

## Behavioral Consequences of hippocampal epileptogenesis in adult male rat

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### Abstract

**Background & Aims:** Behavioral disorders such as anxiety and depression are often experienced by several forms of epilepsy. Since the kindling process, as a model of temporal lobe epilepsy produces behavioral impairment, the present research was designed to evaluate the anxiety and depressive like behaviors induced by hippocampal rapid kindling.

**Material & Methods:** Twenty-one male wistar rats were randomly divided into 3 groups: control (intact animals), sham operation (without any stimulation) and kindled animals. Seven days after electrode implantation in CA<sub>1</sub> region of hippocampus, threshold intensity was determined. In the next day, animals in the kindled group were stimulated in a rapid kindling manner (12 times/day) for six days with the following protocol: 3s train of 50 Hz monophasic pulses of 1ms duration with the threshold intensity at 10 minutes intervals. Finally anxiety and depressive like behaviors were assessed respectively by the elevated plus maze (EPM), open field in the 6th day and forced swim test (FST) in the following day.

**Results:** The results of this experiment showed that the hippocampal kindling increased open arms (OAs) entries percentage ( $P=0.005$ ) and OAs exploration percentage ( $P=0.02$ ), jumping from apparatus and rearing in open field box compared to sham group ( $P=0.006$ ). Also the latency to first immobility in kindled group decreased significantly ( $P=0.000$ ) whereas the duration of immobility increased significantly in comparison to the sham group ( $P=0.000$ ).

**Conclusion:** It seems that kindling enhances excitatory processes or disturbs neuronal inhibition which leads to emotional disturbances such as anxiety and depressive like behavior in animals.

**Keywords:** Forced swim test, Elevated plus maze, Open field, Emotional disorders, Kindling, Epilepsy, Rat

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### Introduction

The prevalence of epilepsy as a convulsive disease is approximately 15 per 1000 (1), and it is often

accompanied by behavior disorders. Temporal lobe epilepsy (TLE) is the most common type of epilepsy emerging by recurrent seizures, originates from the

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temporal lobes of brain, such as hippocampus and amygdala. TLE is associated with a high frequency of psychiatric disorders. The most emotional disturbances are related to depression and anxiety (2). Also 9–37% of epileptic patients experienced depression and 11–25% suffered from anxiety (2, 3).

The etiology of behavior deficits followed by TLE is often multi-factorial and many neurological factors are considered to be reasons for the epilepsy-induced behavior disturbances (4). As mentioned before, hippocampus, amygdala, and piriform cortex are mostly susceptible to seizure initiation and epileptogenesis in TLE (4, 5). A large number of studies indicated that impairment in behavior functions relate to seizure focus especially the hippocampus area that spreads projection to other regions of brain (4,6,7,8). Spread of seizures from hippocampus as identifiable epileptogenic focus to other areas which are participating in mood regulation can cause emotional disorders such as depression and anxiety (7, 8).

Because of the complexity of factors involved in human epileptic patient, the availability of a useful animal model associated with temporal lobe epilepsy is very necessary (4). Kindling is the most universally used model of TLE which can be created by repetitive and low-intensity electrical stimulation of limbic structures including hippocampus (4, 9). At first, conventional electrical kindling described by Goddard et al. (10) and Later, Lothman et al. described rapid kindling protocol which has been investigated on the process of epileptogenesis and changes in interictal emotionality (4, 11).

Several studies use the traditional kindling protocols (once or twice stimulations per day) to investigate the behavioral effects of epilepsy for 10–30 days and the results were controversial. Therefore, the present study designed a procedure to investigate the effect of rapid kindling on anxiety and depressive like behaviors in

adult male rat which renders kindled animals faster than slow kindling.

## Materials and Methods

All experiments of the study were approved by local Ethics Committee of Shahid Chamran University of Ahvaz which were completely in accordance with the guide for the care and use of laboratory animals by the National academy of sciences (EE/97.3.24.49755/scu.ac.ir).

### Animals:

Twenty-one male wistar rats (180–220 g) were obtained from the animal house of Shahid Chamran University of Ahvaz. The animals were kept individually in clear plexiglass 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).

### Surgery:

All rats were anesthetized under intraperitoneal injection of Ketamine (100 mg/kg) and Xylazine (10 mg/kg) mixture and fixed on stereotaxic apparatus (Stoelting Co., USA) (9). After cleaning and shaving the fur on the skull, a straight midline incision was made through the skin with surgical scissors. Then, the periosteum connective tissue on the skull was pushed aside for a clear view of bregma. One tripolar stainless steel electrode (bipolar for stimulating and monopole for recording) was placed in the CA1 region of the right hippocampus at 2.5 mm anteroposterior and 1.8 mm lateral to the bregma and 2.8 mm below the skull using Paxinos and Watson atlas (12). Besides, three stainless steel screws were attached to the skull including one for positioning of a monopolar electrode used as ground and reference electrode in the front of skull. Electrodes were connected to pins and attached to a socket embedded in the skull with acrylic dental cement (9).

### Stimulation Procedures:

Animals were allowed to recover for seven days. After that, the after discharge (AD) threshold was evaluated using a 3 ms monophasic square-wave of 50 Hz which was initially applied at 30  $\mu$ A and it was surged in steps of 10 $\mu$ A with 10 minute intervals between current delivery until inducing ADs for at least 8s. Electrical stimulations were applied using an electro modulator device (Science beam Co., Tehran, Iran) which was connected to a computer to monitor EEG signal alterations using the e-probe software program (13). The rats that exhibited no AD with maximum 150  $\mu$ A were excluded from the experiment. One day following AD threshold determination, animals were stimulated in a rapid kindling manner (12 times/day) for five days with the following protocol: 3s train of 50 Hz monophasic pulses of 1ms duration with the threshold intensity at 10 minute intervals. Finally, anxiety and depressive like behaviors were assessed respectively by the elevated plus maze (EPM) and open field in the 6th day and forced swim test (FST) in the following day.

### Experimental groups:

In the present experiment, 21 rats were divided into three groups: control group which was handled daily (I), sham group which was subjected to the surgical process, but receive no real stimulation (II), and kindled group which was stimulated daily using a 3s train of 50Hz monophasic pulses of 1ms duration with the threshold intensity, applied 12 times daily with 10 min intervals (III). Behavioral progression of the kindling process was scored according to the Racine scale: stage 1: facial clonus, wet dog shakes, and mouth; stage 2: facial movement and head nodding; stage 3: forelimb clonus; stage 4: rearing and tonic extension of forelimbs; stage 5: falling and loss of balance. The kindling stimulations were administered for 6 days (13).

### Behavioral Tests:

#### 1- Elevated Plus Maze Test

The instrument utilized for the EPM test arranges in a + shape and has a gray polypropylene plastic with two

close arms (CAs) and two open arms (OAs) as well as an open platform. The entire maze was 70 cm above the floor. During the experiment, each rat was placed in the center area of the maze with its head directed towards an open arm and was allowed to explore freely the maze for 5 min.

The percentage of entries in the OAs was calculated according to the following equation: the percentage of OAs entries = (number of entries into the OAs/number of OAs + CA entries)  $\times$  100. The percentage of OAs exploration = (the time spent in the open arms / the time spent in both arms of the maze)  $\times$  100. The percentage of OAs exploration and entries determine the level of anxiety in the EPM. The smaller time or entry ratio in open arms indicates the more anxiety levels in rats. After each trial, all arms and the center area of the maze were completely cleaned with an ethanol solution which is an effective odor removal and can reduce bias (14, 15).

#### 2- Open Field Test

An open field box consists of white floor and four transparent Plexiglas-walls (size in cm 45 $\times$ 45 $\times$ 40). Rearing frequency (number of times the animal stood on its hind legs) as a behavioral element was measured. The rearing has also been regarded as an aspect of exploratory behavior in some studies (16, 15), but some 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 studies (17, 18,19).

#### 3- Forced Swim Test

Forced swim test is one of the most common experiments to analyze depressive-related behaviors in rodents. It provides a situation to evaluate the ability of the animal to use active strategies in an inescapable stressful condition; failure to do indicates a depression-related state (15).

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 (5min). A cylinder was filled with water up to a height of 47. Rats were individually forced to swim in the cylinder. Then they were removed from the container and placed in the drying cage. Duration of immobility phase including the passive swimming or floating ability in the water was measured. After each session, the container was washed and refilled with fresh water to avoid any influence on the next rat (16).

#### Statistical Analyses:

Statistical analysis was carried out using SPSS version 21. Data were represented as the mean  $\pm$  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, rearings in the open field, the latency to first immobility and the duration of immobility in FST. The analyses were done two-sided and significance was adjusted at  $p \leq 0.05$ .

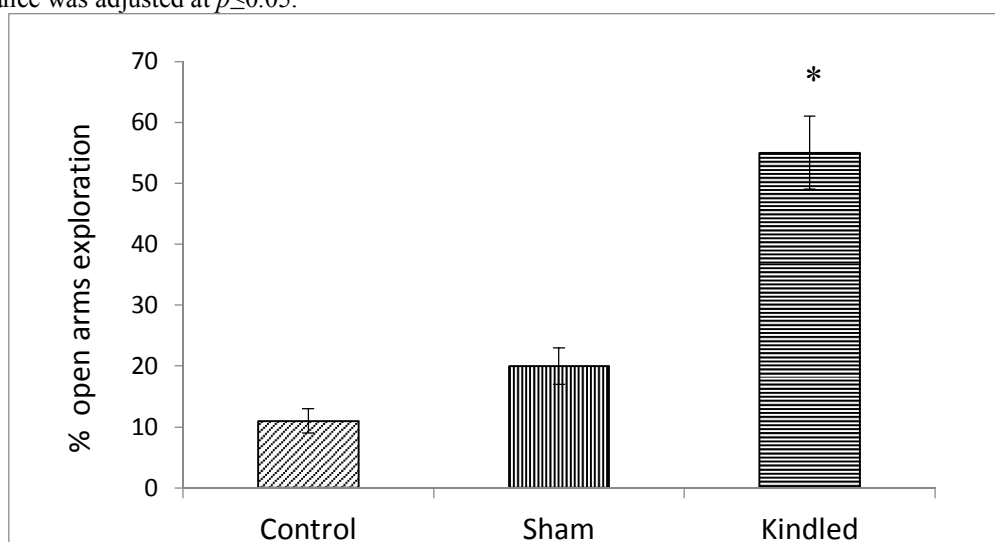
## Results

### 1. Effect of electrode implantation on anxiety and depressive like behaviors

According to the data, there were no significant differences in the percentage of OA entries, exploration of OAs in EPM, rearings in open field, the latency to first immobility, the duration of immobility in FST between the control and sham group. Therefore, the electrode implantation could not affect the behaviors in kindled group compared to sham group (Fig. 1, 2, 3, 4, and 5).

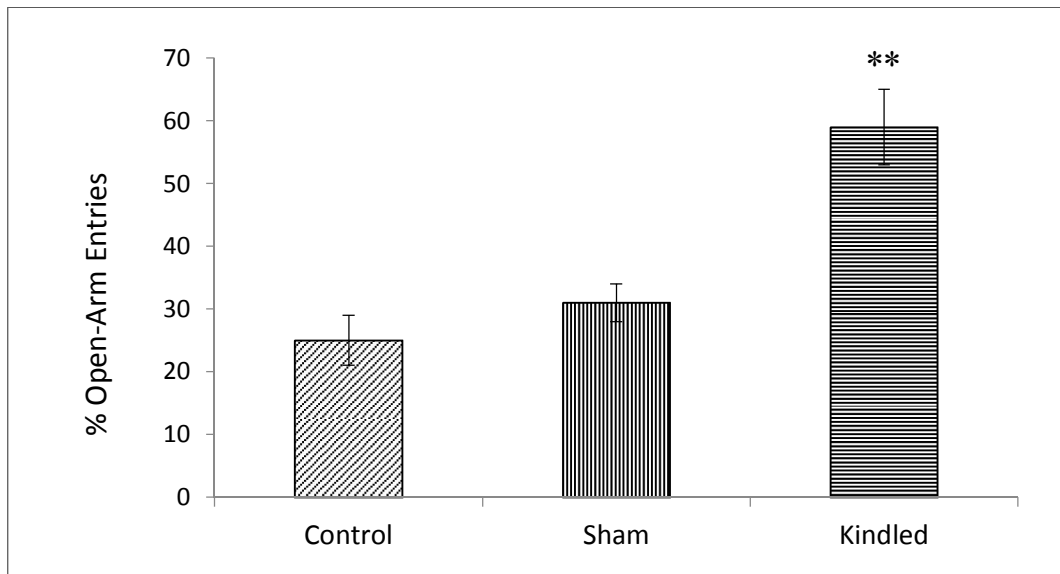
### 2. Effect of hippocampal rapid kindling on anxiety like behavior in elevated plus maze

In the elevated plus-maze test, the percentage of OAs entry ( $p=0.005$ ) and the percentage of OAs exploration significantly increased in the kindled group as compared with the other groups ( $p=0.02$ ) (Fig. 1 and 2). Also, all rats in the kindled group jumped out from the EPM, it seems that the level of fear in rats increased where they were exploring for an escape route from the apparatus.



**Fig. 1.** The effect of kindling stimulations on anxiety like behavior in the EPM.

Values are presented as the Mean  $\pm$  SEM. \* $p \leq 0.05$ . Kindled compared to sham and control groups.



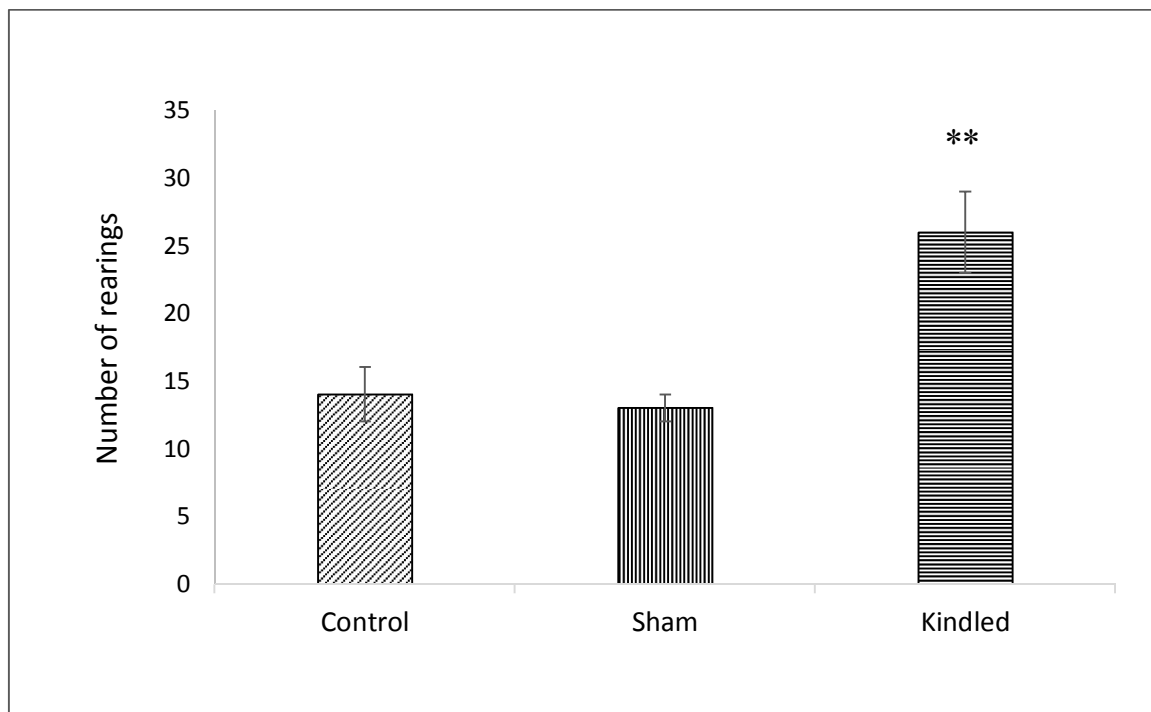
**Fig. 2.** The effect of kindling stimulations on anxiety like behavior in the EPM.

Values are presented as the Mean  $\pm$  SEM. \*\* $p \leq 0.01$ . Kindled compared to sham and control groups.

### 3. Effect of hippocampal rapid kindling on anxiety like behavior in open field

The number of rearings in open field significantly increased in kindled rats in comparison to sham group ( $p=0.006$ )

(Fig. 3).

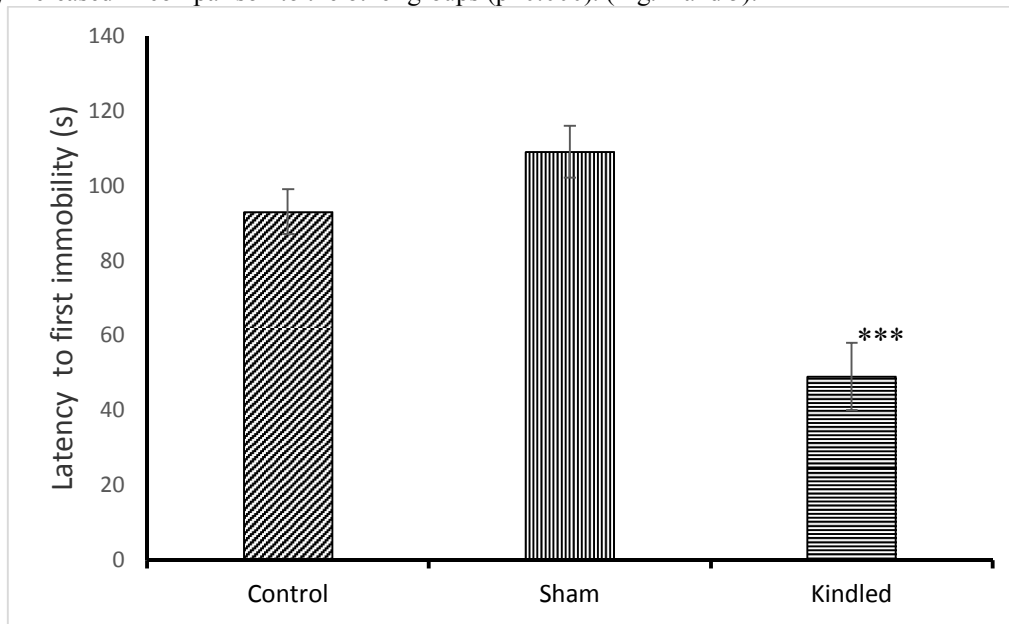


**Fig. 3.** The Effect of kindling stimulation on number of rearings in the open field.

Values are presented as the Mean  $\pm$  SEM. \*\* $p \leq 0.01$ . Kindled compared to sham and control groups.

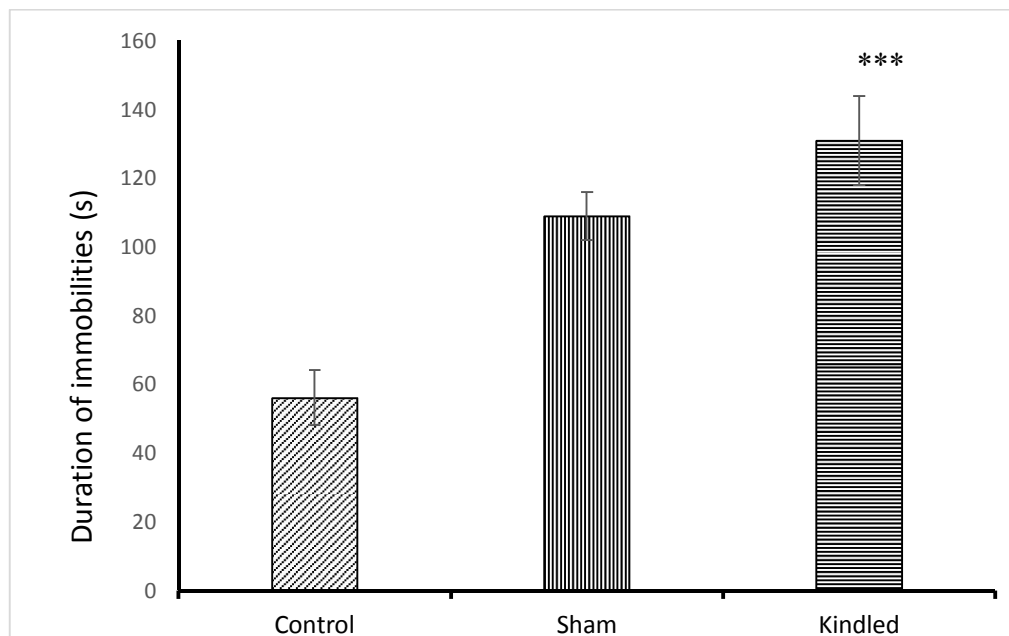
#### 4. Effect of hippocampal rapid kindling on depressive like behavior

In the kindled group, the latency to first immobility significantly decreased whereas the duration of immobility significantly increased in comparison to the other groups ( $p=0.000$ ). (Fig. 4 and 5).



**Fig. 4.** The effect of kindling stimulations on depressive like behavior in the force swim test.

The data are presented as the Mean  $\pm$  SEM. \* $p \leq 0.05$  and \*\* $p \leq 0.01$  and \*\*\* $p \leq 0.001$ . Kindled compared to sham and control groups.



**Fig. 5.** The effects of kindling stimulations on depressive like behavior in the force swim test.

The data are presented as the Mean  $\pm$  SEM. \* $p \leq 0.05$  and \*\* $p \leq 0.01$  and \*\*\* $p \leq 0.001$ . Kindled compared to sham and control groups.

## Discussion

Our data suggested that hippocampal rapid kindling can increase the percentage of entry and time spent on the OAs of the EPM (Fig. 1 and 2). In fact, the surge in these two factors is an important indicator to analyze anxiolytic drug effects. However, since all rats in the kindled group jumped from the EPM and the number of rearings in open field significantly increased in kindled rats (Fig. 3), it may be suggested that the level of fear in rats increased where they were exploring an escape route from the apparatus. Furthermore, animals in the kindled group showed long immobility (despair) in the FST which is an important factor for determining depressive behaviors (Fig. 4 and 5). Our results demonstrated that the rapid kindling of the right hippocampus leads to the exacerbation of the anxiety- and depression-related behaviors.

The hippocampus and amygdala are considered as important origins of temporal lobe epilepsy and also play key roles in anxiety-like behaviors (5). Emotional disturbances induced by temporal lobe epilepsy have been studied extensively. Our results are consistent with Lisa E. Kalynchuk et al. (1997). They found that long-term amygdala kindling in rats can increase OA activity on the EPM and affect their anxiety-related behavior (20).

Similarly, Adamec et al. (1993) reported that short amygdala kindling results in increase of OA activity on the EPM and consequently induces an anxiolytic effect (21).

In contrast to our study Shang-Der Chen et al. (2016) demonstrated that rapid amygdala kindling resulted in a significantly lower frequency entering to open area of either the EPM or the central zone of an open field. Also, the rapid amygdala kindling causes motor seizure and comorbidity of anxiety in rats (22).

Furthermore, it was represented that full kindling of the dorsal hippocampus cannot affect anxiety-like behaviors in either the EPM or an open field task. In fact,

the performance of the kindled animals in the open field task or EPM was not changed (23).

In another study, Jones N. C. et al. (2009) pointed out that rapid amygdala kindling produces an anxiolytic effect in a way that the kindled animals spent much more time in the OAs of the EPM and in the inner areas of the open field task (24). However, few studies investigated the effect of limbic epilepsy particularly kindling on depression-related disorders and the results are controversial. In consistent with our results, Shang-Der Chen et al. (2016) reported that the rapid kindling of right amygdala decreases sucrose intake (anhedonia) in the sucrose intake test and increases immobility (despair) in the FST indicating enhancement of anxiety- and depression-like behaviors (22).

Mazarati et al. represented the surge of immobility time in the kindled animals in the FST as well as depressive-like behaviors following the rapid kindling of the ventral hippocampus (25).

Numerous factors might be involved in the discrepancy between the result of different studies about the effect of the kindling on the behaviors of rats such as intensity of the stimulation current (20, 22), strain of rat (26), number of electrical stimulation, seizure severity under a rapid kindling process (27), regions of applying electrical stimulation (20, 22, 23), hemisphere or nucleus kindled (21), and different measure for the duration of the FST (22).

Since hippocampus affects the excitatory circuits and networks in the amygdala. These areas of the brain play a crucial role in emotional processing in the central nervous system (27). Therefore, the hippocampal kindling can produce changes in the physiological markers of depression and anxiety (28). It seems that kindling increases the secretion of excitatory neurotransmitters and consequently contributes to pathophysiology of some psychiatric disorders such as depression and anxiety (29, 30). To make an example, the kindling- induced enhancement of N-methyl-D-

aspartate (NMDA) receptor function might produce the NMDA receptor hypofunction hypothesis of schizophrenia (31). Besides, the kindling can change the benzodiazepine receptors in specific areas of the hippocampus leading to mediation of emotional behaviors (31). Several mechanisms has been suggested that can contribute to the effect of temporal lobe epilepsy on depression such as impairments in serotonergic, noradrenergic, glutamatergic, and GABAergic transmission (32).

Also, depression seems to be related to a hyperactivation state of the hippocampus. During temporal lobe epilepsy seizures, cortical regions activity changes between hypometabolism and hypermetabolism phase which causes hyperactivity and depression (33).

### Conclusion

These finding suggest that rapid kindling stimulations cause emotional disturbances such as anxiety and depressive like behavior in animals. It seems that kindling enhances excitatory processes or disturbs neuronal inhibition, so leads to the reduction of the seizure threshold and enhancement of anxiety and depression through changing neuronal excitability.

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### Compliance with ethical standards

The authors declare that they have no conflict of interest.

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