Grey-box linear modeling to analyze functional near-infrared spectroscopy-based cerebrovascular reactivity to anodal HD-tDCS in healthy humans

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ABSTRACT

The mechanism of action for the cerebral vasculature hemodynamic response to the electric field effects during transcranial direct current stimulation (tDCS) has been less explored. We postulate that such a mechanistic understanding of the cerebrovascular reactivity (CVR) to tDCS can facilitate adequate tDCS dosing to facilitate cognitive rehabilitation in mild cognitive impairment and dementia as well as motor rehabilitation in stroke and traumatic brain injuries. This study presents a system identification approach to evaluate CVR under high-definition (HD) tDCS using a physiologically constrained model based on a multi-compartmental neurovascular system of vascular smooth muscle, perivascular space, synaptic space, and astrocyte glial cell. The physiologically detailed model generated vessel oscillations in the frequency range of 0.05 – 0.2 Hz driven by nonlinear calcium dynamics. Then, model linearization was performed to develop a grey-box linear model for evaluating the acute effects of HD-tDCS based on the CVR data from healthy humans. CVR was measured using functional near-infrared spectroscopy (fNIRS) with optodes in the vicinity of 4x1 HD-tDCS electrodes. The grey-box linear model response of vessel response through the synaptic potassium pathway was found comparable to known hemodynamic responses. Then, the grey-box linear model was fitted to the CVR to anodal HD-tDCS found from the normalized changes in the fNIRS-total haemoglobin (tHb) concentration (blood volume) in eleven healthy participants. We found that the model pathway from perivascular potassium to the vessel circumference presented the best fNIRS-tHb fit with the least mean square error. The fitted grey-box linear model response presented an initial dip in the vessel response that provided a mechanistic understanding of the fNIRS-tHb changes at the onset of HD-tDCS, which needs to be validated in the future with functional magnetic resonance imaging in conjunction with HD-tDCS.

Keywords—Cerebral Blood Flow, Cerebrovascular Reactivity, Hemodynamics, Near-Infrared Spectroscopy, Neurovascular Coupling, Transcranial Direct Current Stimulation.

Introduction

Cerebral blood flow (CBF) regulation is crucial for normal brain activity where hypoperfusion has been associated with cerebrovascular diseases, including cognitive impairment in various cross-sectional studies¹. Cerebrovascular disease refers to a condition that has an effect on blood vessels and blood supply to the brain. It can happen from a number of causes, like thinning of arteries (atherosclerosis), the formation of arterial blood clots (thrombosis), or formation of blood clots in the vein of the brain (cerebral venous thrombosis). The most common cerebrovascular diseases are: stroke, aneurysms, transient ischemic attack (TIA), carotid, and vertebral stenosis². Since vascular factors are an important contributor to mild cognitive impairment and dementia, that is predicted to increase to 152 million by 2050³; therefore, preventive measures are crucial. CBF is regulated primarily by three mechanisms, cerebral autoregulation that maintains the CBF under changes in systemic blood pressure, cerebral vasoreactivity that is the response to the arterial CO₂ partial pressure changes, and the neurovascular coupling that is the response to the neuronal activity. Transcranial electrical stimulation (tES), particularly transcranial direct current (tDCS), can be a promising method to evoke regional CBF⁴ that can ameliorate hypoperfusion and the related cognitive impairments. A recent study⁵ showed that the spatial distribution of CBF changes correlated with the tDCS-induced electric field distribution found with finite element modeling. Furthermore, the tDCS-induced excitability changes in the left primary motor cortex correlated with the CBF changes in the left sensorimotor region; however, tDCS-induced alterations in cerebral CBF could only partially account for the cortical excitability changes⁵. Here, effects of the electric field on various neurovascular pathways leading to the CBF changes needs to be investigated vis-à-vis cortical excitability changes⁶. Also, bidirectional communication in the neurovascular unit (NVU)⁵⁶ can lead to cortical excitability changes⁵ as a response to the tDCS-induced alterations in cerebral CBF. Moreover, the dosing of the tDCS-induced electric field is crucial for safety since the blood-brain barrier (BBB) dysfunction in more severe cases⁷ that can be worsened by an increased BBB permeability due to tES⁸. In principal accordance, it is important to investigate the underlying mechanism of the hemodynamic response in connection to the tDCS-induced electric field effects on the neurovascular brain tissue, which we term cerebrovascular reactivity (CVR)⁹. In healthy tissue, CVR is a compensatory mechanism where blood vessels dilate in response to the vasodilatory stimulus from tDCS to regulate the resistance to the flow and modifying the cerebral perfusion as per the variation in demands for oxygen and nutrients (i.e., neurovascular coupling)¹⁰. This capacity of blood vessels to dilate is hampered in various cerebrovascular diseases^{11,12}. A remedial function of noninvasive tES approaches, including tDCS, in cerebrovascular disease can be promising due to its ease of use and cost^{13,14}.

The neuro-modulatory consequences of tDCS are understood to be generated due to the induced electric field (and current density) in the cortex by the application of weak direct current through

scalp electrodes causing cortical excitability¹⁵. It is evident from the studies that the induced electric field can change neuronal excitability and evoke responses through the interaction of the electric field with the neuronal structure and orientation. In humans, the application of external electric fields on folded cortical structures produces more unpredictable and variable effects¹⁶⁻¹⁸. The current applied by surface electrodes in tDCS is shunted through the scalp, and cerebrospinal fluid (CSF), and only a fraction of the current reaches to the brain, producing a weak electric field (~ 0.3 V/m per 1mA of applied current)^{19,20}. The persistent electric field is shown to polarize the transmembrane neuronal potential, which in turn can affect excitability²¹, and alter the firing rate of neurons^{22,23}, and modify spatio-temporal brain networks related to information transfer in the brain^{19,24}. The majority of research on tDCS has focused on neuronal effects; however, other cellular targets like axons, dendrites, glia, and vascular cells are known to be affected by the electric field and may accord to neuromodulation mechanisms. Guhathakurta and Dutta²⁵ postulated that the pial arteries and arterioles contain perivascular nerves within their adventitial layer that can be strongly affected by tDCS-induced electric field since the magnitude of the tDCS current density in the cerebrospinal fluid that surrounds pial vessels was found to be almost 10 times of that in the white matter using modeling methods. Therefore, tDCS can have direct stimulation effects on the cerebral neurons, indirect stimulation effects via perivascular nerves, or modulatory effects on non-neuronal cells^{26,27}. Astrocytes, the major non-neuronal glial cells in the brain, are susceptible to small variations in their membrane potential²⁸, and their elongated processes are sensitive to polarization by tDCS²⁹⁻³¹. In animal studies, it has been found that tDCS induces astrocytic calcium waves in the visual cortex, which is known to steer plasticity of visually evoked potentials³². Further, electrically coupled populations of glial cells known as glial syncytium can intensify field polarization in response to tDCS. Glial cells are known to maintain extracellular potassium concentration by the imbalance in their membrane polarity and can cause potassium spatial buffering through tDCS modulation^{26,33}. Likewise, vascular cells such as endothelial cells and arteriolar smooth muscle cells (SMC) seem to be affected by tDCS through modulation of nitric oxide (NO) mediates, alignment and angiogenesis in endothelial cells and polarization of SMC causing vasomotion via metabolites such as potassium ions (K^+), adenosine, NO and calcium ions (Ca^{2+})^{34,35}.

It is considered that neuronal and vascular functions are closely interconnected through neurovascular coupling mechanisms, as evident from studies using functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and, more recently, functional near-infrared spectroscopy (fNIRS). From an anatomical perspective, an integrated system of neurons, astrocytes, and vascular cells forms a neurovascular unit (NVU) that facilitates brain homeostasis and links neuronal and vascular activities at the cellular level^{36,37}. Considering network effects, the changes in neuronal and vascular functions accumulate to cortical activity and hemodynamic responses, respectively. The neurovascular dynamics are complicated phenomena in humans, and it can be hard to uncouple neuronal and vascular effects, including during tDCS³⁸. The association between neuronal activity and hemodynamic responses (i.e., neurovascular coupling) can be indirectly examined through functional neuroimaging techniques such as fMRI, PET, and fNIRS.

The integration of tDCS with functional neuroimaging modalities holds immense promise for throwing light on the underlying neuromodulation processes of stimulation effects. Experimental studies have indicated that tDCS tends to transform neuronal activity through an induced electric field and hemodynamics through modulation of cortical neurotransmitters (like gamma-aminobutyric acid (GABA) and glutamate) during tDCS^{39,40}. In addition, there is evidence of studies on CBF modulation through tDCS^{4,5,41}. Of the available functional neuroimaging technologies, fNIRS, a portable, noninvasive clinically available tool, allows monitoring the local cortical hemodynamic response with reasonable spatial resolution and better temporal resolution compared to the gold standard of fMRI. fNIRS provides a measurement of cerebral oxygenation (changes in concentration of oxygenated-oxy and deoxygenated-deoxy hemoglobin) and blood volume (sum of oxy- and deoxy- hemoglobin)^{42,43}, and is a promising tool to evaluate CVR as evident from several studies^{44,45}. fNIRS provides an indirect measure of CBF during tDCS to evaluate CVR where the advantage over other neuroimaging modalities like fMRI and PET are: portability, better safety, higher temporal resolution, and cost effectiveness⁴⁶. Here, fNIRS can be combined with simultaneous tDCS with no electro-optic interference to measure the hemodynamic response during electrical stimulation. Various studies have evaluated the hemodynamic effects of tDCS using fNIRS in humans in published literature. Merzagora et al⁴⁷ assessed the changes in prefrontal cortical oxygenation related to tDCS using fNIRS in healthy volunteers at rest. A large increase in oxy-hemoglobin concentration was observed in the 10 min period following anodal tDCS compared to baseline levels before tDCS. Muthalib et al⁴⁸ showed that anodal HD-tDCS induced significantly greater increases in oxy-hemoglobin concentration during 10 min of stimulation (2mA) in the sensorimotor cortex region within the vicinity of the 4 x 1 high-definition tDCS (HD-tDCS) montage compared the region outside this boundary; while there were minimal oxy-hemoglobin changes in the contralateral non-stimulated sensorimotor cortex region. The time course of changes in oxy-hemoglobin in the region within the boundary of the HD-tDCS montage showed an initial decrease followed by a steep increase and then a relative plateau for the duration of stimulation. Yaqub et al⁴⁹ evaluated the prefrontal cortex resting-state intrahemispheric and interhemispheric connectivity changes induced by 10min (1mA) anodal 4x1 HD-tDCS in healthy volunteers. Network connectivity changes were observed through analysis on filtered oxy-hemoglobin time-series signal using common graph theory metrics computation. They observed that compared to the pre-stimulation phase, the oxy-hemoglobin concentration levels and resting-state connectivity of the prefrontal cortex increased during and after the stimulation, and moreover, the connectivity changes were greater in the vicinity of the 4x1 HD-tDCS electrodes. Sood et al⁵⁰ extended Dutta et al⁵¹ study, and presented a parameter estimator method using the Kalman filter to evaluate the relation between changes in fNIRS oxy-hemoglobin signal and EEG bandpower signal during anodal tDCS. The time varying poles of the autoregressive model were found to be comparable in all healthy participants; while the zeros of the model exhibited variations across the participants during the stimulation.

Muthalib et al⁴⁸ study established the focality of the hemodynamic response where they found a greater increase in oxy-hemoglobin concentration "within" than "outside" the spatial extent of the

4×1 HD-tDCS electrode as a hemodynamic correlate of the electrical field distribution. Therefore, in this computational modeling study, we will investigate the hemodynamic correlate of the electrical field distribution "within" the spatial extent of the 4×1 HD-tDCS electrode, found by Muthalib et al⁴⁸, using grey-box linear modeling. The increase in regional CBF (rCBF) and oxy-hemoglobin concentration during tDCS are considered to be caused by a mix of two main neurovascular coupling hypotheses (metabolic and neurogenic). The indirect "metabolic hypothesis," which states that an increase in neuronal synaptic activity causes additional energy and oxygen demand, causing various vasodilation agents to send signals to cerebral vasculature for instant vasodilation, resulting in an increase in rCBF and oxy-hemoglobin. This metabolic hypothesis can explain the initial dip in oxy-hemoglobin concentration in the period immediately following tDCS before reaching peak levels, as observed in our previous studies^{48,50}. Other causes for the large increase in oxy-hemoglobin can be explained through direct "neurogenic hypothesis," whereby the direct modulation of neuropeptides and neurotransmitters causes a discharge of various vasoactive agents such as NO for vasodilation and an increase in oxy-hemoglobin⁵². Such a "neurogenic hypothesis" can also be applied to the perivascular nerves during tDCS, e.g., in the pial arteries and arterioles²⁵, which will have a compounding effect to increase oxy-hemoglobin levels.

In this study, we investigated various NVU pathways in the modulation of CVR during HD-tDCS based on grey-box linear systems analysis. Completely data-driven black-box systems approaches provide a correlate of neural and hemodynamic response at an abstract level under the assumption of neurovascular coupling at the cellular level. However, these black-box system approaches do not aim to explicitly capture the underlying mechanisms of action. Considering the evidence of modulatory consequences of tDCS on blood vessels that can be via neuronal and non-neuronal cells, a deeper understanding of the signaling pathways inside the NVU is important for a mechanistic understanding. The current study attempts to model the immediate fNIRS-based CVR effects of tDCS by modeling the intimate relationship between neuronal activity and hemodynamic response that involves the signaling pathways across the compartments of the lumped model of the NVU. This investigation is based on the dynamics of four compartments in the NVU: synaptic space, intracellular astrocyte space, perivascular space, and intracellular space of the arteriolar SMC. CVR for the study is considered as a change in the vessel volume in response to tDCS. In this paper, we analyzed the pilot data from healthy subjects from our prior work⁵⁰ to evaluate the role of various signaling pathways using a grey-box linear systems identification approach that modeled the vessel volume response during tDCS. In this study, the vessel volume changes were related to the blood volume change, which was estimated based on fNIRS total hemoglobin (tHb) change, computed by adding oxy- and deoxy-hemoglobin changes. tHb change is considered as a better measure of the change in the regional cerebral blood volume⁵³ compared to oxy- or deoxy-hemoglobin content taken individually. The signaling pathways modulating vascular response through perivascular space, astrocytes, and direct SMC channels were evaluated and compared using grey-box linear systems' transfer function analysis in eleven healthy participants.

Results

Figure 1. Normalized time course of changes in the total hemoglobin (tHb) concentration (averaged for 4 channels around 4x1 HD-tDCS anode) for eleven healthy volunteers (V1 to V11 and Average) during anodal HD-tDCS (2mA current for 10 minutes, with ramp-up period of 30 seconds)

(A) fNIRS-tHb response during HD-tDCS (experimental data):

Figure 1 shows the change in the normalized tHb concentration obtained during anodal HD-tDCS stimulation from fNIRS channels (optode pairs: C5-CP3, C1-CP3, C5-FC3, and, C1-FC3; shown in Figure 6) around the 4x1 HD-tDCS anode. In the majority of the volunteers, the time course of changes in tHb in the region within the boundary of the HD-tDCS montage showed an initial decrease followed by a steep increase and then a relative plateau for the duration of stimulation.

(B) Physiologically detailed model response:

A physiologically detailed neurovascular coupling model was developed from published literature⁵⁴⁻⁵⁷ (differential equations and parameters are detailed in the supplementary materials) that was simulated using the ODE23TB solver in Simulink (Mathworks, Inc., USA). The results of four simulated pathways are shown in Figure 2; 1: tDCS modulating vessel response through synaptic potassium pathway, 2: tDCS modulating vessel response through astrocytic membrane potential, 3: tDCS modulating vessel response through perivascular potassium currents, and 4: tDCS modulating vessel response through voltage-gated ion current channels on the SMC. Figure 2b shows the output of the grey-box linear model, i.e., the blood vessel circumference, x , for the tDCS driven modulatory current input, shown in Figure 2a. The tDCS effect of the current (applied

in the human experiment), shown in Figure 2a, generated the vasoactive signal for the four pathways in the compartments of the lumped model of the NVU. While solving the model, the vasoactive signals for the pathways were represented as a first-order transfer function of the tDCS input pulse. For pathway 1, the effect of tDCS on synaptic potassium was considered, which drives the neurovascular dynamics leading to the blood vessel response. Similarly, the effect of tDCS on astrocytic membrane potential, perivascular potassium currents, and SMC's membrane potential leading to the vessel response was also evaluated by Pathways 2, 3, and 4, respectively (model details in the supplementary materials), as shown in Figure 2b. Figure 2b shows that all the pathways generated low-frequency oscillations in the frequency range of 0.05 – 0.2 Hz, driven by nonlinear calcium dynamics in various compartments (details in the supplementary materials), in the time course of the vessel response when simulated using a physiologically detailed neurovascular coupling. In this study, we did not investigate the effects of the electric field on the low-frequency (0.05 – 0.2 Hz) oscillations driven by nonlinear calcium dynamics and focused on the initial transient part based on model linearization for grey-box analysis of experimental data.

Figure 2. Physiologically detailed neurovascular coupling (NVC) model showing the input HD-tDCS current (2a) and output vessel circumference response for the four simulated pathways (2b). Pathway 1: tDCS modulating vessel response through synaptic potassium pathway, Pathway 2: tDCS modulating vessel response through astrocytic membrane potential, Pathway 3: tDCS modulating vessel response through perivascular potassium currents, and Pathway 4: tDCS modulating vessel response through voltage-gated ion current channels on smooth muscle cells. As shown in 2b, the four NVC pathways generated low-frequency oscillations in the frequency range of 0.05 – 0.2 Hz in the time course of the vessel response.

Table 1. Transfer functions were obtained through a grey-box linear analysis tool for the four model pathways.

Pathways	First-order transfer function from tDCS current to the vasoactive signal	The linear transfer function of the compartmental neurovascular coupling model	Order of the system
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1. Synaptic Potassium → vessel circumference	$K/(s + 50)$	$\frac{(s+2.371e07)(s+46.5)(s+2.962)}{(s+9.594e06)(s+2.974e04)(s+20.69)(s+15.08)(s+3.3)(s+1.966)(s+1)(s+0.4)(s+0.2446)(s^2 + 9.804s + 95.24)}$	12
2. Astrocytic membrane potential → vessel circumference	$K/(s + 50)$	$\frac{(s+2.371e07)(s+46.5)(s+2.962)}{(s+2.974e04)(s+20.69)(s+15.08)(s+3.3)(s+1.966)(s+1)(s+0.2446)(s+9.594e06)(s^2 + 9.804s + 95.24)}$	11
3. Perivascular Potassium → vessel circumference	$K/(s + 50)$	$\frac{(s+2.371e07)(s+2.962)}{(s+9.594e06)(s+2.974e04)(s+20.69)(s+3.3)(s+1)(s+0.2446)(s^2 + 9.804s + 95.24)}$	9
4. Voltage gated ion channel on SMC → vessel circumference	$K/(s + 50)$	$\frac{(s+2.962)}{(s+9.594e06)(s+20.69)(s+3.3)(s+0.2446)(s^2 + 9.804s + 95.24)}$	7

Figure 3. Model output vessel response (vessel circumference) to the input square tDCS test pulse (2s duration, amplitude 2mA, starting at time zero) is shown for the four pathways in the proposed grey-box linear model (Pathways; 1: tDCS modulating vessel response through synaptic potassium pathway, 2: tDCS modulating vessel response through astrocytic membrane potential, 3: tDCS modulating vessel response through perivascular potassium currents and 4: tDCS modulating vessel response through voltage-gated ion current channels on the smooth muscle cells)

(C) Physiologically detailed model linearization for grey-box analysis of experimental data:

For the grey-box analysis of the experimental fNIRS-tHb data from input tDCS current leading to the output hemodynamic response (normalized tHb changes), model reduction of the four pathways in the physiologically detailed NVC model was performed using Simulink's linear

analysis tool (Mathworks, Inc., USA). This tool allows the linearization of complex nonlinear models at different operating points. In our model reduction and data analysis, we assumed that the perturbation to the lumped NVC model due to tDCS, which is a vasoactive signal approximated by first-order filtering (with arbitrary gain, K , and 20ms time constant) of the current input, will operate near the resting-state baseline conditions; therefore, the linearization of the physiologically detailed model was performed at the initial conditions from published literature models⁵⁴⁻⁵⁷ (details in the supplementary materials). The linearization step approximated the system of nonlinear differential equations around an operating point of the initial resting-state conditions by a linear model. The obtained linear model presented the neurovascular system as a set of input, internal states (compartment variables), and output as transfer functions, which depicted the relationship between input tDCS pulse and output hemodynamic response as a ratio of polynomials (polynomial variable: 's'). The roots of the numerator and denominator polynomial are known as model zeros and model poles, respectively. The poles and zeros are useful in evaluating a system as their values govern the stability and performance of the system. For a stable system, all the poles must have negative real values. The zeros are related to the speed of the response for a given system. Furthermore, the number of poles or degree of the denominator polynomial gives the model order. The transfer function for the four pathways of the grey-box linear model is given in Table 1. All the pathways have an excess of poles over the number of zeros; therefore, the magnitude of the frequency response will tend to zero with an increasing frequency. All the four pathways have complex conjugate poles (from $s^2 + 9.804s + 95.24$ terms) in the stable region. The numbers of zeros are different in the four pathways where the positive real zeros are most likely approximating the time delay in the system. Figure 3 shows the modeled vessel response function of the four pathways' using a square tDCS test pulse (2s, 2mA, starting at time zero (start of the simulation)). Here, Pathway 4 has the fastest response (peaked at 2.231 sec), and Pathway 1 has the slowest response (peaked at 6.431 sec). Here, Pathway 1 acting via the synaptic potassium resulted in the stereotypical time-to-peak⁵⁸ of about 6 seconds.

(D) Grey-box model analysis using experimental fNIRS-tHb data:

The System identification toolbox (Mathworks, Inc., USA) was used to model the time-domain input (tDCS) and output (fNIRS) data from eleven healthy participants. The input time-series was a trapezoidal wave (2mA current intensity applied for 10 minutes with a ramp-up of 30 seconds). The output time-series were normalized values of the fNIRS-tHb changes in the vicinity of the 4 x 1 HD-tDCS electrodes during stimulation (details in the Materials and Methods section, Figure 6). The values of the free parameters (poles and zeros) of the parameterized grey-box linear model were updated using the "Refine Existing Model" approach in the System identification toolbox to improve the fit to the estimation data. In this method, the transfer function for the grey-box model (Table 1) obtained after physiologically detailed model linearization using Simulink's (Mathworks, Inc., USA) linear analysis tool was taken as the initial model to fit the experimental fNIRS-tHb data. This approach was applied to the fNIRS-tHb data from each participant.

Figure 4 shows the simulated outputs (blood vessel circumference change) of the four pathways for each of the 11 participants, while Table 2 shows the mean square error (MSE). The simulated output of all the four pathways demonstrated an initial dip in the tHb concentration (related to the blood vessel circumference in the physiologically detailed model), which was found to be due to the positive zero in the right-half-plane (positive real axis for parameter's' of transfer function) in the parameterized grey-box linear model. This zero in the right-half-plane slowed the time response and resulted in the undershoot response. A positive zero adds to the phase lag in a system wherein the response initially becomes negative or changes direction to that of the required direction before converging in the desired steady state.

Table 2 shows the mean square error (MSE) for each participants' fNIRS-tHb data fitting and its inter-subject variability; while Figure 5 shows the boxplot of the MSE. Pathway 3, from perivascular potassium to vessel circumference, presented the least MSE (median < 5%) across all subjects, as shown in Figure 5.

Figure 4. Simulated grey-box linear model output for the four pathways fitted individually to each of the 11 participants' fNIRS-tHb data. The averaged experimental fNIRS-tHb response across all subjects is also shown with a dashed line. Pathways: 1: tDCS modulating vessel response through synaptic potassium pathway, 2: tDCS modulating vessel response through astrocytic membrane potential, 3: tDCS modulating vessel response through perivascular potassium currents, and 4: tDCS modulating vessel response through voltage-gated ion current channels on the smooth muscle cells)

Table 2. The mean-square error (MSE) for the grey-box model analysis using experimental fNIRS-tHb data of each subject for the four model pathways.

Sub #	Pathway 1 (Synaptic Potassium → vessel circumference)	Pathway 2 (Astrocytic membrane potential → vessel circumference)	Pathway 3 (Perivascular Potassium → vessel circumference)	Pathway 4 (Voltage-gated ion channel on SMC → vessel circumference)
1	0.03194	0.05235	0.2203	0.1321
2	0.1128	0.1422	0.004508	0.07534
3	0.08671	0.1937	0.05579	0.1598
4	0.01857	0.04674	0.07505	0.0679
5	0.06443	0.08512	0.005981	0.1466
6	0.008047	0.1361	0.008353	0.08054
7	0.06033	0.02859	0.02679	0.08053
8	0.09873	0.2548	0.3909	0.1242
9	0.00289	0.03508	0.01706	0.04097
10	0.05032	0.1326	0.00422	0.08931
11	0.05409	0.2009	0.004483	0.08612

Figure 5. Boxplot of the mean square error (MSE) across all the 11 participants' fNIRS-tHb data for the grey-box model fitting of the four pathways. The central red mark indicates the median, and the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively. The whiskers extended to the most extreme data points not considered outliers, and the outliers are plotted individually using the red '+' symbol. Pathway; 1: tDCS modulating vessel response through synaptic potassium pathway, 2: tDCS modulating vessel response through astrocytic membrane potential, 3: tDCS modulating vessel response through perivascular potassium currents, and 4: tDCS modulating vessel response through voltage-gated ion current channels on the SMC).

Discussion

Our study performed a grey-box linear systems analysis of fNIRS-based CVR (change in tHb) to anodal HD-tDCS in healthy humans. The hemodynamic response is crucial for dosing tDCS due to its role in the mechanism of action⁸ that can lead to inter- and intra- subject variability in

neuronal (polarization) response^{17,59}. Furthermore, tDCS can be a promising method to evoke increases in regional CBF⁴ to ameliorate hypoperfusion in cerebrovascular diseases, including cognitive rehabilitation. As shown in Figure 1, the inter-subject variability in the hemodynamic response (tHb changes) was captured using our parametric grey-box linear model; however, there was a large inter-subject variability, as shown in the MSE (see Table 2). Our study illustrated that such a system identification approach using a physiologically constrained linear model can capture the fNIRS-based CVR to anodal HD-tDCS in healthy humans where the pathway from perivascular potassium to vessel circumference presented the least MSE (median < 5%), as shown in Figure 5 (i.e., Pathway 3). Therefore, the primary mechanism of action for the HD-tDCS-induced CVR in this study is postulated to be perivascular potassium that may be a major determinant of the local pial vessel diameter⁶⁰. Guhathakurta and Dutta²⁵ have postulated that the pial arteries and arterioles contain perivascular nerves within their adventitial layer that can be strongly affected by the tDCS electric field. Nevertheless, other pathways can also contribute partially to the hemodynamic response leading to inter-subject variability, as shown in Figure 1, where a weighted sum of the four grey-box linear models of the pathways may provide the best fit to the individual fNIRS-tHb data in our future studies.

The first model pathway considered the effect of tDCS on the synaptic space's vasoactive agent (potassium ions), which then has an influence on vessel response mediated through perivascular and astrocytic compartments. The linear analysis at the model's initial condition for this pathway was represented by seventeen states i.e., differential equations (details in supplementary materials) and presented in linear transfer function form as ratio of polynomials. The order of the transfer function denotes the number of poles in the obtained transfer function. The reduced grey-box model for the first pathway was represented by a twelfth order system. Also, the astrocytic driven and the perivascular potassium-driven pathways were analyzed and were represented by eleventh and ninth order, respectively. For the fourth pathway, the input path considered the influence of tDCS on SMC gated ion channel current. The reduced model for this pathway was represented as a seventh order system. The response function of pathway 1 to the input square test pulse of two seconds (see Figure 3) peaked around 5 sec comparable to the known hemodynamic responses⁶¹. Here, our study attempted to identify the role of various neuronal and non-neuronal pathways using a lumped grey-box model of NVC has on the vascular response to anodal HD-tDCS.

The vessel response for the reduced grey-box linear model of the four pathways (see Figure 3) suggested that the time to peak was least (2.231 sec) when the tDCS vasoactive effects were via the ion gated channels on the SMC when compared to the astrocyte mediated or the synaptic pathways. The reduced grey-box linear model of the four pathways did not investigate the effects of the electric field on the low-frequency (0.05 – 0.2 Hz) oscillations driven by nonlinear calcium dynamics and focused on the initial transient fNIRS-tHb response to tDCS. Therefore, the data was down-sampled to 0.1 Hz to study the slow transient response during 10 minutes (600 sec) of tDCS. However, this significantly reduced the ~0.1 Hz hemodynamic oscillations in the fNIRS-tHb response, also the spontaneous rhythmic 'vasomotor' oscillations in the frequency range of

0.05 – 0.2 Hz⁶². This will be investigated in the future by adding the nonlinear calcium dynamics in our model. Here, ~0.1 Hz hemodynamic oscillations can be related to the synchronization of the intermittent release of calcium within vascular SMC⁶² that was modeled (details in the supplementary materials, see equations 48-49) and shown by a physiologically detailed model response (see Figure 2b). Our prior works have found a significant cross-correlation between log (base-10) transformed electroencephalogram (EEG) band-power (0.5-11.25Hz) and fNIRS oxy-hemoglobin signal in the low frequency (≤ 0.1 Hz) range⁵⁰. Investigation of this ~0.1 Hz oscillatory vessel response vis-à-vis neuronal response (EEG) to tDCS will require a parameterized coupled oscillator model of the physiologically detailed model as future work. Here, the relation of the ~0.1 Hz oscillatory vessel response vis-à-vis neuronal response may be related to the cortical excitability changes to anodal tDCS⁶ due to the involvement of calcium dynamics which needs future investigation. However, the initial dip in the grey-box linear model response (see Figure 4) provided a mechanistic understanding of the initial dip found in the fNIRS oxy-hemoglobin (also, total hemoglobin) signal in our prior work on chronic (>6 months) ischemic stroke survivors⁵¹. Although the shorter (2-3 seconds) initial dip in the fNIRS oxy-hemoglobin can be explained by "metabolic hypothesis" (metabolic mismatch) in health⁶³, which may be longer due to a delayed hemodynamic response in the ischemic brain⁶⁴; nevertheless, longer (~10 sec) initial dip in the vessel response (see Figure 4) can also be due to a greater total hemoglobin or blood volume change in disease⁵¹ that needs further investigation using fMRI in conjunction with tDCS.

Limitations of this study include the methodical limitations of the fNIRS technique⁶⁵. The fNIRS signal acquired with optodes placed on the scalp can represent different hemodynamic signal sources (cerebral versus extra-cerebral) as well as different physiological cause (neuronal versus systemic) that can be evoked by tDCS. Due to the lack of short-separation channels in this pilot study to perform short source-detector regression to remove extra-cerebral hemodynamics, we performed a data-driven principal component analysis to identify the extra-cerebral signal components that explained the greatest amount of covariance across all the spatially symmetrically distributed fNIRS channels. Since we found that the pathway 3 presented the least MSE, as shown in Figure 5, so the fNIRS-tHb signal is postulated to have significant representation from superficial pial vessels. However, differential activation of the oxy-hemoglobin and deoxy-hemoglobin indicated neuronal activation in the cerebral capillaries in the vicinity of the HD-tDCS electrodes (see supplementary materials), which was used as a marker of neuronal cause. During grey-box modeling, the physiologically detailed model was substantially simplified by model linearization that lost nonlinear system dynamics, where calcium dynamics may be important for the understanding of the effects of tDCS on the 'vasomotor' oscillations in the frequency range of 0.05 – 0.2 Hz⁶². The parametrized grey-box linear model was then fitted to the slow transients in the fNIRS-tHb data, where we performed downsampling to 0.1Hz that removed oscillations in that frequency range of 0.05 – 0.2 Hz. Here, our investigation considered the primary or acute effects of anodal tDCS by considering potassium ions as the main vasoactive agent and assumed a lumped response of the NVS. The aftereffects of tDCS were not captured by this proposed model.

In summary, our study presented a preliminary investigation of linear systems analysis using a physiologically-constrained grey-box model that was found useful to explore various pathways in a lumped NVC model related to CVRmodulated by HD-tDCS. The objective was to understand the mechanisms of the fNIRS based CVR to tDCS which can be a therapeutic intervention in cerebral hypoperfusion. Such a mechanistic understanding of the action using fNIRS-tHb data can help in developing an individual safe dose since the electric field can also increase BBB permeability⁸. Furthermore, such grey-box linear systems analysis using fNIRS-tHb data from individuals with pathological conditions⁵¹ can elucidate dysfunction in various pathways of the NVC, i.e., due to the pathological infestation. Here, our proposed linear systems analysis using the grey-box model is amenable to pole-zeros analysis of the transfer function for various pathways of the neurovascular system in health and disease that can also be used to classify the dysfunction.

Materials and Methods

(A) Participants and the Experimental Protocol

Eleven healthy subjects (1 female, 19-45 years old) voluntarily participated in this study that has received Ethics approval by IRB of Euromov (University of Montpellier, France), and in accordance with the Declaration of Helsinki. The subjects had no known neurological or psychiatric history, nor any contraindications to tDCS. In this study, the pilot data from our prior work on online parameter estimation with an autoregressive model⁵⁰ was analyzed with a grey-box model for a further mechanistic understanding of the action.

During the experiment, the subjects were comfortably seated with eyes-open in an armchair with adjustable height. The set-up of the high-definition (HD) tDCS electrodes and NIRS optodes were mounted on the surface of the scalp according to the 10/10 system (see Figure 6). The anodal HD-tDCS (Startim®, Neuroelectronics NE, Barcelona, Spain) was configured in a 4×1 ring montage with the anode placed in the center (C3) in a region overlying the left primary sensorimotor cortex (SMC), and the return electrodes were placed approximately 4 cm away at FC1, FC5, CP5, and CP1 in the 10/10 system (see Figure 6). In the rest of the document, 'HD-tDCS' means 'anodal HD-tDCS' since we only performed anodal tDCS for this human pilot study. Measurements of changes in eyes-open resting-state oxy-hemoglobin (oxy-Hb) and deoxy-hemoglobin (deoxy-Hb) concentrations from the bilateral SMC were made from a 16-channel continuous-wave NIRS system (OxymonMkIII, Artinis Medical Systems, Zetten, The Netherlands) at a sampling frequency of 10 Hz. The receiver-transmitter distance of 3 cm was based on our prior work⁵⁰. The receivers (Rx) were placed on the FC3 and CP3 for the left hemisphere and FC4 and CP4 for the right hemisphere. Transmitters (Tx) were placed diagonally, i.e., at P1, P5, C1, C5, F5, and F1 for the left hemisphere and at P6, P2, C6, C2, F2, and F6 for the right hemisphere, as shown in Figure 6. The HD-tDCS was delivered using PISTIM (Neuroelectronics NE, Barcelona, Spain) electrodes (contact area 3.14cm^2) over the left M1 region to deliver 2mA current for 10 minutes, with ramp up and ramp down period of 30 seconds. Eyes-open resting-state data for changes in oxy-Hb and deoxy-Hb was recorded. The local effect of HD-tDCS was considered for the grey-box modeling,

so the average values of total haemoglobin concentration changes ($= \text{oxy-Hb} + \text{deoxy-Hb}$) were obtained from optodes in the vicinity of 4x1 HD-tDCS electrodes: C5-CP3, C1-CP3, C5-FC3, and, C1-FC3. The criterion to confirm the signal quality is the visual inspection for the presence of cardiac pulsation, in either the time or the frequency domain (peak around 1Hz cardiac frequency). Due to the lack of short-separation channels to perform short source-detector regression to remove extra-cerebral hemodynamics, we performed data-driven principal component analysis (PCA) to identify the signal components that explain the greatest amount of covariance across all the spatially symmetrically distributed 16 channels. This pre-processing of the fNIRS data was performed with HOMER2 (v2.2) routines (hmrIntensity2OD and hmrOD2Conc, respectively) using the modified Beer-Lambert law and their standard pipeline (further details⁵⁰). Specifically, motion correction (hmrMotionCorrectWavelet) and zero-phase bandpass filtering ("hmrBandpassFilt") was performed to extract the frequencies between 0.01 Hz and 0.1 Hz across all the 16 channels. The differential activation of oxy-Hb and deoxy-Hb was found in the vicinity of 4x1 HD-tDCS electrodes: C5-CP3, C1-CP3, C5-FC3, and, C1-FC3 that indicated neuronal activation by tDCS. Therefore, the change in the total haemoglobin concentration (a measure of blood volume) was computed by adding the oxy-Hb and deoxy-Hb time series, which was down-sampled to 0.1Hz to study only the slow transient response during HD-tDCS.

Figure 6. HD-tDCS-fNIRS montage on a 10/10 EEG system. The fNIRS time series data from the transmitter-receiver optode combinations in the vicinity of 4x1 HD-tDCS electrodes (C5-CP3, C1-CP3, C5-FC3 and, C1-FC3) were used for the grey-box modeling.

(B) Grey-box Linear Model Dynamics and Assumptions

The proposed model's block diagram is given in Figure 7. The study uses a physiologically constrained linear model to track the total hemoglobin concentration changes measured using fNIRS due to acute effects of the tDCS electric field and current density in the neurovascular tissue.

The induced current density in the ohmic head model during the application of tDCS was assumed to have a vasoactive influence via a linear transfer function mapping to the vasoactive signal. Prior works have shown the intricate path from tDCS current to the synaptic transmembrane current, considering only excitatory effects that are mapped through a sigmoid function as presented by Molaee-Ardekani et al⁶⁶. Then, the change in the concentration of various vasoactive agents like adenosine, nitric oxide, potassium due to change in synaptic transmembrane current was represented as a vasoactive signal considering first-order Friston's model⁶⁷. In this study, the process of transforming tDCS current to vasoactive signal was implemented in the neuromodulation block (see Figure 7) in the lumped model and was represented by a first-order linear filter, as shown in the equation 1 below. Here, the input signal is tDCS current density ($I_{tdcs}(t)$), operating in the linear part of the sigmoid function, that was mapped to the vasoactive signal ($v(t)$) using a first-order model, as shown in the Laplace domain as:

$$v = \frac{K}{s/\tau + 1} I_{tdcs}(s)$$

where K is arbitrary gain considering the linear part of the sigmoid function, and τ was selected as 20ms since the typical membrane time constant is 10–20ms.

In this study, the lumped model of the neurovascular coupling assumed a system of inputs (vasoactive signal) and outputs (hemodynamic changes in terms of vessel circumference). The vasoactive signal following synaptic transmembrane current affected by tDCS with a 20ms time constant (from equation 1) was assumed as an input to the lumped model of the neurovascular compartments, as shown in Figure 7. The dynamics of the neurovascular unit were considered through chemical signaling mechanisms across four compartments whose details are given in the next section. For modeling the vessel function, a cylindrical component having a unit length along the axis was considered as a lumped model of the blood vessel^{56,57}. The hemodynamic response, i.e., the vessel circumference obtained through the neurovascular model, was mapped to the total hemoglobin (or blood volume) changes through the vessel volume function block. Under the assumption of small changes in the vessel response to tDCS, the changes in the blood volume were assumed to be a function of vessel circumference. The changes in the total hemoglobin were assumed proportional to the cerebral blood volume⁶⁸, which was obtained from the lumped vessel response, i.e., from the proportional changes in the circumference of the lumped blood vessel considering the small change approximations.

Figure 7. Block diagram of the proposed model

(C) Physiologically detailed Neurovascular Compartmental Dynamics

In this study, the mathematical model of a neurovascular unit (greyed box in Figure 7) was developed from published literature⁵⁴⁻⁵⁷ that was for analyzing the neurovascular compartment dynamics. The major signaling pathways considered in these models were: neural synaptic potassium (K⁺) and glutamate to astrocytes, K⁺ signaling between astrocytes, and microvasculature and microvasculature to astrocytes via astrocyte perivascular endfoot⁵⁶. For the present study, the pathway from the neural synaptic activity to the vessel circumference with four compartments, synaptic space, intracellular astrocyte space, perivascular space and, arteriolar smooth muscle cell intracellular space, was used to model the neurovascular unit part of the model. The equations are given in the supplementary material. The model dynamics were represented by seventeen ordinary differential equations in the compartments. The state variables for various compartments of the neurovascular unit used in the present study is given in Table 3.

Compartment			
Synaptic Space	Astrocytic Intracellular Space	Perivascular Space	Arteriole smooth muscle cell (SMC) intracellular space
Potassium concentration in the synaptic space (<i>potassium_s</i>)	Astrocytic Inositol trisphosphate (<i>ip3</i>)	Perivascular potassium concentration, (<i>potassium_p</i>) Perivascular calcium concentration, (<i>calcium_p</i>)	Open KIR (Inward Rectifying Potassium) channel probability (<i>k</i>)
	Astrocytic intracellular calcium concentration, (<i>calcium_a</i>)		SMC Membrane Potential (<i>V_m</i>)
	Gating variable (<i>h</i>)		Open potassium channel probability (<i>n</i>)
	TRPV4 (Transient Receptor Potential Vanniloid Related 4) channel open probability (<i>ss</i>)		Calcium concentration in the SMC (<i>calcium_smc</i>)
	Calcium dependent EET (Epoxyeicosatrienoic Acid) production in the cell (<i>eet</i>)		Fraction of attached cross bridges (<i>omega</i>)
	Open BK(Big Potassium) channel probability (<i>nbk</i>)		Normalized contractile component of length (<i>yy</i>)
	Astrocyte Membrane Potential (<i>V_k</i>)		Mean circumference of the vessel (<i>x</i>)

Table 3. State variables for the lumped model of the neurovascular unit in the physiologically detailed model considering compartmental equations from published literature⁵⁴⁻⁵⁷

The synaptic transmission is assumed to be modulated through the current density induced by tDCS⁶⁹. Then, its vasoactive signal-induced activation of various neurovascular coupling pathways leading to the acute hemodynamic effects of tDCS. Here, studies have shown that potassium ions can act as a potent vasodilator signal that couple local neuronal activity to vasodilation in the brain, and have a major role in cerebrovascular mechanisms^{60,70-72}. Furthermore, studies have shown that the potassium pathway is responsible for the fast onset of

vasodilation compared to the other mediators⁷³⁻⁷⁵. Therefore, to model the immediate effect of tDCS, potassium currents were taken as the primary vasoactive signal in the model. The modulation of potassium through tDCS is represented mathematically as a first-order filter with a time constant of 20ms, as in equation 1.

Figure 8. Schematic representation of the four pathways for grey-box model analysis. The four simulated pathways are, 1: tDCS current density modulating vessel response through synaptic potassium pathway, 2: tDCS current density modulating vessel response through astrocytic membrane potential, 3: tDCS current density modulating vessel response through perivascular potassium currents, and 4: tDCS current density modulating vessel response through voltage gated ion current channels on the smooth muscle cells

(D) Investigation of the Four Pathways: Vessel Response during anodal tDCS

For evaluating vascular response due to tDCS using the physiologically detailed Neurovascular Compartmental Dynamics, four pathways were analyzed: through synaptic space, astrocytic space, perivascular space, and smooth muscle cell space, as depicted in Figure 8. The first pathway is considered modulation of synaptic mediators by the current density induced by tDCS and mediating the effect on the vessel circumference via perivascular and astrocyte compartments through neurovascular dynamics. This is based on the neurovascular coupling mechanism under the effect of current stimulation, where the neuronal activity drives the hemodynamic response. The second pathway considered the change in the membrane potential of the astrocytic compartment, which then drives the vascular response during tDCS. The third pathway is considered modulation of potassium currents in the perivascular space by the current density induced by tDCS. The fourth pathway assumed the direct influence of current density on the smooth muscle cells (SMC) of the vasculature. Here, prior works provided evidence that the voltage-gated potassium channels on the SMC can respond to the electric field^{76,77}, especially in the pial vasculature. In this proposed model for the fourth pathway, an additional input to the

SMC's membrane potential (V_m) was modulated by the electric field (and current density) induced by the tDCS that modulated the voltage-gated potassium channel currents.

Similarly, an additional input to the astrocyte's membrane potential and the perivascular potassium currents accounted for the direct effects of the electric field (and current density) induced by tDCS. All these equations are given in the supplementary material. In this study, our goal was to investigate the role of various neuronal and non-neuronal pathways leading to the CVR to tDCS, as demonstrated by our prior works^{6,50,51}, through an objective physiologically constrained grey-box modeling that is summarized by a block diagram in Figure 8.

(E) Physiologically constrained Grey-box Model Analysis

The physiologically detailed model from Witthoft and Karniadakis⁵⁶ consisted of a set of ordinary differential equations that were implemented in the Simulink environment (Mathworks Inc., USA) to be simplified for this grey-box modeling study. In this lumped model of the neurovascular dynamics, the vascular response was analyzed based on the four pathways (see Figure 8): tDCS current density modulating synaptic potassium, which then influenced vessel response through astrocytes, tDCS current density modulating the astrocytic membrane potential, tDCS current density effects modulating the potassium concentration in perivascular space, and the tDCS current density modulating ion-gated channel current on smooth muscle cells which then modulated the vessel response. The grey-box linear model analysis required simplification of the physiologically detailed model via model linearization under the assumption that the tDCS electric field effects were small, operating at the resting-state baseline conditions of the neurovascular dynamics. With such a physiologically-constrained grey-box linear model, system identification techniques were used to characterize the vessel response based on experimental fNIRS-tDCS data.

The physiologically detailed neurovascular coupling from published literature models⁵⁴⁻⁵⁷ (all the ordinary differential equations and parameters in the supplementary materials) were simulated using ODE23TB solver in Simulink (Mathworks, Inc., USA) as the benchmark of the CVR to tDCS. Then, the model reduction of the four pathways in the physiologically detailed neurovascular coupling model was performed separately using the Simulink (Mathworks, Inc., USA) linear analysis tool. This tool allowed the linearization of complex nonlinear models at the baseline operating point of the physiologically detailed neurovascular coupling⁵⁴⁻⁵⁷. Therefore, the linearized grey-box model was constrained by the individual physiology of the respective four pathways in the physiologically detailed neurovascular coupling model (see Figure 8). Then, the values of the free parameters (poles and zeros) of the parametric grey-box linear model were updated using the "Refine Existing Model" approach in the System identification toolbox to improve the fit to the experimental fNIRS-tDCS total hemoglobin changes to tDCS individually in eleven healthy subjects (detailed in section Participants and the Experimental Protocol).

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Compliance with ethical standards

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