# Micro Cuff Electrode Manufacture for Vagus Nerve Monitoring in Rats


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***Abstract*—This work describes the fabrication of a lab-made cuff electrode intended for rat vagus nerve electroneurography. The cuff electrode is built around 50 µm platinum wires contacts in tripolar configuration, 4mm contact spacing. The body is made of silicone rubber and four surgical threads. In vivo validation allowed to record respiration and cardiac related activity from the vagus nerve. The results show that our electrodes are a suitable low-cost alternative for our preclinical studies.**



***Keywords—micro cuff electrode, manufacture, vagus nerve, monitoring.***


## I. Introduction

Peripherical nerve stimulation and recording is one of the fastest-growing fields of medicine, with treatments and diagnosis for several neural diseases. One of the most common nervous system disorder, treated with neurostimulation, is epilepsy [1]. Epilepsy is very common and it affects more than 50 million people, of which 30% are refractory [2]. Vagus nerve stimulation is an adjunctive treatment whenever epilepsy surgery is not possible. Recently, vagus nerve electroneurogram (VENG) has gained interest with respect to seizure detection for future closed loop stimulation strategies.

Different types of VENG electrode technologies, such as cuff electrodes, flat interface nerve electrodes (FINEs), longitudinal intrafascicular electrodes (LIFEs), transverse intrafascicular multichannel electrodes (TIMEs), Sieve, and Microchannel electrodes offer different selectivity and invasiveness levels [3]. Cuff electrodes are the least selective technology; however, they are the easiest to implant. Also, they are widely used in extraneural recordings in animal studies and are the gold standard in clinical practice. Commercial cuff electrodes (e.g., Microprobe, Cortec, and World Precision Instrument) come with high cost and long delivery time. VENG and VNS studies require enough animals to be involved for statistical significance to be reached [4]–[7], which unfortunately can lead to a large number of implanted electrodes, thus increasing the cost of the study. Therefore, lab-made cuff electrodes may be an alternative to reduce cost and speed up delivery time.

Previous work has proposed low-cost and rapidly manufactured alternatives for peripheral nerve stimulation and recording. However, some of these are for acute experiments only [8], while others are too large (inner diameter>0.5 mm) [9], [10] to be used on the vagus nerve of rats. This paper proposes a VENG lab-made cuff electrode for preclinical studies in rats. We present the cuff electrode manufacture process and an acute in vivo validation.

## II. Materials and Methods

The cuff electrode manufacture process is divided into three parts: manufacturing of (1) the silicone tube, and (2) electrode contacts, (3) assembling the parts. A summary of the process is shown in Fig. 1. The manufactured electrode was subsequently validated in vivo.

### A. Silicone tube

The silicone tube is composed of platinum-cured silicone rubber compound (Ecoflex™ 00-30 A:B 1:1, Pot life: 45 minutes, Cure time: 4 hours). Two resin molds were designed for its molding: the mandrel mold (Fig. 2) and the tube mold (Fig. 3). The mandrel mold defines an inner diameter of 300 µm in the silicone tube. This diameter was chosen according

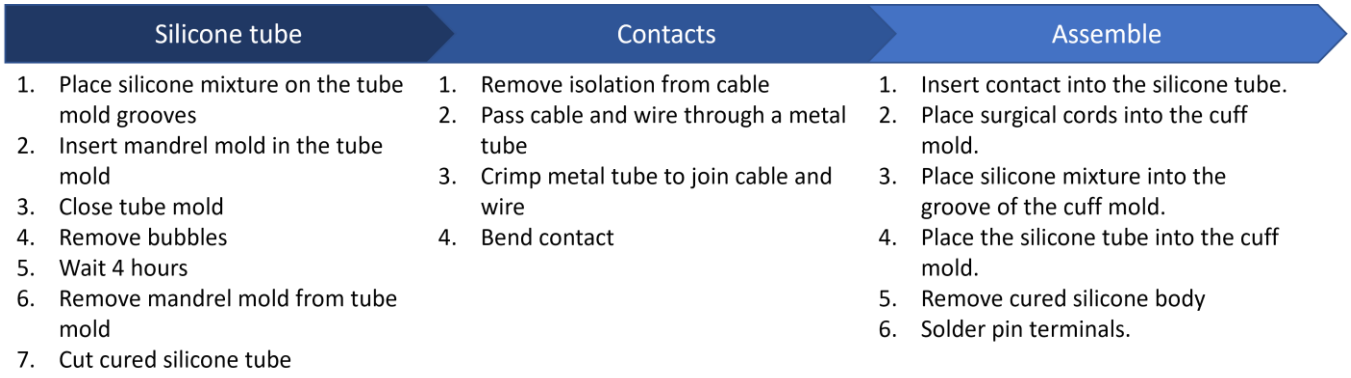


Fig. 1. Diagram of the complete cuff electrode manufacture process.

to morphology studies of the vagus nerve in rats [11]. The mandrel also adds the reference marks necessary to cut the silicone tube (to have a total length of 10 mm) and to have a mark reference for insertion of the wire contacts (4 mm contact spacing). The mandrel (PolyJet Vero material) is printed using 3D printer Eden260VS (Stratasys, Minnesota, USA). The tube mold is made of standard resin, using Elegoo Mars Pro 2 (Elegoo, Shenzhen, China). The tube mold is composed of two parts: the base and the top parts. All the resin parts printed by Elegoo were cured in an UV chamber for 15 minutes, and subsequently baked using a hot plate at 80 °C to prevent curing inhibition of the silicone induced by uncured resin.

The mandrel was dipped in polyvinyl alcohol to make a release layer. Then, the grooves were filled with platinum silicone rubber, and the mandrel was placed onto the base's groove. The mold was closed by placing the top part on the base. The entire mold was maintained closed with clips. Finally, the mold was placed inside a vacuum chamber for silicone degassing for 15 minutes. After 4 hours of curing, the clips were removed, the top part was carefully removed with tweezers. The mandrel surrounded with cured silicone was removed from the mold.

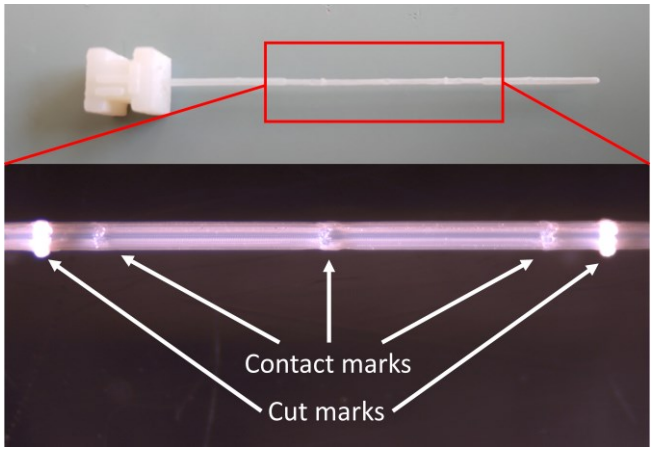


Fig. 2. Mandrel mold. It has a diameter of 0.3 mm at the center, 2 cut marks separated at 10 mm and 3 reference marks for the contacts, separated by 4 mm.

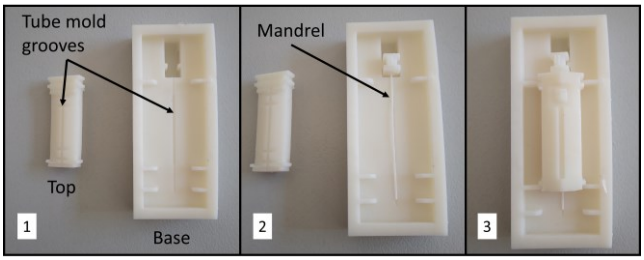


Fig. 3. (1) Tube mold composed of 2 parts: the top and the base. Both have a groove for putting the silicone mixture and for the mandrel placement. (2) Mandrel is placed on the base part. (3) The top part is placed on the base part, closing the mold.

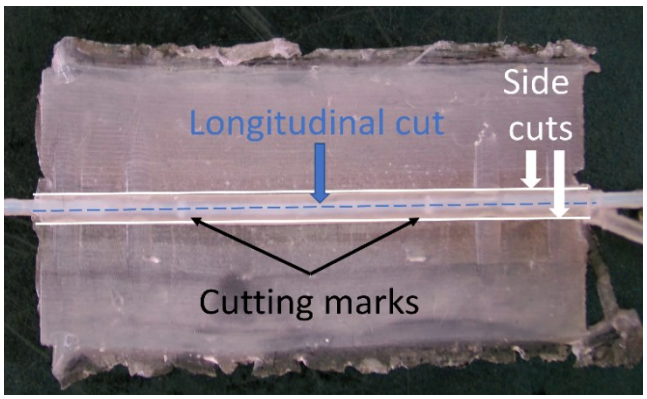


Fig. 4. Cutting the silicone tube. The silicone tube is cut open longitudinally (dashed blue line) and at both sides with a scalpel. Once the silicone tube is removed from the mandrel, it is further trimmed at the cutting marks.

The mandrel was placed in a cut aid to make a longitudinal cut and the excess silicone on both sides was removed. Next, the silicone tube was removed from the mandrel. Finally, the silicone tube was trimmed in length using the cutting marks as a guide (Fig. 4). All this was done in place of buying a simple silicone sheet. Classical cuffs need multilayers with one prestretched for curling.

### *B. Electrode contacts*

The contacts are made of 4 materials: (1) Platinum wire 50um diameter, 99.99% metal-based (Alpha Aesar, Thermofisher) 5mm length, (2) Austenitic stainless steel cable 30 cm length (0.08 mm diameter, Fort Wayne Metal), (3) Stainless steel tube 0.3 mm outer diameter, 0.15 mm inner diameter (Bilaney) 1mm length, and (4) Male pin contact pitch 2.54 mm.

After removal of the cable insulation, the free end of the cable and the platinum wire are passed through the metal tube. Then, the tube was crimped to make contact between materials (Fig. 5). Three contacts were made, named L (left), M (middle), and R (right) contacts, for the tripolar cuff electrode.

A second resin mold was designed and printed to have a reference shape for each contact (Fig. 6). Each contact was

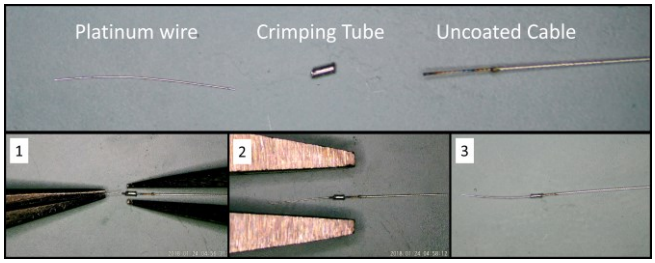


Fig. 5. Contact manufacture: (1) Placing the cable and the wire through the metal tube, (2) Crimping the metal tube (3) Crimped contact.

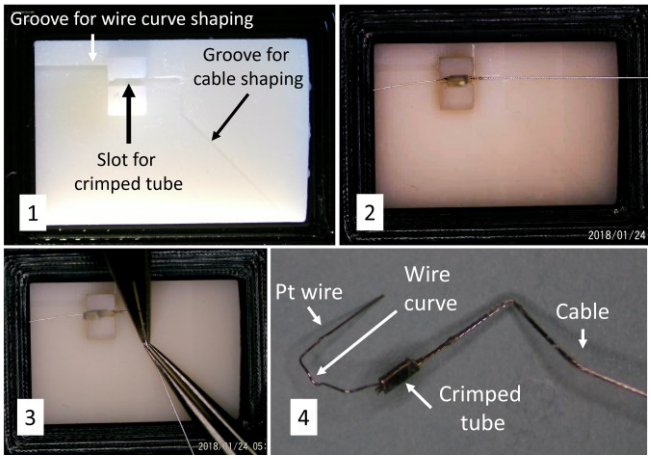


Fig. 6. (1) Bending mold for shaping the left contact. It has a slot to hold the crimped tube while it is bent. The mold has 2 grooves: one is used as a reference in shaping the cable and the other is for shaping the wire curve. (2) Contact placed and fixed on the mold (3) Contact shaping (4) Final contact shape.

bent using tweezers, and finally, each contact was labeled on the distal part.

### C. *Assembling parts*

The wires of each respective contact were inserted with tweezers in the silicone tube, at the marked position. Then, the silicone tube with the contacts were placed on a metal wire with 300µm inner diameter (Fig. 7)

Once the contacts were placed inside the silicone, a third resin mold (Elegoo), named the cuff mold, was used for the final assembly of the cuff. The cuff mold was used to attach the crimped contacts to the silicone tube. Surgical threads were also incorporated into the silicone tube in order to allow closing the cuff tube once implanted (Fig. 8).

A release layer was placed on the cuff mold. Next, the threads were placed in the resin mold, and then they were fixed using tape. Thereafter, the central part of the resin mold was filled with silicone rubber until complete coverage. Next, the silicone tube with the contact was immersed inside the cuff mold with the metal wire. The metal wire protects the inner contacts of being exposed to the silicone mixture, and helps to remove the final mold after the curing process.

Finally, the distal ends of the cables were soldered to male or female metal contacts (2.54 mm pitch). The labels were removed, and the contacts were coded with thermic shrinking tubes: L= black, M= red, R= white (Fig. 9).

### D. *In vivo validation*

The impedance for each contact of the manufactured cuff was measured in saline water.

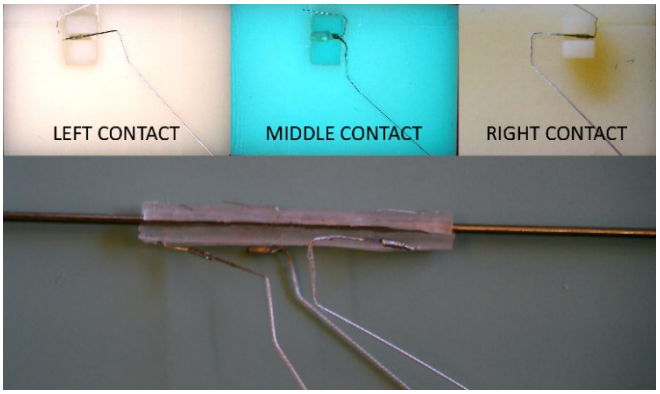


Fig. 7. Contacts placement. Once the contact are shaped, they are placed inside the silicone tube, by passing through the tube in the location where the contact marks are placed.

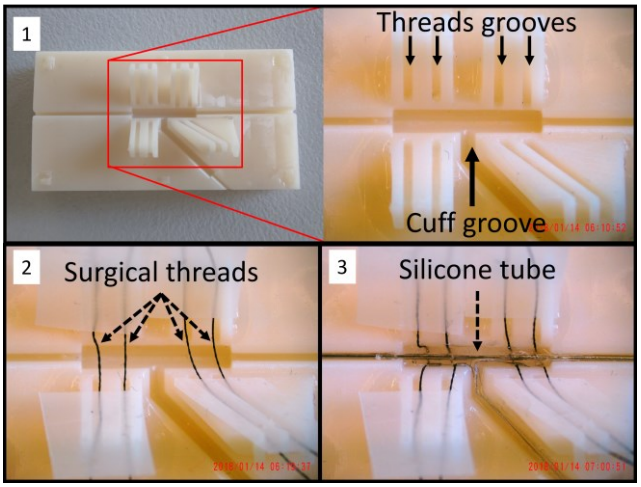


Fig. 8. (1) Cuff mold. It has grooves to receive the surgical threads and slots to hold the silicone tube. (2) The threads are fixed on the grooves using tape. (3) Silicone rubber covers the central part and the silicone tube with contacts is inserted.

An acute measurement was performed on one male Wistar rat (2.5 months and 300g). The rat was injected with Xylazine 7mg/kg and Ketamine 100mg/kg intraperitoneally. The cuff electrode was implanted on the cervical left vagus nerve. This experimental procedure has been approved by the University Health Sciences Sector Laboratory Animal Protection Committee (2018/UCL/MD/001). A detailed description of the surgery was previously reported [12], [13]. VENG and electrocardiogram (ECG) were recorded for 3.5 minutes. The hardware used for signal conditioning for VENG was a Sallen-Key bandpass filter 12-10000 Hz, Gain 900. For ECG,

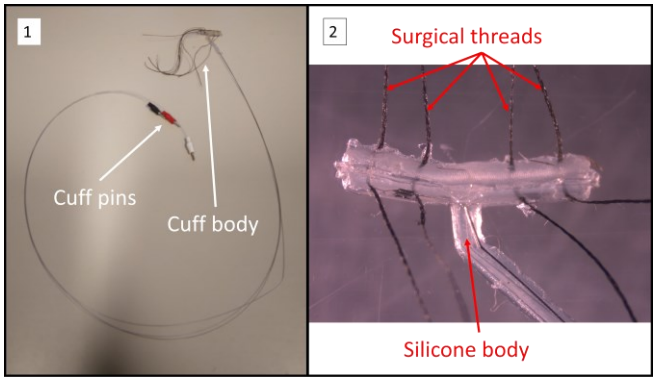


Fig. 9. Manufactured microcuff electrode. (1) General view, (2) Cuff tube zoomed.

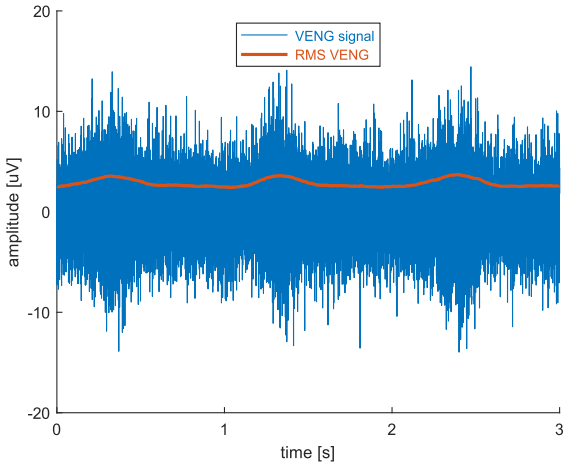


Fig. 10. Raw VENG recording signal. The amplitude changes are the burst related to respiration.

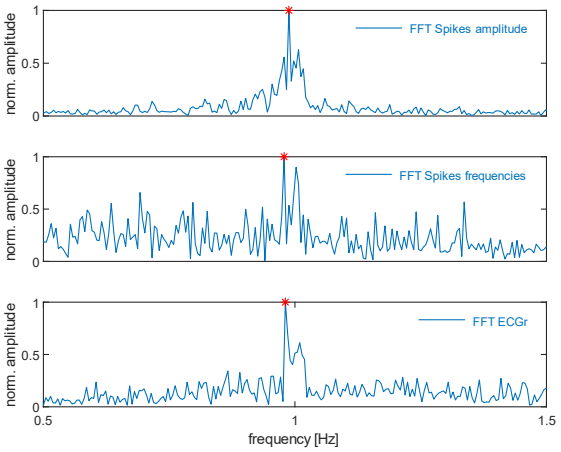


Fig. 11. Frequency spectrum comparison from spikes amplitude (top), spikes frequency (middle) and the respiration derived from ECG (bottom). In the three spectra there is a similar main prominence frequency.

a Sallen-key bandpass filter 15-7000 Hz, Gain 900. The signals were digitized with a USB-6212 multifunction I/O-device (National Instruments, Austin, USA) for an overall resolution of 0.17 µV/bit, using a sampling frequency of 80kS/s for the VENG and 40kS/s for the ECG.

Matlab R2021b was used for signal analysis. VENG signal should contain respiratory and cardiac components to confirm its genuineness. The derived respiration (ECGr) [14], [15] and the heart rate (HR) obtain from the ECG were used as references for the VENG activity. The raw VENG signal was filtered using a digital bandpass filter (2nd order Butterworth 300-3000 Hz). A spike detection algorithm was applied to the VENG signal to check if the spike frequencies and amplitude show the respiratory component. The spike detection algorithm was based on [16]–[18] and already successfully used in VENG [19]. The FFT of the spikes frequencies and amplitudes were calculated to obtain the main prominence frequency. For the cardiac components, the raw VENG signal was filtered using a bandpass digital filter (2nd order Butterworth 20-80 Hz), and the envelope was computed. The resulting signal was called VECG. VECG FFT was obtained to check the main prominence frequency.

## III. Results

The impedance measured in the saline solution was 15kΩ±5 kΩ, in line with our experience with Microbprobe (10kΩ-20kΩ). Our cuff electrode was subsequently successfully implanted. A typical illustration of the VENG trace is given in Fig. 10, which clearly shows respiration bursts. The VENG spike frequency and amplitude spectrums both show clear peaks related to respiration (respectively at 0.978 Hz and 0.988 Hz), close to ECGr frequency (0.981 Hz), as illustrated in Fig. 11. The VECG envelope showed a clear peak at 4.49 Hz, corresponding to 269.42 BPM, also very close to 269.37 BPM HR obtained from the ECG.

## IV. Discussion

We present here a fabrication method for cuff electrodes suitable for cervical implantation and VENG recording in rats. We validated the quality of our cuff electrode by recording acutely VENG and described typical respiratory and cardiac related VENG bursts. The cost of the components is around 8 €, including the wires, cables, tube, and silicone, making it economical for our preclinical studies. The manufacturing time takes around 2.5 hrs (excluding 4 hrs silicone curing time) and as a working labor, considering ~ 65 € per working hour, the price of the cuff increases to ~ 170 €. Considering an initial investment of ~ 20.000 € for the machines (depreciating the equipment only on the electrodes), the cost is recovered after manufacturing ~ 100 cuff electrodes (having as a reference the Microprobe cuff electrode cost), making it economically suitable for clinical studies. Although the 3D printers needed are expensive, the proof of concept is working, and the designs can be adapted for low-cost machines.

## V. Conclusion

In this work we presented a lab-made cuff electrode manufacturing process for vagus nerve monitoring in rats, and its subsequent validation. The cardiac and the respiratory components could be extracted from the VENG signal for testing the reliability of the recordings, showing a high reliability. The cost of the manufacturing process is cheaper compared to the commercial cuff electrode Microprobe.

The suitability for chronic implantation has to be evaluated in future works. Biocompatibility, reusability, signal and mechanical reliability will be evaluated during long term recordings in the context of peripheral nerve monitoring and stimulation in our group [12], [13], [20], [21].

## VI. References

[1] C. Wu and A. D. Sharan, "Neurostimulation for the treatment of epilepsy: A review of current surgical interventions," *Neuromodulation*, vol. 16, no. 1, pp. 10–24, 2013, doi: 10.1111/j.1525-1403.2012.00501.x.

[2] G. B. Mukhopadhyay HK,Kandar CC, Das SK, Ghosh LK, "Epilepsy and its Management: A Review," *J. PharmaSciTech*, vol. 1, no. 2, pp. 20–26, 2012.

[3] E. H. Rijnbeek, N. Eleveld, and W. Olthuis, "Update on peripheral nerve electrodes for closed-loop neuroprosthetics," *Front. Neurosci.*, vol. 12, no. MAY, pp. 1–9, 2018, doi: 10.3389/fnins.2018.00350.

[4] D. M. Woodbury, J. W. Woodbury, and D. M. Woodbury, "Effects of Vagal Stimulation on Experimentally Induced Seizures in Rats," 1990.

[5] M. Takaya, W. J. Terry, and D. K. Naritoku, "Vagus Nerve Stimulation Induces a Sustained Anticonvulsant Effect," *Epilepsia*, vol. 37, no. 11, pp. 1111–1116, Nov. 1996, doi: 10.1111/J.1528-1157.1996.TB01033.X.

[6] D. Sahin, G. Ilbay, M. Imal, O. Bozdogan, and N. Ates, "Vagus

Nerve Stimulation Suppresses Generalized Seizure Activity and Seizure-Triggered Postictal Cardiac Rhythm Changes in Rats," *Physiol. Res*, vol. 58, pp. 345–350, 2009, [Online]. Available: www.biomed.cas.cz/physiolres

[7] R. S. Mclachlan, "Suppression of Interictal Spikes and Seizures by Stimulation of the Vagus Nerve," in *Epilepsia*, 1993, vol. 34, no. 5, pp. 918–923.

[8] E. L. Foldes, D. M. Ackermann, N. Bhadra, K. L. Kilgore, and N. Bhadra, "Design, fabrication and evaluation of a conforming circumpolar peripheral nerve cuff electrode for acute experimental use," *J. Neurosci. Methods*, vol. 196, no. 1, pp. 31–37, 2011, doi: 10.1016/j.jneumeth.2010.12.020.

[9] M. T. Flavin *et al.*, "Rapid and Low Cost Manufacturing of Cuff Electrodes," *Front. Neurosci.*, vol. 15, no. February, pp. 1–11, 2021, doi: 10.3389/fnins.2021.628778.

[10] M. Haugland, "Flexible method for fabrication of nerve cuff electrodes," *Annu. Int. Conf. IEEE Eng. Med. Biol. - Proc.*, no. 1, pp. 359–360, 1996, doi: 10.1109/iembs.1996.656992.

[11] N. A. Pelot *et al.*, "Quantified Morphology of the Cervical and Subdiaphragmatic Vagus Nerves of Human, Pig, and Rat," *Front. Neurosci.*, vol. 14, no. November, pp. 1–19, 2020, doi: 10.3389/fnins.2020.601479.

[12] L. Stumpp *et al.*, "Vagus Nerve Electroneurogram-Based Detection of Acute Pentylenetetrazol Induced Seizures in Rats," *Int. J. Neural Syst.*, vol. 31, no. 7, 2021, doi: 10.1142/S0129065721500246.

[13] L. Stumpp *et al.*, "Recording of spontaneous vagus nerve activity during Pentylenetetrazol-induced seizures in rats," *J. Neurosci. Methods*, vol. 343, no. June, p. 108832, 2020, doi: 10.1016/j.jneumeth.2020.108832.

[14] R. Pallas-Areny, J. Colominas-Balague, and F. J. Rosell, "Effect of respiration-induced heart movements on the ECG," *IEEE Trans. Biomed. Eng.*, vol. v, no. n, pp. 585–590, 1992.

[15] G. Hahn, I. Sipinkova, F. Baisch, and G. Hellige, "Changes in the thoracic impedance distribution under different ventilatory conditions," *Physiol. Meas.*, vol. 16, no. 3A, 1995, doi: 10.1088/0967-3334/16/3A/016.

[16] A. Nonclercq *et al.*, "Cluster-based spike detection algorithm adapts to interpatient and intrapatient variation in spike morphology," *J. Neurosci. Methods*, vol. 210, no. 2, pp. 259–265, 2012, doi: 10.1016/j.jneumeth.2012.07.015.

[17] A. Nonclercq *et al.*, "Spike detection algorithm automatically adapted to individual patients applied to spike and wave percentage quantification," *Neurophysiol. Clin.*, vol. 39, no. 2, pp. 123–131, 2009, doi: 10.1016/j.neucli.2008.12.001.

[18] R. Raffoul *et al.*, "Action Potential Detection Algorithm Adaptable to Individual Nerve and Recording Setup," in *Biomedical Circuits and Systems*, 2022, p. 4.

[19] H. Smets *et al.*, "Analysing vagus nerve spontaneous activity using finite element modelling," *J. Neural Eng*, vol. 18, p. 56008, 2021, doi: 10.1088/1741-2552/abe68f.

[20] J. Cury *et al.*, "Infrared neurostimulation in ex-vivo rat sciatic nerve using 1470 nm wavelength," *J. Neural Eng.*, vol. 18, no. 5, 2021, doi: 10.1088/1741-2552/abf28f.

[21] H. Smets *et al.*, "Chronic recording of the vagus nerve to analyze modulations by the light-dark cycle," *J. Neural Eng.*, no. Under revision.