Chronic Setup System for Continuous Monitoring of Epileptic Rats

Javier Chávez Cerda
BEAMS department
Université Libre de Bruxelles
Brussels, Belgium
javier.chavez.cerda@ulb.be

Elena Acedo Reina
Institute of Neuroscience
Université Catholique de Louvain
Brussels, Belgium
elena.acedoreina@uclouvain.be

Hugo Smets
BEAMS department
Université Libre de Bruxelles
Brussels, Belgium
hugo.smets@ulb.be

Maxime Verstraeten
BEAMS department
Université Libre de Bruxelles
Brussels, Belgium
maxime.verstraeten@ulb.be

Louis Vande Perre
BEAMS department
Université Libre de Bruxelles
Brussels, Belgium
louis.vande.perre@ulb.be

Macarena Díaz Cortés
BEAMS department
Université Libre de Bruxelles
Brussels, Belgium
macarena.diaz.cortes@ulb.be

Pascal Doguet
Synergia Medical SA
Mont -Saint-Guibert, Belgium
pascal.doguet@synergiam.com

Jean Delbeke
Institute of Neuroscience
Université Catholique de Louvain
Brussels, Belgium
jean.delbeke@uclouvain.be

Riëm El Tahry
Institute of Neuroscience
Université Catholique de Louvain
Brussels, Belgium
riem.eltahry@uclouvain.be

Antoine Nonclercq
BEAMS department
Université Libre de Bruxelles
Brussels, Belgium
antoine.nonclercq@ulb.be

***Abstract*— This work presents a setup for chronic monitoring of spontaneous epileptic seizures in rats under kainic acid. The system allows to record the vagus nerve electroneurogram at 40 kS/s and the electroencephalogram at 250 S/s using an USB-6212 multifunction I/O-device. The system includes a video channel (20 fps) controlled by a Raspberry Pi 4 Model B. A slipring allows the rat to move freely. Quick cage cleaning is possible through a movable base. The chronic setup was tested on a Wistar rat after status epilepticus induction, using kainic acid. The system appears to be robust and reliable enough to record status epilepticus, making it suitable for more extended experiments in epileptic rats.**

Keywords—chronic monitoring, epilepsy, kainic acid

I. Introduction

Epilepsy is a prime cause of neurological impairment, affecting around 50 million people worldwide. Among these, more than 30% are resistant to drug treatment [1]. Vagus nerve stimulation (VNS) is being used as an additional treatment for refractory epilepsy. It has previously been shown that an on-demand VNS has a beneficial effect on the seizure reduction [2], [3]. Despite a 30-year experience in the use of VNS for epilepsy, the mechanisms of action of this therapy remain to be fully elucidated [4], [5].

Electroencephalogram (EEG) is the gold standard for the diagnosis and treatment of epilepsy, based on the characterization of electrographic patterns [6], [7]. Also, the vagus nerve electroneurogram (VENG) signal is of great interest as a potential biomarker for early seizure detection, possibly suitable to implement an effective closed-loop VNS therapy, as demonstrated in an acute setting under anesthesia [8], [9].

Translating these results in a chronic setting with awake and free-moving rats will be quite challenging. The main issue are cable damage or disconnection due to the rat's movements [6]. Epileptic rats also tend to be more aggressive and hyperexcited [10]. Handling them and cleaning their cages is thus also more difficult.

Long-term monitoring setups for rodents have been proposed before, including wired [6] and wireless systems [11]. However, the wired system does not include a VENG recording channel. On the other hand, wireless systems that could allow proper monitoring have finite battery life and are costly [12], and may affect the VENG signal due to the high frequency of operation, causing high frequency interferences [13]. This paper presents the design and implementation of our chronic setup system for continuous monitoring in epileptic rats that is also suitable for VENG recordings. An earlier version of this setup has previously been used in a chronic setting in healthy rats [14].

II. Materials and Methods

A. Cage

The cage structure (including its basis) is made entirely of plexiglass (3mm thick). The pieces were cut using a laser cutter (FabKIT MK6, 75W), and all the parts were glued using UV light cure adhesive (Loctite). The cage is rectangular (30x30x45 cm) and allows water delivery through a 1.5 cm hole diameter hole in the front. The cage has a 3 cm open space at the bottom of each side for introduction of the movable basis as in [6]. The 30x30x3 cm basis can pushes away a former basis when changing the litter, While the rat can move from one base to the other. Two screws are used to

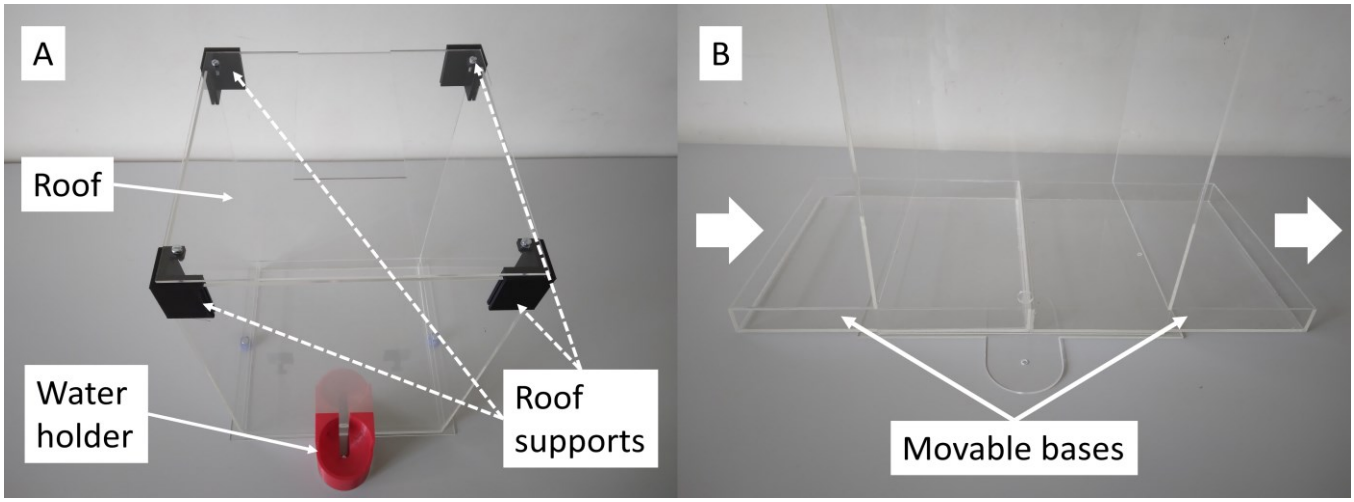

Fig. 1. Cage structure. (a) The cage has a rectangular shape, with a roof included to prevent rats escaping. (b) The cage has a movable base for a faster cleaning, while the rat remains in the cage.

attach the movable basis and prevent the rat from moving it. Also, the cage roof prevents escaping (Fig. 1).

B. Head cup and cables

The implantation process involved the placement of a head cup over the rat scalp for chronic measurements. The head cup contained female pin headers 2.54 mm pitch (male headers are vulnerable to pin torsion) as contacts for EEG and VENG measurements. The head cup is connected to the slipring to prevent cable damage from torsion and to allow free horizontal movement of the rat. The slipring is fixed on a plexiglass support held by two 3D printed holders (Prusa MK3+). Its height can be adjusted manually. In previous studies, the slipring was placed in a balance system, allowing the rat to move vertically as well without tension in the cable. However, according to our experience, the cables were more susceptible to breakage, especially with the constant movement of the epileptic rat under status epilepticus induced by kainic acid (KA) model. In the present system, the slipring was fixed on a support, and the cable is long enough to prevent tension, and is protected against bites as well. The cables from the slipring are connected to a PCB adaptor attached to the plexiglass support and carrying connectors to the amplifiers (Fig. 2).

C. Video

A Raspberry Pi 4 Model B (SDRAM 8GB, Broadcom 2711, Cortex-A-72, 64-bit SoC @ 1.5 GHz) controls the video recording. A Night-Vision Infrared camera (5Mpixel resolution, 30 fps, PIS-1137) is used for motion detection and epileptic seizure validation during day as well as night recordings at 20 fps. The Raspberry Pi (RPi) sends continuously 5-second video clips (.mp4 format) to a computer server through an ethernet connection. The RPi is controlled through a SSH connection using Putty.

D. Signal conditioning and digitalization

Two VENG and two EEG signals can be monitored and processed. For the VENG channels, the pre-amplifiers are placed before the slipring in order to minimize noise, movement artifacts and capacitive interferences. Although important for the low amplitude neuronal signals [15], [16], this precaution was not necessary for the much larger EEG signals, [6], [14]. Therefore, the EEG pre-amps were placed

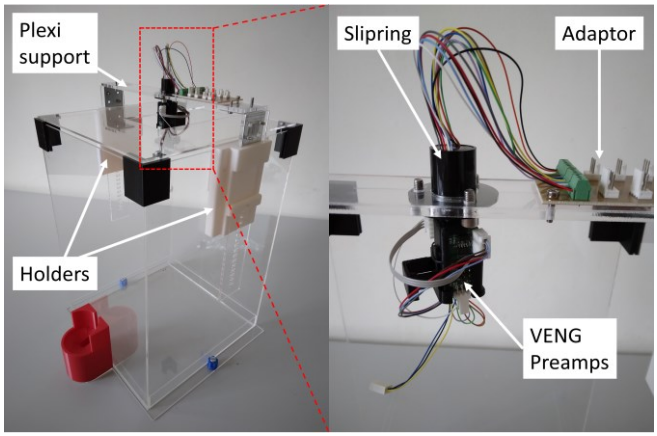

Fig. 2. Electrical connection between the rat and the chronic setup. The setup has a swivel to allow movements of the rat inside the cage. VENG preamplifiers located in front of the swivel reduce noise. At the other end, a PCB adaptor connects the slipring to the amplifiers.

after the slipring, close to the amplifiers. The pre-amps have an input impedance of 10 MΩ, an overall noise of 0.5 µV, a gain of 100 V/V and a RC high pass filter (cut-off frequency of 12 Hz for VENG signals and 0.16 Hz for EEG). The amplifiers contained three stages: amplification (gain 8V/V), isolation, and filtering. An optocoupler was used for circuit protection, and the amplified signal was filtered using a 4th order Butterworth-Bessel 0.05 low pass filter (cut-off frequency of 12kHz for VENG and 80Hz for the EEG). The overall gain for all hardware was 800V/V for VENG and EEG. The preamps and the amplifiers were powered by ±9V (preamplifier, amplifier, and isolation stages) and ±3V (isolation and filtering stages). The amplifiers were placed in printed holders on the cage.

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 40 kS/s for the VENG and 250 S/s for the ECG. One channel is reserved for the synchronization between the video and signal recordings. When the system starts acquiring data, the RPi sends a pulse to turn on a LED in the camera's target. At the same time, that pulse is recorded in the reserved channel. The same pulse is generated when the system ends the acquisition process. Then, a semi-automatic synchronization is implemented by software.

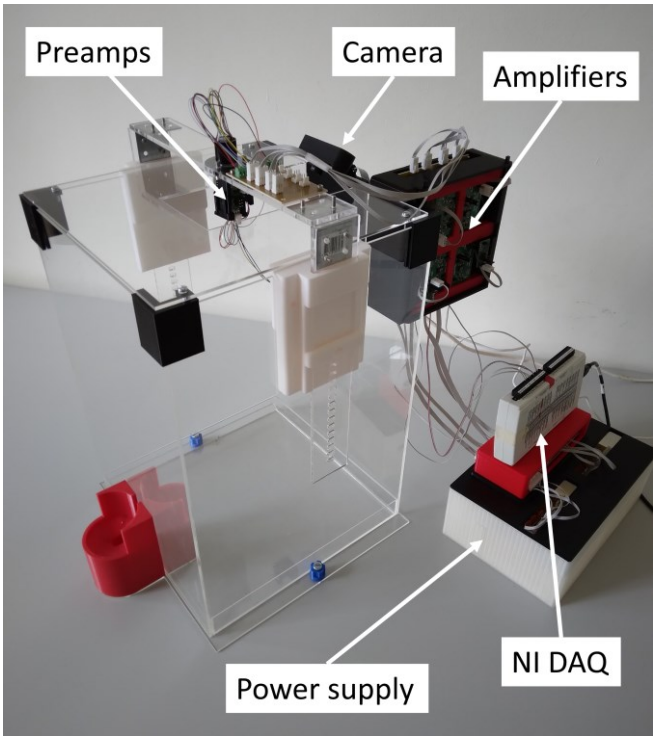

Fig. 3. Chronic setup for continuous monitoring.

For further signal analysis, a 2nd order Butterworth bandpass filter was used to filter the EEG signal (1-80 Hz) and the VENG signal (300-3000 Hz). A complete picture of the setup is shown in Fig. 3.

E. In vivo validation

One male Wistar rat (300g weight, 2 month age) was implanted with a tripolar micro cuff electrode (Microprobe) and epidural electrodes. The rat was injected with Xylazine 7mg/kg and Ketamine 100mg/kg intraperitoneally (i.p.). The micro cuff electrode (Microprobe) was placed on the left cervical vagus nerve, and the epidural electrodes were screwed in the rat's scalp [4]. After two weeks of recovery, the rat was placed in the chronic setup and data acquisition was started. Food and water were available ad libitum.

A baseline of EEG and VENG were recorded for 24 hours before the status epilepticus (SE) induction. The SE procedure was based on [17], [18]. KA was injected i.p., as a single shot of 7.5 mg/kg followed after 45 min by 2.5 mg/kg injections every 30 min until the rat exhibited more than four convulsive seizures per hour. In this experiment, the rat experienced more than four convulsive seizures after the fifth shot of 2.5 mg/kg. This experimental procedure has been approved by the University Health Sciences Sector Laboratory Animal Protection Committee (2018/UCL/MD/001).

III. Results

The rat exhibited generalized seizures during the KA injections, which was confirmed by checking the video recordings and the EEG signals and comparing them with the baseline measurements (Fig. 4). A change in pattern and amplitude can be seen in the EEG signal.

The VENG signal was correctly measured during the baseline and during the SE induction. A change of amplitude can also be observed (Fig. 5).

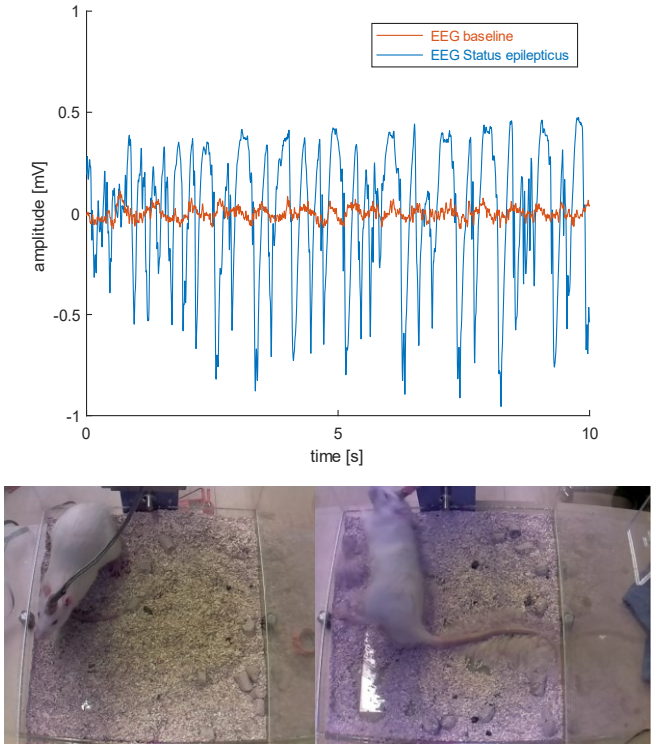

Fig. 4. Top: EEG during baseline versus EEG during seizure. Bottom: Video validation, baseline activity (left) and seizure (right).

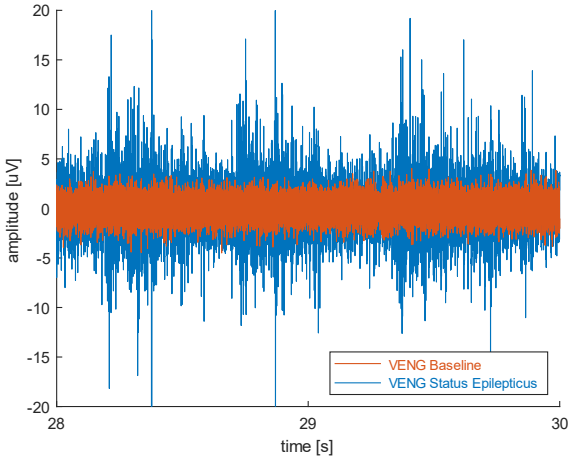

Fig. 5. VENG during baseline activity versus VENG during seizures.

IV. Discussion

The chronic setup presented here is reliable for status epilepticus induction monitoring when the rat exhibits generalized seizures, showing that the cage is suitable for chronic experiments on spontaneous seizures. The cage was ergonomic for the user, and changing the litter was easy and fast, reducing the risk of rat bites or rat escaping.

Based on our previous works [8], [19], [20], spike frequency and amplitude can be extracted from the VENG to characterize the seizure VENG signal and to discard it from a motion artifact. Further analysis could possibly yield a biomarker for seizures. Future studies involving peripheral nerve monitoring and stimulation as already undertaken by our group [14], [21], [22] will benefit from the new tool. VNS experiments can be performed in our chronic setup, offering

long-term VNS therapy efficacy measurements, VNS therapy side effect evaluations, and close loop VNS trials.

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