Measurement of Hippocampal Neural Activity by Radiotelemetry in Unrestrained Piglets

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Abstract
We modified a wireless system to measure hippocampal neural activity in pigs, and tested a handmade microdrive for adjusting the tip of the electrode. Under general anesthesia with halothane inhalation we stereotaxically placed tungsten electrodes (5 MΩ) fitted with handmade microdrives in the temporal hippocampus of male Landrace piglets, and fixed a high-input-resistance transmitter (10 MΩ; 1000-fold amplification) to the skull. Oscillation and saturation were evident in the recording system for several days after surgery. When these phenomena ceased, we successfully recorded the hippocampal electrical activity in four of eight piglets. At 5 or 6 days following surgery, hippocampal electrical activity <0.15 mV in amplitude was observed in resting piglets during the daytime. In this recording, delta (1.0–3.9 Hz) and theta (4.0–7.9 Hz) waves with large amplitude were frequently predominant. However, these activities often alternated with high-frequency and low-amplitude activity, even while piglets were lying down. Manipulation of the microdrive enabled us to reposition the electrodes in the hippocampus 1 week after surgery. This technical development maintains the electrode within the hippocampus to enable neuronal activity with behavioral correlates to be determined in unrestrained piglets.

Discipline: Animal Industry
Additional key words: delta, fast Fourier transformation, Sus scrofa domesticus, theta

Introduction

Pigs often explore an unfamiliar environment by looking, biting, and sniffing as they move around to recognize new surroundings. In addition, they show a high ability at learning the relation between sensory cues and the manipulation of tools for the intake of rewards (associative learning)6,8.

The hippocampus plays a prominent role in memory storage following learning2,9. A study of the hippocampal region of the domestic pig (Sus scrofa domesticus)5 indicates that the temporal pole is larger than the septal pole, and that the highly laminated dentate hilus shows a clear resemblance to that of primates. However, the neural basis underlying the processes of sensing, recognition, learning, and memory in pigs remains unclear.

To reveal the neurophysiological properties of the pig hippocampus, researchers have developed several stereotaxic instruments and atlases of the coordinates of the hippocampus and other brain regions3,10,11. There have been several attempts to use hard-wired systems to re-
cord electrical activity in the pig brain\textsuperscript{1, 4}. However, no such system has yet been developed for the simultaneous recording of neural activity and behavioral changes.

An alternative technique is radiotelemetry, which allows neural activity during behavioral changes to be recorded in animals without the confounding stress of restraint.

In this study, we used our wireless technique\textsuperscript{11} to record hippocampal electrical activities in unrestrained piglets, and tested a microdrive made to adjust the tip of the electrode.

Materials and methods

1. Animal preparation

Seventeen male Landrace piglets weighing between 12 and 15 kg (5–6 weeks old) were used. All animals received humane care as described in the guide for the Care and Use of Experimental Animals of the National Institute of Agrobiological Sciences’ Care Committee. Each piglet was housed and cared for as reported previously\textsuperscript{11}. All surgical interventions and experiments were performed in the Zootron at the National Institute of Livestock and Grassland Science, Tsukuba, Japan.

Piglets were fasted overnight before surgery, but were allowed \textit{ad libitum} access to water. Immediately before the surgery, piglets were sedated by an i.m. injection of xylazine (2 mg/kg) with midazolam (0.5 mg/kg), and then deeply anesthetized by inhalation of 2 % to 3 % halothane and 0.5 to 1.0 L/min nitrous oxide\textsuperscript{11}.

2. Determination of the hippocampal stereotaxic coordinates

We re-examined the stereotaxic coordinates of the dentate area of the temporal hippocampus, which receives olfactory input via the amygdala, in nine piglets. A tungsten electrode (AC impedance 5 MΩ; A-M Systems, Carlsborg, WA, USA) was stereotaxically inserted, and an electrical lesion was made in the temporal hippocampus of the anesthetized piglets at the coordinates (AP–5, H30, L15), which we determined in previous work\textsuperscript{10}, and at new coordinates (AP–4, H30, L14) or (AP–2, H28, L13.5). At the latter coordinates, we found electrical lesion in the dentate area in Nissl-stained sections in preliminary work. Therefore, we used the coordinates (AP–2, H28, L13.5) in targeting the dentate area of the temporal hippocampus.

3. Surgical procedures

1. Transmitter

A radio transmitter (DTT-101, Dia-medical System, Tokyo, Japan) was modified to have high input resistance (10 MΩ) (Fig. 1A). Other specifications and weights were as reported previously\textsuperscript{11}.

2. Microdrive unit

We constructed a microdrive unit (working distance 6–8 mm) from commercially available materials, based on a previous design\textsuperscript{7} (Figs. 1B, C).

3. Implantation of recording electrodes and mounting of transmitter

Before surgery, eight animals were sedated and then deeply anesthetized as described above. After i.m. administration of an antibiotic (synthetic penicillin, 1–1.5 mL), the piglets were secured in the stereotaxic instrument as previously described\textsuperscript{10}. The bregma was the original reference point for the coordinates. Bone wax was applied to the skull to prevent bleeding during surgery. Stainless steel guide cannulae were implanted into each hole at coordinates (AP–2, L13.5) through the dura mater and fixed using dental cement with anchor bolts. After a tiny incision near the lambda, a ground electrode (Ag-AgCl\textsubscript{2}) was inserted under the dura mater.

Tungsten electrodes with microdrives (Figs. 1B, C) were bilaterally and stereotaxically inserted through the guide cannulae into the temporal hippocampus. On the left side, the electrode was fixed at the coordinates (AP–2, H25–28, L13.5), where active neuronal firing was detected on an oscilloscope and by audible output through a speaker. The reference tungsten electrode was placed at the same depth as the recording electrode at coordinates (AP–2, L5). The electrode and adjacent anchor screws were then fixed with dental cement. On the right side, the electrodes were implanted at the same coordinates, but without audible monitoring. The cables of the electrodes and a pair of batteries (each 1.5V, 120 mAh; LR6) were connected to the input pins of the transmitter (Fig. 1A). The transmitter in the housing and all connectors were then carefully fixed to the skull with anchor screws and a plastic protector with dental cement (Fig. 1D). Synthetic penicillin was topically applied to the wound margin. Finally, the batteries were fixed with bandages to the back of the piglet.

4. Recording procedure

Electrical activity was differentially recorded in a Faraday cage\textsuperscript{11} between 10:00 and 12:00, during which time animals are commonly found lying down. During the measurements, an observer recorded the behavior of each animal.

5. Data analysis

The data were digitized at a sampling rate of 10 kHz in the commercial software package WorkBench (Data Wave Technologies, Longmont, CO, USA). The numeric
portions of the data were analyzed by using a fast Fourier transformation (FFT) algorithm from the commercial software packages Labview for Windows (v. 5.0; National Instruments Japan, Tokyo, Japan) and Eight Star and Brain Wave Analysis (Star Medical, Tokyo, Japan).

6. Histochemical identification of electrode position

After the recordings, the animals were deeply anesthetized as described above. A negative electrical current (0.2 mA, 30 s) was applied to each electrode to mark its position. The heads were then perfused with formalin solution (pH 7.4) \(^{10}\). Finally, we identified the locations of the electrical lesions in the Nissl-stained sections as described previously\(^ {10}\).

Results

1. Recording hippocampal electrical activity by radiotelemetry

The electrical activity of the left temporal hippocampus was successfully recorded at the new coordinates (AP–2, H28, L13.5) in four of the eight piglets while they were lying down. In the remaining four piglets, measurement was not performed or was interrupted, because either the animals did not recover from surgery or the protectors and cables were broken during the recovery period.

Among the four successful measurements, marked oscillation and saturation (Fig. 2) were evident for the first several days after implantation in three piglets. These phenomena ceased within the next few days within 4 or 5 days after implantation. No measurement was performed until the oscillation and saturation had ceased.

Examples of the electrical activity of the left temporal hippocampus are shown in Figure 3. The activity was recorded in three piglets while they were lying down. FFT analysis of the raw waves revealed large peaks in the power spectra at frequencies lower than 10 Hz, indicating that slow waves with delta rhythm (1.0–3.9 Hz) and theta rhythm (4.0–7.9 Hz) were predominant in these record-

Fig. 1. Experimental equipments

A: Photograph of the transmitter. B: The process for constructing the microdrive unit with the electrode. C: Photograph of the microdrive. Scale bar represents 1 cm. D: Complete assembly of the electrodes with the microdrives (m, and m\(_2\)), transmitters in housings (h, and h\(_2\)), and a protector (p) for bilateral recordings. Asterisks (*) represent antennae from the transmitters. (F) Frontal view, (L) lateral view.
In addition, spectral peaks were present at higher frequencies ranging from 10 to 20 Hz (Fig. 3A). The electrical lesion produced at the recording electrode was located near the neural cell layer facing the fimbria or in the dentate area (Figs. 3A–C). The lesion produced by the reference electrode on the left side was in the thalamic region or the optic tectum. From one piglet, no lesion produced by the reference electrode could be identified in the sections. The lesion from the recording electrode was found outside the right temporal hippocampus proper.

Figures 4A and B show typical traces of hippocampal electrical activity recorded at coordinates (AP–2, H28, L13.5) while the piglet was lying down. In this measurement, no oscillation or saturation was observed immediately after surgery. At 1 day after surgery, electrical activity with high amplitude and low frequency (<10 Hz; delta and theta) was observed (Fig. 4A(1)). The amplitude of the raw waves was <0.15 mV. In contrast, electrical activity with higher frequency (10–25 Hz) appeared 5 days after surgery (Fig. 4A(2)). The tip of the recording electrode was raised by 0.4 mm by turning a screw under light anesthesia (xylazine injection), and the polarity of the electrical activity reversed (Fig. 4B).

At 1 week after surgery, electrical activity with low frequency and high amplitude often alternated with that of high frequency and low amplitude, even when the piglet was lying down (Figs. 5A, B). Lesions produced after the recordings shown in Figs. 4B, 5A, and 5B were found in the dentate area (Fig. 5C).

Discussion

This study presents a stereotaxic approach to examining the hippocampus of piglets larger than those used in our previous study\(^{10}\), and the development of a wireless technique for recording the hippocampal electrical activity in unrestrained piglets.

1. Predetermined coordinates of the hippocampus based on external skull landmarks

We successfully performed a stereotaxic examination in the larger piglets and using predetermined coordinates based on the external skull structure. Thus, coordinates for the hippocampus can be determined from external skull landmarks when the animals are of the same strain, sex, age, and weight. However, the depth to the temporal hippocampus from the bregma was widely variable, ranging from 28 to 35 mm, when compared with the range from a lateral position of 13.0 to 13.5 mm and an anterior–posterior position of –2 mm to –4 mm from the bregma. A combination of predetermined coordinates and audible monitoring successfully enabled the precise placement of electrodes into the temporal hippocampus of the piglets.

2. Oscillation and saturation in the recording system

Oscillation and saturation in the recording system were evident for several days after surgery in three of the four animals. Possible reasons for these phenomena include the high input resistance (5 MΩ) of the electrode, large amplification (1000-fold) in the transmitter, and less cerebrospinal fluid (CSF) around the electrodes owing to loss during surgery. These phenomena, however, declined and ceased within another a few days, possibly as a result of a gradual decrease in input resistance of the electrode during the recovery period, since the resistance of the electrode was <5 MΩ (1–3 MΩ) at the end of the experiment. In addition, the ground electrode is completely immersed in the CSF and may be electrically stable when the subarachnoidal space becomes filled again with CSF. An alternative way to prevent oscillation and saturation is to lower the degree of amplification in the transmitter: no oscillation and saturation were observed in recordings immediately after surgery with an amplification smaller than 100-fold in the transmitter (Fujiwara and Saito, unpub-
Fig. 3. Examples of electrical activity (raw wave) recorded in the left temporal hippocampus (top) and FFT power spectrum through a Hanning window (bottom) in 3 piglets lying down.

Recordings were performed at 6 or 7 days after surgery. Arrows show the lesions at the tip of the recording electrode. Bars represent 5 mm.
Fig. 4. Changes in the hippocampal electrical activity by manipulating the microdrive
A: Example of electrical activity in the hippocampus of a piglet (1) at 1 day and (2) at 5 days after surgery. The screw of the microdrive has not been turned. B: The tip of the electrode was raised 0.4 mm by turning the screw 1 day after the recording in A (2). Vertical and horizontal scale bars represent 0.5 mV and 0.1 s, respectively.

Fig. 5. Changes in the electrical activity of the hippocampus (A) and compressed power spectral arrays (B; arbitrary units) calculated every 1.28 s (256 data) in a piglet that was lying down.
The raw wave (A) was observed between 38.3 and 41.6 s from the beginning of measurement. The slow wave diminished and was replaced with a faster wave (B) at 40 to 41 s (dashed line), in which the peak lower than 10 Hz transiently declined in the power spectra indicated with open stars. (C) The arrow indicates the lesion at the tip of the recording electrode. Vertical and horizontal scale bars represent 0.5 mV and 0.1 s, respectively.
lished observation), although waveform discrimination was reduced. In a previous study\(^1\), no large oscillation and saturation were observed in electroencephalogram (EEG) measurements using ball-tipped Ag-AgCl\(_2\) electrodes, which have lower resistance than the tungsten, in unrestrained piglets.

3. Use of microdrive for measuring hippocampal electrical activity in unrestrained piglets

This study shows that our handmade microdrive was successful in adjusting the tip of the electrode. Since piglets grow and develop rapidly following weaning, the coordinates of the hippocampus may alter with changes in skull and brain size. The temporal hippocampus is located at least from AP0 to AP–2 in Landrace piglets (~20 kg), but has a larger extension along the vertical axis at these AP levels (Saito, unpublished observation). The working distance (6–8 mm) of the microdrive was calculated from the distance between the dorsal CA3 layer and the bottom of the dentate area, and seems to be sufficient for covering this area vertically. It is possible to keep the electrode in place in the hippocampus for a short period such as 1 week without the aid of the microdrive (Figs. 3, 4A). However, for longer-term measurements, as performed in small animals\(^7\), our microdrive is probably necessary for adjusting the electrode in the pig hippocampus, since the value of the z-axis coordinate (H) becomes larger and changes relative to the x- (AP) and y- (ML) axes. Figure 4B showed the reverse of the polarity in the electrical activity from the temporal hippocampus by turning the screw at 6 days after surgery (see both Figs. 4A(2) and 4B), indicating that our microdrive can move and adjust the electrode in the temporal hippocampus at least within the first week after surgery. However, further examination of our device is needed for long-term use in pigs and for improvement of the automatic adjustment of the electrode.

4. Electrical activity recorded in the temporal hippocampus of unrestrained piglets

This study demonstrates that slow waves associated with the delta and theta rhythms occurred frequently in the hippocampus while the piglets were lying down at rest. The data strongly suggest that the neural mechanisms causing these hippocampal theta and delta rhythms exist in the brain of Landrace piglets, as reported in other small animals\(^5\,12\). Our observation is consistent with previous findings\(^9\) that indicated theta activity (~6 Hz) in the hippocampus in awake pigs determined by using a wired system. Even while piglets were lying down, the slow waves with delta and theta frequencies diminished in the hippocampus (Fig. 5). A previous study by Yamamoto\(^13\) demonstrated in the rabbit hippocampus that its EEG spectra show two peaks in the delta and theta frequency bands, the heights of which change competitively with each other in association with the level of consciousness: the delta-wave peak is higher and the theta-wave peak is lower during sleep, but the opposite during wakefulness. During rest, the heights of both the delta- and theta-wave peaks fall between those during sleep and wakefulness\(^13\). Therefore, transient decreases in the delta and theta powers in unrestrained piglets (Fig. 5) may reflect arousal over very short periods while the piglet is lying down.

In conclusion, this study provides information valuable for the development of techniques for measuring hippocampal neural activity by using a wireless system in Landrace piglets. The use of a microdrive enables the electrode to be maintained in the hippocampus and allows for the neuronal activity to be measured over a longer period, which could cover adaptive and non-adaptive behavioral changes to environments and emotional behaviors in unrestrained piglets.

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References


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