Aim: To investigate the effects of a magnetic field (MF) on febrile seizure latency, seizure duration, and electroencephalographic (EEG) recordings in a rat febrile convulsion model.

Materials and methods: Thirty-six rats were randomly allocated into 1 of 6 groups: sham group (S), febrile convulsion (FC) group without MF exposure, MF group without FC, group exposed to MF before FC (MF + FC), group exposed to MF after FC (FC + MF), and group exposed to MF before and after FC (MF + FC + MF). The rectal temperature after febrile seizure induction, seizure latency, seizure duration, and EEG recordings were recorded for all animals.

Results: Repeated hyperthermic exposure decreased the seizure latency and duration. The effect of the MF was more prominent on seizure duration than on latencies. MF exposure for 10 or 12 days increased seizure latency. MF exposure increased the pathologic theta and delta waves and decreased the beta waves, which are frequently seen in awake animals.

Conclusion: Our results suggest that MF exposure has a negative effect on brain waves, and this effect becomes more evident with prolonged exposure. On the other hand, MF exposure significantly decreased the convulsion durations.

Key words: Febrile convulsion, magnetic field, electroencephalographic recordings

1. Introduction
Epilepsy is one of the most common and serious neurological conditions, characterized by recurrently and repeatedly occurring seizures (1,2). Among these, febrile convulsions (FCs) are the most common seizure type during childhood, affecting 2%–3% of children between the age of 3 months and 5 years (3). On the other hand, it has been shown that 40% of adult patients with hippocampal sclerosis-associated temporal lobe epilepsy have a history of FC, suggesting a causative role for FC that increases the importance of FC treatment (4,5).

Although new antiepileptic drugs have been presented in recent decades, more than 30% of patients with epilepsy are still inadequately treated by currently available medications (6–8). Thus, new novel treatment modalities such as epilepsy surgery, vagus nerve stimulation, ketogenic diet, and magnetic fields (MFs) have received great interest recently (9–12). To our knowledge, many studies with unclear stimulation protocols have been performed to investigate the effects of MF stimulation on different epilepsy models (9,13–15), but there is no research on febrile seizures. Thus, in this study we aimed to investigate the effects of a MF on febrile seizure latency, seizure duration, and electroencephalographic (EEG) recordings in a rat febrile convulsion model.

2. Materials and methods
Although the absolute equivalence in age between rat and human brains is not clear, 5- to 7-day-old rats are suggested to be equivalent only to a full-term newborn infant; the 15-day-old rat brain is equivalent to a human brain at a few months to 1 year old (16), and the 28- to 30-day-old rat to a 2-year-old child (17). Thus, in this study, we used 17-day-old male Wistar albino rats, and they were anesthetized with a combination of xylazine (3 mg/kg) and ketamine (90 mg/kg) given subcutaneously. Following anesthesia, a small area on the top of the rat's head was shaved and the area was cleaned with Betadine.
The rat was placed into a small animal stereotaxic apparatus. After a small midline incision was made on the top of the head, the periosteum was removed and stainless steel screw electrodes were implanted on the dura mater over the cortex, 2 in the frontal region (coordinates with skull surface flat and bregma zero-zero: AP + 1.9; L ± 1.5; 1.5 mm below the dura mater) and a third on the occipital region (Figure 1). Electrodes were attached to the skull with dental acrylic (Figure 1). Following surgery, animals were housed in a temperature-controlled facility of 23 ± 2 °C, with a 12-h light/dark cycle (0700 to 1900 hours) and free access to water and food until the 22nd day. All procedures were approved by the Cumhuriyet University Animal Ethics Committee (Sivas, Turkey) and were conducted in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.1. Febrile convulsion model
A febrile convulsion model was conducted, starting from day 22, for 20 days, applied every other day, which resulted in 10 trials in total. Exposure to hyperthermia was carried out in a glass chromatography tank (30 × 30 × 60 cm) that contained water to a depth such that the animal could stand up-right supported by the side of the tank with only its head above the water level. Exposure to hyperthermia was achieved by maintaining the water in the tank at a temperature of 45 °C by placing it in a temperature-controlled water bath. This temperature (45 °C) does not produce skin damage at exposures of less than 1 h (17). The rats were placed in the water unrestrained for 4 min or until a seizure occurred, once every 2 days, for a total of 10 times (18). The rats in the sham group were exposed to 37 °C water without any FC signs. After that, the rats were immediately removed from the water and placed in an observation chamber at the first sign of seizure onset. The core temperature of the rat was measured before and immediately after exposure to hyperthermia with a lubricated thermistor probe (model BAT-12, Physitemp) inserted into the rectum. The latency to seizure was measured as the interval between the moment the rat was placed in the water and the first sign of seizure onset (usually a myoclonic spasm), and 10 seizure latencies were compared. The seizure duration was measured as the time from seizure onset to the instant when the rat first righted itself and appeared conscious. The state of consciousness was determined by the responsiveness of the rats to one or all of several stimuli (tapping on cage, loud clap, responsiveness to touch, and the movement of a small object before its eyes). At the end of the period of observation, the rat was gently towed dry, placed beneath a heating lamp until its fur appeared free of moisture, and

Figure 1. Stereotaxic EEG recording preapplication. A) Preparation of animal before the operation. B) Three special screw electrodes. C) The screws attached to wires. D) Cable terminators joined to the connector. E) The animal head recovered with acrylic. F) The animal prepared for EEG recording.
then returned to its home cage. The intensity of the seizure was recorded according to the rating scale described by Jiang et al. (17) as follows: 0, no convulsive behavior; 1, facial clonus; 2, head nodding; 3, forelimb clonus; 4, rearing (animal in a standing posture aided by tail and the laterally spread hind limbs showing increased tone); and 5, rearing and falling back. The seizure latency and durations of a total of 10 seizures were compared between all groups.

2.2. Magnetic field exposure
Thirty-six rats were randomly assigned to 1 of 6 groups: sham (S), FC group without MF exposure (FC), MF group without FC (MF), group exposed to MF before FC (MF + FC), group exposed to MF after FC (FC + MF), and group exposed to MF before and after FC (MF + FC + MF). The rats in the MF, MF + FC, FC + MF, and MF + FC + MF groups were exposed to MFs. Every rat in these experimental groups was exposed to a MF every day for 20 days. The rats in the S group were also put in a Plexiglas cage every day with no MF exposure. All devices that could affect the MF were kept away from the experimental area and all rats were fed ad libitum.

Rats were exposed to a MF with 5.0 mT intensity and 50 Hz frequency in a special Plexiglas cage (40 × 17 × 13 cm) (19). The MF was generated in a specially designed device that had a solenoid 500 mm in length and 210 mm in diameter. This magnet was constructed by winding 1400 turns of an insulated soft copper wire, which was 1.4 mm in diameter, on a fiber base (19,20). A MF intensity of \( B = 5 \) mT was measured inside the solenoid using a digital teslameter (Phywe, Gottingen, Germany) with an axial Hall probe (Phywe). The solenoid was always kept in a north-south direction, and its temperature was maintained constant at 25 ± 2 °C. The MF in the solenoid was calculated based on an analytical solution of the field equation derived from the Biot–Savart law. The formula

\[ B = \frac{Ni}{\sqrt{R^2 + L^2}} \]

was used to calculate the MF intensity (19). Using the conditions for the experiment of \( N = 1400 \) turns, \( i = \) current in the device, \( R = 210 \) mm, and \( L = 500 \) mm will give \( B = 5 \) mT.

An electric current (50 Hz) was passed through the device and a time relay was added into the system. In this way, rats were exposed to the alternating MF for 30 min, maintained every 15 min during 540 min of daily exposure. The magnetic exposures were carried out for a duration of 540 min each day between 0800 and 1700 hours.

2.3. Electroencephalographic recordings
EEG recordings were taken for 4 h on the 22nd day and after a total of 20 days MF exposure (IOX Software, version 2.3.2.14, EMKA Technologies, Radon, Ankara, Turkey). The beta, alpha, theta, and delta waves of EEG recordings were analyzed, and power ratios (% total) were calculated and compared.

2.4. Data analysis
Experimental values were presented as means ± SEMs and analyzed by Mann–Whitney U tests and Wilcoxon tests, and by Friedman test when appropriate. A P-value of less than 0.05 was considered to indicate significance. All statistical analyses were performed using SPSS 14.0.

3. Results
3.1. Rectal temperature
The rectal temperature was 36.9 ± 0.3 °C before the febrile seizures and 43.8 ± 0.4 °C immediately after the febrile seizure. The rectal temperature of all groups before febrile seizure was not significantly different. However, the rectal temperature after febrile seizure of the S group was significantly lower than that of all experiment groups (\( P < 0.05 \); Figure 2).

3.2. Seizure latency
There was no significant difference between the latency of the 1st seizures of all groups. The latency of the 2nd seizure in the FC + MF and MF + FC groups was significantly decreased compared with the FC and MF + FC + MF groups (\( P < 0.05 \)). The latency of the 3rd seizure of the MF + FC + MF group was significantly higher than that of the FC + MF group (\( P < 0.05 \)). The latency of the 4th seizure of the FC and FC + MF groups was significantly higher than that of the MF + FC and MF + FC + MF groups (\( P < 0.05 \)). The latencies of the 6th and 7th seizures of the FC group were lower than those of

Figure 2. The rectal temperature of rats before and after the febrile seizure model. a: \( P < 0.05 \), statistically different from the first rectal temperatures of the same group. b: \( P < 0.05 \), statistically different from the first rectal temperatures of the other groups. c: \( P < 0.05 \), statistically different from last rectal temperatures of other experimental groups.
The comparisons of seizure latencies of the FC + MF, MF + FC, and MF + FC + MF groups (P < 0.05). The latencies of 8th and 9th seizures of the FC group were lower than those of the FC + MF group (P < 0.05). The latency of the 10th seizure of the FC + MF group was significantly higher than that of the FC and MF + FC + MF groups (P < 0.05) (Figure 3).

A comparison of seizure latencies of the FC group with each other is shown in Figure 4A. The latency for the 6th seizure was significantly lower than all other latencies except the 9th and 10th latencies (P < 0.05). The 1st latency was significantly higher than the 7th, 8th, and 9th latencies (P < 0.05). The 10th latency was significantly lower than all other latencies except the 3rd, 6th, and 9th latencies (P < 0.05; Figure 4A).

The comparison of seizure latencies of the FC + MF group with each other is shown in Figure 4B. The 1st latency was higher than the 2nd, 3rd, 5th, 9th, and 10th seizure latencies, but lower than the 6th latency (P < 0.05). The 2nd latency was statistically lower than the 4th, 5th, 6th, 7th, and 8th latencies (P < 0.05). The 3rd latency was significantly lower than the 4th, 6th, and 7th latencies (P < 0.05). The 4th latency was significantly higher than the 5th but significantly lower than the 6th latency (P < 0.05). The 5th latency was only significantly lower than the 6th latency (P < 0.05). The 6th latency was significantly higher than the 7th and 8th latencies (P < 0.05). The 8th latency was significantly higher than the 10th latency (P < 0.05; Figure 4B).

The comparison of seizure latencies of the MF + FC group with each other is shown in Figure 4C. The 6th latency in this group was significantly higher than all other latencies except the 1st and 5th latencies (P < 0.05). The 10th latency was significantly lower than the 1st, 5th, and 6th latencies (P < 0.05; Figure 4C).

The comparison of seizure latencies of the MF + FC + MF group with each other is shown in Figure 4D. The 1st latency in this group was significantly higher than the 4th and 10th latencies (P < 0.05). The 2nd latency was statistically higher than the 4th, 9th, and 10th latencies (P < 0.05). The 3rd latency was significantly lower than the 6th latency but significantly higher than the 10th latency (P < 0.05). The 4th latency was significantly higher than the 6th and 7th latencies (P < 0.05). The 5th latency was statistically lower than the 6th latency, and the 6th latency was statistically higher than the 8th, 9th, and 10th latencies (P < 0.05). The 7th latency was significantly higher than the 5th and 9th latencies (P < 0.05; Figure 4D).

3.3. Seizure duration
There was no significant difference between the durations of the 1st seizures of all groups. All other seizure durations (except the 3rd duration) of the FC group were significantly higher than in the FC + MF, MF + FC, and MF + FC + MF groups (P < 0.05). The 3rd, 5th, and 6th seizure durations of the FC + MF group were significantly lower than in the MF + FC and MF + FC + MF groups (P < 0.05). In addition, the 4th durations of the FC + MF group and the
MF + FC + MF group were higher than in the MF + FC and FC + MF groups, respectively (P < 0.05) (Figure 5).

A comparison of seizure latencies of the FC group with each other is shown in Figure 6A. The 1st seizure duration was significantly lower than all other groups except the 2nd and 3rd durations (P < 0.05). The 2nd duration was significantly lower than all other groups except the 1st, 3rd, and 6th groups (P < 0.05; Figure 6A).

A comparison of seizure latencies of the FC + MF group with each other is shown in Figure 6B. The 3rd duration was statistically lower than the 1st and 10th durations (P < 0.05). The 4th duration was statistically lower than the 1st and 10th durations (P < 0.05). The 5th duration was statistically lower than the 9th and 10th durations (P < 0.05; Figure 6B).

A comparison of seizure latencies of the MF + FC group with each other is shown in Figure 6C. The 3rd duration was significantly lower than the 7th, 9th, and 10th durations (P < 0.05). The 4th duration was significantly lower than the 9th and 10th durations (P < 0.05; Figure 6C).

A comparison of seizure latencies of the MF + FC + MF group with each other is shown in Figure 6D. The 3rd duration was significantly lower than the 9th and 10th durations (P < 0.05). The 4th duration was significantly lower than the 9th and 10th durations (P < 0.05).
significantly lower than the 10th duration (P < 0.05; Figure 6C).

A comparison of seizure latencies of the MF + FC + MF group with each other is shown in Figure 6D. The duration of the 1st seizure was significantly higher than the 2nd and 3rd durations (P < 0.05). The 2nd duration was significantly higher than the 3rd (P < 0.05; Figure 6D).

3.4. EEG recordings

There was no significant difference between the first beta power ratios of all experimental groups. The last beta power ratios of all experimental groups were significantly decreased compared with their first beta power ratios (P < 0.05). The last beta power ratio of the S group was significantly different from those of all groups (P < 0.05). The last beta power ratio of the MF + FC group was significantly lower than in the MF group (P < 0.05). The last beta power ratio of the MF + FC + MF group was significantly lower than in the S, FC, MF, and FC + MF groups (P < 0.05; Figure 7A).

There was no significant difference between the first alpha power ratios of all groups. The last alpha power ratios of all experimental groups were significantly increased compared with their first alpha power ratios (P < 0.05). The last beta power ratio of the S group was significantly different from those of all other groups (P < 0.05). The last alpha power ratio of the MF + FC group was significantly lower than in the S, FC, MF, and FC + MF groups (P < 0.05; Figure 7A).

There was no significant difference between the first theta power ratios of all groups. The last theta power ratios of the S, FC, MF, and FC + MF groups were significantly increased compared with their first beta power ratios (P < 0.05). The last theta power ratio of the MF + FC + MF group was significantly higher than in all other groups (P < 0.05) (Figure 7C).

There was no significant difference between the first delta power ratios of all groups. The last delta power ratios of the FC + MF, MF + FC, and MF + FC + MF groups were significantly increased compared with their first beta power ratios (P < 0.05). The last delta power ratios of the S, FC, MF, and FC + MF groups were significantly different from the last delta power ratio of the S group (P < 0.05). The last delta power ratio of the MF + FC group was significantly different from the MF group (P < 0.05). The last delta power ratio of the MF + FC + MF group was significantly different from those of the S, FC, and MF groups (P < 0.05) (Figure 7C).

In the EEG recordings of all groups, there was a dominance of beta waves (92.5 ± 1.6%). The other waves were seen in decreasing ratios as alpha (5.5 ± 1.4%), theta (1.9 ± 0.5%), and delta (0.1 ± 0.1%). In all experimental groups, the ratios of beta waves were decreased, whereas alpha, theta, and delta waves were increased during the experiment. This effect was more prominent in both the FC- and MF-exposed groups.

4. Discussion

One of the most prevalent seizure types during childhood is the febrile seizure, which is usually benign (21,22).
However, it has been shown that these seizures facilitate the development of temporal lobe epilepsy (23,24), and this increases the importance of explaining the pathophysiology of febrile seizures and developing new novel treatment methods to replace inadequate medical treatments. In this study, we investigated the effects of MFs on rectal temperature, febrile seizure latency, seizure duration, and EEG recordings in a rat febrile convolution model.

Tanabe et al. reported rectal temperatures of 75% of their patients at the time of a FC as over 39 °C, and for the remaining 25% as over 40.2 °C (25). The triggering factor for the seizure was the reaching of the highest body temperature, rather than the increasing rate of it (26). The rectal temperatures of all experiment groups were significantly increased in the present study and we thought that the sudden decrease of the rectal temperature after a febrile seizure showed the triggering effect of temperature on febrile seizures.

There are conflicting studies about the effects of MFs on the duration of febrile seizures. Although Klauenberg et al. (27) and Ossenkopp et al. (28) showed the shortening effect of MF on the seizure duration, Keskil et al. (29) found no relation between them. In another study (30), using a similar method to that in our present study, it was shown that the seizure latency decreased but the seizure duration increased. In contrast, there are studies showing the febrile seizure duration increasing (17) or remaining unchanged (23) according to increased seizures. In our study, repeated hyperthermic administration facilitated febrile seizure development, prolonged the seizure duration, and worsened the seizure grades. Additionally, a total of 20 days
of exposure to a MF did not change the seizure latency. Nevertheless, the fact that a MF significantly increased the seizure latency at the 12th day of the experiment shows the maximum effect of MF for 6 exposures. The MF decreased the febrile convulsion duration and this effect was more obvious than its effect on seizure latency.

The similarity of EEG recordings between rats and human has been shown. The EEG recordings of children and young rats showed a high voltage slow wave before convulsions, a wide spike and slow wave with minor symptoms, and rapid rhythmic bursts during convulsions (31). Carpentier et al. (32) reported an increase in the delta band, showing the relationship between cerebral lesion and neuronal loss to be a reliable determinant. In a kindling model, alpha and theta waves were increased in all brain regions except for theta waves in the hippocampus. They suggested the epileptic discharges leading to the thalamus-related facilitations as the cause of increased alpha waves (33). In another study investigating the physiology of increased body temperature, high voltage theta waves at 4–5 Hz originating from parietal and occipital lobes and spreading to all other regions were seen (31). Suppressed and decelerated EEG activities were also recorded after hyperthermic seizures (31) and irregular 5–8 Hz theta waves have been recorded before this seizure type (34).
In the present study, we found a significant increase in alpha, delta, and theta waves, especially in the MF-exposed FC group. Repeated hyperthermic exposure decreased the seizure latency and duration. The effect of a MF on seizure duration was more obvious than the effect on latency. MF exposure for 10 or 12 days increased the seizure latency, but this effect diminished after prolonged exposure for more than 12 days and reversed to control values after 20 days of exposure. Because of the lack of an explanation as to the reason for this effect and due to the limitations of our study, further studies are needed to investigate the effects of MFs with short durations and different intensities on seizure latency. MF exposure increased the pathologic theta and delta waves and decreased the beta waves that are frequently seen in awake situations.

In conclusion, MF exposure has a negative effect on brain waves, and this effect seems to be increased with prolonged exposure. Application of a MF caused a significant decrease in seizure durations. The effect of a MF on seizure duration was more prominent than its effect on latency. This effect was pronounced after long-term application of a MF. Further studies investigating the effects of MFs in the short-term and with different intensities are warranted.

References


