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Full-length Article

Safety parameter considerations of anodal transcranial Direct Current Stimulation in rats

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ABSTRACT

A commonly referenced transcranial Direct Current Stimulation (tDCS) safety threshold derives from tDCS lesion studies in the rat and relies on electrode current density (and related electrode charge density) to support clinical guidelines. Concerns about the role of polarity (e.g. anodal tDCS), sub-lesion threshold injury (e.g. neuroinflammatory processes), and role of electrode montage across rodent and human studies support further investigation into animal models of tDCS safety. Thirty-two anesthetized rats received anodal tDCS between 0 and 5 mA for 60 min through one of three epicranial electrode montages. Tissue damage was evaluated using hemotoxylin and eosin (H&E) staining, Iba-1 immunohistochemistry, and computational brain current density modeling. Brain lesion occurred after anodal tDCS at and above 0.5 mA using a 25.0 mm^2 electrode (electrode current density: 20.0 A/m^2). Lesion initially occurred using smaller 10.6 mm^2 or 5.3 mm^2 electrodes at 0.25 mA (23.5 A/m^2) and 0.5 mA (94.2 A/m^2), respectively. Histological damage was correlated with computational brain current density predictions. Changes in microglial phenotype occurred in higher stimulation groups. Lesions were observed using anodal tDCS at an electrode current density of 20.0 A/m^2, which is below the previously reported safety threshold of 142.9 A/m^2 using cathodal tDCS. The lesion area is not simply predicted by electrode current density (and so not by charge density as duration was fixed); rather computational modeling suggests average brain current density as a better predictor for anodal tDCS. Nonetheless, under the assumption that rodent epicranial stimulation is a hypersensitive model, an electrode current density of 20.0 A/m^2 represents a conservative threshold for clinical tDCS, which typically uses an electrode current density of 2 A/m^2 when electrodes are placed on the skin (resulting in a lower brain current density).

1. Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive method of brain stimulation used to modulate cortical excitability (Nitsche and Paulus, 2000). Conventional tDCS applies a small amount (1–2 mA) of direct current to the scalp using large (25–35 cm^2) electrodes (Brunoni et al., 2012; Woods et al., 2016). Computational and animal models have shown that only a fraction of the applied current reaches the cortex, leading to neuronal polarization and excitability changes in the cortex (Datta et al., 2009a; Marquez-Ruiz et al., 2012; Rahman et al., 2013; Rohan et al., 2015) and hippocampus (Rohan et al., 2015; Kronberg et al., 2017). Given its ability to affect the function of cortical neurons, tDCS has been investigated for a variety of medical and augmentative applications, such as depression (Brunoni et al., 2011; Loo et al., 2012), motor rehabilitation (Edwards et al., 2009), speech rehabilitation (Baker et al., 2010; Fridriksson et al., 2011; Galletta et al., 2015), pain control (Fregni et al., 2006; Dasilva et al., 2012; Castillo-Saavedra et al., 2016), and working memory (Brunoni and Vanderhasselt, 2014). tDCS is considered a safe and well tolerated technique when proper protocols are followed (Bikson et al., 2009; Kasschau et al., 2015; Nitsche and Paulus, 2015; Gbadeyan et al., 2016; Palm et al., 2016; Woods et al., 2016). Nonetheless, as the application of tDCS becomes increasingly commonplace and indications for its use more widespread, additional work on tDCS safety is warranted for supporting basic dosing guidelines (Peterchev et al., 2012; Bikson et al., 2016; Jackson et al., 2016).

Initial safety limitations for tDCS were based upon literature from other electrical brain stimulation techniques. Nitsche et al. discussed safety of tDCS (Nitsche et al., 2003) by referencing safety standards in which pulsating current was applied directly to brain

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tissue (Yuen et al., 1981; Agnew and McCreery, 1987; McCreery et al., 1990; Merrill et al., 2005). In 2009, Liebetanz et al. conducted a canonical study in rodents to better define the minimum dosage at which cortical tissue damage occurs during cathodal tDCS: 0.5 mA for a stimulation duration of 10 min (Liebetanz et al., 2009). The findings reported by Liebetanz are widely cited in tDCS literature and have served as a guide for clinical safety limits (Holland and Crinion, 2012; Brunoni et al., 2013; Truong et al., 2013). Though not extensively tested, Liebetanz suggested the metric of average electrode current density (A/m^2), calculated as the applied current divided by the electrolyte-body contact area – corresponding in their electrode montage to 143 A/m^2 – along with electrode charge density (C/m^2), which multiplies current density by time, as two generalized safety parameters for dosing guidelines (Bikson et al., 2009).

Building upon this framework for rodent safety studies, other tDCS paradigms were evaluated for lesion induction in the mouse (Rueger et al., 2012; Pikhovych et al., 2016a, b), where the lowest current intensity that produced detectable cortical damage was 0.5 mA (220 A/m^2 electrode current density) for both anodal and cathodal tDCS groups (Pikhovych et al., 2016b). However, damage was not consistently observed for 0.5 mA cathodal and 1.0 mA anodal stimulation groups (Pikhovych et al., 2016a, b), which indirectly suggests a role for polarity. More recently, lesions have been reported at an anodal current intensity of 0.6 mA (47.8 A/m^2 electrode current density) (Gellner et al., 2016), suggesting the lesion threshold in rats may be lower than previously reported. Rodent studies evaluating tDCS safety through microglial analysis have shown microglial activation can occur after anodal or cathodal stimulation at 0.5 mA for 15 min (Rueger et al., 2012) (c.f. (Liebetanz et al., 2009). Microglial changes in morphology associated with neurodegeneration after anodal tDCS have been reported at current intensities as low as 0.4 mA (31.8 A/m^2 electrode current density) (Gellner et al., 2016).

Considering the available lesion safety data and the variations in polarity, animal size, and electrode area used across studies, the robustness of average electrode current density (or electrode charge density) as a generalized predictor of injury remains unclear, undermining the use of animal data to support clinical safety thresholds. Indeed, computational current models notably show brain current density is not simply a function of the electrode current density, but also anatomy and details of electrode size and position (Datta et al., 2009a, 2012; Miranda et al., 2009; Saturnino et al., 2015). Therefore, animal models of tDCS safety can benefit from being updated in regards to: 1) variation of stimulation polarity/dose (anodal vs. cathodal); 2) alternative indications of injury (Wachter et al., 2011; Rueger et al., 2012; Wong et al., 2014; Gellner et al., 2016); and 3) the suitability of electrode parameters to set a safety threshold given computational current models show brain current density is not a simple, linear function of the applied current or electrode current density (Datta et al., 2009a, 2012; Miranda et al., 2009; Saturnino et al., 2015).

We initially developed an *in vivo* rodent model of anodal tDCS using a 25.0 mm^2 electrode and evaluated the effect of various stimulation dosages on tissue damage. We evaluated current intensity (0.15–2.5 mA) which span the range of previously established safety limits (Liebetanz et al., 2009; Rueger et al., 2012; Gellner et al., 2016). Ionized calcium-binding adapter molecule 1 (Iba1) activation was also examined as a more sensitive predictor of brain lesion. Brain tissue histology indicated lesions at a lower electrode current density (20.0 A/m^2) than previously reported. Therefore, we systematized our next experiments to critically evaluate this 20.0 A/m^2 limit while controlling the number and area of electrodes (10.6 mm^2 and 5.3 mm^2). Dissociating current intensity from electrode current density (e.g. same current intensity but different electrode current density), combined with high-resolution FEM computational models of current flow in rat, supported testing the hypothesis that brain current density, rather than simply electrode current intensity or electrode current density, predicts the propensity for lesions. This has important implications for how animal (rodent) models of tDCS, especially aimed at safety, are rationalized and applied to develop clinical guidelines.

2. Materials and methods

2.1. 25.0 mm^2 electrode placement surgery

Animals were anesthetized with isoflurane (Piramal Critical Care, Shop Med Vet, Mettawa, IL) using 5% induction and 2–3% maintenance. Animals were treated with standard pre- and post-surgical care. The animal was placed into a stereotaxic apparatus and a caudo-rostral incision was made on top of the head, followed by a lateral incision was made at the shoulders. The periosteum was removed, the skull wiped clean, and a head electrode of 0.25 cm^2 (Valutrode, Axelgaard Manufacturing Co., Fallbrook, CA, 1.25-inch diameter electrode cut to 5 mm × 5 mm) with SignaGel (Parker Laboratories, Fairfield, NJ) was applied to the skull with the center of the electrode resting on the midline 2.5 mm caudal to Bregma (rostro-caudal: 0.0 mm to −5.0 mm). The insulated electrode wire was tunneled subcutaneously and exited the lateral incision made at shoulders. The electrode was held in place by a plastic head clamp which caught on the ridges of the skull (AFRL designed and produced) and two types of adhesives: C&B Metabond Adhesive Luting Cement (Parkell Inc., Edgewood, NY) was applied to the electrode and skull to create an initial bond, followed by acrylic cement (Stoelting, Co. Fisher Scientific, Pittsburg, PA) to bond the electrode to the clamp. Incisions were sutured closed around cement and wire. Animals recovered from surgery for at least 1 week prior to inclusion in experiments. Prior to stimulation, animals were randomly placed into six anodal tDCS treatment groups: 0.15 mA (n = 4), 0.3 mA (n = 4), 0.5 mA (n = 2), 1.0 mA (n = 4), 2.5 mA (n = 3), and sham stimulation (n = 3).

2.2. 10.6 mm^2 and 5.3 mm^2 electrode placement surgery

Animals were prepared as described above and an electrode jacket with a surface area of 5.3 mm^2 (DIXI Medical, Besançon, France) was placed at −2.5 mm Bregma and 2.5 mm left of sagittal suture. The electrode jacket was secured with FUJI I glass ionomer (Dental Wholesale Direct, FL, USA), and a layer of dental cement was placed on top to further secure the electrode. Prior to stimulation, animals were assigned into 2 groups based on electrode placement: 1) a single 5.3 mm^2 electrode placed −2.5 mm Bregma and −2.5 mm left of the sagittal line (n = 6), and 2) two 5.3 mm^2 electrodes placed at −2.5 mm Bregma and 2.5 mm from the midline bilaterally, for a total electrode surface area of 10.6 mm^2 (n = 5). Prior to stimulation, the animals were assigned into stimulation groups based on lesion results from the previous 25.0 mm^2 electrode experiment. The single electrode stimulation group was subdivided into groups based on current intensity: 2.0 mA (n = 1), 1.0 mA (n = 1), 0.75 mA (n = 1), 0.5 mA (n = 2), 0.05 mA (n = 1), and Sham (n = 1). The dual electrode group was also divided into subgroups based on current intensity: 2.0 mA (n = 1), 1.0 mA (n = 1), 0.5 mA (n = 1), 0.25 mA (n = 1), and sham (n = 1).

2.1. tDCS application

2.3.1. 25.0 mm^2 electrode stimulation

Animals were anesthetized with isoflurane (Piramal Critical Care, Shop Med Vet, Mettawa, IL), using a 5% induction and 2–3% maintenance schedule. The reference electrode (8.04 cm^2,