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## **ONEIROS, a new miniature standalone device for recording sleep electrophysiology, physiology, temperatures and behavior in the lab and field**

Bertrand Massot, Sébastien Arthaud, Baptiste Barrillot, Johanna Roux, Gianina Ungurean, Pierre-Hervé Luppi, Niels Rattenborg, Paul-Antoine Libourel

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1        **ONEIROS, a new miniature standalone device for recording sleep electrophysiology,**  
2        **physiology, temperatures and behavior in the lab and field.**

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5

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11

12        **ABSTRACT**

13        **Background:** Sleep is an inactive state of reduced environmental awareness shared by all animals. When  
14        compared to wakefulness, sleep behavior is associated with changes in physiology and brain activity. The nature  
15        of these changes varies considerably across species, and therefore is a rich resource for gaining insight into the  
16        evolution and functions of sleep. A major obstacle to capitalizing on this resource is the lack of a small device  
17        capable of recording multiple biological parameters for extended periods of time both in the laboratory and the  
18        field.

19        **New method:** ONEIROS is a new tool designed for conducting sleep research on small, freely moving  
20        animals. The miniature, standalone system is capable of recording up to 26 electrophysiological signals  
21        (electroencephalogram, electromyogram, electrooculogram, electrocardiogram), metabolic (3 temperature  
22        channels) and behavior via an accelerometer for several days. In addition, the device is equipped with a vibrating  
23        motor which can be used to assess arousal thresholds and to disrupt sleep. The system is available in telemetric  
24        or datalogger configuration useable in the field.

25        **Results:** To demonstrate the efficacy of this tool, we simultaneously recorded for the first time,  
26        electroencephalogram, hippocampal local field potential, electromyogram, electrooculogram, brain, body and  
27        ambient temperature, and 3D accelerometry. We also deprived rats of paradoxical sleep by triggering the  
28        vibrating motor after online recognition of the state. Finally, by successfully recording a pigeon in an 8 m<sup>3</sup> aviary  
29        in a social context with the device in the logger configuration, we demonstrate the feasibility of using the device  
30        in the field.

31        **HIGHLIGHTS:**

- 32        • ONEIROS is a new miniature device with low power consumption dedicated to recording sleep

- 33 • The device can record sleep electrophysiology, physiology, and behavior in the lab or field
- 34 • An integrated vibrating motor can be used to assess arousal thresholds and perform selective paradoxical
- 35 sleep deprivation

36 **KEYWORDS:**

37 Wireless, telemetry, datalogger, electrophysiology, sleep, pigeon, sleep deprivation

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## 73 I. INTRODUCTION

74 Sleep is a vital and complex behavioral state that competes with the time allocated to foraging, courtship,  
75 parental care, and vigilance (Lesku et al., 2012; Rattenborg et al., 2016, 1999). From a behavioral standpoint,  
76 sleep is traditionally defined as an inactive state with reduced responsiveness to environmental stimuli (i.e.  
77 elevated arousal threshold) that is rapidly reversible in response to sufficient stimulation. In many species, sleep  
78 occurs in a species-specific posture and at specific times of the day (Fig. 1. ). The duration and intensity of sleep  
79 increases following sleep deprivation, indicating that it is homeostatically regulated (Campbell and Tobler, 1984;  
80 Piéron, 1913). Initially identified in mammals, two electrophysiological sleep states can be defined during  
81 behavioral sleep: paradoxical sleep (PS) or rapid eye movement sleep (REM sleep) and slow wave sleep (SWS)  
82 or non-REM sleep (NREM sleep) (Aserinsky and Kleitman, 1953; Jouvet et al., 1959). SWS is distinguished  
83 from wakefulness and PS by the presence of high amplitude, low frequency waves in the electroencephalogram  
84 (EEG), reduced heart and respiratory rate, reduced brain and body temperature, reduced muscle tone (compared  
85 to wakefulness), and the scarcity of eye movements. Environmental awareness is lower compare to resting  
86 wakefulness (Fig. 1. ). During PS, the EEG exhibits a desynchronized (low-amplitude, high-frequency) wake-  
87 like pattern. In contrast to wakefulness, PS is associated with tonic skeletal muscle atonia (Jouvet et al., 1959).  
88 This atonia is phasically interrupted by rapid eye movements (Aserinsky and Kleitman, 1953) and other forms of  
89 muscular twitching, particularly in young mammals (Corner, 1977). In addition, heart and respiratory rates  
90 become irregular during PS (Snyder et al., 1964). Brain temperature increases, but all thermoregulatory  
91 mechanisms (i.e. muscle tone, pilo-erection, sweating, and shivering) are abolished (Jouvet et al., 1959;  
92 Parmeggiani, 2003). Finally, both SWS and PS are homeostatically regulated. Total sleep deprivation is  
93 compensated by an increase in the quantity and intensity of SWS (Borbély and Neuhaus, 1979), and selective PS  
94 deprivation is also followed by a recovery period with more PS (Dement, 1960).

95

	Quiet wake	Slow wave sleep	Paradoxical sleep	
<b>Arousal threshold</b>	-	+	+	} Behavior
<b>Homeostasis</b>	-	+	+	
<b>Specific posture</b>	-	+	+	
<b>Cortical EEG</b>	Desynchronized	High amplitude slow waves	Desynchronized	} Electrophysiology
<b>Eye movements</b>	+	-	+	
<b>Muscle tone</b>	-	-	--	
<b>Muscle twitches</b>	-	-	+	
<b>Heart rate variability</b>	+/-	-	+	} Metabolism
<b>Respiratory rate variability</b>	+/-	-	+	
<b>Body temperature</b>	+	-	-	
<b>Brain temperature</b>	+	-	+	

96

97 **Fig. 1.** Table illustrating the main behavioral, electrophysiological and metabolic parameters that covary with  
98 the quiet states (Quiet wake, slow wave sleep and paradoxical sleep) in mammals.

99 Whereas it is largely accepted that sleep is present in animals ranging from jelly fish to the more complex  
100 animals (Campbell and Tobler, 1984; Nath et al., 2017; Omond et al., 2017; Raizen et al., 2008; Siegel, 2008), it  
101 is less clear whether all animals display two sleep states. Of the non-mammalian animals investigated,  
102 unequivocal evidence of mammalian-like SWS and PS was only found in birds (Dewasmes et al., 1985; Heller et  
103 al., 1983; Klein et al., 1964; Rattenborg et al., 2009). Nonetheless, some reports of a PS like state in non-avian  
104 reptiles (Libourel and Herrel, 2016; Shein-Idelson et al., 2016), or twitches during behavioral sleep in  
105 cuttlefishes (Frank et al., 2012) or in bees (Klein et al., 2008) suggest that two sleep states could also be present  
106 in other species.

107 Classically, sleep is studied in laboratory animal models (rats, mice, cats, dogs, fruitflies, and zebrafish) at  
108 various levels of analysis, including genetic, molecular, neuroanatomical, physiological, or cognitive. Several  
109 tools are available to assess these aspects of sleep; for example: optogenetic, immunohistochemical, behavioral  
110 tests, neuronal activity recordings, LFP, EEG, EMG, ECG, EOG, and temperature measurements. However, of  
111 the species in which sleep has been studied (less than 200 of the approximately 66 000 vertebrates), most of  
112 them were studied in to the lab via a tethered device that recorded EEG and EMG. A weakness of this approach  
113 is that using a tether could induce stress by reducing the animal's freedom of movement and precluding the use  
114 of sleeping shelters (Tang et al., 2004). Therefore, wireless alternatives are obviously required to reduce stress  
115 and foster more natural sleep patterns. Since 2000, one logger (Vyssotski, 2005; Vyssotski et al., 2009;  
116 <http://www.vyssotski.ch/neurologger.html>) and several telemetric systems with a limited transmission range  
117 (Lapray et al., 2008; Tang and Sanford, 2002; Weiergräber et al., 2005; Zayachkivsky et al., 2013) have been  
118 developed to record sleep-related EEG and EMG activity in rodents. The telemetry devices could record 1 or 2  
119 channels at a low sampling rate (<1 kHz) for periods lasting from days to months, in the case of devices with

120 capacitive wireless transmission (Tang and Sanford, 2002). Other systems can record more channels (>32) at a  
121 higher sampling rate (>10kHz) needed to record neuronal activity (Harrison et al., 2011; Hawley et al., 2002;  
122 Mohseni et al., 2005; Sodagar et al., 2009; Yin et al., 2014). Nevertheless, with a battery of an acceptable size  
123 for a laboratory rodent to carry, these systems can only record for a few hours, which is insufficient for most  
124 sleep studies wherein disturbing the animals should be minimized. In general, it is essential that a sleep recording  
125 device records for at least 48 hours. Moreover, for comparative studies of species that do not exhibit sleep states  
126 readily comparable to mammalian SWS and/or PS based on EEG and EMG activity alone, it is important to  
127 record as many parameters as possible to facilitate comparison with mammals. Consequently, in the case of  
128 comparative sleep studies, behavioral, electrophysiological and metabolic parameters should be recorded in  
129 order to provide more informative features regarding how an animal sleeps. Unfortunately, none of the existing  
130 devices has enough channels to record multiple EEG, EMG, ECG and EOG channels, LFPs, and brain and body  
131 temperature for at least 48 hours. Moreover, none incorporates a means to evaluate the behavioral criteria of  
132 sleep, such as arousal threshold, reversibility, and homeostasis. Another important constraint is the weight and  
133 the size of the device. Obviously, all devices could record for a month with large batteries. However, most  
134 species are small and cannot carry heavy systems. Therefore, to increase autonomy, the power consumption of  
135 the system should be minimized.

136 In addition to these constraints, if the aim is to record the animal in the wild, other parameters are needed to  
137 describe sleep behavior. In the lab we often record video in conjunction with the electrophysiology, but in the  
138 wild, this cannot be done easily. An alternative is to record the animal's head movement with accelerometry  
139 along the three axes (Rattenborg et al., 2017). Moreover, as the quantity of sleep and the presence of  
140 torpor/hibernation are temperature dependent, it is also important to record naturally occurring changes in  
141 ambient temperature in the wild. However, the major difference between recording in the lab and the wild is the  
142 manner in which the data is stored. In the lab, movement is usually restricted to a small space from which data  
143 can be transferred and stored on a computer via wireless transmission. However, in the field or in large  
144 enclosures (Lesku et al., 2011) that exceed the transmission range of small telemetry devices, the data needs to  
145 be logged on the device (Rutz and Hays, 2009). Currently, only one device available in various versions  
146 (<http://www.vyssotski.ch/neurologger.html>), is small enough, to record EEG and EMG combined with 3D  
147 accelerometry for sleep studies (Vyssotski, 2005). This device has been used successfully to record sleep-related  
148 electrophysiology and behavior in the field in sloths (Rattenborg et al., 2008; Voirin et al., 2014), sandpipers  
149 (Lesku et al., 2012), barn owl chicks (Scriba et al., 2013), and even frigatebirds in flight (Rattenborg et al.,  
150 2016). Although this device opened the door for the first field-based sleep studies and remains a powerful tool  
151 for many field-based sleep studies, the low number of channels (four) and the absence of a device for assessing  
152 arousal thresholds and disrupting sleep limits the scope of the questions that can be addressed with this device.

153 In summary, sleep is a universal and complex state, characterized by behavioral, electrophysiological and  
154 metabolic changes from wakefulness. Currently, multiple tools can assess some features of its phenotype in lab  
155 or more rarely in the wild, but none of them can measure the behavioral, electrophysiological, and metabolic  
156 features of sleep at the same time, in small species, for long periods. A device with these capabilities is needed  
157 for comparative and ecological experiments aimed at understanding the evolution and functions of sleep.  
158 Therefore, we developed ONEIROS (ONE Instrument for Recording Our Sleep), a telemetric/datalogger system

159 designed for sleep studies. This system is small enough to be worn by rats. The device can record 26 referential  
160 channels of electrophysiology (EEG, EMG, ECG, EOG or LFP), three temperature channels, and 3-axial  
161 accelerometry. Moreover, to evaluate the arousal threshold of to enforce sleep deprivation, the system includes a  
162 lightweight, vibrating motor. ONEIROS weighs less than 10g, when configured with a battery capable of  
163 recording for more than 48 hours. To validate the system, we simultaneously recorded for the first time the EEG,  
164 EMG, EOG, 6 LFPs in the hippocampus, the brain and body temperature, and 3D head acceleration of a rat. We  
165 also performed selective PS deprivation on a rat for 6 hours by activating the vibrating motor fixed on the device  
166 when PS was automatically detected via a custom online sleep scoring algorithm (Libourel et al., 2015). We  
167 compared the effect of the deprivation with published data obtained with gentle handling and automated  
168 deprivation methods. Finally, to demonstrate the feasibility of recording in the wild, we recorded a pigeon in a  
169 large aviary with other birds.

## 170 **II. METHODS**

### 171 **1. Surgery and experimental recording conditions**

#### 172 **1.1. Ethical considerations**

173 All experiments were conducted with the 3R principles in animal experimentation and in accordance to the  
174 European Community Council Directive for the use of research animals (86/609/EEC and 2016/63/EU).

#### 175 **1.2. Rat baseline**

176 Under ketamine-xylazine anaesthesia ( $100\text{mg}\cdot\text{kg}^{-1}$  -  $10\text{mg}\cdot\text{kg}^{-1}$  respectively, I.P.), one Sprague Dawley male  
177 adult rat (230g, Charles River Laboratories, France) was placed on a stereotaxic frame (David Kopf Instruments,  
178 USA) and implanted for with sensors for recording the EEG, EMG, and EOG, as well as body and cerebral  
179 temperatures. Following incision of the scalp and removal of the skin, holes were drilled in the skull. EEG  
180 monitoring: two stainless steel screws (Bilaney, Plastics One, Germany) were fixed in the parietal (from bregma:  
181 anterior-posterior (AP), -4mm; medial-lateral (ML), +3mm) and frontal (AP, +3mm; ML, + 1mm) parts of the  
182 skull and two above the cerebellum (AP, -12mm; ML, +3mm) served as references. In addition, a 4-electrode  
183 bundle was placed in the hippocampus for LFP recordings. It was composed by 4-tungsten wires ( $35\mu\text{m}$  in  
184 diameter, Scientific Wire Company, England) with different lengths ( $500\mu\text{m}$  of difference for each). The bundle  
185 was inserted with the following coordinates: AP, - 3.8mm; ML, +1.8mm, and dorsal-ventral, -4mm, to record  
186 from the lower part of the dentate gyrus (longest wire) to the CA1 region (shortest wire) of the hippocampus.  
187 The screws and bundle were fixed on the skull and electrically insulated from one another using acrylic  
188 Superbond (Sun Medical Co, Japan). EMG monitoring: two gold-coated electrodes were inserted into the neck  
189 muscles. EOG monitoring: two wires with gold-coated thin ball ends (1mm in diameter) were bilaterally placed  
190 under the eyelid, close to each eye. The wires were fixed on the skull with Superbond. Cerebral temperature  
191 monitoring: one additional hole was drilled in the occipital part of the skull and a thermistor (GA100K6MCD1,  
192 Measurement Specialties) was inserted close to the brain. The hole was filled with bone wax. Body temperature  
193 monitoring: one thermistor was inserted deeply between the neck muscles and secured with a suture. All wires  
194 were then connected to a head connector (Electronic Interface Board-36-PTB Neuralynx), which was secured to  
195 the skull using Superbond acrylic. Next, dental Paladur cement (Heraeus Kuzler) was applied around the head  
196 connector to protect all of the wires and the connector. At the end of the implantation procedure, the rat received

197 a non-steroidal anti-inflammatory injection (carprofene, 5mg.kg-1, S.C.) and was allowed to recover for 7 days,  
198 during which it was weighed and monitored daily. Then, the rat was housed in a Plexiglas barrel (30cm in  
199 diameter, Blox Usinage Plastique, France) with bedding, food and water *ad libitum* placed in a recording  
200 chamber with a 12h/12h light-dark cycle, ventilation, and a 23°C ambient temperature. ONEIROS was plugged  
201 into the animal's implant and baseline recordings started after 2 days' habituation to the device and the new  
202 environment. Signals were collected using DaqReverse, a custom Matlab (Mathworks, matlab r2016b) program,  
203 and were sampled at 256 Hz except for the temperature and accelerometer which were sampled at 64 Hz.  
204 Vigilance states were scored using SlipAnalysis a custom Matlab program with a 5-s sliding time frame window  
205 according to the following criteria: Active wake (AW) was characterized by desynchronized and irregular low-  
206 voltage and high-frequency (5-9 Hz) EEG activity, sustained EMG neck muscle tone, and movement detected by  
207 the accelerometer. EEG activity was similar during AW and Quiet wake (QW), but QW was differentiated from  
208 AW by the absence of movement. Slow-wave sleep (SWS) was characterized by high-voltage slow-waves (1.5-4  
209 Hz) combined with low muscle tone similar to QW. Paradoxical sleep (PS) was characterized by a very regular  
210 theta rhythm (5-9 Hz) associated with muscle atonia (absence of muscle tone and accelerometer activity).

211

### 212 **1.3. Rat Paradoxical Sleep deprivation**

213 One Sprague Dawley adult rat (male, 270g) was used. The surgical procedure was the same as previously  
214 described. Briefly, four screws for EEGs were fixed on the skull bilaterally over parietal and frontal cortices and  
215 two screws over the cerebellum for references. Two EMG and two EOG electrodes, and brain and body  
216 thermistors were also implanted. At the end of the implantation procedure, the rat received a non-steroidal anti-  
217 inflammatory injection (carprofene, 5mg.kg-1, S.C.) and was allowed to recover for 7 days, during which it was  
218 weighed and monitored daily. The rat was first placed in the recording chamber for baseline recording. Data  
219 were sampled at 128 Hz except for temperature and accelerometry which were sampled at 64 Hz. The baseline  
220 signals were scored and template parameters for each state were extracted from the EEG and EMG for online  
221 sleep scoring (Libourel et al., 2015). To enforce PS deprivation (PSD), we used an online algorithm to detect PS  
222 (Libourel et al., 2015) and a vibrating motor embedded on the ONEIROS device (Fig. 2. A) to awaken the rat  
223 when PS was detected. The vibration intensity was set to 100% and the stimulation duration was 700 ms. After a  
224 6-h period of PSD, the rat was recorded for an additional 6-h recovery (PS recovery - PSR). The time spent in  
225 each vigilance state was quantified during baseline, PSD, and PSR.

226

### 227 **1.4. Pigeon baseline**

228 One adult pigeon (one female, *Columbia livia*, 250 g) was anesthetized using isoflurane, then placed  
229 in stereotactic device (David Kopf Instruments, USA) and instrumented for EEG, EMG, EOG  
230 recordings. For EEG monitoring, four gold-plated, round-tipped (0.5 mm diameter) electrodes (Bürklin,  
231 Germany) were placed over the anterior and posterior hyperpallium (Wulst), a primary visual area. The  
232 electrodes were symmetrically placed, 4 mm apart along the AP axis and 2.5 mm and 3 mm from the midline for  
233 the anterior and posterior electrodes, respectively. Two electrodes were placed above the left and right sides of  
234 the cerebellum served as references for the ipsilateral EEG electrodes. For EOG monitoring, two electrodes were



235 placed in the porous bone cavity behind the top of the eye; the electrodes did not enter the orbit. After  
236 positioning, the EEG and EOG electrodes were secured with dental acrylic. For EMG monitoring, two wire  
237 electrodes were inserted into the nuchal neck muscles. To ensure a good adhesion between the dental acrylic and  
238 the bone, small holes were drilled through the top layer of the cranium, which allowed the acrylic to infiltrate the  
239 bone. The electrodes, cables and the connector were embedded in dental acrylic. At the end of the implantation  
240 procedure, the bird received an intramuscular injection of meloxicam (Metacam 2mg/kg) for post-operative  
241 analgesia. The pigeon was allowed to recover for 24 hours before attaching the data transmitter to the connector  
242 on its head. For the recordings, the device was equipped with a 1 Gb SD card and was attached to the connector  
243 on the bird's head. The pigeon was then placed in an all-female group aviary (2 m x 2 m x 2 m) together with  
244 another three uninstrumented birds. The aviary was equipped with an infrared camera for video  
245 monitoring (Axis M20 Network Camera Series). The EEG, EMG, EOG and 3-axis accelerometry  
246 were recorded in logger mode at 256 Hz for 24 hours. At the end of the recordings, the bird was  
247 recaptured, the device was removed and the data collected from the SD card. All animals had *ad*  
248 *libitum* access to food and water. The aviary had a 12h/12h light-dark cycle and an ambient  
249 temperature of 20°C.

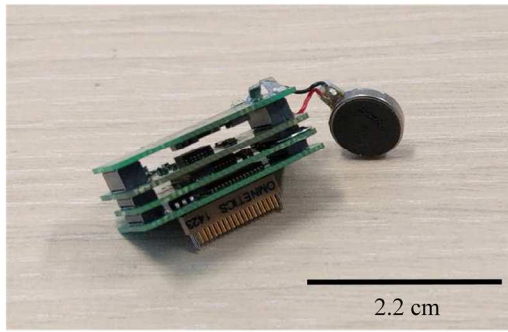
250

## 251 **2. A new device to quantify sleep**

### 252 **2.1. Embedded system for data acquisition**

253 ONEIROS was designed to provide a flexible set of tools, fitting in a tiny device, for the analysis of  
254 sleep in small animals. It includes an integrated, low-power electrophysiology frontend to measure up to 26  
255 biosignals, 3 temperature signals, and 3 accelerometer axes. An additional vibrating motor can be connected to  
256 the system and controlled using either real-time or predefined sequences of variable durations and intensities of  
257 vibration to assess arousal thresholds or prevent sleep. The system can be used either as a data logger, by using  
258 an embedded media storage, or as a telemetry device for real-time monitoring and analysis of the signals. The  
259 overall hardware architecture and software embedded on the system were developed to ensure that it would  
260 match the requirements (number of channels needed and bandwidth) of various possible experimental conditions  
261 and animal species. The size of the entire electronic system is 9 mm x 16 mm x 25 mm and it weighs 4 g.  
262 Together with a small 3 Volts, 150 mAh Li/MnO<sub>2</sub> primary battery of 1.4 g (CP251525, GMB Company Ltd.) it  
263 can be encapsulated in a 28 mm x 18 mm x 15 mm plastic enclosure (Fig. 2. B). The addition of the vibration  
264 motor requires an additional width of 2 mm on one side of the enclosure and a weight of 1 g.

A.



B.



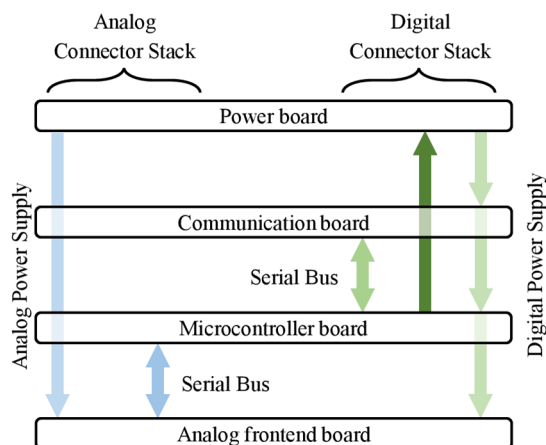
265

266 **Fig. 2.** A. Picture of the device showing the stack of boards with circular vibrating motor connected. B. Picture  
 267 of a rat with the enclosed wireless device on its head.

268 **2.2. Hardware boards**

269 To enable rapid changes and optimizations of the main system functions, we designed small printed circuit  
 270 boards which can be stacked together to form a functional measurement system (Fig. 2. A). The boards are  
 271 interchangeable allowing adaptation of the system to specific experiment conditions. This modularity also allows  
 272 for future improvements of the main functions. In the current version, four boards form a fully working system: a  
 273 power stage, which provides independent power from the battery for the analog and digital boards; a  
 274 communication stage which consists of either an embedded memory storage board, or a wireless data transfer  
 275 board; an analog stage which contains the frontend for the acquisition of biosignals; and, finally, a digital stage  
 276 which controls and synchronizes data acquisition from the analog stage and transfer to the communication stage.  
 277 The connection between each stage is made by stackable connectors on a single side or on both side of the board  
 278 depending on the position of the board in the system (for example, the power board contains connectors only on  
 279 the bottom side as it is the upper stage of the system). The global interconnection between all boards is shown on  
 280 Fig. 3. Any board can be replaced by any other one of the same type, as long as the connectors' placement and  
 281 pinout is retained. To minimize noise on the analog signals, the analog power supply and the serial bus to the  
 282 analog frontend are placed on the first stack of connectors. The digital power supply, control lines and serial bus  
 283 to the digital boards are placed on the second stack of connectors at the opposite of the circuit board. The overall  
 284 functional diagram of the current version of the system is shown on Fig. 3.

285



286 **Fig. 3.** Diagram of the interconnection between hardware modules

### 287 **2.3. Power board**

288 The power board is placed at the top of the system stack, together with the battery. The electronic circuit is  
289 mainly composed of a dual channel boost-converter (LTC3535, Linear Technology Corporation) to provide  
290 constant voltages for analog and digital power supplies from the voltage of the battery, which can vary from 3.2  
291 V down to 2 V at the end of the battery life. Due to the voltages required by the communication and analog  
292 frontend stages, the boost-converter is configured to output 3.3 V for the digital power supply with up to 100 mA  
293 output current (required in case of the use of a micro-SD card in the communication stage). On the other hand,  
294 the second channel of the boost-converter is configured to output 3.6 V for the analog power supply, which is  
295 subsequently fed into a 3.3 V low-noise, low-dropout regulator (LDO). The dedicated LDO (LP5907, Texas  
296 Instruments Inc.) is used to minimize the output ripple of the boost-converter on the analog power supply, as the  
297 amplifier stage of the analog frontend board can be very sensitive to power supply variations and noise. The  
298 power board also contains a light indicator (low power LED) connected to the digital connector stack and can be  
299 controlled by the microcontroller and used to indicate the state of the device. The power switch is a Hall Effect,  
300 bidirectional latch (AN48846B-NL, Panasonic Corporation) which can be opened and closed using a proximity  
301 magnet. This enables control of the power board even when the system is enclosed in a waterproof housing.

### 302 **2.4. Communication board**

303 Two communication boards have been developed and can be exchanged depending on the environmental  
304 conditions of the measurement as well as the requirements of the experiment. The first board is composed of a  
305 micro-SD card interface, and enables the embedded recording of acquired data on a memory card. With this  
306 board, the system is used as an autonomous data-logger system mounted on the animal; the data is retrieved from  
307 the memory card after the experiment. The second board developed is composed of a 2.4 GHz transceiver  
308 (nRF24L01p, Nordic Semiconductors) and an associated radio-frequency (RF) circuit and ceramic chip antenna.  
309 When assembled with the RF communication board, the system is used as a wireless telemetry system and the  
310 data are collected in real-time by using a base-station receiver connected to a computer via a Universal Serial  
311 Bus (USB) cable. As described, the modularity of the system enables it to record in laboratory conditions, with  
312 real-time acquisition of the data, as well as in a natural or semi-natural environment where the animal can freely  
313 move with the device in the data-logger configuration.

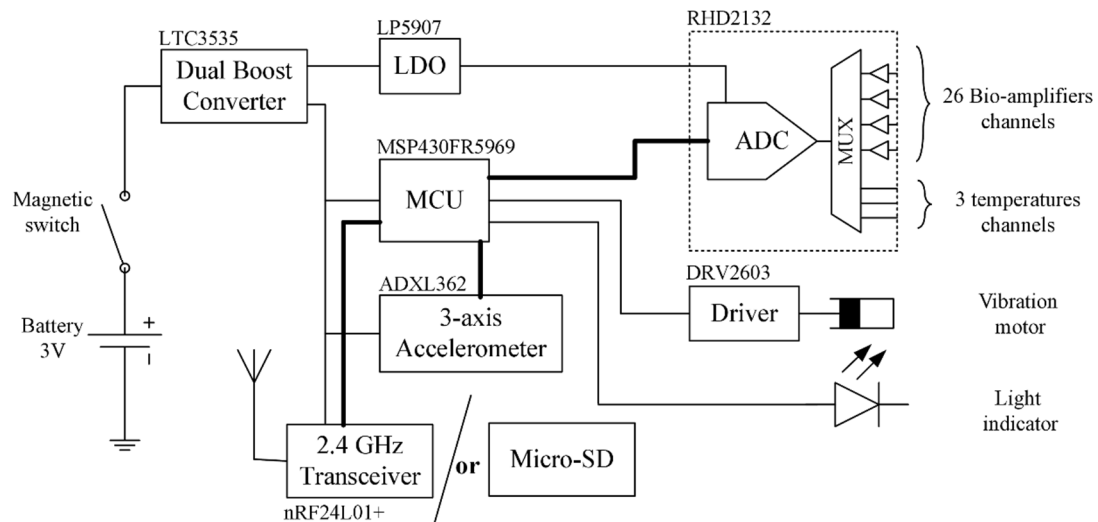
### 314 **2.5. Analog frontend board**

315 The analog frontend board is intended to be placed at the lower stage of the board stack, as the connector  
316 (Omnetics, Dual Row Nano Strip series, 32 contacts) is located on the bottom side of the board for linking the  
317 electronic interface board (EIB) to the electrodes and temperature sensors on the animal. The board is based on  
318 the RHD2132 (Intan Technologies, LLC.) digital electrophysiology interface chip. This low-power, integrated  
319 circuit contains a 16-bit analog/digital converter (ADC), a 32 channels low-noise amplifier with programmable  
320 bandwidths and 3 additional auxiliary inputs. The 3 auxiliary channels are used for temperature measurements  
321 with negative-temperature coefficient thermistors of 100 kOhm at 25 °C. Due to hardware (EIB connector) and  
322 software limitations, only 26 amplified channels are used for electrophysiology measurements which make a  
323 total of 32 channels when combined with temperature (3 channels) and three accelerometer axes. Data  
324 acquisition is triggered independently for each channel, and the ADC result is retrieved using the serial

325 communication bus connected to the micro-controller board. This enables each channel to be independently  
 326 sampled at different rates as described in the software section. This flexibility allows the user to adjust the power  
 327 consumption of the device through changing the sampling rate required for each biosignal, temperature, and  
 328 accelerometer axis.

## 329 2.6. Microcontroller board

330 The microcontroller board contains the microcontroller unit (MCU) and the digital accelerometer integrated  
 331 chip, as well as a driver for the vibrating motor. The accelerometer is an ADXL362 (Analog devices, Inc.), a 3-  
 332 axis MEMS accelerometer with a resolution of 12 bit and an average active consumption of 2  $\mu$ A. The  
 333 microcontroller used in the system is a MSP430FR5969 (Texas Instrument, Inc.). This microcontroller was  
 334 selected due to its very low-power consumption of 100  $\mu$ A/MHz and its flexibility in terms of power  
 335 management (1 active and 3 low-power modes of operation). The software embedded on the microcontroller is  
 336 described in the section 2.7. The microcontroller is connected to three communication serial buses using the  
 337 Serial Protocol Interface (SPI) to control and transfer data from the accelerometer, the analog frontend board,  
 338 and the communication board. Additionally, the microcontroller is directly connected to a universal haptic drive  
 339 (DRV2603, Texas Instruments Corporation) which controls an 80 mA vibration motor that can be connected  
 340 directly to the microcontroller board.



341

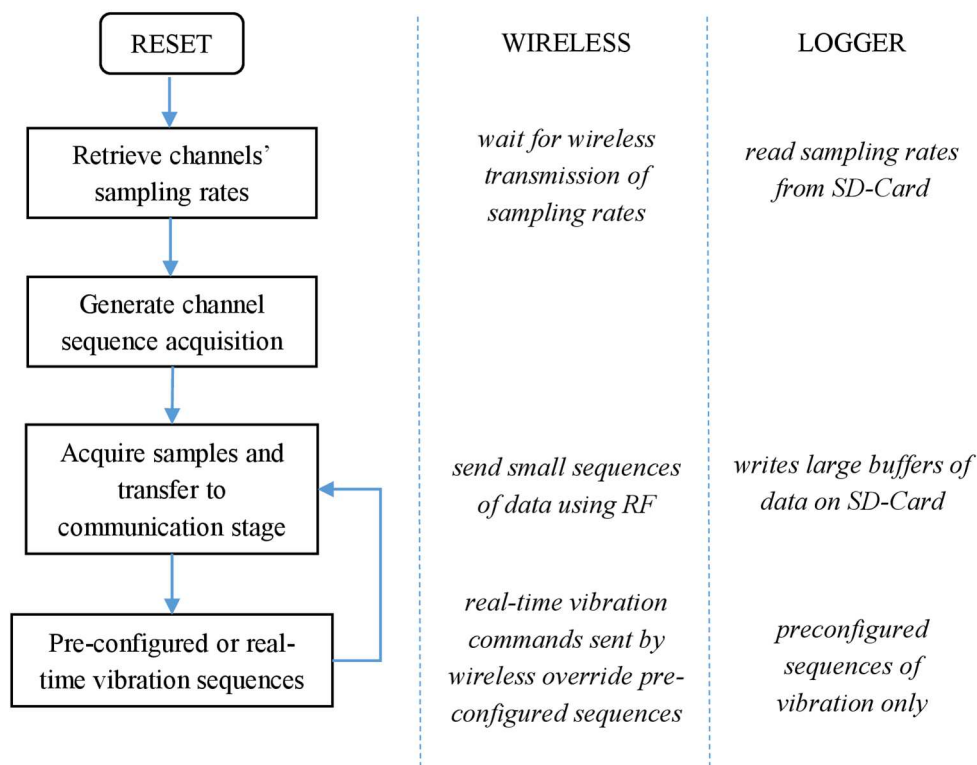
342 **Fig. 4.** Functional diagram of the complete instrumentation

## 343 2.7. Software embedded on the microcontroller

344 The software embedded on the microcontroller was developed using the Code Composer Studio integrated  
 345 development environment (Texas Instrument, Inc.). The software was written in C language, compiled using the  
 346 TI compiler and transferred to the microcontroller with an EZ-FET programmer for the MSP430 microcontroller  
 347 family. The role of this software is to provide flexibility and adaptability of the instrumentation to the specific  
 348 needs of each experiment (i.e. the number of channels used among the 32 channels available, and a dedicated  
 349 sampling frequency for each individual channel). As a result, this method of acquisition has the advantage of

350 minimizing the power consumption of the entire system when less channels or lower sampling rates are required,  
 351 thus extending the autonomy of the device for longer experiments.

352 At startup, the first task of the software is to detect whether the telemetry or the logger board is used for  
 353 recording data and to configure the peripherals accordingly. Then the program enters an infinite loop where three  
 354 different states are executed sequentially: a) retrieving the sampling rate of each channel, b) generating an  
 355 acquisition sequence based on the table of sampling frequencies, and then c) indefinitely acquiring and  
 356 transferring samples (following the generated sequence) until the system is stopped by power down or  
 357 configuration change. If using a telemetry configuration, where data are transmitted to a remote computer, the  
 358 software is able to receive real-time orders to control the vibration motor. In that case, an algorithm executed on  
 359 the remote computer can automatically use the data received from the system to, for example, classify awake or  
 360 sleep states of the animal and then send back vibration commands to induce sleep deprivation or to assess  
 361 arousal threshold. Fig. 5. illustrates the overall execution of the program with annotations regarding telemetric or  
 362 logger specific usages.



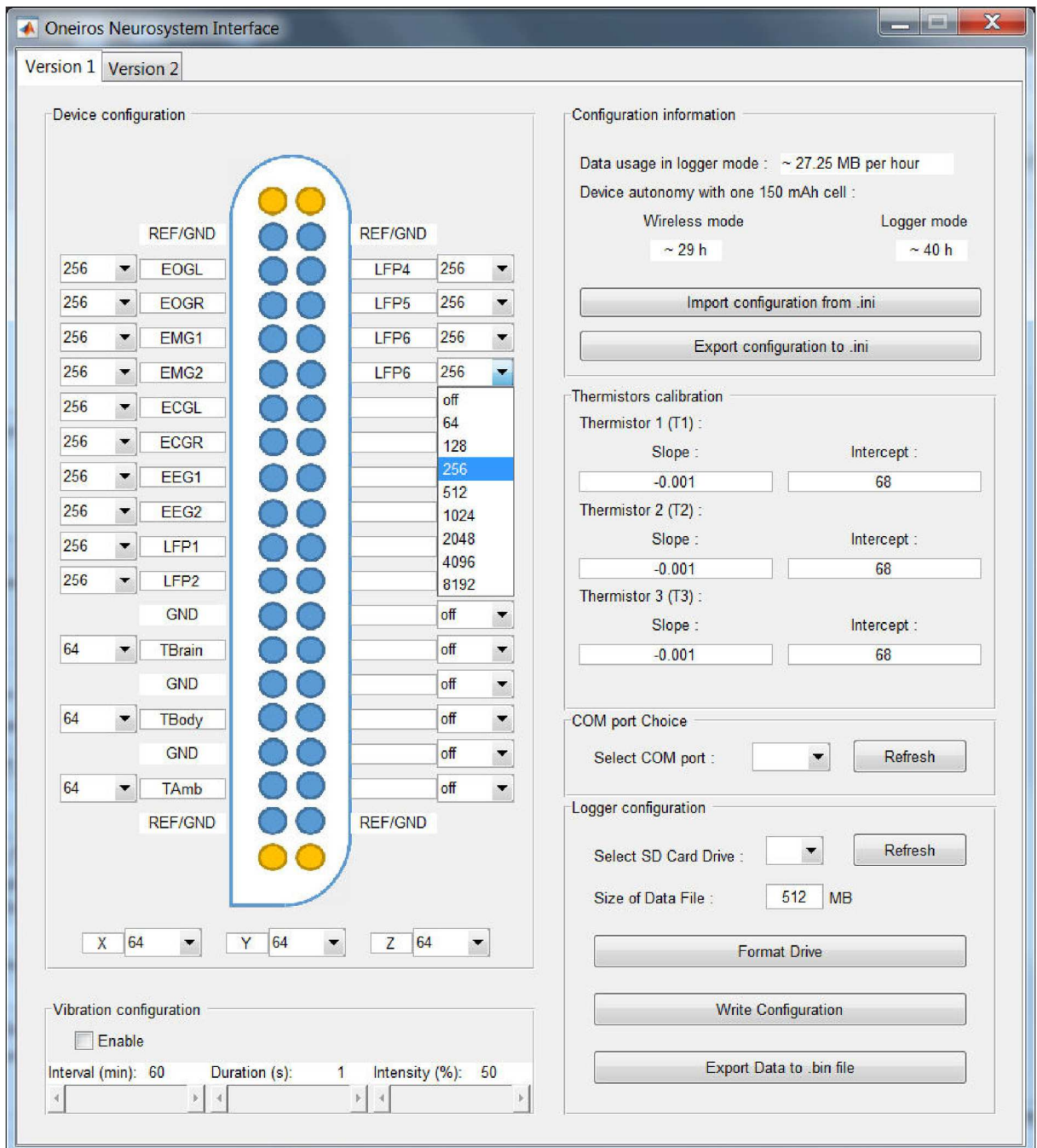
364 **Fig. 5.** Flowchart of the embedded software on the micro-controller unit

### 365 2.8. Signals description and acquisition

366 The device enables the acquisition of 32 different channels of three different types: a) 26 channels for  
 367 biosignals, including a reference channel, b) 3 channels for temperature based on negative temperature  
 368 coefficient (NTC) thermistors, c) 3 channels for accelerometry based on a 3-axis digital accelerometer. Due to  
 369 limited wireless bandwidth, and also to limited power consumption when using the logger configuration, the  
 370 overall maximum sampling frequency is set to 8192 samples per second (sps). This maximum sampling rate can  
 371 be split over the different channels via software (Fig. 6. ) in any combination as long as the sum of all

372 frequencies is equal to or lower than 8192 Hz (for example, 1 channel sampled at 8192 sps or 8 channels  
373 sampled at 1024 sps). Based on the sampling rate defined for each channel individually, the embedded software  
374 automatically generates a sequence of acquisition at a fixed clock rate with respect to every channel frequencies.  
375 For timing precision, a 32.768 kHz crystal oscillator is used with a drift of only 20 ppm or less. Then every  
376 possible sampling frequency that is an integer divisor of this clock (8192, 4096, 2048, 1024, etc.) can be set.  
377 When sent using wireless communication, data are collected using a RF remote receiver connected to a remote  
378 computer using a USB connection. The remote receiver is composed of the same transceiver chip (nRF24L01p)  
379 as the transmitter, and is associated to a PSoC 5LP (Cypress Semiconductors) microcontroller unit which  
380 transmits sampling rate configurations to the system and collects data frames which are subsequently sent to the  
381 computer through USB port. On the host computer, a dedicated driver as well as graphic user interface have been  
382 developed using Matlab (The Mathworks) to facilitate configuration, real-time visualization and control, as well  
383 as storage of the data (Fig. 6. ).

384



385

386 **Fig. 6.** Screen shot of the configuration tool design on Matlab for allocating the sampling rates to the different  
 387 channels.

388

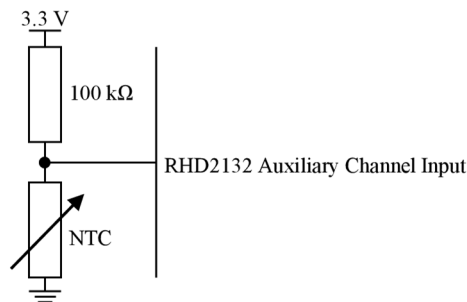
389 **2.9. Biosignals**

390 The 26 unipolar channels for biosignal measurements are acquired through the analog frontend which  
 391 contains one instrument amplifier (referred to a reference electrode) for each channel with a fixed gain of 192  
 392 V/V. The output of all amplifiers can be multiplexed to the input of a single 16-bit analog to digital converter.  
 393 These channels are meant to measure biosignals, such as EEG, ECG, EMG, EOG and LFP. Each channel has a

394 digital precision of 0.195  $\mu\text{V}$  and total voltage range of  $\pm 6.39$  mV. Integrated and configurable analog  
395 bandpass filters prevent aliasing effects and enable DC removal.

### 396 **2.10. Temperatures**

397 The analog frontend contains three auxiliary (not amplified) analog channels used to acquire temperature  
398 measured with NTC, 100kOhm thermistors. The thermistors (GA100K6MCD1, Measurement Specialties) are  
399 polarized with voltage from a voltage divider by using a 100kOhm resistor (Fig. 7. ). Auxiliary inputs are  
400 sampled through the same analog to digital converter as the biosignals, but with a voltage range of 0 – 2.4V.  
401 When using the voltage divider to polarize the thermistor, the linearity error is lower than 1% in the range 15 –  
402 40°C, and variations lower than 0.002 °C can be measured.



403

404 **Fig. 7.** Temperature measurement circuit diagram

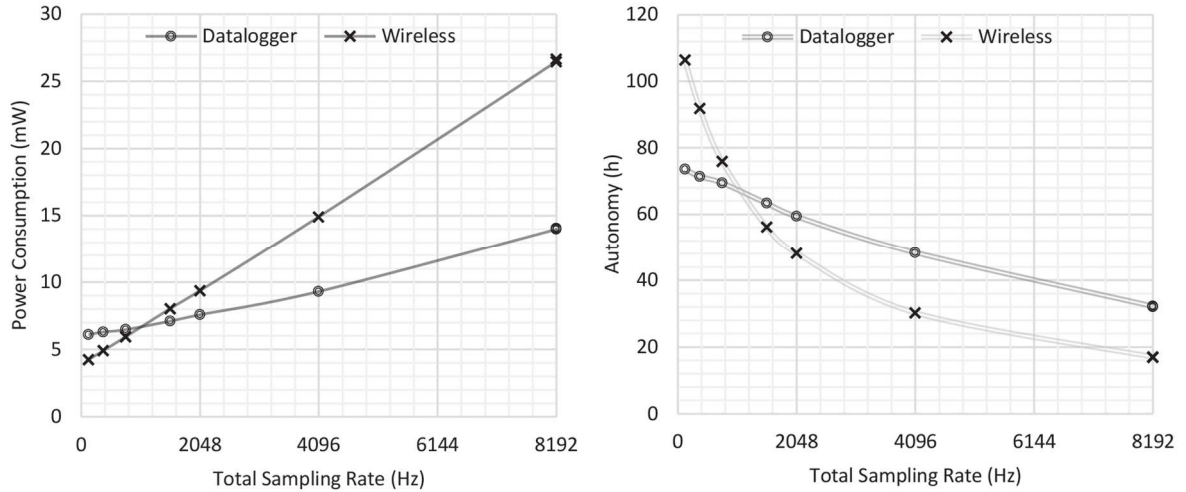
### 405 **2.11. Accelerometry**

406 A 3-axis, 12-bit digital accelerometer is used to assess movement of the animal's head. By using a range of  
407  $\pm 1\text{g}$ , the accuracy of the measurement is 0.001g. Internal bandwidth can be configured depending on the  
408 sampling frequency to avoid aliasing. Data are directly retrieved by the microcontroller through a serial  
409 peripheral interface (SPI).

### 410 **2.12. Electrical characteristics**

411 Although analog signal processing, analog to digital conversion, and digital interfaces with the  
412 microcontroller contribute to the overall power consumption of the system, for the most part, the autonomy of  
413 the system will depend on the communication board. Whether it uses RF communication or SD-Card storage,  
414 this stage has much higher power consumption when handling data. Hence, its consumption depends on the  
415 amount of data collected by the system; i.e. the total sampling rate of the configuration. In order to predict the  
416 autonomy of the system, the overall consumption has been accurately measured for different sampling  
417 frequencies (Fig. 8. ).





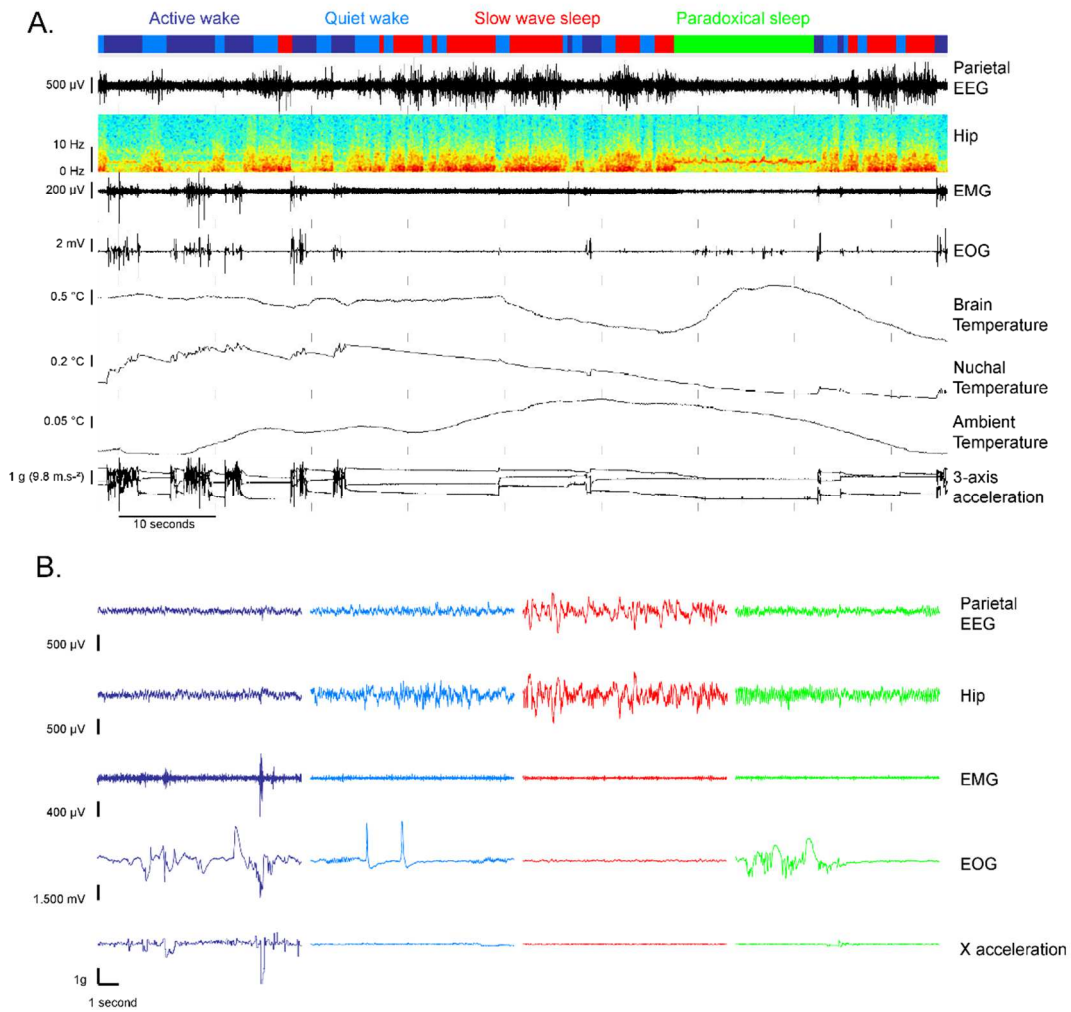
418

419 **Fig. 8.** Power consumption of the system with a 3V supply voltage and expected autonomy with a 150mAh  
 420 primary battery.

421 **III. RESULTS**

422 **1. Multiple parameters recorded in baseline condition with ONEIROS (telemetric version) in a rat**

423 To validate the design of our system (size, weight, signal quality, and autonomy), we first monitored a rat in  
 424 standard lab conditions. Here, for the first time we were able to record at the same time most of the  
 425 electrophysiological parameters that covary with the different states of vigilance. Fig. 9. A. illustrates 90s of raw  
 426 signal recorded with ONEIROS and Fig. 9. B. shows 10s of each state. The characteristics and quantity of each  
 427 state were consistent with previously published data (Fig. 10. ). During active wake (AW: dark blue), EEG and  
 428 hippocampal activity is desynchronized, with the later also showing a sustained theta frequency (around 6Hz, see  
 429 time frequency plot) characteristic of periods of locomotion (see also Fig. 9. and Fig. 10. D.) (Sławińska and  
 430 Kasicki, 1998). The EMG, EOG, and the accelerometer show bursts of intense activity. The brain and nuchal  
 431 temperature are at high levels. During QW (light blue), the animal stops moving, as reflected in the EMG and  
 432 accelerometry recordings. Eye movements become less frequent and the EEG tends to increase in amplitude and  
 433 decrease in frequency when compared to AW (Fig. 10. B). During SWS (red), the EEG is dominated by large  
 434 slow waves (Fig. 9. and Fig. 10. D). During SWS, the EMG remains at a low level, the accelerometry shows  
 435 little variation, indicating the absence of movement, and eye movements are nearly absent. Both temperatures  
 436 tend to decrease during SWS. When the animal falls into PS (green), the parietal EEG and hippocampal  
 437 recording show typical activity in the theta band (Fig. 9. and Fig. 10. D), eye movements occur, the EMG  
 438 becomes atonic, the brain temperature increases to wake levels, but body (nuchal) temperature continues to  
 439 decline (Fig. 9., Fig. 10. C). A small nuchal “twitch” is also visible on the accelerometry signal (Fig. 9. B).  
 440 Although high voltage waves were observed when the animal knocked the device against the cage wall, the  
 441 signals were usually free of artefacts, even during grooming and locomotion.

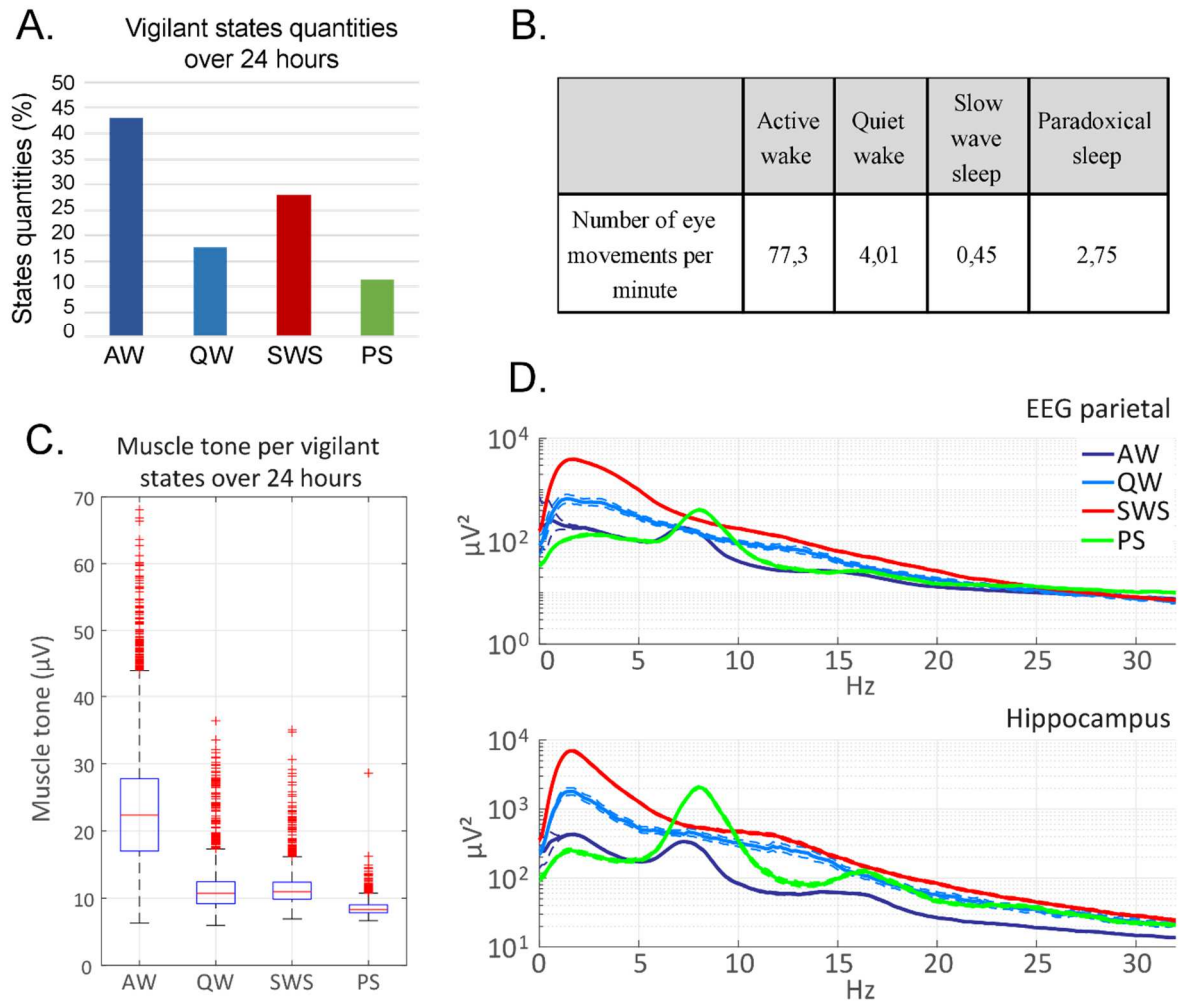


442

443

444 **Fig. 9.** Raw signals obtained with ONEIROS in telemetric mode from a rat during different vigilant states. A.  
 445 90 second recording of all signals. From the top to the bottom; hypnogram illustrating the wake/sleep scoring  
 446 (active wake in dark blue, quiet wake in light blue, slow wave sleep in red, and paradoxical sleep in green);  
 447 parietal EEG; time frequency representation of the hippocampal local field potential (color coded from -131 dB  
 448 in blue to -73 dB in red); EMG with a high pass filter (cutoff frequency 10Hz, order 2); EOG; brain temperature;  
 449 nuchal temperature; ambient temperature; 3 axial accelerometry. B. Representative 10 second examples of the  
 450 parietal EEG, hippocampal LFP, EMG, EOG and acceleration along x axis, occurring during the four states.

451



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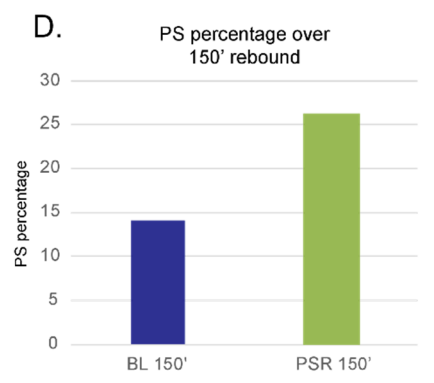
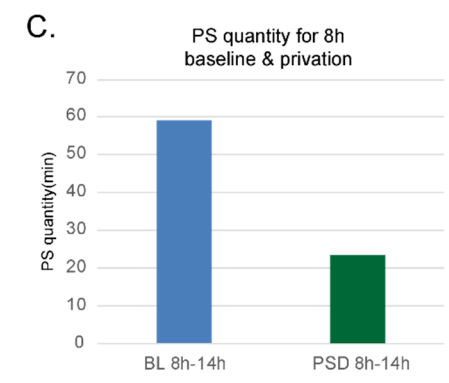
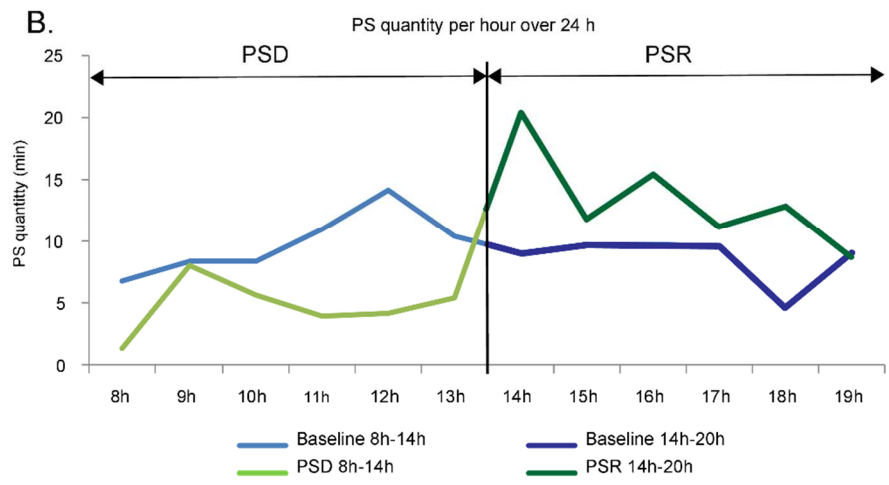
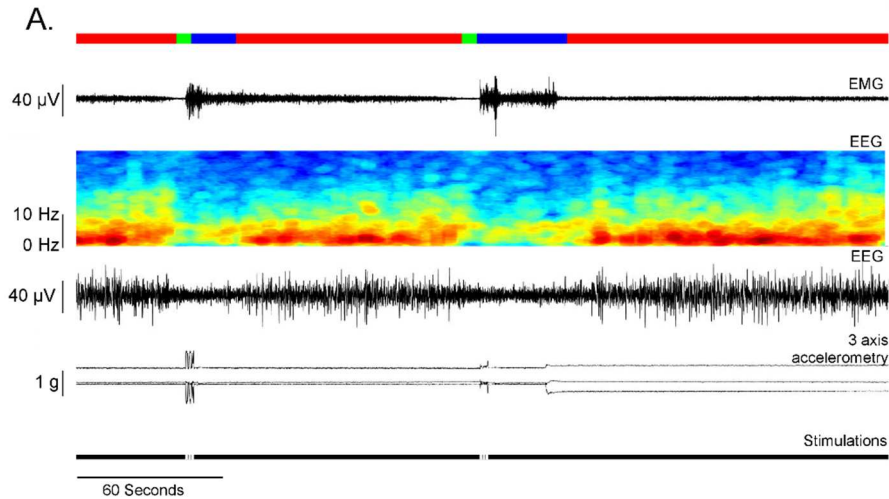
453

454 **Fig. 10.** Results over 24 hours obtained from telemetric recordings in a rat. A. Quantification of the vigilant  
 455 states over 24 hours. B. Number of eye movements per minute per state. C. Distribution of the muscle tone per  
 456 epoch for each state. D. Power spectrum in each state computed for the parietal EEG and hippocampal LFP.

457 **2. Selective paradoxical sleep deprivation in a rat using ONEIROS**

458 The vibrating motor embedded in the ONEIROS device (Fig. 2. A.) was used to evaluate its efficacy in  
 459 suppressing PS for several hours and inducing a homeostatic increase in PS during post-deprivation recovery.  
 460 We performed selective PS deprivation for 6 hours by using a probabilistic online sleep scoring algorithm  
 461 (Libourel et al., 2015). Fig. 11. illustrates the raw signals during the PS deprivation (PSD) experiment (see also  
 462 supplementary video 1). After a few seconds of PS (see atonia and EEG desynchronization), the algorithm sends  
 463 a stimulation (white bar on the bottom bar) that immediately awakens the animal (supplementary video 1). The  
 464 latency to detect PS was around 3-4 seconds. Fig. 11. B. shows the percentage of PS per hour during and after  
 465 the deprivation (in green) compared to baseline conditions (in blue). The deprivation reduced the quantity of PS  
 466 for 6 hours by 60% (58% in baseline to 23% during PSD). Moreover, PSD also induced a rebound of PS (PSR)  
 467 quantity during the recovery period with an increase of 85% over baseline levels (14% in baseline vs 26% during

468 PSR, Fig. 11. D.). Compared to 4 hours of PSD via gentle handling (Ravassard et al., 2016), Fig. 11. E) and  
469 unpublished data from our lab using a mechanical shaking device (Viewpoint S.A., (Libourel et al., 2015), the  
470 quantity of remaining PS during PSD with ONEIROS is higher (5% with ONEIROS compared to 2.6% and  
471 2.7% with the other methods). This is due to the presence of PS episodes during which the stimulations didn't  
472 immediately awaken the animal. However, PS quantities during the recovery were consistent with those obtained  
473 with the other methods.



**E.**

PSD method	PSD Duration	Residual PS	PS Rebound over 150'
Gentle Handling with EEG/EMG Recordings (n = 20) Ravassard et al., 2015, Cereb. Cortex	4h	2.60 +/- 0.3% (vs. 9.5% in BL)	17.90 +/- 0.1%
APSD (n = 4) Unpublished data	6h	2.7 +/- 0.5% (vs. 12.3 +/- 1.2% in BL)	24.6 +/- 2.3% (vs. 10.5 +/- 1.1% in BL)
ONEIROS (n=1)	6h	5% (vs. 16.6% in BL)	26.2% (vs. 14% in BL)

475

476 **Fig. 11.** Paradoxical Sleep deprivation efficiency. A. Hypnogram showing vigilance states (wake in blue, Slow  
477 wave sleep (SWS) in red, and Paradoxical Sleep (PS) in green), raw EMG signal, EEG time frequency and the  
478 associated raw EEG signal, and accelerometry. The bottom black bar shows when a stimulation was sent to  
479 awaken the animal (white bar). B. Percent time spent in PS per hour over the 12-hour baseline (in light blue 8h-  
480 14h and dark blue 14h-20h), PS deprivation period (from 8 to 14h in light green), and PS recovery period (from  
481 14h to 20h in dark green). B. the histogram shows the mean quantity in minutes of PS during 6-hour baseline  
482 (left) compared to the remaining quantity of PS during PSD (right). C. The histogram illustrates the increase in  
483 PS after deprivation by showing the percentage of PS during 6 hours of baseline (left) compared to the 6 hours  
484 after the deprivation (right). D. the table compares the residual quantities of PS during PSD and PSR, during 4  
485 hours of PS deprivation enforced via gentle handling (Ravassard et al., 2016), 6 hours of automated PS  
486 deprivation induced by a custom shaking device (Libourel et al., 2015), and ONEIROS PS deprivation.

487

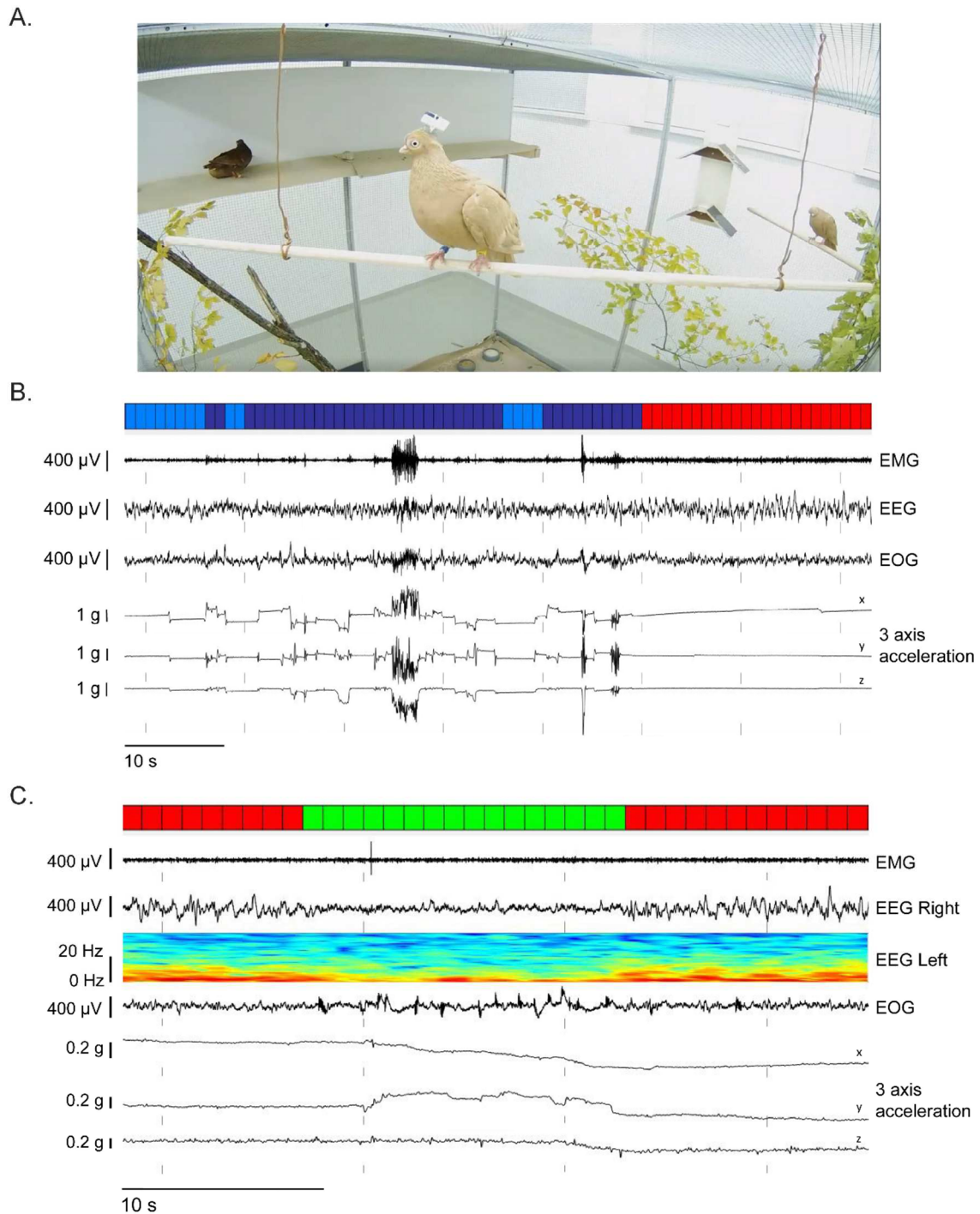
### 488 **3. Multiple parameters recorded in baseline condition with ONEIROS (logger version) in a pigeon**

489 The electrophysiological and behavioral aspects of sleep recorded with ONEIROS in the pigeon were similar  
490 to those recorded in birds using other methods. Fig. 12. illustrates raw recordings with alternating periods of QW  
491 and AW, followed by SWS. The periods of AW were characterized by increased muscle activity and increased  
492 motion (visible on the accelerometry channels), and desynchronized EEG activity. The two peaks at 12 Hz and  
493 24 Hz present on the power spectrum of AW (Fig. 12. A) resulted from head scratching. The periods of QW  
494 were characterized by low muscle activity (Fig. 13. B), comparatively small and infrequent changes in the  
495 accelerometry signal, generated by the birds' head movements, and desynchronized EEG activity. During SWS  
496 the EEG showed increased low frequency activity, when compared to all other states, increased Delta/Gamma  
497 ratio (Fig. 13. C) and the near absence of motion. Muscle tone usually remained at a level comparable to QW.

498 PS was characterized by EEG activation and behavioral signs of reduced muscle tone (e.g. head dropping as  
499 shown in the accelerometry recordings). As in other studies on pigeons and other avian species, the nuchal EMG  
500 rarely showed a reduction in activity, despite the behavioral signs of reduced tone. The number of eye  
501 movements increased during PS when compared to SWS. As previously described in pigeons and other birds  
502 (e.g. Dewasmes et al., 1985; Tobler and Borbély, 1988), eye movements included saccades, as well as faster  
503 oscillations (at 25-30 Hz). Unlike saccades which rarely occurred during SWS, the fast oscillations occurred  
504 during all sleep and waking states. They are thought to disperse oxygen and nutrients in the vitreous humor of  
505 the avian eye by moving a membrane (pecten) that protrudes inside the vitreous humor (Pettigrew et al., 1990).

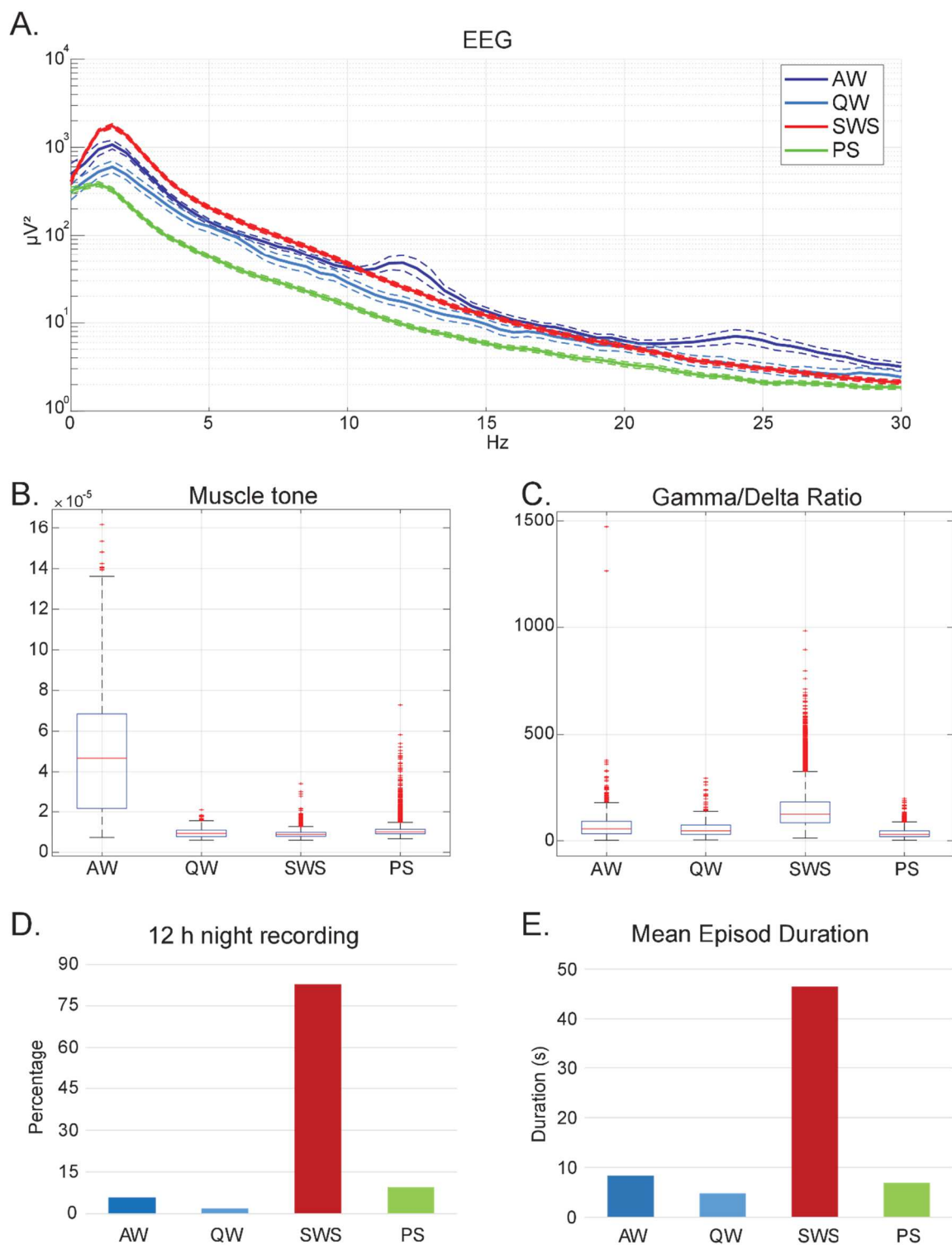
506 Overall, the duration and timing of sleep bouts, as well as the amount of each state was typical for pigeons  
507 (Fig. 13. E). During the 12-h night, the bird spent 82.9% and 9.5% of the time in SWS and PS, respectively (Fig.  
508 13. D).

509



510

511 **Fig. 12.** Raw signals obtained with ONEIROS in logger mode from a pigeon during different vigilant states. A.  
 512 Pigeon wearing ONEIROS (logger version) in the aviary during recording B. Raw signal illustrating the  
 513 transition from wake to slow wave sleep (SWS). C. Raw signal illustrating one paradoxical sleep (PS) episode  
 514 preceded and succeeded by SWS. From the top to the bottom; hypnogram illustrating the wake/sleep scoring  
 515 (active wake in dark blue, quiet wake in light blue, SWS in red, and PS in green); EMG with a high pass filter  
 516 (cutoff frequency 10Hz, order 2); right EEG; for B. only - time frequency representation of the left EEG; EOG;  
 517 3-axial accelerometry.



520 **Fig. 13.** Characteristics of wakefulness and sleep obtained from 12h night recordings of a pigeon with the  
 521 ONEIROS logger. A. Power spectrum in each state computed for right EEG. B. Distribution of the muscle tone  
 522 per epoch for each state. C. Distribution of the Delta/Gamma ratio for each state. D. Percentage of time spent in



523 each state during 12h night recordings. E. Mean episode duration for each state. AW – active wakefulness; QW –  
524 quiet wakefulness; SWS – slow wave sleep; PS – paradoxical sleep. Accelerometry axis direction: x – forward-  
525 backwards, y – lateral, z – vertical.

526

527

## 528 **IV. DISCUSSION**

### 529 **1. Recording electrophysiology, behavior and temperature using a miniature instrument**

530 One of the main reasons for developing ONEIROS was to combine in one small, light wireless device all of  
531 the electronics required to record multiple parameters related to sleep for long periods of time in small animals.  
532 Technically, the main constraints are size, weight, and autonomy. By coupling a frontend designed to record  
533 electrophysiology, an integrated digital accelerometer, a circuit to record temperature from multiple thermistors,  
534 and a low power microcontroller, we were able to build a system for studying sleep in small animals. Our  
535 recordings in baseline conditions in a rat, demonstrated that the size, weight, and autonomy of the system meet  
536 the requirements for recording sleep in the laboratory setting. Indeed, for the first time we were able to record  
537 without artefact in a freely moving animal most of the physiological and metabolic parameters that covary with  
538 sleep states (EEG, EMG, EOG, ECG, LFPs, and brain and body temperature). Moreover, we were also able to  
539 record the posture and the acceleration of the head via a 3 axis accelerometer, as well as ambient temperature.  
540 Our results and analysis demonstrate that an overall view of the classical features of sleep can be obtained with a  
541 single device. For example, the system can simultaneously record 10 electrophysiological channels at 256 Hz,  
542 three temperatures, and three acceleration channels at 64 Hz, for 35 hours with a 150 mAh; or 64 hours with 4  
543 EEG, 1 temperature and 1 acceleration at 128Hz; an infinite number of other recording configurations are also  
544 possible. To our knowledge, in contrast to other commercial and/or published devices, ONEIROS is the only one  
545 that provides such flexibility in the configuration of the channels number and sampling rate, allowing users to  
546 completely customize data acquisition according to their needs.

547 In terms of limitation, while the system is small enough to be worn by rats, it is too large to be used on  
548 smaller animals such as mice. We estimate that the device is only suitable for animals over 100g. Future efforts  
549 should be directed toward reducing the size and weight of the device further, maybe with some compromises in  
550 term of capability and flexibility. Regarding the fields of application, although the system was designed for  
551 recording sleep, it could be used for other neuroscience applications. Behavioral tasks such as mazes, novel  
552 object recognition, and fear conditioning that require freely moving animals, might also benefit from the use of  
553 ONEIROS. Moreover, the device might be useful in studies using animal models of epilepsy wherein long-term  
554 recordings are needed to capture seizure related brain activity and behaviors.

### 555 **2. Sleep deprivation with ONEIROS**

556 For the first time a vibration motor integrated in a wireless recording device has been used to perform  
557 automated, real-time PS deprivation. Our results indicated that the stimulation was intense enough to awaken the

558 animal and thereby induce sleep deprivation. The system effectively reduced PS across a 6-hour period, and  
559 induced a homeostatic increase in PS following deprivation similar to that observed using other methods, such as  
560 gentle handling and shaking the floor of the animal's cage. However, the remaining quantity of PS during PSD  
561 was a bit higher compared to the other methods, likely because the stimulation was less intense compared to  
562 gentle stimulation or cage shaking. This also suggests that it might be difficult to awaken the animal with the  
563 device and settings used during longer term sleep deprivations. Possible solutions to this problem would be  
564 either to increase the duration of the stimulations (700ms in our experiments) or randomized the pattern of the  
565 stimulations. Another possibility would be to encapsulate the vibrating motor inside the dental cement, directly  
566 over the head of the animal.

### 567 **3. Evaluation of the arousal thresholds with ONEIROS**

568 In addition to the main parameters that characterized sleep (electrophysiology, posture, temperature),  
569 including its homeostatic regulation, arousal threshold is also an important feature of sleep. With ONEIROS, the  
570 intensity, occurrence, and duration of the vibrating motor stimulation can also be specified, allowing for the  
571 systematic assessment of arousal thresholds.

### 572 **4. Recording sleep in semi natural environment with ONEIROS**

573 By changing the wireless transmission stack to the logger stack, the device can be quickly transformed from  
574 a lab-based device to a logger suitable for recording sleep in the field. In this regard, ONEIROS does not need  
575 any additional systems to store the data (receiver, computer), as the signals are stored directly on an integrated  
576 SD card. In the logger configuration, sleep can be recorded in wild using methods previously employed with data  
577 loggers having fewer capabilities (Rattenborg et al., 2016). To demonstrate the feasibility of recording sleep in  
578 the field, we implanted a pigeon and recorded its vigilance states in an aviary where other birds were also  
579 housed. The instrumented bird displayed normal behavior including short flights in the 2 x 2 x 2 m aviary. Thus  
580 the system could be used in a completely natural environment, as previously done with a logger with fewer  
581 recording capabilities (Lesku et al., 2012; Rattenborg et al., 2016; Vyssotski et al., 2009). In comparison to this  
582 system, ONEIROS includes the capacity to record temperature, a useful parameter for evaluating the relationship  
583 between ambient temperature, body temperature, and sleep (SWS and PS) and hibernation or torpor under  
584 natural conditions. In addition, it can record more channels, which is necessary when recording sleep in a species  
585 for the first time. ONEIROS provides more sleep parameters combined in a single miniature device than other  
586 devices used for recording into the wild. Future improvements of ONEIROS will include a recording scheduler,  
587 in order to define specific recording periods. For example, this could be used to exclude the post-operative  
588 recovery period, and thereby extend the recording duration capability of the device. Moreover, we plan to  
589 develop waterproofing and other protections necessary for recording in the wild.

590

## 591 **V. CONCLUSION**

592 ONEIROS was developed to record multiple aspects of sleep (behavior, electrophysiology, metabolism)  
593 from animals in the lab and the wild. The goal was to provide researchers with a tool that overcomes the

594 limitations of existing wireless devices. The system provides high flexibility in terms of number of channels and  
595 sampling rate with low power consumption, allowing long-term recordings in small animals (from 100g). By  
596 wirelessly recording a rat under baseline conditions, performing a paradoxical sleep deprivation experiment in a  
597 rat, and logging data under semi-natural conditions in a pigeon, we demonstrated that ONEIROS is a useful tool  
598 for recording sleep under diverse conditions. For the first time EEG, EMG, EOG, ECG, LFPs, 3D acceleration,  
599 brain, body and ambient temperature recording, as well as homeostatic and arousal threshold experiments, can be  
600 conducted with the same system in the lab and in the wild on small animals. By facilitating comprehensive  
601 comparative and ecological studies of sleep, this device may lead to new perspectives regarding the evolution  
602 and functions of sleep.

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## 729 SUPPLEMENTARY VIDEO LEGENDS

- 730 Supplementary Video 1: the video illustrates the online paradoxical sleep deprivation on the rat. From the top to  
731 the bottom: the hypnogram (in red SWS, in green PS, in blue WK), the differential EMG, the EOG, the raw and  
732 time frequency representation of the parietal EEG. The video has been accelerated 2 times. In the top left corner  
733 a red rectangle appears when a stimulation was send.