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FLASH VISUAL EVOKED POTENTIALS IN MICE CAN BE MODULATED BY TRANSCRANIAL DIRECT CURRENT STIMULATION

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Abstract—Transcranial direct current stimulation (tDCS) in humans has been shown to affect the size of visual evoked potentials (VEPs) in a polarity-dependent way. VEPs have been widely employed in mice to study the visual system in physiological and pathological conditions and are extensively used as animal models of neurological and visual disorders. The present study was performed to evaluate whether mice VEPs could be modulated by tDCS in the same manner as in humans. We describe here the effects of 10 min tDCS (anodal, cathodal or no stimulation) on flash-VEPs in C57BL/6 mice under sevoflurane anesthesia. VEP amplitudes of the first major peak (P1) were analyzed before, at 0, 5 and 10 min after tDCS. Compared with no stimulation condition, anodal tDCS increased P1 amplitude slightly more than 25%, while cathodal stimulation had opposite effects, with a decrease of P1 amplitude by about 30%. After-effects tended to reverse toward basal levels within 10 min after tDCS. These results, suggesting polarity-dependent modulation similar to what described in humans of tDCS effects on VEPs, encourage the use of mice models to study tDCS mechanisms of action and explore therapeutic applications on neurological models of disease.

Key words: neuromodulation, transcranial direct current stimulation, visual evoked potentials, C57BL/6 mice, cortical excitability.

In recent years, the non-invasive brain stimulation technique called transcranial direct current stimulation (tDCS) has been shown to painlessly modify human visual cortex excitability in a polarity-dependent way (Antal et al., 2004, 2006). The application of weak direct currents to visual areas modulates visual evoked potential (VEP) amplitudes (Antal et al., 2006), the perception of phosphenes (Antal et al., 2003) and affects contrast sensitivity (Antal et al., 2001). However, the mechanism and site of action of tDCS have not been fully understood and the use of animal models could be of great advantage in this field (Cambiaghi et al., 2010; Fritsch et al., 2010; Liebetanz et al., 2006a,b; Schweid et al., 2008). Previous invasive approaches of cortical direct current stimulation in rats (Bindman et al., 1964; Gartside, 1968) and cats (Creutzfeldt et al., 1962; Purpura and McMurtry, 1965) suggest an effect on cortical excitability by modifications of neuronal plasticity.

Mice have become the most widely used animal models in visual science, and visual evoked potential parameters such as peak amplitude and latencies have been extensively used to test neuronal plasticity over visual areas (He et al., 2006; Lickey et al., 2004; Sawtell et al., 2003). In mice, VEPs can be obtained using implanted epidural electrodes (Mazzucchelli et al., 1995) or subdermal needles (Martin et al., 2006). However, chronically implanted electrodes have the advantage to detect amplitudes without the impedance of the skull and the increased noise and signal variability resulting from subdermal needle placement (Strain and Tedford, 1993). Both flash (Martin et al., 2006; Mazzucchelli et al., 1995) and pattern reversal (He et al., 2006) visual stimulation have been used in rodents, obtaining different waves (Onofrj et al., 1985; Strain and Tedford, 1993). These differences are probably related to the activation of different functional systems of the retina and visual pathway, one responding to the onset of grossly differing stimulus intensities with negligible contrast contest, and the other to differences in contrast at a constant illumination (Strain and Tedford, 1993). In the present paper, we opted for visual stimulation by flash, a technique that is widely used to elicit VEPs in rodents (Hudetz et al., 2009; Mazzucchelli et al., 1995; Onofrj et al., 1985; Ridder and Nusinowitz, 2006; Strain and Tedford, 1993), even in animal model of diseases such as central nervous system myelin pathologies (Gambi et al., 1996; Lehman and Harrison, 2002). Finally, since mouse has been chosen as the preferred model to study plasticity in the visual pathway VEPs (Coleman et al., 2010; Morishita et al., 2010), the possibility to modulate its visual evoked responses by tDCS could be of great importance to study neuroplastic changes in response to weak direct current stimulation.

The major goal of the present report is to investigate whether the application of tDCS on mouse occipital cortex is able to affect visual evoked potentials in a polarity-dependent way, similar to what observed in humans. This is a necessary step in order to subsequently use mouse models for studying the underlying mechanism of action of tDCS in the visual pathway.

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Abbreviations: bpm, beats per minute; dLGN, dorso-lateral geniculate nucleus; (t)DCS, (transcranial) direct current stimulation; VEPs, visual evoked potentials; V1, primary visual cortex.

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EXPERIMENTAL PROCEDURES

Animals

Twelve C57BL/6 naive female mice of 8–12 weeks of age (Charles River, Calco, Italy) were used in this study. They were housed under controlled temperature and on a 12 h light/dark cycle (lights on at 6:30 AM) with free access to chow pellets and tap water. We made all efforts to minimize the number of animals used and their suffering. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and approved by the San Raffaele Institutional Animal Care and Use Committee (IACUC).

Electrodes implant

Deep anesthesia (verified by absence of reaction at tail-pinching) was induced and maintained with sevoflurane (2.5–3%, Sevorane™, Abbott S.p.a. Campoverde, Italy) through a face mask. Temperature during surgery was maintained at 37 °C using a heating pad. For VEPs recording, skin was resected and two epidural stainless steel screw electrodes (0.9 mm diameter) were implanted: active over the right primary visual cortex (V1) (2.5 mm lateral to lambda) and reference over right frontal areas (1 mm lateral and 2 mm anterior to bregma). For tDCS, an epicranial customized plastic tube (inner area=4.5 mm^2) was placed in close proximity to the active occipital VEPs electrode, 1.5 mm lateral and 1.5 mm anterior to lambda. The whole implant was fixed with glass ionomer dental cement. During surgical procedure some Vaseline was put over both eyes of the animals to prevent eye drying or dental cement injury. After surgery, all animals were allowed to recovery 3–6 days before undergoing VEPs recordings.

VEPs

Following overnight dark adaptation, mice were anesthetized with sevoflurane (2–2.5%) in oxygen (30%) and nitrogen (70%) delivered by inhalation through a face mask, in a darkened room with a background luminance of 0.75 lx (measured with a ISO-TECH ILM350 lux meter). Temperature was maintained at 37 °C using a heating pad. Before the experimental tests, mice were allowed to reach a steady state with the anesthetic; the adequate level of anesthesia was verified by checking for the presence of tail-pinching reflex and the absence of the corneal one (Bolay et al., 2000) and by monitoring heart rate frequency, recorded continuously from two subcutaneous needles in right and left forelimbs. This enabled to monitor closely the depth of anesthesia, which is crucial to maintain optimum visual responsiveness (Gordon and Stryker, 1996). For each VEP experiment, two trains of 20 flash stimuli of 10 μs duration and 1 Hz frequency were delivered with a flash photostimulator (intensity 126–231 mJ; Micromed, Mogliano Veneto, Italy) placed 15 cm from the left eye, with bandpass filter 10–80 Hz. Noise controls were produced in the same way but with the flash occluded by an aluminum foil. The mouse primary auditory cortex is, in fact, very close to the primary visual one (Wang and Burkhalter, 2007) and the recording of auditory components is possible, due to the "click" associated with the flash stimulation, as observed by Lehman and Harrison (Lehman and Harrison, 2002). The average of two trains was analyzed before (baseline), immediately (t_0), at 5 min (t_5) and at 10 min (t_{10}) after tDCS. After the end of each experiment, the mouse was put back in its home cage, where it was able to move and fully orient within the following 1–2 min.

The amplitude with respect to baseline and latency from stimulus onset of the three main components of flash-VEPs (N1-P1-N2) (Onofrj et al., 1985; Sato and Adachi-Usami, 2003) were measured at each time point after tDCS. Values were then transformed as percentage of baseline (baseline=pre-tDCS).

tDCS stimulation

tDCS was applied immediately after baseline VEPs recording. For each experiment, the plastic tube for tDCS was filled with saline solution (0.9% NaCl) just prior to the visual stimulation. The counter electrode was a saline soaked sponge (5.2 cm^2) applied over the ventral thorax by using a custom corset, according to published methods (Cambiaghi et al., 2010; Liebetanz et al., 2006b).

Cathodal and anodal tDCS were applied at a current intensity of 250 μA for 10 min by a constant current stimulator (Eldith DS-Stimulator, NeuroConn, Germany). This intensity corresponded to a current density of 5.55 mA/cm^2 (0.25 mA/0.045 cm^2), similar to what used previously in rats (0.2 mA/0.035 cm^2=5.71 mA/cm^2) (Liebetanz et al., 2006b) or mice (Cambiaghi et al., 2010). In order to avoid a stimulation break effect, the current intensity was ramped for 10 s instead of switching it on and off directly (Liebetanz et al., 2006a). In the no stimulation condition, no current was applied but the animal underwent the same manipulations as in the two stimulation conditions and was left anesthetized for 10 min. For each animal, the three experiments were performed at least 3 days apart in random order.

Statistical analysis

Statistical analysis was performed with SPSS statistical software (version 13.0). VEP amplitudes and latencies were entered into a repeated measures ANOVA, using within factors "tDCS condition" (cathodal, anodal and sham) and "time" (t_0, t_5 and t_{10}). When the

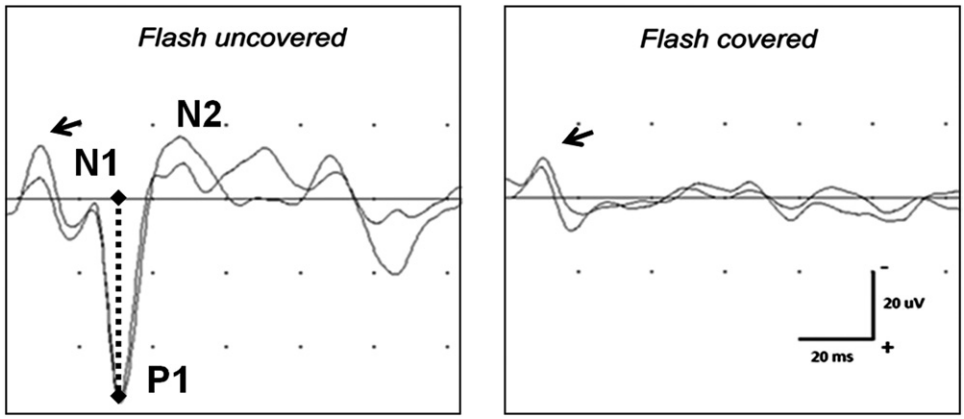

Fig. 1. Example of flash VEPs from a single animal. Left: Dashed line indicates P1 amplitude measure. An early negative peak at about 10 ms (arrow) can be recorded to flash stimulation even when light is completely occluded (right). Each trace represents an average of 20 flash stimuli at 1 Hz, delivered at the onset time of each trace. Note the overlapping of P1 with respect to N1 and N2.