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Intensity-dependent effects of repetitive anodal transcranial direct current stimulation on learning and memory in a rat model of Alzheimer's disease

Xuehong Yu [a], Yiyan Li [b], Huizhong Wen [c], Yinghui Zhang [a], Xuelong Tian [a,*]

[a] Bioengineering College of Chongqing University, Chongqing 400030, China
[b] Department of Electrical and Computer Engineering, University of Nevada, Las Vegas, Las Vegas, NV 89154-4026, USA
[c] Department of Neurobiology, Chongqing Key Laboratory of Neurobiology, Third Military Medical University, Chongqing 400038, China

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ABSTRACT

Single-session anodal transcranial direct current stimulation (tDCS) can improve the learning-memory function of patients with Alzheimer's disease (AD). After-effects of tDCS can be more significant if the stimulation is repeated regularly in a period. Here the behavioral and the histologic effects of the repetitive anodal tDCS on a rat model of AD were investigated. Sprague–Dawley rats were divided into 6 groups, the sham group, the β-amyloid (Aβ) group, the Aβ + 20 μA tDCS group, the Aβ + 60 μA tDCS group, the Aβ + 100 μA tDCS group and the Aβ + 200 μA tDCS group. Bilateral hippocampus of the rats in the Aβ group and the Aβ + tDCS groups were lesioned by $A\beta_{1-40}$ to produce AD models. One day after drug injection, repetitive anodal tDCS (10 sessions in two weeks, 20 min per session) was applied to the frontal cortex of the rats in the tDCS groups, while sham stimulation was applied to the Aβ group and the sham group. The spatial learning and memory capability of the rats were tested by Morris water maze. Bielschowsky's silver staining, Nissl's staining, choline acetyltransferase (ChAT) and glial-fibrillary-acidic protein (GFAP) immunohistochemistry of the hippocampus were conducted for histologic analysis. Results show in the Morris water maze task, rats in the Aβ + 100 μA and the Aβ + 200 μA tDCS groups had shorter escape latency and larger number of crossings on the platform. Significant histologic differences were observed in the Aβ + 100 μA and the Aβ + 200 μA tDCS groups compared to the Aβ group. The behavioral and the histological experiments indicate that the proposed repetitive anodal tDCS treatment can protect spatial learning and memory dysfunction of $A\beta_{1-40}$-lesioned AD rats.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder which is characterized by progressive loss of memory, perception, judgment and movement (Stuchbury & Munch, 2005). According to the statistics (Brookmeyer, Johnson, Ziegler-Graham, & Arrighi, 2007), there are more than 26 million AD patients around the world in 2006. But there are no effective clinical treatments can permanently cure AD (Freitas, Mondragón-Llorca, & Pascual-Leone, 2011; Shafqat, 2008). Nowadays, non-invasive brain stimulations, such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) show great potentials in relieving the AD symptoms by neural modulation (Ahmed, Darwish, Khedr, El Serogy, & Ali, 2012; Bentwich et al., 2011; Freitas et al., 2011). These two techniques are similar in principle, but tDCS is more attractive because of the portability and the lower cost (Gandiga, Hummel, & Cohen, 2006). tDCS modulates the neural activity by injecting a weak direct current into cortex for further polarization of the neurons at the target (Hummel & Cohen, 2006). Studies of anodal tDCS in AD patients are reporting positive results. In a randomized cross-over sham-controlled study (Ferrucci et al., 2008), where anodal, cathodal and sham tDCS were applied to AD patients' temporoparietal cortex, recognition memory was improved by anodal tDCS whereas decreased by cathodal tDCS. Improvements in recognition memory of AD patients were also observed by applying single anodal tDCS over left dorsolateral prefrontal cortex (DLPFC) and left temporal

Abbreviations: AD, Alzheimer's disease; tDCS, transcranial direct current stimulation.

* Corresponding author. Fax: +86 023 65103812.
E-mail addresses: 20121902010t@cqu.edu.cn (X. Yu), liy10@unlv.nevada.edu (Y. Li), just_zhong2006@hotmail.com (H. Wen), 20121913077@cqu.edu.cn (Y. Zhang), xltian@cqu.edu.cn (X. Tian).

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cortex (Boggio et al., 2009). Furthermore, similar results were found and even maintained for at least 4 weeks after repetitive anodal tDCS (Boggio et al., 2012). Beyond human, one study based on AD rats suggested the application of anodal tDCS over the frontal cortex has beneficial effects on restoration of cognitive skill (Yu, Park, & Sim, 2014) which possibly resulted from the potential neuron-protective effect (Kim et al., 2010) and the wide-spread modulatory function in cortical–subcortical network of tDCS (Fregni et al., 2006).

The stimulation parameters, such as polarity, stimulation time and current intensity have diverse influences on the effects induced by tDCS (Nitsche et al., 2008). Based on previous findings, it is commonly agreed that tDCS-induced effects are modulated in a polarity-specific manner (Dockery, Liebetanz, Birbaumer, Malinowska, & Wesierska, 2011; Ferrucci et al., 2008; Fregni et al., 2006; Wachter et al., 2011). Animal studies have demonstrated that anodal stimulation increases and cathodal stimulation decreases the neural excitability, which is consistent with the findings in humans (Cambiaghi et al., 2010). Moreover, stimulation time also has influences on the effects. It was reported that with constant current density, the occurrence and duration of the effects depend on the stimulation time (Nitsche et al., 2008). When tDCS was applied for more than 10 min, a long-term effect in the neural excitabilities can last for more than 1 h (Nitsche & Paulus, 2000, 2001). This effect can last for even longer time if the stimulation was repeated (Benninger et al., 2010; Boggio et al., 2007). In rats, repetitive rather than single tDCS has been proven to improve motor function, and elicit inflammatory and regenerative processes in rat stroke models (Kim et al., 2010; Rueger et al., 2012). Similar repetitive stimulation was found to have antiepileptic effects in epilepticus rats (Kamida et al., 2011). However, the body of existing studies about the intensity-dependent effects of tDCS is rather small, especially in AD rats. Furthermore, the underlying mechanisms still remain unclear and need further investigation.

In this study, repetitive anodal tDCS was applied to the frontal cortex of Aβ-lesioned AD rats in order to evaluate intensity-dependent effects on spatial learning and memory in behavioral and histological levels. In addition, the possible mechanisms, safety and methodology considerations were also discussed.

2. Materials and methods

2.1. Animals

Female Sprague Dawley rats (8 weeks old and weigh 250–320 g) obtained from the Laboratory Animal Center of the Third Military Medical University (Chongqing, China) were employed for all experiments after one-week adaptive cultivation. They were housed in a humidity-controlled environment at 24 ± 1 °C with 12 h–12 h light–dark cycle, and permitted to take food and water freely. Rats were randomly divided into the sham group, the β-amyloid (Aβ) group, the Aβ + 20 μA tDCS group, the Aβ + 60 μA tDCS group, the Aβ + 100 μA tDCS group and the Aβ + 200 μA tDCS group, with six rats in each group.

All animal experiments were conducted following the Guide for the Care and Use of Laboratory Animal of National Institutes of Health (Eighth Edition, NIH, USA).

2.2. Administration and hippocampus injection of $A\beta_{1-40}$

$A\beta_{1-40}$ (No. SCP0037, Sigma, St. Louis, USA) was dissolved into a sterile saline solution at a concentration of 2 μg/μl, and then incubated for one week at 37 °C. Aggregated $A\beta_{1-40}$ was stored in a refrigerator at 4 °C.

The rats in the Aβ group and the tDCS groups were anesthetized by intraperitoneal injection of 10% chloral hydrate (4 ml/mg), then fixed in a stereotaxic apparatus (No. 9084, Chengdu Instrument Plant, Chengdu, China). The hair and the scalp were cut and cleaned with alcohol. According to The Rat Brain in Stereotaxic Coordinates (Paxinos & Watson, 1996), the stereotaxic coordinates of CA1 sub-region in hippocampus are: 3.3 mm posterior to bregma, 2 mm right and left of the sagittal, and 3.5 mm below skull (Figs. 1A and 2A). 5 μl $A\beta_{1-40}$ was injected bilaterally into hippocampus through two small holes (diameter: 0.8 mm) into the skull at a rate of 1.0 μl/min, then the needle was stayed for 5–10 min and withdraw at a rate of 1.0 mm/min. The fascia and skin were sutured and disinfected. The same operation was conducted to the rats in the sham group, but using 5 μl sterile saline instead of using $A\beta_{1-40}$.

2.3. Verification of the injection site

To verify the injection site, an injection test with black ink was conducted. The coronal rat brain section was used to show the injection site in the hippocampus (Fig. 1B).

2.4. Transcranial direct current stimulation

After the drug injection, the electrodes were installed. Based on the previous epicranial electrode protocol (Liebetanz et al., 2006a, 2006b), we modified the electrode with a plastic tube (inner diameter: 2 mm) filled with sponge and copper wire. The anodal

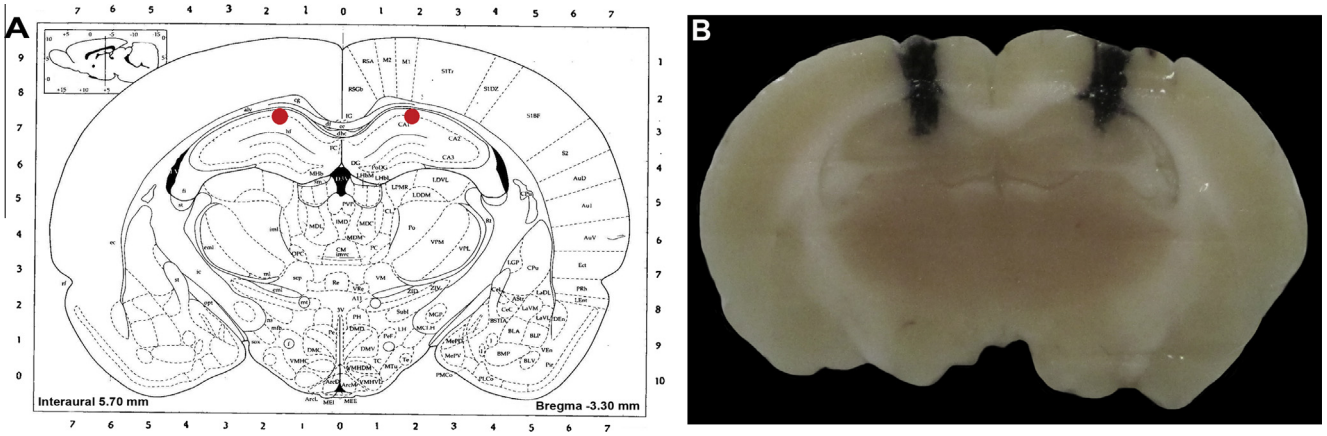

Fig. 1. (A) Schematic of a coronal section from 3.3 mm posterior to bregma, 2 mm right and left of the sagittal of the rat brain (Paxinos & Watson, 1996). It is showing the micro-injection sites (marked by red dots) of $A\beta_{1-40}$ and sterile saline. (B) Verification of CA1 sub-region stained by black ink in a coronal section of the rat's brain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)