Assessment of the Optimal Stimulus Pattern to Achieve Rapid Dorsal Hippocampal Kindling in Rats

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ABSTRACT

Introduction: Although hippocampus is the most famous brain area involved in temporal lobe epilepsy, hippocampal kindling (HK) develops very slowly. Hence, rapid kindling is usually preferred to the traditional kindling and it is widely used. In this article we aimed at finding the optimal stimulus pattern, which yields the fastest HK rate.

Methods: Stimulus patterns with different duration (2, 3, 5 and 10 s) and inter-train interval (ITI) (5, 10 and 30 min) as well as number of trains in 24 h (8 and 12) were exerted to rats' dorsal hippocampus. The stimuli were continued until appearance of 3 consecutive generalized seizures or maximum 7 days stimulations.

Results: While the protocol with train duration of 10 seconds and ITI of 30 min caused the fastest kindling rate and the most growth of after discharges, the protocol with train duration of 5 seconds and ITI of 5 min was the most time-consuming protocol among protocols tested.

Discussion: Rapid HK develops with a time course of days compared to weeks in traditional kindling. Train duration and inter-train interval are key factors for rapid HK. Among the patterns, 12 trains/24h of 50Hz monophasic square wave with 10 seconds duration and 30 min interval between trains, is the best stimulus pattern for eliciting rapid dorsal HK.

1. Introduction

After stroke and Alzheimer's disease, epilepsy with 1% global prevalence, is the most common neurologic disorder (Hirtz et al., 2007). Temporal lobe epilepsy (TLE) is the most common form and drug-resistant epilepsy in adults, with an incidence of more than 30%. Yet, pathophysiology of TLE is not completely understood. The spotlight of TLE often exists in mesial temporal structures, such as hippocampus and/or amygdala, and the seizures are complex partial (Engel, 1996). Regarding behavioral and electrophysiological outline, electrical kindling bears a resemblance to human TLE. Since its introduction by Goddard, kindling has been widely employed as a chronic animal model of TLE (Goddard et al., 1969; Sutula, 1990). Kindling is still extensively accepted as a functional epilepsy model in which altered neuronal response develops in absence of gross morphological damage, such as the one which is seen in many other epilepsy models. This model permits exploring neuronal network contributed in progression of partial seizures to generalized seizures (Racine, 1972).

In traditional kindling, behavioral manifestations of seizures are secondarily developed by daily applying stimulus to one region of limbic system at a rate of once and/or twice daily. At first, behavioral symptoms are local and

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originate from electrical discharge of stimulated region. Then, discharges progress and gradually spread to all regions of the brain and the animal finally became epileptic or allegedly kindled. In traditional kindling, depending on the stimulated region it usually takes 10-30 days to produce a kindled animal.

In a while, rapid kindling approach has been introduced in which daily application of subsequent stimulation with intervals shorter than 12 h produces kindling much faster while it does not have substantial difference in behavioral and electrophysiological profile with traditional model (Lothman et al., 1991; Lothman and Williamson, 1993). Rapid kindling is a model of compressed epileptogenesis where epileptic state can be achieved within a several hours (Lothman et al., 1991; Lothman and Williamson, 1993).

Hippocampus is one of the most important regions involved in TLE. However, it is kindled quite slowly. Indeed, in traditional kindling, more than 30 stimulations into hippocampus are required to produce kindled animal. Concerning time saving, rapid kindling method could be considered as a suitable and functional substitute for traditional kindling. In addition, from phenomenology aspect, the overall response profile of rapid kindling is similar to profile of traditional kindling (Lothman and Williamson, 1993).

There are numerous and growing reports on dorsal hippocampus as a target to develop kindled animals (Musto and Bazan, 2006; Musto et al., 2009; Ishimaru et al., 2010). Furthermore, AD threshold is lower in dorsal CA1 area than in ventral areas (Racine et al., 1977). Therefore, it is of interest to find an optimal protocol providing dorsal hippocampal kindling in a short time. To our knowledge, no data is provided on evaluating the optimal protocol to generate rapid dorsal hippocampal kindling. Based on earlier studies, different stimulation protocols have been used to generate rapid hippocampal kindling.

Parameters used in these protocols include pulse shape (square single or double phase), pulse width (0.1-1 ms), stimulus train frequency (20, 50, 100 Hz), stimulus train duration (1, 2, 5, 10 s), total number of stimulations in each trial during 24 h (6, 8, 10, 12 times) and time interval between each train (Lothman and Williamson, 1993; Musto and Bazan, 2006; Musto et al., 2009; Howland et al., 2007; Mazarati et al., 2007). We evaluated effect of 7 protocols on the rate of dorsal hippocampal kindling. Among these protocols, we focused on different stimulus train duration, number of and interval between stimuli.

2. Methods

2.1. Kindling

Adult male Wistar rats (270-300g, Pasteur Institute of Iran) were used. The study was approved by Ethics Committee of Pasteur Institute and conforms to European Communities Council Directive of 24 November 1986 (86/609/EEC). Rats were implanted with stimulating-recording stainless-steel Teflon-coated electrodes in dorsal CA1 (coordinates; AP, -3.8 mm from bregma; L, -2.2 mm; DV, 2.3 mm from dura) according to the method previously described (Ahmadi et al., 2013). Another electrode was connected to a skull screw, placed above the left cortical surface as earth and differential electrode. After one week, after discharge (AD) threshold was determined for the separate stimulus train of differ-ent protocols as described in Figure 2. Duration of AD (ADD) was measured from the end of stimulus to the end of electrical seizure activity.

AD was identified as paroxysmal discharges with amplitude of at least twice baseline electroencephalographic activity. The stimulation was initially delivered at 50 μA and then increasing stimulus intensity in increments of 50 μA was delivered, until at least 5 seconds of AD was recorded (Ahmadi et al., 2013). Rats were considered fully kindled whenever they showed 3 consecutive stage 5 (generalized) seizure behavior according to Racine classification (Racine, 1972). For each animal, number of stimuli required to reach each of the five behavioral stages (S1-S3 focal seizures, S4 and S5 generalized seizures) as well as ADD were recorded. After completion of experiments, histological evaluation was performed to confirm correct situation of the electrodes (Ahmadi et al., 2013).

2.2. Data analysis

Data are presented as mean ± SEM. Data were analyzed by two ways ANOVA with Bonferroni post-test analysis. In all experiments, $P<0.05$ was considered statistically significant.

3. Results

3.1. Importance of Inter-train interval and train duration parameters for eliciting rapid generalized motor seizures

Figure 1 shows traces of AD recorded from dorsal hippocampus after performing different stimulation protocols at the first day of kindling. The mean AD threshold obtained for the animals was in the range of 150–300 μA.

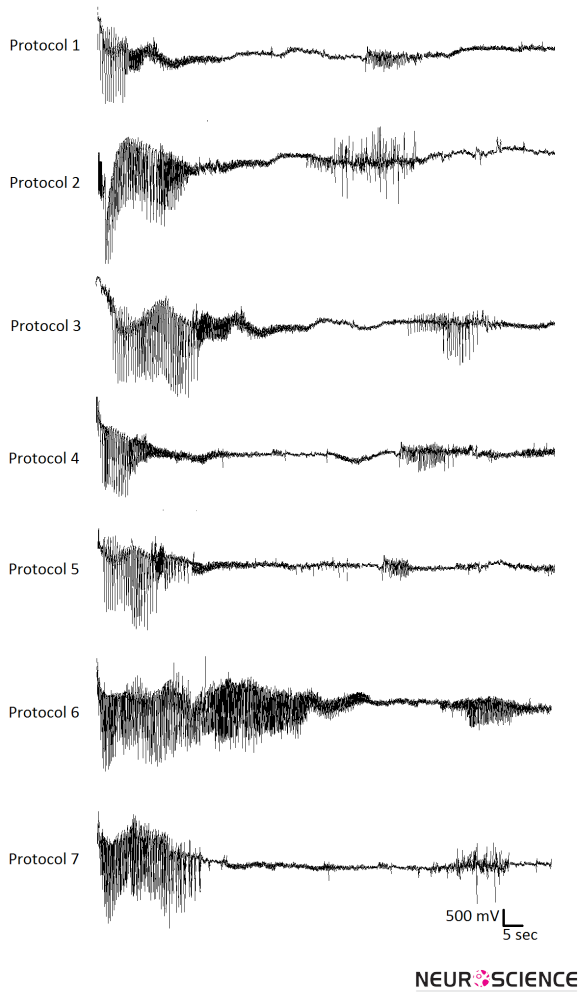


Figure 1. Representative EEG traces of dorsal CA1 at the first day of kindling acquisition induced by different stimulation protocols.

Histological evaluation of the brains confirmed correct position of the electrode in all of the animals.

Two-way ANOVA revealed a significant effect of column factor (different protocols) [$F(6,280)=10.46$; $P<0.001$]. Further analysis using Bonferroni post test revealed a significant difference between protocol 6 and 4, from day 1 to 7 ($P<0.05$) and also between protocol 6 and 1 in the first two days ($P<0.05$) (Figure 2A). Animals treated with protocol 6 showed generalized seizures much earlier than other groups (Figure 2A). On the first day, they showed stage 4 and then responded in a plateau profile on the following days.

On the other hand, protocol 4 failed to produce even stage 2 until 7 days of stimulations. The most prominent differences between these two protocols were in inter-train interval (ITI) and train duration. In protocol 6, 30 min ITI and 10 seconds train duration are included, but these parameters for protocol 4 were 5 min and 5 seconds, respectively. It seems that these parameters are critical for eliciting rapid hippocampal kindling. While after 5 days, protocol 5 and 3 finally produced stage 4, animals treated with protocol 1 and 2 did not become fully kindled. All parameters in protocol 6 were similar to those in protocol 3, other than the stimulus wave form which was biphasic in protocol 3 whereas it was monophasic in protocol 6. Altogether, these data suggest that increasing ITI up to 30 min with monophasic 10 seconds train duration optimally evoked generalized motor seizures in first day of kindling.

3.2. Importance of Inter-train interval and train du-ration for evoking longer after discharge duration

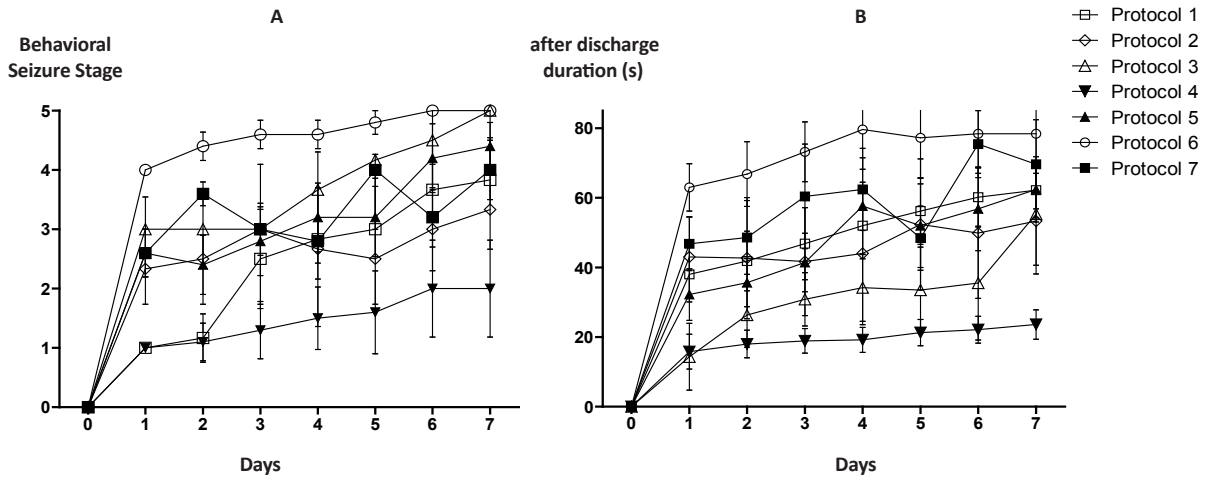
The most efficient approach to evoke long ADD was protocol 6 (Figure 2B). A two-way ANOVA revealed significant difference between different protocols ([$F(6,280) = 17.21$; $P<0.001$]) and post hoc Bonferroni test showed that ADD evoked by protocol 6 (63 ± 6.8 s) was approximately 4 times longer than that of induced by protocol 4 at the first day (15.8 ± 5 s) ($P<0.05$). As noted before, these two protocols differ in ITI and train duration. By experience, we found that by increasing ITI, longer ADD can be achieved so that with 30 min ITI rather than 5 min, durable AD from dorsal CA1 is elicited.

In addition, train duration was a critical factor for evoking long AD as it was 10 seconds for protocol 6 and 5 seconds for protocol 4. The importance of train duration and also train frequency could be more obvious by comparing protocol 1 and 6. ITIs were the same for both protocols (30 min) but train duration for protocol 1 was 2 seconds compared to 10 seconds for protocol 6. However, other parameters may affect AD duration. For instance, as one can see, except for stimulus waveform, the parameters included in protocol 3 and 6 were similar, but protocol 3 failed to elicit long ADD. It seems using monophasic rather than biphasic stimulus is better to achieve very rapid hippocampally-kindled rats.

4. Discussion

The purpose of present study was to find optimal stimulation parameters for achieving rapid hippocampal kindling. In this regard, 12 trains/24 h of 50 Hz monophasic square wave with 10 seconds duration and 30 min interval between trains caused the fastest kindling rate.

Although hippocampus plays a pivotal role in clinical epilepsy, it is experimentally kindled quite slowly. However, in many studies hippocampus is used as a target to create rapid kindling model (Musto and Bazan, 2006;



Protocol	Waveform	Pulse rate (Hz)	Train duration (s)	Number of trains in 24 h	Internal between trains (min)
● 1	Monophasic square	20	2	12	30
■ 2	Monophasic square	50	3	8	10
▲ 3	Biphasic square	50	10	12	30
◆ 4	Monophasic square	50	5	12	5
□ 5	Biphasic square	50	3	8	10
△ 6	Monophasic square	50	10	12	30
◇ 7	Biphasic square	50	5	12	5

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Figure 2. Effect of different stimulus patterns on development of behavioral seizures (A) and growth of after discharges (B) in rat rapid hippocampal kindling. Each point shows mean ± SEM. *P< 0.05 protocol 6 vs. protocol 1 (A) and protocol 6 vs. protocol 3 (B), #P< 0.05, ##P< 0.01 and ###P< 0.001 protocol 6 vs. protocol 4.

Mazarati et al., 2009; Musto et al., 2009; Ishimaru et al., 2010; Nikitidou et al., 2014). Compared to ventral hippocampus, using dorsal hippocampus has the advantage of less damage to the brain tissue due to less depth of implanted electrodes. Moreover, dorsal CA1 has lower AD threshold than ventral areas (Racine, 1977). Therefore, some researchers prefer dorsal hippocampus to generate kindling model (Lothman et al., 1988; Barnes et al., 2005; Musto and Bazan, 2006; Musto et al., 2009). Hence, finding an optimal stimulation pattern for dorsal hippocampal kindling is a major interest.

In this regard, it has been reported that with the same stimulation protocol, animals with dorsal hippocampal kindling develop motor seizure sooner than those with ventral hippocampal kindling (Becker et al., 1997; Barnes et al., 2005). Musto et al. (2006; 2009) achieved dorsal hippocampal kindled mice after 4 days of employing subconvulsive electrical stimulations of 10-s train containing 50-Hz biphasic pulses of 100-μA amplitude at 30-min intervals and six times daily (Musto and Bazan, 2006; Musto et al., 2009). In another rapid kindling study, by applying a stimulus pattern of 10 strain duration, 20 Hz, 1 ms pulse width, square wave monophasic stimuli, and 10 seconds ITI to ventral hippocampus, just 57% of the animals showed stage 4 during first day of stimulations (Mazarati et al., 2007).

In the present study, appearance of a severe limbic seizure during first day stimulation was selected as the condition for a positive day 1 kindling response. Except for the wave form, all parameters in protocol 3 were similar to those in protocol 6, however, in contrast to protocol 6, protocol 3 failed to elicit generalized motor seizures at the first day of implementation. This data is in good agreement with Lothman et al. report in which biphasic stimulation with 50 Hz train and ITI of 5 min yielded mild seizure on the first day (Lothman and Williamson, 1993). In contrast, switching ITI from 5 to 30 min. did not change pattern of responses in day 1.

By setting the rate of stimulation at 30 min interval along with delivering 10 stimuli per day at the outset of day 1 treatment, only mild limbic seizures could be triggered (Lothman and Williamson, 1993). In our study, protocol 4, which efficiently elicits rapid amygdala kindling (Asgari et al., 2014) failed to provoke hippocampal kindling. The reason seems to be related to different vulnerability of amygdala and hippocampus for kindling development as it is known that kindling threshold for amygdala is less than hippocampus and conventional amygdala kindling occurs faster than conventional hippocampal kindling (Musto et al., 2009; McIntyre et al., 1999).

Comparing protocol 1 and 6 revealed that stimulation potency required for kindling depends on both train frequency and train duration, so that less train frequency and shorter train length produce less ADD and corresponding motor seizures.

Consistently, it has been reported that train frequency of 50 Hz rather than 10 Hz induces faster kindling of ventral hippocampal and higher proportion of positive kindled response on day 3 (Lothman and Williamson, 1993). On the other hand, Lothman et al. reported that trains of 20 Hz is the optimal frequency for eliciting maximal dentate activation, accompanied by increased kindling rate (Lothman and Williamson, 1993). In addition to train frequency, we found that ITI is a major determinant for the rate of kindling, and longer ITI consistently elicits longer duration of AD.

There are many experiments using the kindling model that focus on the duration of AD as the main dependent variable (Goddard et al., 1969; Racine, 1972; Morimoto et al., 2004). It has been proposed that AD is characterized by four patterns which develops progressively during kindling and is correlated with corresponding behavioral seizure stages of one to five replicating partial complex and generalized seizures in human (Musto et al., 2009).

In terms of underlying mechanism, it has been suggested that at the onset of kindling, pyramidal neurons and interneurons fire in concert and generate complex burst spikes of AMPA, NMDA, and GABA activation (Morimoto et al., 2004; Ziburkus et al., 2006). By successive stimulations, the fast inhibition process is exhausted (Wendling et al., 2005); this may be involved in down regulation of $GABA_A$ (Liefaard et al., 2009) or $GABA_B$ receptors and, in turn, overcome excitatory activity (Leung and Shen, 2006).

In our study, we found that by protocol 6, rats achieve long AD and corresponding severe motor seizures much earlier than with protocol 4. We found that maximal AD activity is elicited by increasing ITI to 30 min. The underlying mechanism implicated in the rise of AD duration by longer ITI is unknown. Racine et al. evaluated influence of different ITI on amygdala kindling (Racine et al., 1973). They realized that while ITI could be reduced to 1 h without significant alteration of the number of stimuli required to achieve kindling, setting ITI to 30 min increased number of stimuli required for kindling but decreased the time to about 11 h.

In summary, ITI and train duration could be critical parameters for eliciting rapid dorsal hippocampal kindling. While the protocol with 5 min ITI and 5 seconds train duration provokes a very weak response, increasing ITI to 30 min and train duration to 10 seconds produces generalized motor seizures and long AD in the first day of stimulations. With the stimulus protocols examined, kindling takes place in a time frame of 6 h. Moreover, rapid kindling from dorsal hippocampus with 12 monophasic waveform stimulations at frequency of 50 Hz develops quicker than biphasic waveform with the same parameters.

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