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Transcranial focal electrical stimulation reduces the convulsive expression and amino acid release in the hippocampus during pilocarpine-induced status epilepticus in rats

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ABSTRACT

The aim of the present study was to evaluate the effects of transcranial focal electrical stimulation (TFS) on γ-aminobutyric acid (GABA) and glutamate release in the hippocampus under basal conditions and during pilocarpine-induced status epilepticus (SE). Animals were previously implanted with a guide cannula attached to a bipolar electrode into the right ventral hippocampus and a concentric ring electrode placed on the skull surface. The first microdialysis experiment was designed to determine, under basal conditions, the effects of TFS (300 Hz, 200 μs biphasic square pulses, for 30 min) on afterdischarge threshold (ADT) and the release of GABA and glutamate in the hippocampus. The results obtained indicate that at low current intensities (<2800 μA), TFS enhances and decreases the basal extracellular levels of GABA and glutamate, respectively. However, TFS did not modify the ADT. During the second microdialysis experiment, a group of animals was subjected to SE induced by pilocarpine administration (300 mg/kg, i.p.; SE group). The SE was associated with a significant rise of GABA and glutamate release (up to 120 and 182% respectively, 5 h after pilocarpine injection) and the prevalence of high-voltage rhythmic spikes and increased spectral potency of delta, gamma, and theta bands. A group of animals (SE-TFS group) received TFS continuously during 2 h at 100 μA, 5 min after the establishment of SE. This group showed a significant decrease in the expression of the convulsive activity and spectral potency in gamma and theta bands. The extracellular levels of GABA and glutamate in the hippocampus remained at basal conditions. These results suggest that TFS induces anticonvulsant effects when applied during the SE, an effect associated with lower amino acid release.

This article is part of a Special Issue entitled "Status Epilepticus".

1. Introduction

Status epilepticus (SE) is a neurologic emergency that requires immediate management with the purpose to avoid brain injury [1–4]. At present, pharmacological strategies to control SE and its consequences in patients are inadequate. Indeed, the efficacy of diazepam (DZP) and similar first-line abortive SE treatments is incomplete, and SE often continues after administration of these drugs [5,6]. In addition, several drugs applied to stop the SE induce secondary effects such as respiratory depression, sedation, hypotension, and cardiac dysrhythmias [7].

The electrical modulation of the brain induced by strategies such as deep brain stimulation and vagal nerve stimulation has been considered as alternative treatment to control pharmacoresistant epilepsy [8]. However, few neuromodulation strategies have been considered as a treatment option for SE. Low frequency repetitive transcranial magnetic stimulation applied for one-hour sessions for 8 days has been shown to decrease the expression of SE [9]. Other studies indicate that deep brain electrical stimulation (DBS) of areas such as the thalamus may increase the latency to SE under specific experimental conditions [10]. We previously described that the DBS applied in the hippocampus and combined with subeffective doses of diazepam or phenobarbital reduce the expression of lithium–pilocarpine-induced SE [11]. However, experimental evidence indicates that the presence of intracranial electrodes may also facilitate seizure activity [12].

In previous studies, we reported that noninvasive transcranial focal electrical stimulation (TFS) applied via tripolar concentric ring electrodes (TCREs) during the pilocarpine-induced SE in rats was able to decrease

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the seizure expression [13]. We also demonstrated that TFS, combined with subeffective doses of diazepam applied before the induction of the pilocarpine-induced SE, reduces the incidence of mild and severe generalized seizures, an effect associated with a significant reduction of the neuronal damage [14]. These results suggest that TFS represents a noninvasive therapy for SE. However, the mechanism is unclear by which TFS applied during SE is able to reduce the seizure activity and the associated cell loss.

It is known that the neuronal damage induced by SE is associated with glutamatergic excitotoxicity mediated by N-methyl-D-aspartate (NMDA) receptors [15–17]. On the other hand, SE induces a decrease in the inhibitory neurotransmission mediated by γ-aminobutyric acid (GABA) [18–20] and reduces neuroprotective effects produced by benzodiazepines [21]. Accordingly, we speculate that a decrease in the glutamatergic or/and an increase in the GABAergic neurotransmission play an important role in the protective effect of TFS on the SE-induced neuronal damage.

The identification of new noninvasive therapeutic strategies that arrest SE and its consequences represents an important achievement. The present study investigated if TFS is able to reduce the seizure activity and avoid the increase in the glutamate release and/or augment the GABA release in the hippocampus of rats when applied immediately after the establishment of SE.

2. Methods

2.1. Animals

Adult male Wistar rats initially weighing 250–300 g were the subjects of the present study. They were individually housed at 22 °C and maintained on a 12-hour light/dark cycle. Rats had free access to food and water. Procedures involving animal care were conducted in agreement with the Mexican Official Standard (NOM-062-ZOO-1999) and the Ethical Committee of the Center for Research and Advanced Studies (Protocol #222/04).

2.2. Surgery

Rats were anesthetized with a mixture of ketamine (80 mg/kg, i.p.) and xylazine (20 mg/kg, i.m.). Then, a guide cannula attached to a bipolar electrode, consisting of two twisted strands of stainless steel wire and insulated except at the cross section of their tips, was stereotactically implanted into the right hippocampus using the following coordinates from the bregma and skull surface: anteroposterior −5.3 mm, lateral −5.2 mm, depth −7.5 mm [22]. The tip of the cannula was located −4.5 mm below the skull surface. The electrode was attached to male connector pins. A 6.0 mm dia. TCRE was placed in the center of the cranium, as close to 5 mm behind the bregma as possible. Four stainless steel screws were threaded into the cranium over the frontal and cerebellar cortices to fix the electrode assembly to the skull with dental acrylic. Animals were allowed to recover for 7 days before any further manipulation.

2.3. Transcranial focal electrical stimulation

The TFS consisted of 200 μs symmetrical biphasic square charge-balanced constant current pulses at a rate of 300 Hz and at an intensity of 100 to 5000 μA, depending on the experimental protocol (see below). The TFS was applied through the outer ring (external diameter of 6.0 mm) and disc of a TCRE (with the middle ring floating). For this purpose, we used two Grass Technologies S48 square pulse stimulators each with a SIU-C constant current stimulus isolation unit (Grass Technologies, West Warwick, RI). One S48 provided the positive pulses and the other provided the negative pulses.

2.4. Effects of TFS on the afterdischarge threshold and amino acid release

The present experiment was carried out to determine the current intensity necessary to induce changes in amino acid release in the hippocampus of control animals. Rats (n = 8) received daily administration of saline solution (1 ml/kg, i.p.) for 5 days to habituate them to manipulations. Twenty-four hours after the last saline injection, a perfused dialysis probe constructed by ourselves as previously described [23], designed to protrude 3 mm beyond the cannula tip in order to stay in the hippocampus, was inserted into the guide cannula and fixed to the socket with dental acrylic. The active part of the dialysis probe (3 mm) was a polyacrylonitrile membrane (molar weight cutoff of 40,000 D).

The dialysis probe inlet was connected to a syringe assembled to a microperfusion pump (model 1001; BAS) through a dual-channel swivel. The dialysis system was continuously perfused during the entire microdialysis experiment at a rate of 2 μl/min with fresh filter-sterilized artificial cerebrospinal fluid (NaCl 125 mM, KCl 2.5 mM, NaH_2PO_4 0.5 mM, Na_2HPO_4 5 mM, $MgCl_2$ 1 mM, ascorbic acid 0.2 mM, and $CaCl_2$ 1.2 mM; pH 7.4). A 2-hour recovery period was allowed after probe implantation. Following the 2-hour recovery period under basal conditions, the afterdischarge threshold (ADT) was determined to estimate the hippocampal excitability. The ADT was established by administering, in the right ventral hippocampus, a series of stimulations (1 ms rectangular pulses, 60 Hz for 1 s) at 1-minute intervals beginning at 10 μA and incrementally increased in steps of about 20% of the previous current. The threshold was defined as the lowest intensity producing a behavioral change or an afterdischarge with duration of at least 3 s. The afterdischarge duration was recorded from the bipolar electrode implanted into the right hippocampus using an electroencephalograph computer program (GRASS PolyVIEW, Astro-Med. Inc.).

Two minutes after the determination of the ADT, TFS was applied constantly for 30 min with an intensity from 360 to 5000 μA. The ADT was determined for a second time 30 min after the end of the TFS. The dialysates were continuously collected in polypropylene Eppendorf tubes at 10-minute intervals during the TFS application and 30 min after. Then, they were collected at 30-minute intervals for 3 additional hours. Immediately after recovery, dialysates were diluted with 2 M perchloric acid ($HClO_4$) (1:20) and used to determine the concentrations of GABA and glutamate by high performance liquid chromatography (HPLC, see below). The changes in the current necessary to achieve the ADT and the release of glutamate and GABA were correlated with the current intensity for the TFS. At the end of the experiment, rats received an overdose of pentobarbital and were transcardially perfused. The brain was processed for histological verification of the electrode implantation.

A control group (n = 5) was manipulated as described above, except that animals did not receive TFS.

2.5. Effects of TFS on the amino acid released during the SE

2.5.1. SE-TFS group (n = 6)

Rats were manipulated as described above (see Section 2.4.). Following the 2-hour recovery period under basal conditions, animals were administered methylscopolamine (1 mg/kg, i.p.), and then pilocarpine (300 mg/kg, i.p.) was applied 30 min later. Then, continuous behavioral monitoring was carried out to identify the establishment of SE, i.e., when animals presented seizures continuously for longer than 2 min without recovery between them. Five minutes after the establishment of the SE, TFS was applied constantly for 2 h at a rate of 300 Hz and at the lowest intensity to induce changes in the extracellular levels of GABA and glutamate according the previous experiment (see Section 2.4, 100 μA). The dialysates were continuously collected in polypropylene Eppendorf tubes at 30 min intervals under basal conditions (2 h), at the beginning of the SE (first recovery after SE establishment), during the TFS application (2 h), and after it (2 additional hours).

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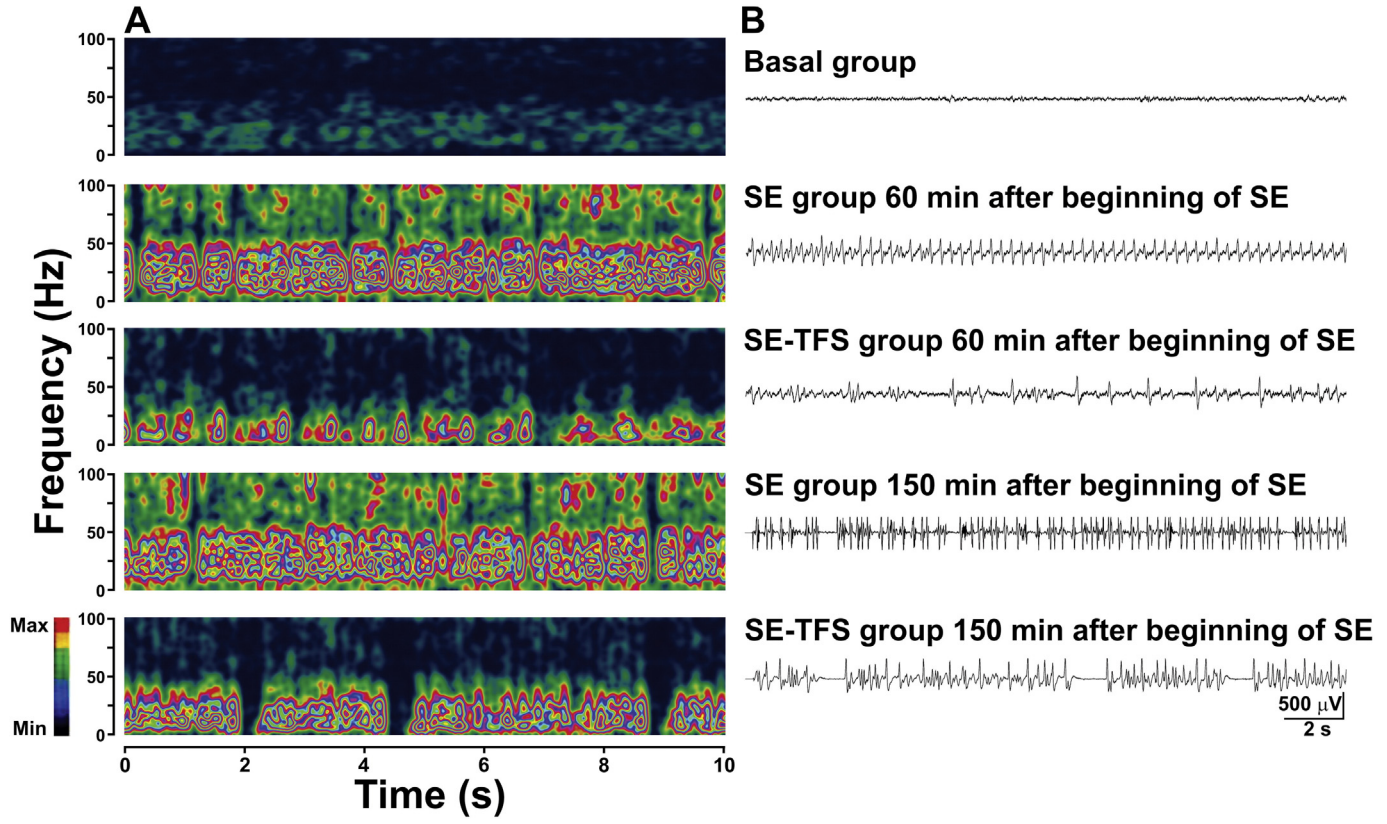

Fig. 1. A) Spectrograms (0.1–100 Hz) and B) electrographic activity obtained from the hippocampus under basal conditions, as well as 60 and 150 min after the beginning of SE of the animals from the SE and SE-TFS groups. In contrast to the SE group, the SE-TFS group presents low electrographic power and decrease in voltage and rhythmic activity during and after TFS. Color bar: red indicates maximum energy whereas blue indicates the lowest energy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Behavioral and electrographic recordings were obtained during the experiment.

2.5.2. SE group ($n = 6$)

Rats were manipulated as indicated previously for the SE-TFS group, except that they did not received TFS.

2.6. High performance liquid chromatography (HPLC)

A solution containing internal standards of GABA (8 ng each in 4 μl) was combined with 16 μl of perfusate-$HClO_4$ mixture, and then mixed with 6 μl of o-phthalaldehyde (OPA) acid for 30 s. The mixture was injected into the solvent stream of an HPLC system 2 min later. The HPLC system consisted of a fluorescence detector operated at an excitation wave of 360 nm and an emission wave of 450 nm. The HPLC fluorometric detection procedure that was followed required that OPA-amino acids and histamine were separated on a reversed-phase 3.9 × 150 mm column (Nova-Pack, 4 μm, C_{18}, Waters®) with Solution A (sodium acetate dissolved in 90% Mili-Q® water and 10% methanol, pH 5.75 with glacial acetic acid) as aqueous solvent, and Solution B (20% Solution A and 80% methanol, pH 6.75 with glacial acetic acid), at a flow rate of 0.5 ml/min, with fluorescent detection (Waters® model 474) following precolumn (Nova-Pack, Waters®) derivatization. The quantification of amino acids was carried out by peak height measurements against standard solutions [24].

2.7. Evaluation of the electrographic activity

The hippocampal electrical activity was recorded with amplifier model P511 (Grass Technologies, West Warwick, RI), and the signals were amplified, band-pass filtered between 0.1 and 100 Hz, digitized at 1000 samples/s, and stored in a hard drive. Off-line spectral analysis using fast Fourier transform (FFT) methods during periods of 1 min at each stage of interest (baseline, 5, 60, and 150 min after the beginning of SE) were performed with computer software developed in the Department of Neuroscience Research of the National Institute of Psychiatry Ramon de la Fuente Muñiz, México [25,26]. Power numerical values corresponding to the different bands were normalized against the maximum power of each band analyzed (0.1–4, 4–8, 8–13, 13–30, and 30–90 Hz).

2.8. Statistical analysis

Values were expressed as mean ± SEM. An unpaired t-test was applied to examine ADT values. The extracellular levels of GABA and glutamate in the course of the microdialysis experiments were analyzed by one-way ANOVA followed by the post hoc Fisher's PLSD test. Pearson's correlation coefficients were estimated to establish the potential impact of TFS on the ADT and release of GABA and glutamate. The power spectrum values were evaluated using an one-way ANOVA test followed by a post hoc Bonferroni's multiple comparison test. In all statistical comparisons, $p < 0.05$ was used as criterion for significance.

3. Results

The histological examination showed that the electrode tips were located within the ventral hippocampus in all experimental groups.

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3.1. Amino acid release and electrographic activity under control and basal situations

Under control (control group) and basal condition (SE and SE-TFS groups), the hippocampal electrographic activity demonstrated prevalence of 0.1- to 4- and 30- to 90-Hz bands (Figs. 1 and 2). The extracellular levels of GABA and glutamate were maintained stable over the experimental period (GABA, 0.36 ± 0.02 μM; glutamate, 0.76 ± 0.08 μM) (data not shown).

3.2. Effects of TFS on the afterdischarge threshold and amino acid release

Under basal conditions, rats showed a homogeneous distribution of their pre-TFS ADT values (173 ± 26 μA ranging from 100 to 300 μA). Once the animals received the TFS, post-TFS ADT values exhibited a nonsignificant increase (189 ± 25 μA, 9.7%, $p < 0.64$), when compared with their pre-TFS values. No significant correlation was detected between the percentage of change on ADT values and the current intensity values for the TFS ($p = 0.1129$) (Fig. 3).

Statistical analysis revealed significant correlations between the current intensity of TFS and the changes in GABA and glutamate release. At current intensities of 2800 μA or lower for TFS, decreased extracellular levels of glutamate and increased GABA were detected, with effects being more evident at the lower intensities (glutamate, 76% decrease at 400 μA; GABA, 90% increase at 620 μA). No significant changes were detected at higher intensities (>4800 μA) (Fig. 3). According to these results and for the subsequent experiments, we decided to apply the TFS at 100 μA during the SE.

3.3. Amino acid release and electrographic activity during the SE

Administration of pilocarpine caused progressive behavioral changes that culminated in SE that occurred at 34.3 ± 5.5 min in 100% of the animals. The analysis of the electrographic activity of the SE group revealed faster, high-voltage rhythmic spikes and an increase in spectral

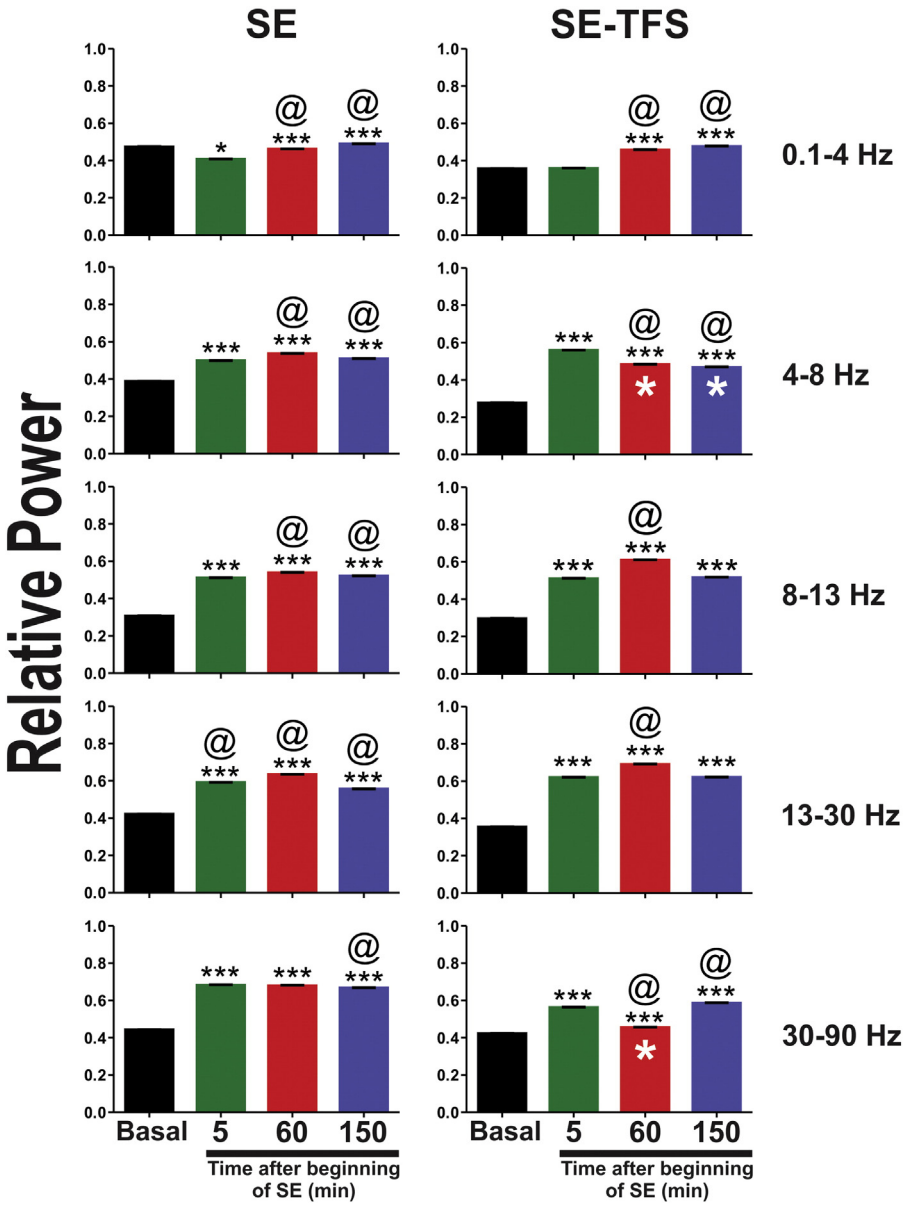

Fig. 2. Relative power spectra densities of different frequencies obtained from electrographic recordings during basal conditions (black bars), and 5 (green bars), 60 (red bars) and 150 min (blue bars) after the beginning of SE of the animals from the SE and SE-TFS groups. Notice a significant decrease in 4- to 8- Hz and 30- to 90- Hz bands during TFS application (white asterisks). *$p < 0.05$; ***$p < 0.001$ when compared with basal condition; @ $p < 0.001$ when compared with the value obtained 5 min after the beginning of the SE. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

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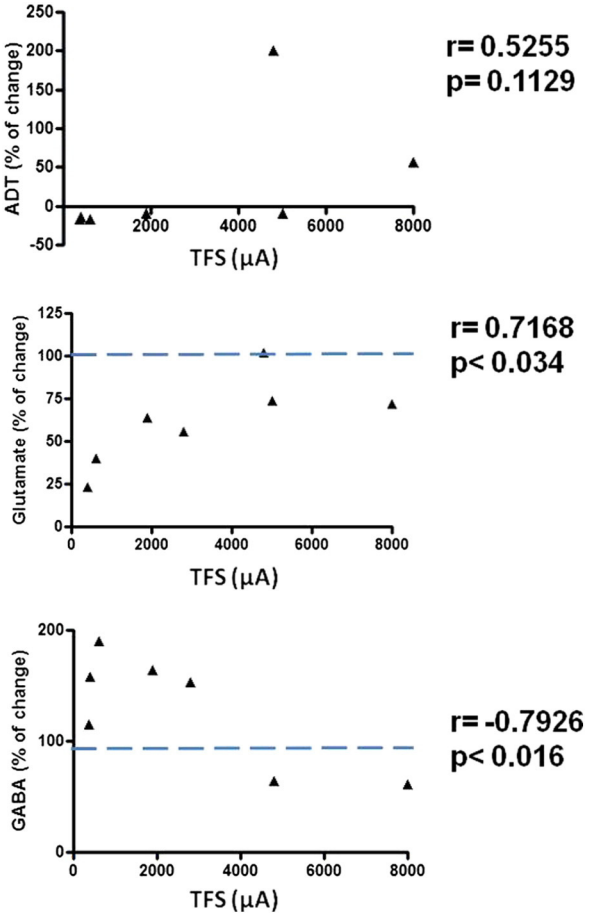

Fig. 3. Correlations between TFS applied at different intensities and the afterdischarge threshold (upper panel), glutamate (middle panel), and GABA (lower panel) extracellular levels in the hippocampus. The values represent the percentage of change when compared with the basal conditions (0% for the ADT and 100% for glutamate and GABA release). Pearson's coefficients and significance are indicated.

potency in 4- to 90-Hz bands, an effect evident at the end of the experiment ($p < 0.001$) (Figs. 1 and 2).

After the initiation of the SE, animals from the SE group demonstrated a significant progressive increase in GABA and glutamate release and reached 120% ($p < 0.001$) and 182% ($p < 0.001$), respectively, by the end of the microdialysis experiment (240 min after the beginning of the SE) (Fig. 4).

3.4. Effects of TFS on the amino acid release and electrographic activity during the SE

When compared with the SE group, the rats receiving TFS (SE-TFS group) demonstrated lower intensity of the behavioral seizure activity, and none experienced wet dog shakes during the SE. During the SE, the analysis of the electrographic activity demonstrated lower voltage and less rhythmic spiking during and after TFS application. In the spectral analysis, the activity showed a lower power of the 4- to 8- and 30- to 90-Hz bands, and an increase in other bands analyzed (0.1–4, 8–13, and 13–30 Hz) ($p < 0.001$) (Figs. 1 and 2).

Concerning the amino acid release, SE-TFS group had a nonsignificant increase of glutamate (44%, $p = 0.182$) and decrease in GABA (24%, $p = 0.154$) at the onset of the SE. Thereafter, no significant changes were detected during (glutamate, 5%, $p = 0.911$; GABA, 21%, $p = 0.171$) and 1 h after the end of the TFS (glutamate, 24%, $p = 0.700$; 15%, $p = 0.716$) (Fig. 4).

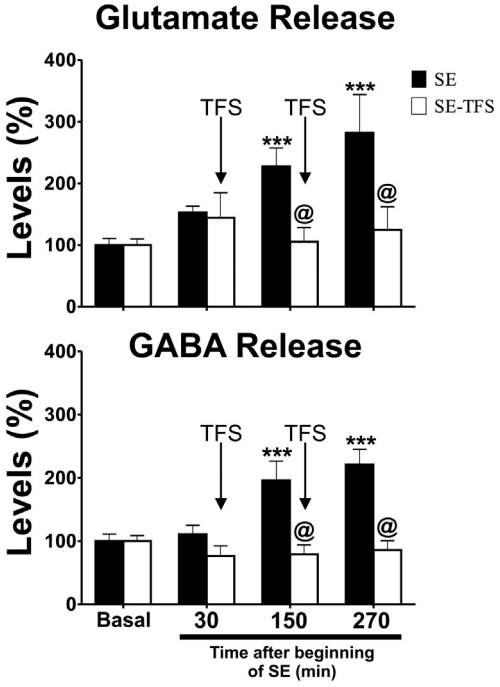

Fig. 4. Glutamate and GABA extracellular levels in the hippocampus of animals from SE and SE-TFS groups, during basal conditions, and 5, 60 and 150 min after the beginning of the SE. Notice that the values of the SE-TFS group during (arrows) and after TFS application are not significantly different when compared with the basal condition. Values are shown as mean ± SEM as percentage of baseline levels (%).

4. Discussion

The present study indicates that TFS applied during SE reduces seizure activity, an effect associated with decreased power in 4- to 8-Hz (theta) and 30- to 90-Hz (gamma) bands in the hippocampus. These results correlated with a significant decrease of SE-evoked increases in GABA and glutamate release.

Our results obtained from control rats revealed that TFS applied at low current intensities results in enhanced GABA and decreased glutamate extracellular levels in the hippocampus. According to these changes, it is expected that TFS protects the animals from the seizure activity when applied before a proconvulsant stimulus in normal animals. However, we found that TFS was not sufficient to modify the afterdischarge threshold in the control group and avoid SE when applied before the pilocarpine injection [14].

It is known that glutamate is released during SE [27,28] and that the elevated levels of this amino acid are involved in the recurrence and spread of seizure activity, and in NMDA receptor-mediated excitotoxicity [29,30]. In the present study, we found that TFS avoided increases in the release of glutamate during the SE, an effect that can explain the reduced seizure activity of animals receiving TFS after the initiation of the ictal event induced by pilocarpine, pentylenetetrazol, and penicillin G [13, 31–34]. On the other hand, the enhanced GABA release during SE may act as a compensatory mechanism to suppress the firing of glutamatergic neurons and to maintain the balance between excitation and inhibition [27,28,35]. Our microdialysis experiments revealed that the extracellular levels of GABA remained without changes when TFS was applied during SE. Although it is possible to assume that this circumstance could contribute to an intensification of the SE, we found reduced seizure activity. This evidence suggests that the anticonvulsant effects induced by TFS depend on the rate of the brain excitability. A similar condition is also observed when phenytoin blocks the development of seizure activity in subjects with high-frequency cerebral firing, while it does not have this effect in subjects with normal brain activity associated with low neuronal firing rates [36].

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The present study supports the notion that the high voltage fast activity in the hippocampus during SE is characterized by the prevalence of delta, theta, and gamma waves [27,30,37–39]. We also found that TFS applied during SE significantly reduced the prevalence of theta and gamma waves and avoided the enhanced release of glutamate. The rhythmically discharging GABAergic interneurons play an important role in the modulation of the theta and gamma oscillations in the hippocampus [40,41] while the long repeated activation of the glutamatergic system alters the theta and gamma rhythmic activity in the hippocampus [42]. It is possible to suggest that the lower rises in glutamate release in the hippocampus are associated with the decreased power of theta and gamma waves and the anticonvulsant effects induced by TFS when applied during the seizure activity [32,33,43].

The TFS may share some mechanisms with other therapeutic or pharmacological strategies. For example, diazepam applied systemically induces an anticonvulsant effect in pilocarpine-induced SE, an effect associated with significant reduction of pilocarpine-evoked increases in hippocampal glutamate release [44]. In addition, diazepam produces an overall slowing of network oscillations [45]. Phenytoin has been suggested to decrease the extracellular levels of GABA and glutamate. These effects have been explained as a result of a reduction in voltage-activated Ca^{2+} entry secondary to the blockade of presynaptic Na^{+} channels, or through a direct action on voltage-dependent Ca^{2+} channels [46–48]. High-frequency electrical stimulation (HFS), a neuromodulatory strategy to reduce seizure activity, has been described to suppress synaptic and nonsynaptic epileptiform activity in the hippocampus. This effect has been explained by "depolarization block" as a consequence of high extracellular levels of K^{+} [49–51] and blockage of Na^{+} current and the Ca^{2+}-mediated responses [52]. High-frequency electrical stimulation applied in the hippocampus during kainic acid-induced seizure activity also results in a decrease of hippocampal glutamate release, while the extracellular levels of GABA are not modified [53].

Future studies should be conducted to determine the mechanisms by which TFS suppresses the seizure activity. However, the present study suggests that TFS can represent a new strategy to abort SE and avoid the subsequent consequences.

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Conflict of interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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