Accepted Manuscript

Therapeutic Effects of Sodium Valproate Combined With Low Frequency Electrical Stimulations on Seizure and Behaviors

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Running Title

Combination therapy in epilepsy

To appear in: Basic and Clinical Neuroscience

Received date: 2018/06/9
Revised date: 2018/11/19
Accepted date: 2018/10/15

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Please cite this article as:


DOI: http://dx.doi.org/10.32598/bcn.9.10.280
Highlights

* Hippocampal rapid kindling can induce several changes in emotional behaviors of rats including anxiety and depressive like behaviors.

* Combination sub effective dose of sodium valproate and LFS have a stronger anticonvulsant effect in comparison with using only sodium valproate or LFS.

* Combination sub effective dose of sodium valproate and LFS significantly decreased emotional disturbance induced by kindling.

Plain Language Summary
Application of antidepressant drugs produces several problems leading to need of alternative agents in emotional disturbances. Since antidepressant drugs increase the seizure risk, alternative treatment like antiepileptic drugs might be useful for patients with epilepsy suffering from psychiatric disorder. Also, all seizures cannot be controlled by antiepileptic drugs in refractory epilepsy. The aim of the present study was to evaluate the behavioral effects of the low dose valproate along with deep brain stimulations during epilepsy. The results of the present experiment showed that deep brain stimulation could increase the effectiveness of sub effective doses of sodium valproate and decrease emotional disturbances induced by seizures.

Abstract

Background: Application of antidepressant drugs produces several problems leading to need of alternative agents in emotional disturbances. Since antidepressant drugs increase the seizure risk, alternative treatment like anti-epileptic drugs (AED) might be useful for patients with epilepsy suffering from psychiatric disorder.

The purpose of this study was to evaluate the behavioral effects of sodium valproate, a none effective dose in seizure treatment (100 mg/kg i.p.) along with application of low frequency stimulations (LFS) during CA1 hippocampal kindling.
**Material and Methods:** 42 male rats were randomly divided into 6 groups including control group which intact animal handled daily (I), sham group which were subjected to the surgical process, but receive no real stimulation (II), saline-kindled group (S.kindled) which were stimulated daily with the following protocol: 3 s train of 50Hz monophasic pulses of 1ms duration applied 12 times a day with the threshold intensity at intervals of 10 minutes where saline was administrated 15 minute before kindling stimulations (III), saline-kindled-LFS group (K4LFS) in which saline was injected 15 minute before kindling stimulations and LFS applied daily after termination of kindling stimulation (IV ), drug-kindle group (Drug100.kindled) where underwent rapid kindling procedure daily where sodium valproate (100 mg/kg) was administrated 15 minute before kindling stimulations(V) and drug-kindled-LFS (Drug100.kindled.4LFS) group in which drug and LFS were administrated respectively before and after kindling stimulations(VI). The behavioral tests were assessed using elevated plus maze, open field and force swim test.

**Results:** Combination of sodium valproate (100 mg/kg) and LFS significantly decreased cumulative seizure severity as compared with kindle group and provides a strong seizure suppressing effect.

Also sodium valproate and LFS increased the percentage of open arms (OAs) entries and the OAs exploration as well as decreased jumping from elevated plus maze and rearing in open field. Furthermore, there was no significant change in the OAs entries and OAs exploration percentages, jumping from apparatus and rearing in open field in Drug100.kindled, K4LFS and Drug100.kindled.4LFS groups as compared with sham group.

There was no significant difference in the latency to first immobility and the duration of immobility in K4LFS groups compare with s.kindled group. In drug-kindled group, the latency to first immobility significantly increased and the duration of immobility decreased as compared with s.kindled. Besides
the latency to first immobility significantly increased and the duration of immobility decreased in drug-kindled-LFS as compared to s.kindled group, but the latency to first immobility was not significantly changed in comparison with drug-kindled groups.

**Conclusion:** Sodium valproate and LFS can modulate the function of the brain regions involved in emotional processing in epilepsy, anxiety and depressive-like behaviors and also decrease emotional disturbances induced by the kindling process.

**Keywords:** Hippocampal kindling, Seizure, Sodium valproate, Emotional disturbances, Low frequency stimulations

1. **Introduction**

Emotional disturbances occur in various medical or neurological conditions (Grunze, 2008). Several comprehensive studies have demonstrated that patients with epilepsy suffer from high levels of anxiety and depression as compared with other neurological diseases (Volcy, 2004; Brandt, & Mula, 2016; Hamid, Ettinger, & Mula, 2011). Although anxiety disturbance might be more common in these patients, most of the attention has been focused on depression. The prevalence of anxiety and depressive disorder has been respectively reported 19% and 11% in patients with temporal lobe epilepsy (Currie, Heathfield, Henson, & Scott, 1971; Beyenburg, Mitchell, Schmidt, Elger, & Reuber, 2005).

Pharmacological therapy dominantly performed for anxiety using benzodiazepine drugs (Von Moltke,., & Greenblatt, 2003). These drugs have been used as the main pharmacological treatment of anxiety over the last 4 decades. Application of benzodiazepine drugs produces several problems leading to the need of alternative agents in anxiety conditions (Liability, 2005). Selective serotonin reuptake inhibitors (SSRIs) can be helpful in treatment of approximately 50-60% of patients with anxiety disorders; however, some patients cannot get complete treatment with SSRIs (Mula, Pini, & Cassano, 2007). Moreover, patients might be addicted to the antidepressant drugs if they use these
drugs for a long time (Suwanee, 2012). In addition, majority of antidepressants drugs reduce seizure threshold, in turn, may induce seizure attacks (Suwanee, 2012; Hill, Coupland, Morriss, Arthur, Moore, & Hippisley-Cox, 2015). For treatment depressive disorder, the initial treatment of depression includes medication and/or psychotherapy. Although it is estimated that 60–70% of patients respond favorably to the initial treatment, other patients are considered to have chronic and refractory forms of depression. Therefore, it is important to explore alternative drug treatment such as AEDs for uncontrolled depression specially in patients with epilepsy (Hamani, & Nóbrega, 2010).

During the past three decades, AEDs have become important parts of the pharmacological treatment of various neurological disorders other than epilepsy (Spina, & Perugi, 2004; Hamid, Ettinger, & Mula, 2011). Antiepileptic drugs might have positive or negative psychotropic effects on patient’s mood (Grunze, 2008; Brodie et al., 2016). Using AEDs such as vigabatrin, phenobarbitone, tiagabine and topiramate associated with mood disturbance (Jackson, & Turkington, 2005). Though the negative effects of these drugs on mood have been frequently reported, their positive effects were addressed for the first time in 1960s (Grunze, 2008). Anti-anxiety effects of AEDs such as carbamazepine, lamotrigine have been reported in various psychiatric disorders (Grunze, 2008).

Valproate is one of the first AEDs and its anticonvulsive characteristics was reported in 1963 (Chateauvieux, Morceau, Dicato, & Diederich, 2010). Recent studies have demonstrated that sodium valproate can produce obvious effects in treating different types of general and partial epilepsy (Chateauvieux, Morceau, Dicato, & Diederich, 2010; Goldenberg, 2010). It can be also served as a reliable option to suppress both generalized and focal seizures (Perucca, 2002).

Sodium valproate as a psychotherapeutic drug has been widely used as a novel method to treat the manic phase in many bipolar patients (Spina & Perugi, 2004). A number of behavioural and clinical studies suggested that sodium valproate can simulate anxiolytic effects of benzodiazepines (Mula, Pini, & Cassano, 2007; Jalilifar, Yadollahpour, Moazedi, & Ghotbeddin, 2017); however, most studies have been
only focused on healthy animal models and unfortunately there has been no reliable evidence to prove the effects of these drugs on the mood of patients with epilepsy.

Most information about the effects of anticonvulsant medicines on neurological diseases except epilepsy is based on case reports and uncontrolled studies and therefore cannot determine the effect and safety of these medications (Spina, & Perugi, 2004).

Since epileptic seizures cause different changes in brain function, the validity of normal animal models for evaluating drugs effect is questionable (Löschter, 2011). Therefore, there is a requirement to use epileptic animals in studying the effect of drugs. Epileptic animals are more susceptible to the behavioral alterations following administration of drugs (Jalilifar, Yadollahpour, Moazedi, & Ghotbeddin, 2017).

Electrical kindling can be induced by daily electrical stimulation in limbic structures and is a useful option to study the process of epileptogenesis and anxiety disturbances in epileptic animals (Jalilifar, Yadollahpour, Moazedi, & Ghotbeddin, 2017).

Brain stimulation has been reported as an efficient technique to suppress epileptic discharges and decrease seizure severity (Laxpati, Kasoff, & Gross, 2014; Zhang et al. 2012). Although many studies carried out on mood-stabilising and anxiolytic effects of valproate, there is a lack of an appropriate study about behavior effects of sodium valproate and LFS, a well effective combined therapy, during hippocampal kindling in rat. Therefore, the purpose of this study was to evaluate the behavioral effects of 100 mg/kg i.p. of sodium valproate, a none effective dose in seizure treatment, along with application of LFS during CA1 hippocampal kindling.

2. Materials and Methods

All experiments and animal care procedures were approved by local ethics committee of Shahid Chamran university of Ahvaz which completely coincides with the guide for the care and use of laboratory animals by the National Institutes of Health publication.
2.1. Animals

Adult male rats (200±20 g) were obtained from the animal house of Shahid Chamran university of Ahvaz. The animals were housed individually in transparent plastic cages with an ambient temperature of (23±2 °C), humidity (50±5%), and a 12-h light/12-h dark cycle (lights on from 7:00 AM).

2.2. Drug

Sodium valproate was purchased from Sigma and was dissolved in physiological saline (0.9% sodium chloride). Animals received sodium valproate or saline at a volume of 1ml/kg body weight daily injections for five days by the intraperitoneal route.

2.3. Surgery

All rats were anesthetized by a ketamine/xylazine mixture (100/10 mg/kg, i.p.) and fixed on stereotaxic apparatus. One tripolar stainless steel electrode (bipolar for stimulating and monopole for recording) was positioned in the right hippocampus using Paxinos and Watson atlas (Paxinos & Watson, 2006) from bregma: anteroposterior: 2.5mm; lateral:1.8mm; vertical: 2.8 below the skull (Stoelting Co., USA). Besides, a monopolar electrode used as ground and reference electrode was attached to the skull with stainless steel crew. Electrodes connected to pins and inserted into a socket and fixed in the skull with acrylic dental cement.

2.4. Stimulation Procedures

Electrical stimulations were applied with an electro modulator device (Science beam Co., Tehran, Iran) which was connected to a computer for monitoring epileptiform ADs using the e-probe software program.
At least one week after surgery, the afterdischarge (AD) threshold was evaluated using a 3 s train of 50Hz monophasic square pulses of 1ms duration which was initially delivered at 30 µA and it was increased in steps of 10µA with 10 minute intervals between current delivery until inducing ADs for at least 8s (Jalilifar, Yadollahpour, Moazedi, & Ghotbeddin, 2017). From all rats implanted with electrodes, we could only use 42 rats because some rats exhibited no afterdischarge until 150 µA. One day following AD threshold determination, the kindling procedure was begun and rats were electrically stimulated at the AD threshold 12 times a day.

2.5. Experimental groups
In the present experiment, 42 rats were divided into six groups: control group which intact animal handled daily (I), sham group which were subjected to the surgical process, but received no real stimulation (II), saline-kindled group (s.kindled) which were stimulated daily with the following protocol: a 3 s train of 50Hz monophasic square pulses of 1ms duration with the threshold intensity, applied 12 times a day at intervals of 10 minutes where saline was administrated 15 minute before kindling stimulations (III), saline-kindled-LFS group (K4LFS) in which saline was injected 15 minute before kindling stimulations and LFS applied daily after termination of kindling stimulation (IV): LFS consisted of four packages at 5-min intervals. Drug-kindle group (Drug100.kindled) received sodium valproate (100 mg/kg) 15 minute before kindling stimulations (V). In drug-kindled-LFS (Drug100.kindled.4LFS) group, drug and LFS were administrated respectively before and after kindling stimulations (VI). Each package contained 200 monophasic square wave pulses of 0.1-ms duration at 1 Hz.

2.6. Experiment
The 100 mg/kg of sodium valproate is a none effective dose in seizure treatment. The present study aimed to investigate the effect of this dose of valproate combined with applying LFS immediately after
termination of kindling stimulation in seizure severity, anxiety and depressive-like behaviors. Seizure severity of the kindling process was calculated according to the scale of Racine: score 1: facial clonus, immobility and rigid posture; score 2: facial movement and head nodding; score 3: forelimb clonus; score 4: rearing with tonic extension of forelimbs; score 5: loss of righting ability and balance (Kalynchuk, 2000). The kindling stimulations were administered until emerging stage 5 seizures (it took 5 days). Thus, the animals treated with sodium valproate or LFS for 5 days. Seizure severity was evaluated through observation of the animal behavior immediately after kindling stimulation. Since all animals were stimulated 12 time per day, cumulative seizure severities were measured (as the summation of seizure severity after daily stimulations). Anxiety and depressive-like behaviors were determined respectively by the elevated plus maze (EPM) and open field tests in the 6th day and forced swim test (FST) in a following day.

2.7 Behavioral Tests

2.7.1 Elevated Plus Maze

The EPM test has been verified to analyze anxiety-related behaviors in rodents. The instrument was plus shape and had two open arms opposite each other and two close arms (CAs). The arms were elevated 70 cm above the floor. During the experiment, each rat was placed in the central platform of EPM facing an open arm and was allowed to explore freely the maze for 5 min (Sestakova, Puzserova, Kluknavsky & Bernatova, 2013).

The numbers of entries and time exploration in each arm and the percentage of entries and time explorations into the OAs were calculated. The percentage of entries into the OAs was evaluated according to the following equation: the percentage of OAs entries was calculated as number of entries into the OAs/number of OAs + CA entries × 100. The percentage of OAs exploration was equal to the time spent in the open arms / the time spent in both arms of the maze × 100 (Rodgers & Dalvi, 1997). Ratio time and entry determined the level of anxiety in the EPM. The smaller the time or entry ratio, the
more anxiety in rats. After each trial, the set up was completely cleaned with an ethanol solution to remove any olfactory cuing (Andreatini, & Bacellar, 1999; Godlevsky, Muratova, Kresyun, Van Luijtelaar, & Coenen, 2014).

2.7.2 Open field test

An open field box consists of white floor and four transparent Plexiglas-walls (size in cm 45×45×40). Rearing frequency (number of times the animal stood on its hind legs) as the behavioral element was measured. The rearing has also been regarded as an aspect of exploratory behavior in some studies, (Rodgers & Dalvi, 1997; Godlevsky, Muratova, Kresyun, Van Luijtelaar, & Coenen, 2014), but some studies suggested that anxiolytic agents decrease the number of rearing. Each of the rats was placed in the center of the box and allowed it to explore the open field for 5 min. After each trial, the set up was cleaned thoroughly with an ethanol solution to remove any olfactory cuing (Ago, Takahashi, Nakamura, Hashimoto, Baba, & Matsuda, 2007).

2.7.3 Forced Swim Test

Forced swim test is one of the most commonly used assays to monitor depressive-related behaviors in rodents. The FST provides a situation to evaluate the ability of the animal to escape from an inescapable stressful condition; the higher amount of immobility duration indicates depression-like behavior (Shumake, & Gonzalez-Lima, 2003).

The test was performed between 9:00AM and 1:00PM. The FST consisted of two sessions, 24 h apart. The first session is known as pre-test stage (it takes 15 min) and the second session is the test stage (5 min). The cylinder was filled with enough water (25°C, depth=47 cm) to ensure that the rats’ hind-paws could not touch the cylinder’s bottom. Rats were individually forced to swim in a plastic cylinder. Duration of immobility including passive swimming or floating in the water without
struggling was measured and they were then placed back in drying cage. After every session, the container refilled with fresh water to avoid any influence on the next rat (Godlevsky, Muratova, Kresyun, Van Luijtemaar, & Coenen, 2014; Slattery, & Cryan, 2012).

2.8 Statistical Analyses

Statistical analysis was carried out using IBM SPSS version 16. Data were represented as the mean ± Standard Error of Mean (SEM). A one-way analysis of variance (ANOVA) followed by a post hoc of a Tukey test was performed to compare the changes in the percentage of entries into the OAs, the percentage of OAs exploration in EPM, the rearings in the open field, the latency to first immobility and the duration of immobility in FST. Moreover, cumulative seizure severity during the kindling process was compared using repeated measures analysis. The analyses were done two-sided and significance was adjusted at $p \leq 0.05$.

3. Results

1- Effects of sodium valproate (100 mg/kg) and low frequency stimulations (LFS) respectively before and after kindling stimulations on seizures induced by hippocampal rapid kindling:

Fig1 sheds light on the effect of 100 mg/kg i.p. of sodium valproate injection on the cumulative seizure severity. As shown in the figure1, this dose cannot affect seizure treatment. Our findings also indicate that LFS leads to decreasing the cumulative seizure severity during hippocampal rapid kindling ($P= .001$). But when these two treatments combined together, the cumulative seizure severity significantly decreased as compared with s.kindled group indicating a stronger anticonvulsant effect in comparison with using only sodium valproate or LFS (Figure1).

2- Effects of electrode implantation in the right hippocampus on anxiety and depressive like behavior:
There was no significant difference in the percentage of entries and exploration of OAs in elevated plus maze, the rearing’s in the open field, latency to first immobility and duration of immobility in FST between control and sham groups. The results also showed that electrode implantation cannot affect anxiety and depressive like behaviors.

3- Effects of kindling stimulations, sodium valproate, LFS and sodium valproate combined with LFS on anxiety-like behavior by elevated plus maze:

The percentages of OAs entries (P=.005) and explorations (P=.02) were significantly increased in s.kindled group as compared with sham group in the elevated plus-maze (Figure 2). Similarly, the jumping from the elevated plus maze increased significantly as compared with sham group (TABLE1). The number of rearing's in open field significantly increased in s. kindled rats in comparison with sham group (P=.006) (Figure 3).

Besides no significant difference in the percentage of OAs entries and explorations were observed between drug-kindled, K4LFS, drug-kindled-LFS and sham groups (Figure 2). Application of LFS, drug and combination of these two treatments decreased the number of jumps from the EPM as compared with the kindled group (TABLE1). The number of rearing's in drug-kindled, K4LFS, drug-kindled-LFS groups was not significantly increased as compared with sham group (Figure 3). These findings showed that there is no significant difference in the anxiety level in these groups compared with sham group, showing that the drug and LFS decreased kindling-induced anxiety.

4- Effects of kindling stimulations, sodium valproate, LFS and sodium valproate combined with LFS on depressive-like behavior in the forced swim test
In s.kindled group, the latency to first immobility decreased as compared with sham group (P=.013). The duration of immobility in the FST was significantly increased in the s. kindled group as compared with sham group (P=.000) (Figure 4).

In drug-kindled group, the latency to first immobility increased as compared with s.kindled group (P=.022) and it was not significant to sham group. The duration of immobility in drug-kindled group significantly decreased as compared with s.kindled group (P=.000) but it was not significantly changed in comparison with sham group (Figure 4).

In K4LFS group, the latency to first immobility increased though not significant as compared with s.kindled group. Moreover, the latency to first immobility in K4LFS was not significantly changed in comparison with sham group. The duration of immobility in K4LFS group decreased as compared with kindled group; however, it was not significant. This duration in K4LFS group significantly changed in comparison with sham group (P=.059) (Figure 4).

In drug-kindled-LFS group, the latency to first immobility was not significantly changed in comparison with sham and drug-kindled groups. Besides, there was significant alteration in latency to first immobility (P=.056) and the duration of immobility (P=.010) in drug-kindled-LFS as compared to s.kindled group (Figure 4).

4. Discussion

The data demonstrated that LFS could increase the effectiveness of sub effective doses of sodium valproate. Our results were consistent with some studies reporting that electrical stimulation of brain combined with antiepileptic which acting on the GABA system can provide a better control of epileptic seizures (Asgari, Semnanian, Atapour, Shojaei, Moradi, & Mirnajafi-Zadeh, 2014). Asgari et al suggested that combination of LFS and barbiturates increases the efficacy of each of these treatments and reduce seizures in amygdala-kindled rats (Asgari et al 2014). Moreover, Cuellar-Herrera et al reported that
application of high-frequency stimulation (HFS) combined with antiepileptic drugs including diazepam, phenobarbital and gabapentin increased the HFS induced anticonvulsant effects (Cuellar-Herrera, Pena, Alcantara-Gonzalez, Neri-Bazan, & Rocha, 2010). In a clinical study showed that applying HFS to the thalamus combined with the drugs improved deep brain stimulation anticonvulsant actions (Kerrigan et al, 2006). However, some antiepileptic drugs like phenytoin, a drug that inhibits voltage-dependent sodium currents, combined with electrical stimulation avoids its protective effects (Asgari et al 2014; Cuellar-Herrera et al. 2010 ). It seems the association of LFS with a sub effective dosage of sodium valproate, caused to increase of the effects of GABA system, to get better protection against seizures (Figure1).

The second part of the study evaluated the effects of sodium valproate and LFS on animal behaviors in the kindling process. The results of the present experiment showed that hippocampal rapid kindling can induce several changes in emotional behaviors of rats including increasing the percentage of OAs entries and explorations, jumping and rearing (Figure 2 and 3). The increase of the percentage of OAs entries and OAs exploration are generally used to analyze anxiolytic drug effects but since all rats in the kindled group jumped from the elevated plus maze and rearing in open field increased, we concluded the level of fear in rats might increase to the extent that they searched for routes to escape from the apparatus which was consistent with Lisa E.Kalynchuk et al results in which kindling produced changes in behavioral and had an anxiogenic effect (Kalynchuk, Pinel, Treit, & Kippin, 1997; Kalynchuk, Pinel, & Treit, 1998).

Our results also indicated that using sodium valproate (100 mg/kg) significantly reduced anxiety-like behavior of rat in the elevated plus maze and open field. Several studies reported the useful effectiveness of anticonvulsant drugs in treatment of psychiatric disorders. Our findings were consistent with the results of Lang AP,2003 and Luisa De Angelis, 1992, in which the anxiolytic-like effects of sodium valproate in the mirrored chamber test and the light/dark aversion test were respectively analyzed. In addition, Kinrys and colleagues demonstrated that sodium valproate can be
a useful option to treat participants with social anxiety disorder (Kinrys & Wygant, 2005). Besides, antiepileptic drugs such as valproate can be used in patients resistant to SSRI via increasing the efficiency of GABA (Van Ameringen, Mancini, Pipe, & Bennett, 2004; Brambilla, Perez, Barale, Schettini, & Soares, 2003). There is a common pathophysiological mechanism for anxiety and epilepsy attacks. Antiepileptic drugs can be helpful in anxiety disorders since AEDs reduce seizures through decreasing the excessive outburst from epileptic neurons, reducing anxiety symptoms and also decreasing neuronal activation within fear circuits (Jones, Salzberg, Kumar, Couper, Morris, & O'brien, 2008).

We also found that hippocampal rapid kindling can increase immobility (despair) in the FST which was consistent with Mazarati et al. 2009, in which rapid kindling increased immobility in the FST and the loss of taste preference towards calorie-free saccharin in immature kindled rats and concluded that brain changes induced by kindling are accompanied by the development of depressive behavior. Our data also suggested that the latency to first immobility increased while the duration of immobility decreased in Drug100.kindled as compared with the kindled group. Thus, sodium valproate had a significant antidepressant effect which was in line with Qiu HM et al, study showing that using valproate leads to decreasing the immobility time in depressive rats. They reported that the antidepressant effect of valproate is related to improving hypothalamic-pituitary-adrenal axis function, elevating the BDNF and 5-HTT expression, decreasing MAO-A and IDO expression of the hippocampus in depressive rats. Valproate decreases catecholamine levels in the brain which are associated with emotional behaviors (Qiu, Yang, Liu, Fei, Hu, & Zhou, 2014; Qiu, Yang, Jiang, Hu, Liu, & Zhou, 2015). Carlson and et al., 2006 suggested that valproate reduces brain infarction and neurological deficits through inhibiting of caspase-3 activation and induction of chaperone proteins. Kindling adversely affects normal serotonergic transmission and produces excessive glucocorticoids, thus contributing to heightened fear and depressed mood (Baf, Subhash, Lakshmana, & Rao,1994; Wilson, McLaughlin, Ebenezer, Nair, & Francis, 2014; Maes, Calabrese, Jayathilake, & Meltzer,1997).
Our results also indicated that there was no significant difference in the percentage of entries and time explorations, jumping from apparatus and the rearing’s between K4LFS, drug-kindled-LFS and sham groups. Besides, in these groups, the latency to first immobility increased while the duration of immobility decreased (p=0.01) as compared with the s.kindled group. According to the results, application of LFS alone or with sodium valproate reduced anxiety and depressive-like behaviors induced by kindling.

Several studies suggested that electrical brain stimulation reduces seizure frequency in epileptic patients and in animal models (Jalilifar et al. 2017; Asgari et al. 2014; Cuellar-Herrera et al. 2010; Esmaeilpour, Sheibani, Shabani, Mirnajafi-Zadeh, & Akbarnejad, 2018) but there is a lack of an appropriate study about effective combined therapy of antiepileptic drugs with LFS and future studies are needed to examine it (Asgari et al. 2014, Cuellar-Herrera, 2010). The results of the study evaluated the effects of LFS combined with sodium valproate on behaviors during rapid kindling. In conclusion, combination of sodium valproate (100 mg/kg) and LFS provides a strong seizure suppressing effect and modulate emotional disturbances.

![Graph showing cumulative seizure severity over days](image)
**Figure1:** Effects of sodium valproate and LFS on cumulative seizure severity: *p≤0.05 and **p≤0.01 and *** ≤0.001 compared to Sham group; Values are mean±SEM.
**Figure 2:** Effects of sodium valproate and LFS on anxiety-like behavior in the elevated plus maze. *p≤0.05 and **p≤0.01 and *** ≤0.001 compared to Sham group; Values are mean±SEM.
**Figure 3:** Effects of sodium valproate and LFS on anxiety-like behavior in the open field. *p≤0.05 and **p≤0.01 and *** ≤0.001 compared to Sham group; Values are mean±SEM.
Figure 4: Effects of sodium valproate and LFS on depressive-like behavior. * p≤0.05 and **p≤0.01 and *** ≤0.001 compared to Sham and # compared to S.Kindled group; Values are mean±SEM.

Table 1: The number of jumps from the elevated plus maze.
Conclusion
Sodium valproate and LFS can modulate the function of the brain regions involved in emotional processing in epilepsy, anxiety and depressive-like behaviors and also decrease emotional disturbances induced by the kindling process.

Ethical Considerations

Compliance with ethical guidelines
All applicable international guidelines for the care and use of animals were followed.

Funding
This research was supported by the Shahid Chamran University of Ahvaz.

Conflict of interest
The authors declare that they have no conflict of interest.

Acknowledgements
We gratefully acknowledge Shahid Chamran University of Ahvaz for supporting this study.

Abbreviations

AD: Afterdischarge
AED: Antiepileptic drug
BDNF: Brain-derived neurotrophic factor
EPM: Elevated Plus Maze

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<th>Group</th>
<th>Number of jumps from the elevated plus maze</th>
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<tr>
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<tr>
<td>Sham</td>
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