Astrocytic calcium activation in a mouse model of tDCS—Extended discussion

Hiromu Monai\textsuperscript{a} and Hajime Hirase\textsuperscript{a,b}

\textsuperscript{a}RIKEN Brain Science Institute, Wako, Saitama, Japan; \textsuperscript{b}Saitama University Brain Science Institute, Saitama, Japan

ABSTRACT
Transcranial direct current stimulation (tDCS) has been reported to be effective for alleviation of neuropsychiatric and neurological conditions as well as enhancement of memory and cognition. Despite the positive effects of tDCS in humans, its mechanism of action remains poorly understood. Recently, we reported that astrocytes, a major glial cell type in the brain, show an increase in intracellular Ca\textsuperscript{2+} levels during tDCS in the cerebral cortex of the awake mouse. This tDCS-induced elevation in astrocytic Ca\textsuperscript{2+} has subsequently been demonstrated to be important for cortical plasticity. In this commentary article, we discuss possible interpretations and implications of our findings from the viewpoint of neuron-glia interactions.

KEYWORDS
astrocytes; G protein-coupled receptors; neuromodulation; noradrenaline; norepinephrine; transcranial direct current stimulation (tDCS)

Introduction
Transcranial direct current stimulation (tDCS) is the application of constant, weak-intensity electrical current to the brain through the skull for a prolonged duration. For humans, typical parameters are a current intensity ranging from 1–2 mA and a duration ranging from 10–30 minutes.\textsuperscript{1} tDCS has been shown to have positive effects on brain function, including memory enhancements, accelerated motor function rehabilitation, alleviation of depressive symptoms, and slower progression of neurodegeneration in Alzheimer disease patients (for reviews, see refs. 2, 3). tDCS can be performed with simple electronics powered by commercially available batteries. Remarkably, while tDCS is practiced in neuropsychiatry and most probably by many curious minds, the mechanism by which these positive effects emerge is not well understood.

Simulation studies and animal experiments have been performed to unveil the underlying mechanisms of tDCS. For example, multiple simulation studies suggest subthreshold depolarization of neurons around the anodal site and hyperpolarization around the cathodal site,\textsuperscript{4–6} confirming the early intracellular recording study that showed neuronal depolarization in rodents.\textsuperscript{7} Learning enhancement has also been confirmed using tDCS in rodents,\textsuperscript{8} which allows for molecular manipulations and in vitro acute brain slice experiments and thus provides a foundation to investigate tDCS mechanisms. As a result, there is a general consensus that tDCS-induced synaptic plasticity is expressed in a manner dependent on the N-methyl-D-aspartate (NMDA) receptor.\textsuperscript{9,10} Furthermore, an influential study demonstrated the involvement of the brain-derived neurotrophic factor (BDNF) and activation of the tropomyosin receptor kinase B (trkB), a receptor for BDNF.\textsuperscript{11} The involvement of BDNF has been further supported by a recent study reporting that tDCS induces epigenetic changes in the BDNF gene locus that lasts for over several days.\textsuperscript{12}

While in vitro acute slice experiments are powerful in assessing synaptic physiology, they are often compromised by truncation of long-range connections, incubation temperature, and inflammatory reactions caused by the preparation. In our recent study, we performed in vivo transcranial calcium (Ca\textsuperscript{2+}) imaging in mice during tDCS and found that astrocytes, a major glia cell type, play a role in tDCS-induced cerebral plasticity. Encouragingly, a mouse behavioral experiment indicated an initial sign of alleviation of depression-like behavior after tDCS.\textsuperscript{13} Here, we extend our
discussion of this original publication to consider how astrocytic activation fits the existing models of tDCS.

**Cortex-wide Ca\(^{2+}\) imaging during tDCS**

We made transgenic mice that have high G-CaMP7 (an improved version of the well-known Ca\(^{2+}\) indicator protein G-CaMP\(^{14}\)) expression in astrocytes and a large population of excitatory neurons in the cortex and hippocampus (G7NG817 mouse\(^{13}\)). Notably, the G-CaMP7 expression of the G7NG817 mouse is sufficient to allow cortex-wide transcranial functional imaging with a standard fluorescence stereo microscope. For example, visualization of single barrel columns and cortex-wide slow oscillations (0.5 to 2 Hz, also known as UP/DOWN states) is feasible in urethane-anesthetized mice. In these cases, the G-CaMP7 signals are predominantly of neuronal origin as they are usually shorter than one second, most likely reflecting action potential-driven Ca\(^{2+}\) influx since GPCR-triggered Ca\(^{2+}\) oscillations are of longer duration. Conversely, cytosolic Ca\(^{2+}\) signals of astrocytes are generally larger in magnitude and longer in duration by at least several-folds because they are triggered by G protein-coupled receptors (GPCRs) that signal to inositol trisphosphate (IP\(_3\)) receptors, leading to the release of Ca\(^{2+}\) from internal stores. Volume-transmitted neuromodulators such as noradrenaline (norepinephrine) and acetylcholine are known to activate astrocytic\(^{15-18}\) and neuronal GPCRs. On the intracellular side, the IP\(_3\) receptor type 2 (IP\(_3\)R2) is the main IP\(_3\) receptor in astrocytes, and the ablation of IP\(_3\)R2 results in the diminishment of large and long-lasting cytosolic Ca\(^{2+}\) elevations in astrocytes.\(^{15,19-22}\) Using G7NG817 mice, we described large and long-lasting cytosolic Ca\(^{2+}\) elevations in astrocytes within several seconds after the onset of tDCS (0.1 mA, for 10 minutes). Importantly, cortex-wide tDCS-induced Ca\(^{2+}\) elevations were not observed in mice deficient of IP\(_3\)R2 (IP\(_3\)R2\(^{-/-}\);G7NG817\(^{+/+}\)),\(^{13}\) suggesting that tDCS-induced Ca\(^{2+}\) elevations are of astrocytic origin.

**tDCS-assisted in vivo sensory plasticity**

One of the core questions in neuron-glia interactions is functional roles of astrocytic Ca\(^{2+}\) signaling in neuronal information processing (for reviews, see refs. 23-26). One of the hypotheses is that astrocytic Ca\(^{2+}\) signaling is involved in synaptic plasticity, owing to the morphological characteristics of astrocytes that surround synapses.

We demonstrated that tDCS enhances sensory-evoked potentials and neuronal Ca\(^{2+}\) responses in the adult cerebral cortex. Interestingly, the Ca\(^{2+}\) response of neuropil was enhanced in superficial layers (layer 2/3) of the cortex, but not in layer 4 where sensory thalamic input arrives. This observation could be related to the phenomenon that layer 4 plasticity disappears as the animal matures, especially after the critical period.\(^{27,28}\) Similar to a human study,\(^{9}\) the tDCS-assisted plasticity in our study is NMDAR-dependent as the plasticity was blocked by AP-5. Remarkably, IP\(_3\)R2 knockout mice did not develop sensory response enhancement by tDCS, suggesting a crucial role of astrocytic Ca\(^{2+}\) elevation.

What is the pathway that connects astrocytic Ca\(^{2+}\) elevation to brain plasticity? There are at least several candidates, which may operate in parallel. First, Ca\(^{2+}\)-dependent gliotransmission—secretion of bioactive molecules from astrocytes—can occur to influence synapses. A wide variety of gliotransmitters have been suggested in literature, including adenosine triphosphate (ATP), glutamate, D-serine, and tumor necrosis factor \(\alpha\) (TNF\(\alpha\)).\(^{29}\) Considering the NMDAR dependency, the NMDAR co-agonist D-serine is a sound candidate, as previously demonstrated in in vitro hippocampal plasticity\(^{30,31}\) and in vivo sensory plasticity in the cortex.\(^{15}\) However, other gliotransmitters such as ATP and TNF\(\alpha\) are also implied in brain plasticity and hence remain as candidate gliotransmitters. Second, astrocytic Ca\(^{2+}\) elevation has been shown to decrease the extracellular ionic balance of potassium (K\(^+\)), leading to an increase in synaptic efficacy.\(^{32}\) Third, astrocytic Ca\(^{2+}\) may be linked to morphological plasticity and integrity of tri-partite synapses.\(^{33}\) Fourth, a change in the extracellular volume and interstitial fluid exchange through astrocytes (i.e. the glumphatic system)\(^{34,35}\) may influence the metaplasticity of synapses. Finally, tDCS on humans has been reported to alter cerebral blood flow in a polarity dependent manner,\(^{36}\) while astrocytic involvement in functional hyperemia is controversial to date.\(^{37,38}\)

Literature suggests that cortical potentiation is preferentially expressed at the anodal site over the cathodal site. A simplenminded explanation is depicted in Fig. 1. In short, astrocytic Ca\(^{2+}\) elevation occurs in wide areas of the cortex, including the contralateral side. This astrocytic Ca\(^{2+}\) elevation could act as a...
permissive factor that allows plasticity at active synapses, such that synapses close to the anode are depolarized and those close to the cathodes are hyperpolarized due to the electric field. Peri-anodal synapses are more susceptible to LTP-type synaptic plasticity, because NMDARs are in part gated by post-synaptic membrane depolarization (Fig. 1).

**Noradrenergic activation and alleviation of depressive behavior**

In our experiments, tDCS-induced Ca$^{2+}$ elevation is blocked by noradrenergic neuron ablation or α-1 adrenergic receptor (A1AR) blockade, suggesting that tDCS results in a noradrenaline release in the cortex. Supposing that noradrenaline release is a direct effect of tDCS, the question arises as to why noradrenaline, but not other neurotransmitters, appears to be responsible to trigger astrocytic Ca$^{2+}$ elevation. We speculate that other transmitters like glutamate, gamma aminobutyric acid (GABA), and acetylcholine (ACh) are secreted as well. However, glutamate and GABA are subject to rapid uptake by respective transporters. ACh is a volume-transmitted neuromodulator; however, its enzymatic breakdown is quite fast due to the abundance of acetylcholinesterase. The efficient elimination of these neurotransmitters from the extracellular space could be the reason why their astrocytic receptors are not activated. More detailed information on cellular morphology and distributions of enzymes, transporters, and receptors will allow simulation studies to predict the dwell times of various neurotransmitters in the extracellular space.

The A1AR is a Gq-type GPCR abundantly expressed astrocytes. For instance, a cell type specific transcriptomic database suggests predominant expression of A1ARs over muscarinic ACh receptors. Also, in vivo Ca$^{2+}$ imaging studies have shown that A1AR is the principal receptor for spontaneous and startle-stimulus-driven Ca$^{2+}$ elevations in astrocytes. A recent study showed that tDCS induces epigenetic changes, including the BDNF locus in the mouse
brain. Considering another recent report that noradrenergic \( \beta \) receptor signaling promotes NMDA-dependent long-term potentiation through epigenetic changes in neurons, tDCS-induced noradrenergic signaling conceivably contributes to neural plasticity and epigenetic modifications, in consort with the astrocyte-mediated signaling.

Noradrenaline and serotonin enhancers (e.g. by inhibiting reuptake) are commonly used to treat depression. In addition to cortical plasticity, we have shown that the same tDCS application (0.1 mA, for 10 minutes) can alleviate a mouse model of depression. This effect lasted for at least several days despite single dose application. Similar to tDCS-assisted cortical plasticity experiments, the antidepressant tDCS effect was dependent on A1ARs and IP\( _3 \)R2s. These results propose the possibility that certain aspects of depression are treatable through noradrenaline signaling. Further, in line with work that showed positive effects of glial ATP transmission, our data support the view that astrocytes are considered to be a potential therapeutic target for depression. Given that astrocytes cover the vasculature by their endfoot processes, astrocytes could have easier access to drugs administered through the bloodstream. Future drug discovery studies may find astrocytic GPCR activation effective in treating mood disorders.

**Adult neurogenesis, cognition and depression**

Enhancements in adult neurogenesis has been implied to be a potential mechanism for long-term tDCS effects such as cognitive enhancement and antidepressant effects (for reviews, see refs. 42-44). Recently, Braun et al. showed that multi-session tDCS facilitated rehabilitation after focal cerebral ischemia and promoted adult neurogenesis in a polarity-independent manner. The duration of positive effects for depressive behavior in our experiment may be related to adult neurogenesis. Among neurotrophic factors for neurogenesis, BDNF has been shown to be related to tDCS, as mentioned earlier. BDNF is also well-recognized to have antidepressant effects. Interestingly, in dissociated hippocampal neuron culture, application of noradrenaline was reported to increase BDNF. Although the origin of tDCS-induced BDNF is yet to be identified, one possibility is the induction of BDNF through activation of adrenergic receptors. Other possible sources of BDNF include microglia and circulation. In addition to neurogenesis, NMDAR-dependent synapse formation of adult-born neurons in the dentate gyrus has been shown to be related to astrocytic Ca\( ^{2+} \) signaling and vesicular release of D-serine. Neurogenesis and the subsequent spine-genesis can be a viable candidate mechanism that explains the long-term effects of tDCS and should be studied in the future by molecular genetics that can manipulate neurogenesis (e.g., ref. 51).

**Scaling to human tDCS**

While we demonstrate that tDCS application resulted in a cortex-wide elevation of Ca\( ^{2+} \) in mice, Ca\( ^{2+} \) elevation was the largest at the anodal site and attenuated with distance. This hints at the possibility that the Ca\( ^{2+} \) elevation is a function of electric field, which has been simulated in recent studies. One potential mechanism for the noradrenaline release is by action potentials reaching at noradrenergic axon boutons. If the applied electric field is strong enough to elicit action potentials in noradrenergic nerve fibers, (which was not monitored in ref. 13), the Ca\( ^{2+} \) response should be more evenly distributed because of the diffuse pattern of noradrenergic innervation. Moreover, other kinds of axons, including the abundant glutamatergic axons, should also produce action potentials; in which case, it would have a profound effect on a mouse’s behavioral state. As a matter of fact, no obvious changes were observed in the frequency of Ca\( ^{2+} \) events in G-CaMP7 positive, presumably glutamatergic, cortical neurons imaged in G7NG817 mice. No obvious behavioral changes were observed during or after tDCS in mice, either. Conversely, we did not find compelling evidence that contradict terminal secretion of neuromodulators by tDCS, although the exact mechanism is yet to be identified. Assuming electric field-dependent neuromodulator secretion, whether tDCS induces cortex-wide Ca\( ^{2+} \) elevation occurs in the human brain would depend on the spread of the electric field. Given Ca\( ^{2+} \) imaging on human brain is not yet possible, we will need to rely on modeling studies to assess the spatial spread of Ca\( ^{2+} \) elevation.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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