Vagus nerve activity based prediction of epileptic seizures in rats

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Abstract
Continuous open loop vagus nerve stimulation (VNS) is an option for the treatment of refractory epilepsy. Animal studies have shown that VNS is able to shorten or even abolish ongoing seizures and that stimulation prior to a pending seizure may prevent the seizure from occurring. VNS therapy might therefore be improved by close loop stimulation, but seizures must then be predicted or detected. The current work investigated the possibility of predicting seizures based on vagus nerve (VN) activity. The envelope of the VN ENG power was synchronized averaged to the R peaks of the ECG and a feature was extracted from this average. Using this feature it was possible to predict onset of pentylenetetrazole (PTZ) induced tonic seizures in 4 out of 5 rats, $97 \pm 59$ s prior to the onset of tonic seizures. The current work indicates that it is possible to predict seizures based on VN recordings. However, further work is needed to evaluate the specificity and the sensitivity to noise of this method.

1 Introduction
VNS has been applied for the treatment of refractory epileptic seizures for more than 20 years. Treated patients typically receive 20-30 Hz pulse trains at a fixed duty cycle (e.g. 30 s every 5 min), at the highest tolerated intensity [1]. In approximate 50% of patients receiving VNS, seizures are reduced by more than 50% [2].

Woodbury and Woodbury showed that VNS was able to either abolish or shorten ongoing epileptic seizures in rats [3]. Takaya and coworkers have later shown that VNS has a seizure inhibiting effect, which may prevent seizures from occurring after ceased stimulation [4]. Given these results, it may be hypothesized that close loop VNS, applied just before or at the early start of seizures, may increase the efficiency of VNS treatment. However, to perform close loop stimulation, a method for prediction or at least early detection of seizures must be established. Possibilities for predicting seizures have been investigated for more than 3 decades and have mainly been based on EEG recordings. However, EEG based prediction methods suitable for clinical applications have not been developed yet [5].

Autonomic changes have also been investigated mainly to elucidate reasons for sudden death in epilepsy. Lathers et al. observed changes in parasympathetic and vagal activity during interictal and ictal periods, which were related to cardiac arrhythmias [6]. Heart rate variability (HRV) has later been investigated as a precursor for seizure onset. Here it was indicated that changes in HRV occur several minutes prior to seizure onset [7]. Seizures have also been associated with an increase in blood pressure [7], which would potentially be accompanied by a change in VN activity. Indeed, Cerati and Schwartz showed in single fiber VN recordings, that firing rate between R peaks increased during increased blood pressure, whereas in the vicinity of the R peak itself no firings occurred [9].

Considering that the current VNS therapy already uses an electrode placed on the VN, an important parasympathetic nerve, it is relevant to investigate if seizures can be predicted based on VN activity. Therefore the objective of this work was to find precursors for the epileptic seizures, based on VN recordings. More specifically, we aimed to develop and evaluate a feature for detecting a potential seizure induced change in VN activity.

2 Methods

2.1 Animal model
The present study was performed on 5 male Sprague Dawley rats, 525-585 g weight. Animals were initially anesthetized by 2 intramuscular bolus doses of ketamine/xylazine (45 mg / 5 mg per kg ). Anesthesia was afterwards maintained by intravenous administration of the same anesthetics (45 mg / 5 mg per kg/hour). Temperature was monitored via a rectal probe, and kept at $37 \, ^\circ C$ using a heating pad. EEG was recorded from 2 stainless steel screws placed in the cranium, 3 mm caudal (active) and 9 mm rostral (indifferent) to the bregma. ECG was recorded from subcutaneous electrodes placed on the front of the thorax. Left VN activity was recorded via bipolar hook electrodes, and the nerve was covered with vaseline to prevent it from drying out. Respiration activity (Resp) was derived from measuring the pressure in an air inflated balloon, mounted in a belt placed around the thorax. In addition, EMG was recorded in one animal from the left styloglossal muscle using stainless steel wire electrodes. All signals were sampled at 20 kHz. After setting up the measurement equipment, the animal was left to stabilize for 15 min before any recordings were performed. Seizures were induced using PTZ diluted in saline, which was infused in the femoral vein at a rate of 10 mg/kg per min over 5 min (total of 50 mg PTZ/kg). After the experiments the animals were killed by an overdose of anesthesia.

2.2 Signal processing and feature description
ECG and ENG recordings were divided into consecutive periods of 20 s. For each of these periods, the VN recording was high pass filtered at 1 kHz, squared and filtered with a 5 ms moving average filter, resulting in the envelope of the ENG power.
The R peaks of the ECG were used for synchronized averaging of the ENG power envelope. This resulted in an average for each 20 s period which emphasizes the VN energy related to the heart beat cycle and reduces VN activities not synchronized to the heart beat (see Fig 3). In the synchronized averaged ENG two intervals were defined: (1) a 80 ms long interval centered on the R peak called “ENG around R” (ENGaR), (2) a 50 ms long interval located from 50 to 100 ms after the R peak, and called “ENG post R” (ENGpR, Fig. 3). The VN feature (VNF) was defined as:

(1) \[ \text{VNF} = \text{mean}(\text{ENGpR}) - \text{mean}(\text{ENGaR}) \]

Subtraction is used to make the feature less sensitive to fluctuation in the general level of VN activity. The standard deviation of VNF baseline (SDBase) was estimated from a 3 min baseline recording. The mean (meanBase) VNF was estimated from the 1 min baseline immediate prior to the onset of PTZ infusion. The normalized VNF (VNFNorm) was then defined as:

(2) \[ \text{VNFNorm} = (\text{VNF} - \text{meanBase}) / \text{SDBase} \]

A seizure was defined as detected when VNFNorm increased to more than 3 (Fig. 4). Seizure onset was defined based on an EEG pattern associated with the onset of tonic seizure (Fig 2).

3 Results

As illustrated in Fig. 1, some VN activity components are related to the heart and to the Resp cycle. Fig. 2 gives an overview of experiment 4, with EEG, EMG, Resp and VN power. Fig. 3 illustrates how the intervals ENGaR and ENGpR are defined and how the ENG power envelope changes during an experiment. Fig. 4 shows the VNFNorm from one experiment.

In 4 out of 5 rats, seizures were predicted 97 ± 59 s prior to tonic seizure onset using the VNFNorm feature. In the last rat the seizure was detected 6 s after tonic seizure onset.

4 Discussion

The tonic phase of PTZ induced seizures were predicted in 4 out of 5 rats. Prediction was based on changes in cardiac related VN activity, which might

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Fig. 1 VN ENG trace together with ECG and Resp. Increased ENG activity is seen both during the heart (a) and the respiration (b) cycles.

Fig. 2 Overview of EEG, EMG, Resp and ENG power (2.5 s bins, ENGPow) in experiment 4. In the EEG trace, “T” indicates the start of the EEG activity associated with the start of a tonic seizure. This is illustrated by the simultaneous onset of tonic activity in the EMG trace. The Resp trace shows some deep breaths (DB), both in the control period and during the PTZ infusion period. Resp pattern becomes shallower and increases in frequency ~1 min before tonic onset. At the tonic seizure onset the rat displays irregular Resp patterns superimposed on a persistent expansion of the thorax. The peaks during DB events and the general changes in ENG power level just before and during seizure shows that ENG power is correlated with Resp.
reflect a response to an increase in blood pressure. Fig. 2 indicates that prediction based solely on ENG power may be possible. However, the ENG power was seen to be correlated with respiration, which is to a large extent voluntary controlled and thus prone to irregular behaviour (e.g. during breath holding and talking). A cardiac related feature was therefore chosen, as there is little voluntary control over the heart.

Novak et al. reported that changes in the HRV became significant ~30 before seizure onset in humans [7]. In our study we were able to detect 4/5 seizures 97 s prior to tonic seizure onset. There are of course some limitations to this pilot study. As no control experiments have been evaluated, the specificity of the prediction algorithm has not been evaluated yet. In addition, the sensitivity to mechanical noise and physiological changes needs further evaluation in awake animals.

5 Conclusion

The results indicate that it is possible to predict the onset of tonic seizures based on cardiac related VN activity. The developed method could be used to improve currently used VNS therapy. However, further work is necessary to investigate the specificity and sensitivity to noise.

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7 Literature


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