A novel method to study the ecological role of sleep in small mammals.

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Abstract

Sleep, is a complex, vital, and universal behavior that strongly differs from mere inactivity. Its ecological role remains, however, largely unknown mostly owing to the lack of methodological tools to record animal sleep states in the wild. By using a small, low power consumption biologger, capable of recording brain activity, body movements, and core physiology, we were able to record and quantify key sleep parameters (circadian distribution, sleep stages and their fragmentation, …), in wild black rats (*Rattus rattus*) in their natural environment over multiple days. We developed a simple, rapid (<1h), surgical procedure using a custom subdermal flexible electrode that provides signal quality equivalent to the cortical electrodes classically used in lab experiments. We also validated a semi-captive procedure, where the animals could be recorded in their own environment, with *ad libitum* access to standardized food pellets, and contact with conspecifics without close interactions. Such a protocol allows for the direct investigation of biotic and abiotic factors, like social interactions, food availability or type, light, temperature, and stress, all of which may strongly impact sleep. By evaluating general behavior and sleep patterns in four wild rats over up to ten days after surgery and by tracking feeding behavior for over a month, we show that the animals do not display any obvious signs of pain or stress and stabilized their sleep patterns two days after manipulation. Altogether this novel method and procedure constitute a unique tool for assessing sleep variability and flexibility and provides a proof-of concept for sleep studies in small (<200 g) wild animals.

Keywords

Sleep, NREM Sleep, REM Sleep, electrophysiology, black rat, *Rattus rattus*, neurologger.

Background

Sleep is not simple rest. It is associated with a low level of vigilance which makes it a risky state [1]. It is complex and can be expressed under multiples forms (drowsiness, Non-Rapid Eye Movement (NREM) sleep, REM sleep, unihemispheric sleep, …) which likely support different functions and adaptations. Moreover sleep is a fundamental and universal behavior that has been described in all animals to date [2] suggesting that it may provide important benefits for animal survival and reproductive success (Fig. 1). Indeed, much effort has been directed towards understanding its mechanisms [3], and various studies demonstrated a strong association between sleep and developmental [4], cognitive [5,6], restorative [7] and maintenance [8] processes, providing an indirect benefit on animal fitness, though an improvement of the animal performances during wake (Fig. 1). On the opposite when animals are sleeping, they are scarifying their vigilance to avoid predation, their time to forage, reproduce, to that state (Fig. 1). In addition, if there are some physiological benefits of sleeping, it has also been demonstrated on laboratory species and human that there is an important physiological cost of not sleeping. Indeed, unlike other resting states, sleep states are also homeostatically regulated, and their disruption, or deprivation, can have deleterious physiological consequences [9,10], leading to possible negative impacts on animal fitness. Altogether, this demonstrates that a fine balance exists between sleep and wake.

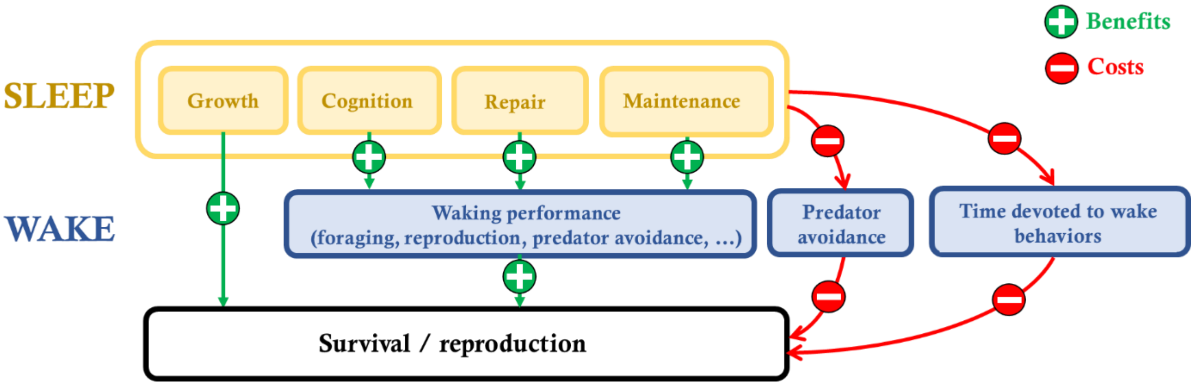
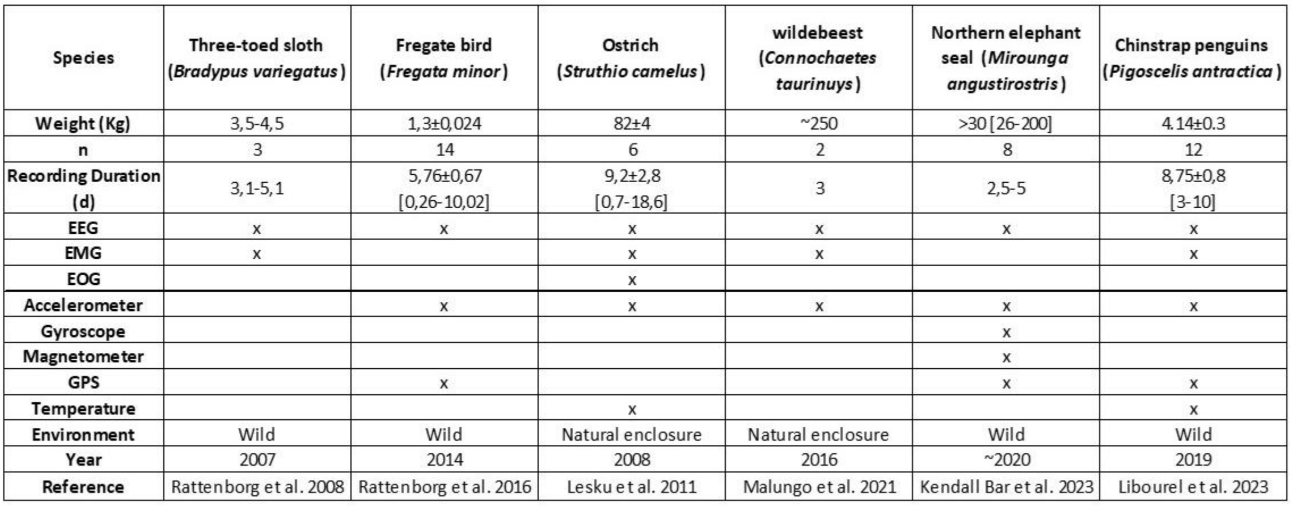


Fig. 1 Schematic representation of the interactions between sleep and wake showing the theoretical benefits and the costs of sleep on animal fitness (survival and reproduction).

Unfortunately, most of the sleep research, been in laboratory environments on laboratory model animal or humans, these efforts failed to inform us on this ecological balance in animal time budget nor on the diversity of sleep adaptations [11–13]. Indeed, only very few studies have been conducted on wild species, in laboratory conditions or *in natura*. A large diversity of sleep expressions was nonetheless reported from those studies on non-conventional animals [2,14], with elephants sleeping 2 hours a day, armadillo up to 18 hours, marine mammals and birds been able to sleep unihemispherically. The quantity of REM sleep also vary across species, European hedgehogs having 3.5 hours of REM sleep a day, whereas horses can have 0.5 hours and bottlenose dolphins seem to have no REM sleep [15]. This highlights the central role of sleep and it substates, but also suggests a large range of adaptations, likely driven by physiological and environmental factors. Understanding those factors may thus provide important cues to decipher the eco-evolutionary role of sleep. Then, more than describing the diversity of sleep expression across the animal kingdom diversity, its flexibility, and variability across and within individuals and populations, should be studied in wild species under natural conditions.

Some experiments, have been able for example to show that parental care could have driven some extreme sleep fragmentation in penguins [16], that foraging in frigate birds [17] and sexual competition in sandpiper birds [18] may induce import reduction of sleep with no obvious physiological cost of this sleep loss. Seasonal variations, that often drive food availability or induce thermal challenge, could also impact the sleep distribution and quantity in barnacle geese [19]. As a chronic source of stress, predation pressure [20], may also be an important driver of the evolution of sleep in natural populations and may have driven the observed variations in sleep characteristics across species. Indeed, variability in some sleep characteristics, such as its daily duration [21], the relative amounts of REM and NREM sleep [22], the daily timing [23], the occurrence of unihemispheric sleep [24], and sleep fragmentation [25], have been putatively correlated with levels of predation pressure.

Those studies are unfortunately too rare to widely understand the ecological role of sleep. This is mostly explained by three factors: I. an understanding of sleep and sleep states (not simply inactivity) requires collecting information on brain activity, and thus relied on dedicated methodologies such as electroencephalography (EEG), II. Sleep, being a rapidly-evolving dynamic state, requires recording techniques with high temporal resolution, III. Sleep is affected by many abiotic (light, temperature, noise, weather, …) and biotic (predation, reproduction, parental care, food resources, parasitism, …) factors which must be controlled to estimate causality and thus requires long-term recording. Despite those limitations, methodologies have already been deployed in the wild to study sleep, with often a trade-off between, the precision needed to disentangle sleep and its substates from inactivity, the recording capacity, the animal size, and the number of individuals [26–29]. Recent studies revealing the adaptive nature of sleep states, for example, have been based on data collected for ten days maximum (usually less) on species weighing more than 1.5Kg (one species), and usually more than 4Kg (Table 1) [16,17,30–33].



Species	**Three-toed sloth (*Bradypus variegatus*)**	**Fregate bird (*Fregata minor*)**	**Ostrich (*Struthio camelus*)**	**wildebeest (*Connochaetes taurinuys*)**	**Northern elephant seal (*Mirounga angustirostris*)**	**Chinstrap penguins (*Pigoscelis antractica*)**
Weight (Kg)	3,5-4,5	1,3±0,024	82±4	~250	>30 [26-200]	4.14±0.3
n	3	14	6	2	8	12
Recording Duration (d)	3,1-5,1	5,76±0,67 [0,26-10,02]	9,2±2,8 [0,7-18,6]	3	2,5-5	8,75±0,8 [3-10]
EEG	x	x	x	x	x	x
EMG	x		x	x		x
EOG			x			
Accelerometer		x	x	x	x	x
Gyroscope					x	
Magnetometer					x	
GPS		x			x	x
Temperature			x			x
Environment	Wild	Wild	Natural enclosure	Natural enclosure	Wild	Wild
Year	2007	2014	2008	2016	~2020	2019
Reference	Rattenborg et al. 2008	Rattenborg et al. 2016	Lesku et al. 2011	Malungo et al. 2021	Kendall Bar et al. 2023	Libourel et al. 2023

Table 1 Comparative table of sleep recordings using EEG conducted on wild species in natural environments (semi captive conditions and wild) and associated methodologies.

The relative important weight of the animals chosen for these studies, as well as the limited recording duration (relative to the animal's ecology and life history) is mostly constrained by the capacity of the neurologgers, i.e. biologgers capable of recording brain activity. Even if the dimension and power consumption of such loggers has significantly decreased over the past 20 years, the volume of data and the battery size still remain a limitation, together with the need to recapture the animals (for data retrieving). Additionally, the stress induced by the capture and the natural variability of sleep states could also be a limitation when interpreting sleep data collected in the wild. A trade-off between, animal size, recording duration, environmental factors, and recapture rate is thus inevitable.

Moreover, if only large animals are recorded for sleep, this could induce a bias, as they represent only a few percentages of the biodiversity [34–36], and the weight been also importantly correlated with other morphological, physiological, behavioral and ecological traits. From a comparative point of view, it would be thus important to develop comparative studies in smaller species (<200g), in particular birds and mammals, as they present a large variety of physiological and ecological adaptations without the potential cofounding adaptations related to large species.

In addition, assessing sleep, does not only involve estimating its quantity. Sleep is a complex trait, with its different elements having potential reciprocal and/or independent benefits and costs. Sleep fragmentation, circadian distribution, homeostasis, depth, infraslow rhythm, asymmetry, and phasic events constitute some of the important elements that could respond to or be modulated by environmental pressures. The homeostatic regulation of sleep for example has been related with seasonality [37] and social pressure [38]. Whereas NREM sleep has been suggested to be mostly involved in restorative [7,39] and cognitive processes [6] REM sleep is thought to be more involved in stress-related behavior [40,41], associated with memory and emotional processing [42,43] thus, potentially responding to predation pressure [44,45]. Fragmentation/drowsiness or unihemispheric sleep, on the other hand, could be an adaptation to maintain vigilance while fulfilling need to sleep [16,46,47].

To bridge the gap, between the fine mechanistic approaches used in laboratory studies, on often inbred model species, and the evolutionary and ecological approaches that attempt to understand sleep on a broader scale, we have developed a novel methodology applicable to small animals (>80g). We demonstrate that this method allows for the characterization of the full phenotype of sleep, and its sub-states, over longer durations, at both the individual and population levels. Our methods not only finely characterize the natural variability of the main sleep traits, but also allows for the estimation of phenotypic flexibility in wild animals in their natural habitats, limiting the potential bias of confounding factors like social interaction, resource availability, or parental care. We deployed our methods successfully on black rats (*Rattus rattus*) in New Caledonia in 2023. The use of rats is particularly relevant as their sleep biology is well described in the lab and a they are present in a wide variety of ecological contexts (i.e. varying climatic conditions, with and without predators or competitors etc…) allowing to assess the impact of ecological factors on sleep phenotypes.

Methods

Capture

Animals were captured in austral winter, June 2023, in New Caledonia, south-west Pacific, in a tropical forest nearby Port-Laguerre (Païta) agronomic research station (22.1037° S, 166.3189° E). Up to six traps (wire cage rodent live-traps with spring door (Pro-trap, ©Connovation NZ) were deployed between 16-17h (Fig. 2). The traps were baited with coconut and butternut. The rats were collected the next morning. In total we equipped four black rats (*Rattus rattus*) (2 females and 2males; 180g, 95g, 197g, 208g). Individuals were included as to balance sex ratio and span various body weights. One 81g female Pacific rat (*Rattus exulans*) was also kept as a control for behavior in semi-captive conditions without implantation.

Fig. 2 Black rats (*Rattus rattus*) trapped in 2023 (Left). Mesh cages constructed on different capture sites (middle and right).

Semi natural enclosures

In order to mitigate the trade-off between ease of monitoring, ease of cage construction and keeping the animals in a similar environment as their natural habitat, we built a 1m^3 cage (1m x 1m x 1m) in galvanized steel mesh with 1mm diameter wire, spaced by 1.3 cm (Fig. 2). The cages were placed 20m from the location where the individuals were captured and were buried 10 cm into the ground. Yet, this design should reduce competition over territories which otherwise could have impacted sleep. A thick layer of vegetation and wood sticks taken from the immediate animal’s habitat were placed into the cage to recreate a familiar natural soil. A plastic food dispenser with a 200g capacity provided food ad libitum and could only be accessed from inside the cage. A flower pot filled with water was buried into the cage and a water bottle was fixed on the cage near the food dispenser. Finally, a cylindrical shelter of 10 cm by 20 cm made of PVC was provided and covered by vegetation and was either placed on the ground or elevated. We also placed two autonomous custom-designed cameras equipped with infra-red lights during the first 6 days of the recording to monitor the animals' behavior. We decided to maintain the animal in semi-captive conditions in order to standardize some of the key parameters that could impact sleep such as food availability and type. Finally, social interactions were maintained as rats could interact with conspecifics through the mesh.

Surgery

Surgeries were conducted in the field under a tent at ambient temperature (27-28°C). After capture, the animal was placed into a small transparent Plexiglas box (10 cm x10 cm 25cm), weighed, and a cotton ball soaked with Isoflurane was introduced in the box. After 1-2 minutes, when the animal showed a reduction in its breathing rate, it was removed from the box and administered a mixture of 80mg/Kg of Ketamine (Ketamil) and 0.5mg/Kg of medetomidine (Medetor) intramuscularly. The animal was then shaved and positioned into a plastic 3D-printed stereotactical frame (https://hackaday.io/project/163510-3d-printed-rodent-stereotaxic-device) adapted to the its size. The ear bars were adapted to be used in pressure on the skull and not into the ear cavity (Fig. 3). A surgical field was positioned over the animal and ophthalmic gel was used to protect its eyes from drying. The skin over the skull was cleaned with physiological serum and betadine. A local analgesic (lidocaine) was injected and sprayed over the skin. After 1-2 min a 2.5cm, antero-posterior incision was made into the skin, which was spread apart with small clamps. The skull was cleaned with H_2O_2 and slightly scratched with the scalped blade after drying. The nuchal muscles (rectus scapitis) were slightly spread with thin round-tip forceps to prepare the insertion of the EMG probe. Using an electrode interface board (EIB) that will later support the logger (Fig. 3), the custom flexible probe we developed (Fig. 3) was then positioned over the skull using the cerebral suture as a reference to position the electrode at the good place. This flexible probe made in FR4 (epoxy resin) with gold plated conductive sites was designed to record the electrical activity of the olfactory bulb, the frontal and parietal cortex as well as the ocular and muscle activity. The use of this flexible probe reduces significantly the invasive nature by keeping the skull intact and thus the duration of the full procedure

(45-60 minutes). We next added some conductive EEG paste (EC2, Natus) on the recording site of our flexible probe (Fig. 4). The electrode was then repositioned and maintained over the skull with a toothpick while a primer dental dement was applied (Ivoclar vivadent, Adhese Universal vivapen) and dried with UV light. When all the electrodes were glued over the skull with a first layer of primer dental cement, a second layer of dental cement (Ivoclar vivadent, Tetric Evoflow A1) was applied. At this time the logger was inserted into the EIB with its 3d printed protective casing and the device was maintained in its final position. This allowed us to fix with dental cement a plastic nut on the skull to maintain the biologger in place with plastic screws (M3, 6mm long). After the EIB and nut were secured in place, the logger was removed, and the electrode, was completely covered in dental cement and the EIB and nut completely secured with dental cement. The skin was then sutured around the EIB connector and betadine was applied over the wound. An anti-inflammatory (0.2 mg/Kg, Melcoxicam) and antibiotic (0.1mL/Kg, Oxytetracycline) were injected sub-cutaneous into the back. The logger in its casing was then started and fixed on the head of the animal. The signal quality was checked for few minutes with the Bluetooth connection and then the logger was programmed to record on its embedded memory. After that, the animal anaesthesia was reversed using 0.25mg/Kg of atipamezole (Reversor). The animal was then placed into its cage where we supervised its awakening. All rats were fully awake after ten to 20 minutes.

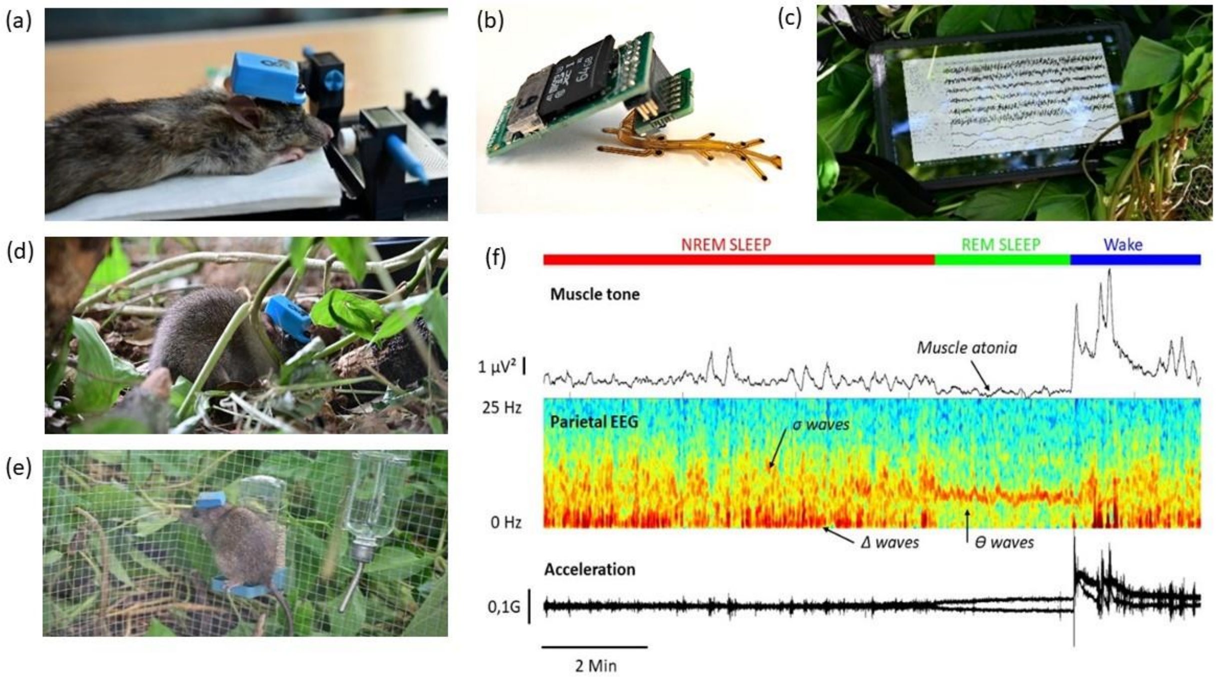


Fig. 3 (a) Anesthetized black rat after the implantation of the logger on the stereotactical frame. (b) Sleep logger connected on the EIB with the flexible electrode. (c) Live recordings of the EEG of a black rat in its cage, collected with Bluetooth on a tablet. (d) Rat carrying the sleep logger in its cage. (e) Rat equipped with the sleep logger over the head, eating in its cage. (f) 10 minutes of recording (muscle tone extracted from the EMG, brain activity from the parietal site represented in time frequency and head movements extracted from the accelerometer) during NREM sleep (red), REM sleep (green) and active wake (blue), showing the typical brain signatures of the different vigilant states (delta, sigma and theta activity), as the muscle atonia typical from REM sleep (right).

Biologger

In order to record cerebral, physiological, and behavioral activity we used a Phynitty PNano logger (Manitty SAS) derived from a previously developed custom-made logger [27,48]. The device itself weighs 1.2g (1.45 with the SD card) and measures 25 x12 mm. The logger can be used in live mode (data collected on a tablet, Fig. 4) or can be programmed (with Bluetooth) to run custom sequences of recording. The logger can record up to six monopolar channels; six EEG (electroencephalograms) in our case, and 3 differential channels. Here we only used two, one for electromyography (EMG) and one for electro-oculography (EOG). The sampling rate (typically 256 Hz for 8 channels) can be customized. In addition to these electrophysiological channels the device records ambient temperature and can have an external channel (light, temperature, …). These additional channels are very useful to quantify environmental variation when recording animals in their own environment. Additionally, a 9-axis inertial measurement unit (IMU) can be activated. The IMU contained a 3D-accelerometer, a 3D-magnetometer and a 3D-gyroscope with customizable acquisition range. The power consumption is proportional to the sampling rate and number of channels activated. In our case, we recorded six EEG, one EMG, and one EOG at 256Hz. In addition, we recorded acceleration and gyroscope data at 64Hz and ambient temperature at 32Hz. The power consumption with this configuration was 2.8mA. We used two battery types: a large battery (EVE EF651625, 700mAh, 8g) and a smaller battery (EVE EF651615, 450mAh, 5g). We could record up to 10 days continuously. However, as the logger allows to schedule the recordings, these can be spread over weeks or months as the power consumption is negligible when the system is not recording. To prepare the logger, we soldered the battery, inserted the SD card, tropicalized it with acrylic resin (APL, Electrolube) and wrapped it into plumber tape before inserted it into its plastic case. The total weight of the logger was 12g (large battery) for the rats weighing over 150g and 9g (small battery) for the lighter individual.

Recordings

Four rats were equipped with our system. We programmed the loggers to record 2-3 days, with all eight electrophysiological channels sampled at 256Hz, the ambient temperature channel at 32Hz and the accelerometer, magnetometer, and gyroscope at 64Hz. The range of the accelerometer was set at +/- 2G and the gyroscope at 125dps. The four rats were set up after the surgery into their cage. We also monitored a non-implanted rat into a cage to evaluate the impact of the logger on animal *behavior*. After that the initial recording session the animals were recaptured and sedated with a cotton of isoflurane, to check the data and set up a new set of recordings of one day every second/third day. After that last manipulation the animals were left into their cage, their food consumptions were evaluated by checking the food dispenser every four to six days when the food dispenser and bottle of water were refilled. Upon each visit, we also checked the animal status and position. The experiment was stopped after 30 days, the animals were weighed without the logger and the data were collected from the SD card. The final weight gain/loss was evaluated as well as the signal quality and the proportion of the different vigilant states over the days of recording. Three states (Wake, NREM and REM sleep) were scored in 5-second epoch bouts based on the electrophysiological signals [48]. Every complete 24-hour recording without artefacts was analyzed. The evolution of the vigilance states quantities (wake, NREM and REM sleep), as well as their distribution, were used to estimate the possible disturbances due to the semi-natural conditions. Indeed, we were expected to see sleep perturbations because of the new environment and stress cause by the capture, manipulation and surgery recovery. But as sleep is highly genetically driven [49,50], it should then stabilize when the environmental conditions remain stable ‘‘which should be the case in our semi captive conditions if the weather is stable). We also computed the percentage of inactivity to compare with the true proportion of sleep, in order to determine to what extend an accelerometer itself can be or can’t be a reliable way to estimate sleep. To do this, we extracted the percentage of inactivity based on the norm of the three channels of the acceleration, filtered with a high pass filter (cut off frequency 1Hz, order 10). The mean of the acceleration was computed per each 5-second epoch. We used as a threshold to score inactivity and activity which was the mean acceleration that separated true sleep from true wake. The videos (when available) were also inspected to look at the animal’s *behavior* to detect potential sign of stress/anxiety or abnormal *behavior*.

Results

The flexible probe: surgery

Usually, surgeries to record animal sleep states typically require drilling small holes into the skull to insert microelectrodes, which allows to record a stable EEG over days or even months. In addition, electrodes are implanted into the nuchal muscles to detect the muscle atonia during REM sleep and differentiate sleep from wake. Electrooculogram (EOG) is further used to monitor the phasic ocular activity occurring during REM sleep. The use of the flexible electrode design employed here enabled us to refine the surgery and refrain from drilling holes. This made it easier and quicker to implant animals in the field, and shortened the recovery process. Of the four animals implanted, three recovered well and were recorded throughout the whole experiment. One rat did not survive. From the video we never observed this rat eating or drinking. In addition, the second night after the surgery it rained hard. Because the shelter was positioned on the bottom of the cage we suspect that the animal died of hypothermia. This animal was also the only one for which a large battery was used which may have impacted its recovery. Although we can exclude post-surgery infection, multiple factors likely contributed to the rapid decline and death of this animal. Lastly, we validated on three individuals that the procedure used (i.e. gluing the electrodes to the skull) was adequate to record for at least 30 days.

The flexible probe: signal quality

Using a flexible probe was a challenge as the sensors are directly in contact with the brain, which could possibly lead to an attenuated signal (decreased signal-to-noise ratio). In addition, the use of a flexible probe could be less stable over time and the amplitude may change after few days of recording, which would be a problem to consistently analyze the data. Yet, our recordings showed excellent signal quality, consistent across channels, mostly artefact free, with a desynchronized EEG in the frontal and parietal area when the animal is awake. The left and the right hemisphere also display similar signal which is expected in this species. When the animal was active a typical Theta (5-9Hz) oscillation occurred in the EEG allowing us to identify and score that state. During NREM sleep and REM sleep we also observed the classical electrophysiological features of each state including spindle activity, slow waves, hippocampal theta and phasic theta when phasic activity occurs during REM sleep (Fig. 3). As opposed to the robust change in the gamma band of intracranial olfactory bulb LFP signals between wake and sleep [51], the surface olfactory bulb activity did, not significantly change between wake and sleep in the gamma band (50–70Hz). EOG was recorded for the first time with a flexible probe, but the signal was not consistent across individuals. This is likely due to the electrode position and the fact that the measure was a differential between the left and the right EOG. However, in some case the EOG provided a usable signal when ocular activity occurred during wake and REM sleep. The EMG was of sufficient quality to detect muscular bursts of activity but also muscle atonia when the animal switched from NREM sleep to REM sleep (Fig. 3f). This signature is very useful to develop automated state scoring as the muscle tone is one of the rare features that differentiate NREM sleep from quiet wake (including freezing). In addition, to these signals, we were also able to collect information on animal movement thanks to the 3-axis accelerometer, 3-axis gyroscope, and 3-axis magnetometer. Those sensors could be used to identify movement, head position, head direction (magnetometer), but also grooming, or freezing when associated with brain activity. Lastly, thanks to the gyroscope we were also able to distinguish the breathing rate during the resting phase which could be of interest in some cases. Altogether, the quality of our recording revealed our ability to identify a wide panel of vigilant states, and in particular sleep states (Video1). The quality of the signal is also sufficient and stable enough across at least 10 days of recording to develop automated sleep scoring and extract with precision state quantity, distribution, and fragmentation. In addition, some specific features, associated with cognition (like spindles activity), sleep depth (delta power) or REM sleep (phasic theta), as well wake substates (quiet wake, active wake, grooming, freezing, …) could be easily identified over multiple days. When comparing sleep (scored with EEG/EMG electrodes) to the proportion of inactivity based on the acceleration alone, a measure often used as a proxy of sleep [52–55], we revealed an overestimation of 14.6 ± 8.9 % of the sleep quantity. This comes from the misclassification of quiet wake as sleep when using only acceleration or actimetry. This mistake could be even more important if the animal is freezing.

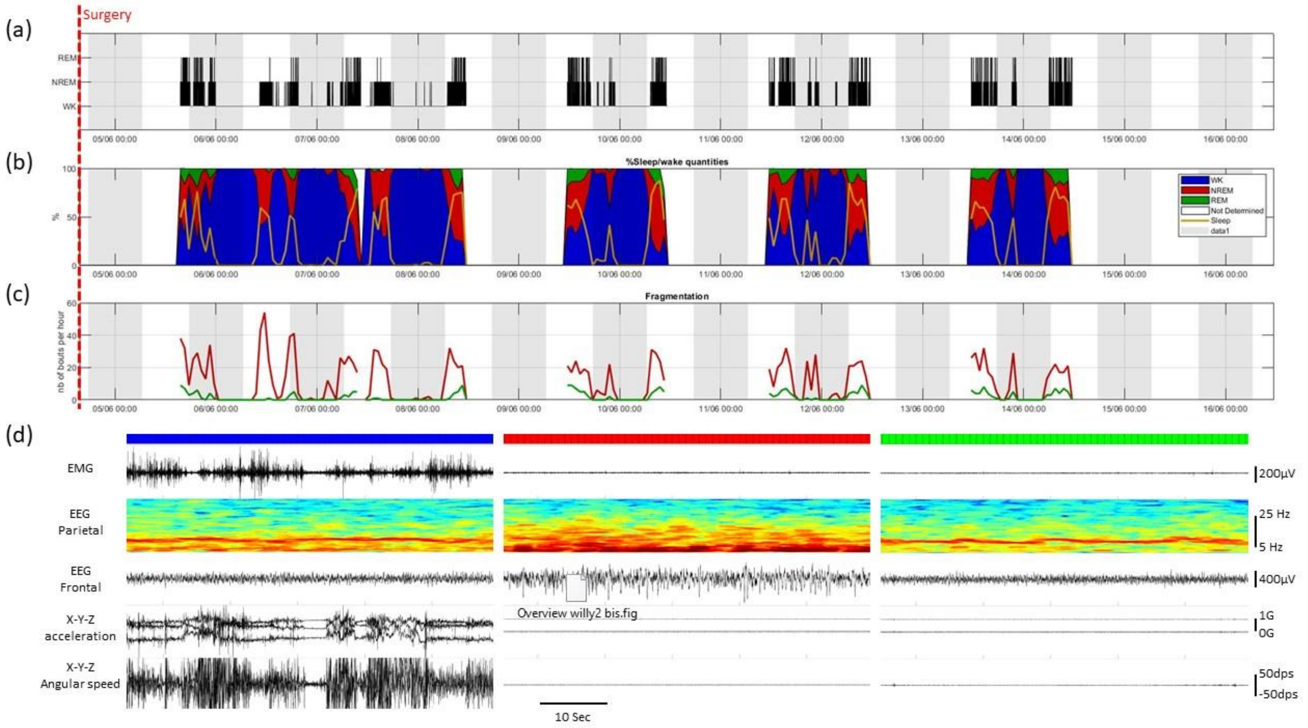


Fig. 4 (a) hypnogram representing the evolution of the sleep states across the recording days. Because not all days were complete and scored, and because we scheduled the logger after the 8/06 to record one day every two days there is some missing values (b) Evolution of the wake and sleep states quantities per hour (wake–blue; NREM sleep–red; REM sleep–green) showing that the sleep state quantities stabilized two days after the surgery. (c) Number of wake, NREM, and REM sleep bouts per hour; showing a more fragmented sleep the first days after the surgery. (d) Illustration of the signal collected over 60 seconds during each vigilant state, from the top to the bottom: EMG; Parietal EEG represented in normalized color-coded time frequency (Blue=low spectral power, red= high spectral power); EEG frontal; 3-axis acceleration in; 3-axis angular speed collected from the gyroscope.

Behavior

Thanks to the cameras we placed around the cages, we were able to monitor the animal’s behavior. Animals adapted quite fast to the semi-captivity. They quickly found the shelter, where they built a nest with vegetation available in the cage (Video 1). They also quickly found food and water. We were able to observe the rats eating and drinking in the 5-10 hours after the surgery. The animals also climbed on the mesh and ran on the branches inside the cage (Video 2). Finally, in one animal we also observed some social interaction with a conspecific lasting most of the night (Video 3). Stress in rats is often characterized by excessive grooming, time spent in the shelter, or freezing. Excepting the first days when we visit the animals (Video 4), those states were not observed during our recordings. The animals were also not observed biting the mesh. After the first session when the animals were recaptured, none displayed lesions that could be related to repetitive attempts to escape, which could sometimes be observed in trapped animals. The three individuals did not lose weight (respective weight gain: 0g; 8g; -1g) after two-tree days post-surgery when we retrieved the first data suggesting they recovered quickly from the intervention.

Sleep monitoring

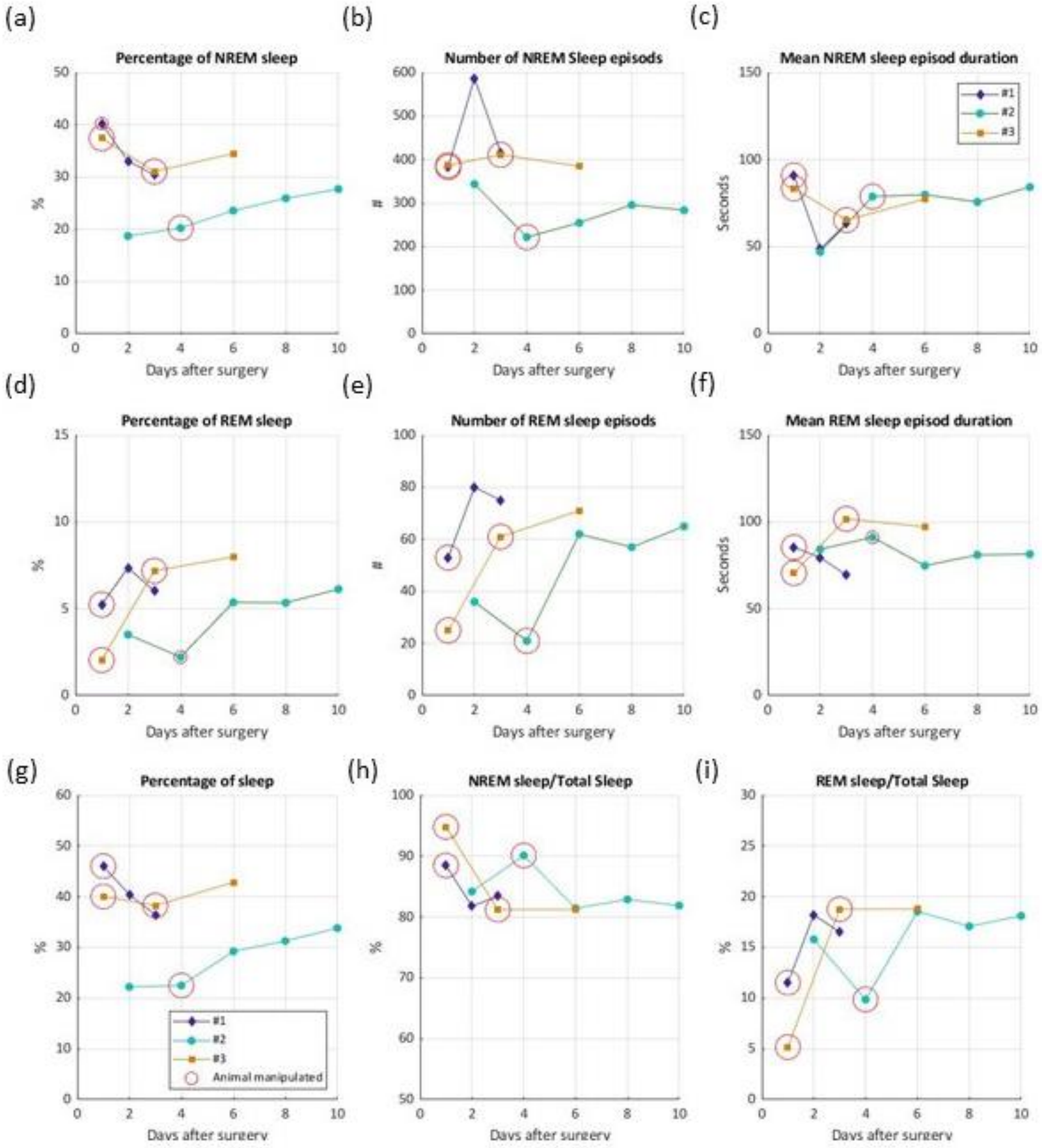


Fig. 5 Evolution of the daily quantity (a), number of episodes (b) and mean episode duration (c) of NREM (a-c), and REM sleep (d-f). The percentage of sleep (NREM plus REM sleep) (g) and the relative quantity of NREM (h) and REM (i) sleep. Each animal is color-coded. The red circles indicate days that the animals were manipulated in the previous 24hours (surgery; data retrieval).

Thanks to the EEG, the EMG, and the acceleration data we were able to score vigilant states of three rats over three to ten days. The first couple of days after the animals were manipulated, the quantity of NREM sleep tends to be high whereas the quantity of REM sleep decreased (Fig. 5A and D). The total proportion of sleep (NREM and REM sleep) is variable across individuals the first days after manipulation. However, we observed that the relative quantities of NREM and REM sleep tend to stabilized two days after manipulation (Fig. 4, 5). Indeed, the coefficient of variations (CV = std/mean) of the three rats decreased to 20.7% for the total sleep time, 1.4% for NREM and 6.4% for REM sleep when they were not been manipulated the day before, vs 22.6%, 5.3% and 29.4% if we include all the recording days. When looking at the total sleep time intra-individual variation, the CV also becomes lower ([7–17%] vs 20.7%) suggesting a stable individual phenotype. When comparing the intra-individual variation of NREM and REM sleep relative to the total sleep time compared to the global CV the differences are even smaller suggesting that these traits are highly conserved across individuals. In addition, the highest variations of REM sleep quantities relative to the total sleep time are found when the manipulation days are not removed (6.4% vs 29.4%). Lastly, the mean bout duration more than the number of bouts also tends to stabilize after manipulation.

Long-term monitoring

Our four animals (three carrying a logger, one without), were followed for a month after the surgery (Fig. 5). Animals kept their loggers at all times even when it was not recording data. The monitoring of the food consumption shows that all individuals were eating 19.6g ±3.3 of food every day (around 22g for the animal weighing more than 150g and 17g for the lightest one (80g). The animals, during most of the recording, were always observed in their shelter when the experimenter came to fill the food dispenser. The rats were also reactive when the experimenter checked the animal's status by touching the shelter. However, between the 28th and the 5th of July (date of the two-last visits) one animal was found lethargic (weight loss 20%) and two animals were found dead, including the animal without a logger (weight loss 16% and 1.2%), while the remaining animal was still in good health (no weight loss). Because the animals were eating properly for the whole month we looked at the weather conditions (Fig. 6). The data reveal an increase in the precipitation and drop of the temperature at night in between these days (Fig. 6). We hypothesize that animals may have suffered from hypothermia. It is also important to note that after mid-June (around 2 weeks after the surgery) the food dispensers were generally found empty which suggest that the animals were eating more during this second part of the experiment. We also had to change one food dispenser as intruder rats were chewing on it to get food. The animal that survived was the only one that had a high-mounted shelter.

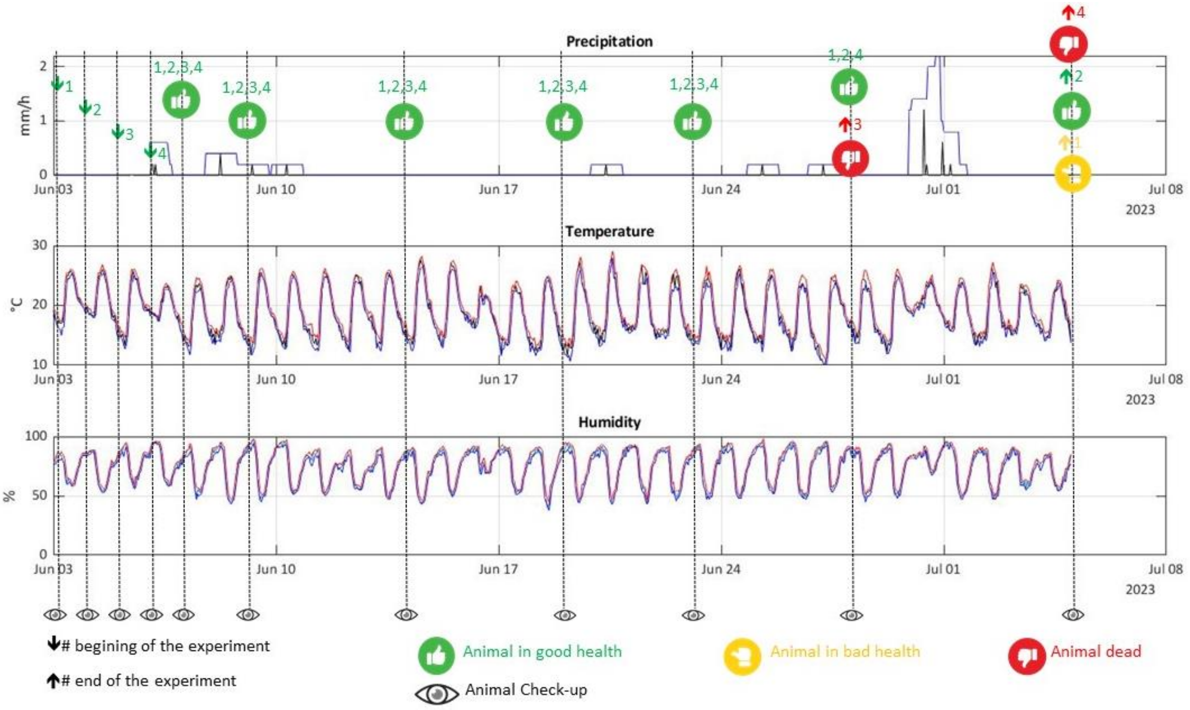


FIG. 6 Timeline of the experiments showing the evolution of the weather conditions including precipitation (Top, in black the average hourly precipitation, in blue the average daily precipitation), the temperature (middle) and the humidity (bottom) collected from la Tountouta weather station (Lat: -22.0173°, Long: 166.222333°, Alt: 37m) which is at 18km from the recording site. For temperature and humidity, the black line is the mean, the blue is the minimal and the red line the maximal value over an hour. The animal status (color-coded) was evaluated periodically until the end of the experiment the 5th of July. Animal 1 to 3 underwent surgery whereas animal 4 was our control.

Discussion

Sleep assessment in the wild

A clear trade-off between the need to sleep and the need to remain awake or interact with the environment exists. However, there is an important pressure to sleep, likely because of physiological needs. Understanding how animals manage this need remains crucial to better understand how animals survive, especially in a changing world. To date, very few studies have been able to investigate sleep in the wild, and most of them focusing on large species, whereas small species constitutes the majority of the biodiversity. This necessary condition to such studies is to monitor the animal's brain activity, as sleep strongly differs from mere inactivity [16,17,30,31,33]. Until now, this was not possible in small animals (~100g) as it was challenging to design and implement lightweight bio logger, as well as methodologies to perform surgeries in the field. Here, we showed that using inactivity as a proxy of sleep in rats is inaccurate. Specifically, the quantity of sleep is overestimated by around 15% but this could be higher if the animal displays specific behavior, such as freezing, notably when hiding from a predator (Video 4). Thus, our results show that, to decipher the role of the sleep and its substates, recording the cerebral activity is unavoidable. Yet, EEG can provide significant insights into how sleep varies across individuals and populations and adapts to environmental changes or physiological needs. Some limitations, however, remain when trying to record animal brain activity longitudinally. One is the reduced autonomy of the loggers and the need to recapture the animal as it is impossible to transmit the large volume of data collected wirelessly. Measuring the low amplitude (few microvolt) of the EEG and minimizing movement artefacts to maintain a stable signal across days are also challenging, especially for small animals. By developing a less invasive methodology, relative to typical lab-based methods involving a flexible probe and a small programmable logger we were able to obtain excellent data on 3 small animals with a good and stable signal quality. This allowed us to record the complete sleep expression, as well as the complete panel of waking behavior, within a relatively long timeframe as the recording can be delayed or schedule intermittently. This opens new avenues to understand the ecological role of sleep in nature and its response to environmental changes. Some limitations however still remain, especially in terms of size and weight. In the future, we expect that the battery capacity will help to overcome this limitation, but for now the sampling rate could be decrease to record for longer periods of time as well as the number of channels and the recording days could be delayed spread across a longer period.

Semi-captive vs. captive recordings vs. free-moving animals

The vast majority of sleep studies are currently conducted under laboratory conditions, often in inbred strains of mice and rats and under artificial (un-ecological) conditions (stable temperature and light cycle, small cage, often tethered to assess EEG). Although this has resulted in an important body of knowledge on the mechanisms of sleep in these animals, the lack of genetic diversity and environment complexity, prevents us from addressing ecological and evolutionary questions. On the opposite, recording non-conventional wild animals, in their habitat, can reveal the true flexibility and adaptive nature of sleep, but this remains technically challenging in particular because of the high number of possible confounding factors.

Whether we should bring those animals into the lab, keep them in semi captive conditions, or record them in the wild remains an open question as it exists trade-offs between those conditions. In the lab, wild animals may be exposed to intense stress as they will be exposed to a novel environment and removed from their natural habitat, but, this would however allow to record their sleep continuously under standardized conditions reducing the bias of external factors. Unfortunately, in these conditions the sleep phenotype may reflect the stress response on sleep traits and may hinder the assessment of natural individual variability. One key advantage is that all individuals would be recorded in the same conditions. On the other hand, when recording sleep in the wild, we are limited by the need to recapture the animal, and important confounding factors that cannot be controlled or quantified exist. This is the case of the social interactions, food type and availability, health condition, predation exposure, etc. Even if ideally, it would be better to record sleep in completely free and wild animals a high unexplained variability may exist, especially regarding the limited number of days/weeks for which small animals can be recorded. A good alternative is to record animals in semi-captive condition in their own environment as done here. The advantages are partial control over factors like food or social interactions coupled to high-quality recordings under reduced stress conditions. The main disadvantage is the possible stress induced by the semi-captive conditions. This approach, however, allowed us to characterize the baseline full sleep expression. In addition, by recording animals in semi-captive conditions we can record for longer periods of time if batteries are changed. Another important concern when recording animal in semi -captivity, is the possible stress induced by limiting the freedom of the animal. Our new method, however, demonstrated that after a period of manipulation, when sleep is possibly impacted with modifications (in NREM and REM duration in particular), the stress tends to be minimal as the animals were displaying normal behavior, including eating, exploring, and sleeping in quantities that tended to stabilized after few days. We cannot completely reject the fact that some level of stress is present but because our animals do not show stereotypic behaviors like excessive grooming or repetitive biting the mesh, nor displayed any lesions on the snout, this strongly suggests that our recording conditions are well-adapted to understanding the diversity of sleep expression in different rat populations. Recording small animals sleep in semi-captive conditions also opens new perspectives for testing the flexibility in response to various stressors, like simulating the presence of a predator, changing the diet, or moving the cage into a new territory with other pressures, thus allowing us to get more insight into the flexibility of sleep.

Possible improvements

This experiment, was the first one to date, allowing to record precisely sleep, in small wild animals, in their own environments. Indeed, all information collected on small wild mammals and birds were done by bringing the animal in lab conditions [2,22,56,57]. Only large species (>1Kg) were recorded in wild or semi captives conditions [16,58]. Despite the fact that our new method and technology fills the gap between the lab and the field in small species, some improvements could be done. To prevent intruder rats to try to eat the food of our experimental rats, the food dispenser could in metal or installed in the center of the cage, and its capacity could be increased to avoid filling it too regularly. In addition, we lost three rats of the four after important rains and temperature drops. This could be prevented by installing a small roof over the shelter and by installing the shelter way from the bottom of the case, for instance in an elevated position. We can also provide more bedding to the animal to let it construct a more insulating nest, thus reducing hypothermia risk. However, those solutions tend to decrease the natural aspects of the experiments and are the dark side of limiting the freedom of the animal. A more ecological solution would be to construct larger cages, allowing the animals to have access to more natural shelters, bedding … In addition, testing the reliability of our recording methods, specifically the flexible electrode in standardized conditions in the lab is still to be to ensure that the signal is stable over longer durations (months). The recording of the olfactory bulb is not mandatory as our data shows that the signature is not useful for sleep scoring in wild rats in semi-captive conditions. EOG also did not provide any consistent information and could thus be removed if the objective is not to quantify the density of eye movements during REM sleep. Alternatively, when EOG would be of interest, measuring its local activity with a differential between two electrodes over one eye instead of in-between the two eyes would provide a more accurate and stable signal. Finally recording the ECG by connecting two small wires to our EIB could bring information related to energy expenditure, or stress. Indeed, heart rate itself and heart rate variability have been used for long time to assess these two parameters. Finally, it could be of interest to use more precise tracking methods such as RFID microchips to determine how much time the animal spends in its shelter, spends eating or foraging as using a camera is sometimes difficult in the wild, especially for long term recordings, though this would add to the total weight of the implant.

Conclusions

Because sleep is a vital and complex state its ecological role remains poorly understood. It remains thus crucial to develop methods to characterize its variability and flexibility across populations and individuals under different environmental conditions. By providing a fast, and minimally invasive surgical procedure and a recording technology adapted to small species, we demonstrated the first proof-of-concept for sleep studies on small animal in the wild on a wide range of animals. We demonstrated that rats adapt well to the recording conditions and to the semi-captivity and that an assessment of the complete sleep phenotype in wild populations is feasible. Sleep is as important as feeding, reproduction, and avoiding predation, but its direct role in species survivals remains unknown. The novel method presented here for small species of mammals, opens new perspectives in our understanding of the ecological drivers of the evolution of this enigmatic state.

Abbreviations

EEG: electroencephalogram; EMG: Electromyogram, EOG: Electrooculogram, NREM sleep: non-rapid eye movement sleep, REM Sleep: rapid eye movement sleep

Supplementary information

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Author contributions

P.-A.L. conceived, designed and supervised the project. B.M. & P.-A.L. Conceived the electrophysiological instrumentation. A.D. & P.-A.L. conceived the flexible probes. E.V and F.B. provided support regarding the logistics in New Caledonia. E.V. & W.W brought valuable insights on rat behaviors. S.A & P.-A. L. set up the surgical procedure. A.B. & P.-A.L. capture the rats, build the mesh cages, did the surgeries and collected the data. S.A scored the data. F.B. & W.W. did the weekly check-ups. P.-A.L. & S.A. analysed the data. A.B. & P.-A. L. discussed and interpreted the results. P.-A.L.

prepared the figures and wrote the first version of the manuscript. All authors reviewed and revised the initial draft of the manuscript.

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Availability of data and materials

Data will be available upon request

Declarations

Ethical approval and consent to participate

The surgical procedure was adapted and refined from a validated procedure used in lab (APAFIS #33624-2021102712475018 v6). The first experiment was supervised by a veterinarian, conducted in agreement with the local authority (DAVAR), and the specific laws (in particular Lp. 240-2 and Lp. 243-4 from the country law n° 2017-12 from the 23rd of august 2017).

Conflict of Interest statement

The authors declare no competing interests.

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Figures, table and video captions

Fig. 1 Schematic representation of the interactions between sleep and wake showing the theoretical benefits and the costs of sleep on animal fitness (survival and reproduction).

Fig. 2 Black rats *(Rattus rattus*) trapped in 2023 (Left). Mesh cages constructed on different capture sites (middle and right).

Fig. 3 (a) Anesthetized black rat after the implantation of the logger on the stereotactical frame. (b) Sleep logger connected on the EIB with the flexible electrode. (c) Live recordings of the EEG of a black rat in its cage, collected with Bluetooth on a tablet. (d) Rat carrying the sleep logger in its cage. (e) Rat equipped with the sleep logger over the head, eating in its cage. (f) 10 minutes of recording (muscle tone extracted from the EMG, brain activity from the parietal site represented in time frequency and head movements extracted from the accelerometer) during NREM sleep (red), REM sleep (green) and active wake (blue), showing the typical brain signatures of the different vigilant states (delta, sigma and theta activity), as the muscle atonia typical from REM sleep (right).

Fig. 4 (a) hypnogram representing the evolution of the sleep states across the recording days. Because not all days were complete and scored, and because we scheduled the logger after the 8/06 to record one day every two days there is some missing values (b) Evolution of the wake and sleep states quantities per hour (wake–blue; NREM sleep–red; REM sleep–green) showing that the sleep state quantities stabilized two days after the surgery. (c) Number of wake, NREM, and REM sleep bouts per hour; showing a more fragmented sleep the first days after the surgery. (d) Illustration of the signal collected over 60 seconds during each vigilant state, from the top to the bottom: EMG; Parietal EEG represented in normalized color-coded time frequency (Blue=low spectral power, red= high spectral power); EEG frontal; 3-axis acceleration in; 3-axis angular speed collected from the gyroscope.

Fig. 5 Evolution of the daily quantity (a), number of episodes (b) and mean episode duration (c) of NREM (a-c), and REM sleep (d-f). The percentage of sleep (NREM plus REM sleep) (g) and the relative quantity of NREM (h) and REM (i) sleep. Each animal is color-coded. The red circles indicate days that the animals were manipulated in the previous 24hours (surgery; data retrieval).

Fig. 6 Timeline of the experiments showing the evolution of the weather conditions including precipitation (Top, in black the average hourly precipitation, in blue the average daily precipitation), the temperature (middle) and the humidity (bottom) collected from la Tountouta weather station (Lat: -22.0173°, Long: 166.222333°, Alt: 37m) which is at 18km from the recording site. For temperature and humidity, the black line is the mean, the blue is the minimal and the red line the maximal value over an hour. The animal status (color-coded) was evaluated periodically until the end of the experiment the 5th of July. Animal 1 to 3 underwent surgery whereas animal 4 was our control.

Table 1 Comparative table of sleep recordings using EEG conducted on wild species in natural environments (semi captive conditions and wild) and associated methodologies.

Additional file 1: Video 1. A wild black rat, equipped with its sleep logger, in its experimental cage. The video shows the animal and the corresponding signal during a transition from wake to sleep (NREM and REM sleep). From the top to the bottom: Live video of a black rat in its experimental cage recorded in infra rad below a color-coded hypnogram (blue is wake, red is NREM sleep, Green is REM sleep, Cyan is freezing), EMG signal (filtered with a high pass filter at 10Hz order 10), parietal EEG Left signal (filtered with a high pass filter at 1Hz order 10), Frontal left EEG signal represented in a color-coded time frequency (Blue: low spectral power, Yellow: high spectral power); ambient temperature recorded from the thermistor on the logger; Axis acceleration. The video accelerated 5 times, shows a 30 sec windows with the vertical black line in the middle corresponding to the current image.

Additional file 1: Video 2. A wild black rat, equipped with its sleep logger, in its experimental cage, jumping, running climbing and eating to the food dispenser. The signals are the same as in the Video 1 and the video, also accelerated 5 times, shows a 30 sec windows with the vertical black line in the middle corresponding to the current image.

Additional file 1: Video 3. A wild black rat, equipped with its sleep logger, in its experimental cage interacting with two conspecifics at night. The signals are the same as in the Video 1 and the video, also accelerated 5 times, shows a 30 sec windows with the vertical black line in the middle corresponding to the current image.

Additional file 1: Video 4. A wild black rat, equipped with its sleep logger, in its experimental cage interacting, showing a freezing state just before and during our intervention to install the food dispenser and fulfil its bottom of water the day after the surgery. This video show at what point inactivity could not be consider at sleep especially when the animals are exposed to a proximal danger. The signals are the same as in the Video 1 and the video, also accelerated 5 times, shows a 30 sec windows with the vertical black line in the middle corresponding to the current image.