Video Article

Noninvasive EEG Recordings from Freely Moving Piglets

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Abstract

The method allows the recording of high-quality electroencephalograms (EEGs) from freely moving piglets directly in the pigpen. We use a one-channel telemetric electroencephalography system in combination with standard self-adhesive hydrogel electrodes. The piglets are calmed down without the use of sedatives. After their release into the pigpen, the piglets behave normally—they drink and sleep in the same cycle as their siblings. Their sleep phases are used for the EEG recordings.

Video Link

The video component of this article can be found at https://www.jove.com/video/58226/

Introduction

Piglets are an emerging model system for neuroscience[1]. In order to strengthen translational research, we invented a method to record non-invasive, clinical EEGs from unrestrained piglets[2] (**Figure 1** and **Figure 2**). Two prerequisites for a translational use of EEG recordings, regarding EEG patterns associated with cortical maturation, are a non-invasive methodology, comparable to the clinical setting, and the abstinence of sedatives or anesthesia. The one-channel telemetry system[3] in combination with self-adhesive electrodes can be fixed in about 5 min. Afterward, the piglets will recover quickly from the handling procedure and synchronize their feeding and sleeping behavior to that of the other piglets and the sow.

Even though there are already attempts to use non-invasive EEG recordings from sedated animals[4], most electroencephalography studies from animals are conducted with invasive approaches. These methods have side effects regarding inflammatory processes around the implanted electrodes[5,6] and, in most cases, they require a social separation of the animals due to the external components of the implanted EEG system. Hence, the translation of these data to the clinical context is difficult. The need for translational approaches is becoming clear by the fact that it is still not known how a "normal" brain maturation during the early cortical development is represented by clinical, non-invasive electroencephalography[7]. This knowledge gap is caused by technical challenges associated with EEG recordings from preterm babies[8]. In animal model systems, patterns of early cortical development are better accessible, since most animals are born with a "preterm brain" in comparison to human cortical development[9]. Besides conserved patterns of cortical development across species[2], it has recently been shown that EEG recordings from preterm babies can also predict the individual clinical outcome during later life[10,11]. The method described here is especially useful for the translational aspects of developmental neuroscience.

Protocol

All procedures were approved by the local ethics committee (#23177-07/G10-1-010/G 15-15-011) and followed the European and the German national regulations (European Communities Council Directive, 86/609/ECC; Tierschutzgesetz).

All animal procedures were performed in accordance with the Medical Center of the Johannes Gutenberg-University Mainz animal care committee's regulations.

1. Setup

1. Prior to the experiment, check for any line noise and find an adequate location for the set-up and the antenna. Line noise is visible as a 60 or 50 Hz sine wave.
   NOTE: The placement of the antenna and especially the distance between the transmitter and the receiver depends on the transmission strength of the system. The system used here is adjustable. It was adjusted to a relatively low power, with an approximately 3 m transmission

range. Additionally, the metallic fences in the pigpen can dampen the signal and cause interferences. In this case, it is necessary to place the antenna inside the metal cage.
2. Use a cable drum to supply the set-up with line power. Connect the laptop, the receiver unit, and the analog-to-digital converter (if necessary) for the specific telemetry system that is being used.
NOTE: The telemetry system used here sent digital data to the receiver. This might be different for other systems.
3. Place the electrodes, the adhesives, the Q-tips, and the wipes, as well as the mixing blocks, on a separate table.
4. Prepare the electrodes with short cables. To do so, cut the electrodes and solder them again with a length as short as possible, depending on the size of the animal. The cables must be long enough to connect the desired recording positions on the head with the telemetric EEG unit, transmitting the data. Cables that are too long must be recoiled and covered with skin adhesive silicone elastomer. Longer cables that must be recoiled make the silicone patch bigger and heavier.

2. Piglet

1. Catch a piglet by grabbing it at the leg or at the thorax. Hold it and be aware of any defecation or urination.
2. If necessary, mark the piglet with a number.
3. Wrap the piglet in a towel. The piglet will calm down. Be aware of overheating the piglet.
4. Hold the piglet with one hand at the body or forearm. Use the other hand to hold the snout. Be aware of overheating the piglet and make sure it is free to breathe properly.

3. Electrodes

1. Have a second person attach the electrodes.
2. Clean the skin from dirt with water or ethanol. If necessary, shave the head.
3. Remove any dead skin cells with an abrasive EEG gel and a Q-tip. Remove the abrasive gel afterward. Alternatively, use sandpaper.
4. Fix the self-adhesive electrodes at the desired location. Place the ground electrode above the cerebellum (between the ears) and the reference electrode on the nose. Place the recording electrode at the desired location.
NOTE: In this case, a unipolar recording was performed, because the reference was placed at a neutral position (nose). There is no standardized system available for piglets until now. Here, a parietal recording position was used (between the eye and the ear) on the right brain hemisphere.
5. Connect the cables to the telemetry unit. Turn the unit on. Depending on the telemetry system used, this might be a magnetic switch or a radio frequency wake-up signal.
6. Cover the telemetry unit and all cables as well as all electrodes with two-component skin adhesive silicone rubber (see **Table of Materials**). By mixing equal amounts of both components, the curing time will be in the range of 1 min. Eyes and eyelashes should not be covered with the rubber.
7. Wait until the silicone rubber is completely cured.
8. Place the piglet back in the pigpen.
9. Observe the piglet to see if it is showing signs of discomfort over a longer period of time (several minutes).

4. Measurement

1. Wait until the piglet has recovered and starts to synchronize its behavior with that of its siblings (feeding, playing, sleeping), usually after 30 s (**Figure 1**).
2. Wait for sleep phases, if desired. The recording time depends on the specific scientific question. Here, 10 min recording sessions were used.
3. If the telemetry unit is covered by more than 2 other piglets, the signal might be too low for the receiver. Gently push the piglets away if they are sleeping on top. Be aware of the sow; it might react aggressively.
4. Start the recording with the data acquisition software (see **Table of Materials**).

5. Finish

1. After the recording (usually several hours), catch the piglet again as has been described in step 2. Be aware of the sow; it might react aggressively.
2. Gently lift the silicone rubber at one edge. Then, remove the whole patch of silicone rubber containing the electrodes and the telemetry unit. Be careful with the piglet's eyes.
3. Place the piglet back in the pigpen.

Representative Results

We were able to record typical EEG patterns associated with non-REM sleep, like spindle bursts or delta brushes, from freely moving piglets (**Figure 1** and **Figure 2**). We were mostly interested in representative patterns during non-REM sleep, but phases of REM-like sleep[12] with a very low amplitude have also been recorded (**Figure 3**). The physiology and the amount of REM sleep differ across species[13]. Short REM phases in the range of a few minutes are typical for pigs[14]. Good recording quality was also available during feeding (lactation) (**Figure 4**). As for playing behavior, strong muscle activity leads to muscle artifacts; however, filters have been designed to extract EEG bands. Further possible analytical tools are calculations of power spectral density or analytical tools focusing on network activity, as, for example, phase-amplitude coupling. These analytical tools can also be used with single-channel EEG recordings.

Figure 1: A sleeping piglet with the telemetric EEG system. Please click here to view a larger version of this figure.

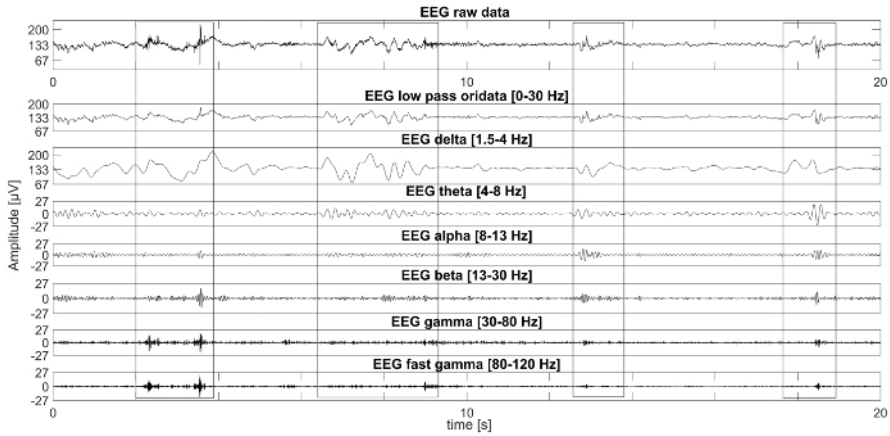

Figure 2: Typical EEG patterns recorded during the sleep phase of a freely moving piglet, resting beside its siblings. Four events are highlighted by boxes. From left to right, the first box shows typical patterns elicited by muscle activity; for example, short twitches during sleep, characterized by high amounts of fast gamma activity (above 80 Hz) and a burst-like appearance. The second box shows a delta brush-like episode, characterized by delta activity with superimposed activity in the theta and alpha range. The third and fourth boxes show short episodes of gamma bursts (sleep spindle-like events), characterized by frequency components in the alpha, beta, and gamma bands. Please click here to view a larger version of this figure.

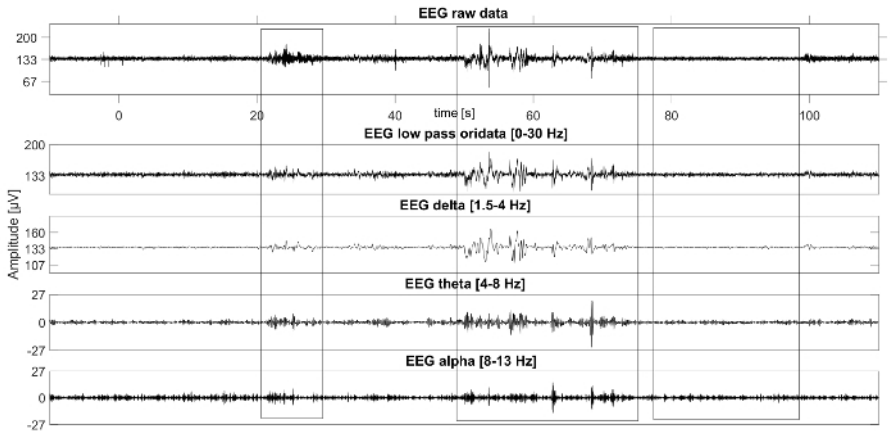

Figure 3: REM phases during sleep. The first and the second box show slow-wave sleep phases. The third box indicates a low-amplitude EEG phase of approximately 20 s in duration. Please click here to view a larger version of this figure.

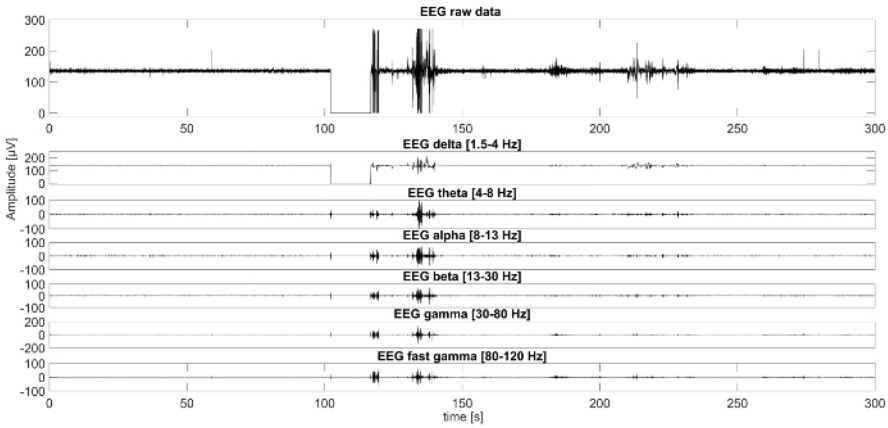

Figure 4: A 10 min EEG recording trace of a drinking piglet (lactation). The trough after 100 s is due to a short loss of radio communication between the transmitter and the receiver of the EEG recording system. Afterward, there are some muscle or movement artifacts. Muscle artifacts are characterized by very high delta band amplitudes. In contrast, EEG activity is characterized by a gradual decline of amplitude power from slow to fast waves (see, for example, between 200 and 250 s). Please click here to view a larger version of this figure.

Discussion

A critical step in the protocol is the adequate skin contact with the electrodes, especially the ground electrode, to achieve stable recordings with low noise. Furthermore, since piglets are very agile, it is important to cover the whole system with silicone rubber to protect the electrodes and the telemetry unit. Furthermore, if the experiments are conducted in a stable with a slatted floor, be cautious with small devices or connectors.

In case of an inadequate grip of the self-adhesive hydrogel electrodes, try to remove the abrasive cream as much as possible. An alternative way to remove the outer dead skin layer is using sandpaper. A pretreatment with alcohol is not mandatory. Dirty skin can also be cleaned with water. Do not use plaster strips instead of body double silicone to fix the telemetric system. Plaster strips can cause skin irritation and massive signs of discomfort. Piglets get rid of the system very fast in that case.

A limitation of the method is the stability of hydrogel electrodes. They may dry after a few hours, resulting in a loss of recording quality. Furthermore, as with all EEG techniques, strong movements are usually associated with muscle artifacts on the recording trace (as seen in **Figure 4**). Additionally, a good placement of the antenna is important to reduce artifacts which may result from a poor receiver signal. Metal fences can also cause high-frequency interferences, resulting in a loss of data sequences. A solution for this issue is to place the antenna inside the metal fence in an optimal position. The optimal position of the antenna can only be evaluated in the field by trial and error. The sow and the other piglets do not lead to artifacts, because the piglets wearing the system usually do not allow other piglets to manipulate their head. This might be different for other species.

The method is especially significant for translational approaches in neuroscience. With this technique, it is possible to use non-invasive recordings without sedation. The resulting EEG recordings are very similar to the clinical setting. The technique might open up new possibilities to characterize preterm EEG maturation by means of animal model systems. Beyond that, many neuroscientific questions, especially regarding cortical field potentials, might also be examined by this noninvasive technique. Hence, the method has the potential to reduce the number and severity of animal experiments in the field of neuroscience.

For the future, recordings with more than one electrode are planned. A prerequisite is the miniaturization of the electrodes. Furthermore, the long-term stability of the electrodes is an issue for future innovations.

Disclosures

The authors have nothing to disclose.

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