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Neurovascular-modulation: A review of primary vascular responses to transcranial electrical stimulation as a mechanism of action

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ABSTRACT

Background: The ubiquitous vascular response to transcranial electrical stimulation (tES) has been attributed to the secondary effect of neuronal activity forming the classic neurovascular coupling. However, the current density delivered transcranially concentrates in: A) the cerebrospinal fluid of subarachnoid space where cerebral vasculature resides after reaching the dural and pial surfaces and B) across the blood-brain-barrier after reaching the brain parenchyma. Therefore, it is anticipated that tES has a primary vascular influence.

Objectives: Focused review of studies that demonstrated the direct vascular response to electrical stimulation and studies demonstrating evidence for tES-induced vascular effect in coupled neurovascular systems.

Results: tES induces both primary and secondary vascular phenomena originating from four cellular elements; the first two mediating a primary vascular phenomenon mainly in the form of an immediate vasodilatory response and the latter two leading to secondary vascular effects and as parts of classic neurovascular coupling: 1) The perivascular nerves of more superficially located dural and pial arteries and medium-sized arterioles with multilayered smooth muscle cells; and 2) The endothelial lining of all vessels including microvasculature of blood-brain barrier; 3) Astrocytes; and 4) Neurons of neurovascular units.

Conclusion: A primary vascular effect of tES is highly suggested based on various preclinical and clinical studies. We explain how the nature of vascular response can depend on vessel anatomy (size) and physiology and be controlled by stimulation waveform. Further studies are warranted to investigate the mechanisms underlying the vascular response and its contribution to neural activity in both healthy brain and pathological conditions – recognizing many brain diseases are associated with alteration of cerebral hemodynamics and decoupling of neurovascular units.

Introduction

The neuronal mechanisms underlying the acute effect and after effect of Transcranial Electrical Stimulation (tES) have been extensively explored [1–4]. The acute effect of tES has generally been attributed to up- or down-regulation of spontaneous firing rate, oscillations, neurotransmitter or neuromodulator activity, synaptic efficacy or connectivity, or plasticity [5].

In parallel with the exploration of tES modulatory effect on neuronal elements, understanding of the intricate relationship between neurons and adjacent non-neuronal structures particularly glial cells and brain vasculature forming the neurovascular units (NVU) and their close functional interdependence known as "Neurovascular Coupling" (NVC) has advanced considerably [6]. Moreover, reliance on NVC is the foundation for functional neuroimaging techniques such as fMRI that captures the vascular response associated with neuronal activation [7]. Following neuronal activation, a cascade of biochemical and bioelectrical events result in a secondary vasodilation to increase the cerebral blood supply, meeting the metabolic demand and clearing byproducts of neuronal activity. Thus, traditionally the vasodilatory response is deemed to be secondary to neuronal activity [8].

Various studies have demonstrated fMRI Blood-Oxygen Level Dependent (BOLD) response changes during and after tES and, as

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typical in imaging studies, attributed the vascular changes to responses to neuronal stimulation [9–11]. A dose-dependent cerebral blood flow enhancement has also been observed with tES, which is not fully explained by a secondary vascular response [12–17]. Inter-individual variability in neuronal and network-level responses reported across tES studies remains largely unexplained if the primary electro-neural effect is considered the only relevant effect of tES [18].

We propose that these tES imaging studies could in fact demonstrate a direct vascular response to tES, and that tES may even reverse the neuronal-vascular recruitment order. Demonstration of a direct vasodilatory effect of electrical stimulation on vasculatures such as the middle cerebral artery, dural arteries, and skin blood vessels span decades [19–21]. Furthermore, according to the vasculo-neuronal coupling model, the autoregulatory vascular response to changes of intramural cerebral blood flow (CBF) pressure may proceed and even influence the neuronal response suggesting an intricate interplay between neuronal and vascular components of NVU [22,23]. Therefore, it is not only conceivable but also highly likely that in addition to neurons and neuronal networks, tES affects the nearby non-neuronal structures of the NVU, particularly vasculature, which then leads to secondary changes in neuronal activity.

Nonetheless, the order and extent by which electrical stimulation affects the components of the neurovascular unit in both healthy brains with a functional NVC and also in diseased brains with impairment of NVC, remain to be elucidated. Which cellular elements are activated by neuromodulation is critical to understanding the mechanism of action, even if final outcomes depends on inter action across many cell types. This review is concerned with the direct electrical stimulation of vascular function. We considered both experiments that demonstrated vascular responses in a coupled system (with complex bidirectional cascades between neurons, glial cells, and vasculature) and experiments where structural/functional changes in vasculature in response to electrical stimulation were directly assessed or even isolated from neurons and glia. The former is confounded by any significant direct effect of stimulation on neurons, inevitably triggering a secondary coupled vascular response- which, while critical to understand the mechanisms of tES, is not our primary interest here. The latter provides unambiguous evidence for direct vascular stimulation considering that a large portion of electrical current in tES first reaches the outer surface of meninges consisting of dural and pial layers and concentrates in the cerebrospinal fluid of the subarachnoid space where pial and penetrating vasculature reside, hence, providing an opportunity for a primary vasculo-modulatory effect.

The current flow pattern, and resulting regional electric fields, have been characterized for tES at a macroscopic (tissue) scale [24,25]. At a meso/micro-scopic scale, the current density delivered transcranially concentrates in: A) the cerebrospinal fluid of subarachnoid space where cerebral vasculature resides after reaching the dural and pial surfaces [26] and B) across the blood-brain-barrier (BBB) after reaching the brain parenchyma [27]. At the capillary scale, the electric field across the BBB (endothelial cells) was predicted to be 400x of the brain parenchyma, meaning BBB fields >100 V/m during conventional tES [27]. The concentration of electric field around/across various vascular structures gives further impetus to study the direct effects of vascular stimulation.

Further knowledge of the non-neuronal effect of tES is of paramount importance in understanding of the mechanisms underlying the acute effect and after-effects of tES in various brain conditions. In addition, the knowledge of the tES direct vascular effect opens an avenue for new therapeutic indications for use of tES in brain diseases with abnormal neurovascular coupling such as ischemic cerebrovascular diseases or neurodegenerative disorders with impairments of brain clearance mechanisms, such as in Alzheimer's Disease.

Therefore, in this review, we aim to study the existing knowledge and body of evidence demonstrating the direct response of vascular elements to electrical stimulation and response of coupled neurovascular units to tES. But first, we will review the anatomical structures of NVU with functional neurovascular coupling.

Anatomy and function of cerebrovascular tree and neurovascular unit components

There are four main components of distal cerebrovascular tree branching off the internal carotid artery and large intracranial Circle of Willis arteries: pial arteries, penetrating arterioles, intraparenchymal arterioles, and the capillaries. Both the pial arteries and medium-sized penetrating arterioles contain peri-vascular nerves and multilayered ($\geq$3) smooth muscle cells (SMC) with strong contractile capabilities. In the transition from medium size penetrating to small intraparenchymal arterioles, the arteriolar vasculature loses the peri-vascular nerves while keeping 1–2 layers of SMC with modest contractile activity. Finally, the capillaries at the most distal segment of the cerebrovascular tree do not contain peri-vascular nerves and SMC are also replaced with pericytes (Fig. 1). Given the difficulty of isolating and studying the pericytes due to the lack of specific marker that is expressed uniquely by true pericytes, their contractile capability has remained controversial [28–31]. Small intraparenchymal arterioles and capillaries are the microvasculature involved in the formation of the blood-brain barrier (BBB). They are the only vascular components in direct contact with cerebral neurons of neurovascular units (NVU).

In addition to the microvasculature, the neurons and glial cells are the other cohabitants of NVU. These three main components of NVU are in constant interaction to maintain the cerebral tissue homeostasis. This close intricate relationship, also known as neurovascular coupling (NVC), ensures the maintenance of neuronal energy supply and clearance of its metabolic by-products during neuronal activity by increasing cerebral blood flow (CBF) [32]. Neural activity results in a cascade of metabolic and electrical changes in the vicinity of the NVU, notably the synaptic glutamate release and an increase in extracellular K^+. Synaptic glutamate activates both neurons and astrocytes via N-methyl-D-aspartate (NMDA) and metabotropic Glutamate receptors (mGlutR) respectively, resulting in increased intracellular Ca^{+2} and activation of Ca^{+2} dependent enzymes, cyclooxygenase 2 (COX2) and nitric oxide synthase (NOS). These neuronal and astrocytic enzymes activity will result in the production of potent vasodilators, prostaglandin E2 (PGE2), a byproduct of arachidonic acid metabolism, and nitric oxide (NO). Simultaneously, the increased extracellular K^+ caused by neuronal depolarization will be siphoned by astrocytic endfeet to vascular smooth muscle cells resulting in vasodilation. In addition, lower oxygen concentrations at NVU results in accelerated glycolysis and astrocytic lactate production, which also reflexively dilates blood vessels. Meanwhile, 20-Hydroxyeicosatetraenoic acid (20-HETE), another byproduct of arachidonic acid metabolism released from astrocytes, has been shown to constrict the vessels in NVU resting states with low demand and high oxygen concentration [6].

In addition to glutamate, the neuronal metabolic byproducts such as carbon dioxide, adenosine and lactate, vasoactive neurotransmitters released from interneurons (GABA, acetylcholine, vasoactive intestinal peptide), and afferents of central pathways originating from locus coeruleus, nucleus basalis, and raphe nucleus also play roles in regulating cerebrovascular tone and

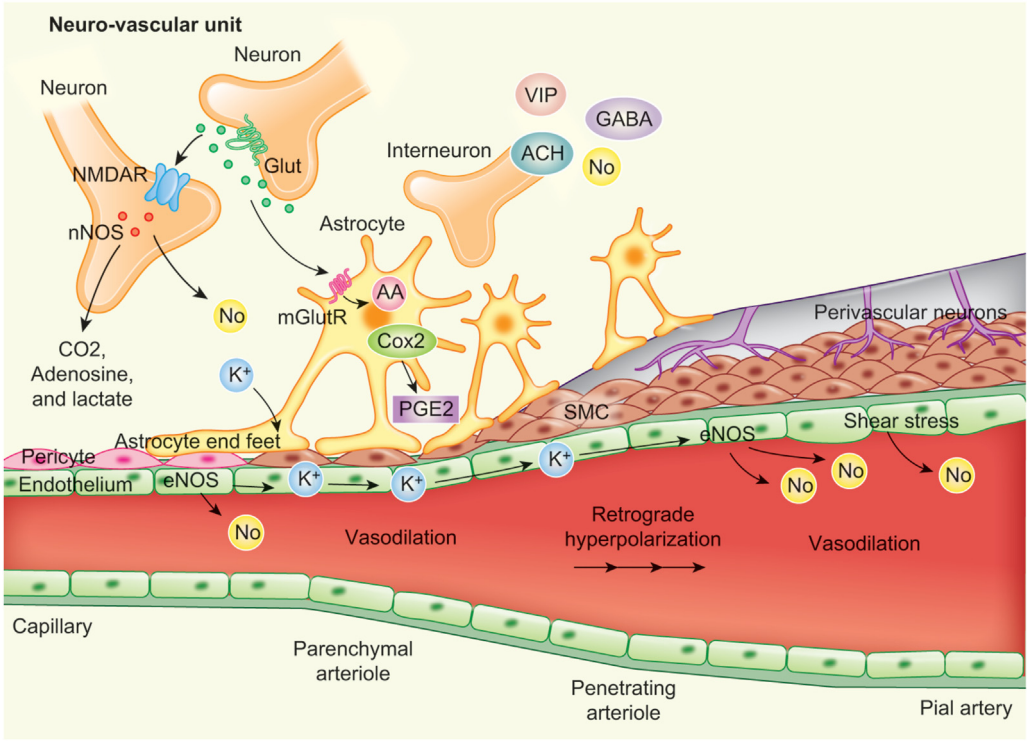

Fig. 1. Components of Neurovascular Unit (NVU) and the cellular and biochemical elements involved in neurovascular coupling (NVC) and functional hyperemia. Dilation of upstream pial arteries is necessary for the functional hyperemia of NVC. The glutamate released from pre-synaptic neuronal excitation, the vasodilatory metabolic byproducts of post-synaptic neuronal excitation (CO2, Adenosine, lactate, K^+), and the vasoactive neurotransmitters of interneurons (VIP, ACH, NO, GABA) result in endothelial hyperpolarization directly or via astrocytic endfeet (PGE2 and siphoned K^+). The vasodilatory signal retrogradely propagates from parenchymal capillaries and arterioles to larger penetrating arterioles and pial arteries resulting in a secondary vasodilation. The mechanical forces such as increase in shear stress also participate in the retrograde transmission of vasodilatory signals to pial arteries resulting in activation of eNOS and release of endothelial NO (flow-mediated vasodilation).
The biochemical elements involved in vasoconstriction and the afferents of central pathways are not shown in this figure for simplification purposes. AA: Arachidonic Acid; ACH: Acetylcholine; CO2: Carbon Dioxide; COX2: cyclooxygenase-2; eNOS: Endothelial Nitric Oxide Synthase; Glut: Glutamate; K: Potassium; NO: Nitric Oxide; NMDAR: N-methyl-D-aspartate Receptor; nNOS: Neuronal Nitric Oxide Synthase; PGE2: Prostaglandin E2; VIP: Vasoactive Intestinal Peptide.

hemodynamic changes related to NVC. Fig. 1 depicts the various pathways involved in NVC.

The afferents of central pathways abutting on astrocytic endfeet of NVU and the vascular wall insert their effect on vascular tone through neighboring neurons, astrocytes, or via a direct effect on vasculature [33]. For instance, the global vasoconstrictive effect of norepinephrine released from locus coeruleus has been shown to mediate the CBF redistribution required for local NVC-induced functional hyperemia [34]. On the other hand, norepinephrine contributes to the enhancement of local CBF by activating a broad network of glutaminergic pyramidal cells, GABAergic interneurons, and astrocytes, suggesting the complex role of central pathways in regulating the cerebrovascular hemodynamics [35].

The locations in the cerebrovascular tree that participate in NVC activity modulating CBF have been a matter of debate. It has been shown that capillary pericytes and endothelial cells will transmit the neuronal induced vasodilatory signals to larger penetrating arterioles and pial arteries with SMC in their vessel walls via a retrograde propagation of signals [36]. (Fig. 1) This vasomotor activity will be conveyed to upstream pial vessels via calcium-activated K^+ channels and NO. To avoid a flow steal phenomenon from interconnected vascular territories, the vasodilation of pial arteries is necessary for increasing the downstream CBF at the capillary level and fulfilling the cerebral tissue metabolic demand [37]. However, a vasodilatory response to neuronal activity is in excess of the metabolic needs of neurons resulting in a functional hyperemic state [38]. It has been shown that this exaggerated vascular response influences other members of NVU and has modulatory effects under the vasculo-neural coupling (VNC) model [22,23]. This neuromodulatory effect, suppressive or excitatory depending on the involved neural network and its state of activity, is indicative of the intricate interdependence of NVU components with multidirectional interactions between hemodynamics, neurons, and glia.

In addition to this vasomotor response from downstream deep neural activity, the pial arteries with a multilayered SMC also respond to the activity of their adjoining perivascular nerves. This rich perivascular innervation originates from peripheral parasympathetic and also, to a lesser extent, sympathetic ganglions [33]. The main vasodilatory neurotransmitters of the perivascular nerves originating from trigeminal and sphenopalatine ganglions are calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), and NO [39–41]. CGRP is well-known as a powerful vasodilator in various tissues, including the cerebral cortex, which plays a role in pathological conditions such as vasodilatory response to cortical spreading depression of migraine and cerebral ischemia [42]. CGRP induces vasodilation via ATP-sensitive K^+ channels (K^+_{ATP}) and NO [43].

CGRP is also the main vasodilatory neurotransmitter of dural arteries, the most superficially located vasculature feeding the dural surface of meninges. Dural arteries such as the middle meningeal artery branching off the extracranial carotid artery also contain peri-vascular nerves and multilayered SMC like pial arteries. These dural vessels play a role in intracranial cerebral perfusion only under pathological conditions such as acute or

chronic intracranial arterial steno-occlusive diseases via dural arteriolar anastomoses with cortical vessels [44].

In addition to neuronal and metabolic influences on pial arteries, pial vessel diameter also changes according to the local intraarterial mechanical and electrical forces [45]. The mechanical forces consist of fluid shear stress and intramural pressure, causing a myogenic response [46].On one hand, the intravascular fluid shear stress activates endothelial mechanosensitive calcium channels and releases endothelial nitric oxide that results in vasodilation. On the other hand, the intramural pressure causes a myogenic response by stretching the SMC membrane resulting in membrane depolarization and vasoconstriction, although the release of other substances such as 20-HETE has been shown to play a role [47]. Interestingly, the endogenous electrical field generated by the stream of blood flow influences the arterial diameter by increasing the secretion of NO from endothelial cells [48,49].

In physiological conditions, a balance between these local forces and neuronal and metabolic influences maintain a basal vascular tone that meets the cerebral tissue metabolic and oxygenation demands and, while on the contrary, neurovascular uncoupling occurs in pathological conditions, particularly cerebral ischemia, Alzheimer's disease, and traumatic brain injury resulting in compromise of NVU vascular supply [50–53].

Direct response of vascular compartments to electrical stimulation

The direct response of different vascular wall elements to electrical current that is dose and frequency-dependent has been shown by various studies (summarized in Supplemental Table 1). Direct vascular response to electrical current is shown in two vasculature sizes: 1) Large arteries and medium-sized arterioles including circle of Willis, dural, and pial arteries, cerebral penetrating arterioles, and skin arterioles with perivascular neural structures and multilayered SMC; and 2) Microvasculature of BBB consisting of small parenchymal arterioles and capillaries lacking perivascular neural structures and multilayered SMC.

Large and medium-sized vascular response to electrical stimulation-preclinical studies

Direct arterial and medium-sized arteriolar response to electrical current has been assessed in dural arteries, pial arteries and arterioles. This response has been shown to originate from the activation of perivascular nerves evoked by electrical current [43,54,55]. Kurasawa et al. electrically stimulated the exposed dura matter of anesthetized rats with 30 s of rectangular pulses, ranging in intensity and frequency of 10–20 V and 5–10 Hz, respectively [54]. They observed a robust vasodilatory response of the dural middle meningeal artery in a dose and frequency-dependent manner. A similar response was also suggested after isolating the dura from the underneath pial vessels.

In two studies assessing the effect of transcranial electrical stimulation (tES) on dural and pial arteries in healthy rat models, tES evoked strong vasodilation of the middle meningeal artery and pial arteries [43,55]. Peterson and colleagues delivered 10 s of electrical current with 5 Hz frequency, via a bipolar electrode placed over a thinned cranial window of rats with 200 μm distance from dural vessels. The intensity of the tES was gradually increased until a maximal vascular response was observed. They demonstrated an increase in the diameter of the middle meningeal artery and pial arteries by 119.1 ± 6.9% and 96 ± 14%, respectively. This response was abolished with CGRP- inhibitor indicative of CGRP-dependent vasodilation [55].

In a similar study by Gozalov et al. using the same stimulation protocol, tES evoked an increase in dural and pial arteries diameter by 81 ± 11% and 20 ± 7%, respectively. The response was abolished with a K^{+}_{ATP} channel inhibitor, indicating the role of the K^{+}_{ATP} channel attributed to the vasodilatory response to tES. The K^{+}_{ATP} channel inhibitor also abolished the vasodilatory response to CGRP without tES, eliciting the role these channels play in mediating the CGRP-induced vasodilation [43]. In both studies, the response was less in pial than dural arteries. In the studies mentioned above, the CGRP released from electrically stimulated perivascular nerves relaxing the arterial smooth muscle cells via activation of K^{+}_{ATP} channels was deemed to be the underlying mechanism for neurogenic vasodilation.

Changes in vascular diameter evoked by directly exposing vessels to an electrical current have been elucidated in the large circle of Willis arteries in preclinical studies [20,56,57].

Harder et al. exposed the middle cerebral artery of cats to electrical current once transmurally at the adventitia and then intraluminally at the endothelial surfaces [20]. Electrical current in the form of anodal direct current (DC) that passed transmurally through the adventitia caused significant vasodilation with a strong positive correlation with intramural pressure. At higher intramural pressures, the vasodilatory response was greater in magnitude. In contrast, the intraluminal stimulation of blood vessels through the endothelial surface resulted in vasoconstriction. The vasodilatory nature of transmural electrical stimulation was attributed to release of VIP from adventitial nerves enhanced by distention of the artery at higher intramural pressures. An increased release of neurotransmitters from perivascular nerves under greater intramural pressure has been shown in other studies [56]. Although no clear mechanism of action for the observed depolarizing nature of intraluminal stimulation was proposed, different responses from various layers of vessel wall SMC were suggested with the SMC layers closer to adventitial mediating vasodilation and the SMC closer to endothelium involved in vasoconstriction. Moreover, the presence of an intact endothelial surface was necessary only for the intraluminal vasoconstriction and not for the vasodilation implicating an endothelial independent neural mechanism. On the contrary, in a study stimulating the endothelial lining of an isolated hamster's feed artery, intraluminal passage of anodal DC with an anode directly across the feed artery and cathode positioned in the adventitia resulted in vasodilation [57]. An intact endothelial lining was found to be necessary for this endothelium- dependent vasodilation and was attributed to the relaxation of SMC via myoendothelial coupling independent of perivascular nerves. Nonetheless, given the proximity of the active electrode to the arterial wall used in these studies, the achieved current density in the vessel wall is much higher than that seen with tES.

Large-medium sized vascular response to electrical stimulation-human studies

The dermal arterioles, as part of cutaneous “microcirculation”, contain perivascular nerves and multilayered SMC enabling them to dilate or constrict in response to different stimuli such as electrical field or thermal stimulation. Therefore, the skin response to electrical stimulation is an aggregate response of these medium-sized arterioles and small-sized capillaries.

The vascular response of dermal arterioles to electrical current has been investigated in humans. In fact, multiple human studies have shown a substantial non-polarity dependent vasodilatory response of skin blood vessels to electrical stimulation in healthy subjects [21,58–60]. Moreover, the skin vasodilatory response to tES is observed in practice, and skin irritation and warmth are the most reported adverse effects of tES in humans [61,62].

In a study using iontophoresis, Durand et al. tested the vascular effect of anodal square-wave pulses on healthy volunteers forearm skin using laser Doppler flowmetry (LDF) [58]. An anodal current of 0.1 mA for 30–120 s resulted in significant vasodilation. The anodal stimulation was applied either in a single session or two consecutive sessions with a 5 min resting period in between. The segmented application resulted in an amplified vascular response compared to a single delivery of comparable total charge suggestive of increased sensitivity to the electrical current by the first period of current application. Release of PGE2 from afferent nerve endings and secondary release of other vasodilatory peptides in response to PGE2 were deemed responsible for the initial slow vasodilation after a single stimulation and the abrupt vasodilation of greater amplitude following a second session, respectively. These cell-mediated pathways underlying the sensitization mechanism with repetitive stimulation result in long-lasting cumulative effects of electrical stimulation on afferent nerve endings. Lastly, in a study comparing the influences of anodal and cathodal DC on skin blood flow, a non-polarity dependent vasodilatory response was suggested by showing the vasodilatory effect of both cathodal and anodal DC and even the cathodal influence was six times larger than anodal DC [59].

The only human studies of direct cerebrovascular response to tES have assessed the effect of transcranial direct current stimulation (tDCS) on vasomotor reactivity (VMR) of the middle cerebral artery, a large basal artery, using transcranial Doppler ultrasound (TCD) [63,64]. Cerebrovascular VMR reflects the vasodilatory capacity of cerebral vasculature, mainly at the arteriolar level, in response to internal molecular signals such as hypercapnia, hypoxia, and increase in pH or external vasodilatory stimuli. VMR can be estimated by measuring the CBF changes using perfusion imaging or measuring the cerebral blood velocity changes of large basal arteries as a surrogate for CBF [65]. However, the cerebral blood velocity is greatly influenced not only by changes in CBF, but also by the diameter of the sonicated blood vessel. Therefore, the change in the velocity is only equivalent to CBF changes if the diameter of the assessed vessel remains unchanged [66].

Vernieri et al. showed bihemispheric changes in TCD measured VMR after 15 min of tDCS with a C3-ipsilateral shoulder montage. The post-stimulation VMR diminished when the anode was used as the center electrode and increased when the center electrode was a cathode compared with pre-stimulation VMR [64]. Involvement of central sympathetic pathways originating from locus coeruleus in the pons or cervical sympathetic ganglions influencing the basal vascular tone was deemed responsible for the changes in VMR. This hypothesis was supported by observed changes in bilateral VMRs and heart rate variability likely related to a greater than usual electrical field concentration in the brain stem or cervical region caused by the extracephalic reference electrode. Nonetheless, considering the complex role the sympathetic nervous system plays in regulating the tone of the entire cerebrovascular tree and blood redistribution during neuronal activation [33–35], the direct effect of extracephalic stimulation montage on vessel diameter and CBF warrants further investigations via optical and perfusion imaging techniques.

In another tDCS study using a conventional bicephalic montage (M1-SO), no change in VMR or autonomic function was observed after 15 min of tDCS [63].

Response of microvasculature/blood-brain barrier (BBB) to electrical stimulation-preclinical studies

A recent set of preclinical studies have investigated the direct influence of tES on small arterioles and capillaries participating in the formation of the blood-brain barrier (BBB) [67,68].

Shin et al. applied 20 min 0.1–1 mA of anodal direct current to rat brain using epicranial electrodes and showed that tDCS increased the gaps between endothelial cells resulting in enhancement of the blood-brain barrier permeability. This direct effect on endothelium was diminished with NO inhibitors, which strongly indicated the role of NO mediating the direct current's vascular effect. Moreover, secretion of other peptides such as vascular endothelial growth factor (VEGF) from endothelium influencing its permeability was also suggested [67].

In a similar study by Fu et al. using the same high resolution multiphoton microscopic technique, 20 min of 1 mA anodal tDCS transiently increased the substance transport in the extracellular matrix of brain tissue [68]. This increase in solute diffusivity in rat brain tissue was associated with decreased density of the extracellular matrix, and, interestingly, was more significant for macromolecules, in-dependent of their charge.

In addition to the increased in endothelial gap junctions space and modulation of the extracellular matrix, other factors such as electroosmosis phenomenon and NO-dependent vasodilation induced by direct current have been shown to be associated and even mediate the enhanced BBB permeability effect [69].

Response of microvasculature/blood-brain barrier (BBB) to electrical stimulation- human studies

Limited work has been done on the influence of tES on BBB in humans, particularly at the microscopic level. We found one safety study that assessed the effect of tES on BBB integrity in humans using MRI with gadolinium [16].Nietzsche et al. delivered 1 mA of cathodal and anodal tDCS to 10 healthy subjects using a C3-SO montage and current density of 0.03 mA/cm2 while simultaneously obtaining MRI with gadolinium. They assessed the safety of tDCS in terms of new MRI lesions and radiographic evidence of severe BBB disruption induced by direct current. No MRI lesion or abnormal contrast extravasation, indicative of a critical disruption of BBB integrity, was noted after 13 and 9 min of anodal and cathodal stimulation, respectively.

Coupled neuro-vascular response to transcranial electrical stimulation

Changes in CBF in response to electrical stimulation have been mainly attributed to the secondary vascular response following a primary direct neural stimulation according to the classic neurovascular coupling (NVC). Due to a highly integrated coupled neurovascular system, it is difficult to distinguish a secondary vascular response from a primary vascular influence; however, it is highly likely that the immediate changes in CBF are in fact a primary phenomenon arising from various cellular components of large, medium, and small sized vessels influenced by the electrical field first reaching the dural and pial surfaces and then concentrated by the cerebral vasculature. The next sections review CBF responses to electrical stimulation, as a marker of NVC, with special focus on tDCS.

In the studies, testing tDCS specifically, the terms “anodal” and “cathodal” reflect the hypothesized brain target – namely, if the nominal brain target is nearer the anode or cathode electrode [70]. This convention is adopted here, noting there is always both an anode and cathode, and the position of the so-called “return” electrode will impact activity under the so-called “active” electrode [71].

Most studies have regarded the tDCS evoked hemodynamic response as a surrogate for neuronal activity and have argued for primary neuronal stimulation influencing the local vasculature via NVC- i.e., anodal-induced neuronal excitation causing secondary

vasodilation and cathodal-induced neuronal inhibition resulting in vasoconstriction. However, studies have reported an immediate transient increase in CBF underneath both cathode and anode electrodes and some demonstrated a substantial enhancement of micro- and macro-vascular perfusion immediately following tES initiation indicative of a direct influence of electrical stimulation on vascular tone and/or a simultaneous astrocytic-mediated effect on both neuronal and vascular elements via release of astrocytic potassium into the NVU extracellular space [12–17].

Coupled neurovascular response to transcranial electrical stimulation-preclinical studies

Bragina et al. studied the effect of anodal tES in rat models of TBI with two-photon laser microscopy technique. They also assessed the effect of tES on regional CBF and tissue oxygenation with near infrared spectroscopy (NIRS) [72]. Anodal tDCS of 0.1 mA intensity for 15 min was delivered to the contused brain region via active and reference electrodes placed directly on the craniectomy site and chest, respectively. The stimulation was repeated daily for four consecutive days at 3-day intervals spanned over four weeks. A strong enhancement of microvascular perfusion and tissue oxygenation in association with an increase in arteriolar vessel diameter was observed immediately after applying 0.1 mA of A-tDCS to the contused brain region resulted in neurological improvement. Interestingly, similar CBF enhancing vasodilatory response was also evident in their non-TBI models receiving tDCS. Although not directly tested, release of NO from endothelium or other vasodilatory neuropeptides from astrocytes was suggested as contributory factors to this effect. Astrocytic response to electrical stimulation has been investigated in the past and plays a major role in the regulation of vascular tone [73,74].

Similarly, Han et al. applied 0.2 mA of anodal tES to rat somatosensory cortex for 10 min with simultaneous NIRS monitoring. They demonstrated an immediate increase in oxyhemoglobin concentration that continued to increase towards the end of the stimulation and gradually decreased and returned to pre-stimulation levels 20 min post-tDCS [12].

In addition to the studies showing the enhancing effect of anodal stimulation on cerebral perfusion, cathodal tDCS-induced changes in cerebral hemodynamics using functional photoacoustic microscopy have also been shown. Liu and colleagues investigated the neuroprotective effect of cathodal tDCS, with and without peripheral stimulation, on different markers of neural activity, hemodynamics, final infarct volume, and behavioral outcomes in photothrombotic focal ischemia rat models. C-tDCS, 0.4 mA in intensity for 20 min (20.37 A/m2 density), was delivered immediately following the induction of ischemia via an epicranial electrode placed over the skull, 5-mm anterior and 3-mm lateral to the bregma, and reference electrode attached to the animal's belly. Their results indicated that standalone C-tDCS immediately enhances the cerebral blood volume and oxygen saturation, particularly at regions upstream to the ischemic cortical vessel, and significantly improves the neuronal activity measured via somatosensory evoked potential. While C-tDCS alone did not have a statistically significant effect on final infarct volume or behavioral recovery, C-tDCS plus peripheral limb stimulation resulted in a smaller final infarct volume after 48 h and significant long-term recovery [14].

In contrast to the studies mentioned earlier showing an immediate enhancement of CBF with both cathodal and anodal tDCS, in two preclinical studies monitoring the effects of tDCS with LDF, CBF diminished after cathodal tDCS and increased with anodal tDCS consistent with a secondary vascular phenomenon related to classic NVC [75,76]. In healthy rat models, Wachter et al. showed a polarity specific CBF modulation measured using LDF after delivering 15 min of tDCS via an epicranial electrode of 3.5 mm^2 placed 2 mm behind the coronal suture and 4 mm lateral to sagittal suture and reference electrode attached to the animal thorax. The tDCS induced CBF changes were measured via LDF probe starting at the end of stimulation for 30 min and compared between anodal and cathodal stimulations across three different intensities of 0.025, 0.05, and 0.1 mA. tDCS resulted in a polarity-dependent, dose-dependent CBF changes with A-tDCS resulting in CBF enhancement and cathodal tDCS causing the CBF to decrease, with both changes being greater at higher intensities [75]. Mielke et al. demonstrated similar results after testing the CBF effect of a single 15-min session of cathodal tDCS using similar electrode locations as the previous study but various intensities delivered via different electrode sizes in healthy rat models. They observed a long-lasting regional reduction in CBF underneath the cathode and areas distant from the stimulated region dependent on the electrode surface size. These studies did not assess the immediate effect of electrical stimulation on CBF, and they instead captured the after effect of tDCS on CBF in a post-tDCS epoch [76].

Neuro-vascular units response to transcranial electrical stimulation-human studies

The neurovascular coupling-mediated changes in cerebral perfusion in response to tDCS has been investigated via perfusion MRI and CT techniques such as arterial spin labeling (ASL) and CT perfusion [77,78].

In two human studies conducting conventional tDCS of the primary motor cortex with M1-contralateral SO montage and simultaneous CBF monitoring using ASL, a polarity and intensity dependent effect on CBF was observed with anodal stimulation enhancing and cathodal tDCS decreasing the local CBF of the primary motor cortex. These CBF changes were mainly attributed to the primary neuronal effect on local vasculature via NVC with anodal-induced neuronal excitation causing secondary vasodilation and cathodal-induced neuronal inhibition resulting in vasoconstriction. However, both studies observed an initial transient increase in motor cortex CBF with both anodal and cathodal tDCS before further increasing in the case of anodal and declining in cathodal tDCS.

Zhang et al. demonstrated an increase of 17% in local CBF after anodal stimulation and a smaller 6% initial increase after cathodal stimulation of the motor cortex before decreasing to below baseline levels using conventional tDCS with an average current intensity of 1.4 mA [78].

Jamil et al., using conventional tDCS with intensities ranging from 0.5 to 1 mA, demonstrated a dose-dependent increase in CBF with anodal stimulation and a tendency towards an initial CBF enhancing effects with cathodal stimulation of the motor cortex [77].

Both studies observed a subsequent decline in CBF to levels lower than baseline in case of cathodal and persistent increase in CBF with anodal tDCS. Although the ultimate polarity dependent CBF altering effects of tDCS could be attributed to a secondary vascular response to neural activity, the initial CBF enhancing effects of both anodal and cathodal stimulation cannot be explained by this phenomenon. Therefore, a primary vascular response to electrical stimulation that is eventually modified to match the stimulated brain network's neuronal activity was suggested as a candidate explanation.

In another study by Dutta et al., the NVC induced hemodynamic response to anodal tDCS was captured using simultaneous NIRS and EEG recordings. Anodal tDCS was delivered to patients with chronic middle cerebral artery stroke with a Cz-SO montage and a

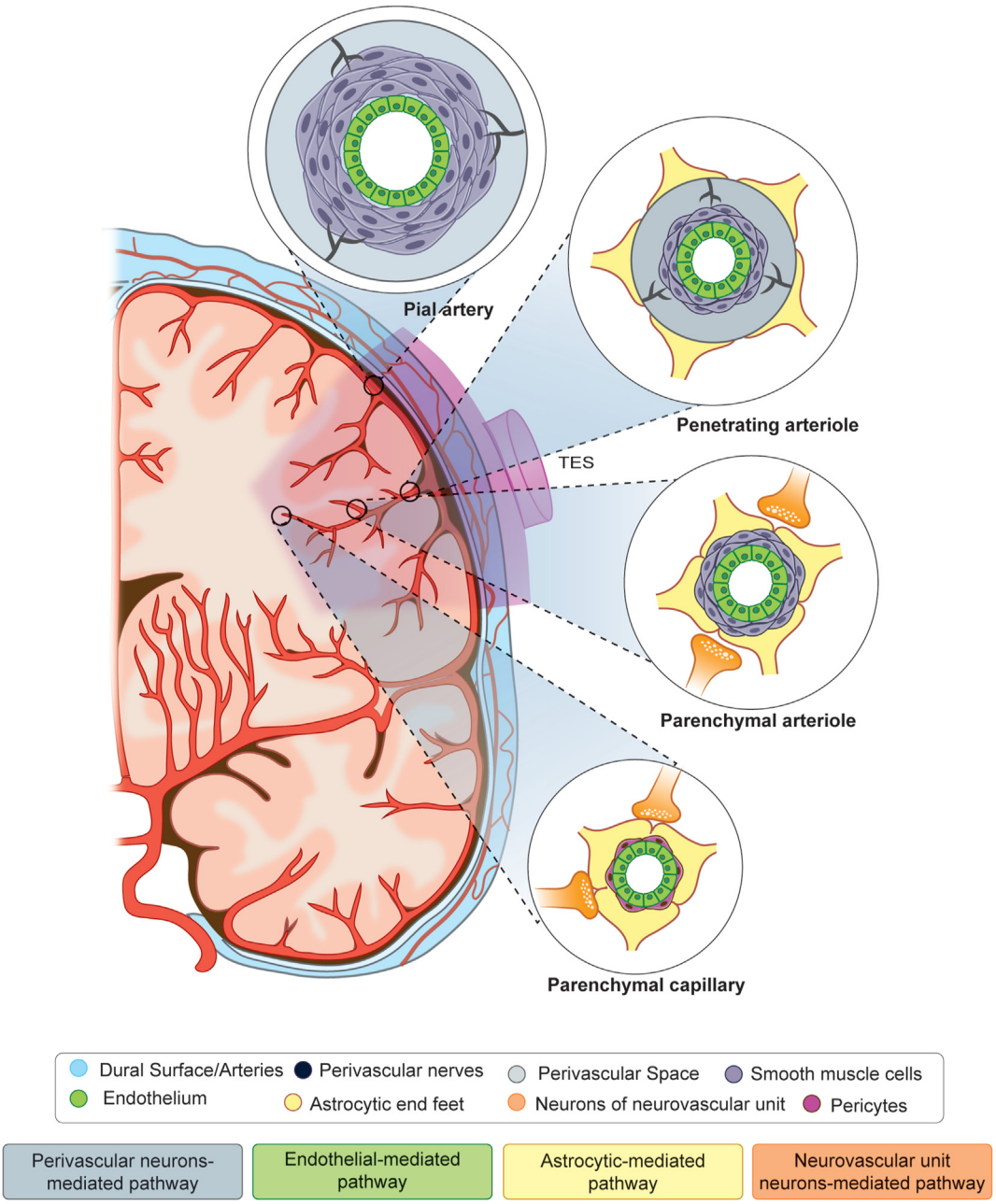

Fig. 2. Demonstration of four mediators of vascular response to transcranial electrical stimulation: 1) Perivascular neuron-mediated pathway (Gray); 2) Endothelial-mediated pathway (Green); 3) Astrocytic-mediated pathway (Yellow); and 4) Neurovascular unit neuron-mediated pathway (Orange).
The electrical field concentrates in the cerebrospinal fluid of subarachnoid space where dural and pial vasculature and blood-brain barrier (BBB) reside (in color purple). Pial arteries and penetrator arterioles with multilayered smooth muscle cells (color brown) and rich perivascular nerves (color black/gray) predominantly contribute to the perivascular neuron-mediated vascular response. The endothelial lining of all vessels (color green) responds to electrical field via the endothelial-mediated pathway. Astrocytes (color yellow) and neurons (color orange) of NVU play roles in a secondary vascular phenomenon and as part of classic neurovascular coupling. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

current density of 0.526 A/m^2 for 0–30 s. They demonstrated an immediate hemodynamic response with a transient dip followed by an increase in oxyhemoglobin with anodal stimulation. Study authors postulated a simultaneous vascular and neuronal response to potassium released from glial cells in response to stimulation [79].

Finally, Trofimov et al. applied anodal tDCS to the left motor cortex of patients with traumatic brain injury while monitoring the CBF with CT perfusion [80]. They observed a significant increase in cerebral blood flow and cerebral blood volume and decreased mean transit time of contrast material through the stimulated tissue. An NVC-induced vascular response could not account for the dramatic changes of cerebral perfusion; therefore, authros postulated a direct effect of tDCS on vascular tone via NO release versus astrocytic mediated vasodilation was postulated by authors.

Discussion

Various preclinical and some clinical evidence suggest both primary and secondary vascular effects of electrical current. The vascular response originates from different cellular elements and

Table 1
Contribution of the four mediators of vascular response across large, medium, and small-sized vasculature.

	Perivascular neuron-mediated pathway	Endothelial-mediated pathway	Astrocytic-mediated pathway	NVU neurons-mediated pathway
Dural Arteries*	✓	✓		
Pial Arteries	✓	✓		✓**
Penetrating Arterioles	✓	✓	✓	✓**
Parenchymal Arterioles		✓	✓	✓
Capillaries		✓	✓	✓

NVU: Neurovascular Unit

* Dural arteries do not normally contribute to cerebral blood flow unless under pathological conditions such as intracranial arterial (i.e. carotid or middle cerebral artery) steno-occlusive diseases via anastomosis to cortical vessels.

** The neuronal-induced vasodilatory signals are transmitted from capillary pericytes and endothelial cells to larger penetrating arterioles and pial arteries via a retrograde propagation of signals.

vascular compartments and may vary depending on the size and anatomy of the vessel exposed to electrical stimulation and the also the type, shape, and strength of the electrical field.

Four cellular elements have been identified driving the effects with endothelium and perivascular nerves mediating a direct primary response to tES, and astrocytes and neurons of NVU resulting in a secondary vascular response: 1) Perivascular neuron-mediated response resulting from stimulation of perivascular nerves releasing vasoactive peptides; 2) Endothelium-mediated response involving release of vasoactive peptides, activation of ion channels, and changes in blood-brain barrier permeability; 3) Astrocyte-mediated response from stimulated astrocytes releasing vasoactive substances in response to electrical stimulation independent of primary neural activity of NVC; and 4) Neurons of neurovascular unit (NVU)-mediated response leading to traditional neurovascular coupling.

According to most studies assessing the vascular effect of tES, a primary non-polarity-dependent immediate vasodilatory response is suggested. The direct vasodilation via perivascular neurons-mediated pathways likely arises from dural and pial arteries and penetrating arterioles with multilayered SMC and abundant perivascular nerves that predominantly secrete vasodilatory peptides of calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP) from their rich parasympathetic innervation that outnumbers the sympathetic counterpart. In addition, given that these vessels are more superficially located closer to the meninges and skull, they receive a much higher current density than the deeper vasculature and neuronal tissue. Furthermore, due to their larger innate diameter, higher transmural pressure, and stronger endogenous electrical field, electrical current is likely to have a larger enhancing effect on perivascular neurons and the endothelial lining also rich with predominantly vasodilatory neuropeptides and ion channels such as NO, PGE2, and K^+_{ATP}, compared to smaller parenchymal arterioles and capillaries [20,56]. However, the contribution of the endothelial lining of parenchymal microvasculature of the BBB to the primary vascular response is also of great interest, particularly at higher current densities as shown in preclinical studies [67]. The transient non-injurious increase in solute diffusivity and permeability of BBB in response to concentrated direct current in the subarachnoid space changes the neuronal and glial microenvironment and hence directly influences NVU neurons. Nonetheless, a human safety study of tDCS has not shown any BBB disruption due to direct current [16].

Pial vessels also play a significant role in the secondary vascular response to neurovascular coupling. Given the inherently coupled and highly interdependent neurovascular system, their initial vasodilatory response eventually integrates with the astrocytic and neurovascular coupling mediated effects after the electrical current activates or inhibits a brain network. This explains the immediate CBF enhancing effect of both cathodal and anodal tDCS prior to their modulation of the motor network's neural activity in humans. Fig. 2 summarizes the four mediators of vascular response to tES. Table 1 summarizes the contribution of various cellular components to large, medium, and small-sized Vascular Response to tES.

Although the precise order by which these cellular compartments are influenced by electrical current remains unclear, some of these responses likely occur in parallel due to the highly integrated neurovascular system in physiological conditions. However, in pathological conditions where there is decoupling of neurovascular units, this initial vascular response to tES may be amplified or sustained due to lack of downstream neuronal feedback, and it may even have a substantial secondary influence on neural activity via vasculo-neural mechanisms. This theory also explains the variability of responses to tDCS seen across studies stimulating different neural networks and the differences of effects between healthy individuals and participants with pathological conditions affecting NVC or cerebrovascular hemodynamics. For example, the steno-occlusion of large to medium arteries of circle of Willis in cerebrovascular diseases redirects the blood flow to dural and pial anastomotic collateral vessels causing increased forced shear stress [81,82]. These changes in the local mechanical and electrical forces of pial vessels may amplify the vasodilatory response to electrical current, increasing perfusion to the ischemic tissue via collateral enhancement. Alzheimer's disease (AD) is another example of a

pathological condition with impairment of NVC due to microvascular alterations causing reduced cerebral blood flow [32,38,83–85]. Cerebral hypoperfusion has been shown to initiate and or contribute to beta-amyloid aggregation, and attenuated hemodynamic response to neural activation may diminish the paravascular drainage system and induce beta-amyloid elimination failure [86–92]. Therefore, we speculate that the tES-induced vascular response could potentially have a therapeutic effect in AD by enhancing the cerebral hemodynamics and/or increasing the clearance of beta-amyloid and other pathological neuropeptides.

Future perspectives

Many tES studies have used hemodynamic imaging only as a marker of neuronal activation, but very few have focused on understanding the precise spatial and temporal properties of vascular responses. Ultimately, future research may disambiguate the neuronal versus vascular response to tES and further elucidate their order of activation and magnitude of contribution to neurovascular modulation. For instance, by incorporating optical, quantitative perfusion, and blood-brain barrier imaging techniques, future studies could investigate depth-specific responses of various vascular compartments to tES and assess their temporal relationship to neuronal and glial activation [93–96]. Furthermore, preferential activation of different cerebral vascular compartments by electrical fields of various shapes and depths remains to be elucidated.

Lastly, studying the effects of neurovascular decoupling and dampened hemodynamic response due to neurological diseases or simply aging on these nuances of tES-induced primary vascular response is also of paramount importance.

Understanding “which cellular elements” are activated by stimulation, has long underpinned the understanding and optimization of neuromodulation techniques [97–99], including tES [5]. Measuring “target engagement” during human trials then underpins rational intervention development and validation [1,100]. Neuronal versus vascular targets evidently represent distinct neuronal targets and associated biomarkers of engagement, with essential implications for tES outcomes.

In conclusion, a direct vascular effect of transcranial electrical stimulation is highly suggested based on various preclinical and clinical studies. This direct vascular effect may influence the neuronal activity reversing the role of vasculature from secondary responders to primary modulators of neuronal response to electrical current. However, further studies are warranted to investigate the exact mechanisms involved in the vascular response and its contribution to neural activity in both healthy brains and pathological conditions with decoupling of neurovascular units.

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Declaration of competing interest

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M. Bikson (MB)- The City University of New York holds patents on brain stimulation with MB as inventor. MB has equity in Soterix Medical Inc. MB consults, received grants, assigned inventions, and/or serves on the SAB of Boston Scientific, GlaxoSmithKline, Mecta, Halo Neuroscience, X.

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Appendix A. Supplementary data

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