

# Bursts of high-frequency repetitive transcranial magnetic stimulation (rTMS), together with lorazepam, suppress seizures in a rat kainate status epilepticus model



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## ABSTRACT

**Background:** Status epilepticus (SE) is a condition of prolonged or recurrent and often drug-resistant seizures where nonseizure therapy remains an important unmet need. Repetitive transcranial magnetic stimulation (rTMS) is emerging as a means to suppress seizures but has not been extensively studied in models.

**Objectives:** We aimed to test the antiepileptic potential of high-frequency rTMS in SE. As a step toward eventual coupling of rTMS with antiepileptic pharmacotherapy, we also tested whether high-frequency rTMS in combination with a low (ineffective but less likely to cause a side effect) lorazepam dose is as effective as a full lorazepam dose in suppressing seizures in a rat SE model.

**Methods:** EEG was recorded to measure epileptic spike frequency in the rat kainate SE model. Epileptic spikes were counted before, during, and after either high-frequency rTMS treatment alone or high-frequency rTMS treatment in combination with lorazepam, a firstline SE treatment.

**Results:** We found that rTMS alone decreases epileptic spike frequency only acutely. However, combinatory treatment with half-dose lorazepam together with rTMS was as effective as a full lorazepam dose.

**Conclusion:** We report that high-frequency rTMS has modest antiepileptic potential alone but acts in complement with lorazepam to suppress seizures.

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## 1. Introduction

Transcranial magnetic stimulation (TMS) is a method for focal non-invasive brain stimulation in which small intracranial electrical currents are induced by a fluctuating extracranial magnetic field. Low-frequency ( $\leq 1$  Hz) repetitive TMS (rTMS) induces a lasting reduction in cortical excitability and, when applied interictally, raises seizure threshold and reduces seizure frequency by mechanisms similar to those of long-term depression (LTD) of excitatory synaptic strength [1,2].

Although less well-established, there is also the potential for ictal rTMS to terminate ongoing individual seizures [3,4]. For abortive seizure therapy, as opposed to interictal increase of seizure threshold, rTMS anticonvulsant mechanisms may relate more to its capacity to interrupt ongoing neuronal activity as can be seen, for instance, with functional impairment of cortical function in human subjects [5–7]. Ictal rTMS

has been applied clinically in several cases of human epilepsy partialis continua, with seizures terminated in less than half of the subjects [4, 8–10]. However, interpretation of data from these few human trials is limited since a broad range of rTMS protocols have been employed in a small and heterogeneous population of patients. Thus, the differential effect of altering ictal rTMS paradigms has not been formally tested and remains a gap in knowledge. Indeed, the partial efficacy of ictal rTMS in humans justifies translational use of animal seizure models, allowing for systematic testing of rTMS protocols in homogeneous subjects [1].

Relevant to the present project, seizure interruption by rTMS may occur with high-frequency ( $\geq 10$  Hz) stimulation trains. Although prolonged high-frequency rTMS (hf-rTMS) trains enhance cortical excitability via long-term-potential-type mechanisms [11,12], short hf-rTMS trains are more likely to interrupt ongoing cortical processes [5–7] and could interrupt seizures in status epilepticus (SE). The feasibility of seizure suppression by hf-rTMS is also supported by the termination of ongoing seizures by the responsive neurostimulation (RNS) system using electrical high-frequency cortical stimulation [13–15].

Since many patients do not respond to initial pharmacologic SE treatments, there remains a need for more efficacious treatment options

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without the risk of respiratory compromise. For instance, lorazepam (LZP), the firstline benzodiazepine anticonvulsant used clinically in SE, is appreciably limited by its sedative and respiratory-depressive side effects [16,17]. Thus, we tested whether hf-rTMS can suppress seizures in a rat kainic acid (KA) SE model. Further, as rTMS is unlikely to be applied in the absence of anticonvulsant medications in a realistic clinical setting [18], we tested whether hf-rTMS can complement LZP therapy in a rat SE model and enable seizure suppression with a lower LZP dose, which may mitigate LZP-related side effects.

## 2. Materials and methods

### 2.1. Animals

Male adult Sprague–Dawley rats (200–230 g) were maintained under a 12-h light–dark cycle with food and water ad libitum. Following experiment completion, all animals were euthanized with CO<sub>2</sub>. All procedures were approved by the Boston Children’s Hospital IACUC and were in accordance with its guidelines.

### 2.2. Seizure induction

Nonconvulsive seizures were triggered by KA (15 mg/kg, i.p.) in rats previously anesthetized with urethane (1.2 mg/kg, i.p.) [19] (Fig. 1). Urethane anesthesia enables continuous EEG seizure monitoring [19] and rTMS in immobilized subjects with minimal effect on GABAergic transmission at the low doses used in this study [20–22]. The onset of seizures (with at least a 0.1-Hz spike frequency) was apparent on EEG about 1 h after KA injection. All animals were closely observed during the entirety of the procedure, and no distress or change in respiratory rate was detected.

### 2.3. EEG

One-channel continuous EEG was acquired with subdermal wire electrodes (Ives EEG Solutions, Newburyport, MA) after each animal was anesthetized, as previously described by our group [23]. The EEG signal was digitized at 400 Hz, bandpass filtered at 1–70 Hz, and recorded for post hoc analysis.

### 2.4. TMS

Repetitive transcranial magnetic stimulation was delivered with a Magstim Rapid stimulator (Magstim, Whitland, UK) and a modified

figure-of-eight coil with an angle of 158° between the wings (outside diameter of each wing: 70 mm) centered overhead. Verum TMS was delivered at 80% machine output (MO) intensity in excess of the motor threshold for rats to increase the likelihood of stimulating the subcortical limbic structures (the source of KA seizures), whereas sham rTMS was delivered at 8% MO intensity. Each rat received 10 rTMS trains of 3-s duration at 20 Hz with a 30-s intertrain interval with a single 5-min session. This protocol was chosen to mimic clinical hf-rTMS applications and also corresponds to the technical limits of most stimulators [24,25].

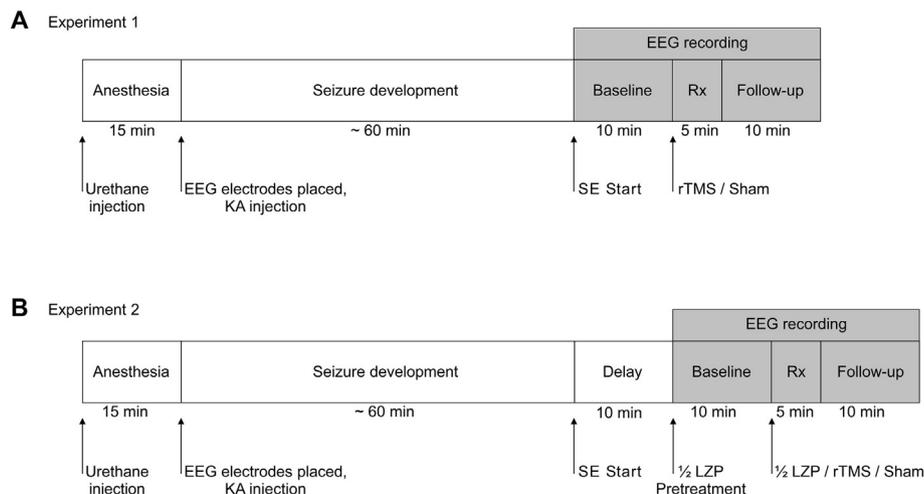
### 2.5. Experimental design

In Experiment 1, we tested the antiepileptic effect of hf-rTMS in reducing the epileptic spike frequency (Fig. 1A). Rats were divided into three groups to receive (1) KA and verum rTMS (N = 9), (2) KA and sham rTMS (N = 9), or (3) sham rTMS with no KA (N = 6). The TMS treatments were delivered immediately after baseline EEG recording (i.e., 10 min after SE development; see EEG analysis).

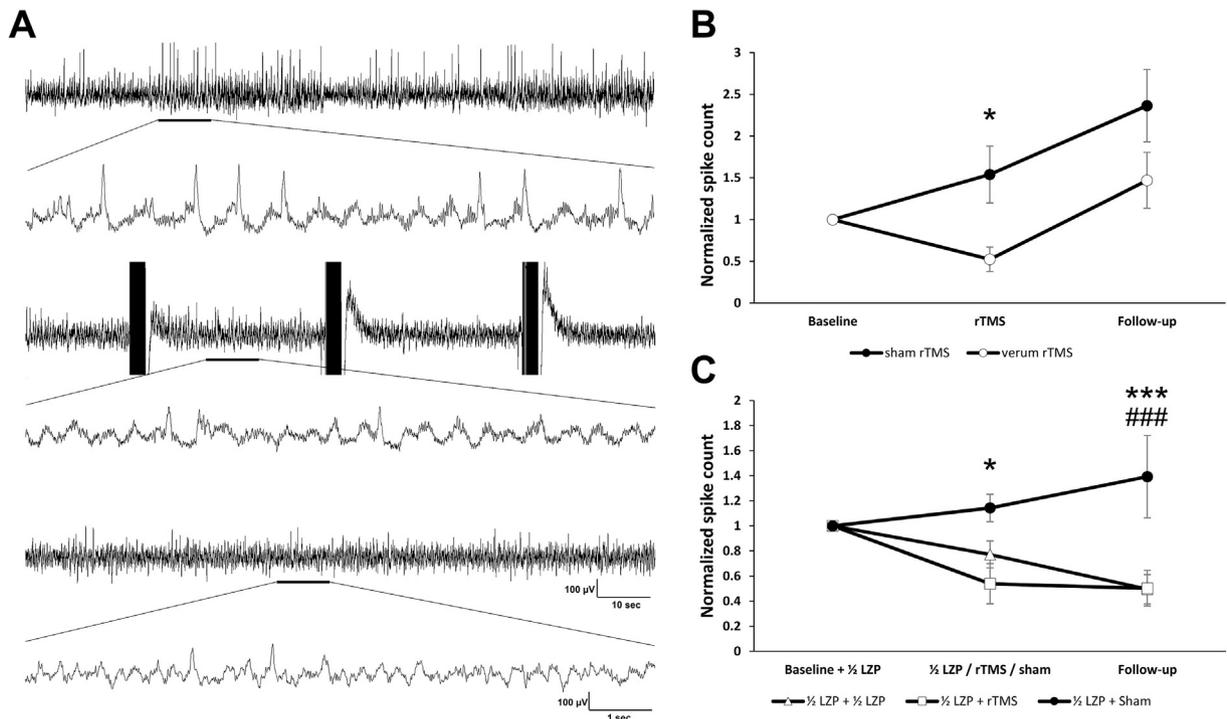
In Experiment 2, we aimed to test the antiepileptic effect of rTMS in conjunction with LZP (Fig. 1B). In dose–response pilot experiments (N = 6), we identified 1.0 mg/kg as an effective (operationally defined as ≥50% reduction in epileptic spike frequency) LZP dose to suppress SE in this model. All animals were pretreated with half of the effective LZP dose (½ LZP = 0.5 mg/kg) to test the hypothesized combinatory effect with rTMS. To match the Experiment 1 protocol and initiate the treatment 10 min after SE onset, the ½-LZP pretreatment was delivered 10 min after SE onset (“Delay” block, Fig. 1B). Following ½-LZP pretreatment, rats were divided into three groups to receive (1) a second ½-LZP dose to mimic clinical SE treatment (N = 10), (2) rTMS as in Experiment 1 (N = 10), or (3) sham rTMS (N = 7). Similar to the first experiment, treatment (additional ½ LZP or verum rTMS or sham rTMS) was administered immediately after the 10-minute baseline EEG recording.

### 2.6. EEG analysis

EEG was analyzed for number of epileptic spikes during the 10-min baseline, 5-min treatment, and 10-min follow-up periods. Representative EEG traces are shown in Fig. 2A. The baseline recording started as soon as the 0.1-Hz EEG spike frequency was established. Automated EEG spike detection was performed using a custom algorithm developed in Matlab (v 8.0, Mathworks, Natick, MA) as recently described [26].



**Fig. 1.** Timeline of experiments 1 (A) and 2 (B). Latency of each experimental stage is presented in minutes; the EEG in both experiments was recorded during baseline, treatment (Rx), and follow-up. See [Materials and methods](#) for further details.



**Fig. 2.** (A) Representative 2-min EEG traces of a seizure (top panel), during verum rTMS (middle panel), and a follow-up full LZP dose (bottom panel). Note the TMS artifact in the middle panel. Insets show 10-s EEG traces with representative spike-and-wave discharges. (B) Average spike count in the TMS-only experiment shows a decrease in spike frequency during the treatment which relapses at the follow-up. (C) Average spike count in the TMS + LZP experiment shows comparable reductions of spike frequency with either two  $\frac{1}{2}$ -LZP doses or a single  $\frac{1}{2}$ -LZP dose followed by rTMS. \* $p < 0.05$  and \*\*\* $p < 0.001$  as compared to TMS groups; ### $p < 0.001$  as compared to the  $\frac{1}{2}$ -LZP +  $\frac{1}{2}$ -LZP group.

## 2.7. Statistical analysis

Results are presented as mean  $\pm$  SEM. Spike count was normalized to baseline condition in both experiments. Data were analyzed using repeated-measures ANOVA, followed by Fisher's LSD post hoc test.

## 3. Results

### 3.1. Spike suppression by rTMS

In the first experiment (Fig. 2B), repeated-measures ANOVA with group as a between-subjects factor and treatment stage as a within-subject factor revealed a significant main effect of group ( $F(1, 16) = 4.99$ ,  $p = 0.04$ ) and treatment stage ( $F(1, 16) = 21.44$ ,  $p < 0.001$ ). While spike frequency continuously increased in sham-treated animals during the experiment, post hoc analysis showed that TMS-treated animals had significantly reduced spike counts during treatment ( $p = 0.041$ ).

### 3.2. Complementary treatment with lorazepam and rTMS

In the second experiment (Fig. 2C), repeated-measures ANOVA with group as a between-subjects factor and treatment stage as a within-subject factor revealed a significant main effect of group ( $F(2, 23) = 6.22$ ,  $p = 0.007$ ) and a significant interaction between the two factors ( $F(2, 40) = 3.66$ ,  $p = 0.042$ ). Post hoc analysis showed a significant decrease in spike frequency during treatment in the  $\frac{1}{2}$ -LZP + rTMS group and at follow-up after treatment in both the  $\frac{1}{2}$ -LZP +  $\frac{1}{2}$ -LZP (full LZP dose) and  $\frac{1}{2}$ -LZP + rTMS groups.

## 4. Discussion

We have demonstrated for the first time, to our knowledge, that ictal trains of hf-rTMS can reduce epileptic spike frequency in a rat SE model.

One plausible mechanism of this effect is interruption of ongoing neuronal activity similar to cortical functional impairment in human subjects [5–7].

We note that hf-rTMS alone, while it decreased seizures during stimulation, did not prevent relapse at the follow-up period, suggesting that longer or additional treatment may be needed to sustain the therapeutic hf-rTMS effect.

Further, we discovered that hf-rTMS in conjunction with half of a therapeutic LZP dose is as effective as a full LZP dose. We view this finding as an important step toward eventual systematic testing of combinations of electrical and pharmacologic SE treatments, as has been done with other transcranial neurostimulation modalities [23,27]. While recognizing the preliminary nature of our findings, we do propose the possibility that hf-rTMS may be used to reduce benzodiazepine requirement in SE management. If confirmed and translated to humans, this would lead to reduced morbidity because of less sedation and respiratory suppression.

The mechanism by which hf-rTMS suppressed seizures in our experiment will need further study. Recent preclinical experiments indicate that TMS delivered as intermittent theta-burst stimulation decreases GABA-interneuron-mediated cortical inhibition in mice [20], and continuous theta-burst stimulation in humans enhances GABA-mediated short-interval intracortical inhibition in humans [28]. However, there is no evidence that bursts of hf-rTMS, as delivered here, modulate GABAergic transmission. Thus, mechanisms other than increased GABAergic inhibition above the increase conferred by LZP injection may explain the antiepileptic hf-rTMS effect. Accordingly, we propose a complementary and distinct antiepileptic mechanism where hf-rTMS-mediated disruption of ongoing cortical activity contributes to desynchronization of ongoing spike-and-wave discharges [5–7].

One limitation of our study is that KA-induced seizures were subclinical, represented by separate spikes, and did not produce more clinically relevant spike clusters. This led to an arbitrary spike frequency threshold of 0.1 Hz to start baseline recordings. To address more clinically relevant outcomes, testing of additional animal SE models is needed. Another

limitation of our rat TMS study is direct stimulation of a relatively larger brain region than in humans, which restricts its clinical translation. Yet, despite these limitations, our findings suggest the seizure-suppression potential of hf-rTMS and the combinatory antiepileptic mechanism of hf-rTMS and LZP.

### Conflict of interest

Authors report no conflict of interest.

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