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Long-term synchronized electrophysiological and behavioral wireless monitoring of freely moving animals

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Abstract

Parallel electrophysiological recording and behavioral monitoring of freely moving animals is essential for a better understanding of the neural mechanisms underlying behavior. In this paper we describe a novel wireless recording technique, which is capable of synchronously recording *in vivo* multichannel electrophysiological (LFP, MUA, EOG, EMG) and activity data (accelerometer, video) from freely moving cats. The method is based on the integration of commercially available components into a simple monitoring system and is complete with accelerometers and the needed signal processing tools. LFP activities of freely moving group-housed cats were recorded from multiple intracortical areas and from the hippocampus. EMG, EOG, accelerometer and video were simultaneously acquired with LFP activities 24-h a day for 3 months. These recordings confirm the possibility of using our wireless method for 24-h long-term monitoring of neurophysiological and behavioral data of freely moving experimental animals such as cats, ferrets, rabbits and other large animals.

Keywords

Electrophysiology; movement; accelerometer; sleep/wake

Introduction

A better understanding of the correlation between certain patterns of neural network activities and the corresponding behavior is necessary but it is limited by the available recording technologies. A simple behavioral experiment investigating activity changes during transition between states of vigilance can be carried out on head-restrained animals (Timofeev et al., 2001), which do not allow for a natural investigation of behavior. Electrophysiological recordings from animals placed into small chambers gives more opportunities for uncovering the relation between behavioral patterns and neural activities (Buzsaki, 2010). However with wired recordings the animals are not absolutely freely moving (Lashgari et al., 2012; Marlinski et al., 2012; Wolansky et al., 2006). In contrast,

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wireless recordings from freely moving animals can provide substantial new electrophysiological data, acquired in various behavioral conditions, which cannot be investigated in the same species in head-restrained or tethered recording conditions (Eliades and Wang, 2008; Roy and Wang, 2012). Modern wireless systems enable recordings up to 64 channels, but even with heavy batteries, the maximal recording time is limited to 6 hours (Szuts et al., 2011).

The wireless monitoring system described in this paper was developed to record electrophysiological signals and behavioral patterns from freely moving cats in a controlled laboratory environment. Previous studies conducted on cats in isolated small size chambers demonstrated a very fragmented pattern of sleep (Bowersox et al., 1984; Lancel et al., 1991; Ruckebusch and Gaujoux, 1976; Tobler and Scherschlicht, 1990). It was unclear to what extent these experimental conditions influenced the exact pattern of sleep-wake alterations in these species. The proposed system can be used to answer this question.

The described wireless system records multichannel neurophysiological data from various cortical and deep brain areas, acquires other electrophysiological signals such as electrooculogram (EOG) and electromyogram (EMG). It also records animals' daily movement activities with two accelerometers in addition to a video camera. Furthermore, we developed a signal processing method; enabling to report the activity and inactivity time a cat spends in each hour of the 24 -h periods.

The combination of commercially available and custom-built components created a lightweight system, allowing continuous 24-hour recording of electrographic and movement activities.

Methods

Animals and electrode implantation

All experiments were conducted according to the protocol approved by the animal care committee of Laval University. Laboratory adult male cats were housed in colonies under a standardized environment. The employees had limited access to the room (floor: 230 × 300 cm; height: 250 cm) (Fig. 1A). On weekdays, cleaning the room, playing with the cats and providing ample food and water for fulfilling the ad libitum needs were carried out between 09.00–10.00 h and 15.00–16.00 h. On weekends, the same tasks were accomplished once a day between 11.30–12.30 h. The room was illuminated on a 12-h light-12-h dim schedule (light: 07.00–19.00 h, dim: 19.00–07.00 h), and kept at 22°C. Laboratory cats (n=4) were implanted with cortical, deep brain, EMG and EOG electrodes under sterile surgical conditions. The anesthesia was initiated by the IV administration of the mixture of ketamine (15 mg/kg), buprenorphine (0.01 mg/kg), acepromazine (0.3 mg/kg) and glycopyrrolate (0.011 mg/kg). For preventing the chance of infection, cefazolin (50 mg/kg) antibiotic was injected. For post-operational recovery anafen (2 mg/kg, 2–3 times for 3 days), baytril (5 mg/kg, 1 time for 7 days), metoclopramine (0.4 mg/kg) and cyproheptadine were given for the animals. The associative (AC), visual (VC), somatosensory (SS) and motor (MC) cortices were implanted with electrodes, which were fabricated from 200 µm in diameter Teflon coated stainless steel wires (A-M systems, Sequim, WA, USA). The electrodes were inserted 1 mm deep in the cortex through the dura mater with manipulators. A coaxial bipolar stainless steel electrode (FHC, Bowdoin, ME, USA) was inserted into the hippocampus according to stereotaxic coordinates (A 4, L 5.5, H 8). The holes on the cranium, around the implanted probes, were filled with a quick drying silicone elastomer (Kwik-Sil, World Precision Instruments, Sarasota, FL, USA). The silastic layer minimizes tissue growth on the dura, prevents the leakage of cerebrospinal fluid and provides stability to the implanted electrodes for long-term recordings (Roy and Wang, 2012). Silver wires

were implanted into the frontal (over frontal sinus, close to cribriform plate) and occipital bones (over cerebellum) around the midline for reference. The EOG was acquired with two silver ball electrodes ($d=380\text{ }\mu\text{m}$, A-M system), which were inserted into the holes drilled in the inferior surface of orbital plate of frontal bone over the superior and lateral extents of the eye. The EMG was recorded with Teflon coated stainless steel electrodes ($d=250\text{ }\mu\text{m}$, Sigmund Cohn Corp., Mount Vernon, USA) implanted into the neck muscles on each side. Ten-twelve stainless steel screws were screwed into the cranium on both sides and a 3–5 mm thick dental cement layer was applied for covering the cranium in order to provide stable fixation for electrodes and the recording system. A 7 mm thick Teflon plate ($d=25.5\text{ mm}$, SmallParts, Logansport, IN, USA) was fabricated and placed on the dried dental cement over the midline of the skull. An Omnetics connector (Omnetics Connector Corp., Minneapolis, MN) was embedded to the plate, which was protected by a 30 mm high, cut piece of a plastic syringe (60 cc/ml, Terumo Medical, Somerset, NJ, USA) (Fig. 1B). The bottom part of the acetal lid of the protective housing included a low-power, 3-axis accelerometer (ADXL-330, Analog Devices, Norwood, MA, USA). The bandwidth of that accelerometer was limited to 0–50 Hz by connecting $C_x=C_y=C_z=0.1\text{ }\mu\text{F}$ capacitors to the 3 output channels of the accelerometer (Fig. 1C). $R_1=R_2=R_3=499\text{ k}\Omega$ and $R_4=R_5=R_6=1\text{ k}\Omega$ were connected to the circuit in order to reduce the amplitude of the original output signals. To prevent the amplifier from saturation, the gain was set to a small value (1/500) because the input range of the wireless headstage transmitter was 4 mV V_{p-p} . All leads were connected to the Omnetics connector. The different signals were transmitted by a telemetry system (NeuroWare W-series, Triangle Biosystems Inc., Durham, NC, USA), which was plugged to the Omnetics connector and protected by the housing. A small sized and high energy density battery (LS14250, Saft, Bagnole, France), which was mounted on top of the lid of the protective housing, provided energy for the wireless head-stage for 72 hours. A standard battery (CR1632, Panasonic Corp., Secaucus, NJ, USA), placed on the external side of the protective housing, supplied energy for the accelerometer on the bottom of the lid. A dental acrylic cap was built around the protective housing for stabilizing the implant in place (Fig. 1B). A commercial web camera (Agama V-1325R, Genius KYE Systems Corp., Taipei, Taiwan) was mounted on the wall of the animal room in order to provide behavioral data synchronized with the electrophysiological recordings. Continuous accelerometer, video and electrophysiological recordings were carried out for up to 3 months. In addition to the head accelerometer, the movement activity patterns of laboratory cats ($n=4$) were recorded (40 Hz sampling rate) with a small size, USB equipped 3-axis accelerometer (X6-2 mini, GCDC Data Concepts, Waveland, MS). It was placed into a small plastic box, which was attached to a harness and worn on the back of the animal (Fig. 1A). The accelerometer was fixed in place inside the box preventing false detection of movements. It was replaced every day (replacement time less than 30 seconds) and the replacement was carried out during the scheduled entrance times.

Data sampling and analysis

Over a two-week period the cats were adapted to the animal room and to wearing the harness. The wireless data recording started immediately at the end of the surgery. LFP, EOG, EMG and the head accelerometer signal were pre-amplified, filtered (0.8 Hz–7 kHz, -3 dB), and wirelessly transmitted by the 15-channel headstage. The data was then sampled with the PowerLab data acquisition system (PL3516, AD instruments, Colorado Springs, CO, USA) at 1 kHz for 3 months 24-h a day. In some experiments we implanted high impedance (5–7 M Ω) tungsten electrodes (FHC, Bowdoin, ME, USA), to record extracellular spiking activity. In this case the sampling rate was set to 20 kHz. The corresponding and synchronized video (30 fps) recording was achieved with the same system through the Labchart software (Labchart 7.3, AD instruments, Colorado Springs, CO, USA). The recording system with the connected laptop (Tecra R840, Toshiba, Irvine,

CA, USA) was placed into a cart with closed doors inside the animal room (Fig. 1A). The data transfer occurred at the same time as animal handling.

We developed a Matlab (Mathworks Inc., Natick, MA, USA) script that allowed to: (a) cut the midnight-to-midnight 24-h accelerometer signal from two consecutive recordings, (b) calculate the derivative of the vector sum of the 24-h x-, y- and z-axis data (40 Hz sampling rate) and (c) sum the values in each consecutive seconds. By running this script we obtained the 24-h movement data of a cat with a second precision.

The recorded signals were copied from the hard drive of the acquisition laptop to external hard drives every day. Filtering the LFPs (0.5–500 Hz), the MUA (0.3–10 kHz), the EMGs (100–500 Hz), the EOGs (0.5–300 Hz) and the head accelerometer signal (0.5–50 Hz) as well as the FFT analysis of the 24-h electrophysiological data was carried out with the LabChart software.

Manual sleep scoring was carried out according to the standard criteria (Ursin, 1971). The polygraphic data was scored for 30-s epochs by analyzing the cortical and hippocampal LFPs, the LFP power densities of the delta and theta ranges, and the video recordings. Video recording was performed only during the light period.

Results

The implanted (n=4) cats provided synchronized electrophysiological and behavioral data 24-h a day for months. The continuous recordings allowed assessing distinct behavioral patterns and associating those with electrophysiological features. We investigated the natural sleep-wake cycle of freely moving cats, however the dataset includes many other behavioral patterns, as interaction with other cats or information on sensory and motor processing.

A signal segment from several cortical, hippocampal, EOG, EMG and accelerometer channels are shown on Figure 2A. The distinct features of the natural sleep-wake cycle (Wake, REM and SWS) were scored. In agreement with previous descriptions (Ursin, 1971), the wake state was characterized by high frequency and low-amplitude LFP activities on each channel. The muscular tone (EMG recordings) and eye movements (EOG recordings) depended on the activity level. REM was characterized by hippocampal theta, rapid eye movements and absent EMG activity except occasional twitches. SWS was characterized by slow waves in cortical and the hippocampal LFP recordings, inactive EOG, and decreased and stable amplitude EMG activities. Figure 2A shows simultaneously appearing changes of activity recorded with accelerometers and the EMG. Occasionally, when the animal voluntarily moved to the metal cage located within the recording room, the connection between the wireless emitter and receiver was temporally lost, but recovered on all channels within 30 sec. The back accelerometer provided artifact-free data on the daily behavior of the laboratory cats.

Using high impedance electrodes enabled recordings of spiking activities of neurons (Fig. 2B). As expected in these recordings, the neuronal firing was associated with depth-negative deflections of the LFP. Because the electrodes were fixed, the ability to detect spiking activities generally deteriorated within 7–10 days from the end of the surgery, but occasionally could be recorded 3 months after the implantation of electrodes. The quality of the LFP recordings was stable over long periods of time. To quantify the quality of recordings we computed standard deviation of signal during activated LFP states (waking) for several months. Over the investigated period of time, the standard deviation of signal did not show any trend for an increase or decrease, suggesting stable quality of recordings (Fig. 1D).

Figure 2C shows that during the 24 hours the cat has multiple alterations of active and inactive periods (bottom, red). Based on the cortical and hippocampal LFPs, their delta and theta frequency range analysis, the EOG and EMG signals as well as the video recording we assessed the 24-h hypnograms of freely moving laboratory cats. Superposition of processed accelerometer signal with the hypnogram clearly demonstrates that only waking state was associated with movements of the animal while movement activities were absent during both slow-wave and REM sleep stages. Muscle twitches during REM sleep were detected with the accelerometers.

Discussion

Here we present a new system that enables simultaneous wireless recording of electrographic and movement activities of cats. With the option of the battery that we used, an uninterrupted recording lasted for up to 72 hours. The system provides LFP data, multiunit activities and activity measured with accelerometers. Because we used fixed electrodes, which likely were encapsulated after several days, the quality of recording of unit activities deteriorated with time. Using simple portable manipulators (Kloosterman et al., 2009) can dramatically increase the duration of unit recordings.

Our study was done on cats. An identical system can be used on other laboratory animals of similar size: rabbits, ferrets, monkeys etc. Smaller animals (e.g. rats) can also be monitored, however the battery for these animals has to be smaller, which will significantly limit the duration of continuous electrographic recordings. The use of large accelerometers (e.g. X6-2 mini, GCDC data concepts) on smaller animals is impractical, because of their size combined with the amplifier, battery, electrodes etc. will definitely disturb the normal behavior of the animals. However, the accelerometer described in Figure 1B and related text, can be used on animals as small as mice.

Multi-electrode recordings from freely moving rodents and large-bodied animals are becoming more and more widespread (Buzsaki, 2010). The current interest is to investigate electrographic activities during particular behavioral states, which are minimally disturbed by experimental settings. Wireless transmission systems coupled with video recordings provide essential tools for these kinds of experiments. However, if quantitative assessment of a particular behavioral task is needed and this type of behavior occurs rarely, then the experimenter has to manually scan multiple hours of recordings to identify a few of these particular states. Multiple forms of behavior are accompanied with particular forms of muscle contractions, which may be recorded with accelerometers and analyzed automatically. Here we demonstrate a good correspondence of accelerometer data with detection of sleep and waking states (Fig. 2A). The other forms of behavior (walking, chewing, drinking, scratching, seizure activity etc.) can be recorded and automatically detected with the described accelerometer. We have not analyzed these behavioral patterns yet, but a simple observation of recorded data indicates that these behavioral patterns produce distinct and consistent signal. We conclude that the described system is a useful tool to investigate uninterrupted behavior of animals of a size comparable with cats.

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Non-standard abbreviations

AC	Associative cortex
ACC	Accelerometer
EMG	Electromyogram
EOG	Electrooculogram
FFT	Fast Fourier Transform
FHC	Frederick Hear Corporation
GCDC	Gulf Coast Data Concepts
HC	Hippocampus
LFP	Local Field Potential
MC	Motor cortex
MUA	Multi-Unit Activity
R	Right
REM	Rapid Eye Movement
SWS	Slow Wave Sleep

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Highlights

- We developed a system for parallel electrophysiological, video and movement recording
- The system enables 24-hours/day long-term recordings from freely moving animals
- The system was used on cats to collect data on sleep/wake activities
- The system can be used to study unrestrained behavior of laboratory animals

Figure 1. Experimental approach for parallel wireless electrophysiological and movement recordings

(A) The wireless recording environment. (B) The cross-sectional view of the head showing spatial arrangement, fixation and protection of the implanted electrodes, wireless amplifier, custom-made accelerometer, and batteries. (C) The schematic diagram of the accelerometer. (D) Sample of 5 sec LFP traces collected during waking state in different days (left) and standard deviation of LFP traces collected over 72 days (right). Red dots on right panel correspond to traces on the left panel.

Figure 2. Synchronous electrographic and accelerometer recordings during three states of vigilance of an unrestrained cat

(A) Synchronized Electroencephalogram (LFP), Electrooculogram (EOG), Electromyogram (EMG) and accelerometer (Acc. I and Acc. II) recordings during the different states of the natural sleep-wake cycle of a freely moving laboratory cat. The states of vigilance are indicated at the top. LFP was recorded from two cortical areas [motor cortex (MC), associative cortex (AC)] and the hippocampus (HC). The accelerometer I was mounted on the back and the accelerometer II on the head. Note the parallel increase in EMG and accelerometer activities. Signal segments highlighted with grey and marked with stars (REM, WAKE, SWS) on the central figure are expanded below. A period of active waking is shown in the right panel. The back accelerometer signal has arbitrary unit. The threshold (red line) separates inactivity from activity. (B) LFP (black) and MUA (blue) recorded from the right MC during REM, WAKE and SWS. The shown recording was obtained from permanently implanted electrodes 90 days after the surgery. (C) The 24-h accelerometer signal (red), the hypnogram (black) and the hourly percentage of Wake (blue), REM (green) and SWS (yellow) states based on manual scoring. Note that the hypnogram and the

processed accelerometer signal have very similar patterns. Horizontal bars with stars on top indicate the regulated entry times of personnel.