**Title:** Effect of High-Frequency Electrical Stimulation of Olfactory Bulb on Spontaneous Excitatory Post-Synaptic Currents in Hippocampal CA1 Pyramidal Cells of Kindled Rats

**Running Title**: Olfactory Bulb Stimulation and Hippocampal EPSP in Seizure

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**Abstract**

**Introduction:** Application of deep brain stimulation (DBS) has been considered as a new therapeutic manner for neurological disorders, including epilepsy, treatment. However, the precise procedures underlying the anticonvulsant action of high-frequency stimulation (HFS) remain unclear. In this study, we explored the impact of applying high-frequency stimulation in the olfactory bulb on alterations observed in spontaneous excitatory postsynaptic currents (sEPSCs) in kindled animals.

**Methods:** Male rats underwent a kindling procedure involving semi-rapid electrical stimulation (six stimulations/day) of the dorsal CA1 area. HFS (130 Hz) was administered in full kindled rats at four times (i.e., 5 min, 6 h, 24 h, and 30 h) after the $6^{th}$ (i.e., the last) kindling stimulation (kindled+HFS group). Subsequently, the impact of HFS on EPSCs was evaluated in pyramidal cells in CA1 area of the hippocampal slices from kindled subjects using whole cell patch clamp technique.

**Results:** In kindled animals sEPSC amplitude increased ($p<0.001$), while the sEPSC inter-event interval decreased compared to the control group ($p<0.05$). Application of HFS in the olfactory bulb of fully kindled rats resulted in a reduction in sEPSCs amplitude and an increase in the sEPSCs' inter-event interval. There was not any significant difference in membrane potential and input resistance in kindled animals compared to control group. Notably, application of HFS in kindled animals restored the observed changes, so that no significant change was observed in mentioned parameters when kindled+HFS compared with the control group.

**Conclusion:** These findings suggested that the olfactory bulb may be considered a viable DBS target in epilepsy treatment, and HFS application may mitigate the increase in sEPSCs occurrence following seizure in kindled animals.

**Keywords:** Epilepsy; Deep brain stimulation; Olfactory bulb; Hippocampus; Seizure

**Highlights**

- sEPSC amplitude increased, while the sEPSC inter-event interval decreased in kindled animals.
- Applying DBS in the olfactory bulb reduced the sEPSC amplitude in kindled rats.
- Applying stimulation in the olfactory bulb of kindled rats increased the inter-event interval of sEPSC.
- No significant difference was observed in eveluated parameters between control and kindled animals that received DBS.
- Inhibition of sEPSCs in hippocampus may be a mechanism for anticonvulsant action of DBS.

**Plain Language Summary**

Epilepsy is among the most widespread neurological disease in all populations. Some epilepsy patients are drug resistive and new manners need to find for their treatment. Deep brain stimulation (DBS) is a novel potential manner for treatment of these patients. However, to use this method, it is necessary to find its mechanism of action. In the present study, we showed that when DBS is applied in olfactory bulb, the excitatory synaptic currents in the hippocampus may be reduced. These currents have critical impact in seizure generation and propagation in the hippocampus. Therefore, reducing the excitatory post-synaptic transmission in the hippocampal neurons is among the basic mechanism of DBS anticonvulsant action.

## 1. Introduction

Approximately 50 million people worldwide suffers from epilepsy, making it the third most prevalent chronic neurological condition (Ghosh et al., 2021). Despite the widespread occurrence of epilepsy, there remains a lack of definitive manners to treat the epilepsy. While anticonvulsant drugs serves as the predominant approach in epilepsy patients, its effectiveness lies in suppressing seizures rather than addressing the fundamental brain irregularities responsible for epilepsy (Macdonald & Kelly, 1995). Consequently, there is a need to explore novel methods aimed at mitigating the adverse effects of seizures on the brain.

The kindling model represents a progressive evolution of focal seizures followed by focal to bilateral tonic seizures, triggered by repetitive electrical or chemical stimulation (Cheng et al., 2020). The mechanism underlying epileptogenesis is thought to involve the increase in the activity of brain's excitatory pathways, which includes heightened glutamate transmission. These changes may lead to an imbalance of neuronal excitation/inhibition. The increment in glutamate receptors' activity plays a significant role in both the initiation and propagation of epileptic discharges throughout the kindling process (McNamara et al., 1988b).

DBS has undergone extensive clinical investigation as a treatment modality for various brain disorders, including epilepsy, Parkinson's disease and pain management, addiction, stroke recovery, obsessive–compulsive disorder, and depression (Kuhn et al., 2011; Sandoval-Pistorius et al., 2023). One potential mechanism of DBS is its ability to induce depressive effects, and therefore, preventing neuronal over-firing, particularly when applied at a suitable pattern.

DBS is usually applied at high frequencies (i.e. high-frequency stimulation: HFS) and 130 Hz is one of its most common frequency in seizure control in both clinical and experimental studies (Covolan et al., 2014; Lee et al., 2006; Wyckhuys et al., 2010). It has been proposed that there are three potential mechanisms of HFS on neuronal activity: inhibition, excitation

and modulation. Previous researches indicated that the neuronal firing within the neural circuits is reduced by applying HFS. This may occur through activation of inhibitory synapses (Alhourani et al., 2015), or by depleting of excitatory neurotransmitters (Iremonger et al., 2006). Nonetheless, some animal experiments and through mathematical simulations demonstrated the HFS-induced elevation in the neuronal firing rate (Deniau et al., 2010a). These controversies in HFS effectiveness on neuronal activity may be explained by the third possible mechanism, i.e., modulation. HFS may alter the patterns of neuronal firing or the rhythms of neural circuits, rather than solely modifying the rate of neuronal firing (Florence et al., 2016; Herrington et al., 2016; McConnell et al., 2012). Enhancing the pre-synaptic inhibition is also among the proposed DBS anticonvulsant mechanism. This enhancement may occur through hyperpolarization of the somas and dendrites of the neurons following stimulation. Or, it may be through depolarizing-induced blockage of neurotransmitters (Deniau et al., 2010b; Montgomery Jr & Gale, 2008).

Knowing little about the precise anticonvulsant mechanisms of DBS, made it difficult to be widely used in epilepsy treatment (Chiken & Nambu, 2016). Therefore, these mechanisms need to be investigated. In this investigation we attempted to study the impact of applying high-frequency-DBS (HFS) on alterations of excitatory-post synaptic currents (EPSCs) in hippocampal CA1 neurons in kindled animals.

## 2. Materials and methods

*Animals*

In this experimental investigation, 17 male Wistar rats were individually housed in animal cages (each rat was kept separately in one cage) maintained at 22-25˚C room temperature, and subjected to a 12 h light and 12-h dark cycle with lights on at 6:00 am and off

at 6:00 pm. The animals aged 2-4 months old (200-280 g) at the time of surgery and had access to food and water with no limitation.

*Experimental design*

We assessed how HFS influenced synaptic currents in fully kindled animals. Subjects were divided into four groups: a) control group (6 rats) in which animals underwent the surgical procedure but were not subjected to either HFS or kindling stimulations; b) control+HFS group (3 rats) in which animals underwent similar experimental procedures to the control group but received HFS; c) Kindled group (5 rats) that received kindling stimulation and 48 h after the last kindling stimulation and achieving full kindled state, the brain slices were prepared from the hippocampus for patch clamp recording (these animals did not receive DBS and d) kindled+HFS group (3 rats) that received HFS after achieving full kindled state and their hippocampal slices were prepared similar to the manner explained for the kindled group; Slices were prepared in the control and control+HFS groups, at a comparable time duration similar to the kindled and kindled+HFS groups.

In different groups, the number of cells was 13 cells/12 slices in control group, 9 cells/5 slices in control+HFS group, 10 cells/9 slices in kindled group and 8 cells/5 slices in kindled+HFS group.

*Surgery*

The rats underwent deep anesthesia by a combination of ketamine/xylazine (Sigma, England, 100/10 mg/kg, i.p. injection) and were securely positioned in a stereotaxic apparatus in a horizontally flat skull configuration. The animal skull was exposed following a cut in the scalp. A bipolar electrode (for stimulation) along with a monopolar electrode (for recording) were carefully inserted into the hippocampal CA1 area of the right hemisphere. The coordination of these bipolar and monopolar electrodes was: 5.3 mm posterior and 5.2 mm lateral to the bregma, and 6.5 mm below dura. In addition, a monopolar recording electrode,

These electrodes served for both kindling stimulations and afterdischarge recording. Additional bipolar stimulating electrodes were inserted into the right and left olfactory bulbs (coordinated as: 7.5 mm anterior to bregma, 1.0 mm lateral to right or left, and 1.6 mm below the dura) for HFS. The stainless-steel electrodes were coated by teflon (A-M Systems, Inc., WA, USA, 127 μm in diameter). The electrodes were checked to be completely insulated in their length and only their tips was not insulated. Additionally, a stainless-steel screw was connected to the skull (over the occipital cortex) and attached to a monopolar electrode as a reference and/or ground.

To induce kindled seizures in animals, afterdischarge (AD) threshold was measured by applying a 1 ms square pulse (50 Hz), for 2 s (Ghafouri et al., 2016a). At the first step the stimulus was administered at the intensity of 30 μA. The epileptiform spikes that had an amplitude higher than baseline×2 and their frequency was bigger than 1 Hz, were considered as ADs. If ADs were recorded for at least 8 s, the applied stimulating current was considered as AD threshold. If AD were not recorded, the intensity of stimulation was augmented in 10 μA steps with intervals of at least 10 min until the ADs were recorded for 8 s or more (Khodadadi et al., 2022). The occurrence of ADs following stimulation of animal at AD threshold is a critical phenomenon in the kindling model of seizure. Therefore, ADs recording is necessary to confirm proper development of the kindling procedure.

*Kindling induction*

As explained previously (Khodadadi et al., 2022), the threshold intensity for AD generation threshold was determined after a post-surgery recovery period of at least seven days. Each animal received electrical stimulations for 6 times/day at the intensity equal to AD threshold. The interval between consecutive stimuli wwas 20 min. After each kindling stimulation, the AD spikes were recorded through recording electrode from the CA1 area through a data acquisition system (BIODAC ES1721, TRITA Health Technology CO., Tehran,

Iran) that was connected to PC. The Racine's scale (Racine et al., 1977) was used to quantify the severity of seizure behaviors. According to this scaling method, stage 0 was considered as no convulsions, stage 1 as facial clonus, stage 2 was head nodding, stage 3 was unilateral forelimb clonus, stage 4 was determined as bilateral forelimb clonus, and stage 5 was considered as rearing, falling, and generalized convulsions. The fully kindled state was achieved when the animal displayed at least one stage 5 seizure scale in three consecutive days.

*Applying HFS in olfactory bulb*

To investigate the impact of applying HFS in olfactory bulb on hippocampal kindled seizures, fully kindled rats underwent four sets of bilateral HFS in the olfactory bulb using stimulating electrodes. The first set of HFS was administered in full kindled animal immediately after the last kindling stimulation once the ADs were finished. The second set of HFS was delivered 6 hours later. The third set of HFS was applied on the subsequent day (24 h after the first HFS), and the fourth set of HFS was administered at 6 h after the third HFS. Each set of HFS contained 4 trains of monophasic 0.1 ms square pulses. The duration of each train was 200 seconds, with an inter-train interval of 100 seconds. These trains were applied as high-frequency, at 130 Hz, therefore, each train contained 26,000 pulses. HFS parameters were obtained based on previous studies (Ghafouri et al., 2016b; Sadeghian, Salari, Azizi, Raoufy, Shojaei, Kosarmadar, Zare, Rezaei, Barkley, & Javan, 2020).

*Whole-cell patch clamp recording*

Whole-cell patch clamp recordings was run in dorsal and ventral hippocampal slices. The procedure of brain slice preparation was alike to previous study (Ghafouri et al., 2016a). Briefly, rats were decapitated after anesthetized with $CO_2$. Then, the brain's right hemisphere was quickly detached and put in a cutting solution that was coled by ice. The cutting solution had 2.5 mM KCl, 2 mM $MgCl_2$, 26.2 mM $NaHCO_3$, 1 mM $NaH_2PO_4$, 0.5 mM $CaCl_2$ and 238 mM sucrose, and 11 mM D-glucose. This solution was bubbled by carbogene (95% $O_2$- 5%

$CO_2$) continuously and its osmolarity was in the range of 290-300 mOsm. For hippocampal slices preparation a  a vibratome (1000 Plus Sectioning System, Vibratome, MO, USA) was used and slices with 300 μm thickness were prepared. Then, the right hippocampus isolated from other parts of the brain and transferred to artificial cerebro-spinal fluid (ACSF) that was consisted of 3 mM KCl, 125 mM NaCl, 25 mM $NaHCO_3$, 1.25 mM $NaH_2PO_4$, 2 mM $CaCl_2$, 10 mM D-glucose and 1.3 mM $MgCl_2$. This solution (290-300 mOsm) was also bubbled with 95% $O_2$- 5% $CO_2$ and pH was adjusted to 7.2-7.35 by NaOH (1mM). The hippocampal slices were incubated at 35˚C for at least 1 hour. Slices were kept at room temperature (23-25˚C) before transferring to the recording chamber. All mentioned chemicals were bought from Sigma, England.

A plexiglas recording chamber was put in a fixed-stage upright microscope (Axioskop 2 FS MOT, Carl Zeiss, Germany). The chamber was continuously (1.5-2.5 ml/min) perfused with ACSF.  All recordings were done at 23-25˚C (room temperature). By using an IR-CCD camera (IR-1000, MTI, USA) and a water immersion objective lens (×40 ), the CA1 pyramidal neurons were visualized. The neurons with a pyramidal shape that had a smooth, low-contrast appearance were selected for recording. Patch clamp recording was run in a whole-cell configuration in voltage clamp mode. Borosilicate glass microelectrodes (1.5 mm outer diameter, BF150-86-10, Sutter Instruments, USA) were prepared by a horizontal puller (P-97, Sutter Instruments, USA) and were used for spontaneous EPSCs (sEPSCs) recording. The microelectrodes were filled with intracellular solutions for spontaneous EPSCs (sEPSCs) recordings. The internal solution contained 20 mM KCl, 135 mM K-gluconate, 0.2 mM EGTA, 10 mM HEPES, 7 mM disodium-phosphocreatine, 0.3 mM NaGTP, 2 mM MgATP and 1 mM QX-314.

The microelectrodes had a resistance of 5-8 MΩ, and the series resistance during recording was 18 - 30 MΩ. In the case of neurons that the changes in series resistance during

sEPSC recording was higher than 20%, the obtained data were discarded. Capacitance compensation was performed during recordings. A Multiclamp 700B amplifier and a Digidata 1440 (Molecular Devices, CA, USA) were used for recording. The low-pass filter was 3 kHz and the sample rate was 10 kHz. The recorded signals were stored on a PC and data were offline analyzed by MiniAnalysis and pCLAMP10 software.

The impact of applying HFS on CA1 pyramidal neurons sEPSC was investigated in kindled animals by recording in voltage clamp mode for 5 min. During the sEPSC recording, the holding potential was -52 mV that was equal to $GABA_A$ reverse potential to omit the GABAergic inhibitory currents. The $GABA_A$ reverse potential obtained by adding CNQX (AMPA receptor antagonist, 20 μM, Tocris Bioscience, England) and AP-5 (NMDA receptor antagonist, 50 μM, Tocris Bioscience, England) to ACSF. At this situation, the I-V curve of GABAA receptors was obtained and the $GABA_A$ reverse potential was calculated (Sadeghian, Salari, Azizi, Raoufy, Shojaei, Kosarmadar, Zare, Rezaei, Barkley, Javan, Fathollahi, & Mirnajafi-Zadeh, 2020). sEPSCs were recorded at least 10 min after achieving the whole-cell configuration. The amplitude and inter-event interval of sEPSPs were calculated.

*Statistical analysis*

Obtained data were presented as mean ± SEM. Statistical analysis was conducted using GraphPad Prism (Ver 6.01, GraphPad Software, Ca, USA). To assess the impact of applying HFS on various parameters of sEPSC, we employed one-way ANOVA followed by Tukey's post-hoc test for comparing different sEPSC's parameters among different groups. The significant difference was considered when the measured *p-value* was less than 0.05.

## 3. Results

The number of stimulation days to achieve full kindled seizures was not significantly different among the kindled (7-12 days) and kindled+HFS (7-12 days) groups. The AD

threshold was also in the similar range (60-80 μA) in two groups. Additionally, there was not any significant difference in the duration of AD following the first stimulation at the AD threshold between kindled (93.19±6.86 s) and kindled+HFS groups (86.15±4.86 s). These findings showed no significant differences in seizure susceptibility among two experimental groups.

In the first experiment the effect of HFS administration was investigated on sEPSC in hippocampal pyramidal cells of kindled animals. One-way ANOVA showed that there was not any significant difference in input resistance and resting membrane potential of ventral and dorsal hippocampal CA1 pyramidal cells between different groups (Fig.1A, B, C, D). However, there was a significant increase in amplitude of sEPSCs in ventral hippocampal CA1 pyramidal cells in kindled rats compared to the control group ($P<0.001$). Also, the inter-event interval of sEPSCs in pyramidal cells in kindled rats decreased significantly compared to the control group ($P<0.05$). Application of HFS in kindled+HFS group restored the changes in sEPSC amplitude and inter-event intervals and there was no significant difference between kindled+HFS and the control group. Application of HFS alone had no significant effect on these parameters compared to the control group (Fig.2B, D).

All above mentioned parameters were also evaluated in the dorsal hippocampal slices. The amplitude of sEPSCs increased significantly in dorsal hippocampal CA1 pyramidal cells in kindled rats compared to the control group ($p<0.001$). The inter-event interval of sEPSCs in pyramidal cells in kindled rats was also decreased significantly compared to the control group ($p<0.01$). These parameters restored to control values following applying HFS in kindled+HFS group. There was not any significant difference between kindled+HFS and the control group Application of HFS alone had no significant effect on these parameters compared to the control group (Fig.2C, E).

## 4. Discussion

Our results suggested that application of HFS in the OB of kindled animals restored the changes in sEPSC parameters in CA1 pyramidal neurons of the dorsal and ventral hippocampus. sEPSCs serve as indicators of the excitability of the nervous system and affect the spontaneous firing in neurons (Meyer et al., 2008). Evaluating the parameters of sEPSCs provides a suitable approach to investigate the excitability of the nervous system. Increasing evidence indicates the participation of the N-methyl-d-aspartate (NMDA) subtype of glutamate receptors in seizure progression. The special voltage-sensitive action of NMDA glutamate receptors involves in regenerative qualities of synaptic transmission, causing increased depolarization and burst firing, much like what is seen in epileptiform discharges (Herron et al., 1986; McNamara et al., 1988a; Mei et al., 2020; Nowak et al., 1984). Accordingly, it may suggest that applying HFS in the OB reduced the kindling -induced increment in neuronal excitability in both dorsal and ventral hippocampus.

It has been shown that OB has a strong connection with ventral hippocampus through entorhinal cortex (Vanderwolf, 1992). It means that, high frequency electrical stimulation of OB may modulate the neuronal activation of hippocampal neurons indirectly. Our previous study showed that applying DBS in OB has anticonvulsant effect of hippocampal kindled seizures (Khodadadi et al., 2022). Therefore, restoring the sEPSCs parameters in hippocampal CA1 neurons may be considered as a possible mechanism of anticonvulsant action of DBS when is applying in the OB.

The cellular mechanism of this effectiveness, as well as the effect of DBS on synaptic transmissions in both excitatory and inhibitory synapses, is unknown. It may be postulated that following the application of HFS, the release of GABA and glutamate from presynaptic terminals increases. Glutamate causes depolarization of the postsynaptic neuron terminal through AMPA receptors, and this depolarization is reduced by the inhibition caused by the

stimulation of $GABA_A$ receptors. With the continuation of stimulation, the inhibitory mechanisms are lost and depolarization continues, and secondarily, it may lead to NMDA receptors activation and the entry of calcium through voltage-dependent calcium channels, and as a result, the subsequent discharge waves begin (Morimoto et al., 2004). Therefore, it was a limitation of the present study that we could not find the mechanism of HFS action. Accordingly, it can be suggested to use a calcium voltage-dependent channel antagonist or a GABA receptor antagonist to determine their roles in HFS effects on seizure. Moreover, the effect of HFS may also be related to activation of microglia and their anti-inflammatory effects, which has been involved in epilepsy (Chen et al., 2020; Peng et al., 2019).

Also, HFS increases the release of GABA by activating $GABA_A$ receptors on the cell body, dendrites and axonal ends of GABAergic neurons (Mantovani et al., 2009). HFS leads to reduction of excitability, inhibition of epileptic activities and inhibition of the nerve network of the target tissue (Bikson et al., 2001). However, more investigations have to be conducted to shed light on the HFS mechanism(s) of action on sEPSCs.

The findings of this study revealed a notable increment of sEPSCs' amplitude and decrement in sEPSC' inter-event interval in CA1 pyramidal neurons in the kindled rats. These changes indicated a rise in the sEPSC's occurrence and therefore, an increase in glutamatergic transmission in the hippocampal CA1 area in kindled animals. These results are in line with previous studies that indicated a significant changes in the NMDA responses of granule cells in the dentate gyrus and pyramidal neurons in hippocampal CA3 region (Kraus et al., 1994) are profoundly altered following chronic epilepsy (kindling) (Hellier et al., 2009). The observed increase in glutamatergic synaptic transmission in the present study, as well as in previously mentioned reports, could be associated with alterations in the function of NMDA and AMPA receptors. These alterations may arise from changes in opening duration or alterations in the $Mg^{2+}$ blockade of NMDA receptors, upregulation of glutamatergic receptors

or an increase in the affinity of these receptors (Ekonomou et al., 2001; Yeh et al., 1989) in epileptic neurons (Ghasemi & Schachter, 2011). Therefore, according to the observed changes in the amplitude of excitatory currents, postsynaptic mechanisms are probably involved. However, since our results showed the change of inter event interval in the ventral part, then pre-synaptic mechanisms can be involved too.

Interestingly, the changes in sEPSCs were observed in both dorsal and ventral hippocampus. The OB has many connections with ventral hippocampus (Vanderwolf, 1992) and the role of hippocampus in odor emotion and odor learning exerts through ventral hippocampus (Fanselow & Dong, 2010). Therefore, it may expect that the anticonvulsant action of DBS in OB exerts via ventral hippocampus. However, significant changes of synaptic currents in dorsal hippocampus revealed that applying DBS in OB has a widespread action in all hippocampal regions. Considering the important role of dorsal hippocampus in seizure propagation (Fujita et al., 2014), the restoring effect of DBS on dorsal hippocampal neurons had an important role in DBS anticonvulsant action.

Our findings indicated that applying HFS to the olfactory bulb at frequency of 130 Hz effectively reduced excitability in the hippocampal slices of kindled animals. As HFS is generally inhibitory, one hypothesis is that the antiseizure effect of HFS may be mediated by the inhibition of pro-seizure glutamatergic neurons (Wang et al., 2020). Further, results obtained through whole cell recording indicated that HFS in the OB significantly decreased the excitatory currents mostly through decreasing the amplitude and increasing the inter-event intervals of EPSCs. These effects shows that both presynaptic and postsynaptic mechanisms may be involved in inhibitory effects of HFS in kindled animals.

## 5. Conclusion

Analyzing the obtained data of the present experiments indicated that administration of HFS in kindled rats prevented the increment of spontaneous glutamatergic transmission in hippocampal CA1 pyramidal neurons. This action may be involved in the anticonvulsive action of HFS in kindled animals. In addition, considering the functional and anatomical connectivity between olfactory bulb (as a major part of olfactory system) and the hippocampus and given the fact that stimulation of olfactory bulb is possible through the olfactory epithelium stimulation, the olfactory system may be a noninvasive and suitable target for applying DBS as an anticonvulsive agent in epilepsy patients.

## Ethical considerations

*Compliance with ethical guidelines*

All procedures related to experimental work and animal care were done in agreement with international protocols governing the use of animals, and were approved by the Tarbiat Modares University Ethical Committee for Animal Research by IR.MODARES.REC.1399.088 ethical code. Measures were taken to decrease the number of animals and any distress experienced by them.

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*Conflict of interest*

The authors declared no conflict of interest.

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Fig 1.

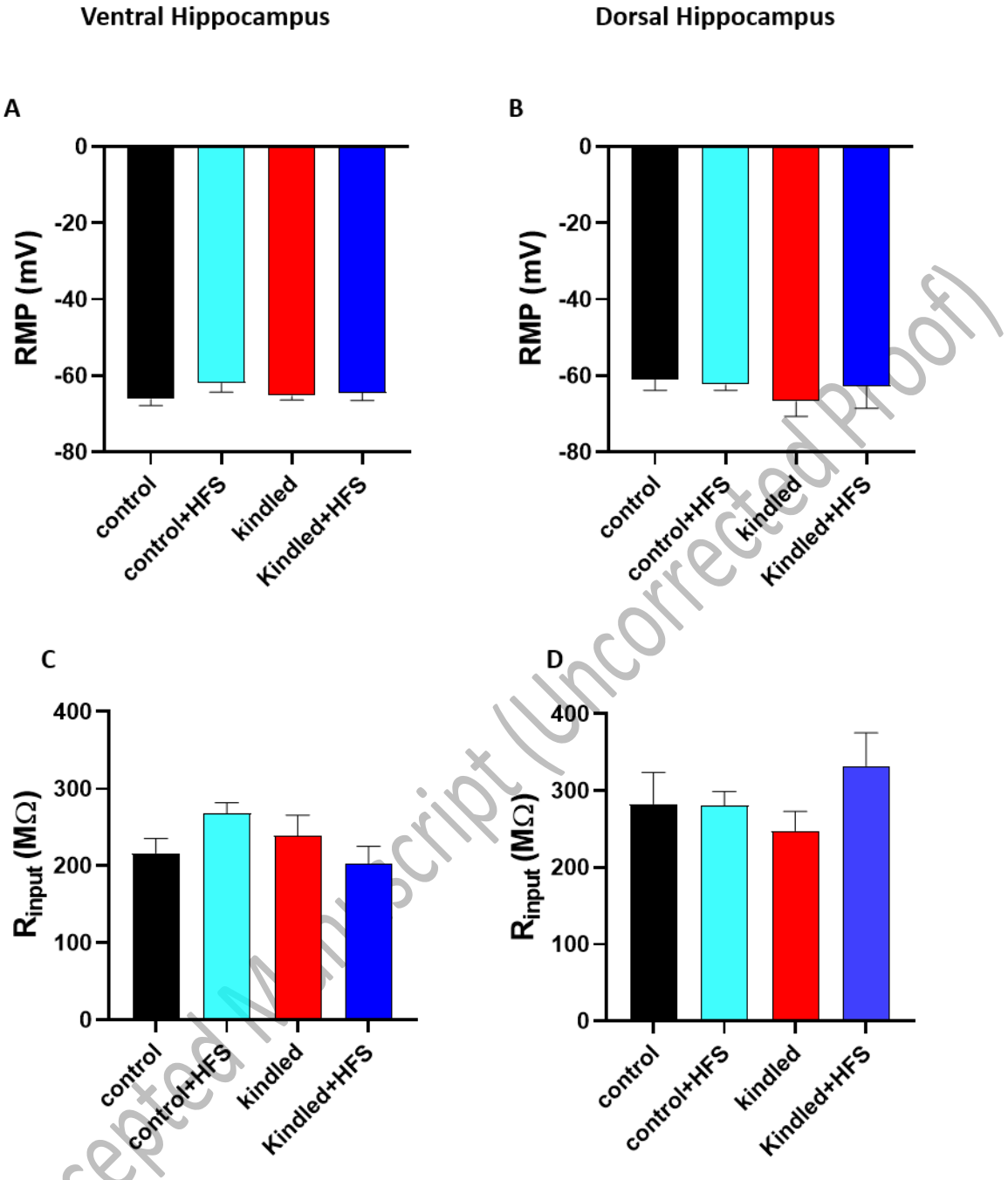




**Fig.1**: A-D, plots show resting membrane potential and input resistance in ventral and dorsal CA1 hippocampal slices. No significant changes existed in resting membrane potential and input resistance in different groups. Data are shown as mean ± SEM.

Fig 2.

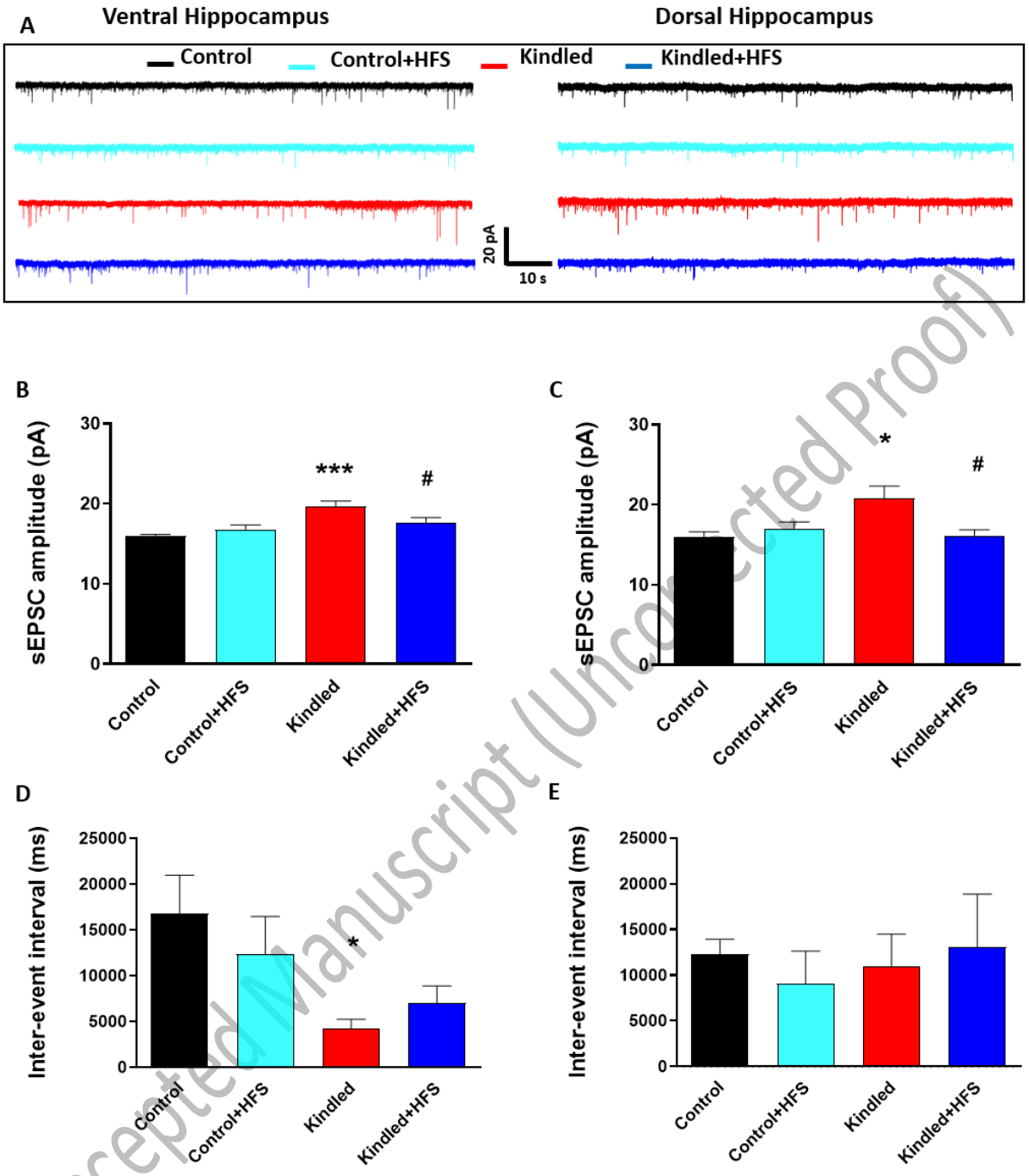




**Fig. 2**: Effect of high-frequency stimulation (HFS) application on kindling-induced changes in spontaneous excitatory post-synaptic currents (sEPSCs) in ventral and dorsal CA1 hippocampal slices. A. Sample records of sEPSCs in different experimental groups. B-E. Amplitude and inter-event interval of sEPSCs in different experimental groups. A significant increase in amplitude and decrease in the inter-event interval existed in the kindled compared to the control group. Application of HFS in kindled+HFS animals prevented the changes in these parameters. Data are shown as mean ± SEM. * P<0.05 and ***P<0.001, compared to control group; # P<0.05 compared to kindled group.