induced seizures.

5. Conclusion

We have developed a method to record spontaneous VENG spiking activity during PTZ induced seizures in rats and demonstrated the genuine neural origin of the recorded signals.

In order to extract an early biomarker from the vagus nerve for seizure detection and develop closed loop VNS, further experiments are necessary to better understand how seizures affect VENG activity during seizures.

CRediT authorship contribution statement

Lars Stumpf: Conceptualization, Methodology, Investigation, Writing - original draft. Hugo Smets: Methodology, Writing - review & editing. Simone Vespas: Resources, Writing - review & editing. Joaquín Cury: Methodology, Writing - review & editing. Jean Delbeke: Conceptualization, Methodology, Writing - review & editing. Emmanuel Hermanes: Resources, Writing - review & editing. Christian Sevcencu: Resources, Writing - review & editing. Thomas N. Nielsen: Resources, Writing - review & editing. Antoine Nonclercq: Conceptualization, Methodology, Writing - review & editing, Supervision. Riem El Tahry: Conceptualization, Methodology, Writing - review & editing, Supervision.

Acknowledgements

The research was supported by the Walloon Region - Pôle Mecatech (Project NEUROPV). We thank Prof. Christian Gestreau of the Institute de Neurosciences des Systèmes at Aix-Marseille University for supplying us with crucial information about the anatomy and function of the vagus nerve and Prof. Anne Vanhoestenberghe of the Aspire CREATE - Centre for Rehabilitation Engineering and Assistive Technology at University College London for her valuable help in VENG signal acquisition.

References

Falcone, Jessica D., et al., 2018. A wraparound microwire electrode for awake, chronic interfacing with small diameter autonomic peripheral nerves. bioRxiv, 402925.