Original Research Paper

Electrophysiological evidence on epileptiform activity enhanced by electrical stimulation of teeth in rats

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The aim of this study was to evaluate the possible effects of electrical stimulation (ES) of tooth on penicillin-induced epileptiform activity in rats.

Experiment was realized on 24 adult male Sprague Dawley rats. Rats were assigned three groups [stimulation group (SG), penicillin group (PG), and penicillin + stimulation group (PSG)]. In SG, ES was only applied. Ten pulses of electrical current were delivered to the teeth for a duration of 2 milliseconds at 1-second intervals from a stimulator. Currents were applied in the range of 40–240 μA with 40 μA steps. Electrocorticography (ECoG) recordings were taken before and after ES. In PG, ECoG recordings were taken before and during the injection of penicillin. In PSG, after epileptiform activity was induced, ES was applied and ECoG recordings were taken as in SG. All the data were analyzed with Student's *t* test. Applied currents did not cause any epileptiform activity in SG. When the PSG was compared with the PG it was seen that the spike frequency of epileptiform activity increased in a statistically significant way after application of 240 μA ($P < 0.05$). On the other hand current application caused an increase in the spike amplitude of the PSG compared with the amplitude of the PG, but it was not statistically significant.

We concluded that ES of tooth with high current can trigger epileptiform activity in rats. For this reason, further research is required to evaluate the effects of ES of tooth for pulp testing on epileptic human subjects and antiepileptic drug users.

Keywords: Electric pulp testing, Pulp, Epilepsy, Epileptiform activity, Electrical stimulation

Introduction

The assessment of pulp vitality is an essential diagnostic procedure in endodontics. For this reason, several testing methods such as sensibility and vitality tests are available. Pulp sensibility tests include thermal tests (heat and cold stimuli), electric pulp tests, and test cavity and anesthetic tests.[1,2] The most commonly used tests in clinical practice are thermal and electric pulp tests. Pulp vitality tests include laser Doppler flowmetry, pulse oximetry, and tooth temperature measurement.[1] Pulp sensibility tests monitor pulp status by measuring the neural responses whereas pulp vitality tests measure the amount of vascular circulation. Electric pulp tests are based on stimulation of sensory nerves, and rely on subjective assessments and comments from the patient.[3] Electric pulp testers are widely used diagnostic tools for this purpose. They deliver a current sufficiently enough to overcome the thickness of enamel and dentin resistance and stimulate the myelinated sensory A-delta fibers at the pulpodentinal junction. The unmyelinated sensory C fibers of the pulp do not respond because greater current is needed to stimulate them.[4]

Epilepsy is a chronic neurological disorder characterized by recurrent spontaneous seizures caused by episodic abnormal electrical activity in the brain.[5] It affects 1–3% of the population and almost 10% of the population will have one or more seizures at some time in their life.[6] Most epileptic seizures are due to discharges generated in cortical and hippocampal structures, although subcortical structures are also involved in some seizure types.[7] Gamma-aminobutyric acid (GABA) is an important inhibitory neurotransmitter in the human brain that plays a major role in both physiological and pathological processes. Decrease of GABAergic inhibition may be involved in neuronal hyperexcitability, which can lead to seizures.[8] Several initiators (e.g. stress, sudden excitement, and emotional states) can trigger epileptic

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seizures in individuals with epilepsy.[5,9] Therefore, some actions performed during the dental treatment may be also one of these initiators.

To date, to our knowledge, there has been no literature published on the relationship between the electrical stimulation (ES) of pulp sensory nerves and epileptic seizures. The purpose of this study was to evaluate the possible effects of ES of tooth for pulp testing on penicillin-induced epileptiform activity in rats.

Material and Methods

Animals

The Declaration of Helsinki recommendations and internationally and locally accepted principles were followed for the care and use of the animals in this study. Twenty-four adult male Sprague Dawley rats weighing 225–280 g were used in this study. All described procedures were approved by the local ethics committee (KTU Animal Care and Ethics Committee, 2010/47). Animals were housed in groups of 3–4 and were allowed free access to food and water, except for the short time that the animals were removed from their cages for the experiments. All animals were kept in a temperature controlled ($22 \pm 1^{\circ}C$) environment on a 12-hour light/dark cycle.

Rats were randomly divided into the following groups. Each group was composed of eight rats.

Stimulation group ($n = 8$); ES was only applied. Two electrodes were used to stimulate the teeth of rats. Electrocorticography (ECOG) recordings were taken before and after stimulation.

Penicillin group ($n = 8$); penicillin was injected intracortically into the left sensorimotor cortex for initiating epileptiform activity. Electrocorticography recordings were taken before and during the injection of penicillin.

Penicillin + stimulation group ($n = 8$); after epileptiform activity was induced by penicillin, electrical stimuli were applied after the 40 minutes passing the penicillin injection and ECoG recordings were taken as in SG.

Induction of epileptiform activity

The animals were anesthetized with intraperitoneal injection of urethane (1.25 g kg^{-1}, i.p.) and additional doses were applied when necessary. After incision of the skull, the head of the animal was fixed by using a stereotaxic apparatus (Harvard Instruments, South Natick, MA, USA) (Fig. 1). The left cerebral cortex was exposed by craniotomy (5 mm posterior to bregma and 3 mm lateral to sagittal sutures). The epileptic focus was generated by 500 international units (IU) penicillin G potassium injection, 1 mm beneath the brain surface by a Hamilton microsyringe type 701 N (22s ga, bevel tip) with an infusion rate 0.5 μl/minute. During the experiment, liquid vaseline (37°C) was added drop by drop to the surface of the cerebral cortex. Body temperature was maintained at 37°C with a feedback-controlled heating system (Homeothermic Blanket Control Unit, Harvard Apparatus, MA, USA). All contact and incision points were infiltrated with procaine hydrochloride to minimize possible sources of pain.

Electrical stimulation of tooth

Electrical stimulation of teeth and the recording of epileptiform activity were carried out using an electrophysiological data acquisition system (PowerLab 16/30, AD Instruments, Castle Hill, Australia). Two alligator clip electrodes were used to stimulate the right maxillary and mandibular incisor teeth of rats. Each tooth was isolated with rubber-dam and dried thoroughly with cotton pellets prior to testing. The tip of the electrode was lightly coated with fluoride gel (APF gel thixotropic, Deepak Pro., Miami, FL, USA) and applied to the labial surface of the tooth in the incisal third, midway mesiodistally (Fig. 1). Ten pulses of electrical current were delivered to the incisor teeth for a duration of 2 milliseconds in duration at 1-second intervals, from a stimulator on a PowerLab 16/30 data acquisition system. *Currents* were applied in the range of 40–240 μA with 40 μA steps and 10 minutes of breaks in between.

Electrophysiological recordings

Electrocorticography recordings were made in urethane anesthetized animals. Two Ag–AgCl ball electrodes were placed over the left neocortex. The first electrode was placed on the primary motor cortex and its coordinates were 2 mm lateral to sagittal suture and 1 mm anterior to bregma. The second electrode was placed on the secondary visual cortex mediomedial area and its coordinates were 2 mm lateral to sagittal suture and 5 mm posterior to bregma. These recording electrodes were stabilized on the cortex surface by two different electrode holders (Fig. 1). The common reference electrode was fixed on the right pinna. Multi-channel data acquisition system (PowerLab 16/30, AD Instruments) was used to record the electrical activity of cortex. The signals from the electrodes were amplified and filtered with a 0.1–50 Hz bandpass filter (BioAmp, AD Instruments, Castle Hill, Australia). They were digitized at a sampling rate of 1024 Hz. All recordings were stored on a computer. The frequency and amplitude of epileptiform activity was analyzed off line. Spikes were automatically detected and counted using amplitude threshold detector and counting module of LabChart software (AD Instruments). Spike frequency was found by counting the number of spikes with amplitudes greater than three-fold baseline activity. Spike amplitude was measured automatically as the voltage change from peak to peak.

Based on previous experience, the following exclusion criteria were applied: (i) the spontaneously occurring spike discharges in the baseline ECoG

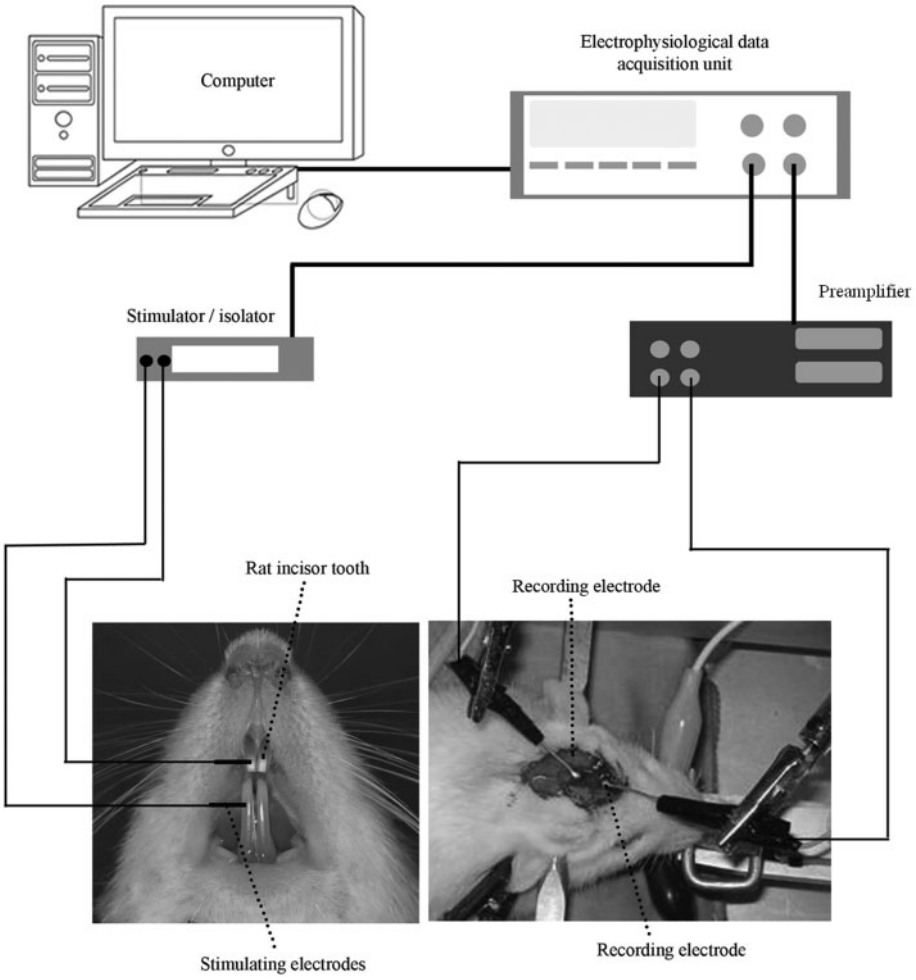


Figure 1 Demonstration of experimental set-up.

activity (ii) the ineffective penicillin injection for epileptiform activity (iii) superficial cerebral cortical hemorrhages, (iv) animal's death.

Statistical analysis

In the present study, no animals were excluded from the data analyses as they met the inclusion criteria. All statistical procedures were performed using SPSS (v16.0, SPSS Inc., Chicago, IL, USA) statistical software program. Statistical analysis was performed using Student's *t* test for independent samples. Values are expressed as the mean $\pm$ SEM. Statistical significance was set at $P < 0.05$.

Results

Representative ECoG recordings before and after penicillin administration or ES were showed in Fig. 2. Baseline ECoG activities of each animal recorded before the intracortical administration of penicillin or the application of ES did not have spike type activity (Fig. 2A). Currents at 40, 80, 120, 160, 200, and 240 μA did not cause epileptiform activity on animals in SG (Fig. 2B). Intracortical injection of penicillin (500 IU) induced an epileptiform ECoG activity characterized by bilateral spikes or spike wave complexes (Fig. 2C). The epileptiform activity began approximately within 3 minutes after penicillin application and lasted 3–5 hours It reached a constant level as to frequency and amplitude in maximally 30 minutes (Fig. 2C). Stimulations were also performed during epileptiform activity induced by penicillin in PSG (Fig. 2D). The means of spike frequency and amplitude were 28 ± 3 spike/minute, 1233 ± 283 μV, respectively at the 30 minutes after injection of penicillin in PG. After 55 minutes, the means of spike frequency and amplitude were 23 ± 2 spike/minute, 916 ± 218 μV, respectively (Tables 1, 2, and Fig. 3). Thirty minutes after penicillin injection and immediately prior to the 40 μA current applications, the means of spike frequency and amplitude were 26 ± 2 spike/minute, 1001 ± 167 μV, respectively in PSG. After 55 minutes of this measurement and then after 240 μA current application the means of spike frequency and amplitude were 29 ± 1 spike/minute, 1116 ± 210 μV, respectively (Tables 1 and 2). When PSG compared with the PG it was seen that the spike frequency of epileptiform activity increased in a statistically significant way after application of 240 μA ($t = 2.609$; $P = 0.021$, Fig. 2). On the other hand current application caused an increase in the amplitude of the spike of PSG, but it was not statistically significant.

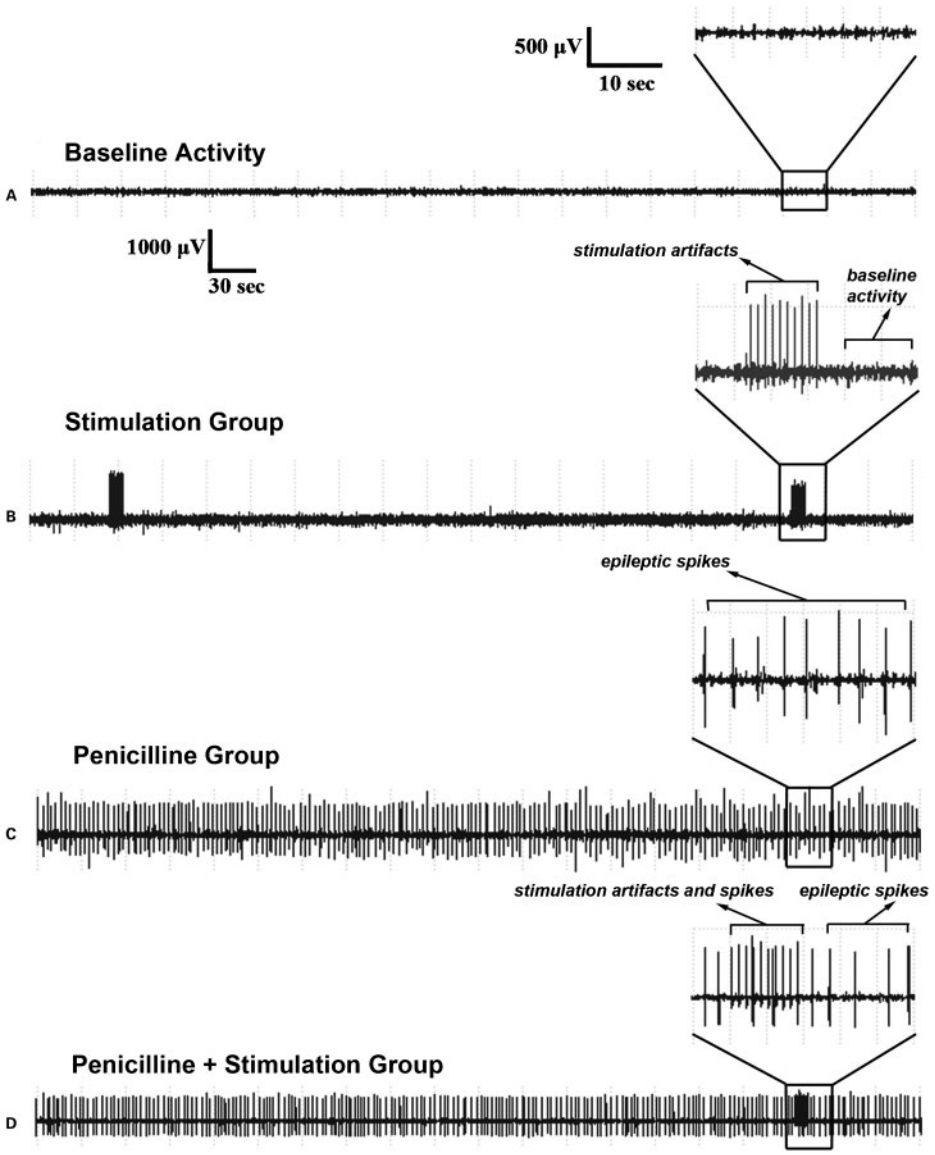


Figure 2 Representative electrocorticography (ECoG) recordings before and after penicillin administration or electrical stimulation (ES). (A) Baseline ECoG activity before the administration of intracortical penicillin or stimulation. (B) ECoG activities after ES of tooth in the stimulation group (SG). (C) Intracortical injection of penicillin (500 IU) induced epileptiform activity on ECoG in the penicillin group (PG). (D) ECoG activities after penicillin injection and ES in the penicillin+SG (PSG).

Discussion

Accurate diagnosis is very important for endodontists to correctly treat endodontic diseases. Various testing methods and tools have been developed for this purpose. One of them, electric pulp tests are based on stimulation of sensory nerves. Electric pulp tester is widely used tool for this testing. The principle of the electric pulp tester is to raise the electrical potential through the enamel and dentine into the pulp to provoke a measurable response from the pulp.[10] Electrical stimulation of nerves within the pulp depends on the rate of current increase, its power (voltage or current), duration, frequency, and current density.[2,10] To date, electric pulp testers have been used with different peak current intensity ranges of 200, 250 μA or 50 mA.[11–13] In this study, we used the current intensities in the range of 40–240 μA to stimulate the rat incisor teeth.

Studies[14,15] showed that epileptic patients tend to have poorer oral health and receive less adequate dental treatment in comparison with non-epileptic patients. The number of decayed and missing teeth, the degree of abrasion, and periodontal indexes are significantly worse in epileptic patients.[14,15] They also have significantly fewer restored and replaced teeth than the general population. For this reason, epileptic patients can refer to dental clinics for treatment of their dental problems. Clinicians must have some information and experience about epileptic patients because they have special needs during dental treatment. According to the literature, stress, sudden excitement, emotional states, sleep deprivation, fatigue, alcohol, fasting, high-dose caffeine intake, visual or other some sensory and reflex stimulus (e.g.: hot water, noise, and vibration), and menstruation can trigger epileptic seizures in individuals with

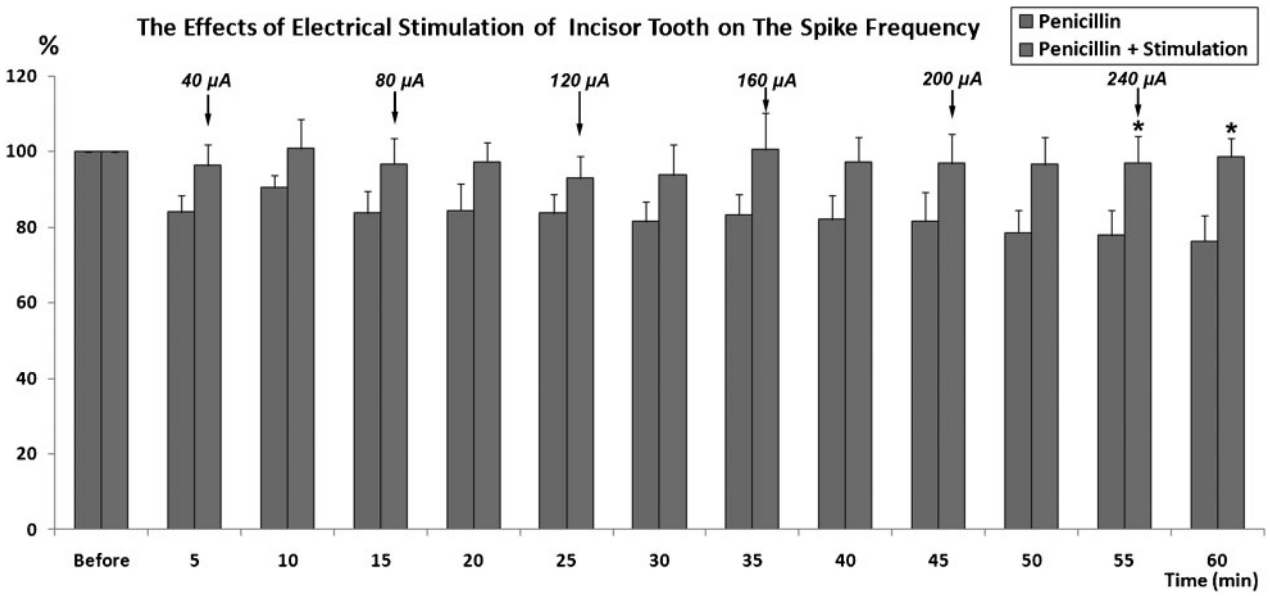


Figure 3 The effects of electrical stimulation (ES) of incisor tooth on the mean spike frequency of penicillin-induced epileptiform activity. When penicillin + SG (PSG) compared with the penicillin group (PG) it was seen the spike frequency of epileptiform activity increased in a statistically significant way after administration of 240 µA ($P < 0.05$). The percentage frequency of epileptiform electrocorticography (ECoG) activity value depends on both the frequency of epileptiform ECoG activity before and after the stimulation as it is defined as: Frequency Value% = *The mean of spike frequency after substance administered* × 100 The mean of spike frequency before substance administered.

epilepsy.[5,9] To minimize the risk of an epileptic seizure during a dental visit we must avoid the initiator dental actions as mentioned above. Electrical stimulation of tooth for pulp vitality can be one of the initiator to trigger an epileptic seizure. To our knowledge, there has been no literature published on the relationship between the ES of pulp sensory nerves and epileptic seizures. The purpose of this study was to evaluate the possible effects of ES of tooth for pulp testing on penicillin-induced epileptiform activity in rats. According to our results, applied currents did not cause any epileptiform activity in SG. When PSG compared with the PG it was seen that the spike frequency of epileptiform activity increased in a statistically significant way after application of 240 µA. On the other hand current application caused an increase in the spike amplitude of PSG, but it was not statistically significant.

In this study, rubber-dam cover was used to isolate the teeth prior to testing to ensure pulpal rather than neighbors and the gingivae nerve fibers stimulation. Testing with an electric pulp tester requires electrode contact onto tooth surface. The tooth region of high neural density will have a relatively fast and strong response and require the least electric current.[16] In

Table 1 The effects of electrical stimulation (ES) of incisor tooth on the spike frequency

Time (minutes)	Stimulation (µA)	Penicillin group (spike/minute)	Penicillin + stimulation group (spike/minute)
Before	Without	28 ± 3	26 ± 2
5	40	23 ± 2	25 ± 2
15	80	22 ± 2	25 ± 2
25	120	22 ± 1	24 ± 2
35	160	22 ± 1	26 ± 3
45	200	22 ± 2	25 ± 3
55	240	23 ± 2	29 ± 1*

Statistical analyses were performed using Student's *t* test. Values are expressed as mean ± SEM.

Table 2 The effects of electrical stimulation (ES) of incisor tooth on the spike amplitude

Time (minutes)	Stimulation (µA)	Penicillin group (µV)	Penicillin + stimulation group (µV)
Before	Without	1233 ± 283	1001 ± 167
5	40	1278 ± 325	991 ± 165
15	80	1073 ± 233	1025 ± 178
25	120	1048 ± 247	1001 ± 177
35	160	1012 ± 229	1047 ± 179
45	200	1004 ± 232	1073 ± 184
55	240	916 ± 218	1116 ± 210

Statistical analyses were performed using Student's *t* test. Values are expressed as mean ± SEM.

permanent teeth, pulp horns contain the highest concentration of neural elements, which decrease gradually in cervical and radicular regions of the pulp.[16,17] Studies[17,18] have reported the appropriate electric probe placement positions for each tooth group (anterior teeth, premolars, and molars). Bender *et al.*[18] showed that placing the electrode at the incisal edge of anterior teeth evoked a response with the least amount of electrical current. In this study, the tip of the electrode was placed as Bender *et al.*[18] did and fluoride gel was used for conductance.

Experimental epilepsy models for the development of antiepileptic drugs and epilepsy research have played a very important role.[19] Systemic and local application of penicillin G is an experimental model commonly used to produce epileptiform activity in cerebral cortex.[20,21] Penicillin is a broad spectrum antibiotic that is effective against a number of pathogenic bacteria. Epileptogenic effects of penicillin were first reported in 1945 by Johnson and Walker.[22] It causes epileptic activity by selectively blocking GABA-associated postsynaptic inhibition.[23] Application of penicillin to the neocortex results in synchronous discharge of neurons, which bears an electrophysiological resemblance to human focal interictal epileptic discharges.[24] Interictal epileptiform discharges can occur in various forms, such as spikes, polyspikes, and sharp waves and are believed to be the result of summated membrane events from abnormally hypersynchronous neurons within epileptic tissue.[25] Therefore, we used penicillin-induced epilepsy model to evaluate the possible effects of ES of tooth for pulp testing on penicillin-induced epileptiform activity in rats in the present study.

Conclusion

Within the limitations of this study, it was found that ES of tooth with high current can trigger epileptiform activity in rats. This study was an animal study, thus it may not reflect the clinical situations. For this reason, further researches are required to evaluate the effects of ES of tooth for pulp testing on epileptic human subjects and antiepileptic drug users.

Disclaimer statements

Contributors Kürşat Er: executive, experiment, writing. Mehmet Yildirim: experiment, writing. Tamer Taşdemir: experiment. Metahan Akca: experiment. Ismail Abidin: experiment.

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Conflicts of interest The authors have no declared financial interests in any company manufacturing the types of products mentioned in this article.

Ethics approval All described procedures were approved by the local ethics committee (KTU Animal Care and Ethics Committee, 2010/47).

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