

Effects of COX and 5-LOX Pathways on Different Seizure Models

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Research Article

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Abstract

Purpose

This study aimed to examine the relationship between epilepsy and COX/5-LOX inflammation pathways in the penicillin and pentylenetetrazole (PTZ) induced epilepsy models.

Methods

For this purpose, forty-two albino male Wistar rats were used in this study. In the penicillin and PTZ-induced epilepsy models, epileptiform activity was induced by injection of penicillin (500 IU, i.c) and PTZ (35 mg/kg, i.p, three times a week), respectively. Licofelone (20 mg/kg, i.p), a dual inhibitor of COX/5-LOX, and esculetin (20 mg/kg, i.p), a 5-LOX inhibitor, were given. In the penicillin-induced epilepsy model, ECoG activity was recorded for 180 min. In the PTZ-induced epilepsy model, both ECoG activity was recorded and behavioral parameters were performed.

Results

In the penicillin groups, both licofelone and esculetin decreased the mean spike frequency and amplitude during the experiments. In the PTZ groups, licofelone (20 mg/kg, i.p) was more effective than esculetin (20 mg/kg, i.p). Licofelone showed its protective effects both in ECoG activity and in behavioral parameters. Esculetin was less effective when compared to licofelone.

Conclusion

The electrophysiological and behavioral data from the present study indicated that inflammation pathways might have a crucial role in controlling epileptiform activity in rats. Licofelone might be a valuable candidate in advanced studies.

1. Introduction

Epilepsy is characterized by repeated spontaneous bursts of neuronal hyperactivity and high synchronization in the brain (Trinka et al., 2015). In general, epileptic seizures predicate a disequilibrium between inhibitory (GABAergic) and excitatory (glutamatergic) neurotransmission. However, the mechanism of epileptogenesis is still unclear (Vezzani et al., 2013). In many cases, antiepileptic drugs (AEDs) can partially control seizures and help patients to maintain their everyday lives. Although advanced in antiepileptic drugs and research, seizures occur in approximately 30% of patients with epilepsy (Rahim et al., 2021). In this manner, many factors have essential roles in the pathophysiology of epilepsy, and we must consider these, including inflammation, in the treatment of epilepsy.

Inflammation is a complex process, and in general, it is the defense state against stimuli such as injury, trauma, microbial activity, and toxin in the body (Paudel et al., 2018). Initially, metabolized arachidonic acid through cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) pathways play a critical role in epilepsy

and inflammation. Cyclooxygenase is the critical enzyme that converts arachidonic acid to prostaglandins (PGs) (Dhir et al., 2006). Arachidonic acid causes some alterations and inflammatory mediators' production on microglia, astrocytes, and brain capillary endothelial cells. The activation of inflammatory mediators such as COX-2 and nuclear factor kappa B (NF- κ B) and the over-production of down-stream inflammatory factors including interleukin (IL)-1 β , IL-6, tumor necrosis factor (TNF)- α , and prostaglandin E2 (PGE2) contribute to the pathophysiology of seizures (Wang et al., 2018). Emerging evidence suggests that inflammatory pathways play a crucial role in the pathophysiology of epilepsy (Vezzani et al., 2015).

Although there are various reports regarding the increase of cyclooxygenase enzyme following seizure activity (Takemiya et al., 2003), some studies have conflicting results. Selective and non-selective COX inhibitors can cause a delay in the progress of seizure (Tanaka et al., 2009), increase the seizure threshold (Akula et al., 2008), or worsen the seizure activity (Taskiran et al., 2017). These effects can vary according to ligand type, seizure type, the dose of agents, injection time, etc. In this manner, the acute or chronic phase of inflammation might have a vital role in the pathophysiology of epilepsy.

The effects of the 5-LOX pathway on epilepsy are not as effective as the COX pathway; thereby, the 5-LOX pathway has received less attention. Kim et al. showed that 5-LOX inhibitors do not have protective effects alone, but when aspirin, a COX-2 inhibitor, and esculetin, a 5-LOX inhibitor, were combined, the protective effects against neurotoxicity appeared in the PTZ-induced seizure (Kim et al., 2008). Similarly, Acetyl-11-Keto- β -Boswellic Acid (AKBA), a 5-LOX inhibitor, did not affect the seizure activity in the kainic acid-induced epilepsy. Whereas, AKBA+indomethacine increased the seizure latency (Bishnoi et al., 2007).

A number of animal studies showed that both COX and 5-LOX pathways have contradictory results (Akula et al., 2008; Tanaka et al., 2009; Taskiran et al., 2017). In addition, there can be different effects on seizures in terms of acute or chronic phase inflammation. With this background, the present study was aimed to explain the effects of COX and 5-LOX in the penicillin and PTZ-induced epilepsy models.

2. Materials And Methods

2.1. Animals

In the present study, forty-two male Wistar Albino rats (180-250 g, 8 weeks old) were used. The animals were maintained under standard laboratory conditions with a 12/12-h light/dark cycle, 60 \pm 3% humidity level, and 22 \pm 4°C constant room temperature. They had ad libitum access to water and food. All experiments were conducted between 09:00 and 17:00 h. The animals were purchased from Erciyes University Experimental Research and Application Center. This study was approved by the Ethical Committee for Animal Experiments at Erciyes University (Approval number: 17/143).

2.2. Experimental design

The animals were divided into the following groups:

Group 1. Penicillin (500 IU, 2.5 µl, i.c.) + Saline group (2 ml, i.p.) (n=7)

Group 2. PTZ (35 mg/kg, i.p.) + Saline group (2 ml, i.p.) (n=7)

Group 3. Penicillin (500 IU, 2.5 µl, i.c.) + Licofelone group (20 mg/kg, i.p.) (n=7)

Group 4. Penicillin (500 IU, 2.5 µl, i.c.) + Esculetin group (20 mg/kg, i.p.) (n=7)

Group 5. PTZ (35 mg/kg, i.p.)+ Licofelone group (20 mg/kg, i.p.) (n=7)

Group 6. PTZ (35 mg/kg, i.p.) + Esculetin group (20 mg/kg, i.p.) (n=7)

2.3. Drugs

In the penicillin-induced epilepsy model, the seizure activity was triggered by penicillin G potassium (I.E., Ulagay Pharmacy) (500 units, i.c). After the stabilization of ECoG activity in 30 minutes, licofelone and esculetin were administered. In the PTZ-induced epilepsy model, licofelone and esculetin were given 30 min before the administration of PTZ. In this study, licofelone (20 mg/kg), a COX/5-LOX dual inhibitor, and esculetin (20 mg/kg), a 5-LOX inhibitor, were used as chemical agents. All chemicals were bought from Sigma Aldrich and dissolved in artificial cerebrospinal fluid. Doses of the drugs were selected based on the studies in the literature.

2.4. Surgical procedure and drug administration

2.4.1. Penicillin-induced epileptiform activity

The animals were anesthetized with urethane (1.25 g/kg, i.p.) and fixed in a rat stereotaxic frame. A midline incision was made through the scalp. Two stainless-steel screw electrodes were implanted on the left somatocortex skull bone (first screw 3 mm lateral and 4 mm rostral to bregma, second screw 3 mm lateral and 4 mm caudal to bregma). A third hole was opened for penicillin injection (3 mm lateral and 3 mm rostral to bregma), and Penicillin G Potassium was injected using Hamilton microsyringe type 701N. Epileptiform activity occurred approximately within 3–4 min. In about 25–30 min, spike frequency and amplitude of the epileptiform activity became stable and continued for about 4-5 h. Licofelone (20 mg/kg) and esculetin (20 mg/kg) were performed 30 min after penicillin injection. ECoG activity was recorded by PowerLab 16/SP (AD Instruments, Australia) for 180 min after drug injections (Figure 1). Electrophysiological records were analyzed with a software program (LabChart v8), and the data were transferred to MS Excel.

2.4.2. PTZ-induced epileptiform activity

In the PTZ-induced epilepsy model, the ECoG recording process is slightly different from the penicillin-induced epilepsy model (Figure 1). Licofelone and esculetin were administered 30 min before PTZ injection. PTZ (35 mg/kg) was administered for the kindling process three times a week (Monday, Wednesday, and Friday), and animal behavior was monitored to evaluate the behavioral parameters. In the kindling process, the Fisher and Kittner seizure scales were used (Fischer and Kittner, 1998) (Table 1).

After having reached at least five seizures of stage 3-5, fully kindled animals were anesthetized with ketamine/xylazine and fixed in a rat stereotaxic frame. The surgical process and the implantation of the electrodes were performed as described in 2.4.1. All animals were allowed to recovery for seven days. After a seven-day post-surgery recovery period, rats were placed separately in glass cages and ECoG activity was recorded for 30 min. In this model, the seizure score, the number of needed injections for kindling, first myoclonic jerk (FMJ), and total spikes/30 min were determined in awake rats.

Table 1
Fischer and Kittner seizure scale.

Stage 0	no evidence of convulsive activity
Stage 1	weak head nodding
Stage 1,5	mild forelimb clonic activity
Stage 2	myoclonic body jerks, clonic forelimb convulsions without rearing
Stage 2,5	frequent clonic forelimb convulsions, short (incomplete) rearing
Stage 3	severe bilateral forelimb clonus (>10 s) with full rearing (Kangaroo position)
Stage 3,5	rearing and falling in addition to severe bilateral forelimb clonus
Stage 4	generalized clonic convulsions with rearing and falling down episodes or jumps
Stage 4,5	generalized clonic-tonic seizures with loss of righting reflex (tonic extension of the forelimb)
Stage 5	generalized clonic-tonic seizures and status epilepticus (>2 min)

2.5. Evaluation of ECoG activity and behavioral parameters

In the penicillin-induced epilepsy model, ECoG activity was recorded for 180 min after licofelone and esculetin injections. Mean spike frequency and amplitude were determined every 10 min over a period of 180 min. For evaluation of ECoG recording, mean amplitude and spike frequency in the ten min between the 20th and 30th minute before penicillin administration were considered 0th control value. In the calculation of percentage, the following formula was used:

$$\frac{\text{Mean spike frequency or amplitude after drug administration}}{\text{Control value, 0}^{\text{th}}\text{min}} \times 100$$

In the PTZ-induced epilepsy model, ECoG activity was recorded for 30 min and total spike count were evaluated for 30 min. Behaviors of the rats were monitored to evaluate the seizure score, the number of needed injections for kindling, and the first myoclonic jerk (FMJ).

2.6. Statistical analyses

All statistical analyses were performed by GraphPad Prism 8 software. Electrophysiological data were analyzed using the LabChart program (Version 8). Values are expressed as means \pm S.E.M. The Shapiro-Wilk test was applied to determine the normality of data. In the penicillin groups, data were analyzed using two-way ANOVA followed by post hoc test for multiple comparisons. In the PTZ groups, behavioral and ECoG data were analyzed using one-way ANOVA. Results were considered significant at confidence limits of $p < 0.05$.

3. Results

3.1. Evaluation of ECoG recordings in the penicillin-induced epileptiform activity

Intracortical injection of penicillin (500 units/ 2.5 μ l) triggered epileptiform activity about 3-4 min after injection. Approximately 30 minutes after the penicillin administration, the activity reached a constant level, and this activity lasted about 4-5 h. Licofelone and esculetin were administered 30 min after penicillin and recorded for 180 min.

The mean spike and amplitude of the epileptiform activity in the penicillin group were 36.06 ± 3.25 spike/min and 1.152 ± 0.241 mV/min within 30 min after saline injection, respectively. The spike frequency and amplitude of seizure activity showed a small decrease during experiments and this seizure activity continued until the end of the experiment ($p < 0.05$). In the Penicillin+Licofelone group, the mean spike frequency and amplitude of the seizure activity were 27.18 ± 2.64 spike/min and 0.823 ± 0.24 mV/min, respectively ($p < 0.05$). In the Penicillin+Esculetin group, the mean spike frequency and amplitude of the seizure activity were 30.15 ± 4.58 spike/min and 0.913 ± 0.35 mV/min, respectively ($p < 0.05$). In the acute model of epilepsy, all ligands affected the seizure activity and decreased the spike frequency and amplitude at the end of the experiment. The mean spike frequency and amplitude of ECoG recordings of the animals are shown in Figure 2 and 3. Representative traces of ECoG recordings of the animals are shown in Figure 4. The mean spike frequency of each group is given in Table 2.

Table 2

Mean spike frequency (spikes/min) of the penicillin-induced epileptiform activity in study groups.

Groups	0th	60th	120th	180th
Penicillin + Saline	32,9 \pm 3	38,1 \pm 4	32,4 \pm 4	30,5 \pm 3
Penicillin + Licofelone (20 mg/kg)	35,4 \pm 2	30,9 \pm 2	27,5 \pm 3	25,2 \pm 4
Penicillin + Esculetin (20 mg/kg)	35,6 \pm 4	33 \pm 2	30,1 \pm 2	29,1 \pm 3

Data are presented as mean \pm SEM. Licofelone group was statistically significant between 10 and 60 minutes compared to the penicillin+ saline group.

3.2. Evaluation of seizure scale, number of injections, and FMJ in the PTZ-induced epileptiform activity

In the PTZ group, the mean number of needed injections for kindling was 4.22 ± 0.66 ($p=0.04$) (Figure 5). According to the Fischer and Kittner seizure scale, the seizure severity stage was 4.01 ± 0.23 in the PTZ group ($p<0.0001$) (Figure 5). The seizure severity was found significantly lower in the esculetin group (3.32 ± 0.16 , $p=0.12$) and the licofelone group (2.32 ± 0.11 , $p=0.003$) compared to the PTZ group. In terms of the number of injections, similar results were seen in the seizure severity stage. In the licofelone group, the mean number of injections for kindling was significantly increased (18.20 ± 1.82) compared to the PTZ and esculetin groups ($p=0.002$). In the esculetin group, the number of needed injections was 8.6 ± 1.72 compared to the PTZ group ($p=0.186$). In the PTZ group, the onset of FMJ was 143.2 ± 7.08 sec ($p<0.0001$). In the licofelone group, FMJ was significantly increased at 787.8 ± 70.04 ($p<0.001$). In the esculetin group, FMJ was 183.9 ± 17.08 sec ($p=0.948$). Licofelone was more effective than the esculetin group in terms of all parameters (Figure 6).

3.3. Evaluation of ECoG recordings in the PTZ-induced epileptiform activity

In the PTZ model, before the implantation of electrodes, seizure scores, the number of injections, and FMJ were examined from video recordings. Later, ECoG activities were recorded for 30 min. In the PTZ group, the total spike count was 2495 ± 145.9 spike/30 min ($p=0.0087$). Both licofelone and esculetin affected the seizure activity and decreased the total spike at 809 ± 42.55 and 1302 ± 221.45 spike/30 min, respectively ($p=0.007$, $p=0.019$). Representative traces of ECoG recordings of the animals are shown in Figure 7.

4. Discussion

In the present study, licofelone, a dual inhibitor of COX and 5-LOX receptors, and esculetin, a 5-LOX inhibitor, were studied in two experimental models of epilepsy. Both licofelone and esculetin decreased the seizure activity in penicillin-induced epilepsy, while licofelone was more effective in preventing PTZ-induced seizure activity.

Previous studies revealed that metabolized arachidonic acid has an important role in epilepsy and the inflammation process. Arachidonic acid causes some alterations resulting production of inflammatory mediators on microglia, astrocytes, and brain capillary endothelial cells. Inflammation is an essential condition in epileptic mechanisms, both in terms of being the primary mechanism involved in forming epilepsy and further triggering current seizures. There are many studies in the literature, but these studies include conflicting results.

Results of the present study showed that both licofelone and esculetin decreased the spike frequency and amplitude of the seizure activity in the penicillin model. Both of them showed anticonvulsant activity with similar results in the acute model of epilepsy. In our previous studies, aceclofenac (10 and 20 mg/kg) and aspirin (150 and 500 mg/kg), COX-2 inhibitors, have conflicting results in the penicillin-induced epilepsy model. While aspirin has protective effects at low and high doses, aceclofenac used for rheumatoid arthritis in the clinic has proconvulsant effects (Tasdemir et al., 2018; Taskiran et al., 2017). Previous studies revealed that pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α might affect seizure activity through glutamatergic, GABAergic, and NMDA receptors (Młodzikowska-Albrecht et al., 2007; Roseti et al., 2015; Viviani et al., 2003). TNF- α is the most critical cytokine in the acute phase response to inflammation (Varela et al., 2018). Moreover, TNF- α exhibits a dual role in the pathophysiology of seizures, exerting pro-convulsive effects through TNF receptor 1 (TNFR1) and anti-convulsive effects via TNF receptor 2 (TNFR2) (Balosso et al., 2013). This may explain the conflicting results between the early stage (acute effect) inflammation and epilepsy.

There are not enough studies conducted with acute models and inflammation. Related studies showed that these conflicting results might be due to the ligand type, dosage, used epilepsy model, activated inflammatory regulators, the complexity of the inflammatory process, and the time of occurrence. In the present study, promising results were gained with ECoG recordings and behavioral observations in the PTZ-induced epilepsy model. Especially, licofelone appears to have more effects that are protective in the chronic model in terms of all parameters. As mentioned in the results section, licofelone decreased the seizure severity and total spike count while increasing the number of needed injections for kindling and time for FMJ. It is suggested that licofelone has anticonvulsant activity with all these parameters and is worth investigating with advanced studies. Compatible with our results, licofelone was examined in different experimental epilepsy models. In another study, licofelone has anticonvulsant activity at 10 mg/kg and above in mice in a PTZ-induced model (Payandemehr et al., 2015). Another study demonstrated that 10 mg/kg licofelone had similar activity in the Lithium-pilocarpine model (Eslami et al., 2016). However, while the two studies were compatible with our study, it was observed that not all of them had an altogether terminating seizure activity. There are also compatible studies with other COX inhibitors in the literature. Akula et al. reported that rofecoxib (2 and 4 mg/kg) increased the seizure activity threshold but not at 1 mg/kg in PTZ-induced epilepsy (Akula et al., 2008). Another study showed that 2 mg/kg celecoxib has protective effects against PTZ induced seizures (Oliveira et al., 2008). Dhir et al. found that nimesulide (2.5 mg/kg) and rofecoxib (2 mg/kg) increased the mean onset time of convulsions, decreased the duration of clonus, and decreased the mortality rate in bicuculline and picrotoxin-induced convulsions in mice while not in 1 mg/kg nimesulide and rofecoxib. On the other hand, these inhibitors did not affect the seizure activity in maximal electroshock-induced seizures (Dhir et al., 2006). Administration of PTZ may affect the brain-blood barrier (BBB), leading to the structure's disruption (Cacheaux et al., 2009). With this breakdown, especially in the PTZ-induced model, TGF- β , an inflammatory regulator, may have a key role in epileptogenesis.

Conflicting results with COX-2 inhibitors that act as a proconvulsant are confusing in epilepsy. Pretreatment with nimesulide augmented seizures and increased the mortality rate from approximately

10–69% (Kunz and Oliw, 2001). In the temporal lobe epilepsy of pilocarpine, parecoxib (10 mg/kg), a COX-2 inhibitor, has been found to cause neuronal damage in both the hippocampus and the piriform cortex (Polascheck et al., 2010). The opposite results can be seen by changing the injection time in the same study. Kunz and Olive examined pre-treatment and post-treatment of nimesulide (10 mg/kg) in the kainic acid-induced seizure. They found that nimesulide after the kainic acid had better effects on seizures compared to the pre-treatment of nimesulide (Kunz and Oliw, 2001).

The other group in the present study was the 5-LOX inhibitor, esculetin. According to the obtained data, the effects of esculetin were seen in ECoG activity. In behavioral parameters, the results were not statistically significant. However, esculetin exhibited anticonvulsant activity in this study. In the literature, studies with both esculetin and other 5-LOX inhibitors are very limited. In these studies, 5-LOX inhibitors were not effective in controlling seizures. However, 5-LOX inhibitors have protective effects when administered in combination with other COX inhibitors. Kim et al. examined the combined effects of COX inhibitor (aspirin) and 5-LOX inhibitors (NS-398 and esculetin) in a kainic acid-induced seizure model. They found that aspirin given with 5-LOX inhibitors has protective effects against neurotoxicity after the injection of kainic acid, but not given alone (Kim et al., 2008). In another study, Acetyl-11-Keto- β -Boswellic Acid (AKBA), a 5-LOX inhibitor, were not effective in kainic acid-induced seizure. When AKBA combined with other COX inhibitors, it increased the seizure latency (Bishnoi et al., 2007). Although esculetin was administered alone in our study, we think that the main effect of co-administered COX and 5-LOX inhibitors shows affinity to the COX pathway.

5. Conclusion

Epilepsy is the most common and important neurological disorder in the world. Inflammation is an important physiological process that is very critical in the body. The possible adverse effects of anti-inflammatory drugs should be considered, especially in patients with epilepsy. In the present study, both acute and chronic application of inhibitors revealed that inflammation pathways might have a vital role in the pathophysiology of epilepsy. Licofelone is more effective than esculetin. Licofelone will be beneficial in epilepsy in further studies where quantities and changes of pro-inflammatory cytokines will be determined.

Declarations

Ethical Approval

This study was approved by the Ethical Committee for Animal Experiments at Erciyes University (Approval number: 17/143)

Consent to Participate

N/A

Consent to Publish

N/A

Author Contribution

MT conceived and designed research. MT conducted experiments, analyzed data and wrote the manuscript. The author declares that all data were generated in-house and that no paper mill was used.

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Competing Interests

The author has no conflicts of interest.

Availability of data and materials

If requested, raw data will be presented as an excel file.

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Figures

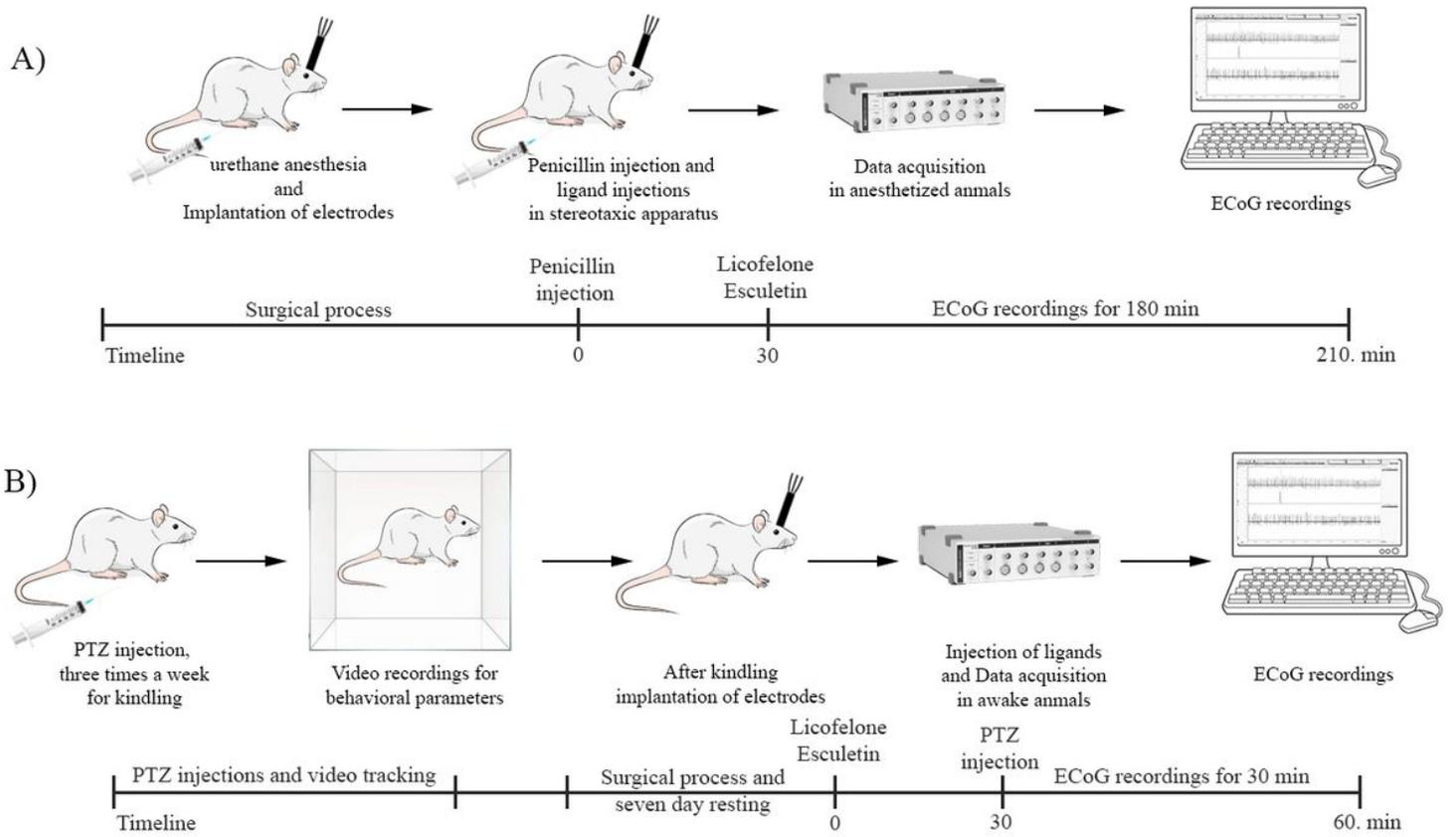


Figure 1

Experimental procedure. **A)** Penicillin-induced epilepsy. **B)** PTZ-induced epilepsy.

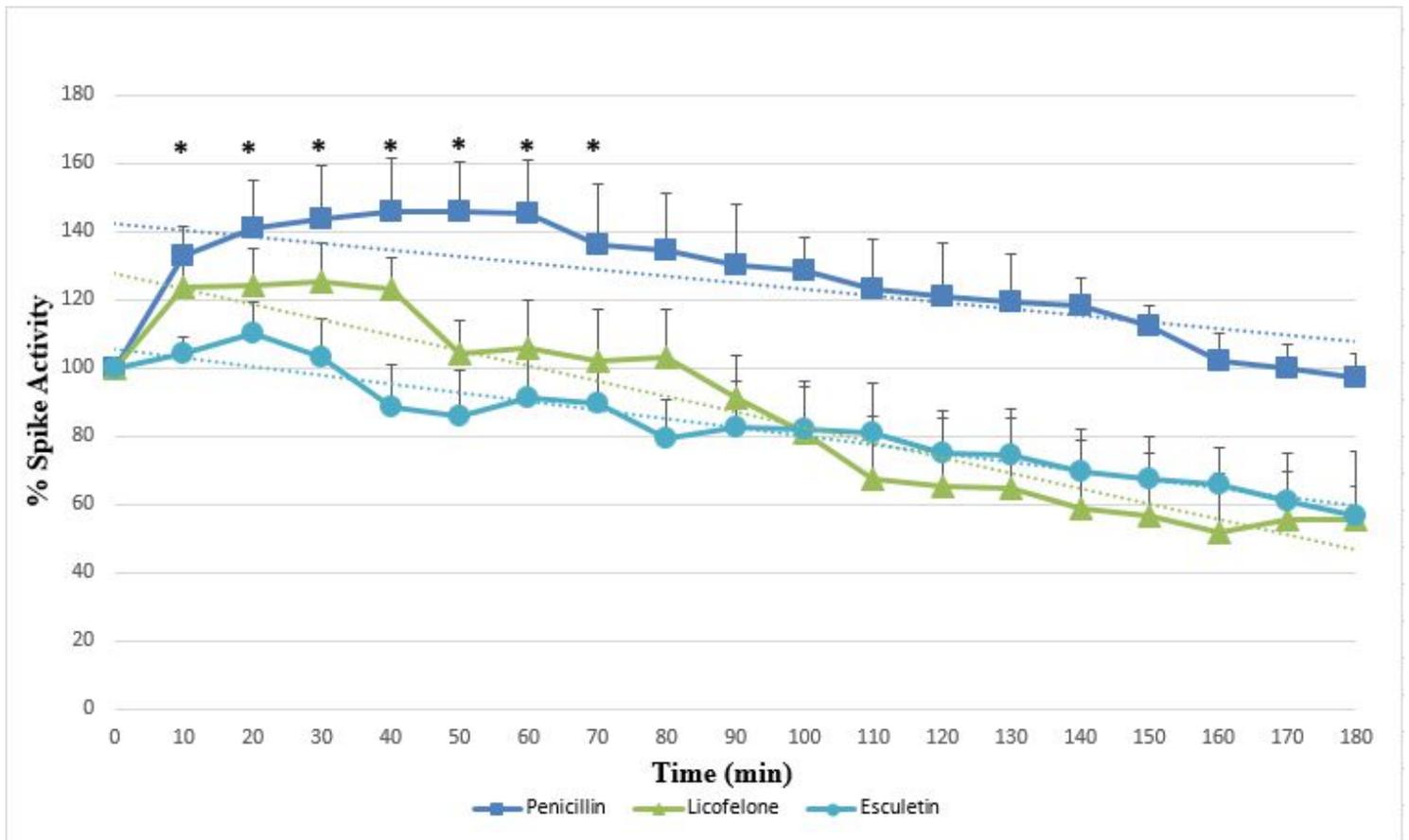


Figure 2

Mean spike frequency percentages of groups on penicillin-induced epilepsy. Licofelone and esculetin decreased the mean spike frequency of the epileptiform activity. Significant effects appeared between 0-70 min in licofelone and esculetin groups after the injection of penicillin. (*; in licofelone $p < 0.05$, *; in esculetin $p < 0.05$). The results are expressed as mean \pm standard error (SEM).

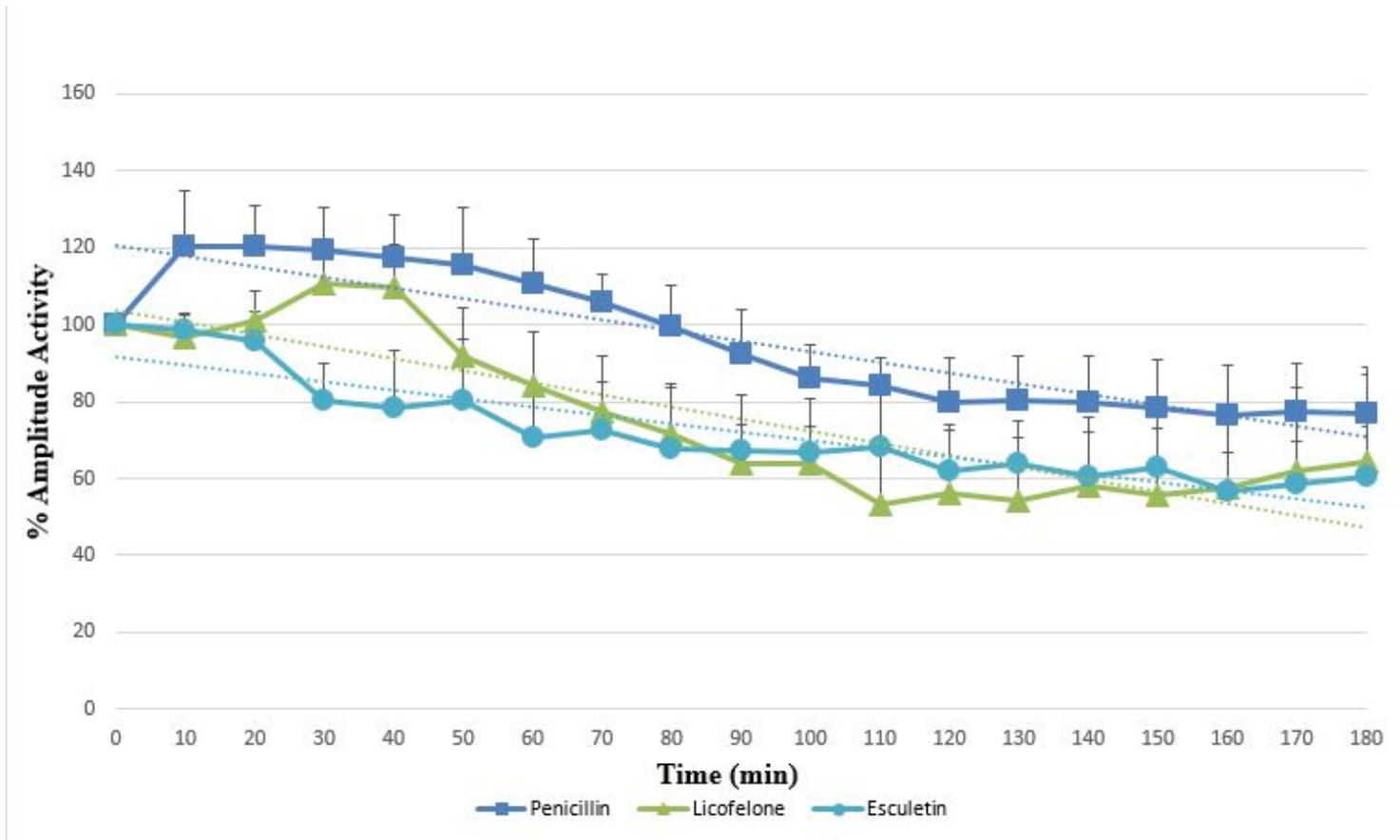


Figure 3

Mean amplitude percentages of groups on penicillin-induced epilepsy

Licofelone and esculetin decreased the mean amplitude activity for 180 min. No significant results were observed ($p > 0.05$). The results are expressed as mean \pm standard error (SEM).

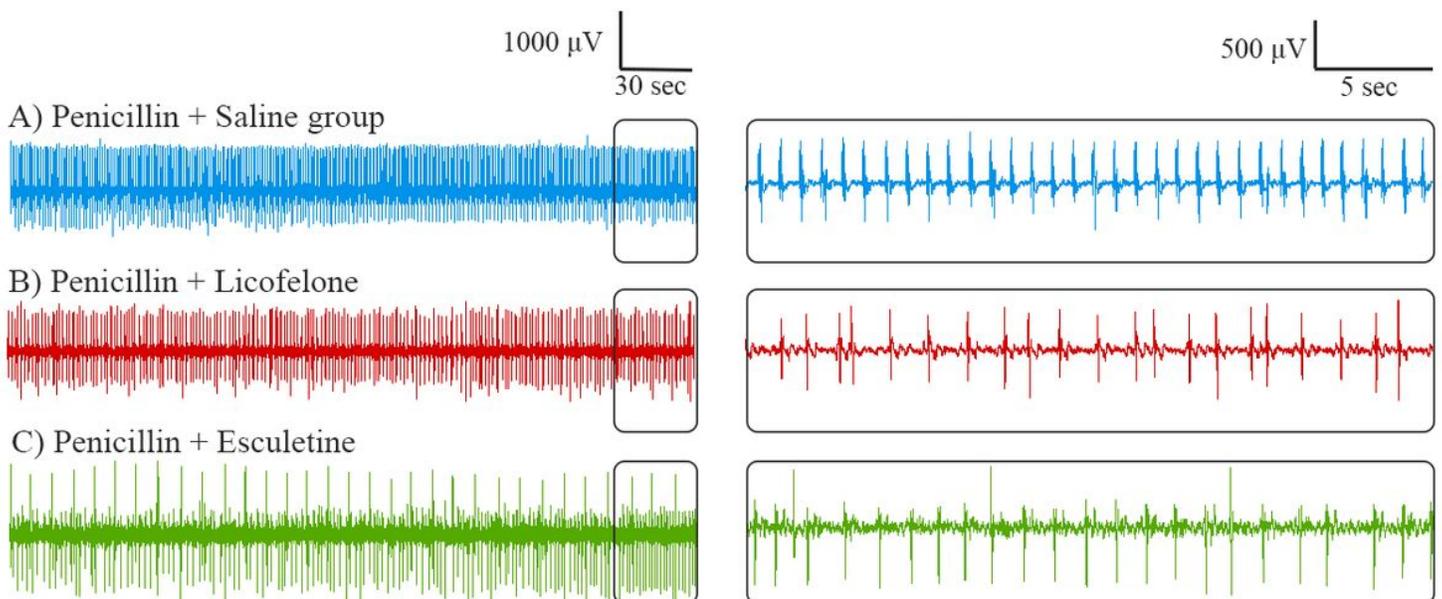


Figure 4

Representative traces of ECoG recording. (A) Penicillin + Saline (control group); (B) Penicillin + licofelone (20 mg/kg); (C) Penicillin + esculetin (20 mg/kg).

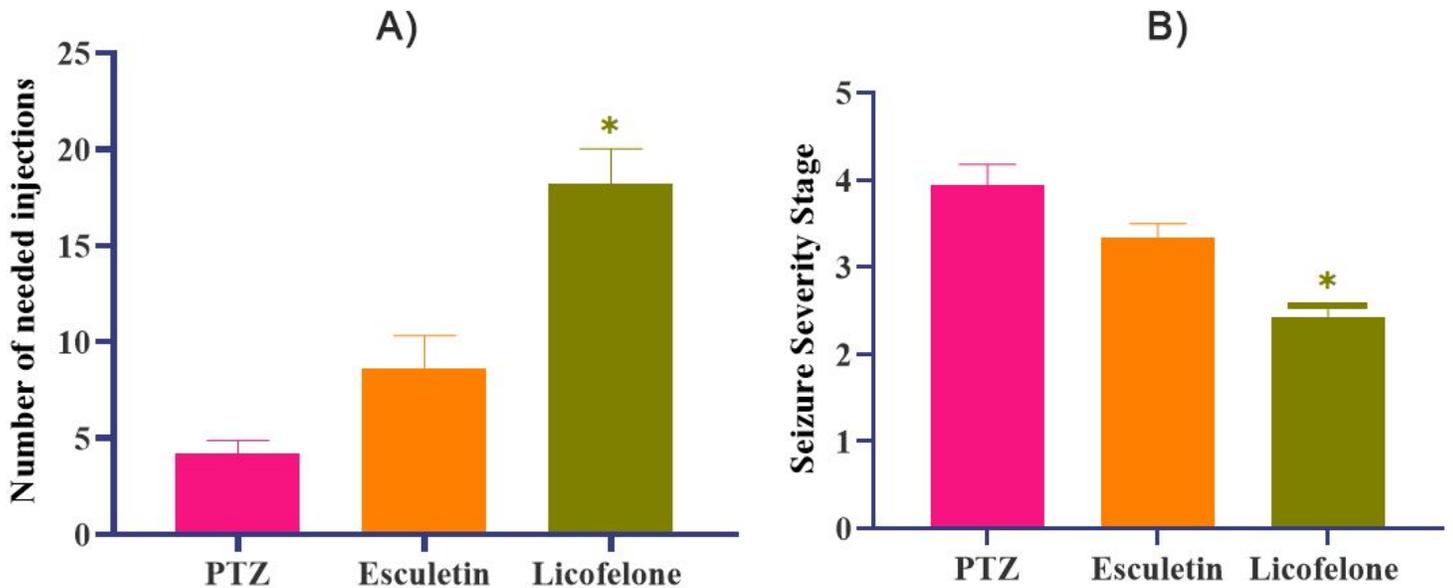


Figure 5

The number of PTZ injections and seizure severity score on PTZ-induced epilepsy. **A)** In the PTZ group, the animals were kindled with approximately five PTZ injections. Although the number of injections increased in the esculetin group, it was not statistically significant. Licofelone increased the number of needed injections for kindling (*, $p < 0.05$). **B)** Fischer and Kittner seizure scale. Although esculetin decreased the seizure severity stage, it was not statistically important. Licofelone decreased the seizure severity (*, $p < 0.05$). The results are expressed as mean \pm standard error (SEM).

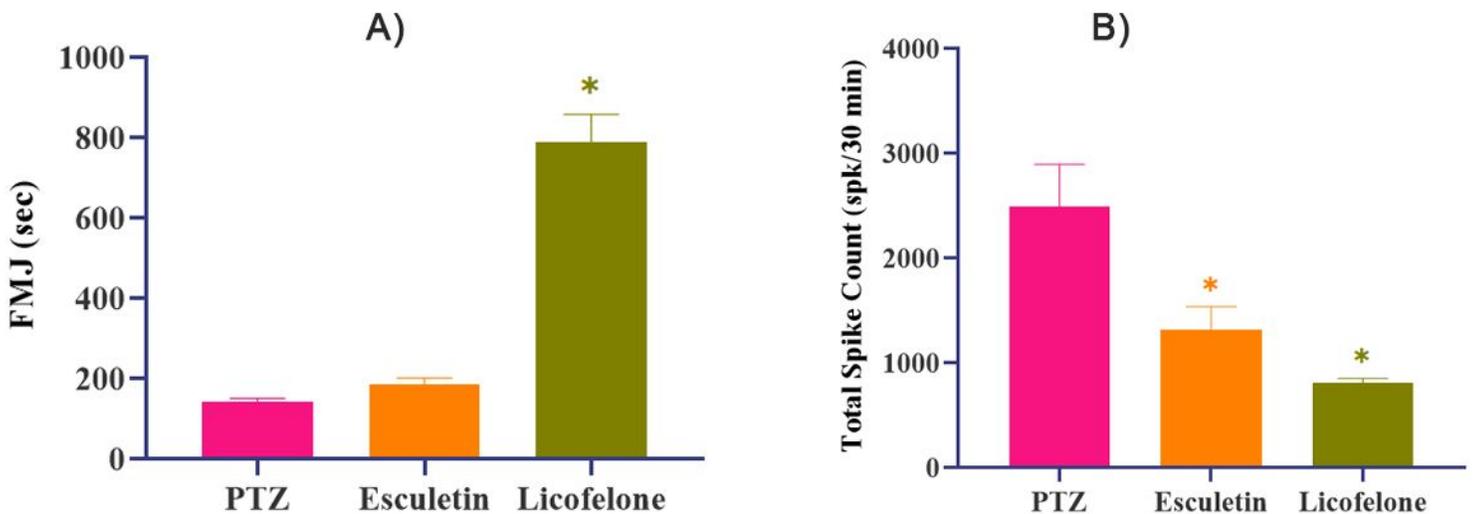


Figure 6

First myoclonic jerks (FMJ) and total spike count on PTZ-induced epilepsy. A) Time for first myoclonic jerks (FMJ). In the PTZ and esculetin groups, first myoclonic jerks were seen approximately 200 sec. after PTZ injection. Licofelone increased the time for the first myoclonic jerks at about 800 sec (*, $p < 0.05$). **B)** Total spike count for 30 min. Esculetin and Licofelone decreased the total spike count for 30 min compared to the PTZ group (*, $p < 0.05$). The results are expressed as mean \pm standard error (SEM).

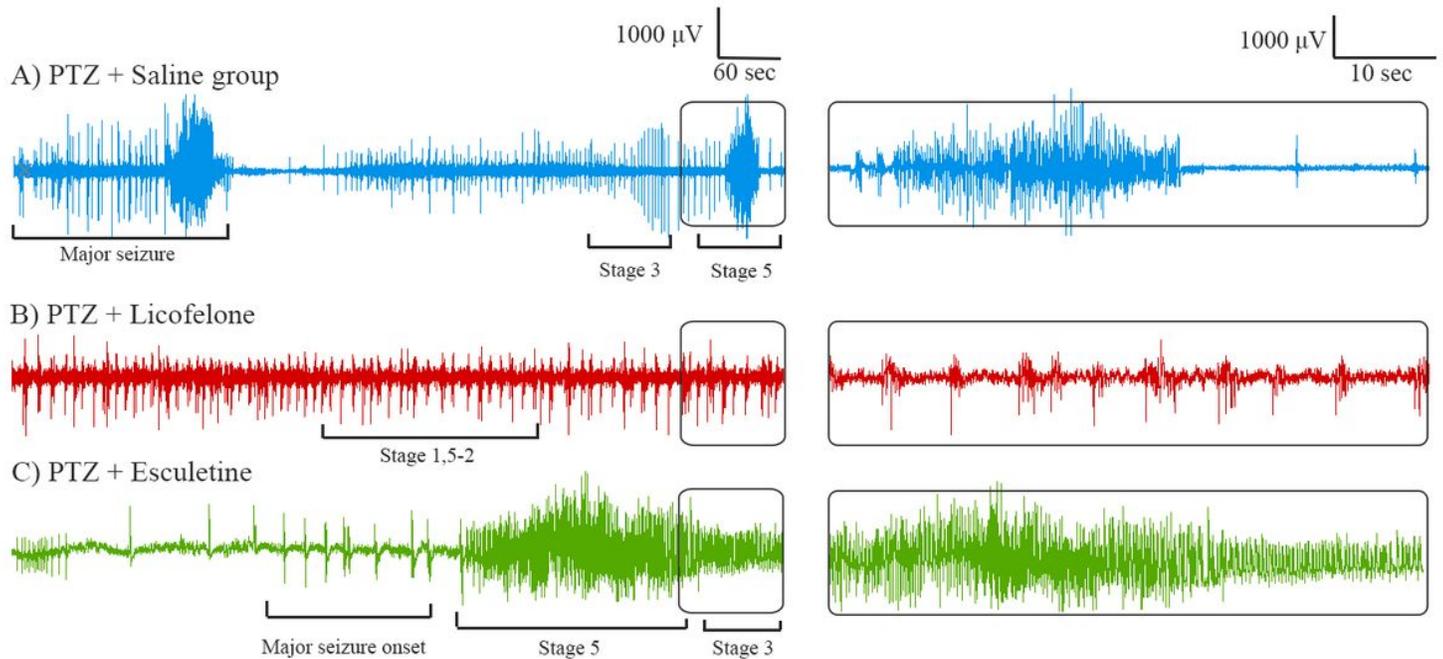


Figure 7

Representative traces of ECoG recording. (A) PTZ + Saline (control group); **(B)** PTZ + licofelone (20 mg/kg); **(C)** PTZ + esculetin (20 mg/kg).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [PTZRawData.pzfx](#)
- [PenicillinRawData.xlsx](#)