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## Effects of Anodal Transcranial Direct Current Stimulation (tDCS) Preconditioning Combined with Arachydonilcyclopropylamide (ACPA) on Exploratory Locomotion in Male Mice

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### Abstract

**Background:** When confronting with an unfamiliar environment, animals exert orderly and complex behaviors called exploration. Locomotion is the most important part of exploratory behavior, but the principles of this behavior have not been fully understood yet. Here we studied the effects of the frontal region preconditioning with right and left frontal anodal transcranial direct current stimulation (tDCS) combined with the cannabinoid CB1 receptor agonist, arachydonilcyclopropylamide (ACPA) on locomotion in NMRI male mice. **Materials and Methods:** This study was carried out with 12 groups of NMRI mice (each group consisted of 8 mice), which were divided into 3 categories of ACPA alone, right, and left frontal anodal tDCS combined with ACPA. Anodal tDCS (with a current intensity of 0.2 mA for 20 minutes) was performed one day prior to ACPA intraperitoneal injection (0.01, 0.05, 0.1 mg/kg) and 15 minutes after injection the exploratory locomotion test was carried out. **Results:** The data showed that right frontal anodal tDCS combined with 0.01 and 0.05 mg/kg of ACPA and left frontal anodal tDCS combined with 0.05 mg/kg ACPA increased exploratory locomotion. **Conclusions:** Our finding suggested that combined implementation of right and left anodal tDCS and ACPA exerted anxiolytic properties and could increase exploratory related locomotion. [GMJ. 2016;5(4):173-79]

**Keywords:** Anodal Stimulation Transcranial Direct Current Stimulation; Arachydonilcyclopropylamide; Locomotion; Exploratory Behaviors

### Introduction

When animals confront new environments, they perform orderly yet complicated repertoire of spontaneous behaviors, which is called exploration. Although many studies were carried out to investigate the exploration principles, so far, these principles are not sufficiently understood in rodents [1].

Locomotion, which is the animal's movement from one location to another, is the foremost component of exploratory behavior. Investigating locomotor activity provides valuable insight into plenty of research areas such as drugs pharmacological effects, drug efficacy prediction, and locomotor abnormalities, also curiosity, emotionality, arousal, and exploratory states [2]. Transcranial direct current

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stimulation (tDCS) is a non-invasive, cheap, and harmless neuromodulatory tool which has been currently shown its effectiveness in different disorders. Also, recently tDCS has shown encouraging results in brain activity modulation and its resultant behavioral alterations [3]. Furthermore, several studies indicated that tDCS could influence and improve motor activity, for instance, a study showed that anodal tDCS had the potential to enhance the effectiveness of gait training in chronic stroke [4]. In other study patients with ischemic white matter lesions showed gait and balance improvement after implication of anodal tDCS combined with physical therapy [5], also it was shown that anodal tDCS could improve motor learning and working memory. On the other hand, frontal anodal tDCS affect cognitive functions like working memory [6], attention, learning [7]. Moreover, frontal region tDCS affected risk-taking behaviors, for example in a study performed on humans, left anodal/right cathodal tDCS over the dorsolateral prefrontal cortex (DLPFC) caused high-risk decision-making [8].

Cannabinoids have a regulatory role not just in fundamental physiological processes such as motor coordination, food intake, and pain perception, but also in cognitive functions such as learning and modulate emotional responses. Cannabinoids exert its effects by two receptors CB1 and CB2. The CB1 cannabinoid receptor is G-protein coupled, and the most of the endocannabinoid brain signaling are mediated by it [9]. Drugs which activates brain CB1 receptors induce alterations in motor behaviors, these alterations are dose-dependent and can be motor stimulating or motor inhibiting [10]. Lately, on account of endocannabinoids (eCB) anxiolytic effects, they have been considered seriously as a companion therapy for treating different anxiety-related disorders [11]. Also, a study revealed that endocannabinoids could help eliminating aversive memories [12]. Fox *et al.* suggested complex effects of CB1 receptor agonist alterations on novelty exploratory behavior in the male rat which was dependent on age and dosage of CB1 agonist [13].

In the present study, we investigated the effect of right or left frontal region preconditioning

(stimulation one day prior to the test) by anodal tDCS combined with ACPA on exploratory locomotor activity.

## Materials and Methods

### *Animals*

Experimental groups consisted of 8 male NMRI mice, which (weigh 25–30 g) were obtained from the University of Tehran (Tehran - Iran) animal house. A total of 12 groups, which divided into 3 sets of four groups (ACPA alone, right and left frontal anodal tDCS combined with ACPA) were used. Every experimental group was kept in plastic cages and at a controlled temperature of  $22\pm 2$  °C under a 12/12-h light/dark cycle with free availability of food and water. Behavioral tests were done during the light phase of the light/dark cycle and one hour before the initiation of the trial subjects transferred to the test room undisturbed. The study was approved and performed by the Ethics Committee of the Faculty of Science of the University of Tehran, which corresponds to the national guidelines for animal care and use.

### *Drug*

We obtained ACPA (selective CB1 receptor agonist) from Tocris (Tocris, Cookson Ltd., UK). The drug was dissolved in anhydrous ethanol at a concentration of 5 mg/ml and then diluted into the required doses using saline (0.01, 0.05, and 0.1 mg/kg). The acquired doses were injected intraperitoneally.

### *Stereotaxic Surgery*

Subjects were anesthetized by intraperitoneal injection of a solution contained ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg). Their skulls were uncovered and custom made epicranial electrode with 2.1 mm internal diameter and 1.7 mm void space (for providing 3.5 mm<sup>2</sup> effective contact area after filled with saline) were fixed over the designated areas of skull, which were corresponded to right and left frontal region (stereotaxic coordinates for the right and left frontal region was 1 mm anterior from bregma and 1 mm from the sagittal suture to the right and left respectively [14]. All subjects were given 5 days to recover from surgery.

### Transcranial Brain Stimulation

We used Active Dose II unit made in Activatek company-Taiwan and custom made epicranial electrode with the aforementioned specifications which were placed over the right or left frontal (Figure-1) region served as anode electrode, as well as a 9.5 cm<sup>2</sup> carbon rubber in the soaked sponge cover, which was put on the subject's chest and served as cathode electrode. By implementing this setup, we reduced the electrical current diversion and therefore, achieved safe and efficient stimulation [15]. We excavated the brain of tDCS group subject's and sliced them by vibroslicer; then we examined them under microscope. No abnormalities were seen in their frontal cortex region.

### Locomotion Test

The locomotion apparatus (BorjSanat Azma Co, Tehran, Iran) comprised of clear perspex container box (30 cm × 30 cm × 40 cm high). The apparatus has a gray perspex panel (30 cm × 30 cm × 2.2 cm thickness) with 16 photocells which separated the box into 16 equal-sized squares. Locomotor activity was recorded as the number of crossings from one square to another during 5 min [10, 13, 16].

### Experimental Design

Our experimental groups consisted of eight mice. Intraperitoneal injection of ACPA carried out 15 min before the test phase (10 ml/kg), while control groups received the vehi-

cle. The timeline of this study is shown in the Figure-2.

### Data Analysis

Data was analyzed by SPSS version 19 using one- or two-way analysis of variance (ANOVA) and Tukey's post hoc test. The  $P \leq 0.05$  was set as the significance level. In all experiments, only significant data were shown.

## Results

### Effects of ACPA On the Exploratory Locomotion Behavior

One-way ANOVA analysis of locomotion showed that ACPA effect was significant ( $F_{3,28} = 3.229$ ,  $P = 0.037$ ). The post hoc analysis revealed that ACPA at a dose of 0.1 mg/kg increased exploratory locomotion (Figure-3, left panel).

### Combined Effect of Right Frontal Anodal tDCS and ACPA On the Exploratory Locomotion Behavior

Two-way ANOVA analysis of locomotion showed that the main effect of group was not significant, while tDCS main effect ( $F_{1,56} = 33.84$ ,  $P = 0.0001$ ) and interaction effect ( $F_{3,56} = 3.85$ ,  $P = 0.014$ ) were significant. The post hoc analysis revealed that right frontal anodal tDCS combined with ACPA at doses of 0.01 and 0.05 mg/kg increased exploratory locomotion behavior (Figure-3).

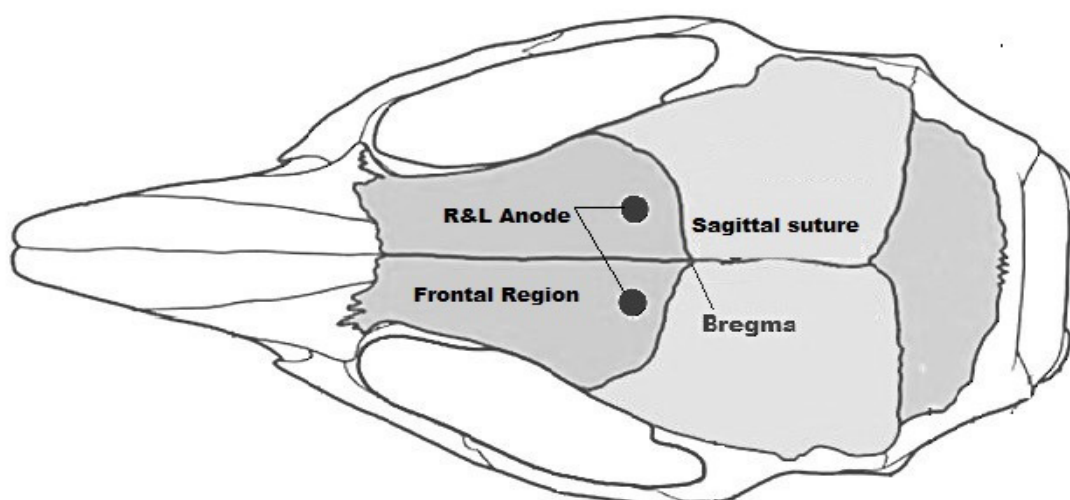


Figure 1. Epicranial Electrode Position on Mice Cranium

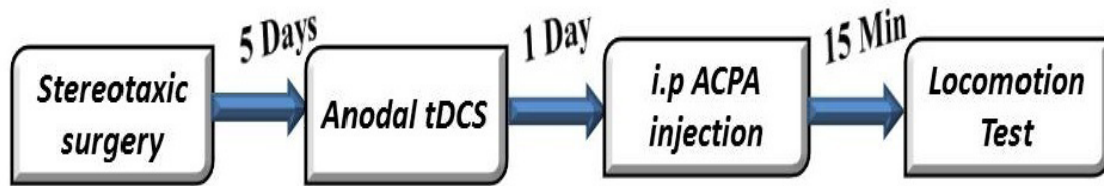
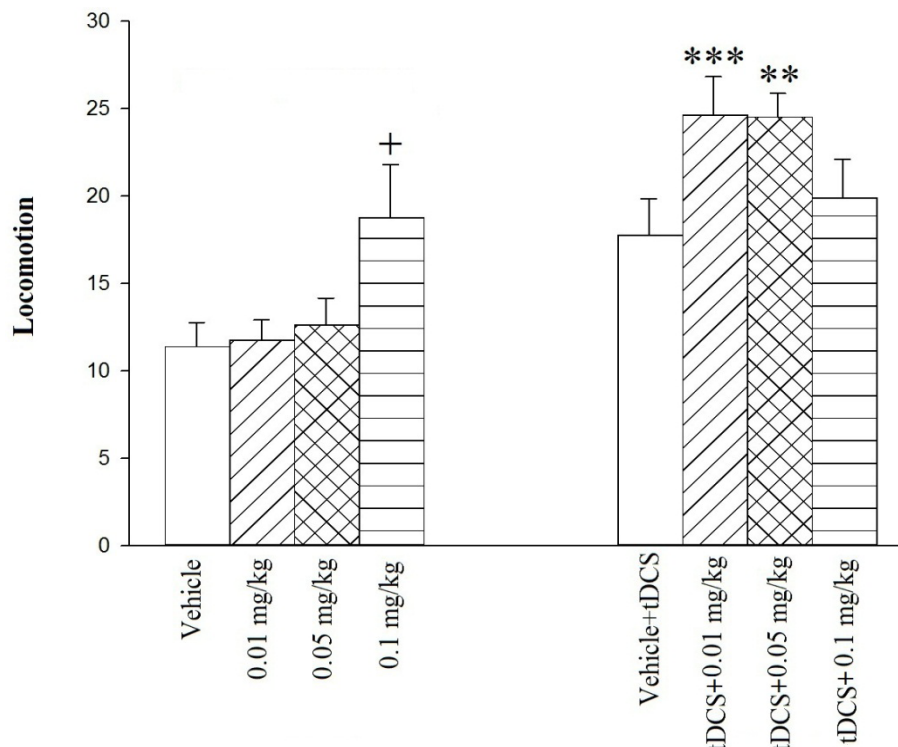


Figure 2. Study Timeline

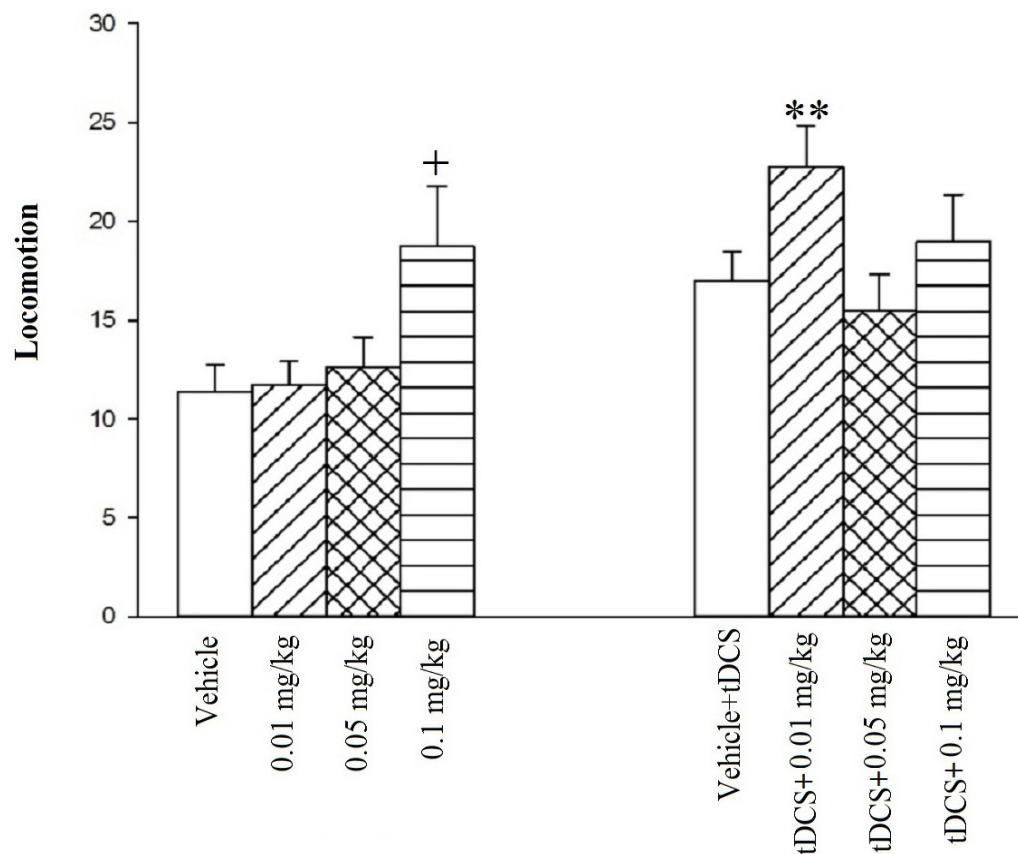


**Figure 3.** Presents the combined effect of right frontal anodal tDCS and ACPA on exploratory locomotion, which consists of two panels. Left panel indicates the effect of ACPA and right panel demonstrate the combined effect of right frontal anodal tDCS and ACPA on exploratory locomotion. One-way ANOVA was used to compare the effects of different doses of ACPA with vehicle (left panel), while two-way ANOVA was used to compare the combined effect of right frontal anodal tDCS plus ACPA with ACPA alone. The animals received tDCS one day and vehicle of ACPA (10 ml/kg) or different doses of ACPA (0.01, 0.05 and 0.1 mg/kg) 15 minutes prior to the test. Values are expressed as mean±S.E.M (n = 8 in each group). + P<0.05 different from vehicle group in left panel; \*\*\* P<0.001 and \*\* P<0.01 different from the respective group in the left panel.

#### *Combined Effect of Left Frontal Anodal tDCS and ACPA On the Exploratory Locomotion Behavior*

Two-way ANOVA analysis of locomotion showed that the main effect of tDCS was not significant, while the group main effect (F1,56

= 5.99, P = 0.001) and interaction effect (F3,56 = 6.92, P = 0.0001) were significant. The post hoc analysis revealed that left frontal anodal tDCS combined with ACPA at a dose of 0.01 mg/kg increased exploratory locomotion behavior (Figure-4).



**Figure 4.** Presents the combined effect of left frontal anodal tDCS and ACPA on exploratory locomotion, which consists of two panels. Left Panel indicates the effect of ACPA and right panel demonstrate the combined effect of left frontal anodal tDCS and ACPA on exploratory locomotion. One-way ANOVA was used to compare the effects of different doses of ACPA with vehicle (left panel), while two-way ANOVA was used to compare the combined effect of left frontal anodal tDCS plus ACPA with ACPA alone. The animals received tDCS one day and vehicle of ACPA (10 ml/kg) or different doses of ACPA (0.01, 0.05 and 0.1 mg/kg) 15 minutes prior to the test. Values are expressed as mean±S.E.M (n = 8 in each group). + P<0.05 different from vehicle group in the left panel and \*\* P<0.01 different from the respective group in the left panel.

## Discussion

Our findings indicated that ACPA only at the highest administered dose (0.1 mg/kg) affected exploratory locomotion, this is in line with earlier reports that revealed CB1 receptor agonists had complex effects on exploratory behaviors which were age and dose dependent [10]. We could deduce that ACPA at 0.1 mg/kg had an anxiolytic effect and consequently augmented the risk-taking behaviors, this augmentation in turn increased the incidence of exploratory behaviors.

It was shown that the medial prefrontal cortex-amygdala-hippocampus circuit (mPFC-

AMY-HPC) had a regulatory role in anxiety behaviors, including risk-assessment via theta oscillations [17], besides, a recent study has shown that anodal tDCS over mPFC in humans increased frontal-midline theta oscillations [18]. Hence, it could be assumed that by utilizing anodal tDCS alone exploratory behaviors could be modulated, but our findings demonstrated that in mice, single tDCS stimulation one day prior to locomotion test with current intensity of 0.2 mA for 20 min duration did not cause any significant difference in exploratory locomotion behavior either in the right or left frontal regions.

Moreover, preconditioning a circuit before



applying any other intervention alter the known outcome of that intervention, since the homeostatic mechanisms maintain plastic changes within a beneficial physiological range to preserve the network stability [19], in other words, the response of a network is state-dependent. Preconditioning with tDCS has been examined in several studies, for instance, Tae Gun *et al.* showed that priming by tDCS followed by repetitive transcranial magnetic stimulation (rTMS) could cause better motor performance in stroke patients [20]. Fregni *et al.* showed that combining tDCS prior to repetitive electrical stimulation (ES) that imitates the effects of rTMS could significantly modify the effects of ES alone on cortical excitability in cortical spreading depression (CSD) [21]. Also, Moloney *et al.* described that rTMS primed with tDCS effectively modulate the pain thresholds [22], to sum up, preconditioning with tDCS could reverse the known effects of other interventional modalities such as rTMS [23].

Our data demonstrated that right and left frontal anodal tDCS one day before the locomotion test at the intensity of 0.2 mA and 20 min duration combined with ACPA administration reversed the ACPA effects on exploratory locomotion, which means tDCS combined with ACPA at a dose of 0.1 mg/kg had no significant effect on exploratory locomotion. While priming with tDCS increased exploratory locomotion at lower doses of ACPA (for right frontal tDCS at 0.01 and 0.05 mg/kg, and for left frontal anodal at 0.01 mg/kg) these results were consistent with aforementioned findings. Furthermore, we could suggest that right fron-

tal anodal tDCS had a stronger effect on exploratory locomotion than left frontal anodal tDCS.

Due to novelty the of tDCS studies in animals, there is no electrical charge distribution map available in animals and this case mice, the limitation of this study was that we could not explicitly delineate the amount of electrical charge delivered in different parts of the brain. In order to gain a better insight in this area, series of correlated studies should be performed to make this map.

### Conclusion

Frontal region tDCS preconditioning together with ACPA administration increased the locomotion activity which might be resultant of their anxiolytic effects. To understanding the mechanism underlying this combined effect call for more in-depth investigation.

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### Conflict of Interest

The authors do not declare any conflict of interests.

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