Video Article

Manipulation of Epileptiform Electrocorticograms (ECoGs) and Sleep in Rats and Mice by Acupuncture

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Abstract

Ancient Chinese literature has documented that acupuncture possesses efficient therapeutic effects on epilepsy and insomnia. There is, however, little research to reveal the possible mechanisms behind these effects. To investigate the effect of acupuncture on epilepsy and sleep, several issues need to be addressed. The first is to identify the acupoints, which correspond between humans, rats, and mice. Furthermore, the depth of insertion of the acupuncture needle, the degree of needle twist in manual needle acupuncture, and the stimulation parameters for electroacupuncture (EA) need to be determined. To evaluate the effects of acupuncture on epilepsy and sleep, a feasible model of epilepsy in rodents is required. We administer pilocarpine into the left central nucleus of the amygdala (CeA) to simulate focal temporal lobe epilepsy (TLE) in rats. Intraperitoneal (IP) injection of pilocarpine induces generalized epilepsy and status epilepticus (SE) in rats. Five IP injections of pentylenetetrazol (PTZ) with a one-day interval between each injection successfully induces spontaneous generalized epilepsy in mice. Recordings of electrocorticograms (ECoGs), electromyograms (EMGs), brain temperature, and locomotor activity are used for sleep analysis in rats, while ECoGs, EMGs, and locomotor activity are employed for sleep analysis in mice. ECoG electrodes are implanted into the frontal, parietal, and contralateral occipital cortices, and a thermistor is implanted above the cerebral cortex by stereotactic surgery. EMG electrodes are implanted into the neck muscles, and an infrared detector determines locomotor activity. The criteria for categorizing vigilance stages, including wakefulness, rapid eye movement (REM) sleep, and non-REM (NREM) sleep are based on information from ECoGs, EMGs, brain temperature, and locomotor activity. Detailed classification criteria are stated in the text.

Video Link

The video component of this article can be found at https://www.jove.com/video/54896/

Introduction

Epilepsy is a common neurological disorder in which recurrent seizures occur throughout a patient's lifespan. Most epileptic recurrences can be well-controlled by anti-epileptic drugs (AEDs). However, about 30% of epileptic patients develop refractory epilepsy[1]. Epilepsy causes sleep disturbances, which can further exacerbate recurrence. Evidence demonstrates that epilepsy may either disrupt sleep at night or may cause excessive daytime sleepiness[2,3]. Our previous studies further indicate that epilepsy occurring at zeitgeber time (ZT) 0, *i.e.*, the beginning of the light period in the light:dark cycle, decreases sleep; this is mediated by corticotropin-releasing hormone (CRH), a homeostatic factor. Epilepsy at ZT13 (the beginning of the dark period) enhances the expression of another homeostatic factor, interleukin-1 (IL-1), which increases sleep. Sleep circadian rhythms are altered when epilepsy occurs at ZT6, the middle of the light period[4,5]. On the other hand, sleep problems further exacerbate the progression and recurrence of epilepsy[6]. Based on the aforementioned evidence, we try to reveal an optimal therapeutic method to simultaneously control epilepsy and prevent sleep disruptions in epilepsy patients. We previously found that electroacupuncture (EA) with a 10-Hz stimulation frequency, in which a certain amount of current is delivered into the acupoint through a stainless-steel needle, successfully suppresses electrocorticogram (ECoG) epileptic activities and epilepsy-induced sleep disturbances[7]. EA with a 100-Hz stimulation frequency further deteriorates epileptic activities and sleep disruptions in rats[8,9]. This successful experiment depends on three factors: firstly, a feasible epileptic animal model; secondly, a method for sleep recording and analysis in rodents; and thirdly, the accurate performance of acupuncture and the accuracy of the acupoint locations.

Epilepsy has been categorized into two major types: focal epilepsy and generalized epilepsy. We are interested in focal temporal lobe epilepsy (TLE), generalized epilepsy, status epilepticus (SE), and the recurrence of spontaneous generalized epilepsy. Therefore, different manipulations are applied to create suitable epileptic models for our experiments. To establish focal TLE, a low dose of pilocarpine is administered into the left central nucleus of the amygdala (CeA). To verify this model, six ECoG electrodes are implanted on the frontal (F1 & F2), parietal (P1 & P2), and

occipital (O1 & O2) lobes in both the left and right hemispheres, and another two reference electrodes (R1 & R2) are placed over the cerebellum in both hemispheres. An additional microinjection guide cannula is surgically implanted into the left CeA (AP, 2.8 mm from bregma; ML, 4.2 mm; DV, 7.8 mm relative to bregma). The coordinates are adapted from the Paxinos and Watson rat atlas[10]. If the focal TLE is successfully induced, only the recording from the electrode on left parietal cortex (P1), which is near the left CeA, should acquire the dominant epileptiform ECoGs, with no significant epileptiform ECoGs recorded from the other ECoG electrodes. Intraperitoneal (IP) injections of pilocarpine into rats induce generalized epilepsy and SE, but this can be fatal. Five IP injections of pentylenetetrazol (PTZ) with a one-day interval between each injection successfully induce spontaneous generalized epilepsy in mice and also ensure the mice's survival. Two wire ECoG electrodes are implanted into the frontal and parietal cortices in the mice to receive ECoG signals and to verify spontaneously recurrent epilepsy.

Polysomnography (PSG) is a comprehensive method to record physiological changes that occur during sleep, and it can objectively classify sleep into different stages of non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. PSG records parameters of body functions, including brain waves (electroencephalogram, EEG), eye movements (electrooculogram, EOG), skeletal muscle tones (electromyogram, EMG), heart rhythms (electrocardiogram, ECG), and blood oxygen levels and respiratory parameters. In rats, we record ECoGs, EMGs, cortical temperature, and locomotor activity to classify vigilance states into wakefulness, NREM sleep, and REM sleep. Sleep analysis in mice is conducted using ECoGs, EMGs, and locomotor activity results. Rats are surgically implanted with three ECoG screw electrodes at the frontal, parietal, and contralateral cerebellar cortices by stereotaxic surgery. Post-acquisition determination of the vigilance states (wakefulness, NREM sleep, and REM sleep) is conducted according to the parameters acquired from the ECoGs, EMG, brain temperature, and locomotor activity. Detailed criteria for categorizing the animal's behavior in both rats and mice are described in the protocol.

Both rats and mice need to be anesthetized with a low dose of zoletil (25 mg/kg), which is half the dosage of anesthetics normally administered during stereotaxic surgery, before performing manual acupuncture or EA. This dosage allows animals to wake up 30 to 35 min after the injection. Either manual acupuncture or EA is performed at the beginning of the dark period, with a constant time period of 30 min, and each animal is consecutively treated for two to three days. Stimulating EA currents are delivered into a particular acupoint through a stainless-steel needle that is inserted into the acupoint. The stimulus current is a train of biphasic square pulses, in which the pulse duration is 150 ms and the stimulation intensity is 1 mA. If a dry needle is used for manual acupuncture, the needle inserted into the acupoints is twitched 10 times every 5 min. The difficult part of manual acupuncture or EA is to localize the acupoints in rodents. The location of acupoints in rats or mice is similar to their anatomical location in humans. For example, the bilateral Fengchi acupoints are located 3 mm away from the posterior median line on the neck, between the two ears, which is similar to its anatomical location in humans[11]. Furthermore, the acupoints with low impedance on the skin can be further confirmed. Sham acupuncture or sham EA manipulation is necessary for acupuncture or EA experiments. Sham acupuncture or sham EA should be performed at a non-acupoint located close to the acupoint, such as near the axilla[12].

To successfully investigate the effects of acupuncture or EA on epilepsy and epilepsy-induced sleep disruptions, the following factors must be in place: a feasible epileptic animal model, the precise analysis of epileptiform ECoGs and the recurrence of epilepsy, a method to classify vigilance states, and the accurate performance of acupuncture or EA in rodents.

Protocol

All experimental protocols are approved by the Institutional Animal Care and Use Committee (IACUC) of National Taiwan University.

1. Stereotaxic Surgery for Implanting ECoG Electrodes, EMG Electrodes, Brain Thermistor, and Injection Guide Cannula

1. **For rats (250 - 350 g, 6- to 8-week-old Sprague-Dawley rats)**
    1. Anesthetize the rats by IP injection with 50 mg/kg zoletil. Confirm the proper depth of anesthesia by observing a lack of response after pinching the hind paw. Apply eye ointment, shave the fur, and sterilize the skin with povidone-iodine solution and 75% ethanol. Inject an antibiotic (penicillin G) to prevent infection.
    2. Prepare scalpels, scissors, hemostats, gauzes, and cautery machine for surgery. Sterilize surgical gear and gauzes by an autoclave and the cautery by 75% ethanol.
    3. Place an ear bar into the ear canal and mount the rat to the stereotaxis.
    4. Using a scalpel, make an approximate 2-cm midline incision on the skull along a line between two the ears, moving caudally. Clip the skin flaps with hemostats to expose the skull and remove the tissue over the skull using a scalpel.
    5. Drill eight holes (F1, F2, P1, P2, O1, O2, R1, and R2), each approximately 0.7 mm in diameter, on the skull with a rotary tool. Screw eight ECoG electrodes on the frontal, parietal, and occipital lobes and the cerebellum in both the left and right hemispheres. These electrodes are used for focal epilepsy detection.
        1. Use the following coordinates for the recording electrodes: frontal (F1 and F2): +2.0 mm anterior to bregma and +2.5 mm from the midline, parietal (P1 and P2): -2.0 mm anterior to bregma and +3.0 mm from the midline, and occipital (O1 and O2): -5.5 mm anterior to bregma and +3.0 mm from the midline.
        2. Place two reference electrodes (R1 and R2) over the cerebellum (-11.0 mm anterior to bregma and +4.0 mm from the midline).
    6. In a separate groups of rats, drill three holes and place two screw EEG electrodes over the right frontal (F2) and parietal lobes (P2) of the cortices with the same coordinates as described in step 1.1.5.1. Place a third EEG electrode over the left cerebellum (R1), which serves to ground the animal and reduce signal artifacts. These electrodes are used for confirming generalized epilepsy and analyzing vigilance stages.
    7. Separate the neck skin and muscle and insert two EMG electrodes into the neck muscle.
    8. Drill another hole on the skull and place a microinjection guide cannula into the left CeA (AP, 2.8 mm from bregma; ML, 4.2 mm; DV, 7.8 mm relative to bregma) in rats. The coordinates are adapted from the Paxinos and Watson rat atlas[10].
    9. Drill a bigger hole (with a diameter of 1.6 mm) on the skull and insert a calibrated 30-kV thermistor on the surface of the parietal cortex, which will be cemented later, to monitor the cortical temperature in rats.

10. Use gauze and cautery to stop bleeding when it occurs.
11. Route the insulated leads from the ECoG electrodes and EMG electrodes to a pedestal and connect the thermistor to the tether. Cement the pedestal and guide cannula to the skull with dental acrylic.
12. Treat the incision topically with polysporin (bacitracin zinc/polymyxin B sulfate) to prevent infection. Give the animals both ibuprofen and penicillin G in water for one week after surgery.

2. **For mice (20 - 30 g, 6- to 8-week-old C57BL/C mice)**
    1. Anesthetize the mice by IP injection with 50 mg/kg zoletil and confirm the proper depth of anesthesia by observing a lack of response after pinching the hind paw. Apply eye ointment. After shaving the fur, sterilize the skin with povidone-iodine solution and 75% ethanol. Inject an antibiotic (penicillin G) to prevent infection.
    2. Place an ear bar into the ear canal and mount the mouse to the stereotaxis.
    3. Using a scalpel, make an approximate 1.5-cm midline incision on the skull along a line between the two ears, moving caudally. Clip the skin flaps with hemostats to expose the skull and remove the tissue over the skull with a scalpel.
    4. Poke two holes on the skull with surgical scissors and place two wire ECoG electrodes on the right frontal lobe (F2: +2.0 mm to bregma and +1.5 to the midline) and left parietal lobe (P1: -3.0 mm to bregma and -2.5 mm to the midline).
    5. Separate the neck skin and muscle and insert two EMG electrodes into the neck muscle.
    6. Connect the insulated leads from the wire ECoG electrodes and EMG electrodes to the female terminals and to a 2.54-mm connector, and then cement to the skull with dental acrylic.
    7. Treat the incision topically with polysporin (bacitracin zinc/polymyxin B sulfate) to prevent infection. Give the animals both ibuprofen and penicillin G in water for one week after surgery.

3. Allow all animals to recover for seven days prior to the initiation of the experiments.
4. House rats or mice separately, in individual recording cages, in the isolated room where the temperature is maintained at 23 ± 1 °C and the light:dark (L:D) rhythm is controlled in a 12:12-h cycle (40 W x 4 tubes illumination). Provide food and water *ad libitum*.
5. Connect the ECoG, EMG, and thermistor through a tether to the amplifiers one week after surgery to start the recordings.

2. Establishment of Focal TLE Epilepsy, SE, and Spontaneously Recurrent Epilepsy

1. **For rats**
    1. Administer 0.5 µL of pilocarpine (2.4 mg/µL) into the left CeA through the injection guide cannula using a microinjection pump. The injection rate should be set at 0.2 µL/min to induce focal TLE.
    2. IP inject 300 mg/kg of pilocarpine to induce generalized epilepsy with recurrent SE.

2. **For mice**
    1. IP administer PTZ (0.035 mg/g mouse bodyweight) at a particular ZT point every other day. Five consecutive injections will cause the development of spontaneously and recurrently generalized epilepsy.

3. **For both rats and mice**
    1. Amplify the ECoG signals by 5,000 and filter the analog bandpass between 0.1 and 40 Hz.
    2. Use an A/D converting board to convert the analog ECoGs signals to digital signals with a 128-Hz sampling rate.
    3. Use a software for visual scoring and analyze the onset and the duration of epilepsy. Measure the time scale in order to represent the duration.
    4. Define ECoG epilepsy by the appearance of epileptic spikes with amplitudes greater than 2 mV and with durations of more than 30 s[13].

3. Classification of Vigilance States

1. **For rats**
    1. Determine the vigilance states by using the parameters acquired from ECoGs, EMG, brain temperatures, and locomotion within a 12-s episode of recording. Connect the ECoG, EMG, and thermistor through a tether to the amplifiers one week after surgery to start the recording. Score the states with a custom-made software according to steps 3.1.5 - 3.1.7.
    2. Measure locomotor activities using an infrared motion detector, integrate the signals every 1 s, and store the signals.
    3. Measure cortical temperature and store the signals.
    4. Classify vigilance states according to our previously defined criteria[14].
    5. Score wakefulness by using the following characteristics: small-amplitude ECoGs with high-frequency spectra, higher delta power (0.5 - 4.0 Hz), and lower theta power (6.0 - 9.0 Hz); dominant locomotor activity; high EMG activity; and gradually increasing cortical temperature.
    6. Score NREM sleep by using the following characteristics: delta-wave dominant ECoGs with large amplitudes, declined EMG activity, reduced cortical temperature, and no locomotor activity.
    7. Score REM sleep by using the following characteristics: decreased amplitude of ECoGs with the dominant theta frequency, suddenly increased cortical temperature, minimal EMG activity, and low locomotor activity with body twitches.

2. **For mice**
    1. Repeat the classification of vigilance states in the mice as conducted in the rats, except there is no cortical temperature recorded from the thermistor.

4. Performance of Manual Acupuncture and EA in Rats

1. The rat is anesthetized for the EA protocol.
2. Locate the acupoint by anatomy and confirm low skin impedance at the acupoint. The light is flashing when detect the low skin impedance. Note: Fengchi acupoints are located 3 mm away from the posterior median line between the two ears, on the neck.
3. Insert stainless-steel needles into the acupoints at a depth of 2 mm.
4. Twitch the inserted needles 10 times every 5 min.
5. Using a functional electrical stimulator, deliver a train of biphasic pulses (150-μs duration each) with an intensity of 1 mA to the acupoints through the needle.
6. Perform a sham acupuncture or sham EA as a control.

Representative Results

There are different rat and mouse models to satisfy the needs of different epilepsy types. To induce focal TLE, 0.5 μL of pilocarpine (2.4 mg/μL) is administered into the left CeA. The predominant epileptiform ECoGs are acquired from the ECoG electrode on the parietal lobe of the left hemisphere (**Figure 1A: b**), and rare epileptic activities are picked up from the rest of the ECoG electrodes (**Figure 1A: a**, **c**, **d**, **e**, and **f**) when the pilocarpine is administered. Epileptiform ECoGs are primarily recorded immediately after pilocarpine administration. These results are adopted from [8]. These results indicate the successful induction of focal TLE in rats after the direct injection of low-dose pilocarpine into the CeA.

Within 5 min, the IP injection of 300 mg/kg pilocarpine induces severe cholinergic effects on behavior, such as piloerection, salivation, red eyes, shivering, and facial automatisms. The severity of these behavioral signs gradually increases until an ECoG generalized seizure occurs (**Figure 1B**, blue box). SE also occurs following the generalized epilepsy (**Figure 1B**, red box). These results are adopted from[13]. Our results suggest that an IP injection of 300 mg/kg pilocarpine is a reliable method to induce ECoG-documented generalized epilepsy and SE. However, the survival rate after SE development is between 15% and 20%. The PTZ-kindling model of epilepsy in mice is used to develop spontaneously and recurrently generalized epilepsy. PTZ at a dose of 0.035 mg/g mouse bodyweight is IP injected at a particular ZT point (*e.g.,* the beginning of the dark period, ZT13) every other day, and each injection is separated by a one-day interval. There are no epileptiform ECoGs found during the dark period after the 1st injection of PTZ (**Figure 1C: a**) or during the following dark period, when it is an off day for injection (**Figure 1C: b**). No significant epileptic activity is found after the 2nd, 3rd, and 4th injections (data not shown here). However, epileptiform ECoGs are induced after the 5th PTZ injection (**Figure 1C: c** and **d**), along with spontaneously and recurrently generalized epilepsy (**Figure 1C: e** and **a'**).

We classified vigilance states into wakefulness, NREM sleep, and REM sleep according to the criteria we mentioned in the protocol. Wakefulness is visually scored by desynchronized ECoGs with low amplitude and high frequency. The power density values in the delta frequency band (0.5 - 4 Hz) are generally greater than those in the theta frequency band (6 - 9 Hz) during wakefulness, and more high frequency spectra (> 10 Hz) are found. Wakefulness exhibits a high amplitude of EMG and lots of locomotor activity. Furthermore, cortical temperature gradually increases when the vigilance state transits from either NREM sleep or REM sleep to wakefulness (**Figure 2**). NREM sleep is characterized by synchronized ECoGs with high amplitude and low frequency. The power density values are dominant in the delta frequency band. The EMG amplitude gradually declines, and no locomotor activity is exhibited during NREM sleep. Cortical temperature decreases when the vigilance state transits from wakefulness into NREM sleep (**Figure 2**). During REM sleep, the ECoG wave is desynchronized, the amplitude is reduced, the predominant EEG power density occurs within the theta frequency (6.0 - 9.0 Hz), EMG activity is the lowest, phasic body twitch is observed, and cortical temperature rapidly increases (**Figure 2**).

We demonstrated the distinct effects of EA on epileptic activity when EA stimulates the Fengchi acupoints with either a high-frequency stimulation (100 Hz) or a low-frequency stimulation (10 Hz). In **Figure 3A**, a microinjection of 0.5 μL of pilocarpine (2.4 mg/μL) into the left CeA induces focal TLE, as mentioned previously (B). However, 100-Hz EA of bilateral Fengchi acupoints exacerbates the pilocarpine-induced epileptiform ECoGs (C). These results are adapted from [8]. In contrast, 10-Hz EA of bilateral Fengchi suppresses the pilocarpine-induced epileptiform ECoGs (**Figure 3B: C**). These results are adapted from [7]. Administration of pilocarpine into the left CeA also suppresses NREM sleep during the light period of the 12-h light:dark cycle (**Figure 4A** & **4B**). The reduction of NREM sleep during a few hours of the dark period is primarily due to the effect of anesthesia, and is not a direct effect of EA[15]. Application of 100-Hz EA further deteriorates the pilocarpine-induced suppression of NREM sleep (**Figure 4A**). In contrast, a 10-Hz EA of bilateral Fengchi acupoints increases NREM sleep *per se* during the dark period and blocks the pilocarpine-induced reduction of NREM sleep during the light period (**Figure 4B**). These results are adopted from [9].

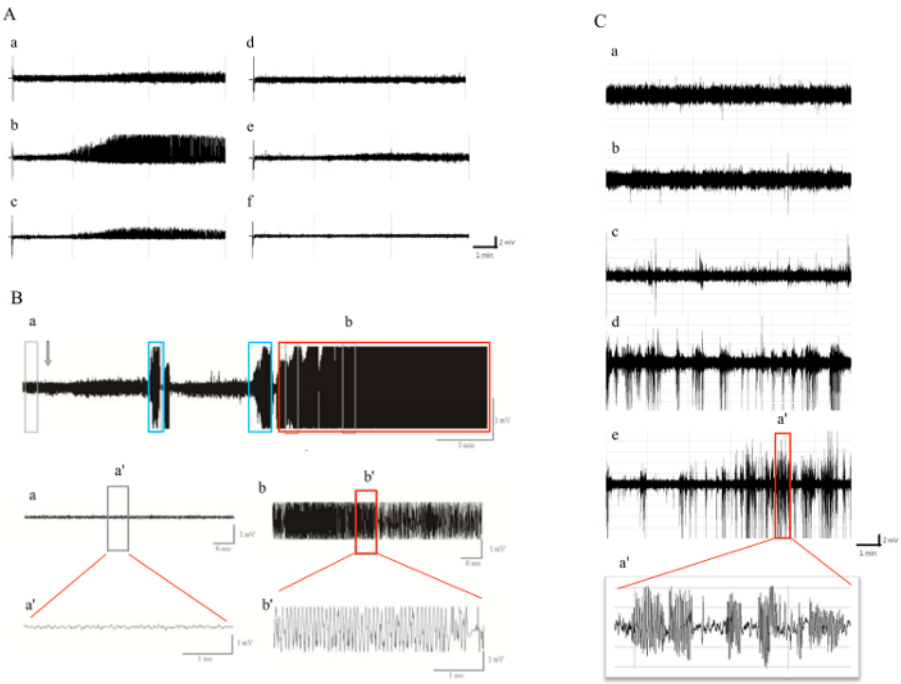

Figure 1: Different Epilepsy Models in Rats and Mice. A demonstrates the focal TLE in rats. **B** elucidates the generalized epilepsy with SE in rats. **C** indicates the spontaneous and recurrent epileptiform ECoGs. **(A)** (a), (b), (c), (d), (e), and (f) represent the ECoG signals recorded from the electrodes placed on the left frontal, left parietal, left occipital, right frontal, right parietal, and right occipital cortices, respectively. **(B)** The arrow indicates the IP injection of pilocarpine. The grey box (a) demonstrates the baseline ECoGs obtained before the pilocarpine injection. The blue box shows the epileptiform ECoGs of generalized epilepsy. The red box (b) represents the SE. The ECoG signals extracted from (a) and (b) are shown in (a') and (b'). **(C)** (a) represents the ECoGs acquired after the 1st PTZ injection and (b) represents the ECoGs recorded the day after the 1st PTZ injection in mice. Epileptic activities occur after the 5th PTZ injection (c) and afterwards. The recurrent epileptic ECoGs that occur the day after 5th PTZ injection are shown in (d). The recurrent epileptic ECoGs recorded 5 days after the 5th PTZ injection are shown in (e). The figure at the bottom (a') represents the ECoGs extracted from the red box. Please click here to view a larger version of this figure.

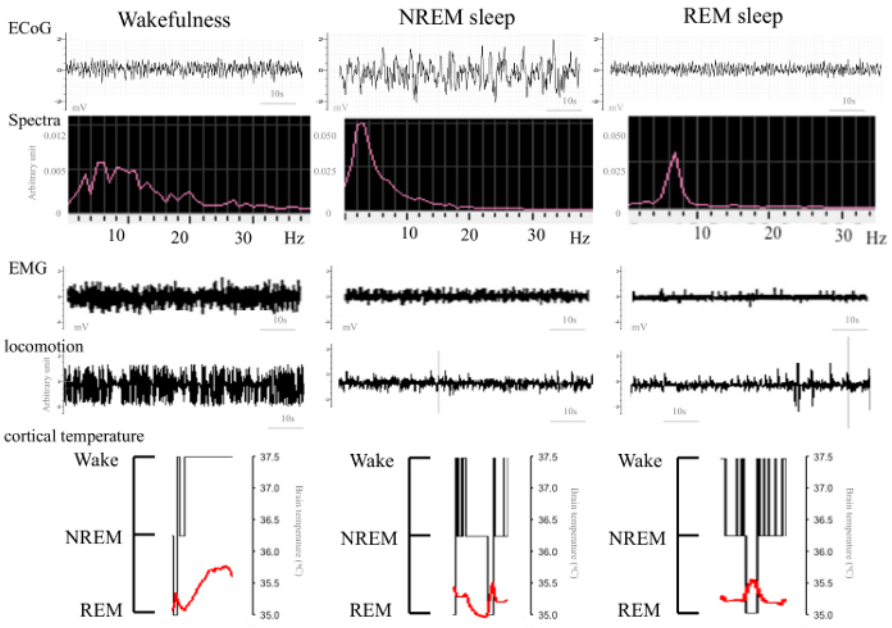

Figure 2: Classification of Vigilance States: Wakefulness, NREM Sleep, and REM Sleep. The vigilance states of rats are classified by the parameters from the ECoGs, ECoG spectra, EMGs, locomotor activity, and cortical temperature. The vigilance states of mice are characterized by the same parameters, except cortical temperature is not applicable.
Please click here to view a larger version of this figure.

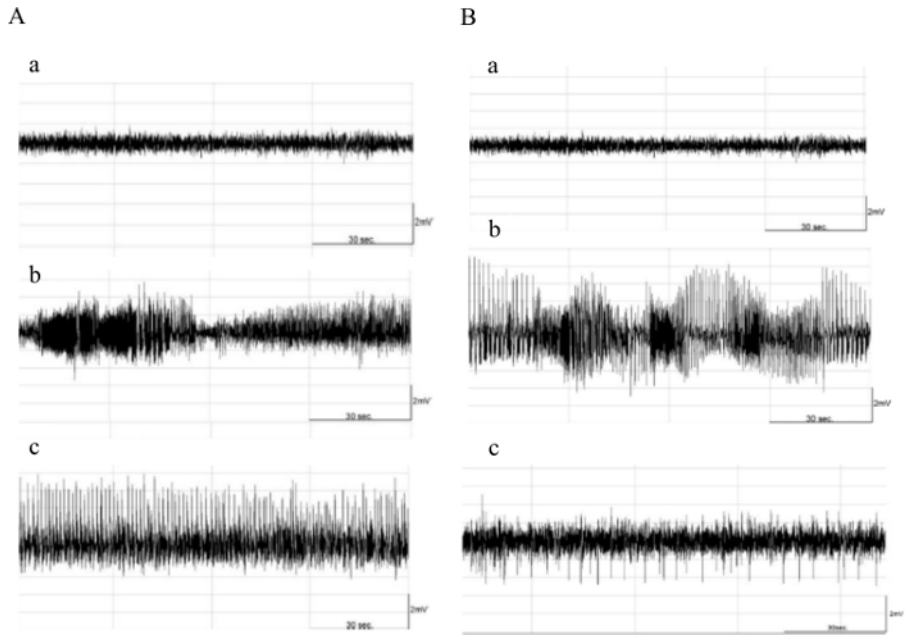

Figure 3: The Effects of 100-Hz and 10-Hz EA on Epilepsy. In **(A)**, the first ECoG trace demonstrates the baseline ECoGs (a). Microinjection of pilocarpine into the left CeA induces focal TLE (b). Application of 100-Hz EA at bilateral Fengchi acupoints exacerbates the epileptiform ECoGs (c). In **(B)**, the first ECoG trace demonstrates the baseline ECoGs (a). The microinjection of pilocarpine into the left CeA induces focal TLE (b). The application of 10-Hz EA at bilateral Fengchi acupoints suppresses the epileptiform ECoGs (c). Please click here to view a larger version of this figure.

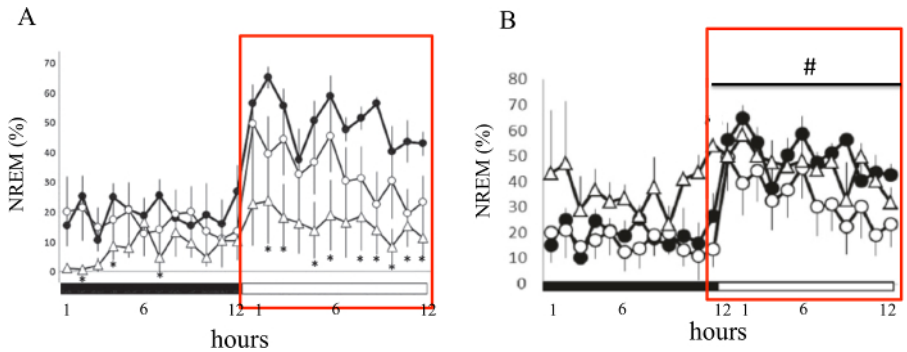

Figure 4: The Effects of 100-Hz and 10-Hz EA on Epilepsy-induced Sleep Disruptions. (A) Focal TLE induced by the microinjection of pilocarpine into the left CeA reduces NREM sleep during the light period. 100-Hz EA further deteriorates the epilepsy-induced sleep disturbance. **(B)** The application of 10-Hz EA blocks the epilepsy-induced reduction of NREM sleep during the light period. Furthermore, 10-Hz EA enhances NREM sleep during the dark period. Values are represented as mean ± SEM. The black circle represents the values obtained before the microinjection of pilocarpine, the open circle depicts the data acquired after the pilocarpine injection, and the open triangle demonstrates the values obtained after EA stimuli. The black bar represents the dark period of the 12-h light:dark cycle, and the white bar depicts the light period. * represents the statistical difference between the 100-Hz EA + pilocarpine group and the control (ANOVA, $P < 0.05$) and # indicates the statistical difference between the 10-Hz EA + pilocarpine group and the pilocarpine group (ANOVA, $p < 0.05$). Please click here to view a larger version of this figure.

Discussion

Choosing a feasible epilepsy animal model is essential for each experimental purpose. One of our goals is to elucidate the effects of EA on epilepsy suppression. EA is an alternative medicine that may exhibit therapeutic effect in epilepsy and has been documented in ancient Chinese literature. However, there is a lack of scientific evidence to prove it. To determine the effects of EA on epilepsy, we primarily focused on the effects of EA on mild focal epilepsy, rather than on severe generalized seizure or SE. Our previous study used a focal TLE model in rats to demonstrate the effects of low-frequency (10 Hz) and high-frequency (100 Hz) EA of bilateral Fengchi acupoints on pilocarpine-induced focal TLE and focal TLE-induced sleep disruptions[7-9]. Our results suggest that 10-Hz EA of bilateral Fengchi acupoints suppresses focal TLE and sleep disruption, whereas 100-Hz EA exacerbates both focal TLE and TLE-induced sleep disturbances. Another example is to demonstrate the effects of deep brain stimulation on refractory epilepsy, especially generalized seizure and SE. We determined that unilateral deep brain stimulation of the anterior nucleus of the thalamus (ANT) with high-frequency and low-intensity currents successfully inhibits the recurrence of generalized epilepsy and the duration of SE[13]. We also developed spontaneously and recurrently generalized epilepsy using the PTZ-kindling method in mice. This PTZ-kindling model can be widely applied to epilepsy studies. The critical step for establishing these epilepsy models is the use of the appropriate dosages. A microinjection of larger amounts of pilocarpine into the CeA may develop secondary generalized epilepsy. IP administration of more than 300 mg/kg pilocarpine induces generalized epilepsy and SE; however, most rats did not survive after the SE

developed. In addition, doses lower than 280 mg/kg could not successfully establish generalized epilepsy and SE. This same situation happens with the PTZ-kindling model. Larger doses of PTZ may cause SE, but many mice do not survive. In contrast, low doses of PTZ require more injections to establish spontaneously recurrent epilepsy.

The classification of different vigilance states in rats and mice is primarily based on features of polysomnography recorded from humans. We modified the protocol in order to analyze sleep-wake activity by the values recorded from ECoG, spectral distribution, EMG, locomotor activity, and cortical temperature. The characteristics of ECoG, spectral distribution, EMG, locomotor activity, and cortical temperature recorded from wakefulness and NREM sleep are similar to those acquired from humans. However, REM sleep is sometimes ambiguous and difficult to classify in rodents. Most literature describes REM sleep in rodents according to its ECoGs and EMGs. The theta frequency band of ECoGs is predominant and the muscle tone of EMG is lowest when rats or mice are in REM sleep. In order to increase the confidence of scoring REM sleep, we further recorded locomotor activity and cortical temperature. Phasic body twitch is observed and cortical temperature rapidly increases when rats enter REM sleep. Locomotor activity and cortical temperature, in addition to the ECoGs, spectral distribution, and EMGs, enhance the accuracy for REM sleep analysis. The more parameters that are included, the more precise classifications can be made.

Two main points have always been criticized when researchers perform manual acupuncture or EA experiments. The first issue is how researchers identify and confirm the acupoints in rats. The meridian system and acupoints in humans are well established, however there is no meridian system and acupoint map confirmed in rodents. Therefore, the localization of corresponding acupoints in rodents is determined according to the relative anatomical location. For example, Fengchi acupoints (GB 20) are located in the depression between the upper portion of the musculus sternocleidomastoideus and the musculus trapezius in humans. We found the related anatomical location in rodents and further confirmed it by measuring low skin impedance. The impedance of an acupoint is lower than that of the surrounding skin. The second issue is how the acupuncture doses are controlled during the experiment. If manual acupuncture is used for the experiment, the manipulation of the twitching needle should be performed by the same researcher in the same way for every manipulation. It would be easier to control the acupuncture dose if EA is applicable, as EA ensures a consistent stimulation frequency and current intensity. Furthermore, in analgesia, EA exhibits more effectiveness than manual acupuncture[16]. However, manual acupuncture and EA with low-frequency and high-frequency stimulation at the same acupoint may activate different brain regions[17], which may imply different underlying mechanisms.

In summary, this paper demonstrates several chemical-induced epileptic models in rats and mice, including focal TLE, generalized epilepsy, SE, and spontaneously and recurrently generalized epilepsy. We also established the traditional and rapid electrical-kindling epileptic model in rats. If readers are interested in the electrical-kindling model, please refer to References 4 and 5. We also provide the criteria to analyze sleep-wake activity in rats and mice. To evaluate the effect of EA on epileptic activity and sleep, we describe how to localize the related acupoints in rats and how to perform the acupuncture, and we also present some representative results to demonstrate the effects of EA on epilepsy and epilepsy-induced sleep disruptions.

Disclosures

The authors have nothing to disclose.

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