Spatiotemporal after-effects of transcranial direct current stimulation on sensory-evoked activity in rat S1: A pilot VSDI study.

Sandrine Chemla, Wing KL Witharana and Bruce L McNaughton

Canadian Centre for Behavioural Neuroscience (CCBN), University of Lethbridge, 4401 University Drive Lethbridge, Alberta T1K 3M4, Canada.

Abstract

For decades, transcranial direct current stimulation (tDCS) has been used to manipulate cortical excitability and plasticity of the human brain. Promising therapeutic results, such as depression treatment, have emerged and are extensively studied today. However, the underlying cortical mechanisms of this electrical stimulation remain unclear. Here, we propose to use voltage-sensitive dye imaging (VSDI) on rat primary somatosensory cortex to study the after-effects of tDCS on neuronal population activity. We found that after repetitive anodal tDCS, sensory-evoked VSD responses were significantly increased in amplitude and spatial extent of activation, counteracting the sensory adaptation process observed in control rats. Combining tDCS and VSDI, offers an excellent tool for observing in real-time subthreshold tDCS effects over a large cortical area, when applied to the animal brain.

Keywords: Transcranial direct current stimulation, Voltage-sensitive dye imaging, Rat somatosensory cortex, Evoked VSD dynamics, Sensory adaptation.

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Introduction

Cortical excitability of the human brain can be increased or decreased using brain stimulation techniques. One of the methods currently used for that purpose is weak transcranial direct current stimulation (tDCS). This portable, non-expensive and non-invasive technique has recently received new interest in the field and has given promising results mainly in clinical neuropsychiatric and stroke recovery studies [1-3]. However, the fundamental mechanisms underlying these changes in cortical excitability, as well as their after-effects on sensory processing remain mainly unknown. Concurrently with the comeback of this age-old technique, the voltage-sensitive dye imaging (VSDI) method is a technological breakthrough since it offers the possibility to visualize in real-time the activity of large cortical neuronal populations with high spatiotemporal resolution [4-6]. Indeed, based on voltage-sensitive dyes that instantaneously change their fluorescence yield in proportion to membrane potential changes, VSDI allows the study of spatiotemporal dynamics of cortical processing at the mesoscopic level. Combining these two methodologies could help to reveal the neuromodulatory after-effects of tDCS on cortical population activity. This is indeed an issue of significant importance in this field [7], promising improvements in the therapeutic use of electrical stimulation.

Transcranial electrical stimulation using direct current is a very old technique, first used to investigate the source of "animal electricity". More than two centuries later, neuroscientists are still using this methodology to enhance brain function. The principle of this brain stimulation technique is very simple: The application of weak electrical currents through the scalp modulates cortical activity through neuronal membrane excitability [8,9]. Over the last past decade, there has been a re-emergence of the tDCS technique, both in clinical and research studies (see [10], for a review on the subject). In humans, tDCS is used in the treatment of psychiatric disorders, such as depression (see [1,11-13], for a review), as well as in improving patient recovery after stroke (see [14-17] for a review). The mechanism generally accepted is that anodal stimulation pushes neuronal resting potentials closer to the activation threshold and therefore increases tissue excitability, whereas cathodal stimulation inhibits cell firing and decreases excitability. Lately, fundamental research has focused on tDCS as a modality either to improve sensorimotor performance [18-20], or to create temporary cortical dysfunction [21,22]. Once again, anodal
and cathodal stimulations are respectively associated with increase and decrease in cortical excitability. However, studies are far from being consistent, the effects often being region specific and the underlying connectivity being complex. Furthermore, it remains unclear if these observed changes in excitability have a functional consequence.

Here we studied the spatiotemporal after-effects of anodal tDCS on rat somatosensory cortex, using VSDI. We recorded and studied hind-limb sensory VSD responses to brief tactile stimuli, before and after tDCS periods. We observed that repetitive anodal tDCS enhances the spatiotemporal dynamics of sensory VSD responses. In comparison, control rats (i.e. not electrically stimulated) exhibit sensory response adaptation, i.e., attenuation of VSD responses amplitude after repeated sensory stimulation. These results suggest that anodal tDCS enhances a cortical mechanism that counteracts sensory adaptation. Similar to adaptation [23], tDCS may modulate the balance between excitatory and inhibitory circuits.

Materials and Methods

Surgical Preparation and VSD Staining

Eight female Brown Norway-Fisher hybrids rats (2 months old) were used in this study. All surgical procedures were performed according to the National Institutes of Health guidelines and Canadian Council for Animal Care (CCAC) regulations under the guidance of the University of Lethbridge Institutional Animal Care committee. The rats were anesthetized with urethane (0.75 mg/kg) and placed in a stereotaxic apparatus, while their temperature was maintained at 37ºC by a heating pad and their electrocardiogram was closely monitored on an oscilloscope throughout the experiment to ensure a constant level of anesthesia. After marking the position of the required opening area, a metal head-plate was attached to the skull of the animal with dental acrylic. A 8 × 8 mm in diameter cranial window was drilled above both cortical hemispheres (Figure 1A, bregma 2.5 to -5.5 mm, lateral -4 to 4 mm). The dura mater was then carefully removed and the RH1691 dye (Optical Imaging) was allowed to stain the cortex for 2 h. Finally, the cortex was rinsed to remove the unbound dye and covered with 1.5% agar and a glass coverslip.

Imaging Voltage-Sensitive Dye Signals in Response to Hind-Limb Sensory Stimulation

Voltage-sensitive dye (VSD) signals were recorded from a focal plane ~ 300 µm below the pia matter by a MICAM ULTIMA CMOS camera (SciMedia) and frames were collected every 10 ms. The camera was mounted at the top of an Olympus MVX10 Macro Zoom System microscope equipped with epifluorescence, and a 100 W halogen lamp gated by a shutter (Uniblitz) provides excitation light. The latter was filtered with a 630 ± 15 nm band-pass filter, reflected onto the cortex by a 650 nm dichroic, and the epifluorescent image was collected after a 665 nm long-pass filter. Hind-limb stimulation was delivered for 50 ms duration by a custom-made vibrating device (100 Hz). Imaging data were acquired triggered to the EKG signal, and alternate trials were either with (evoked response) or without (blank response) hind-limb stimulation. Five sequences of 20 trials (10 evoked responses and 10 blank responses randomly interleaved) were collected (C, S1, S2, S3 and S4). Stacks of images were stored on hard-drives for off-line analysis with MATLAB R2014a (MathWorks), using the Optimization, Statistics and Signal Processing Toolboxes. In each block, the evoked response was computed in two successive basic steps. First, the recorded value at each pixel was divided by the average value before stimulus onset (frames 0 division) to remove slow stimulus-independent fluctuations in illumination and background fluorescence levels. Second, this value was subsequently subtracted by the value obtained for the blank condition (blank subtraction) to eliminate most of the noise due to heartbeat and respiration.

Figure 1. A: Schematic showing the rat skull after craniotomy for fluorescent imaging and screw implantation for electrical stimulation. B: tDCS mechanism. The pink area represents the rat brain, while the green area is the hindlimb primary somatosensory area. s+ and s- represent the two stimulation electrodes. Dotted black line outlined the region of the craniotomy as shown in A.
**Transcranial Direct Current Stimulation**

Transcranial direct current stimulation was delivered by a linear stimulus isolator (A395, World Precision Instruments) using two screws as transcranial stimulation electrodes. The active electrode is placed in the skull in front of the imaging chamber, while the grounded reference electrode is placed behind it (Figure 1A). Therefore, the constant current flow field passes through the entire brain (Figure 1B). The 8 rats were divided into two groups: the stimulated group (n=4) and the control group (n=4). On stimulated rats, transcranial direct current stimulation was applied for 5 min at 280 µA current intensity, in between hindlimb stimulation sequences. The stimulation intensity and duration were chosen considering the safety limits reported in a previous tDCS studies on rats [24]. An oscilloscope controlled constant current flow, and anodal stimulation was the main condition of the present study.

**Observations**

Our goal was to investigate the spatiotemporal changes of somatosensory evoked VSD responses elicited by tactile hind-limb stimulation after repetitively stimulating the rat using tDCS.

To examine these changes before and after tDCS, we recorded with VSDI the dynamic activation of the hind-limb cortical area after a 50 ms vibratory stimulation of the contralateral hind-limb. Figure 2 shows the spatiotemporal VSD activity representative of the two groups of rats, either receiving tDCS in between tactile

![Figure 2. Spatiotemporal effect of tDCS on sensory-evoked VSD activity in response to a brief left hindlimb tactile stimulation. Each image corresponds to the normalized (with respect to the maximal amplitude of the control sequence C) ∆F/F (single frame, time of acquisition in milliseconds is indicated on top, 0 is the time of hindlimb stimulation) averaged over 10 consecutive trials. A: Control rat. A 5 min period of time is left between sequences (before S1, S2, S3, S4). B: Electrically stimulated rat. A 5 min period of tDCS is applied between sequences (before S1, S2, S3, S4)
stimulation sequences (tDCS group, Figure 2B) or not (control group, Figure 2A). Time of acquisition in milliseconds is indicated on top of the frames, with time 0 representing the onset of the tactile stimulation. For control rats (top), a 5 min period of time followed every 10 trials, while for electrically stimulated rats (bottom), a 280 µA anodal tDCS was applied to the rat skull for the same period of time. We observed that the spatiotemporal dynamics of the somatosensory VSD response changed after repetitive tDCS periods.

The trial-to-trial analysis of sensory-evoked VSD responses reported in Figure 3 (left panel, average across 4 animals in each group), offers a finer representation of the tDCS after-effects over time of acquisition (x-axis) and trials (y-axis). The averaged time-course of each 10-trial sequence is reported in the right panel, after normalization by the maximum amplitude of the control sequence (C, black traces). We observed that control rats were characterized by a gradual decrease in response amplitude from sequence C to S4 (Figure 3A, right panel), while electrically stimulated rats exhibited an increase in response amplitude after repetitive tDCS periods (Figure 3B, right panel), reaching a plateau (S3) before decreasing back to a previous state (S4).

Figure 3C reports the averaged normalized peak fluorescence for both groups (n=4), revealing a significant increase in amplitude, when comparing control vs. tDCS groups, already after the first tDCS period (S1, t-test with p<0.01). The effect remains significant when comparing the third and fourth sequences (S3 and S4, t-test with p<0.05), suggesting that tDCS counteracts the adaptation process observed in control rats after repeated sensory stimulation.

We then investigated the spatial effects of repetitive tDCS by calculating the normalized area of activation of the somatosensory VSD responses in both groups of rats (Figure 3D), before (C) and after repetitive tDCS (S1, S2, S3, S4). The area of activation was computed as the number of pixels in the contralateral hemisphere whose mean over the first 100 ms of the response (see Figure 2) is greater than 2.7 times the standard deviation of the baseline, and converted to mm by multiplying by 0.0064, i.e., the area of one pixel in mm². We finally normalized each value relative to the area of activation calculated for the control period (C, black histogram). We observed that repetitive anodal tDCS induces a net significant increase in activated area already after the first period of stimulation (S1, t-test with p<0.05). The effect, although reduced, remains significant after the third period of electrical stimulation (S3, t-test with p<0.05), once again suggesting a counter-action of tDCS to sensory adaptation observed in control rats.

**Figure 3.** Trial to trial analysis of the somatosensory VSD responses as a function of time (average across 4 animals in each group). Each line of the matrices depicted on the left is a single trial, which represents the averaged activity over the activated hemisphere (contralateral). The hind-limb stimulus was presented for 50 ms (vertical gray shaded area) at time 0. A: Control group (n=4). A 5 min period of time is left every 10 trials. B: tDCS group (n=4). A 5 min period of tDCS is applied every 10 trials. Averaged normalized somatosensory responses of each sequence (C, S1, S2, S3, S4) as a function of time are reported on the right panel. C: Averaged data for normalized peak fluorescence and D: normalized spatial extent of activation of the somatosensory VSD responses (n=4 for both control and tDCS groups).

Error bars indicate SEM and statistical significance was calculated using an unpaired two-sample Student t-test: *P<0.05; **P<0.01; ***P<0.005
Discussion

We used voltage-sensitive dye imaging on rat primary somatosensory cortex to study the spatiotemporal aftereffects of repetitive transcranial direct current stimulation on cortical population activity. After repetitive anodal tDCS, evoked-sensory VSD responses showed a significant increase in amplitude and spatial extent of activation. Interestingly, our observations are in accordance with results obtained in humans [25] during tDCS stimulation over the hand motor cortex. In comparison, control rats exhibit sensory response adaptation, i.e., attenuation of VSD responses amplitude after repeated sensory stimulation. Heiss et al. [23] have proposed that sensory adaptation of S1 neurons is caused by a shift in the balance between excitation and inhibition. Our results therefore suggest that tDCS is likely to disrupt this balance, counteracting sensory adaptation.

Regarding the fact that the rats were anesthetized with urethane, one can naturally question whether the observed tDCS effects might be rather explained by a change of cortical arousal state of the animal. Indeed, under urethane, strong sensory stimulation causes the cortex to go from synchronized to desynchronized state [26,27], which is accompanied by a non-theta to theta transition in the hippocampus and heart rate modulations [28,29]. Although we did not record from the hippocampus, we carefully monitored heart rate of the animal throughout each experiment, ensuring a very stable anesthesia over time and ruling out any acceleration in heart rate when comparing stimulated to control rats (data not shown).

VSDI offers the possibility to manipulate and concomitantly observe mesoscopic networks of neurons with a high spatio-temporal resolution. A recent study from Molaee-Ardekani et al. [30] presented a similar approach aiming at studying the effects of tDCS on neuronal systems, using a combination of computational modeling and electrophysiology. They recorded local field potentials from the barrel cortex of rabbits, with and without tDCS, and showed that their neural mass model of the cortex coupling with an externally applied electric field accurately reproduced the experimental evoked responses. We believe that VSDI recordings provide the advantage of broader scale analysis of tDCS effects on cortical activity, and could give some insight into how tDCS affects propagation of activity in the cortical space. However, electrophysiological recordings will be useful if one wants to understand mechanisms at the cellular level and to discriminate effects in different cortical layers.

Although the cortical alterations induced by tDCS can be observed and investigated using VSDI, it is not presently possible to have direct access to the current flow. In human studies, several head models have been developed in order to simulate the current flow and density in the entire human brain. Finite element methods derived from MRI data have been the major tool for predicting current flow through the brain during tDCS ([31-33], among others). These models represent a required advancement for using tDCS in clinical human treatment; however they do not provide information on the underlying neuronal activation. To our knowledge, only a few models have addressed this issue at the mesoscopic level [30,34]. We believe that tDCS neural mechanisms investigations could also benefit from coupling a model of the electric field (possibly inspired by the model of extracellular field potentials developed by Bedard et al. [35,36]) to multi-compartment biophysical models of individual neurons, such as developed by Chemla and Chavane [37].

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Correspondence to: Sandrine Chemla, Institut de Neurosciences de la Timone, UMR 7289, CNRS and Aix-Marseille Universite, 27 Bd Jean Moulin, 13385 Marseille, France.

Tel: (33) 491324027 Fax: (33) 491324056 E-mail: sandrine.chemla@univ-amu.fr