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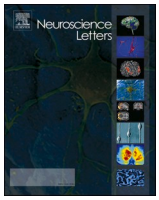

Different paradigms of transcranial electrical stimulation induce structural changes in the CA1 region of the hippocampus in a rat model of Alzheimer's disease

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ABSTRACT

One of the prominent sign of Alzheimer's disease (AD) is structural changes in the hippocampus. Recently, the new methods used to treat this disease is transcranial electrical stimulation (tES). This study evaluated the effect of four primary standards of tES, including tDCS, tACS, tRNS, and tPCS on beta-amyloid 25–35 (Aβ25-35)-induced structural changes in the CA1 region of hippocampus in male rats. For this purpose, rats weighing 250–275 g were selected, the cannula was embedded reciprocally into the hippocampi. Aβ25-35 (5 μg/ 2.5 ml/ day) was infused reciprocally for four continuous days. Then, animals were then given tES for 6 days. Subsequently, structural changes in the hippocampal CA1 were evaluated using the stereological method. Aβ25-35 resulted in loss of neurons ($P < 0.01$) and decreased hippocampal volume ($P < 0.05$). However, the administration of tES paradigms prevented these changes. The results proposed that through the improvement of hippocampal cell number and volume, tES paradigms can retain efficiency in remediating structural impairments in AD. From this, it can be concluded that other tES paradigms besides tDCS can also be considered for the treatment of AD.

1. Introduction

Alzheimer's disease (AD) is a common brain neurodegenerative disease that causes dementia in older adults. During aging, the hippocampus undergoes structural and molecular changes that can impact cognitive function [1]. The hippocampus is one of the foremost critical parts of the brain influenced by AD. A significant change in the size of the hippocampus is used to diagnose AD [2]. Patients with mild to moderate AD have a significantly smaller hippocampus compared to the normal elderly [3]. In AD, the pathological burden spreads systematically throughout the brain. Neuropathological studies have shown that neurofibrillary tangle pathology in AD begins in the transentorhinal and entorhinal areas of the brain, then spreads to the subiculum and CA1, and finally to the CA2 and CA3 areas of the hippocampus before invading the neocortex [4]. Three-dimensional analysis of the hippocampus showed specific atrophy in the subregional of hippocampus in the brains of patients with AD [5]. Hippocampal atrophy in AD is not a uniform process, but rather a subregion-specific one that closely follows the known anatomical trajectory of the neurofibrillary tangles [6].

Hippocampus CA1 deficiency plays a key role in AD [7]. In rodents, more studies have been performed in this area than in other areas of the hippocampus. Memory impairment in AD is due to the high sensitivity of CA1 cells to the toxicity of the disease [8]. The best-known hallmark of AD is memory deficits associated with decreased hippocampal volume due to neuronal and synaptic dysfunction in the CA1 region and entorhinal cortex [8–11]. Previous evidence has shown that Aβ-induced

Abbreviations: AD, Alzheimer's disease; Aβ25-35, Beta-amyloid 25–35; BDNF, Brain drived neurotrophic factor; DC, Direct current; MRI, Magnetic resonance imaging; NFTs, Neurofibrillary tangles; NSC, Neural stem cell; tACS, transcranial alternative current stimulation; tDCS, transcranial direct current stimulation; tES, transcranial electrical stimulation; tPCS, transcranial pulsed current stimulation; tRNS, transcranial random noise stimulation.

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degeneration of neurons proceeds through an apoptotic pathway that contributes to the neuronal loss associated with AD [12]. Ethical constraints in cognitive studies have led us to use noninvasive techniques to identify neural, cognitive, and behavioral correlations [13].

Transcranial electrical stimulation (tES) is a new, safe, non-invasive method of altering electrical activity and function in the brain using two electrodes placed on the scalp [14]. TES includes some different paradigms, including 1. Transcranial direct current stimulation (tDCS) 2. Transcranial alternating current stimulation (tACS) 3. Transcranial random noise stimulation (tRNS) 4. Transcranial pulsed current stimulation (tPCS). Transcranial Direct Current Stimulation (tDCS) is a well-known neuromodulation technique based on active electrode polarization for corticospinal excitability changes. TDCS causes membrane depolarization with Anodal stimulation or membrane hyperpolarization with cathodal stimulation [15]. The use of tACS as a neuromodulation technique allows for the modification of cortical excitability by delivering alternating biphasic pulses with balanced electrical charges [16]. The application of tRNS, a type of neuromodulation similar to tACS, involves the use of a low-frequency alternating current that induces random changes in the intensity and frequency of the current flow [17]. In tPCS, the current is interrupted by two parameters: pulse duration (PD) and Interpulse interval (IPI) [18]. Among these paradigms, tDCS has been the most extensively studied. Previous studies have appeared that tDCS could be a potential helpful instrument for the treatment of many drug-resistant disorders including postoperative motor impairment [19], post-stroke aphasia, epilepsy [20], chronic pain [21], and Parkinson's disease [22]. Previous studies have shown that tDCS can modulate synaptic plasticity, oxidative stress, neuroinflammation, and autophagy [23–26]. Therefore, tDCS is a new method for treating diseases with impaired neuroplasticity [27]. It has also been shown that the application of tDCS can improve memory in patients with AD [28,29]. In addition to human studies, animal studies have shown the effects of different paradigms of electrical stimulation in Alzheimer's models. A study conducted in 2018 demonstrated the effects of different paradigms of tES improve learning and memory in a rat model of AD in the Morris water maze and novel object recognition tasks [30,31]. Kim et al showed that repeated tDCS have functional and histologic changes in the rat stroke model [32]. In a recent study, tDCS ameliorated cognitive impairment by preventing morphological changes in the hippocampus induced by vascular dementia [33]. Recently, Heidarzadegan et al. revealed that different paradigms of tES improved motor function impairment and striatum tissue injuries in an intracerebral hemorrhage rat model [34].

Given that histological, cellular, and molecular studies of different paradigms of stimulation, especially in animals, have not been conducted extensively, this study helps to clarify the histological effects of tES paradigms. Because of the destructive effects of Aβ on the structural changes of the hippocampus and the neuroprotective effects of tES, in this study, the effect of different tES paradigms on the structural changes in hippocampal CA1 induced by Aβ (25–35) was investigated using the stereological method.

2. Methods

2.1. Animals and grouping

In this study, adult male Sprague-Dawley rats were utilized. Animal weight was 250–275 g. The animals were kept in a controlled environment with a specific light–dark cycle at room temperature (25 ± 2 °C) with lights on at 7:00 AM. Food and water were provided ad libitum. The experimental protocols and animal care followed by the ethics committee of Shiraz University of Medical Sciences and were based on the NIH guidelines (IR.SUMS.REC.1395.S974). A total of 56 rats were used in the study, divided into 7 groups with 8 rats in each group; the sham group, the Aβ group, the Aβ + anodal tDCS group, the Aβ + tACS group, the Aβ + tRNS group, the Aβ + tPCS group. The rats were anesthetized with an intraperitoneal injection of ketamine and xylazine, with a dosage of 100 mg/kg and 10 mg/kg, respectively. The study did not provide details on the purpose of the anesthesia. The rats were positioned in a stereotaxic frame, and cannulae were inserted into their hippocampus based on the Paxinos brain atlas. The coordinates used for the cannula placement were AP − 3.8, ML ± 2.2, DV − 2.7. Additionally, a plastic tube was attached to the right frontal cortex (1 mm anterior to the coronal fissure) for electrical stimulation. The cannula and plastic tube were embedded into to the skull using stainless steel screws and acrylic cement. Aβ peptide (25–35) (Sigma, USA) at a concentration of 2 μg/μl was dissolved in sterile distilled water and then stored at − 70 °C. The Aβ25-35 was aggregated in vitro by incubating it at 37 °C for 4 days. The drug was injected using a 10 μl Hamilton syringe connected to the injection cannula by a polyethylene tube. The injection of Aβ25-35 and its vehicle (sterile distilled water) were done bilaterally at a concentration of 5 μg/2.5 μl/day for four consecutive days [35] (Fig. 1).

2.2. Induction of electrical stimulation

Plastic tubes placed on the surface of the skull on the day of surgery are filled with sponges were moistened with saline solution. The rats were covered with a towel and the electrodes were inserted. The anodal and cathodal electrodes were placed the plastic tube above the right frontal cortex and on the ventral thorax with a corset respectively. To minimize contact impedance, the sponges within the plastic tubes were moistened with saline solution before applying electrical stimulation [24]. During the induction of electrical stimulation, the rats were awake and could easily move. TES procedure involved applying electrical stimulation for one week from day 5 (One day after last Aβ injection), with each session lasting 20 min. The current intensity used was 200 μA, and the current intensity was ramped for 10 s. The frequency used in tACS was 30 Hz and the frequency was used in tRNS randomly selected between 1 and 200 Hz. In the case of tPCS, Frequency was 5 Hz with a Duty cycle of 75 % (the percentage of one period in which a signal or system is active) was used [34]. Sham stimulation was conducted in both the sham and Aβ groups. Electrodes were placed, but no stimulation was applied during the sham stimulation (Fig. 2).

2.3. Tissue preparation

The hemispheres were placed on the cryostat specimen disk and then fixed with OCT (Cryoprep, American master, USA), and coronal sections with a thickness of 50 μm were obtained in the range from the beginning to the end of the hippocampus based on the atlas of Paxinos. The sections were placed on gelatinized slides and dried, after which Giemsa staining (Merck, Germany) was performed [36].

2.4. Stereological study

To calculate the hippocampal CA1 volume, as shown in Fig. 3a, using a stereomicroscope, the CA1 area was determined based on the Atlas of Paxinos. Ten slices containing the CA1 region were selected at a given distance and the CA1 surface area was calculated using the Cavalieri principle [37,38]. The total area of CA1 in the total sections was multiplied by the distance between the two sections and the volume of the CA1 region was calculated.

$$EstV = \sum p \times a_p \times d$$

Pyramidal neurons were counted using an optical dissector [39]. In this method, a frame with a particular dimension is determined. The numerical density (NV) of the pyramidal neurons was calculated using the following formula:

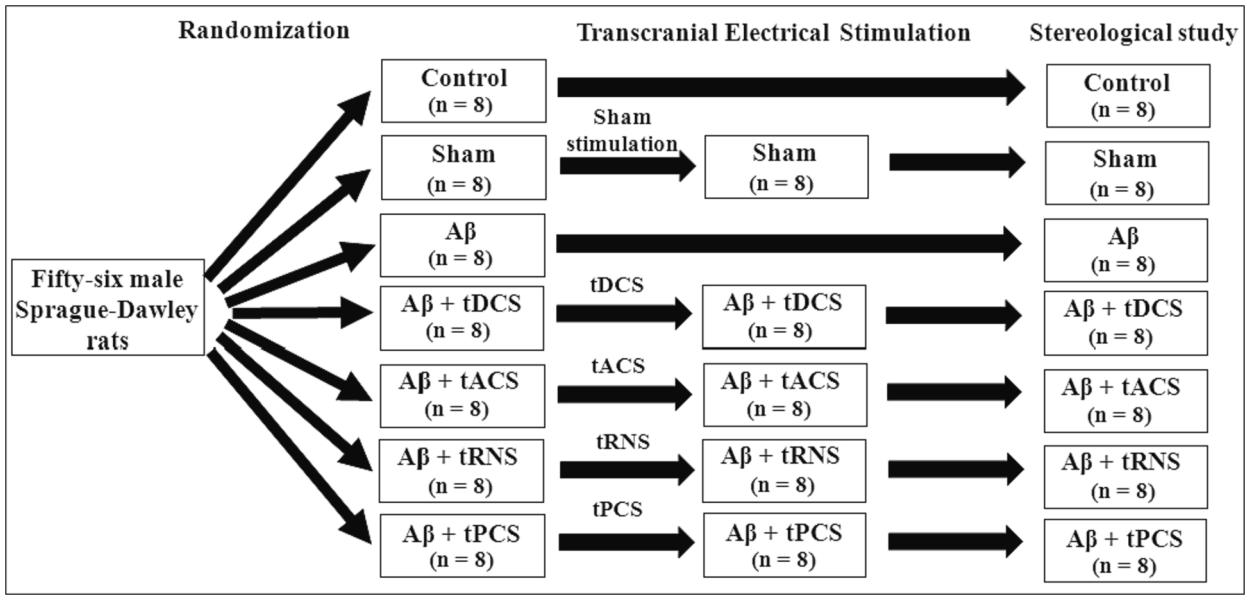
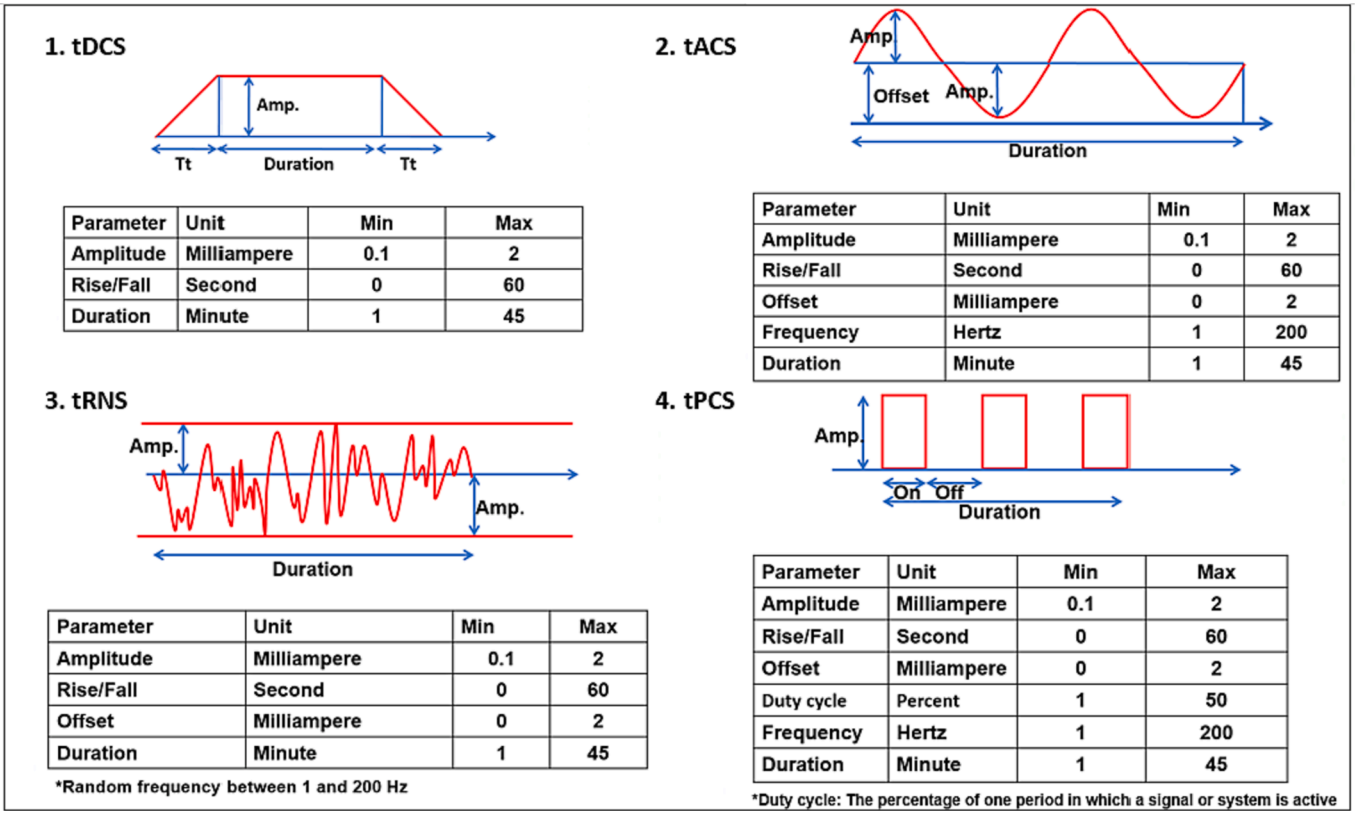


Fig. 1. The schematic experimental protocol.



Parameter	Unit	Min	Max
Amplitude	Milliampere	0.1	2
Rise/Fall	Second	0	60
Duration	Minute	1	45

Parameter	Unit	Min	Max
Amplitude	Milliampere	0.1	2
Rise/Fall	Second	0	60
Offset	Milliampere	0	2
Frequency	Hertz	1	200
Duration	Minute	1	45

Parameter	Unit	Min	Max
Amplitude	Milliampere	0.1	2
Rise/Fall	Second	0	60
Offset	Milliampere	0	2
Duration	Minute	1	45

*Random frequency between 1 and 200 Hz

Parameter	Unit	Min	Max
Amplitude	Milliampere	0.1	2
Rise/Fall	Second	0	60
Offset	Milliampere	0	2
Duty cycle	Percent	1	50
Frequency	Hertz	1	200
Duration	Minute	1	45

*Duty cycle: The percentage of one period in which a signal or system is active

Fig. 2. Schematic of different paradigms of transcranial electrical simulations and their adjustable parameters.

$$\mathrm{Nv(Pyramidal\ neurons)} = \frac{\sum Q^{-}}{\sum P \times \left(\frac{a}{f}\right) \times h} \times \frac{t}{BA}$$

where ΣQ is the number of neurons counted, "h" is the investigated thickness, ΣP is the number of frames counted in the CA1 region, "a / f", is the frame area, "t" is the average thickness of the section, and "BA" is the thickness adjusted on the microtome, (50 μm). The total number of pyramidal neurons was calculated by multiplying the cell density by the CA1 volume.

2.5. Data analysis

All stereological analyses and decoding were performed in a blinded manner. Statistical analysis was performed using the Prism 8 software. The results are presented as the mean standard error of the mean (S. E. M). In all statistical comparisons, a $p < 0.05$ is considered a significant difference.

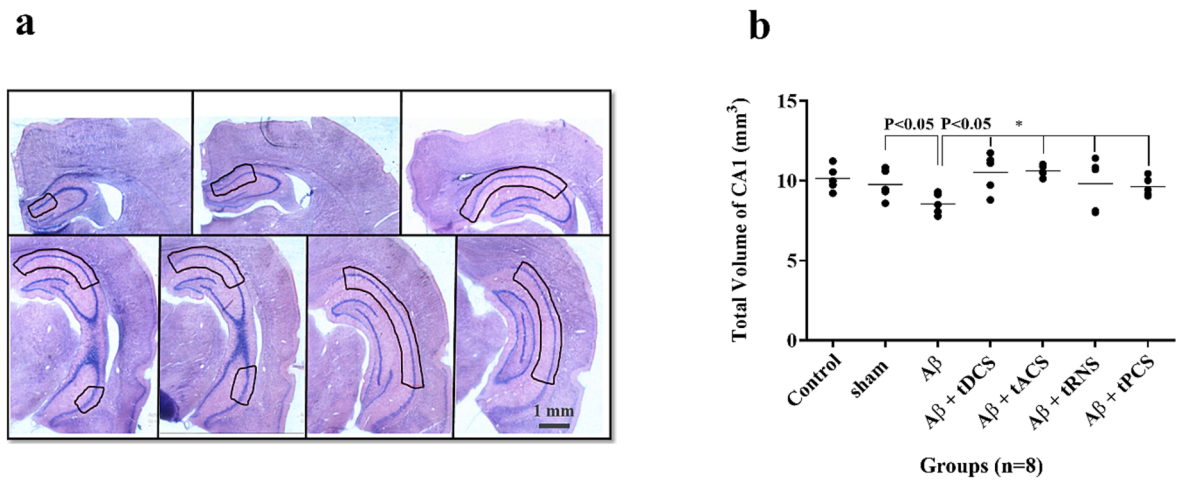


Fig. 3. The effect of vehicle, Aβ 25–35 or (and) tDCS; tACS; tRNS; tPCS on the volume of the CA1 region of the hippocampus. The CA1 region in different cross-sections of the hippocampus in Giemsa staining to calculate the total volume of the CA1 region; scale bar = 1 mm for all sections (a). Total volume of CA1 in study groups (b). Values are expressed as the mean ± SEM. *P < 0.05, indicates the difference between the sham, Aβ + tDCS, Aβ + tACS, Aβ + tPCS, Aβ + tPCS, and the Aβ group (b) (n = 8).

3. Results

The CA1 volume of hippocampus in the study groups is shown in Fig. 3. As can be seen from the graph, the sham-operated rats showed no significant differences from the control group in CA1 volume. The CA1 volume in the Aβ group shows a significant decrease by 12 % (P < 0.05) compared to the sham and control groups. There was no significant decrease in the CA1 volume in tDCS, tACS, tRNS, and tPCS groups. In addition, no significant difference was observed between these groups that received electrical stimulation

The cell population in the study groups are shown in Fig. 4. The sham-operated rats showed no significant differences from the control group in pyramidal cell number. However, the total number of pyramidal cells in the Aβ group were reduced 41 % (P < 0.01) compared to those in the sham group. In addition, the data showed that there was no decrease in cell population in the tDCS, tACS, and tRNS groups compared to the Aβ group. Further analysis revealed that although electrical stimulation was not effective in preventing cell loss in the tPCS group, the volume of this region remained unchanged compared to that in the Aβ group.

4. Discussion

In this study, we evaluated the neuroprotective effects of different

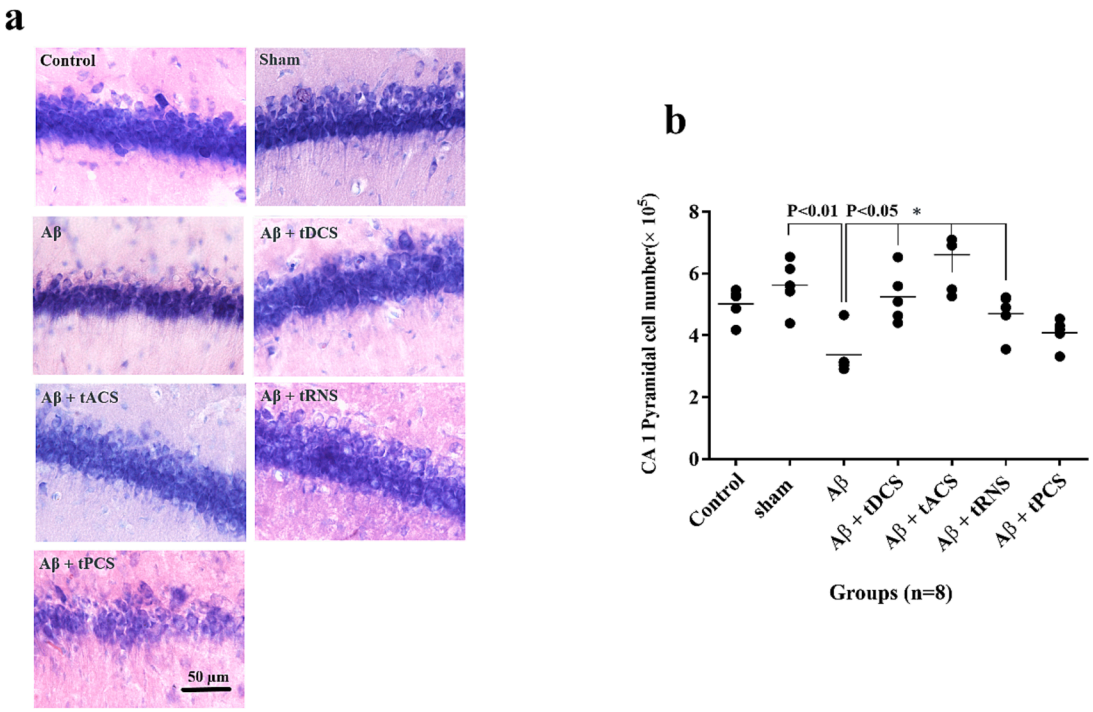


Fig. 4. The effect of vehicle, Aβ 25–35 or (and) tDCS; tACS; tRNS; tPCS on the number of pyramidal cells in the CA1 region of the hippocampus. The representative light microphotographs are shown for CA1 pyramidal neurons, Scale bar = 50 µm for all images (a). Values are expressed as mean ± SEM. P < 0.01 represents a difference between the Aβ- and vehicle-treated groups. P < 0.05, represents the difference between the Aβ + tDCS, Aβ + tACS, Aβ + tPCS, and the Aβ group. *P < 0.05, represents the difference between Aβ + tACS, Aβ + tDCS, and Aβ + tPCS (b) (n = 8).

tES paradigms against neuronal cell loss and hippocampal shrinkage in a rat model of AD. Treatment with different tES paradigms in Aβ25–35-induced AD can prevent neuronal loss and decrease hippocampal CA1 volume. The results of this study showed that these types of electrical stimulations prevented Aβ-induced structural changes in the rat hippocampus. A hippocampal injection of Aβ25–35 is used to create an AD model [40,41]. The Aβ25-35-induced neurodegenerative process and neuronal loss have been demonstrated in the hippocampal CA1 [42]. In our study, CA1 pyramidal neurons in the hippocampal formation showed susceptibility to Aβ25–35 toxicity. The progressive loss of neurons is a hallmark of neurodegeneration that causes cognitive deficits. Administration of Aβ25-35 resulted in a decrease in the hippocampal volume, particularly in the CA1. Human AD studies have found that hippocampal volume is reduced even in the early stages of AD and may correlate with impairments in spatial memory [43]. Stoub et al. reported a decrease in hippocampal volume using MRI that correlated with reduced declarative memory in amnesiac patients with mild cognitive impairment [44]. It is important to diagnose this volume reduction, which is associated with the impairment of declarative memory and occurs before AD. Rodent memory may exhibit a higher susceptibility to ultrastructural changes compared to human memory [45]. The memory impairment seen in Aβ25-35 treated mice may be due to the significant loss of CA1 pyramidal cells. In the present study, beta-amyloid reduced the volume and pyramidal cells of the hippocampal CA1 and all four electrical stimulation paradigms were able to reverse the effect of beta-amyloid on the volume of the hippocampus. However, in the case of pyramidal cell count, only three electrical stimulation paradigms (tDCS, tACS, and tRNS) were able to prevent cell number reduction induced by Aβ, and tPCS did not have this effect. The previous study showed that tDCS significantly increases neural stem cell (NSC) counts due to the facilitated proliferation and migration of endogenous NSCs [46]. Recent findings revealed that tDCS stimulates the activation of neural stem cells in rats, leading to amplified proliferation and motility [47]. Kaviannejad et al., showed that tDCS rescues neurons by suppressing apoptotic signaling pathways and enhancing anti-apoptotic signaling pathways by reducing Bax and caspase-3 levels and increasing Bcl-2 expression [48]. Another study showed that anodal tDCS prevents a decrease in the number of hippocampal cells induced by Aβ1-40 [49]. Previous studies have attributed the effects of tDCS to a variety of factors, including changes in membrane potential polarity, NMDA-dependent plasticity, induction of glutamate release, increased intracellular calcium concentration, increased BDNF levels, and anti-inflammatory and anti-apoptotic effects [50]. A recent study has shown that repeated electromagnetic field stimulation reduces the level of secreted beta-amyloid peptides [51]. Considering that the exact mechanism underlying the effects of tES is not clear, these paradigms may exert their protective effects through such a mechanism. However, further molecular studies are required to confirm these findings. The results of our study confirmed the effect of anodal tDCS on the number of cells and showed that tACS and tPCS also had positive effects in this case. No study has been conducted on the effect of other paradigms on the cell number or volume of brain structures, and we showed the effect of other paradigms in this case. Past studies have shown tACS and tRNS can affect cognitive processes by altering brain oscillations [52–54]. However, the exact cellular and molecular mechanisms involved have not yet been elucidated. The results of the stereological study were consistent with those of the behavioral tests in our previous study, which showed that different paradigms improve behavioral performance [30,31]. In addition to tDCS, these three paradigms have positive effects on the volume and number of hippocampal cells. These results confirm the effect of structural changes in the hippocampus, especially CA1, on cognitive function, and determine the importance of this region.

5. Conclusion

In general, this study indicates that Aβ25–35 leads to neural cell loss and hippocampal shrinkage. Treatment with tES paradigms likely reduces cell loss and hippocampal shrinkage. Since the administration of tES paradigms prevented hippocampal cell loss and volume, it can be suggested that tES paradigms improve memory by affecting these changes. The current study suggests that other stimulation paradigms may be effective in treating structural impairments. Based on the evidence presented, it is reasonable to expect that, in addition to the use of transcranial direct current stimulation (tDCS) in the treatment of neurodegenerative diseases, other stimulation paradigms may also be effective in treatments of these diseases. Further studies are required to make these methods available in a clinical setting.

6. Ethics approval and consent to participate

All experimental protocols were approved by ethics committee of Shiraz University of Medical Sciences and were based on the NIH guidelines (IR.SUMS.REC.1395.S974).

7. Consent for publication

Not applicable.

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Competing interests

The authors report no declarations of interest.

CRediT authorship contribution statement

Amir Hossein Zarifkar: Conceptualization, Investigation, Writing – review & editing. **Asadollah Zarifkar:** Data curation, Writing – original draft. **Sepideh Safaei:** Methodology, Visualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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