STUDY PROTOCOL Version 1.1

The impact of transcranial direct current stimulation (tDCS) on the visual function of proliferative diabetic retinopathy patients

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1. Scientific background

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that can modulate cortical activity. It involves the application of a weak direct electrical current (1-2mA) through the intact scalp using surface electrodes to stimulate the brain. TDCS modulates neuronal activity via alterations of the neuronal membrane potential, leading to prolonged synaptic efficacy changes. In the motor cortex, anodal (positive current) stimulation results in subthreshold depolarization and increases the likelihood of neurons firing, while cathodal (negative current) stimulation hyperpolarizes neurons and decreases the likelihood of their firing (Nitsche *et al.*, 2003; Stagg & Nitsche, 2011). In other words, cathodal and anodal stimulation decreases and increases cortical excitability, respectively. The excitability modulation induced by tDCS is considered a potential alternative intervention to modulate motoric and cognitive function in healthy individuals and patients with neuropsychiatric disorders. Indeed, due to its relatively low cost, ease of use and safety profile, the application of tDCS moves from basic research towards clinical applications. Preliminary evidence showed the effectiveness of tDCS in the treatment of migraine, epilepsy and stroke complications (Brighina *et al.*, 2013; D *et al.*, 2018; Elsner *et al.*, 2018; San-Juan *et al.*, 2018), as well as attention-deficit hyperactivity disorder, depression and schizophrenia (Szymkowicz *et al.*, 2016; Salehinejad *et al.*, 2019a, 2019b; Zandvakili *et al.*, 2019).

In recent years, it has become apparent that diseases such as diabetes mellitus (DM) can alter function and structure in tissues not typically associated with complications such as the brain (Seaquist, 2015). Vascular and Alzheimer's dementia are more common in patients with type 2 diabetes, whereas neurocognitive changes such as reductions in measures of motor speed and psychomotor efficiency can be seen in adults with type 1 diabetes (Ott *et al.*, 1996; Nathan, 2014). The majority of these complications were attributed to diabetic neuropathy secondary to microvascular injuries involving small blood vessels that supply the nerves. In the eyes, this condition initially produces no symptoms and is called non-proliferative diabetic retinopathy (NPDR). However, the persistent lack of blood flow leads to ischemia which in turn promotes the growth of tiny abnormal blood vessels (neovascularisation) and fibrous growth in the retina and surrounding vitreous fluid. This stage is called proliferative diabetic retinopathy (PDR) and is the leading cause of irreversible blindness worldwide. Although diabetic retinopathy is commonly considered a vascular disorder and clinical staging are based on the severity of vascular abnormalities, mounting evidence supports early neural dysfunction in these individuals. For example, contrast sensitivity (CS) which is the ability to distinguish between finer and finer increments of light versus dark (contrast) has long been known to be reduced in diabetics who have not yet developed clinically-apparent retinopathy, and these CS deficits can become more severe as the disease progresses (HOWES *et al.*, 2007; HYVÄRINEN *et al.*, 2009). Existing literature indicates that CS losses may be associated with structural

changes (e.g. retinal ganglion cell layer thinning) of the inner-retina that similarly affect the magnocellular (MC) and parvocellular (PC) pathways (Gualtieri *et al.*, 2011; Montesano *et al.*, 2017). However, a study suggests that high levels of noise within the visual system may, at least in part, limit CS in diabetic patients (McAnany & Park, 2018). Therefore, it is counter-intuitive that inhibiting or decreasing noise within the visual system might modulate the post-retinogeniculate cortical processing of retinal signals and improve PDR patients' CS.

There is a proposal that most diseases that affect the retina impair visual function by increasing internal noise (Pelli *et al.*, 2004; McAnany *et al.*, 2013; McAnany & Park, 2018), and these patient groups seem to benefit from the reduction of visual cortical excitability using tDCS. For instance, the unilateral application of cathodal tDCS inhibits visual evoked potentials (VEPs) amplitudes and improves visual acuity in Amblyopic patients (Bocci *et al.*, 2019). In addition, patients with mild myopia also exhibit an improvement of uncorrected visual acuity and contrast sensitivity after transcranial random noise stimulation (tRNS), another brain stimulation method that can enhance the signal-to-noise ratio in the cortical network (Camilleri *et al.*, 2014). So far, the inhibitory effect of cathodal tDCS is not tested yet on PDR patients. Therefore, in the present study, we aimed to test the hypothesis that inhibiting the visual cortex's excitability using cathodal tDCS stimulation can downregulate cortical noise and improve PDR patients' visual function.

2. Name and description of the investigational device

2.1 Transcranial direct current stimulation (tDCS)

For tDCS, a 1mA current will be delivered via a rectangular saline-soaked surface sponge electrodes connected to a battery-driven, constant-current DC-stimulator (ELDITH DC-stimulator, NeuroConn, Germany). The electrodes' surface area measured 35 cm^2 with a current density of approximately 0.043 mA/cm^2. The stimulating (cathode) electrode will be placed over the Oz EEG electrode locations (International 10-20 EEG System) which correspond to the midpoint of the left and right primary visual cortex (V1). The reference (anode) electrode will be positioned over the right shoulder. For the real stimulation conditions, the current will be delivered for 10 minutes and slowly ramped-up and down for 10 seconds at the start and end of stimulation, respectively. The impedance during stimulation will be maintained below 10kΩ in order to minimize the tingling skin sensation. The same amount of current will be applied in the sham stimulation condition but only for 30 seconds and automatically turned off. This will ensure effective blinding with regards to the stimulation conditions because the participants experienced a similar skin sensation during sham that is indistinguishable from the real stimulation conditions.

2.2 Early Treatment Diabetic Retinopathy Study (ETDRS) chart.

De Venecia will conduct the visual acuity testing using the standard ETDRS chart before and immediately after stimulation. The Best-Corrected Visual Acuity (BCVA) will be measured first in the right eye, while the left eye was occluded then vice versa. To prevent the patient from getting familiar with the chart, two different charts will be used to test the eyes (Chart 1: right eye, Chart 2: left eye). The patients have to slowly read the letters on the chart from top to bottom, letter-by-letter, and beginning with the first letter on the top row. If a patient misread >2 letters on a line, we will stop the procedure and add 0.02 log unit (for every misread letter) to the logMAR score of that line. Patients who fail to read the letters will be given a logMAR score of 1.9 for the ability to count fingers, 2.3 for detecting hand motion, 2.7 for light perception, and 3.0 for the absence of light perception. High logMAR score is indicative of worsening vision.

2.3 Color and Numerosity discrimination

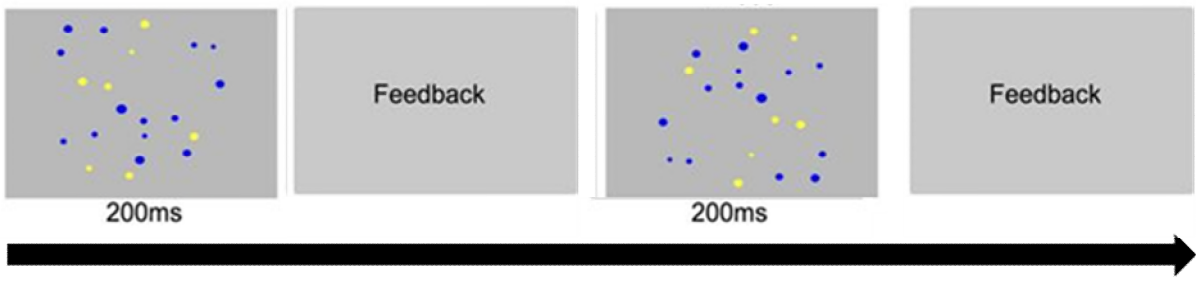

Figure 1. Time course of the color and numerosity discrimination task.

In this task, the patients will be presented with a set of intermixed, computer-generated yellow and blue dots. The dots will be presented for 200 ms, and the patients have to indicate which set contained more dots as quickly and accurately as possible. The patients have to press the computer keyboard to respond (letter “B” for blue and letter “N” for yellow) using their right index finger. The next trial will appear after a button press. The task will be divided into 3 blocks with 20 trials per block (total of 60 trials). The patient’s reaction times (RTs) and accuracy will be measured for each trial. RT is defined as the time the dots are presented until the pressing of a button. The patients will perform the task before and after stimulation.

3 Risks and benefits of the investigational device and the experiment

The results will be communicated to the participants upon request after the study is completed and will be presented in person. The methods applied in this protocol are used in our laboratory, and in many laboratories worldwide in thousands of healthy participants, and patients, without major adverse events, when exclusion criteria were respected (Poreisz *et al.*, 2007; Bikson *et al.*, 2016). TDCS, when applied in long-lasting sessions, from our own experience, can result in tiredness and a slight headache. Therefore, we have limited the duration of the sessions necessary for the conduction of the study. If side-effects occur, the

respective participant will be medically treated, and observed, until complete remission. Quality, time of occurrence, duration, intensity, frequency, treatment, severity, and association with the stimulation will be documented.

4 Aims and hypotheses of the experiment

Aim: To investigate the impact of cathodal tDCS stimulation of PDR patients' primary visual cortex in visual acuity and numerosity discrimination.

Hypothesis: Decrease in cortical noise will improve the patient’s visual acuity and numerosity discrimination task performance.

5 Structure of the study

5.1 General information

In the present study, we will recruit proliferative diabetic retinopathy patients. Participants must be at least 18 years of age and right-handed. The study will be carried out using a randomized sham-controlled between-subject design. Twenty-two participants will be recruited and will be randomly assigned to the real or sham stimulation group. In the study, an experimental session starts with a detailed explanation of the study and task to each patient. Then, the patients will be asked to sign a written informed consent. Subsequently, we will determine the participant’s baseline performance by letting them perform the color and numerosity discrimination task. After the baseline performance, the primary visual cortex's location will be determined using the 10-20 EEG coordinates. The tDCS electrodes will be attached, and the stimulation will be started. Immediately after stimulation, the participants will perform the same task.

5.2 Test persons

In accordance with the safety aspects of non-invasive brain stimulation techniques and study-specific requirements, the following inclusion and exclusion criteria are defined:

5.2.1 Inclusion criteria for the selection of test persons:

(1.) Clinically diagnosed PDR patients.

(2.) Voluntary participation and capacity to consent

(3.) Right-handedness (Edinburgh Handedness Test)

(4.) At least 18 years of age during the experiment.

5.2.2 Exclusion criteria for the selection of test persons:

(1.) Other co-morbid conditions such as chronic or residual neurological, psychological, and psychiatric disorders (esp. epilepsy, schizophrenia, mania or depression)

(2.) History of head injury with loss of consciousness

(3.) Intracerebral ischemia/history of cerebral bleeding

(4.) Metal implants in the head and neck area (e.g. post-operative clips)

(5.) Electronic implants (pacemakers, cochlear implant, deep brain stimulator)

(6.) Pregnancy or breastfeeding

(7.) Alcohol or drug addiction

(8.) Local or global aphasia

(9.) Any legal reason why the candidate cannot participate

(10.) Participation in another scientific or clinical study within the last 8 weeks

6. Data management

The principal investigator will perform the statistical analysis of data. All statistical procedures will be validated by another member of the team. The data will be captured electronically and backed up on a central server that is additionally secured using the software. Records of the data will also be available as copies on a hard drive. Data will be kept at least 5 years and possibly longer, depending on the longest applicable standard.

7. Procedure to obtain informed consent

During the interview, the investigator will outline the purpose and potential risks of the study. It must be explicitly stated that the participant has volunteered for the study and that he/ she may revoke his/ her consent at any time during the study and terminate their participation without giving any particular reason. If the participant gives a reason, then this should be noted and signed within the study materials. During the interview, the participant will receive a consent form and the following important information:

- Nature and purpose of the study

- Privacy Policy

- Conditions to be complied with

- Instructions as to the Course of the Study

The consent will be given in writing and documented in a standardized consent form signed by the physician and the test person. As part of the inclusion criteria, only interested participants who have the capacity to consent will be included in the study.

8. References

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