Physiology and Pharmacology

Physiol Pharmacol 19 (2015) 1-13 **www.phypha.ir/ppj**

Original Article

Effect of repeated transcranial magnetic stimulation during epileptogenesis on spontaneous activity of hippocampal CA1 pyramidal neurons in rats

Amir Shojaei[1], Saeed Semnanian[1], Mahyar Janahmadi[2], Homeira Moradi[1], Seyed Mohammad Firoozabadi[3], Javad Mirnajafi-Zadeh[1*]

1. Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Medical Physics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Abstract

Introduction: Considering the antiepileptogenic effects of repeated transcranial magnetic stimulation (rTMS), the effect of rTMS applied during amygdala kindling on spontaneous activity of hippocampal CA1 pyramidal neurons was investigated.

Materials and Methods: A tripolar electrode was inserted in basolateral amygdala of Male Wistar rats. After a recovery period, animals received daily kindling stimulations until they reached stage 5 seizure. In one group of animals, rTMS at frequency of 1 Hz were applied to hippocampus once daily at 5 min after termination of kindling stimulations. 24 h after the last kindling stimulation, spontaneous activity of CA1 pyramidal neurons of the hippocampus was investigated using whole cell patch clamp technique.

Results: Kindling-induced seizures resulted in increment of spontaneous activity of hippocampal CA1 neurons, but application of rTMS during amygdala kindling prevented it. Moreover, rTMS administration inhibited the kindling-induced enhancement of afterdepolarization (ADP) amplitude and action potential duration.

Conclusion: Results of this study suggest that rTMS exerts its anticonvulsant effect, in part, through preventing the amygdala kindling-induced increase in spontaneous activity and excitability of hippocampal CA1 pyramidal neurons.

Keywords:
Epilepsy;
Kindling;
Transcranial magnetic stimulation;
Action potentials;

Received:
7 Aug 2014
Accepted:
6 Jan 2015

***Correspondence to:**
J. Mirnajafi-Zadeh
Tel:
021-82883865
Fax:
021-82884555
Email:
mirnajaf@modares.ac.ir

Introduction

Epilepsy is a chronic brain disorder in which a person has repeated seizures over time. It affects millions of people worldwide and has remained one of the most common neurological conditions (Shorvon, 1996). Despite many advances in epilepsy research, seizures fail to come under control in many patients with epilepsy (20 to 40% of the overall epileptic population) (Leppik, 1992, Sander, 1993, Sillanpaa and Schmidt, 2006). Temporal lobe epilepsy is the most common form of human epilepsy with intractable seizures originating from the medial or lateral temporal lobe

(Devinsky, 1991). Surgical removal of epileptogenic tissue, particularly the hippocampus, often results in pharmacologically controlled seizures after surgery (Wiebe *et al.*, 2001, Spencer, 2002, Spencer and Burchiel, 2012), implying that the hippocampus plays a crucial role in the drug-resistant temporal lobe epilepsy.

The failure of current therapies in controlling the refractory epileptic seizures highlights the need for new, effective, and safe treatments. Transcranial magnetic stimulation (TMS) is a novel technique for noninvasive stimulation of brain through intact scalp (Barker *et al.*, 1985). It is a widely used method in research of human brain physiology and a therapeutic tool for some drug-resistant neuropsychiatric disorders (Dayan *et al.*, 2013).

It has been postulated that low frequency repetitive TMS (rTMS; ≤2 Hz) decreases, while high frequency rTMS (≥5 Hz) increases the cortical excitability (Pascual-Leone *et al.*, 1994, Chen *et al.*, 1997, Tergau *et al.*, 1997, Kim *et al.*, 2004). Furthermore, low frequency rTMS has been shown to have diverse degrees of seizure control in both epileptic patients (Tergau *et al.*, 1999, Menkes and Gruenthal, 2000, Theodore *et al.*, 2002, Misawa *et al.*, 2005, Fregni *et al.*, 2006, Joo *et al.*, 2007, Santiago-Rodriguez *et al.*, 2008) and in laboratory animals (Ebert and Ziemann, 1999, Fleischmann *et al.*, 1999, Rotenberg *et al.*, 2008, Mongabadi *et al.*, 2013). Focal epilepsies are particularly ideal for the application of rTMS due to a delimited zone of increased excitability (Santiago-Rodriguez *et al.*, 2008).

Considering the ability of rTMS as a potential therapeutic manner for epileptic patients, it is necessary to explore neuronal effects associated with its anti-epileptic outcome. Understanding these effects will also be useful in determining the probable side effects following application of rTMS. It has been suggested that long-term depression- or depotentiation-like mechanisms may be responsible for rTMS effects (Chen *et al.*, 1997, Hallett, 2000, Kobayashi and Pascual-Leone, 2003, Tokay *et al.*, 2009, Dayan *et al.*, 2013).

Kindling is a widely studied animal model of temporal lobe epilepsy in which repetitive focal electrical stimulation of the brain induces progressive seizure activity culminates in tonic-clonic convulsion (Sutula, 1990). Our recent study showed that application of rTMS can prevent kindling-induced synaptic potentiation (Yadollahpour *et al.*, 2014). It has been shown that synaptic potentiation is accompanied with changes in electrophysiological properties of neurons (Hallett *et al.*, 1999, Wagner *et al.*, 2009, Pell *et al.*, 2011). These changes can be observed in areas involved in seizure generation or propagation of amygdala kindling, including the hippocampus. Previous studies showed that CA1 region of the hippocampus is among the most important areas involved in the propagation of amygdala kindling-induced seizures (Dasheiff and McNamara, 1982, Mirnajafi-Zadeh *et al.*, 2002).

Regarding the inhibitory effect of rTMS on epileptogenesis and considering the important role of CA1 pyramidal neurons in spreading of the amygdala kindling-induced seizures, in the present study, we investigated whether application of rTMS during amygdala kindling procedure can prevent the kindling-induced changes in electrophysiological properties of hippocampal CA1 pyramidal neurons during amygdala kindling.

Materials and methods

Animals

Adult male Wistar rats (1 month old) obtained from Pasteur institute of Iran were maintained in a colony room kept at 23±2°C temperature on 12:12 light:dark schedule (lights on at 7:00 A.M) and permitted free access to food and water. All experiments were performed in accordance with the ethical guidelines set by the “Ethical Committee of Faculty of Medical Sciences, Tarbiat Modares University”

Amygdala semi-rapid kindling

Animals underwent stereotaxical surgery under ketamine/xylazine mixture (100/10 mg/kg, i.p.) anesthesia and were chronically implanted with a tripolar electrode in the basolateral amygdala of right hemisphere (coordinates: A, 2.5 mm; L, 4.8 mm from Bregma and 7.5 mm below dura) according to the atlas of Paxinos and Watson (2006). The electrode

consisted of three twisted stainless steel, teflon coated wires, 127 µm in diameter, insulated except at their tips (A-M Systems, Inc., WA, U.S.A.). Two stainless steel screws were also positioned in the skull above the frontal and occipital cortices as reference and ground electrodes. All electrodes were connected to pins of a lightweight multichannel miniature socket and fixed to the skull with dental acrylic.

After a postoperative recovery period of 7 days, the rat was transferred to a recording box. Animal's socket was connected to a flexible, shielded cable and the rat was allowed to move freely in the recording box. Electrical stimulation for semi-rapid kindling consisted of a 3 s train of 50-Hz monophasic square waves (1 ms) at afterdischarge threshold, delivered twelve times daily at inter-train intervals of 5 min as described previously (Mohammad-Zadeh *et al.*, 2007, Mohammad-Zadeh *et al.*, 2009). To determine the afterdischarge threshold, the stimulating currents were initially delivered at 10 µA and then its intensity was increased in increments of 10 µA at 5 min intervals. The minimum intensity that was sufficient to induce the afterdischarges for at least 8 s was selected as the afterdischarge threshold and used for kindling stimulation. Responses were amplified and digitized (at 10 kHz) using a PC-based data acquisition system (D3111 Data Aqcuisition, ScienceBeam Co., Iran) and custom-designed software and were continuously monitored and stored on disk. The duration of afterdischarges following twelve daily stimulations was considered as daily afterdischarge duration. The progression rate of kindling was monitored by recording the behavioral seizure stage and afterdischarge duration following each stimulation. Behavioral seizures were scored according to Racine's classification (Racine, 1972): stage 1, facial clonus; stage 2, head nodding; stage 3, forelimb clonus; stage 4, rearing and bilateral forelimb clonus; stage 5, rearing and falling. The animals considered as kindled when they exhibited stage 5 seizure.

rTMS application

For rTMS application, the animal was fixed in a special designed restrainer and underwent a CO_2-stunning procedure to prevent the head movements. The CO_2-stunning procedure had no significant effect on the kindling parameters. The head was sufficiently accessible to allow close positioning of the coil. The coil was positioned with its facial plane tangential to the scalp without direct contact, with its plane of symmetry at an approximate 45˚ angle to the mid-sagittal line, and with its facial plane 5 mm above the parieto-temporal region of scalp. The coil was fixed in this position and kept unchanged until the end of stimulation. rTMS was applied as biphasic pulses using a magnetic simulator (Magstim Ltd, Whitland, Wales, UK). Magnetic stimulations were given by a figure-8-shaped coil of 25 mm diameter (inner diameter, 14 mm; outer diameter, 43 ± 2 mm; turns, 14 ± 1). The stimulation intensity was adjusted at the 90% of motor threshold.

The motor threshold was determined by applying a single pulse stimulation which was adjusted for evoking motor response in the hind limb muscles. The motor response was detected by visual inspection (Yadollahpour *et al.*, 2014). rTMS was applied 5 minutes after cessation of last kindling stimulation as one train of pulses at the frequency of 1 Hz for 4 minutes. rTMS pattern was selected according to our previous study (Yadollahpour *et al.*, 2014). Although its duration seems unusually short (especially compared to the duration of rTMS administration in human which is usually about 15 min or more), however, rTMS exerts significant anticonvulsant effects when applied in short durations (Ebert and Ziemann, 1999, Rotenberg *et al.*, 2008).

Whole-cell patch clamp recording

24 h after the last kindling stimulation and/or rTMS application, rats were killed by decapitation while anesthetized with ether. Then, the right hemisphere were rapidly removed and submerged in ice-cold cutting solution containing (in mM) 2.5 KCl, 0.5 $CaCl_2$, 2 $MgCl_2$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 238 sucrose and 11 D-glucose bubbled with 95% O_2- 5% CO_2. The osmolarity was adjusted to 295-300 mOsm. Transverse slices (400 µm) were cut using a vibrotome (1000 Plus Sectioning System, Vibratome, MO, USA). Subsequently, the right hippocampus were dissected out and transferred to standard ACSF (that was continuously bubbled with 95% O_2- 5% CO_2) containing (in mM) 125 NaCl, 3 KCl, 1.25 NaH_2PO_4, 25

$NaHCO_3$, 10 D-Glucose, 2 $CaCl_2$, 1.3 $MgCl_2$. The osmolality was in the range of 290-300 mOsm and pH was adjusted to 7.3-7.4 by NaOH 1 M. Slices were then incubated for 1 h at 35°C and then stored at room temperature (23–25°C) until they individually transferred to a submerged recording chamber.

Recording chamber was mounted on a fixed-stage upright microscope (Axioskop 2 FS MOT; Carl Zeiss, Germany) and continually were perfused at 1.5-2.5 ml/min with standard ACSF at room temperature (23-25°C). CA1 pyramidal neurons were visualized using an IR-CCD camera (IR-1000, MTI, USA) with a 40x water immersion objective lens. Neuronal somas were selected for recording based on their relatively pyramidal shape and smooth, low-contrast appearance. Whole cell patch clamp recordings were made under current clamp condition. Recording microelectrodes (1.5 mm outer diameter, borosilicate glass, GC150-11; Harvard Apparatus, UK) were pulled with a horizontal puller (P-97, Sutter Instrument, USA) and filled with intracellular solution containing (in mM): 115 K-gluconate, 20 KCl, 10 HEPES, 2 EGTA, 10 disodium-phosphocreatine, 2 MgATP and 0.3 NaGTP. pH was adjusted to 7.25-7.30 and osmolality was in the range of 285-290 mOsm. Electrode tip resistance in the bath was typically 4 to 6 MΩ, and series resistance ranged from 12 to 25 MΩ. Cells were rejected if series resistance changes were more than 20% during experiment. Capacitance compensation and bridge balance were carried out. Data were low-pass filtered at 3 kHz and acquired at 10 kHz with a Multiclamp 700B amplifier equipped with Digidata 1440 A/D converter (Molecular Devices, CA, USA). The signal was recorded on a PC for offline analysis using the Axon pClamp 10 acquisition software. After establishment of a giga seal (more than 2 GΩ), the whole-cell configuration was attained simply by applying a brief suction.

To investigate the effect of daily rTMS treatment during amygdala kindling on firing properties of CA1 pyramidal neurons, we recorded spontaneous activity of CA1 pyramidal cells for 5 min in current clamp mode (I=0) at least 5 min after achieving the whole cell configuration.

Experimental groups

Following the postoperative recovery period, animals were randomly allocated into four groups. In kindled group (n=6), animals received daily kindling stimulations until they achieved stage 5 seizure. In kindled+rTMS (KrTMS) group (n=6), daily rTMS was applied 5 min following termination of kindling stimulations. The animals in rTMS group (n=3) were treated with daily rTMS alone. The number of stimulation days in KrTMS and rTMS groups was equal to the mean stimulation days required to achieve a stage 5 seizure in kindled group. Another two groups, including sham-operated (n=3) and naive (non-operated) (n=3) rats were also used. As, no significant difference was found between the data of these two groups, their data were pooled and considered as control group (n=6). All of the measured parameters in whole cell recordings were obtained from slices derived from the above mentioned numbers of animals.

Measured parameters

The occurrence of spontaneous action potentials and their amplitude and half-width, amplitude of after depolarization (ADP), maximum rise and decay slopes were examined. Action potential amplitude was determined as the peak voltage with respect to the baseline of 10 ms before the peak of the action potentials. Action potential half-width was computed as the width of spike at one half of its maximum amplitude. Maximum rise slope was measured as the maximum slope of rising phase and maximum decay slope was calculated as maximum incline of falling phase of the spike. ADP amplitude was computed as the peak voltage of ADP with respect to the baseline. Single spikes were used for determining the action potential characteristics. We also utilized phase-plot analysis to characterize action potential features among various experimental groups. In phase-plot analysis, the rate of change in membrane potential with respect to time (dV/dt) is plotted as a function of membrane potential. Various components of action potentials are clearly reflected in phase-plot curve (Fig. 1).

Statistical analysis

All results were expressed as mean ± standard error of mean (S.E.M.). Statistical analysis was performed using GraphPad Prism version 6.01 for Windows (GraphPad Software, Ca, USA). The parameters were compared by one-way ANOVA followed by post hoc Tukey test. A value of less than 0.05 was considered statistically significant.

Results

Animals of kindled group (n=6) achieved stage 5 seizure after 6±0.44 days. The mean seizure stage in kindled group was 4.75±0.25 on day 6 of experiment. rTMS application in KrTMS group (n=6) significantly prevented development of behavioral seizures, so that the mean seizure stage in this group was 1.83±0.17 on day 6 of experiment.

Results of the intracellular recording indicated that amygdala kindling significantly enhances spontaneous action potential firing of CA1 pyramidal neurons in kindled (25 cells from 6 animals) compared with control group (25 cells from 6 animals). rTMS application in KrTMS group (26 cells from 6 animals) inhibited kindling-induced increase in spontaneous activity of CA1 pyramidal neurons (Fig 2 A and B). No neurons in rTMS group exhibited spontaneous activity, thus, the action potential properties were examined in

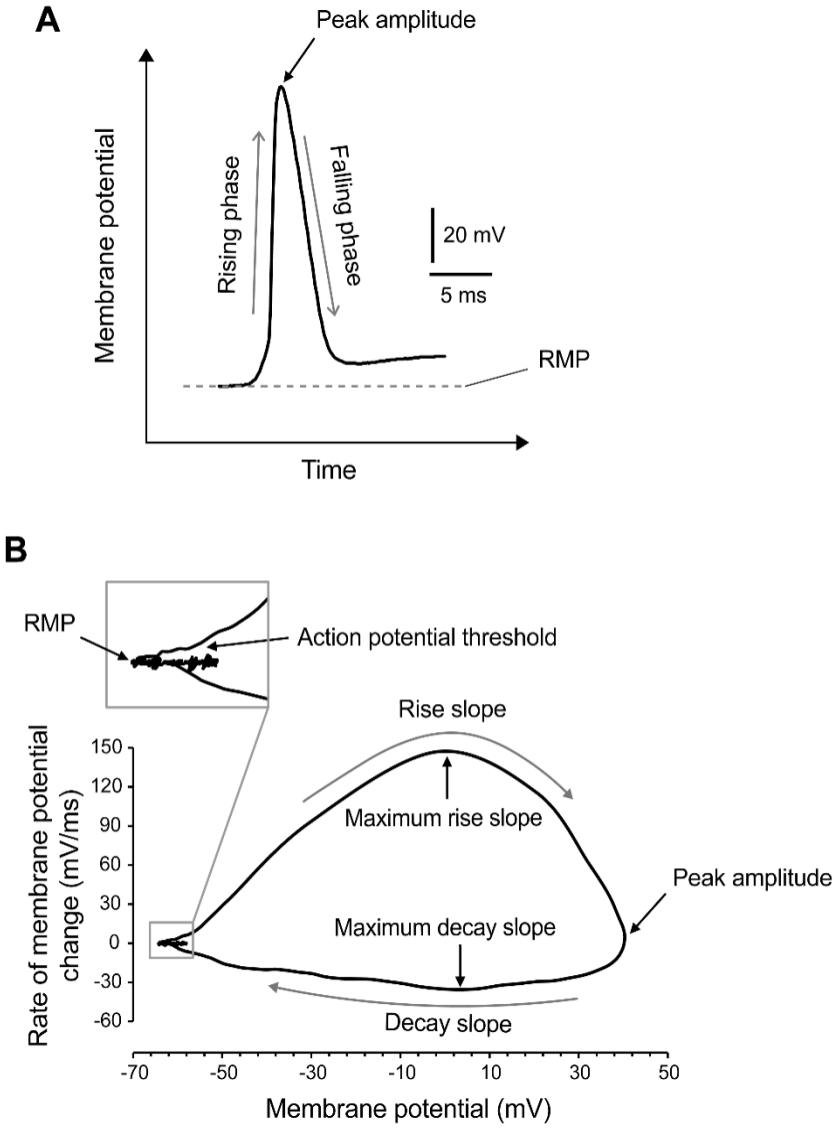

Fig 1: Phase-plot analysis of action potentials. A illustrates various components of a normal action potential recorded from CA1 pyramidal neurons in hippocampus. B shows phase-plot representation of the action potential in A. Different parameters of action potentials has been illustrated on this phase-plot curve.

other 3 groups.

Enhancement of multiple spike occurrence (Fig 3 A) and increase in burst activity is another characteristic of neuronal hyperexcitability, therefore, we explored incidence of these events in the experimental groups.

Obtained results showed that kindling procedure increased the occurrence of multiple spikes. There were 9 complex spikes including 4 doublets, 2 triplets and 3 quadruplets in kindled group (n=25 cells from 6 animals), while there were only 2 multiple spikes in control group (n=25 cells from 6 animals) (Fig 3 B). rTMS application in KrTMS group prevented the occurrence of multiple spikes so that there was only one triplet in this group (n=25 cells from 6 animals).

Measuring the ADP amplitude revealed that this parameter increases following kindling stimulations

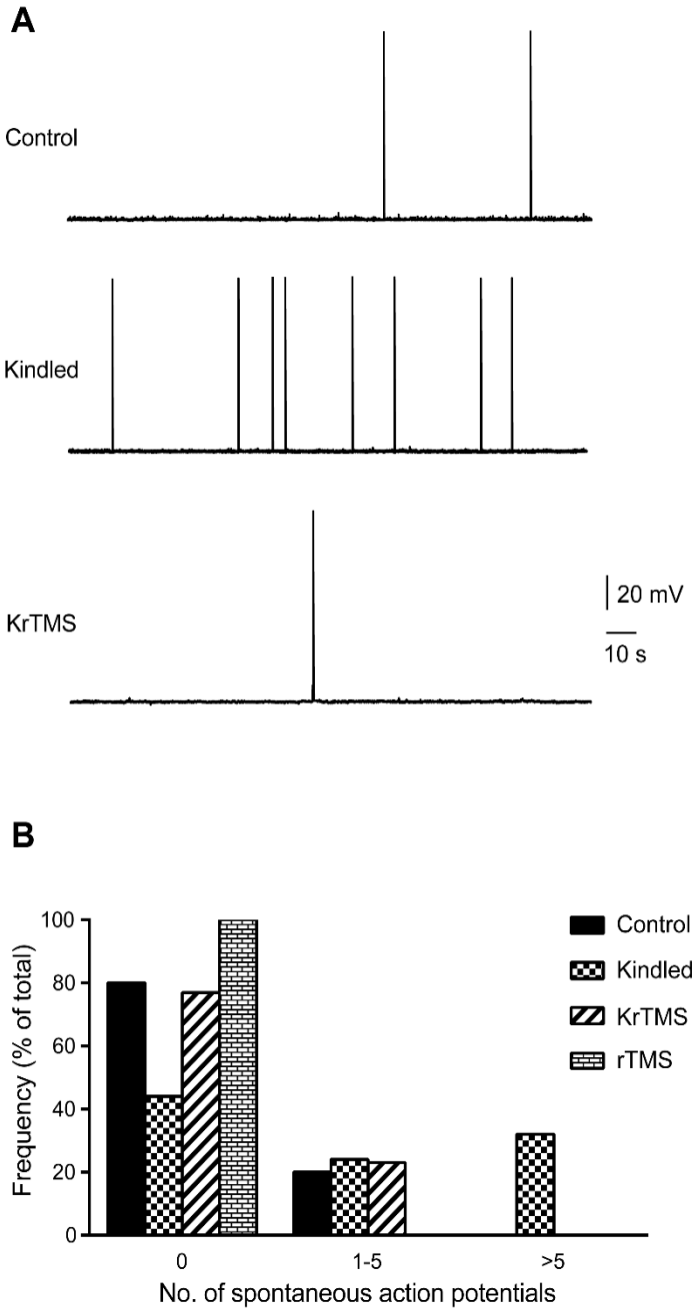

Fig 2: The effect of rTMS applied during amygdala kindling on spontaneous firing activity of CA1 pyramidal neurons. A, A sample record of spontaneous activity of neurons in various experimental groups. B shows the percentage of neurons fired different ranges of spontaneous action potentials in different experimental groups during the 5 min of recording. “0” means no action potential exhibited during the time of recording.

and there was significant variation in this parameter among kindled (15.11±1.23 mV, 9 cells from 6 animals) and control (9.54±1.06 mV, 6 cells from 4 animals) groups ($p<0.05$, Fig 3 C and D). rTMS administration in KrTMS group (5 cells from 4 animals) significantly inhibited the kindling-induced increase in this parameter (6.78±1.62 mV, $p<0.01$, Fig 3 C and D).

Amygdala kindling significantly decreased action potential amplitude from 108.7±0.73 mV in control (n=5 cells from 4 animals) to 94.01±3.00 mV in kindled group (n=11 cells from 6 animals) (Fig 4 A and C). rTMS application significantly prevented the kindling-induced reduction of this parameter in KrTMS group (105.5±3.56 mV; 6 cells from 4 animals; $p<0.05$ compared to kindled group) (Fig 4 A and C).

Maximum rise slope of action potentials significantly decreased in response to kindling stimulations in kindled group (131.2±10.94 mV/ms, n=11 cells from 6 animals) compared to control group (203.3±14.81 mV/ms; 5 cells from 4 animals, $p<0.01$) (Fig 4 B and D). rTMS treatment inhibited the decrease in this parameter so that a significant difference was observed between KrTMS (232.1±20.42 mV/ms, n=5 cells from 4 animals) and kindled group ($p<0.001$) (Fig 4 B and D).

A significant decrease was also observed in maximum decay slope of action potentials in kindled (-34.16±1.86 mV/ms, 10 cells from 6 animals) compared to control (-43.34±3.20 mV/ms, 5 cells from 3 animals) group ($p<0.05$). rTMS application in KrTMS group (-57.58±2.04, 6 cells from 4 animals) not only significantly precluded kindling-induced reduction of this parameter ($p<0.001$ compared to kindled), but also increased this parameter compared to control group ($p<0.01$, Fig 4 B and E).

As a result of kindling stimulations, action potential half-width was significantly ($p<0.01$) prolonged in neurons from kindled group (2.70±0.10 ms; 13 cells

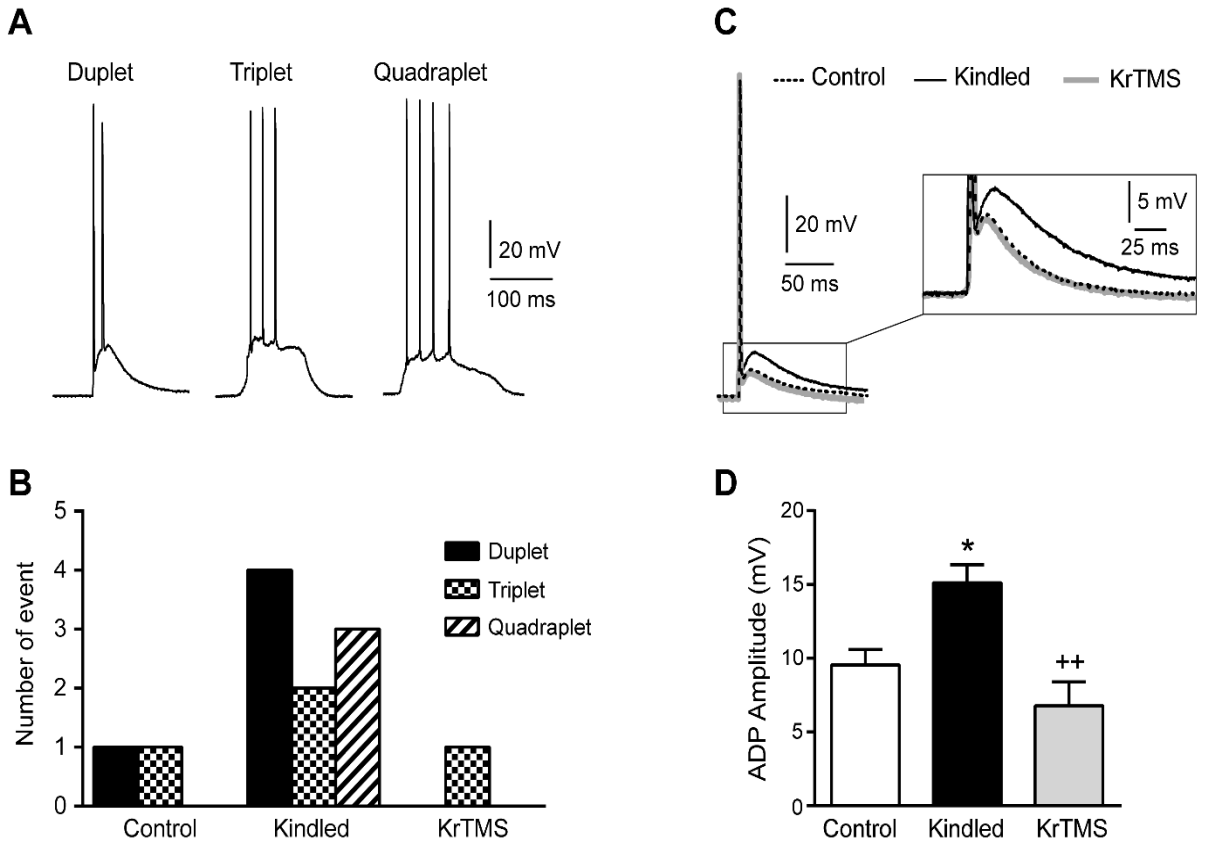

Fig 3: Effects of rTMS application on incidence of multiple spike discharge and ADP amplitude during the kindling acquisition in CA1 pyramidal neurons. A) Representative samples of duplet, triplet and quadruplet spikes recorded in this study. B) Frequency of multiple spikes incidence among various experimental groups. C) Comparing samples of ADPs recorded in different experimental groups. D) Quantitative comparison of ADP amplitude. *$p<0.05$ compared to control and ++ $p<0.01$ compared to kindled, mean±SEM.

from 6 animals) compared to that of control group (2.17±0.14 ms; 5 cells from 4 animals). This parameter was significantly prevented to be lengthened in KrTMS group (1.71±0.04 ms; 6 cells from 4 animals, p<0.001 compared to kindled group) (Fig 4 B and F).

As fig 5 indicates, phase plot representation of action potentials in kindled group showed distinct differences compared to that of control, reflecting the decrease in

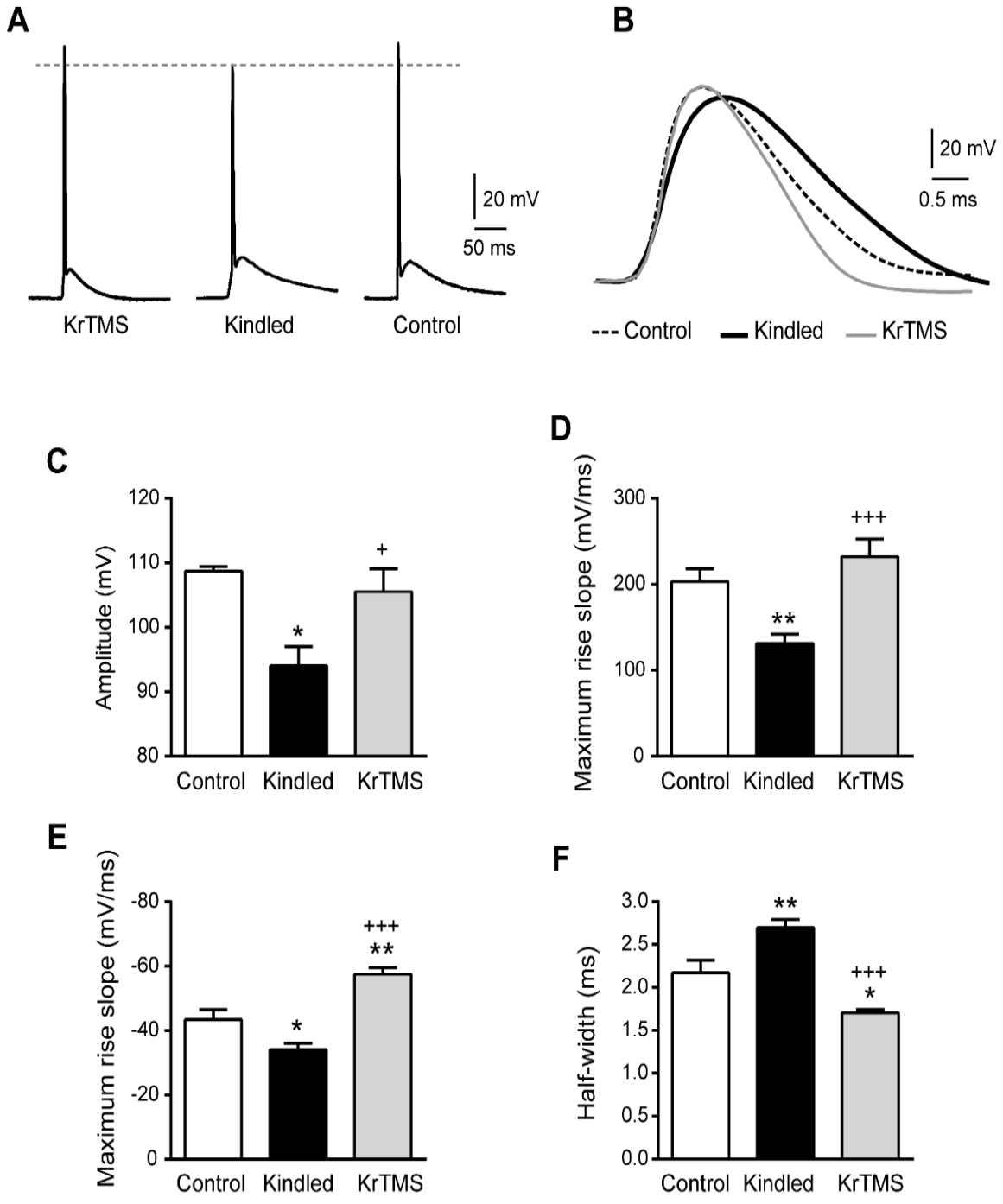

Fig 4: Effects of rTMS treatment following kindling stimulations on properties of spontaneous action potentials in CA1 pyramidal neurons. A) Representative traces of action potentials recorded form various experimental groups. The dashed line shows the spike amplitude of kindled group to simplify comparing the amplitude between different groups. B) Superimposed typical traces of action potentials in CA1 pyramidal neurons. D-F show, respectively, quantitative comparison of amplitude, maximum rise slope, maximum decay slope and half-width of action potentials in different groups. *p<0.05 and ** p<0.01 compared to control; +p<0.05 and +++p<0.001 compared to kindled; mean±SEM.

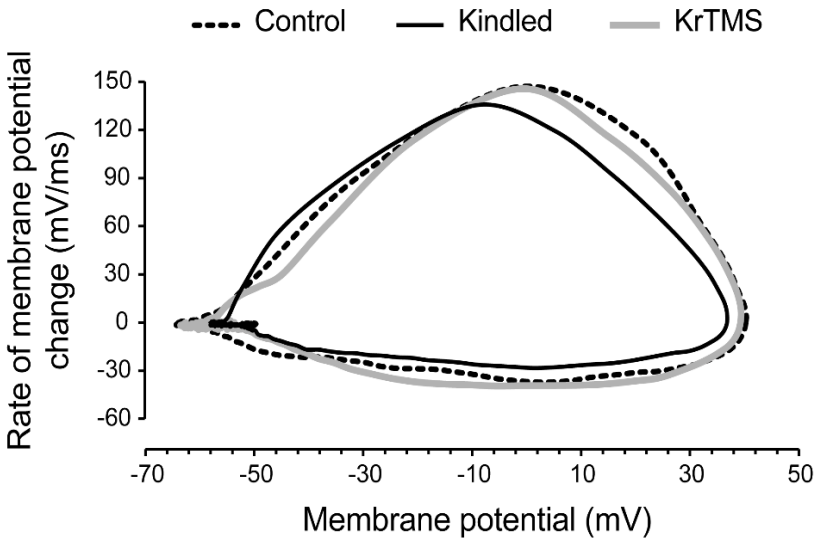

Fig 5: Comparing the phase-plot representative of action potentials in different experimental groups. As can be observed in this figure, rTMS application almost prevented kindling-induced changes in action potential dynamics.

amplitude, maximum rise slope and maximum decay slope of action potential following kindling stimulations. Interestingly, action potential phase plot deflection in KrTMS group was almost similar to that of the control group, indicating application of rTMS prevented kindling-induced changes in action potential dynamics.

Discussion

Results of the present study provided direct cellular evidence that rTMS application prevents kindling-induced changes in spontaneous activity of CA1 pyramidal neurons. These results are consistent with our previous study which showed that administration of low frequency rTMS during kindling process has an inhibitory effect on epileptogenesis (Mongabadi *et al.*, 2013, Yadollahpour *et al.*, 2014) accompanied with the suppression of kindling-induced potentiation of perforant path-evoked population spikes in the dentate gyrus of awake rats (Yadollahpour *et al.*, 2014).

Amygdala kindling enhanced the excitability of CA1 pyramidal neurons as evidenced by an increase in the spontaneous action potential discharges. This parameter is dependent on both neuronal intrinsic ionic conductance and excitatory and/or inhibitory synaptic inputs (Kaczmarek and Levitan, 1987, Urban *et al.*, 2012). rTMS application suppressed the excitatory effect of kindling on spontaneous activity. This inhibitory effect may be resulted from magnetic stimulations impact on intrinsic ionic conductance of CA1 pyramidal neurons or because of preventive effect on kindling-induced changes of synaptic inputs activity (Kaczmarek and Levitan, 1987), so that rTMS reduced the neuronal excitability. Similar mechanisms are likely involved in rTMS group resulting in no spike firing.

Occurrence of multiple spikes was enhanced by kindling stimulations. Although these spikes are normally observed in spontaneous activity of CA1 pyramidal neurons and they have an important role in learning and memory processes by hippocampus (O'Keefe, 1976, Otto *et al.*, 1991), but the increase in frequency of these events is due to kindling-induced hyperexcitability of neurons. Incidence of these events in CA1 pyramidal neurons is attributed to ADP that follows each spikes (Azouz *et al.*, 1996). When the amplitude of ADP is large enough to reach the action potential threshold, it triggers spike(s) (Azouz *et al.*, 1996). These multiple spikes increase neurotransmitter release from presynaptic terminals, and then amplify output signals from the neurons (Borst and Sakmann, 1999). In the present study, ADP amplitude was significantly increased by kindling epileptogenesis and it can be considered as the main cause for the increase in the number of multiple spikes. It has been shown that persistent sodium currents and R-type

calcium currents participate in ADP generation in CA1 pyramidal neurons (Azouz *et al.*, 1996, Chen *et al.*, 1997, Metz *et al.*, 2005, Yue *et al.*, 2005). Furthermore, inhibition of dendritic A-type potassium currents has been reported to strengthen burst activity through amplifying the ADP amplitude (Magee and Carruth, 1999). In addition to these currents, dendritic D-type potassium currents also influence ADP. Metz et all (2007) reported that activation of this current decreases ADP amplitude and reduces the burst activity by neurons (Metz *et al.*, 2007). Therefore, we hypothesize that, the increase in persistent sodium currents and R-type calcium currents and the decrease in A-type and D-type potassium currents may account for the increase in both ADP amplitude and incidence of multiple spikes by kindling epileptogenesis in CA1 pyramidal neurons. On the other hand, the preventive effect of rTMS on multiple spike firing and ADP amplitude enhancement may be accomplished by inhibiting the kindling effect on these currents.

Our recent study showed that kindling epileptogenesis depolarizes resting membrane potential (RMP) and increases membrane input resistance of CA1 pyramidal neurons (Shojaei *et al.*, 2014). More depolarized RMP results in reducing the voltage difference between membrane potential and action potential threshold. Moreover, increment of membrane input resistance causes more changes in the membrane potential magnitude in response to a given synaptic currents. These alterations enhance neuronal sensitivity to excitatory synaptic inputs and increase the likelihood of triggering action potentials in response to these currents, and therefore, augment neuronal excitability. In addition, it has been shown that amplitude and frequency of excitatory synaptic inputs in baso-lateral amygdala increase following kindling achievement (Shoji *et al.*, 1998). Moreover, expression of voltage-gated sodium channel has been reported to increase by kindling stimulations (Blumenfeld *et al.*, 2009). This finding can explain the enhanced spontaneous firing of action potentials following amygdala kindling.

Amplitude and maximum rise slope of action potentials reduced by kindling stimulations. Rising phase of action potentials is mediated by voltage-gated sodium channels (Bean, 2007), and thus, magnitude of these parameters is largely dependent on the voltage-gated sodium channel activity. It has been reported that inactivation curve of these channels shift to more hyperpolarized membrane potential due to kindling development (Vreugdenhil and Wadman, 1992). In addition, as mentioned before, resting membrane potential of CA1 pyramidal neurons shift to more depolarized value by kindling stimulations (Shojaei *et al.*, 2014). These modifications cause the inactivation of a fraction of voltage-dependent sodium channels at voltages near to resting membrane potential, so that they will not be accessible for action potential generation. As a consequence, the low number of available sodium channels may account for the kindling induced decline in amplitude and maximum rise slope of action potential.

rTMS application in KrTMS group precluded the kindling-induced reduction of these parameters. This finding suggests that rTMS exerts its antiepileptogenic effect, in part, by preventing the modulation of voltage-gated sodium channels during the kindling development; however, further studies are needed to confirm this hypothesis.

It has been demonstrated that repolarization of action potentials at low frequencies is mainly mediated by A-type potassium currents (Storm, 1987). Hence, attenuation of maximum decay slope in kindled group indicates alteration of the A-type potassium channels which may be as a result of changes in their expression or kinetics. Previous studies have shown that these currents play an important role in regulation of neuronal excitability and abnormality in their functions results to the hyperexcitability and incidence of seizure activity (Segal *et al.*, 1984). Application of rTMS in KrTMS group showed that magnetic stimulations not only inhibited the kindling induced-decline in the activity of these channels, but also increased their function.

Action potential half-width was prolonged following kindling stimulations. Magnitude of this parameter depends on the balance between some input and output currents (Bean, 2007). As we discussed above, both maximum rise and decay slope of action potentials reduced due to kindling epileptogenesis. Duration of action potential is an important factor in determining the amount of calcium influx to

presynaptic terminal (Borst and Sakmann, 1999, Bean, 2007). It is believed that the 4th power of calcium concentration in presynaptic terminal is translated to the amount of neurotransmitter release (Borst and Sakmann, 1999, Dayan *et al.*, 2013). Broadening the action potentials, especially at high frequencies such as during seizure activity, heightens the calcium influx to the presynaptic terminal and cause a several-fold increase in neurotransmitter release (Borst and Sakmann, 1999). These alterations can facilitate propagation of amygdala-kindled seizures through the hippocampus. Interestingly, rTMS application inhibited the increase in action potential half-width following kindling stimulations. This finding suggests that rTMS treatment may exert its anti-epileptogenic effect by prevention of broadening the action potentials. Results of phase-plot analysis revealed that action potential dynamics is altered due to propagation of seizures. However, rTMS application prevents the kindling-induced changes in characteristics of spikes, giving them features that are close to normal action potentials.

In summary, finding of the present study provide direct electrophysiological evidences demonstrating rTMS application prevents amygdala kindling-induced changes in spontaneous activity of CA1 pyramidal neurons. Results of this research are in agreement with the studies which introduce rTMS as a therapeutic tool for control of seizures.

Acknowledgments

This study is part of the PhD thesis of the first author in physiology that has completely performed in physiology department of Tarbiat Modares University. This research was supported by grants from Iran National Sciences Foundation (INSF) (Grant # 92040251).

Conflict of interest

All authors declared that there is no conflict of interest.

References

Azouz R, Jensen MS, Yaari Y. Ionic basis of spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. J Physiol 1996; 492: 211-23.

Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. Lancet 1985; 1: 1106-7.

Bean BP. The action potential in mammalian central neurons. Nat Rev Neurosci 2007; 8: 451-65.

Blumenfeld H, Lampert A, Klein JP, Mission J, Chen MC, Rivera M, et al. Role of hippocampal sodium channel Nav1.6 in kindling epileptogenesis. Epilepsia 2009; 50: 44-55.

Borst JG, Sakmann B. Effect of changes in action potential shape on calcium currents and transmitter release in a calyx-type synapse of the rat auditory brainstem. Philos Trans R Soc Lond B Biol Sci 1999; 354: 347-55.

Chen R, Classen J, Gerloff C, Celnik P, Wassermann EM, Hallett M, et al. Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. Neurology 1997; 48: 1398-403.

Dasheiff RM, McNamara JO. Intradentate colchicine retards the development of amygdala kindling. Ann Neurol 1982; 11: 347-52.

Dayan E, Censor N, Buch ER, Sandrini M, Cohen LG. Noninvasive brain stimulation: from physiology to network dynamics and back. Nat Neurosci 2013; 16: 838-44.

Devinsky O. Interictal behavioral changes in epilepsy. In: Devinsky O, Theodore WH, editors. Epilepsy and behavior. New York: Wiley-Liss; 1991. p. 1–21.

Ebert U, Ziemann U. Altered seizure susceptibility after high-frequency transcranial magnetic stimulation in rats. Neurosci Lett 1999; 273: 155-8.

Fleischmann A, Hirschmann S, Dolberg OT, Dannon PN, Grunhaus L. Chronic treatment with repetitive transcranial magnetic stimulation inhibits seizure induction by electroconvulsive shock in rats. Biol Psychiatry 1999; 45: 759-63.

Fregni F, Otachi PT, Do Valle A, Boggio PS, Thut G, Rigonatti SP, et al. A randomized clinical trial of repetitive transcranial magnetic stimulation in patients with refractory epilepsy. Ann Neurol 2006; 60: 447-55.

Hallett M. Transcranial magnetic stimulation and the human brain. Nature 2000; 406: 147-50.

Hallett M, Wassermann EM, Pascual-Leone A, Valls-Sole J. Repetitive transcranial magnetic stimulation. The International Federation of Clinical Neurophysiology. Electroencephalogr Clin Neurophysiol Suppl 1999; 52: 105-13.

Joo EY, Han SJ, Chung SH, Cho JW, Seo DW, Hong SB. Antiepileptic effects of low-frequency repetitive transcranial magnetic stimulation by different stimulation durations and locations. Clin Neurophysiol 2007; 118: 702-8.

Kaczmarek LK, Levitan IB. Neuromodulation: the biochemical control of neuronal excitability. USA: Oxford University Press; 1987.

Kim YH, Park JW, Ko MH, Jang SH, Lee PK. Facilitative effect of high frequency subthreshold repetitive transcranial magnetic stimulation on complex sequential motor learning in humans. Neurosci Lett 2004; 367: 181-5.

Kobayashi M, Pascual-Leone A. Transcranial magnetic stimulation in neurology. Lancet Neurol 2003; 2: 145-56.

Leppik IE. Intractable epilepsy in adults. Epilepsy Res Suppl 1992; 5: 7-11.

Magee JC, Carruth M. Dendritic voltage-gated ion channels regulate the action potential firing mode of hippocampal CA1 pyramidal neurons. J Neurophysiol 1999; 82: 1895-901.

Menkes DL, Gruenthal M. Slow-frequency repetitive transcranial magnetic stimulation in a patient with focal cortical dysplasia. Epilepsia 2000; 41: 240-2.

Metz AE, Jarsky T, Martina M, Spruston N. R-type calcium channels contribute to afterdepolarization and bursting in hippocampal CA1 pyramidal neurons. J Neurosci 2005; 25: 5763-73.

Metz AE, Spruston N, Martina M. Dendritic D-type potassium currents inhibit the spike afterdepolarization in rat hippocampal CA1 pyramidal neurons. J Physiol 2007; 581: 175-87.

Mirnajafi-Zadeh J, Mortazavi M, Fathollahi Y, Alasvand Zarasvand M, Reza Palizvan M. Effect of transient hippocampal inhibition on amygdaloid kindled seizures and amygdaloid kindling rate. Brain Res 2002; 954: 220-6.

Misawa S, Kuwabara S, Shibuya K, Mamada K, Hattori T. Low-frequency transcranial magnetic stimulation for epilepsia partialis continua due to cortical dysplasia. J Neurol Sci 2005; 234: 37-9.

Mohammad-Zadeh M, Mirnajafi-Zadeh J, Fathollahi Y, Javan M, Ghorbani P, Sadegh M, et al. Effect of low frequency stimulation of perforant path on kindling rate and synaptic transmission in the dentate gyrus during kindling acquisition in rats. Epilepsy Res 2007; 75: 154-61.

Mohammad-Zadeh M, Mirnajafi-Zadeh J, Fathollahi Y, Javan M, Jahanshahi A, Noorbakhsh S, et al. The role of adenosine A1 receptors in mediating the inhibitory effects of low frequency stimulation of perforant path on kindling acquisition in rats. Neuroscience 2009; 158: 1632-43.

Mongabadi S, Firoozabadi SM, Javan M, Shojaei A, Mirnajafi-Zadeh J. Effect of different frequencies of repetitive transcranial magnetic stimulation on acquisition of chemical kindled seizures in rats. Neurol Sci 2013; 34: 1897-903.

O'Keefe J. Place units in the hippocampus of the freely moving rat. Exp Neurol 1976; 51: 78-109.

Otto T, Eichenbaum H, Wible CG, Wiener SI. Learning-related patterns of CA1 spike trains parallel stimulation parameters optimal for inducing hippocampal long-term potentiation. Hippocampus 1991; 1: 181-92.

Pascual-Leone A, Valls-Sole J, Wassermann EM, Hallett M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. Brain 1994; 117 (Pt 4): 847-58.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. New York: Academic press; 2006.

Pell GS, Roth Y, Zangen A. Modulation of cortical excitability induced by repetitive transcranial magnetic stimulation: influence of timing and geometrical parameters and underlying mechanisms. Prog Neurobiol 2011; 93: 59-98.

Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol 1972; 32: 281-94.

Rotenberg A, Muller P, Birnbaum D, Harrington M, Riviello JJ, Pascual-Leone A, et al. Seizure suppression by EEG-guided repetitive transcranial magnetic stimulation in the rat. Clin Neurophysiol 2008; 119: 2697-702.

Sander JW. Some aspects of prognosis in the epilepsies: a review. Epilepsia 1993; 34: 1007-16.

Santiago-Rodriguez E, Cardenas-Morales L, Harmony T, Fernandez-Bouzas A, Porras-Kattz E, Hernandez A. Repetitive transcranial magnetic stimulation decreases the number of seizures in patients with focal neocortical epilepsy. Seizure 2008; 17: 677-83.

Segal M, Rogawski MA, Barker JL. A transient potassium conductance regulates the excitability of cultured hippocampal and spinal neurons. J Neurosci 1984; 4: 604-9.

Shojaei A, Semnanian S, Janahmadi M, Moradi-Chameh H, Firoozabadi SM, Mirnajafi-Zadeh J. Repeated transcranial magnetic stimulation prevents kindling-induced changes in electrophysiological properties of rat hippocampal CA1 pyramidal neurons. Neuroscience 2014; 280: 181-92.

Shoji Y, Tanaka E, Yamamoto S, Maeda H, Higashi H. Mechanisms underlying the enhancement of excitatory synaptic transmission in basolateral amygdala neurons of the kindling rat. J Neurophysiol 1998; 80: 638-46.

Shorvon SD. The epidemiology and treatment of chronic and refractory epilepsy. Epilepsia 1996; 37: S1-S3.

Sillanpaa M, Schmidt D. Natural history of treated childhood-onset epilepsy: prospective, long-term population-based study. Brain 2006; 129: 617-24.

Spencer D, Burchiel K. Selective amygdalohippocampectomy. Epilepsy Res Treat 2012; 2012: 382095.

Spencer SS. When should temporal-lobe epilepsy be treated surgically? Lancet Neurol 2002; 1: 375-82.

Storm JF. Action potential repolarization and a fast after-hyperpolarization in rat hippocampal pyramidal cells. J Physiol 1987; 385: 733-59.

Sutula TP. Experimental models of temporal lobe epilepsy: new insights from the study of kindling and synaptic reorganization. Epilepsia 1990; 31: S45-S54.

Tergau F, Naumann U, Paulus W, Steinhoff BJ. Low-

frequency repetitive transcranial magnetic stimulation improves intractable epilepsy. Lancet 1999; 353: 2209.

Tergau F, Tormos J, Paulus W, Pascual-Leone A, Ziemann U. Effects of repetitive transcranial magnetic stimulation (rTMS) on cortico-spinal and cortico-cortical excitability. Neurology 1997;48: A107.

Theodore WH, Hunter K, Chen R, Vega-Bermudez F, Boroojerdi B, Reeves-Tyer P, et al. Transcranial magnetic stimulation for the treatment of seizures: a controlled study. Neurology 2002; 59: 560-2.

Tokay T, Holl N, Kirschstein T, Zschorlich V, Kohling R. High-frequency magnetic stimulation induces long-term potentiation in rat hippocampal slices. Neurosci Lett 2009; 461: 150-4.

Urban KR, Waterhouse BD, Gao WJ. Distinct age-dependent effects of methylphenidate on developing and adult prefrontal neurons. Biol Psychiatry 2012; 72: 880-8.

Vreugdenhil M, Wadman WJ. Enhancement of calcium currents in rat hippocampal CA1 neurons induced by kindling epileptogenesis. Neuroscience 1992; 49: 373-81.

Wagner T, Rushmore J, Eden U, Valero-Cabre A. Biophysical foundations underlying TMS: setting the stage for an effective use of neurostimulation in the cognitive neurosciences. Cortex 2009; 45: 1025-34.

Wiebe S, Blume WT, Girvin JP, Eliasziw M, Effectiveness, Efficiency of Surgery for Temporal Lobe Epilepsy Study G. A randomized, controlled trial of surgery for temporal-lobe epilepsy. N Engl J Med 2001; 345: 311-8.

Yadollahpour A, Firouzabadi SM, Shahpari M, Mirnajafi-Zadeh J. Repetitive transcranial magnetic stimulation decreases the kindling induced synaptic potentiation: effects of frequency and coil shape. Epilepsy Res 2014; 108: 190-201.

Yue C, Remy S, Su H, Beck H, Yaari Y. Proximal persistent Na+ channels drive spike afterdepolarizations and associated bursting in adult CA1 pyramidal cells. J Neurosci 2005; 25: 9704-20.