Radiotelemetry recording of electroencephalogram in piglets during rest

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Abstract

A wireless recording system was developed to study the electroencephalogram (EEG) in unrestrained, male Landrace piglets. Under general anesthesia, ball-tipped silver/silver chloride electrodes for EEG recording were implanted onto the dura matter of the parietal and frontal cortex of the piglets. A pair of miniature preamplifiers and transmitters was then mounted on the surface of the skull. To examine whether other bioelectrical activities interfere with the EEG measurements, an electrocardiogram (ECG) or electromyogram (EMG) of the neck was simultaneously recorded with the EEG. Next, wire electrodes for recording movement of the eyelid were implanted with EEG electrodes, and EEG and eyelid movements were simultaneously measured. Power spectral analysis using a Fast Fourier Transformation (FFT) algorithm indicates that EEG was successfully recorded in unrestrained piglets, at rest, during the daytime in the absence of interference from ECG, EMG or eyelid movements. These data indicate the feasibility of using our radiotelemetry system for measurement of EEG under these conditions.

Keywords: Radiotelemetry; Electroencephalogram (EEG); Fast Fourier Transformation (FFT); Power spectra; Eyelid movement; Landrace piglets

1. Introduction

The electroencephalogram (EEG) reflects spontaneous cerebral cortical activity. The amplitude and rhythm of the EEG is greatly altered in a characteristic manner dependent upon the state of consciousness [1,2]. EEG methodology has been extensively used in both scientific study and clinical diagnosis. For instance, EEG recording and analysis has been performed to investigate the influence of pharmaceutical drugs [3,12–14,36], the effects of chronic stress on sleep [4], the relation of EEG activity to behavior in the normally behaving monkey [11], as well as to diagnose disorders or diseases of the brain [5–10]. Thus, EEG recording and analysis has contributed to the understanding of brain function in man and animals.

When EEG activity is measured via standard techniques through a wired system, animals are constrained by tethering to lead wires or must wear backpacks attached to a frame. By use of a hard-wired recording system, the duration and frequency of EEG recording sessions, as well as opportunities to simultaneously monitor behavioral parameters, are restricted. Previous studies have been performed using hard-wired methodology to record EEG signals in the pig [15–19], but most of the EEG measurements were performed while the animals were quiet, sleeping, or immobilized. In pigs, long-term EEG recordings are rare and are difficult to perform using a hard-wired system since pigs are sensitive to restraint [20–26] and are expected to be physically active during long-term measurement. Thus, it is desirable to develop a recording technique in which long-term EEG data can be collected in unrestrained conditions for this animal.

Recent technical developments have markedly increased the opportunity to develop radiotelemetry monitoring of body temperature, blood pressure, electrical signals (for example,
electrocardiogram (ECG), EEG, and electromyogram (EMG)), and locomotor activity in monkey and laboratory animals [11,27–33]. Radiotelemetry systems are thought to be essential in recording EEG and other electrical signals in animals without the confounding influence of restraint stress. Previous studies have been published regarding radiotelemetry recording in the lamb [34] and chicken [35]. However, to our knowledge, the system has not been developed for pigs. In this study, we develop a radiotelemetry system for recording of EEG signals in pigs. The system was tested in unrestrained Landrace piglets during the daytime at rest.

2. Materials and methods

2.1. Animal preparation

Eleven male Landrace piglets (Sus scrofa domesticus) weighing between 12 and 15 kg (at 5–6 weeks of age) were used in this study. All animals received Humane Care as described in the guide for the Care and Use of Experimental Animals (National Institute of Agrobiological Science Care Committee). The pigs were housed in a cage (1.2 m × 0.8 m) in a shielded room at 23 °C, 60 % in humidity, light 6:00–18:00 h, dark 18:00–6:00 h for about 1 week prior to use. All surgical interventions and experiments were performed in Zootron, National Institute of Livestock and Grassland Sciences, Tsukuba.

2.2. Surgery

2.2.1. Implanting electrodes and mounting miniature telemetry devices

Pigs were fasted overnight but were allowed ad libitum access to water prior to surgical procedures. All surgical procedures were carried out using sterile technique during which respiration, body temperature and ECG was continuously monitored. Immediately prior to surgery, animals were sedated by intramuscular injection of xylazine (2 mg/kg; Bayer, Tokyo) and midazolam (0.5 mg/kg; Yamanouchi Pharmaceuticals, Tokyo). Once a deep plane of anesthesia was achieved through inhalation of 3–4% halothane and 0.5–1.0 l/min nitrous oxide, animals were placed in a handmade holder that supported the head. The sagittal and coronal sutures of the skull were exposed through a midline incision from a dorsal approach and the landmark bregma noted. The electrode positions were marked on the skull over the frontal, parietal, and occipital cortices. Holes were manually drilled completely through the bone at each location with care taken not to penetrate the dura matter. The recording and reference electrodes (ball-tipped silver/silver chloride electrodes; open circles) were symmetrically implanted across the sagittal or coronal sutures between 0.8 and 1.2 cm and covered the frontal and parietal cortices. EEG was measured differentially between LR and LF (LR-LF) and between RR and RF (RR-RF). The position of the ground electrode (Lambda) was shown by head arrow and G. Closed circles indicate the bilateral positioning of implanted wire electrodes for recording eyelid movement.

A pair of miniature telemetry devices (modified DTT 101, each 1 cm × 0.8 cm, Diamedical System, Tokyo), which amplify and relay EEG signals to the receiver, was also mounted on the skull. The specification of the device was as follows: impedance (input resistance) 1 MΩ, amplification 1000 times, frequency 1 Hz–10 kHz, weight 10 g, single channel, sending frequency 75–105 MHz. The thin cables from the electrodes were connected to input-pins of the devices. The devices and all connectors were sealed and packed into a handmade plastic housing. The devices and all connectors were sealed and packed into a handmade plastic housing. Finally, the housing of the devices were carefully fixed with anchor screws and dental cement. A protector was fixed on the skull. An antibiotic (Mycillin sol, synthetic penicillin, 1–1.5 ml; Meiji Seika, Tokyo) were administered within 30 min of beginning surgical procedures.

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Four pigs were additionally instrumented with electrodes for recording the ECG (EEG + ECG) by attachment of surface electrodes to the skin of the left chest and back. In three other piglets, stainless steel wire EMG electrodes were bilaterally implanted to the dorsal musculature of the neck (EEG + EMG). Finally, in four separate piglets, fine wire electrodes were bilaterally sewed in the skin of the upper eyelids to record movement of the eyelids (EM) after implanting the muscular antibiotics (Mycillin sol, synthetic penicillin, 1–1.5 ml; Meiji Seika, Tokyo) were administered within 30 min of beginning surgical procedures.
EEG electrodes (EEG+EM; Fig. 1). Reference electrodes were sutured to the outer corners of the eyes. For amplification and relay of the ECG, EMG or eyelid movement signals, a second battery-powered miniature telemetry device (DTT 201, Diamedical System, Tokyo) was attached to the back. Specifications of the telemetry device were similar to that of the EEG recording device except for the bandwidth of frequency 0.3 Hz–20 kHz, and lower amplification capacity (100 times).

Following surgical procedures, animals were kept in the surgical room and given inhaled oxygen until they awakened. Once sternal and alert, the animals were then transferred to their cages in the shielded room. The animals received intramuscular injection of antibiotics for 4–5 days after operation and were observed for an additional 1–2 days before recordings.

2.3. ECG, EEG and EMG recordings

The pigs were placed inside a Faraday cage to reduce 50-Hz interference within the shielded room. Recording equipment for ECG, EEG and EMG signals were arranged outside of the Faraday cage. Electrical signals were detected with antennae and transmitted to receivers (time constant 0.3 s, high-cut filter off) through cables. The signals were further magnified with a conventional amplifier (AB610J, Nihon Koden, Tokyo; bandpass filter 0.08–100 Hz). The 50-Hz notch filters were not used. The signals were then stored onto a digital audio recorder (DAT 1204, Sony, Tokyo) for subsequent off-line analysis. A video camera and monitor were used to provide the experimenter with a view of the animal’s behavior during the recording period. The EEG was differentially recorded during the daytime postprandial period from 10:00 to 12:00 h, a time during which animals commonly fall asleep. After recordings, the animals were sedated by intramuscular injection of ketamine (5 mg/kg body weight; Sankyo, Tokyo) and midazolam (0.5 mg/kg body weight; Yamanouchi Pharmaceuticals, Tokyo) and then sacrificed by a rapid intravenous injection of overdose of sodium pentobarbital (50–100 mg/kg body weight, Abbott Laboratories, North Chicago, IL). The surface of the dura matter was examined for any lesions that may have been sustained during recordings.

2.4. Data analysis

The data were reproduced and digitized at a sampling rate of 500 Hz. Power spectra were obtained by calculation using a Fast Fourier Transformation (FFT) algorithm in epochs of 4 s (2048 points) from 0 to 250 Hz, using commercial software packages (WorkBench, Data Wave Technologies, Longmont, CO; Eight Star and brain wave analysis, Star Medical, Tokyo). The data were shown as a compressed spectral array, if necessary. The transformed data of the EEG were then divided up into the frequency bands of, delta (1–3.9 Hz), theta (4–7.9 Hz), alpha (8–12.9 Hz) and beta (14.1–25 Hz), and the value of the relative power (%) for each wave was obtained at each 4 s.

3. Results

The present data were obtained 7–10 days after surgical operations.

Fig. 2. (A) Example of EEG and ECG records obtained from a piglet while lying at rest. EEG was bilaterally recorded from frontal–parietal electrodes. The traces in (A) started at the point shown by the arrowhead in (B). The ECG activity was recorded by means of the A–B lead. R shows the R-wave in the ECG. (B) The compressed spectral arrays of the EEG and ECG recordings. Magnification of each spectral array is ×1 (arbitrary unit).
Fig. 2A illustrates a typical trace of EEG and ECG simultaneously recorded from a piglet at rest (EEG + ECG). The amplitude of the raw waves of the EEG was less than 150 μV. Most notably, EEG tracings revealed no apparent peak (notch) corresponding to the R-wave of the ECG. The respective power spectral changes by FFT analysis of the EEG and ECG are shown in Fig. 2B. The power spectral array of EEG activity shows the appearance of slow waves with the delta and theta frequencies and replacement of these slow waves with the alpha or beta frequency. In contrast, each power spectrum of the ECG revealed a nearly identical pattern corresponding to the rhythmic heartbeats. The rhythm of the R-wave of the ECG was approximately 2 Hz. Thus, the spectral array of the EEG is quite different from that of the ECG in both the pattern and time course of each other. Successful recording of the EEG in the absence of cardiac electrical artifacts was performed in all four piglets of this group.

Fig. 3. (A) Example of EEG and EMG records obtained from a piglet while lying at rest. EEG was bilaterally recorded from frontal–parietal electrodes. The EMG activity was recorded from the dorsal cervical musculature. The traces in (A) started at the point shown by the arrowhead in (B). (B) The compressed spectral arrays of the EEG and EMG recordings. Magnification of each spectral array is ×1 (arbitrary unit).

Fig. 4. Example of EEG recording in the absence of eyelid movement obtained from a piglet while lying at rest. Relative power of each activity (delta, theta, alpha and beta) are concomitantly calculated and shown at the bottom of each panel.
Power spectral analysis of simultaneous EEG and EMG recordings of the neck was performed in a separate group of piglets (EEG + EMG, n = 3). Fig. 3A illustrates a typical trace of the EEG and EMG simultaneously recorded from a piglet while lying at rest. In this posture, subtle EMG activity was recorded, but there was no visible change in the power spectral array (Fig. 3B). However, temporal changes in the EEG rhythm were obtained (Fig. 3B). In the lying posture, the EMG further diminished and was negligible (data not shown). Further examination of the data was performed in order to determine if eyelid movement (EM) interferes with the EEG recordings (EEG + EM, n = 3) while the piglets were lying down, since blinking is known to cause large artifact in the EEG trace. Since rest periods varied among piglets, recording times ranged from 30 min to 90 min during a 2-h observation period. Figs. 4 and 5 illustrate typical tracings of EEG activity and eyelid movement simultaneously measured in a piglet. In the absence of eyelid movement, slow waves with a large amplitude appeared in the EEG (Fig. 4). In this case, the power of the delta and theta activities became larger than the alpha and beta. When eyelid movement did occur, faster
waves with small amplitude were recorded. There was no visible artifact in the EEG trace (Fig. 5). While the eyelid movement was present, the power of the alpha and beta activities was stronger than others. Through power spectral analysis, spectral changes of the EEG were observed independent of those from eyelid movement (Fig. 6). Thus, data indicate that successful recording of the EEG can be achieved free from eyelid movement artifact.

4. Discussions

In the present study, we developed a wireless EEG recording system for use in unrestrained, male Landrace piglets. By using this system, we demonstrated the ability to successfully record EEG activity, free from artifact, in the presence of other bioelectrical signals in the piglets, while lying at rest.

4.1. Transmitters

We initially considered implantable, miniature radio-telemetry devices for use in the piglets in this study. The implantable transmitter is commercially available and has the advantage of reducing surgical damage or wounds to the animals [29]. However, we felt the implantable transmitters available for small laboratory animals might be inadequate for use in the piglets, due to the short range of transmission (20–30 cm). The transmitter we used in the present study has enough transmission range for sending the EEG, ECG and EMG activity from the unrestrained piglets to the antennae in the experimental room. The transmitter is reusable and can be processed to appropriate modifications for other uses including that of an implantable one. By use of the present transmitter, the EEG signals from the piglets can be collected for up to 4 weeks. Since the transmitter has enough capacity to send the signals within the room, the EEG of larger animals can be lead by rearrangement of the equipment for adequate husbandry and measurement.

4.2. Evaluation of artifacts in the EEG signals

In the present study, prior to collecting data, we examined whether EEG could be adequately recorded without interference from bioelectrical activity produced from cardiac or skeletal muscle in the piglets. Raw EEG traces shown in Figs. 2–5 show no evidence of recorded ECG, neck EMG or the mechanical movement of the eyelids. Power spectra of the EEG had quite a different pattern from those of the ECG, neck EMG and eyelid movement of the piglets (Figs. 2B, 3B and 6). The amplitude of the cardiac and skeletal muscle electrical activity was approximately 1 mV and greater; however, that of the EEG was less than 150 μV (Figs. 2–5). It is expected that when higher amplitude electrical signals such as those from cardiac and skeletal muscle penetrate into the EEG trace, large peaks may appear in the power spectra as well as visible marks in the raw trace, corresponding with those of the ECG or EMG. The present study indicates that the EEG signals were successfully recorded without disturbance from the cardiac and neck muscle electrical activity, or eyelid movements while the piglets were lying down.

4.3. EEG activities in the piglets during rest

Data recordings indicate that the delta and theta activities became dominant in the absence of eyelid movement in the piglets (Fig. 4). Larger power of the delta and theta activities in the EEG has been recorded during sleep in piglets (1–2 weeks of age) [15] and pigs (3–4 months of age) as well as infant monkeys [11]. These activities were replaced with faster, alpha and beta activity once eyelid movement appeared (Fig. 5), suggesting arousal while the animals were at lying at rest. The frequency profiles of the EEG with or without the eyelid movement in this situation seem to be very similar to those recorded during arousal or sleep as described in previous studies [15,16].

Sleep stages have been classified according to the frequency and amplitude of the EEG, the presence or absence of eye movement on the electrooculogram (EOG) and neck muscle electrical activity [1,2]. The trace of eyelid movement in the present study mainly reflects mechanical movement, but may not represent the EOG itself. To classify the sleep stages, simultaneous measurement of the EEG, EOG, and neck EMG activity is required. This would be feasible since the present system can be adapted for simultaneous measurement of these types of bioelectrical signals by increasing the number of recording channels or by enabling the device to perform multi-channel processing.

In conclusion, in the current study, we are the first to describe the use of a custom-designed radiotelemetry system to record cortical electrical activity in unrestrained Landrace piglets during rest. By use of our system, EEG activity can be recorded in a wireless manner, in the absence of artificial interference from ECG, neck EMG and eyelid movements. The present system can most likely be adapted to record EEG activity in piglets in studies investigating the effects of sleep, restraint stress and physiological–behavioral correlates. However, careful examination of recorded data is necessary in order to collect reliable EEG activity during behavioral changes.

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