

Original Research

# Detection of pentylenetetrazol-induced seizure activity in the 19–21 Hz beta range using a magnetic coil induction method

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## Abstract

**Introduction:** A force transducer or automatic scoring system is not sufficient to detect small or fine seizure activity. To improve previous assessments of epileptic behavior, a novel coil method was developed to detect an early behavioral marker for epileptic seizures. **Methods:** The present study used the  $\gamma$ -aminobutyric acid (GABA) receptor antagonist pentylenetetrazol (PTZ) to induce seizure activity and epileptic behavior in mice. A coil method was used to detect motor seizures consisting of small amplitude 19–21 Hz muscle contractions. **Results:** Seizure activity in the 19–21 Hz range detected by the coil method was positively correlated with generalized clonic seizures with a kangaroo posture after PTZ administration. GABA receptor agonist valproic acid and ethosuximide decreased PTZ-induced 19–21 Hz seizure activity. The pattern of the amplitude ratio (%) of 19–21 Hz seizure activity after administration of the GABA<sub>A/C</sub> receptor antagonist picrotoxin was similar to the group that was treated with PTZ but different from the group that was treated with the nonselective muscarinic receptor agonist pilocarpine. The coil method detected 19–21 Hz seizure activity after PTZ administration. However, the force transducer method did not detect 19–21 Hz seizure activity. **Conclusions:** The coil method was more sensitive than the force transducer method for detecting epileptic behaviors. The findings may indicate a novel behavioral marker that can be detected by the coil method to reveal epileptic seizures, thus improving our understanding of the brain mechanisms of action and specific brain waves that are associated with PTZ-induced 19–21 Hz seizure activity.

**Keywords:** coil method; epilepsy; pentylenetetrazol; valproic acid; ethosuximide

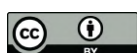
## 1. Introduction

Epilepsy is a chronic neurological disease that is caused by the occurrence of repeated unprovoked seizures. Epilepsy has a prevalence of approximately 1% in the population worldwide, and epileptic patients suffer from seizure symptoms [1,2]. The diagnosis of epilepsy has been defined by the International League Against Epilepsy (ILAE) and International Bureau for Epilepsy (IBE). Epileptic seizures are thought to consist of abnormal excessive or synchronous neuronal activity in the brain [3]. Therefore, the definition of epilepsy requires the occurrence of at least one episode of an epileptic seizure. Seizures are one of the essential symptoms for a diagnosis of epilepsy.

Seizures involve a combination of electrical and behavioral responses that induce chemical, molecular, and anatomical changes [4]. Seizure activity can be accelerated by stressful events [5]. Many behavioral assessments have been used to detect epileptic seizures in animal models of epilepsy [6–10]. One typical method is to perform video recordings for analyzing epileptic behaviors. Convulsive and nonconvulsive epileptic behaviors are then classified and scored according to specific criteria [9–13]. However, this method has some limitations with regard to detecting epileptic behaviors. Such issues involve subjective judgments and individual bias in assessing epileptic seizures. To

overcome this shortcoming, this method of analysis is often combined with various equipment, such as force transducers and video recordings, to automatically detect epileptic behavior [8]. Nevertheless, these assessments typically cannot detect small or fine movements that might be associated with epileptic behavior. The present study used a magnetic coil induction method to detect small movements. This method utilizes a permanent magnet that induces current changes in a coil. The voltage changes in the coil are amplified to assess changes in movement [14]. This method has a high temporal and spatial resolution in real-time to achieve the detection of smaller or finer movements [15–17]. The coil method has been applied to test movements and motor function in models of Parkinson's disease [18,19], neuropathic pain [14], peripheral nerve stimulation [15], and eye-tracking [16]. However, no studies have used the coil method to date to detect epileptic seizures. We compared this novel coil method with the conventional force transducer method to measure seizure activity and epileptic behavior.

To improve assessments of epileptic behaviors, the present study used the coil method to automatically detect epileptic behavior. We evaluated the validity of a specific behavioral index (i.e., 19–21 Hz seizure activity) for defining pentylenetetrazol (PTZ)-induced seizures. We also in-



investigated the relationship between the brain waves and the fine clonus 19–21 Hz seizure activity. Lastly, we sought to determine whether the coil method is more sensitive than the transducer method in measuring 19–21 Hz seizure activity. We found that 19–21 Hz seizure activity could be detected by the coil method, and this epileptic behavior was associated with both  $\gamma$ -aminobutyric acid (GABA) mechanisms and electroencephalographic (EEG) waves.

## 2. Material and methods

### 2.1 Animals

Male C57BL/6 mice (8 weeks old and 25–30 g) were housed in an air-conditioned room under a 12 h/12 h light/dark cycle at a controlled room temperature ( $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) and humidity (50%) with free access to food and water. All of the experiments were performed in accordance with the guidelines of the Academia Sinica Institutional Animal Care and Utilization Committee and all NIH guidelines for the care and use of animal subjects were followed.

### 2.2 Drugs

Pentylenetetrazol, pilocarpine (PIL), and picrotoxin (PTX) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All three drugs were used to induce seizures. Pentylenetetrazol was dissolved in sterile water and injected intraperitoneally at 50 mg/kg as a single injection [20]. Pilocarpine and PTX were dissolved in sterile water and injected intraperitoneally at 300 and 5 mg/kg, respectively, as a single injection [21,22]. Ethosuximide (ESM) and valproic acid (VPA) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used to suppress seizures. The doses of ESM were 200 mg/kg (ESM-200 group), 400 mg/kg (ESM-400 group), and 600 mg/kg (ESM-600 group). The doses of VPA were 200 mg/kg (VPA-200 group), 300 mg/kg (VPA-300 group), and 400 mg/kg (VPA-400 group). All of the drugs were dissolved in distilled water and intraperitoneally administered 30 min before PTZ administration to induce seizures. The injection volume for drug administration was 0.1 mL/25 g. The doses of each drug were based on previous studies [20,21].

### 2.3 Video recording of animal behavior

Behavior was recorded by a video camera and classified and scored according to criteria from Itoh's convulsion scale [9,10]: no. 0 (normal), no. 1 (immobilization), no. 2 (short myoclonic jerks), no. 3 (myoclonic jerks), no. 4 (generalized clonic seizures with a kangaroo posture), and no. 5 (generalized tonic-clonic seizures with a loss of posture tone).

### 2.4 Magnet-induced current coil system

For the magnet-induced current coil system, we referred to the method of the previous study [17]. The NdFeB magnet was attached to the belly of the mouse's body. The coil was wrapped with a round copper wire (1000 turns) and

enclosed by a ring of aluminum in a cylindrical tube (25 cm outer diameter, 45 cm height). Voltage in the coil was induced by a change in the electromagnetic field associated with the magnet's movement (0.8 cm outer diameter, 0.2 cm thickness). The voltage in the coil was in accordance with Faraday's law of electromagnetic induction. The voltage depends on the acceleration of the magnet. The voltage was also influenced by the direction of the magnet's movement. Vertical movements more efficiently changed the voltage compared with horizontal movements. The output voltage signals in the coil were amplified by a differential alternating-current amplifier (Model 1700, A-M system, Carlsborg, WA, USA) and a data acquisition system (Axon Digidata 1322A, Axon Instruments, Union City, CA, USA). The currents that resulted from the animal's movements were recorded on a hard drive for off-line analysis.

### 2.5 Surgery and EEG/behavioral recording

The mice were anesthetized with 4% isoflurane in 100%  $\text{O}_2$  and then placed in a stereotaxic apparatus. The animals were maintained under anesthesia with 1.6% isoflurane in 100%  $\text{O}_2$ , and body temperature was maintained at  $36.5\text{--}37.5^{\circ}\text{C}$  during surgery with a homeothermic blanket system (Model 50-7079, Harvard Apparatus, Holliston, MA, USA). For EEG recordings of the cortex, a small hole was drilled in the skull (the hole was centered 1.5 mm anterior to bregma and 2.0 mm lateral to the midline), and silver-ball electrodes were placed in the hole. The electrodes were implanted in the epidural space. Stainless steel screws were used as the reference and ground electrodes and fixed to the front and back of the skull. All of the electrodes were connected with a connector (catalog no. A79014, Omnetics Connector Corporation, Minneapolis, MN, USA). The self-curing dental acrylic powder was used to affix the Omnetics connector to the skull. After surgery, the mice were placed in cages to recover for 1 week and observed to ensure that no untoward effects of surgery occurred.

One week after surgery, an elastic bandage was used to attach the magnet to the mouse's body, perpendicular to the peritoneal cavity. The silver-ball electrode was connected with the Omnetics connector to a computer via chronic head stages (RA16CH, TDT, Alachua, FL, USA). Individual mice were then placed inside the test chamber, and the camera was adjusted to ensure that the full chamber could be captured in the videos. Five minutes later, the behavior was videotaped, and EEGs were recorded before and after intraperitoneal PTZ administration. The experimental mice were acutely treated with a convulsive dose of PTZ (50 mg/kg). Control mice received the volume injection of 0.1 mL/25 g for the normal saline solution with an intraperitoneal injection. EEGs and convulsive behavior were recorded for 30 min. All of the data, including brain responses and the video recordings, were stored on a hard disk for off-line analysis.

## 2.6 Transducer system

According to a previous study [22], a box was suspended on a force transducer (Model FT 10, Grass Instruments, Cary, North Carolina, USA). A damper was used to restrict the horizontal movement of the chamber, so vertical movements of the chamber were recorded as the animal's movement. The output signals from the transducer were connected to a data acquisition system (Axon Digidata 1322A, Axon Instruments, Union City, CA, USA). Vertical displacement of the box that was caused by the animal's movements was converted to a digital signal and recorded on a hard disk for off-line analysis.

## 2.7 Data analysis

All EEG and behavioral recordings were subjected to fast-Fourier transform (FFT) to a spectrum of 0–50 Hz. Amplitude ratio (%) is calculated as the power amplitude of each frequency band divided by the summation of power amplitude for 0–50 Hz. The amplitude ratio (%) was then calculated for each frequency band. Behavioral ratio (%) was calculated by the formulation  $[100 * (\text{the numbers of the specific behavior responses} / \text{the numbers of all behavioral responses})]$  (%). The measured behavioral responses included the following: baseline: resting and walking; No. 1: immobilization; No. 2: short myoclonic jerk; No. 3: myoclonic jerk; No. 4: generalized clonic seizures with a kangaroo posture; and No. 5: generalized tonic-clonic seizure with loss of posture tone. Note that, “short myoclonic jerk” was the head nodding, facial and forelimb clonus; however, “myoclonic jerk” was the continuous myoclonic jerk tail rigidity; that is, Straube tail [9,10]. One-way analysis of variance (ANOVA) was performed to compare the amplitude ratio (%) of the 19–21 Hz frequencies of the sham, PTZ, ESM-200, ESM-400, ESM-600, VPA-200, VPA-300, and VPA-400 groups, followed by the Fisher Least Significant Difference (LSD) *post hoc* test. Eight mice were then randomly selected for 30 min of behavioral recordings. The correlation of amplitude ratio (%) of the 19–21 Hz frequencies was analyzed for different types of epileptic behaviors. Pearson correlations were calculated between brain waves (EEG)-induced voltage and epileptic behavior-induced coil voltage (No. 3 [myoclonic jerks] and No. 4 [generalized clonic seizures with a kangaroo posture]) for different frequencies of seizure activity (all frequencies [0–50 Hz], alpha [8–12 Hz], delta [1–4 Hz], theta [4–8 Hz], and beta [12–30 Hz]).

## 3. Results

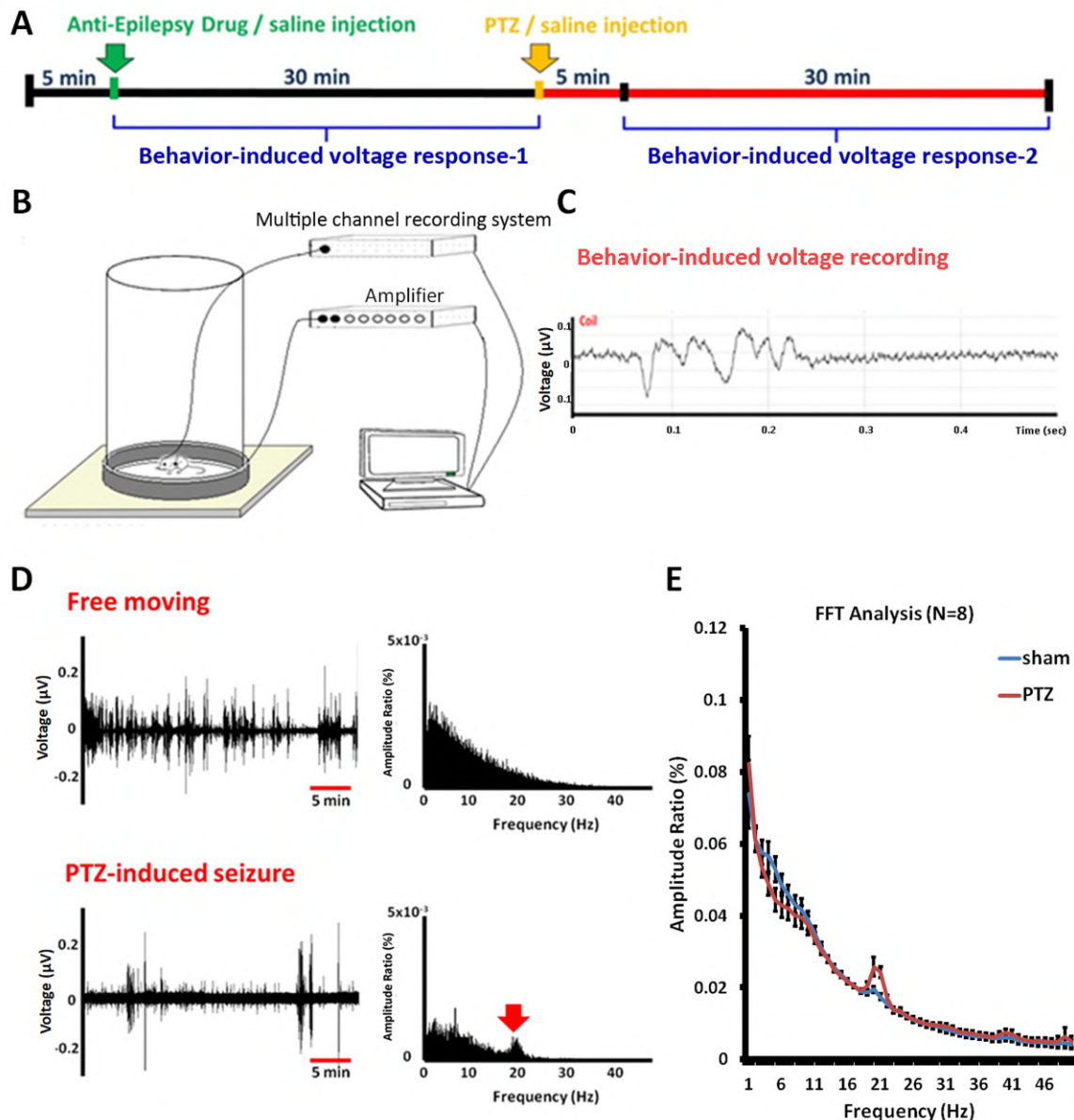
The timeline of the behavioral experiment that assessed the effects of anti-epilepsy drugs and saline on PTZ-induced seizure activity is shown in Fig. 1A. The recording system included the coil, EEG recording devices and a magnet (Fig. 1B). A schematic diagram of the time series of voltage changes in the coil system during behavioral recordings is shown in Fig. 1C. Fig. 1D (upper panel) shows

different electrical currents in freely moving mice. The data were transformed into amplitudes for different frequencies. The specific FFT pattern of PTZ-induced seizure activity for the 20 Hz frequency (red arrow) is shown in Fig. 1D (lower panel). The PTZ group ( $n = 8$ ) produced a significantly increase in 19–21 Hz frequency compared to that of the sham ( $n = 8$ ) group in Fig. 1E.

The anti-epilepsy drugs ESM and VPA were administered to examine which doses decreased 19–21 Hz waves and reduced seizure activity (Fig. 2A,B). Based on the amplitude ratio (%) for the 19–20 Hz frequency range, there was a significant effect of group in the amplitude ratio ( $F_{4,115} = 7.34, p < 0.05$ ). PTZ significantly increased the 19–21 Hz amplitude ratio (%) in the PTZ group compared with the saline group ( $p < 0.05$ ). The 19–21 Hz amplitude ratio (%) in the ESM-400 and ESM-600 groups significantly decreased compared with the PTZ group ( $p < 0.05$ ), with no difference between the ESM-200 and PTZ groups ( $p > 0.05$ ; Fig. 2C). We then evaluated the effect of VPA on PTZ-induced seizure activity. There was a significant effect of group ( $F_{4,118} = 8.99, p < 0.05$ ). The 19–21 Hz amplitude ratio in the PTZ group significantly increased compared with the saline group ( $p < 0.05$ ). The VPA-200 group exhibited a significant increase in the 19–21 Hz amplitude ratio (%) compared with the PTZ group ( $p < 0.05$ ). The 19–21 Hz amplitude ratio (%) in the VPA-400 group significantly decreased compared with the PTZ group ( $p < 0.05$ ; Fig. 2D). These results indicate that PTZ increased seizure-like 19–21 Hz waves, based on the amplitude ratio (%), compared with the saline group, thus indicating PTZ-induced seizure activity. The high and middle doses of ESM decreased the 19–21 Hz amplitude ratio (%), whereas the lower dose of ESM did not affect the 19–21 Hz amplitude ratio (%). The high dose of VPA decreased the 19–21 Hz amplitude ratio (%) compared with the PTZ group. These results indicate that both VPA and ESM decreased PTZ-induced seizure activity.

A typical example of coil waves of the different types of behaviors for PTZ-induced seizures is shown in Fig. 3A (left). Fig. 3A (right) shows these waves as FFT data. The 19–21 Hz frequencies occurred only with generalized clonic seizures with a kangaroo posture (Fig. 3A, red arrow). We also analyzed the behavior ratio (%) for normal behavior (e.g., resting and walking) and PTZ-induced epileptic behavior (no. 1, no. 2, no. 3, no. 4, and no. 5). The incidence of normal behavior was 27.68% of overall behavior, and the incidence of epileptic behavior was 72.32% ( $n = 8$ , Fig. 3B, left). Within the 19–21 Hz frequency range, the proportion of generalized clonic seizures with a kangaroo posture (no. 4) was 84.21% ( $n = 8$ , Fig. 3B, right).

We examined specific behavioral features (generalized clonic seizures with a kangaroo posture [no. 4]) that occurred during 19–21 Hz seizure activity. Fig. 4A (left panel) shows coil waves that were associated with general-

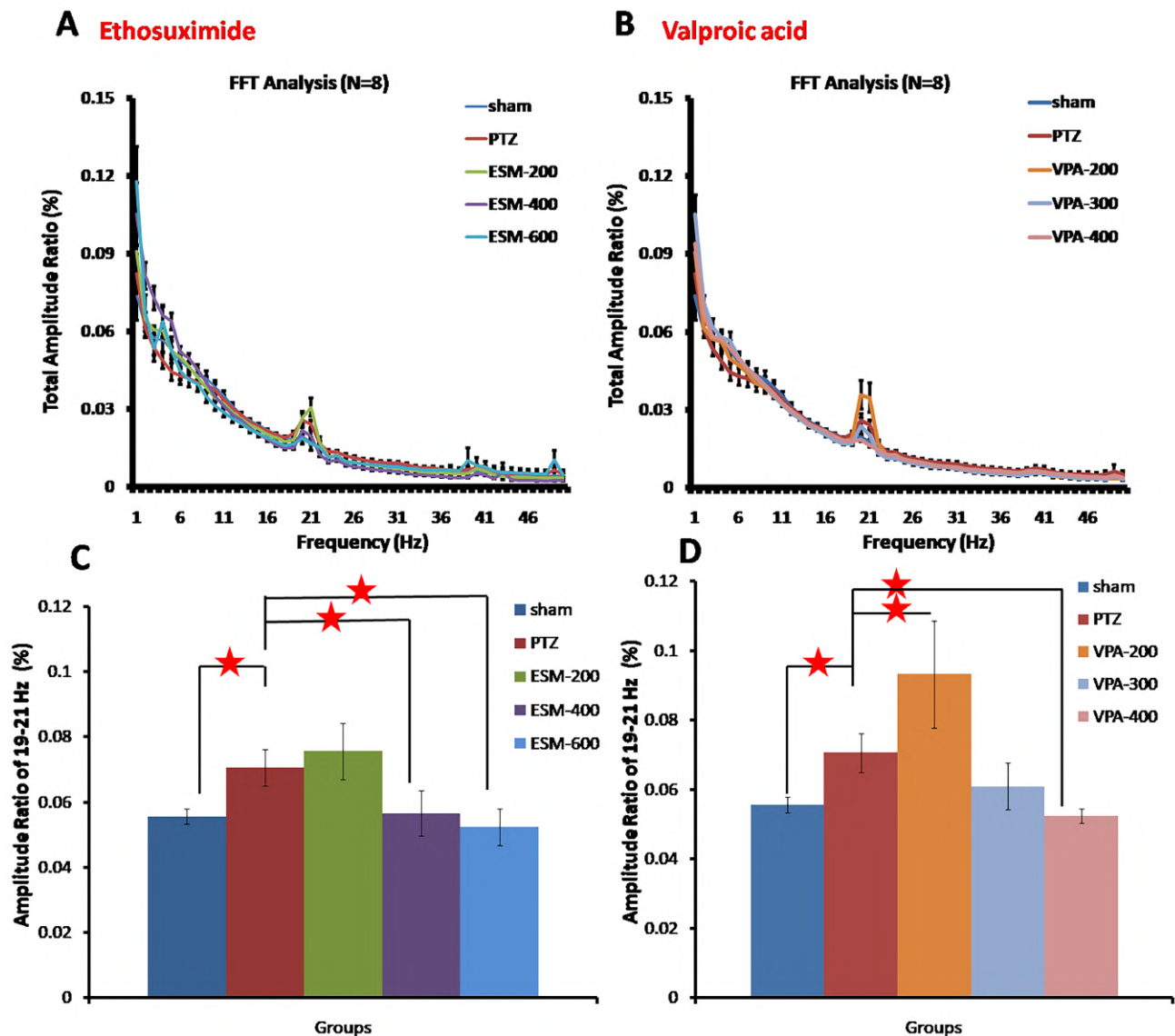


**Fig. 1. Experiment protocol and recording system.** (A) Timeline of anticonvulsive drug/saline and PTZ administration in the behavioral experiment. (B) Overview of the proposed system. (C) Example of behavior-induced voltage recording. (D) Evaluation of behavior-induced voltage response before and after PTZ administration. The mice were allowed to move in the observation box freely. Fast-Fourier transform (FFT) of the freely moving recording waveform showed a curve from the upper left (low frequency) to the lower right (high frequency), and a specific peak (red arrow) occurred after PTZ-induced seizures. (E) Prominent 19–21 Hz seizure activity occurred after PTZ treatment.

ized clonic seizures with a kangaroo posture, and the coil wave associated with a severe jumping in blue line and quiver wave in red line. Fig. 4A (right panel) shows an image of the mouse during an episode of seizure activity. In Fig. 4B, the blue line shows the expanded waveform that was associated with severe jumping (left panel), and the FFT analysis did not produce a 19–21 Hz frequency peak (right panel). In Fig. 4C (left panel), the red line shows the

expanded quiver waveform after severe jumping. The FFT analysis showed a significant frequency peak during 19–21 Hz seizure activity (Fig. 4C, right panel). Overall, 19–21 Hz seizure activity was observed during the time interval that is indicated by the red line after PTZ administration, and 19–21 Hz seizure activity did not occur during the time interval that is indicated by the blue line.

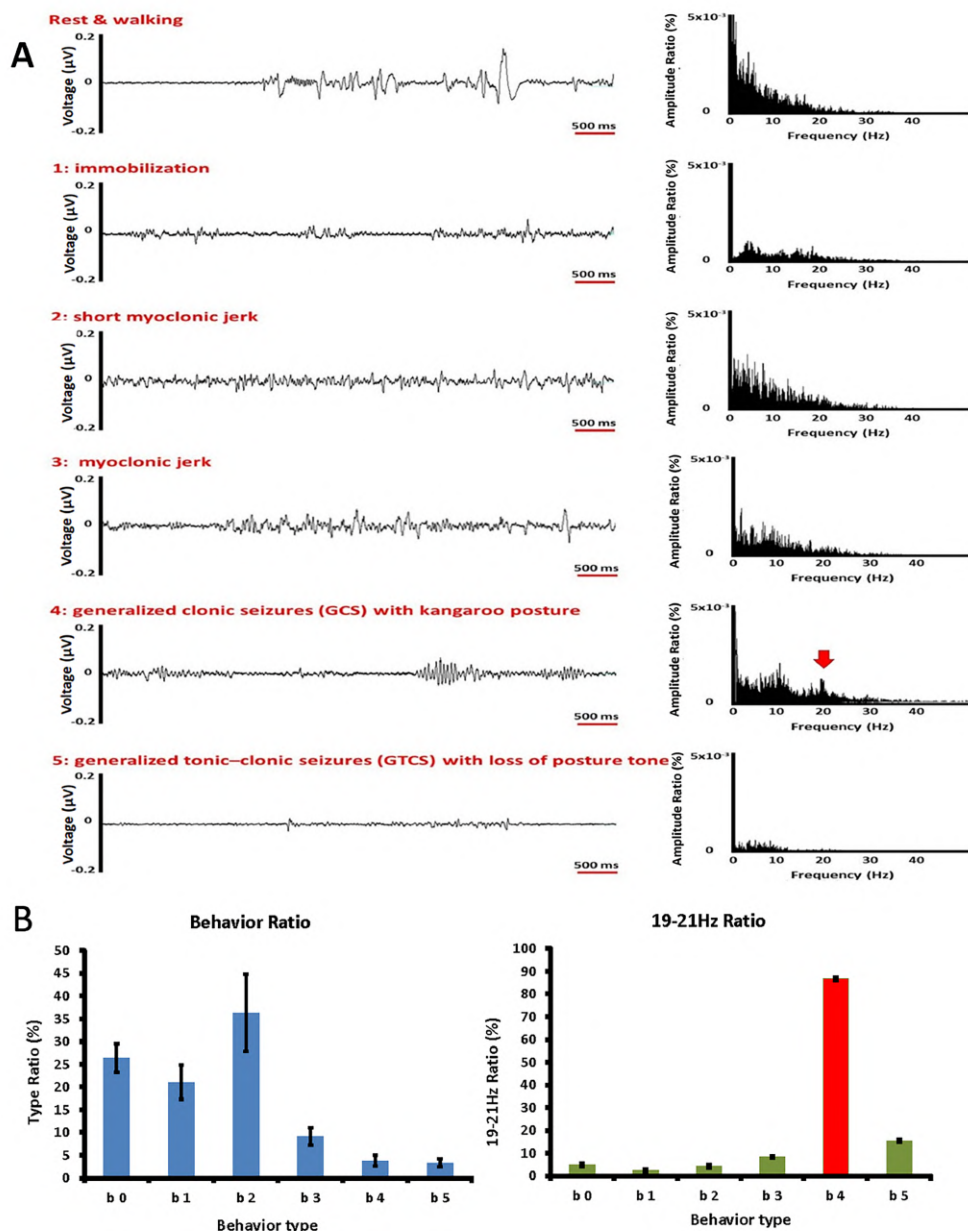




**Fig. 2. Influence of anticonvulsant drugs on the FFT analysis.** The anticonvulsant drugs ESM and VPA blocked 19–21 Hz seizure activity. (A) Ethosuximide (200, 400, and 600 mg/kg). (B) Valproic acid (200, 300, and 400 mg/kg). (C,D) To verify that the specific peak of 19–21 Hz seizure activity resulted from epileptic behavior, the anticonvulsant drugs ESM and VPA were administered to determine which doses reduced epileptic seizures and diminished the specific peak during 19–21 Hz seizure activity. \* $p < 0.05$ , significant differences when compared to the PTZ group.

Pearson correlations were analyzed for the 19–21 Hz seizure activity and EEG data. The sham group exhibited significant positive correlations between 19–21 Hz seizure activity and 1–50 Hz ( $r = 0.73$ ,  $p < 0.05$ ; Fig. 5A, left), 1–4 Hz (delta;  $r = 0.46$ ,  $p < 0.05$ ) and 4–8 Hz (theta;  $r = 0.31$ ,  $p = 0.05$ ), with no correlation between 19–21 Hz seizure activity and 8–12 Hz (alpha;  $r = -0.10$ ,  $p > 0.05$ ) or 12–30 Hz (beta;  $r = -0.06$ ,  $p > 0.05$ ; Fig. 5A, right). Moreover, significant correlations occurred between myoclonic jerks (no. 3) and 1–50 Hz ( $r = 0.53$ ,  $p < 0.05$ ; Fig. 5B, left) and 12–30 Hz (beta;  $r = 0.25$ ,  $p < 0.05$ ), but there were no correlations between myoclonic jerks and 8–12 Hz (alpha;  $r = 0.06$ ,  $p > 0.05$ ), 1–4 Hz (delta;  $r = 0.26$ ,  $p > 0.05$ ) or 4–8

Hz (theta;  $r = 0.06$ ,  $p > 0.05$ ; Fig. 5B, right). Furthermore, a significant correlation occurred with high-frequency beta waves (20–30 Hz), but not with low-frequency (12–16 Hz;  $r = 0.02$ ,  $p > 0.05$ ) or middle-frequency (16–19 Hz;  $r = 0.04$ ,  $p > 0.05$ ) beta waves. Significant correlations occurred between generalized clonic seizures with a kangaroo posture (no. 4) and 1–50 Hz ( $r = 0.49$ ,  $p < 0.05$ ; Fig. 5C, left), 8–12 Hz (alpha;  $r = 0.26$ ,  $p < 0.05$ ) and 12–30 Hz (beta;  $r = 0.40$ ,  $p < 0.05$ ), and there were no correlations between generalized clonic seizures with a kangaroo posture and 1–4 Hz (delta;  $r = 0.20$ ,  $p > 0.05$ ) or 4–8 Hz (theta;  $r = 0.20$ ,  $p > 0.05$ ; Fig. 5C, right). Furthermore, significant correlations occurred with low-frequency ( $r = 0.31$ ,  $p < 0.05$ )

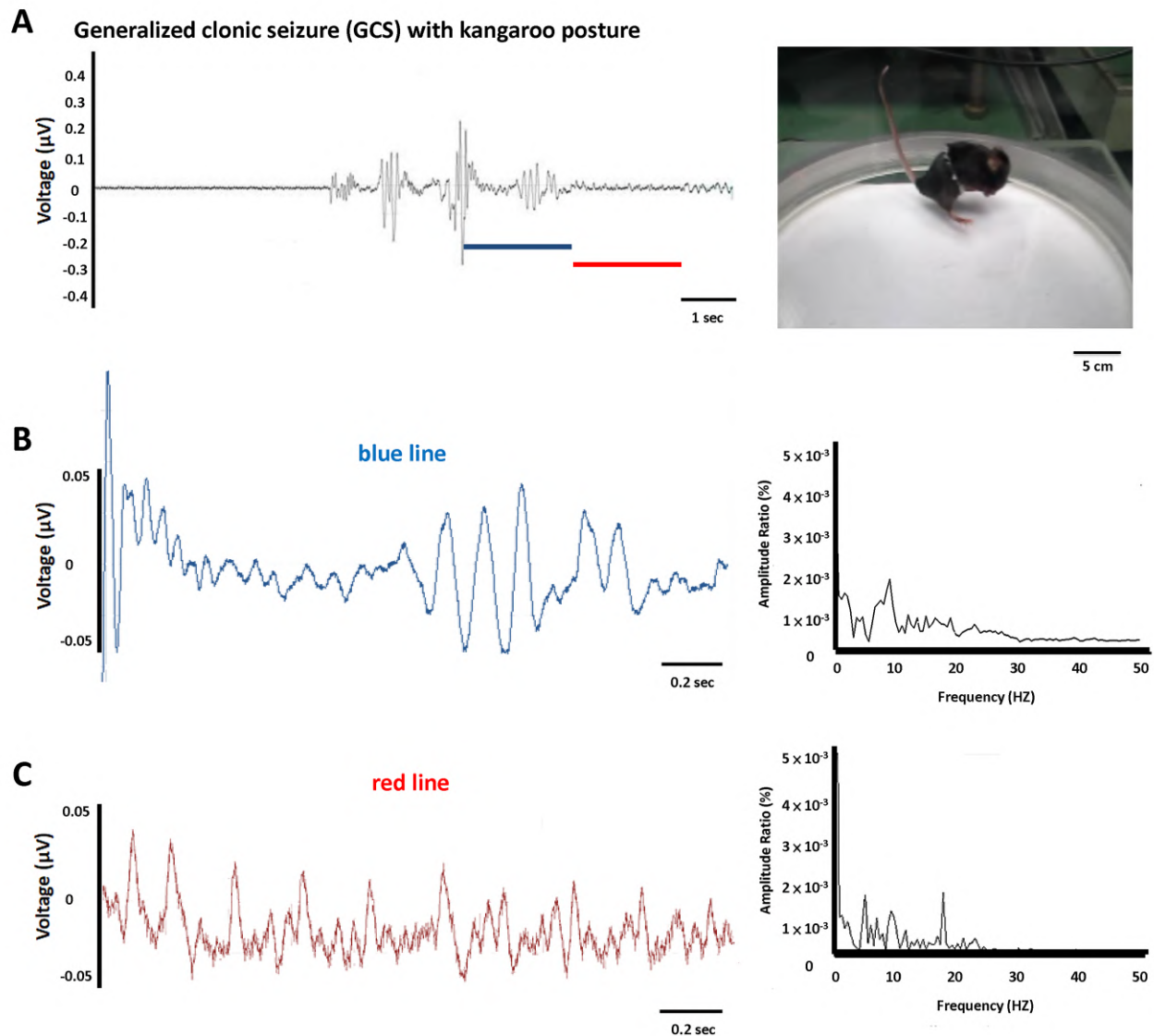


**Fig. 3.** The relationship between various behaviors and amplitudes ratio (%) of different frequency (Hz). (A) Various behaviors were scored from the video recordings. The frequency band distinguished epileptic behaviors from spontaneous body movements. (B) The 19–21 Hz frequency range of seizure activity mainly occurred during generalized clonic seizures with a kangaroo posture (no. 4).

and high-frequency ( $r = 0.58$ ,  $p < 0.05$ ) beta waves, but not with middle-frequency beta waves ( $r = 0.20$ ,  $p > 0.05$ ). In summary, myoclonic jerks (no. 3) and generalized clonic seizures with a kangaroo posture (no. 4) showed a significant positive correlation with high-frequency beta waves (20–30 Hz).

The epilepsy drugs PTX and PIL were also used to induce seizures. Fig. 6A,B show coil waveforms and FFTs for PTX and PIL. Fig. 6C shows that PTZ and PTX but not PIL induced very similar patterns of the amplitude ratio (%) from low to high frequencies. The PTZ and PTX groups but not the PIL group exhibited 19–21 Hz seizure activity.

We compared the pattern of frequencies that were induced by PTZ between the coil and transducer methods. Fig. 7A shows the ways in which all of the systems were integrated for the coil and transducer methods for multichannel EEG recording. Fig. 7B shows synchronous recording waveforms for the coil and transducer methods. Fig. 7C shows that the transducer method did not detect 19–21 Hz seizure activity after PTZ administration. The coil method detected 19–21 Hz seizure activity. These findings indicate that the coil method is more sensitive than the transducer method in detecting a specific frequency of seizure activity.



**Fig. 4. Analysis of generalized clonic seizures (GCS) with a kangaroo posture.** (A) Behavioral responses with generalized clonic seizures during seizure activity. (B,C) A specific 19–21 Hz band (red line) occurred after a dramatic jump (blue line).

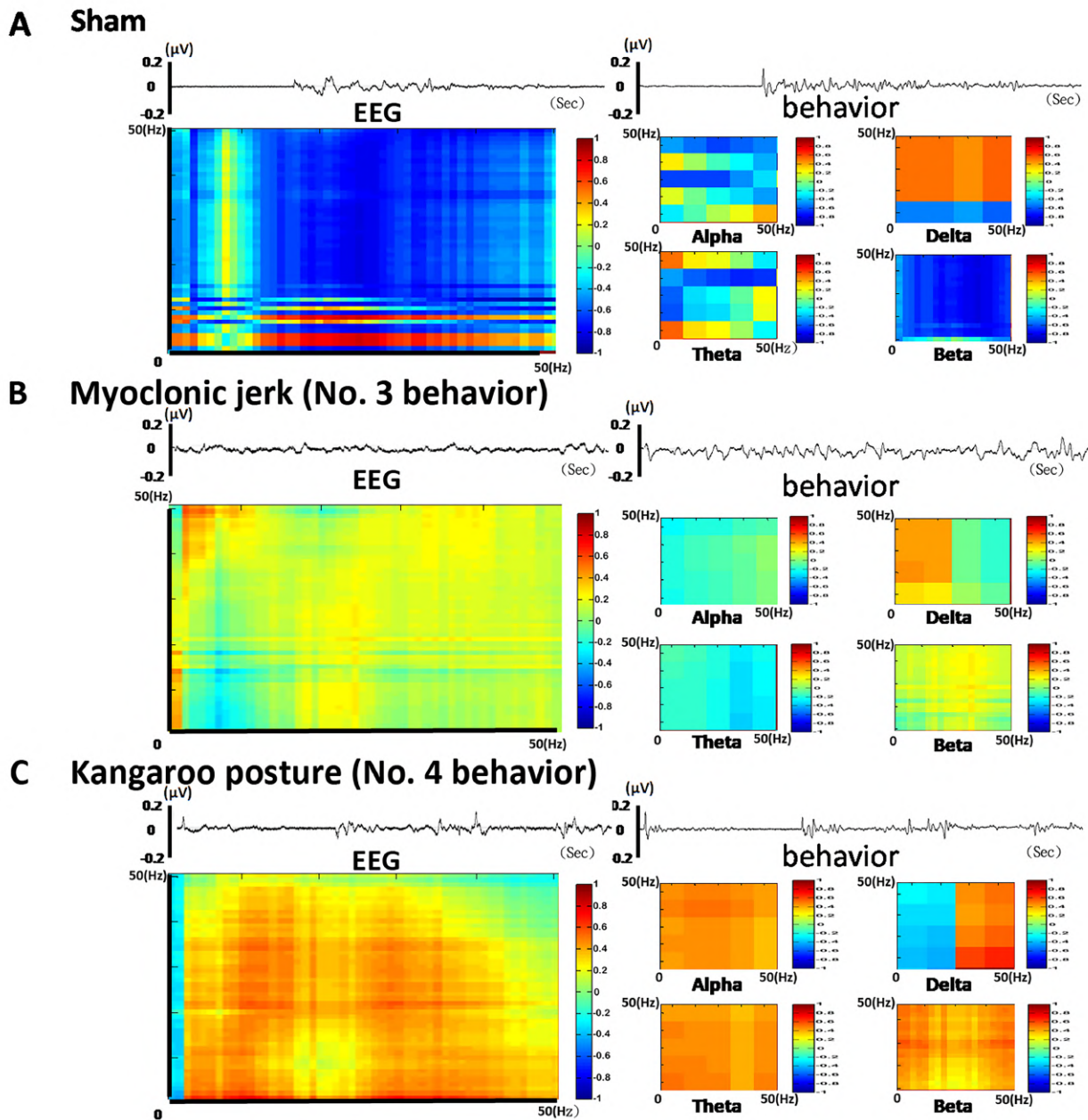
## 4. Discussion

The present study used the coil method to measure magnet-induced voltage changes in different drug-induced epileptic behaviors in mice. The noncompetitive GABA receptor antagonist PTZ induced 19–21 Hz seizure activity, and 19–21 Hz seizure activity was positively associated with generalized clonic seizures with a kangaroo posture (no. 4). Moreover, 19–21 Hz seizure activity was positively associated with 20–30 Hz high-frequency beta waves that were related to myoclonic jerks (no. 3) and generalized clonic seizures with a kangaroo posture (no. 4).

### 4.1 Epilepsy and 19–21 Hz seizure activity: a novel behavioral marker

Many behavioral scoring systems and indices can effectively assess epileptic behaviors in animal models [6,8–

10,12,13,23–26]. For example, some studies have measured the number of seizures and the duration of epileptic behaviors to indicate seizure activity after PTZ administration [6,8,23,25]. Such assessments are often used to determine seizure activity, but they are rough estimations and cannot delineate different types of epileptic behaviors. The application of Itoh's convulsion scale to animal models also sums all scores for different epileptic behaviors to confirm the seizure strength of PTZ-induced epileptic behaviors [9,13,27]. Racine's convulsion scale [12], Malhotra's convulsion scale [26], and Rathor's convulsion scale [24] have used similar concepts to define different categories of epileptic behaviors and seizure strength. These scales provide a more detailed characterization and dissociation of different types of epileptic behaviors, but scoring these different types of epileptic behaviors is more complicated.

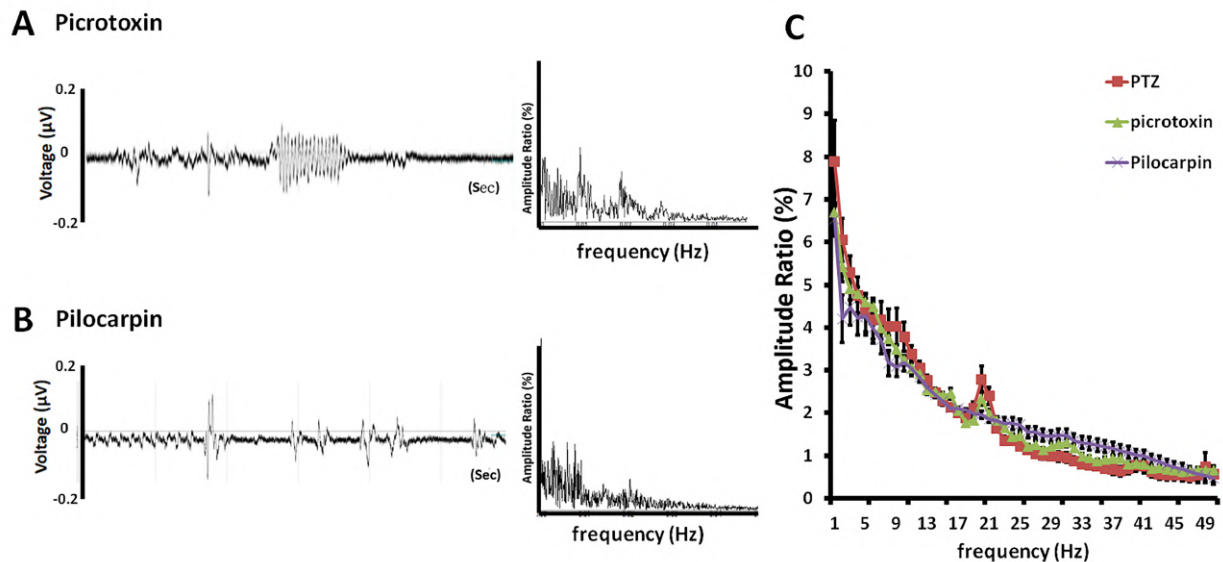


**Fig. 5. Correlations testing between EEG (0–50 Hz) and epileptic behaviors for the sham, myoclonic jerk (no. 3), and generalized clonic seizures with a kangaroo posture (no. 4) condition.** (A) The sham group depicted significant correlations occurred at 0–50 Hz, 1–4 Hz (delta), and 4–8 Hz (theta;  $p = 0.05$ ), but non-significant correlation occurred at 8–12 Hz (alpha) or 12–30 Hz (beta;  $p > 0.05$ ). (B) The myoclonic jerks (no. 3) depicted significant differences occurred in 0–50 Hz and 12–30 Hz (beta;  $p < 0.05$ ), but non-significant differences occurred at 8–12 Hz (alpha), 1–4 Hz (delta), or 4–8 Hz (theta;  $p > 0.05$ ). (C) The generalized clonic seizures with a kangaroo posture (no. 4) depicted significant differences occurred at 0–50 Hz, 8–12 Hz (alpha), and 12–30 Hz (beta;  $p < 0.05$ ), but non-significant differences occurred at 1–4 Hz (delta) or 4–8 Hz (theta;  $p > 0.05$ ).

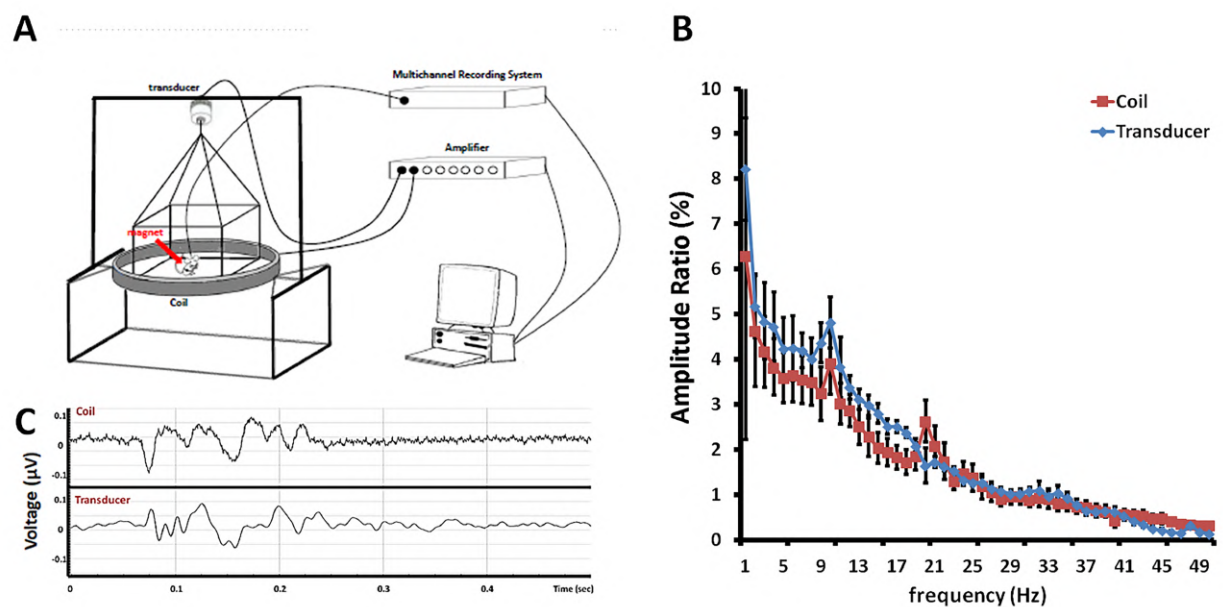
The present results indicated that 19–21 Hz seizure activity was positively correlated with generalized clonic seizures with a kangaroo posture (no. 4), reflected by Itoh's convulsion scale, after PTZ administration. The time analysis indicated that 19–21 Hz seizure activity occurred with a delay after PTZ administration. No studies of which we are

aware have reported correlations between epileptic behavior and 19–21 Hz seizure activity. Our findings indicated that 19–21 Hz seizure activity, assessed by the coil method, might be a novel behavioral marker of epileptic behaviors.





**Fig. 6.** Seizures-induced by pentylenetetrazol (PTX) and pilocarpine (PIL) and 19–21 Hz frequency analysis. (A,B) Fast-Fourier transform analysis of seizures induced by (A) pentylenetetrazol (PTX) and (B) pilocarpine (PIL). (C) The PTZ and picrotoxin groups but not the pilocarpine group exhibited 19–21 Hz seizure activity.



**Fig. 7.** Comparison of frequency patterns of seizure activity induced by PTZ administration using the coil and transducer methods. (A) Overview of the proposed system that included a coil and transducer. (B) The coil system can record the responses of mice at the same time. (C) Comparison of amplitude ratio (%) between the coil and transducer methods after PTZ-induced seizures.

#### 4.2 Relationship between 19–21 Hz seizure activity and $GABA_A$ receptor system

The present study sought to determine the brain mechanisms that are involved in 19–21 Hz seizure activity. The coil method detected 19–21 Hz seizure activity after PTZ administration. Pentylenetetrazol is a  $GABA_A$  receptor antagonist. Another  $GABA_{A/C}$  receptor antagonist, PTX, exerted a similar pattern of the amplitude ratio (%) for all frequencies of seizure activity. However, this pattern of the

amplitude ratio (%) after PTZ administration was different from the nonselective muscarinic receptor agonist PIL, particularly in the 19–21 Hz frequency range. VPA reduced 19–21 Hz seizure activity. Overall, these findings suggest that the  $GABA_A$  receptor is just one of the mechanisms probably involved, but other mechanisms may be involved in the pathogenesis of the 19–21 Hz seizure activity [28,29]. However, the cholinergic system was not involved in the 19–21 Hz seizure activity.

Anticonvulsant drugs have two main mechanisms of action for treating epileptic behavior [30,31]. One mechanism involves GABAergic neurotransmission. For example, VPA has been shown to increase GABAergic neurotransmission and reduce PTZ-induced seizures in animal models. Valproic acid also suppresses patients' partial seizures and generalized seizures [32]. Another mechanism involves the blockade of neuronal ion channels. For example, ESM has decreased T-type calcium channel currents in the thalamus to induce seizure activity [33]. Ethosuximide has been reported to ameliorate symptoms of generalized seizures in epilepsy patients [32]. Previous studies have suggested that ESM and VPA might share a similar mechanism of action that decreases the threshold of T-type calcium channel currents to reduce epileptic seizures [33,34]. The mechanism of action of ESM and VPA might partially overlap to treat symptoms of epileptic seizures. A previous human study of childhood absence seizures suggested that ESM and VPA were more effective than lamotrigine to ameliorate symptoms. Moreover, ESM had fewer adverse effects on attention function [35]. Thus, many mechanisms of action appear to mediate symptoms of epileptic seizures. The present study indicated that the highest dose of ESM (600 mg/kg) and VPA (400 mg/kg) decreased 19–21 Hz seizure activity that was induced by PTZ. The present results are consistent with a previous study that showed that ESM and VPA reduced other epileptic seizure behaviors, such as PTZ-induced clonic seizures [36]. On the other hand, the present result showed that the low dose of VPA (200 mg/kg) facilitated 19–20 Hz seizure activity induced by PTZ. Obviously, this evidence conflicts with the data of the highest dose of VPA (400 mg/kg). In particular, the present results showed that the low dose of VPA enhanced PTZ-induced seizure activity. The data were consistent with the previous clinical findings [37,38]. For example, the EEG study appeared that VPA administrations facilitated absence epilepsy [37]. Moreover, fewer patients with absence seizure increased the frequency of seizure activity after VPA treatments [38]. Therefore, some cases with VPA administrations revealed opposite results. Therefore, it is a crucial issue that why the different doses of the VPA administration show opposite results for affecting PTZ-induced seizure activity? Does VPA appear to have a dose-dependent effect on PTZ-induced clonic seizures? This emerging issue should be scrutinized in further studies.

#### 4.3 Analysis of EEG recordings and 19–21 Hz seizure activity

The specific brain waves that are associated with epileptic behaviors remain unknown. For example, patients with temporal lobe seizures exhibit 1–2 Hz slow-wave delta activity in bilateral frontal and parietal cortices. Moreover, 1–2 Hz slow-wave delta activity in bilaterally parietal cortices was correlated with temporal lobe seizures [39]. How-

ever, Jan *et al.* [40], suggested a different brain wave pattern in temporal lobe epilepsy patients. These authors suggested the occurrence of irregular 2–5 Hz lateralized activity, 5–10 Hz sinusoidal waves, and continuous epileptic discharges in start-stop-start ictal rhythms in temporal lobe epilepsy patients [40]. Extra-temporal seizures (including in frontal, central, parietal, occipital, and middle brain areas) have been shown to be associated with diverse types of brain waves. These brain waves and frequencies include beta, alpha, theta, and delta frequency ranges [41]. A focal beta frequency discharge was linked to the early stage of frontal lobe epilepsy [42]. Serafini *et al.* [43], suggested that ~20 Hz frequency brain waves (rhythmic EEG spike potentials) were associated with myoclonic jerks, which is consistent with the present results. In the present study, 19–21 Hz seizure activity was positively associated with 20–30 Hz high-frequency beta waves that were related to myoclonic jerks (no. 3) and generalized clonic seizures with a kangaroo posture (no. 4). The relationship between specific brain waves and epileptic behaviors needs to be further clarified.

#### 4.4 Comparison of coil and transducer methods

The present study compared the coil method and force transducer method with regard to their ability to detect epileptic seizures. The coil method utilizes a magnetometer coil to detect small and fine behavioral responses, based on changes in electromagnetic fields. The coil method requires that a magnet be attached to the mice. When the animal's movement causes the induction of a magnetic field, the coil device can detect voltage changes, and then these signals are transformed by an amplifier. The coil method provides high temporal and spatial resolution in real time and, consequently, detects smaller and finer movements [15,18,19,44]. In the present study, the coil method detected 19–21 Hz seizure activity after PTZ administration. The force transducer method did not detect 19–21 Hz seizure activity. These findings are consistent with previous studies [15,18,19,44]. Overall, the coil method detected a novel behavioral marker of epileptic seizures that could have clinical implications.

#### 4.5 Limitations and further research

The present study developed a coil method to detect seizure activity in 19–21 Hz frequencies. The 19–21 Hz seizure activity is a critical behavioral marker for detecting the occurrence of seizure behavior. Some limitations and issues should be addressed in further studies. For example, the magnet size should be kept in miniature, and the magnet weight was required to be minimum to avoid interfering with the animal's behaviors. The diameter of the coil can be increased to allow more space for the animal to move. But it may reduce the sensitivity of the magnetic field detection. There is the possibility that other behavioral frequencies are associated with different types of seizure be-

haviors. The coil method may detect another early behavioral symptom in neurodegeneration diseases or neurological disorders such as Parkinson's disease or Alzheimer's disease. Thus, further studies need to investigate how to apply the coil method in detecting other neurological diseases.

On the other hand, the present result showed that the low dose of VPA (200 mg/kg) enhanced PTZ-induced 19–20 Hz seizure activity compared to the PTZ group. The result was opposite to the anticipatory effect. The reason may be that VPA activated various mechanisms but not the specific mechanism of the GABA<sub>A</sub> receptor. For example, these mechanisms included several voltage-operated channels, GABA clearing enzymes and GABA transporters, the IP3 (inositol triphosphate) pathway, etc. [29]. Therefore, the crucial issue has emerged: one of these mechanisms in the lower dose of VPA facilitated PTZ-induced 19–20 Hz seizure activity. It should be investigated in further studies.

## 5. Conclusions

The present study has developed a novel coil method to improve the deficits of previous method in the assessments of epileptic behaviors. The coil method was more sensitive than the force transducer method for detecting epileptic behaviors. Seizure activity that occurs in the 19–21 Hz frequency range, detected by the coil method, may be a novel marker of epileptic behavior for clinical implications.

## Abbreviations

ANOVA, analysis of variance; EEG, electroencephalographic; ESM, Ethosuximide; FFT, fast-Fourier transform; GABA, the  $\gamma$ -aminobutyric acid; LSD, Significant Difference; PIL, pilocarpine; PTX, picrotoxin; PTZ, pentylenetetrazol; VPA, valproic acid.

## Author contributions

HCL and WJC contributed to the conducted experiments, performed data analysis and interpretation. ACWH and BCS performed data analysis and interpretation and wrote the manuscript.

## Ethics approval and consent to participate

All of the experiments were performed in accordance with the guidelines of the Academia Sinica Institutional Animal Care and Utilization Committee and all NIH guidelines for the care and use of animal subjects were followed (protocol #: 18-12-1252).

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## Conflict of interest

The authors declare no conflict of interest.

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