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Graviceptive Neglect treatment in acute stroke using transcranial Electrical stimulation clinical trial (GravNegElect): rationale, objectives, and design

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*Grav*iceptive *Neg*lect treatment in acute stroke using transcranial *Elect*rical stimulation clinical trial (*GravNegElect*): rationale, objectives, and design.

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Abstract

Background: Stroke is one of the leading causes of disability globally and is often related to poor functional recovery and a high risk of falling. These outcomes correlate closely with an impaired perception of visual verticality (VV), a frequent condition referred to as visual graviceptive neglect (V-GN). Our prior findings indicate that post-stroke VV is most frequently rotated away from the lesioned hemisphere in the roll plane. Furthermore, we demonstrated that a focal transcranial direct current stimulation (tDCS) montage (known as high-definition tDCS; HD-tDCS) can induce a remarkable and sustained rotation away from the stimulated hemisphere when positioned over the temporo-parietal junction (TPJ), a key integrative cortical hub for VV. Therefore, we hypothesize that stimulating the contralesional TPJ in sub-acute stroke individuals will correct their V-GN. **Methods:** This phase IIa, randomised, double-blind, sham-controlled clinical trial aims to determine whether HD-tDCS can safely correct V-GN in individuals who have experienced a stroke affecting either hemisphere. Ancillary aims are to investigate whether HD-tDCS influences ocular torsion; explore neuroanatomical characteristics related to V-GN; investigate the relationship between EEG functional connectivity and V-GN; and determine the cerebrovascular reactivity effects of HD-tDCS. A total of 28 individuals diagnosed with acute or early sub-acute unihemispheric ischemic stroke (50% with right hemisphere lesions) will be randomized to receive six 20-minute sessions (conducted within 2 days) of either 2mA cathode-centre HD-tDCS or sham stimulation over the contralesional TPJ. VV will be assessed using the bucket method. The primary outcome is the change in VV from baseline to immediately after the intervention protocol, while the secondary outcome is VV assessed at 1 month post-intervention. Fundus photography (ocular torsion), vascular reactivity using transcranial Doppler, and resting state EEG (functional connectivity) will be assessed at the same time-points as VV. Structural and resting state functional MRI will be performed 2-15 days after stroke onset. Fractional anisotropy within the V-GN network regions-of-interest (ROIs) and transcallosal TPJ tract will be correlated with baseline VV and V-GN response to intervention. **Discussion:** This trial has the potential for a significant public health impact by providing foundational clinical and physiological evidence to develop future clinical interventions for V-GN.

Keywords: Graviceptive Neglect; Verticality Perception; Non-invasive Brain Stimulation; High-Definition Transcranial Direct Current Stimulation.

Trial registration: ClinicalTrials.gov NCT06747676. Registered on 12 December 2024. Version 2.

Introduction

Stroke is the third leading cause of disability globally, and evidence-based rehabilitation interventions to improve the burden of stroke remain limited [1–4]. One critical symptom after stroke for which there is no established treatment is graviceptive neglect (GN), the abnormal verticality perception observed after brain lesions in either hemisphere that can manifest across visual, haptic, and postural perceptual modalities [5–8]. This condition may present with or without its severe behavioural manifestation, lateropulsion [9].

Visual GN (V-GN) is the incapacity to visually judge the orientation of the body or environment without external reference cues [10–15]. Without visual input, haptic GN (H-GN) refers to the inability to manually position an object (such as a stick or rod) in a vertical orientation, whereas postural GN (P-GN) is defined as the incapacity to correctly perceive the upright orientation of one's own body [16,17]. In general, the error in the verticality perception is tilted to the contralesional side after supratentorial lesions [5,18–20]. Due to lack of awareness, individuals with GN typically do not report an abnormal sense of upright, which may remain undetected unless appropriately assessed [21].

Descriptions of GN frequency vary across studies, and V-GN is the best-studied verticality-perception modality. After recent cerebral hemispheric strokes, Yelnik et al. [5] observed V-GN in 57% of individuals (65% right lesions; 50% left lesions), while 89 to 94% of individuals with brainstem lesions were reported by Dieterich and Brandt [22,23].

Individuals with GN tend to align their bodies with their perceived vertical reference, leading to postural imbalance and loss of autonomy, with increased risk of falls [8,24]. It is worth noting that postural balance relies on multimodal integrative neurological functions [7,18]. Robust evidence suggests that GN can have a major impact on global functionality measured using the modified Rankin

Scale and Functional Independence Measure [8,25]. As well, GN has been characterized as a strong independent factor for balance recovery after stroke [7,8,25]. Most recently, lateropulsion was characterised as the primary cause of balance and gait disorders at the subacute stage after stroke [26]. Yet to date, there are no effective methods to therapeutically improve this prevalent disorder, as no Phase III pivotal trials have yet confirmed the efficacy of potential treatments [9].

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation (NIBS) strategy that involves weak electrical current delivered via scalp-surface electrodes and modulates the neuronal firing rate (without overt synaptic activity) [27,28]. Typically, tDCS induces an increase in excitability under the anode electrode and a decrease under the cathode electrode [29]. The neuromodulatory effects of NIBS can be observed following direct targeted stimulation by local effects or distant areas of its neural network, with or without direct axonal projections [30,31].

NIBS has been extensively used in experimental neurology to induce a transient disruption of a function ("virtual lesion") in healthy subjects mimicking clinical symptoms in several neurological domains [31–37]. Consequently, if a brain area is related to one behaviour, specific NIBS targeting involved areas can result in a transient influence on the performance of that behaviour. Moreover, the "Interhemispheric Rivalry Model" proposes that excitatory and inhibitory NIBS protocols can be applied to restore interhemispheric excitability imbalance after brain lesions [38,39].

In stroke individuals, the severity and functional recovery of spatial perception disorder (spatial neglect, SN) were reported to be directly related to the interhemispheric (transcallosal) excitability imbalance [40]. Yet, the variability of lesions and their respective neuroplastic adaptive mechanisms after brain damage requires predictive algorithms to develop patient-centred therapeutic strategies.

We followed the model used to develop NIBS SN treatment, based on the "Interhemispheric Rivalry Model" that progressed from the induction of a "virtual-lesion" after a single session leading to reversible effects in healthy subjects [39–43], subsequent testing a single-session protocol in participants after stroke [44], and later develop a treatment using an exposure of multiple NIBS sessions that resulted in longer-lasting clinical effects [45]. Similar to the response described in individuals with SN after stroke, either an excitatory NIBS protocol over the lesioned hemisphere or an inhibitory NIBS targeting the contralesional hemisphere could plausibly be effective to treat GN after stroke [17,46–52].

The choice of an inhibitory protocol on the contralesional side would be considered safer for individuals in the post-stroke acute and early subacute phases and more effective for targeting the intact cortex.

The temporoparietal junction (TPJ) is a rational target region in post-stroke GN, as it is a critical hub for multisensory integration and processing [53]. Essential for verticality perception and postural control [54–56], the TPJ typically exhibits right-hemisphere dominance [15,57–59]. Targeting the TPJ, we recently demonstrated that a single session of conventional tDCS could manipulate visual verticality perception [60] of healthy participants and postural control [61] of post-stroke participants in a polarity-dependent manner during and after conventional tDCS over TPJ bilaterally (bipolar-balanced montage) [62]. An alternative approach involves high-definition tDCS (HD-tDCS), which employs small-diameter gel electrodes in a concentrated configuration to achieve a more focused stimulation field [63]. This technique holds significant promise for neurorehabilitation because it can elicit more pronounced and longer-lasting changes in cortical excitability compared to conventional tDCS [64,65].

Through sham-controlled studies, we advanced our investigation by using HD-tDCS over the TPJ, sparing the vestibular receptors to induce a top-down influence and a high-level process of modulation [66]. Our HD-tDCS protocol demonstrated good safety and tolerability, alongside significant behavioural and physiological effects [66]. More recently, we reported the consistent induction of transient, clinically significant V-GN with large effect sizes in healthy adults following 20 minutes of stimulation to either the right or left hemisphere [67].

Consequently, the current study builds upon these foundations. This phase IIa randomised, double-blind, controlled clinical trial primarily aims to demonstrate the preliminary efficacy of HD-tDCS in treating V-GN and to refine the parameters necessary for a definitive phase III trial. Furthermore, we seek to confirm the safety and tolerability of this HD-tDCS application specifically within a post-stroke population.

Visual verticality perception arises from the multisensory integration and processing of information from the eyes, head, body, and environment [68–71]. The brain interprets retinal images and integrates them with other sensory information to maintain accurate upright perception [72,73]. Consequently, V-GN can be influenced by ocular torsion, frequently presenting alongside ocular torsion deviations following subcortical brain lesions [74]. However, it remains unclear whether cortical areas can also

modulate ocular torsion [75]. Therefore, an ancillary aim of this clinical trial is to investigate whether HD-tDCS influences ocular torsion in stroke survivors with V-GN.

As part of a biomarker investigation, we will explore neuroanatomical characteristics to establish relationships among V-GN severity, the extent of TPJ damage, and, more broadly, the putative V-GN Network (V-GNnet) [23,76,77]. Our study will also evaluate the intervention response relative to the transcallosal TPJ tract. Since this addresses a longstanding gap in the literature, the theoretical framework for the transcallosal tract draws on recent evidence on spatial perception and other brain functions in healthy and post-stroke participants. These findings implicate transcallosal integrity as a relevant predictor, supporting that interhemispheric dynamics underlie the response to neuromodulation [78–82].

Furthermore, the application of HD-tDCS has the potential to induce plastic changes in both the directly stimulated regions and the interconnected neural networks. Such effects may restore functional connectivity, re-establish disrupted pathways, and ultimately foster new connections that support the recovery of impaired functional networks [83,84]. In post-stroke individuals, specific resting-state EEG (rsEEG) features could facilitate the diagnosis and evaluation of V-GN, enabling topographic mapping of the target cortical areas. Quantitatively, rsEEG analysis provides an objective estimation of the modulated cortical territory and the pathways likely affected. Additionally, post-stimulation rsEEG recordings will enable the assessment of HD-tDCS outcomes by comparing pre- and post-intervention connectivity through dynamic network analysis. Consequently, as an exploratory objective, this study will investigate the associations between EEG functional connectivity, baseline V-GN, and the subsequent response to neuromodulation.

HD-tDCS may influence the cerebrovascular system through a direct effect of the electric current on the brain's blood vessels and an indirect effect, which occurs when the modulated neuronal activity changes the cerebral blood flow via neurovascular coupling [85,86]. Under the same HD-tDCS montage of our behavioural clinical trials, we found no evidence of change in middle cerebral artery blood flow velocity (MCA-BFv), ipsi or contralateral to the 2-minute HD-tDCS protocol. These results suggest that HD-tDCS-induced behavioural effects may be independent of a vascular response leading to gross regional blood flow change and support the safety of our protocol [87]. However, the need for further

exploration is acknowledged, given that the potential vascular response to brain electric fields might fluctuate over time and that effects of longer-duration stimulation, such as that used therapeutically, might differ. The available literature is limited, and no study has analysed the effects of HD-tDCS over the TPJ on cerebral blood flow post-stroke to date. Thus, we will determine the bilateral cerebrovascular reactivity effects of HD-tDCS for 20 minutes, using transcranial Doppler Ultrasound in post-stroke individuals.

Study objectives

The primary aim of this study is to analyse the effects of HD-tDCS on V-GN in individuals after sub-acute stroke. We hypothesize that active cathode HD-tDCS will reduce V-GN compared to sham stimulation, with verticality correction shifting away from the targeted hemisphere. As secondary analyses, we plan to explore the effects of HD-tDCS on ocular torsion after sub-acute stroke; determine whether the structural integrity of V-GNnet and transcallosal TPJ tract, and V-GNnet rs-fMRI functional connectivity predicts response to HD-tDCS; explore how baseline interhemispheric rsEEG functional connectivity relates to response to HD-tDCS; and assess the effects of HD-tDCS on bilateral vascular reactivity in individuals with subacute stroke.

Methods

Ethics

The study protocol was approved by the Institutional Review Board of our institution (CAAE: 74877723.4.0000.5440) and will follow Standard Protocol Items Recommendations for Interventional Trials (SPIRIT) guidelines for nonpharmacologic trials. All participants will provide written informed consent. This study was registered on http://www.clinicaltrials.gov (NCT number, NCT06747676).

Protocol design

This is a phase IIa randomised double-blinded controlled clinical trial with a parallel design and

two arms. The overall data collection structure is shown in Figure 1.

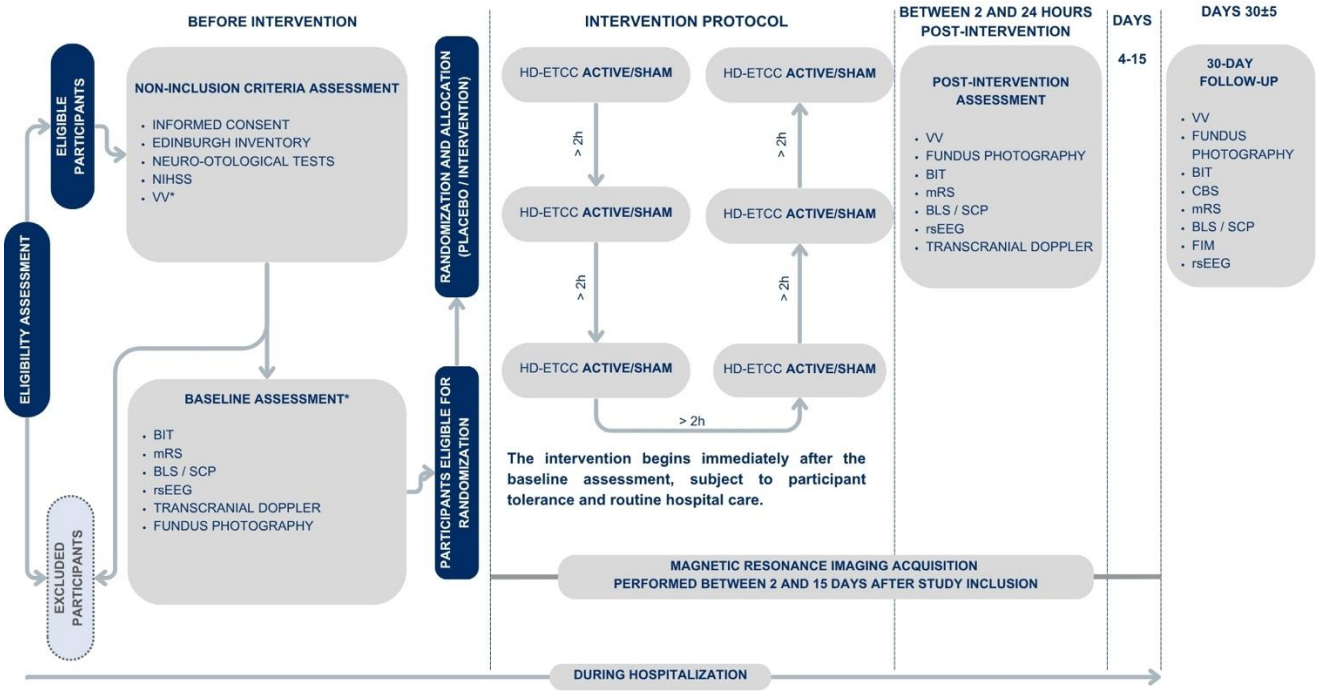


Figure 1. Timeline and overall structure of data collection.

Participants

The study will include 28 individuals following acute or early sub-acute unihemispheric ischemic stroke (14 subjects per arm; 50% with right hemisphere lesions in each group). Table 1 summarizes the expected sociodemographic characteristics of the study population. Post-stroke individuals will be screened upon hospital admission, and systematic screening will follow our established Visual Vertical Stroke Registry protocol. On a daily basis, the research assistant will review medical records of all admitted individuals against the following inclusion criteria: adults aged 18–80 years; ischemic stroke (excluding cerebellar and brainstem lesions) confirmed by neuroimaging and clinical assessment; individuals with previous stroke are eligible if the modified Rankin score is less than 1 and symptoms are unrelated to vestibular or postural control deficits; clinically stable (stable vital parameters for ≥24 hours; no chest pain in the previous 24 hours; no significant arrhythmia; no evidence of deep vein thrombosis); normal or corrected-to-normal vision; naïve to and blinded regarding the HD-tDCS intervention and study objectives; able to provide informed consent (participant or legal representative); and able to comply with the intervention and evaluation schedule.

The study will employ the following exclusion criteria: migraine; pregnancy; cardiac pacemaker or other metal implants; history of seizures; claustrophobia or inability to undergo MRI; other neurological disorders; psychiatric disorders; sensitive scalp or previous brain surgery; previous experience with HD-tDCS; and uncontrolled medical conditions (e.g., end-stage cancer or renal disease).

Participants meeting inclusion and exclusion criteria will be further assessed for the following non-inclusion criteria: left or mixed-handedness (determined by Edinburgh Handedness Inventory); peripheral vestibular deficits identified on neurotological examination (oculomotor tests, head shake test, head thrust test, Dix-Hallpike test, Pagnini-McClure test); previous musculoskeletal disorders affecting postural alignment and balance (e.g., moderate-to-severe scoliosis, torticollis); global or Wernicke's aphasia; and absence of V-GN.

Table 1. Expected sociodemographic characteristics of the study population.

Non-clinical participant characteristics	
Characteristic	**The population expect to be included**
Age	Adults aged 18 to 80 years. In line with the age distribution of stroke, more than 50% of the sample is expected to be aged over 50 years [88,89] .
Sex	Individuals of both sexes — female and male.
Gender	Women and men, including transgender individuals, as there is evidence of increased stroke risk in this group [90] .
Race, ethnicity and ancestry	Recruitment will aim to reflect the ethnic diversity of the local community, matching the composition of the geographical area studied.
Socioeconomic status	The trial will aim for broad participation across the full socioeconomic spectrum, from the least to the most deprived groups.
Geographic location	Participants will be recruited from acute care hospitals, including inpatients, and the trial will aim to include residents of both urban and rural areas.

Clinical characterisation of participants

The clinical characterisation of the enrolled participants will comprise the following instruments: National Institutes of Health Stroke Scale (NIHSS); Functional Independence Measure (FIM); Behavioural Inattention Test (BIT); Burke Lateropulsion Scale (BLS) and Scale for Contraversive

Pushing (SCP); Edinburgh Handedness Inventory. Figure 2 presents the participants' timeline according to the SPIRIT guidelines, detailing the schedule of enrolment, interventions, and assessments.

	TRIAL PERIOD							
	Enrolment		Post-randomization					Close-out
TIMEPOINT	$-t_i$ to 0	0	t_1	t_2	t_3	t_4	t_5	
ENROLLMENT:								
Eligibility assessment	X							
Informed consent	X							
Non-inclusion assessment	X							
Baseline assessment		X						
Randomization		X						
INTERVENTIONS:								
Active HD-tDCS			↔					
Sham HD-tDCS			↔					
ASSESSMENTS:								
Post-intervention assessment					X			
Follow-up assessment							X	
MRI acquisition*			↔		↔			
Safety			X	X				

Figure 2. SPIRIT figure. Participants' timeline: schedule of enrolment, interventions, and assessments. HD-tDCS, high-definition transcranial direct current stimulation. MRI, magnetic resonance imaging. t1 and t2: six sessions of HD-tDCS delivered over two days; t3: 2 to 24 hours after completion of the intervention protocol; t4: up to 15 days after study inclusion; t5: 30 days after the intervention.

Sample size calculation

The sample size calculation of our clinical trial will follow the pragmatic recommendations of Whitehead et al. [91] because the proposed study will be the first to assess the efficacy of transcranial electrical stimulation on visual verticality disorder in stroke individuals; our feasibility investigation in two individuals does not provide enough data for a sample size calculation; and our primary outcome

measure (VV_o; a difference between T0 and T1) is quantitative. Thus, considering a two-sided alpha of 0.05, estimated power of 80%, a standardised difference of 0.6, an inflation factor with 80% upper confidence limit, the present trial will require 12 participants per arm (two arms; total sample size of 24 participants) (for review see [91]). The increase in sample size due to attrition or the missing-data procedure will be 16% for the present study. Therefore, the total sample size will be 28 participants (14 per arm).

Intervention

Neuromodulation will be applied using a 3x1 ring electrode configuration (HD-tDCS) at 2 mA for 20 minutes. The surround electrodes will be placed on P4, C4, and T4 for right-hemisphere stimulation, and on P3, C3, and T3 for left-hemisphere stimulation. The centre electrode will be positioned at the circumcenter of the surround electrodes, which is slightly dislocated from the standard CP6 in the right hemisphere and CP7 in the left hemisphere (Figure 2A) [66,67]. The circumcentre position was chosen relative to the standard EEG coordinates because our prior electrostatic modelling using Finite Element (FE) methods for the HD-tDCS protocol demonstrated that the cortical electric field and current density would be largely confined to the boundaries of the surround electrodes and centred over the temporoparietal junction (TPJ) [92]. During stimulation, the participant, seated upright, will perform tasks assessing verticality perception, each lasting 5 minutes. The first task will involve using the non-affected hand to position tilted figures so that they align with the vertical lines on a whiteboard (Figure 2B). The second task will involve aligning the head vertically with a line presented in the mirror positioned approximately 2 metres in front of the participant, after the examiner has tilted the participant's head (Figure 2C). A 5-minute interval will be maintained between the two tasks.

Direct current (DC) will be generated by a 1x1 DC stimulator and then split into the 3 high-density Ag/AgCl sintered ring electrodes (Soterix Medical). Participants will be randomised to receive six sessions over 2 days, with intervals of at least 2 hours between sessions, of either the active cathode centre or the sham cathode centre condition. For appropriate comparison, the sham stimulation condition will consist of the same electrode positioning, with a ramp-up to 2 mA over 30 seconds and a subsequent ramp-down over 30 seconds. Baseline data will be collected prior to stimulation.

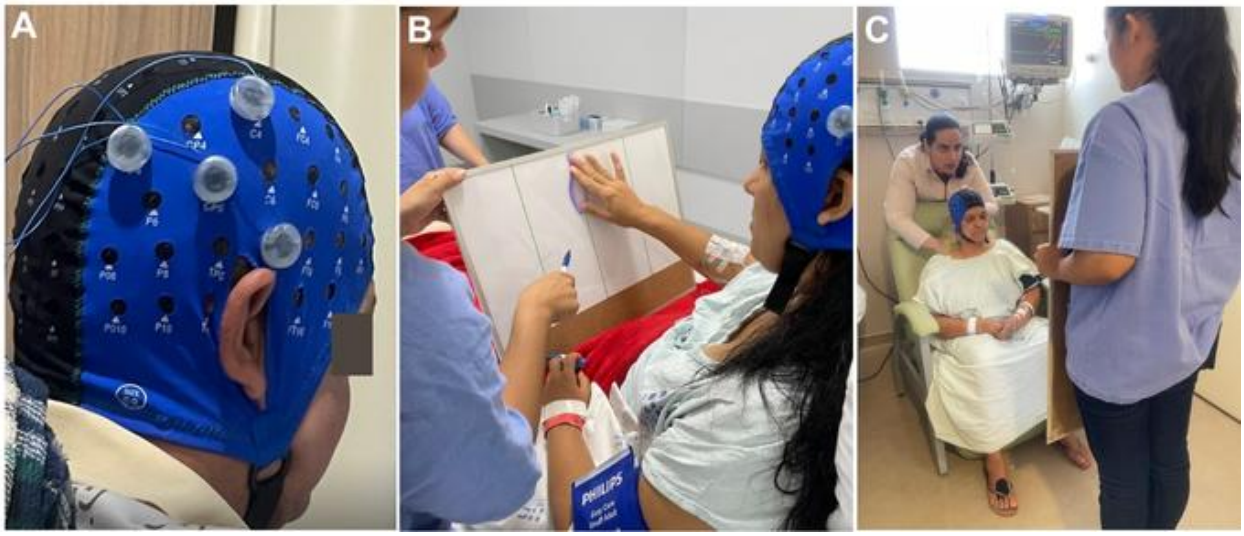


Figure 2. Cathodal HD-tDCS applied over the contralesional temporoparietal junction. **(A)** 3 × 1 HD-tDCS montage. **(B)** Non-affected hand task: positioning tilted figures to align with vertical lines on a whiteboard. **(C)** Head alignment task: aligning the head vertically with a line presented in a mirror positioned approximately 2 m in front of the participant after the examiner has tilted the participant's head. The participants in the images have provided informed consent for their publication.

Randomisation

The randomisation sequence will be built using R Project for Statistical Computing (Statistical Analysis System, version 9.4). Block randomisation with different block sizes will be used for HD-tDCS conditions, stratified to include 50% of right- and 50% of left-hemisphere stroke lesions in each arm.

Blinding and allocation concealment

Participants, outcome assessors, investigators, and the statistician will be blinded to the intervention. One research assistant will input the randomised HD-tDCS condition for each participant in the HD-tDCS device, while the other research assistant will be the outcome assessor.

Safety and tolerability

After each HD-tDCS session, the assessor will ask all enrolled participants to rate their

discomfort on a visual analogue scale from 0 to 10. Participants will also be instructed to report any adverse effects they experience to the research assistant after the intervention session, if they are related to the study protocol.

The adverse effects will be classified by the principal investigator as serious or non-serious, and as study-related, possibly study-related, or not study-related. All adverse effects will be reported to the Institutional Review Board (IRB) within 48 hours of knowledge of the event. We expect no serious adverse effects, such as seizures, hospitalisations, or unanticipated problems related to the intervention, because the stimulation protocol is within the safety limits described in the literature [93–95].

Stopping Rule

Participants would be required to discontinue the study if any serious adverse effects related to the stimulation protocol occur. Specific caution will be exercised with regard to the occurrence of a seizure or scalp burn at the site of the electrodes.

Data safety and management

All case report forms, whether paper-based or stored in Research Electronic Data Capture (REDCap), and containing confidential participant information, will be identified only by the participant's initials and a unique study number. All confidential information will be transferred and stored in anonymised form. In addition, all researchers involved in the study will receive training to ensure the protection of participants' rights and the integrity of the research project. Data sharing will comply with HIPAA regulations and institutional policies.

Feasibility

Non-adherence will be determined in two different ways: (a) if the participants fail to participate in the appointment for the intervention session, (b) if participants ask to interrupt the intervention in the middle of the stimulation. The non-adherence frequency will be observed. We will assume a conservative attrition rate of 16% in the sample size calculation for the present study.

To improve adherence, the following strategies will be implemented: (a) phone calls to

participants or their caregivers before each visit, (b) financial support for transportation to each study visit, (c) specialized training for staff to enhance communication and interviewing skills with patients, and (d) flexibility in scheduling sessions, including options for weekends.

We will use descriptive statistics to analyse the study feasibility through the following measures:

1. The number of eligible participants of the targeted population;
2. Recruitment rate within the study period (randomised participants divided by the number of months spent to complete the recruitment);
3. Retention (proportion of study-related dropouts);
4. Adherence (proportion of compliant participants to the study protocol).

Outcome Measures



Figure 3. Outcome measures assessment protocol in the GravNegElect trial. **(A)** Primary outcome: visual verticality perception assessed using the bucket test. The participant is seated upright, and angular deviation is measured with an inclinometer attached to the bottom of the bucket and recorded by an examiner. **(B)** Magnetic resonance imaging (MRI) is used to assess structural and functional connectivity and is acquired within 2 to 15 days after study inclusion. **(C)** Electroencephalography (EEG) is used for functional connectivity analysis. **(D)** Transcranial Doppler ultrasound is used to assess middle cerebral artery blood flow velocity for vascular reactivity analysis. **(E)** Fundus photography is performed with the participant seated upright and the head positioned on a chin rest for ocular torsion analysis. The participants shown in the images provided informed consent for publication.

Visual Verticality Perception (primary outcome)

VV will be determined using the bucket test described previously [66,96]. In addition to being extensively validated and accepted, this method was found to be better tolerated by individuals in the acute and early subacute post-stroke periods compared to virtual reality-based techniques in our institution. In brief, to perform the assessments, each participant will remain seated with back and foot support, with the trunk and head in a perfectly upright position, and visualise a black line (10.5 cm long, 0.4 cm wide) positioned at the centre of the bucket base, viewed internally. On the exterior surface of the bucket base, a calibrated digital inclinometer (precision ± 0.01°) will be used to indicate the angles. The examiner will manually rotate the bucket slowly in clockwise (+VV) and counterclockwise (–VV) directions while participants look into the bucket. Participants will verbally report when the line appears upright. The examiner who rotates the bucket and monitors head position will be blinded to inclinometer readings, while a second examiner records VV results (Figure 3A). All participants will complete at least 4 practice trials to control for learning effects. The VV assessment will consist of ten trials: 5 beginning at the upper edge of the line in the clockwise direction and 5 in the counterclockwise direction, presented in random order with a random initial angle.

The VV result will be expressed using 3 indices: "orientation" (VV_o; primary outcome measure) expressed by VV trial average of real numbers (with positive and negative values); "uncertainty-absolute" ($VV_{u\text{-}ab}$; exploratory outcome measure), as the mean of absolute values (without sign); and "uncertainty-standard deviation" ($VV_{u\text{-}sd}$; exploratory outcome measure), as the SD of the VV_o. The normal range for participant selection was defined as -2.5º to 2.5º for VV_o [69]. $VV_{u\text{-}sd}$ and $VV_{u\text{-}ab}$ followed a similar criterion of 2.5º. V-GN for each index (V_o-GN; $V_{u\text{-}ab}$-GN; $V_{u\text{-}sd}$-GN) was considered present when its measurement was outside the established VV normal range.

VV will be assessed at the following time-points: **T0**: baseline (before the first HD-tDCS session); **T1**: post-intervention (next day after the last HD-tDCS session); **T2**: 1-month post-stroke follow-up. The primary outcome measure will be the VV_o difference of **T0** (baseline) with **T1** (post six treatment sessions). Baseline to follow-up (**T0-T2**) will also be analysed secondarily. Spatial perception (using the behavioural inattention test) will be assessed alongside VV_o to explore the association between 'spatial' and 'verticality' perception changes across the intervention period, as well as lateropulsion.

Visual Verticality Perception analysis

A graphical investigation will be made to analyse the data distribution. Since we expect the raw data to meet the assumptions of normality, linearity, and homoscedasticity, parametric approaches are expected to be applicable. The mean change from baseline (T0) observed after stimulation protocol (T1) between groups will be analysed using a two-sided t-test for error in perception of verticality (VV_o; primary outcome). A similar analysis will compare the VV_o mean changes from T0 to T2 (secondary outcome). Primary analysis will use the intention-to-treat population, and secondary the per-protocol population. Missing data will be handled using the last observation carried forward method.

As an exploratory analysis, a linear mixed-effects model will be used to investigate the effects of high-definition transcranial direct current stimulation on VV over time. VV degree will be considered as the outcome variable, and the individual characteristics/responses as a random effect. The time and condition (cathode centre or sham) will be treated as fixed effects. The comparison among means will be made through orthogonal contrasts. The hypotheses involved in the contrasts will be tested using the t-test. Since the hypotheses will be defined a priori, no adjustments for multiple comparisons will be performed [97]. The two-sided testing hypotheses are that there will be a difference between sham and active conditions at each time point of assessment, and between baseline time points and the stimulation time points among stimulation conditions. Graphical analysis and repeated-measures correlation [98,99] will be used to assess the relationship between verticality (VV) and spatial (BIT) perceptual changes across the intervention period.

In all tests, a 5% level of significance will be used (two-sided). Statistical analyses will be performed using R software.

The calculation of effect size between each comparison described above will be performed by

$$Cohen's\ d = \frac{\bar{x}_1 - \bar{x}_2}{s_{pooled}}$$

where $\bar{x}_1$ and $\bar{x}_2$ are the sample means of the tDCS conditions, and s_{pooled} will be estimate by

$$s_{pooled} = \sqrt{\frac{s_1^2(n_1 - 1) + s_2^2(n_2 - 1)}{n_1 + n_2 - 2}}$$

where s_1^2, s_2^2, n_1 and n_2 are the respective variance and sample size of the tDCS conditions. The effect size will be calculated by dividing the mean estimated difference between baseline and the other time-points by the SD for each HD-tDCS condition[99]. Effect size will be considered small when d=0.2, medium when d=0.5, and large when d=0.8 [100].

Neuroimaging investigation (predictive factor)

Magnetic resonance imaging (MRI) acquisition will be performed on a Philips Ingenia 3.0T scanner (Philips Healthcare, Best, The Netherlands) equipped with a 16/32-channel system and a 60 cm bore diameter. The maximum field of view was 50 × 50 × 45 cm³. Images will be acquired using a dStream Head 32-channel coil and MR Systems Achieva dStream software, Release 5.7 (2021-10-04) (Figure 3B). MRI data will be collected 2 to 15 days after stroke onset. Lesions will be identified on 3D T1, and investigated using Axial T2, FLAIR, and DWI. The MRI acquisition protocol will include the following sequences: a) 3D T1 - weighted images (TR=9.7ms; TE=4ms; flip angle=12 degrees; matrix=256×256; FOV=256mm; thickness=1mm; providing an isotropic voxel of 1 mm3); b) Axial T2 (3mm thickness); c) 3D FLAIR (matrix=256x256; FOV=256mm; thickness=1mm; providing an isotropic voxel of 1mm3); and d) TOF 3D will be acquired in axial orientation using the following parameters: field of view, 20 cm; TR/TE, 25/3.5 ms; flip angle, 20°; matrix, 528x270; voxel size, 0.38x0.74x1.1 mm; reconstruction voxel size, 0.2x0.2x0.55 mm; acquisition time, 7m 5s; e) DTI: will be performed with 32 gradient directions, consisting of 72 axial images, 2mm thickness, with FOV=256x256mm (128x128 matrix, 2mm isotropic voxel), TR= 8391ms, TE=65ms and b factor=1000 s/mm2; f) Processing for the seed-to-voxel and ROI-to-ROI analyses will be performed using the CONN Toolbox (v22.a). The standard CONN preprocessing pipeline will be used for the preprocessing steps, including realignment, centring, slice-timing correction, artifact detection (ART), and segmentation and normalization to the MNI standard of functional volumes; followed by centring, segmentation, and normalization to the MNI standard again, but this time for structural volumes. Finally, spatial smoothing of the functional data is performed using a Gaussian kernel of 8 mm [101].

We will analyse the structural integrity of the V-GN brain regions. Each participant's DTI metrics will be calculated, including the primary outcome fractional anisotropy (FA) and other exploratory

outcome measures (radial diffusivity (RD), axial diffusivity (AD), and mean diffusivity (MD). For transcallosal temporoparietal tract analysis, fibre tractography will be performed with default values, as described by Yeatman et al. 2012 [102]. Non-invasive transcranial neuromodulation response has been related to the integrity of interhemispheric connections between the stimulated cortical areas [78–82]. Thus, we will assess the structural properties of the segmented Posterior Parietal and Temporal corpus callosum fibres of the participants as a response factor of our HD-tDCS protocol over the TPJ. Leukoaraiosis will not be an exclusion criterion, but we will explore if there is an association between the presence and severity of leukoaraiosis (assessed by Fazekas scale) and the alteration of the V-GN [76].

Lesion Analysis

The lesion images will be examined for possible oedema, which may distort lesion mapping [57]. The lesion boundary will be performed blinded to the study proposal. The other Axial T2, FLAIR, and DWI sequences will be used as references during analysis as needed. The lesion will be visually determined, and a mask will be created manually on an individual MRI scan using MRIcroN (https://www.nitrc.org/projects/mricron). The 3D reconstruction of the lesion will be checked for imperfections and discrepancies by a second, trained operator [103,104].

Structural integrity

Preprocessing procedures will be performed using QSIPrep 0.13.0RC2 based on Nipype 1.6.0 [104,105]. T1- weighted (T1w) image will be corrected for intensity non-uniformity (INU) using N4BiasFieldCorrection, and for skull-stripped using antsBrainExtraction. OASIS will be used as a target template [106]. Spatial normalization to the ICBM 152 Nonlinear Asymmetrical template version 2009c will be conducted through nonlinear registration with antsRegistration [107] using brain-extracted versions of both T1w volume and template (ANTs 2.3.1). Brain tissue segmentation of cerebrospinal fluid (CSF), white matter (WM), and gray matter (GM) will be performed on the brain-extracted T1w [108] using FAST (FSL 6.0.3:b862cdd5).

DWI data analysis

The images with a b-value less than 100 s/mm^2 will be treated as a b=0 image. MP-PCA

denoising will be applied with a 5-voxel window (MRtrix3's dwidenoise; https://www.mrtrix.org)[109]. After MP-PCA, B1 field inhomogeneity will be corrected using dwibiascorrect from MRtrix3 with the N4 algorithm[106]. After B1 bias correction, the mean intensity of the DWI series will be adjusted so all the mean intensity of the b=0 images match across each separate DWI scanning sequence. FSL (version 6.0.3:b862cdd5) will be used for head motion correction and Eddy current correction[110]. Eddy current correction will be configured with a q-space smoothing factor of 10, a total of 5 iterations, and 1000 voxels to estimate hyperparameters. A linear first-level model and a linear second-level model will be used to characterize Eddy current-related spatial distortion. Q- space coordinates will be forcefully assigned to shells. Field offset will be separated from participant's movement. Shells will be aligned post-eddy. Eddy outlier replacement will be conducted[110]. Data will be grouped by slice, including values from slices determined to contain at least 250 intracerebral voxels. Groups deviating by more than four standard deviations from the prediction will have their data replaced with imputed values. Final interpolation will be performed using the Jac method. Several confounding time-series will be calculated based on the pre-processed DWI (framewise displacement (FD) using the implementation in Nipype). The head-motion estimates calculated in the correction step will also be placed within the corresponding confounding file. Slicewise cross-correlation will also be calculated. The DWI time-series will be resampled to ACPC, generating a pre-processed DWI run in ACPC space with 1.2mm isotropic voxels. For more details on the pipeline, see the section on workflows in QSIPrep's documentation [111,112]. Structural integrity mean values of the V-GNnet will be extracted with nltools using the function extract_roi (nltools 0.4.4, https://nltools.org/).

The pre-processed DWI will be used as input for whole brain tractography using Python Automated Fiber Quantification (pyAFQ;https://github.com/yeatmanlab/pyAFQ). The fiber tractography will be performed with default values as described in Yeatman et al. 2012[102]. The white matter mask will be defined using fractional anisotropy (FA) values greater than 0.3, with a deterministic streamline-tracking algorithm (STT) at a 1 1-mm fixed step size. Two standard termination criteria will be used: tracking is halted if (1) the FA estimate d at the current position is below 0.2 and (2) the minimum angle between the last path segment and next step direction is greater than 30°.

The whole-brain tractogram of each participant will then be registered to manually segmented

whole-brain streamlines using the Streamline-based Linear Registration (SLR) method[113]. The registered streamlines will be clustered to identify bundles based on the Minimum average Direct-Flip (MDF) distance between model bundle centroids using QuickBundles. The RecoBundles method[113] will be implemented using Dipy 1.4.1 (http://dipy.org)[112]. The model bundle will be the Posterior Parietal and Temporal subdivisions of the Corpus Callosum (CC), defined with two ROIs placed in homologous regions of each hemisphere to segment the callosal fibers[102]. Mean values of FA and MD metrics will be extracted using TracVis 0.6.1 (http://trackvis.org/).

Means, medians, standard deviations, and interquartile intervals will be used to describe continuous variables. The neuroimaging study will be conducted in all participants included (n=28). Based on previous studies with the same purposes across different perceptual neural networks [114–116], we estimate that the planned sample size will be suitable for the present neuroimaging study and provide reliable results. The investigation of the relevant biomarkers for intervention response (H2) will be conducted in the participants who underwent active HD-tDCS intervention (n=14).

While we acknowledge the multicollinearity of post-stroke visual verticality disorder and recovery, univariate correlation analyses are recommended in the present study due to the sample size. Thus, we will use the Spearman correlation test to investigate the correlation of mean V-GNnet FA with baseline VV in participants after stroke (H1), and to analyze the correlation of the transcallosal temporoparietal tract integrity (summation of the segmented Posterior Parietal and Temporal corpus callosum fibres mean FA) with VV intervention response (degrees of VV correction at T7 and at T8 from baseline).

Further analysis will be performed to explore the correlation of baseline VV and of VV response to neuromodulation with mean structural integrity values (FA, radial diffusivity, axial diffusivity, and mean diffusivity) of each V-GNnet region-of-interest, and mean FA and volume of the segmented Posterior Parietal and Temporal corpus callosum fibres.

Resting State functional MRI connectivity

The rs-fMRI seed-to-voxel and ROI-to-ROI analyses, the preprocessing and analysis will be performed using the CONN Toolbox (v22.a). The standard CONN preprocessing pipeline will be used for the preprocessing steps, including realignment, centring, slice-timing correction, artifact detection

(ART), and segmentation and normalization to the MNI standard of functional volumes; followed by centring, segmentation, and normalization to the MNI standard again, but this time for structural volumes. Finally, spatial smoothing of the functional data is performed using a Gaussian kernel of 8 mm [101].

After preprocessing is completed, the next step involves noise removal through linear regression and temporal band-pass filtering. Consequently, seed-to-voxel maps are created using seeds defined by the Harvard-Oxford Atlas and AAL3 Atlas, related to the ventral visual (VV) system, particularly the regions of the inferior frontal gyrus (IFG), central opercular cortex (COC), insular cortex (IC), and planum polare (PP) [101]. Functional maps of Pearson correlation are then generated for these regions, which will later be correlated with findings from diffusion, VV, and BIT results. ROI-to-ROI correlation matrices will also be created for the whole brain, for resting-state functional networks, and for regions related to the V-GN [117].

EEG functional connectivity (exploratory investigation)

Resting-state EEG (rsEEG) data will be collected from participants in a seated position, first with their eyes open for 5 minutes and then with their eyes closed for 5 minutes, using EEG-fMRI Brain Products® equipment (64 channels) at a sampling frequency of 500 Hz (Figure 3C). Recordings will be performed on the day before stimulation, 1 day after the end of the stimulation protocol, and 30 days after completion. Before data collection, all channels will be referenced to the vertex with an electrical impedance below 50 kΩ.

After data collection, the EEG signals will be filtered with a high-pass filter at 0.1Hz, and the reference will be set to an average reference. The method of generalized partial directed coherence, described by Baccalá and Sameshima (2001) and formulated from the multivariate autoregressive model, is based on Granger Causality and the generalization of directed coherence (DC) by Saito and Harashima (1981) and will be used for functional connectivity analysis. This method provides directionality for information flow across four or more channels and can be used as a directional estimator of brain connectivity. We will explore whether functional connectivity parameters, primarily the debiased weighted phase lag index (dwPLI), predict response to neuromodulation and whether

there is a difference in this outcome between the active stimulation and placebo groups. Dynamic temporal statistical models developed by our group (Nascimento et al., Information Sciences, 2020; Frontiers in Neuroscience, 2020) will also be used and further improved for the analysis of EEG functional connectivity.

Middle cerebral artery vascular reactivity (exploratory investigation)

We will assess middle cerebral artery blood flow velocity (MCA-BFv) using a portable transcranial Doppler ultrasound system, the Viasonix Dolphin 4D device, equipped with the Viasonix Dolphin XF Robotic Helmet (Figure 3D), which enables simultaneous bilateral assessment. The exam will be performed by experienced neurologists in awake participants, resting comfortably in a supine position, with minimal external audiovisual stimulus. The transducers will be placed over the transtemporal bone window, assessing the middle cerebral artery at an average depth of 55mm (±3mm).

The assessment of cerebral vascular reactivity will be carried out through the breath-holding index (BHI) and apnea test (AT), immediately before and right after the first intervention. In the BHI, the patient will be lying in a supine position, and they will be asked to hold their breath for up to 30 seconds after a normal exhalation. The BHI will be calculated as follows:

$$\mathrm{BHI} = \frac{\mathrm{Vapnea} - \mathrm{Vstart}}{\mathrm{Vstart} - \mathrm{tapnea}} \mathrm{x100}$$

where V= velocity, t=time in seconds.

The Index will be considered normal if = 1.2 ‡ 0.6 %/s. After the MCA-BFv returns to the baseline value, the AT will be performed. In this test, the patient will be asked to hyperventilate for 40 seconds and then hold their breath for at least 30 seconds. The MCA-BFv values during maximum hypo and hyperventilation will be compared to determine the difference between them. An impairment in cerebral reactivity will be considered if this difference is greater than 15% [118,119]. For this assessment, we will use the same TCD setup mentioned above. The use of the robotic helmet will make the analysis more homogeneous, preventing the vessel's insonation from being interrupted during the analysis, either due to patient movement or even the evaluator's movement, thus making the assessment more

accurate and with fewer biases. Furthermore, the data can be analyzed continuously, increasing sensitivity for the detection of any kind of ultrasonographic alteration, as well as allowing the elaboration of more reliable statistical analyses to understand the phenomenon of vasoreactivity. The primary outcome measure of the cerebral hemodynamics clinical trial will be the difference between baseline and after the stimulation, both sides. Time-series analysis of MCA-BFv as a continuous variable will be explored.

Ocular torsion (exploratory investigation)

Following the clinical assessments at T0, T1, and T2, we will acquire non-mydriatic fundus photographs of each eye using a portable retinography equipment (Eyer2, Phelcom Technologies; Figure 2E). During the procedure, participants will be seated with their head in a perfectly upright position, stabilised by a slit-lamp chin rest.

To evaluate ocular torsion, we will measure the disc-fovea angle (DFA), which is widely recognised in the literature as the gold standard. Because retinal anatomical landmarks remain constant, DFA measurements derived from conventional fundus photography demonstrate low intra- and inter-observer variability, ensuring highly reliable and reproducible data [120–122].

The DFA will be calculated from a single, well-focused image using standard image analysis software (version 1.54g, ImageJ; National Institutes of Health, Bethesda, MD, USA). To determine the optic disc centre, a manually adjusted rectangle will be drawn around its borders, and the intersection of two diagonal lines within this rectangle will mark the exact centre. The fovea was identified semi-automatically, based on the calculation of the centre of the macular region, previously delineated by image analysis assisted by software. The angle of cyclorotation will then be measured between a horizontal line intersecting this centre point and a line connecting the optic disc centre to the fovea.

All images will be analysed by an experienced examiner, blinded to participant information, who will extract one measurement per eye. We will report the ocular torsion angle for each eye individually and calculate the binocular cyclorotation (BCR) using the formula:

$$\mathrm{BCR} = \frac{\mathrm{CR\ right\ eye} - \mathrm{CR\ left\ eye}}{2}$$

Consistent with established literature, a torsional deviation equal to or greater than 1.9 degrees from zero will be classified as pathological [73,123].

Discussion

V-GN represents a silent impairment present in the majority of stroke survivors, and it currently lacks effective treatment. Non-invasive neuromodulation, particularly tDCS, is an emerging technique of considerable clinical and research interest. While tDCS offers potential as a treatment in stroke recovery, further formal controlled studies are required. The current clinical trial aims to provide evidence for focal effects of HD-tDCS on physiology, its association with clinically relevant behavioural changes, and its critical dependence on stimulation polarity and intensity.

Modulation of cortical excitability that outlasts the stimulation period can be achieved by applying trains of transcranial magnetic stimuli, or a low-intensity electric field held for tens of minutes. Transcranial electrical stimulation is appealing as a potential therapeutic modality largely due to its low cost, safety and practicality; it has been most extensively studied using weak direct currents (tDCS). Despite over two decades of scientific work on tDCS, there remains a pressing need for well-controlled trials using contemporary technology to facilitate clinical translation, especially given the increasing interest from the scientific community, government organizations, patients, clinicians and the media.

The technological innovation presented in this trial builds on several years of work by the investigative team and collaborators to design, model, physiologically validate, and implement targeted HD-tDCS. This approach provides a relatively circumscribed current distribution to the cortical region of interest. The application of the high-definition tDCS represents an innovation in the field, particularly applied over the temporo-parietal junction (TPJ). Based on historical lesion mapping, the TPJ is known to be an integrative hub in the human brain for vestibular and visual information, which contributes to the perception of visual verticality, a dominant reference during daily function.

Our translational innovation lies in the specific cortical target and the remarkable manipulation of visual verticality perception in the intact brain. Clinically, the innovation is the application of our neuromodulation method in a highly prevalent yet incompletely understood phenomenon in post-stroke

disability. V-GN severely impacts functional recovery, postural balance, and falls risk, yet it currently has no targeted confirmed treatment. Furthermore, while the majority of neuromodulation studies focus on the chronic post-stroke phase, it is generally accepted that applying rehabilitative strategies early after stroke can better capitalize on the critical period for brain plasticity.

The rationale for implementing multiple intervention sessions was based on studies utilizing tDCS for the treatment of spatial neglect, which demonstrated enhanced efficacy following repeated applications [124]. Furthermore, clinical benefits have been observed when neuromodulation strategies are applied twice daily [125–127]. Despite these findings, a consensus regarding the optimal number of sessions required to induce maximal effects has yet to be established [128,129].

Our decision to target the acute/early subacute phase (in-hospital stage) was based on three primary considerations: 1) prevalence: graviceptive neglect is highly frequent during this window [130], observed in 72% of eligible patients at our institution (unpublished data); 2) recovery potential: the acute phase is characterised by significant potential for functional recovery [131]; 3) clinical access: implementing the protocol during acute hospitalisation addresses a critical logistical reality in low-and middle-income countries (LMICs), where the hospital stay often represents the sole opportunity for patients to receive rehabilitation [132,133].

As the average length of stay for this cohort at our centre is five to seven days, clinical feasibility was a primary consideration in designing the protocol. Accordingly, the intervention was structured to begin as soon as clinical stability is reached, provided at least 24 hours have elapsed since stroke onset, and comprises six sessions conducted over two consecutive days. A minimum interval of two hours between sessions was maintained to ensure compatibility with standard hospital care routines while providing a sufficient window for neuromodulatory effects [134]. We acknowledge the methodological limitation regarding the variability of intervals between sessions, as well as between the intervention and outcome assessments. However, this pragmatic approach was the only viable alternative to ensure the study's feasibility and to provide evidence for an intervention that can be realistically replicated within the clinical practice of public hospitals in LMICs [135].

From the neuroanatomical perspective, the innovation lies in investigating the graviceptive neglect brain network using the microstructural analysis of diffusion tensor imaging (DTI) in patients

after stroke, and correlating these findings with the response to neuromodulation [136]. We will also provide evidence characterizing changes in functional connectivity related to V-GN in post-stroke participants, thereby expanding understanding of the underlying neurophysiological mechanisms. The investigation of rs-fMRI connectivity may shed light on the internal model of verticality and its potential to predict response to neuromodulation [117]. In addition, the superior temporal resolution of the proposed rs-EEG investigation will provide a novel neuroanatomical perspective on cortical connectivity [137]. We seek to demonstrate how targeted neuromodulation via HD-tDCS can induce modifications in brain network dynamics, favouring both perceptual and functional connectivity recovery.

Visual verticality perception is related to ocular torsion in subjects with peripheral vestibular disorders [123], brainstem lesions [138], and thalamic infarctions [139]. However, a 1994 study reported participants with supratentorial stroke lesions with a dissociation between VV and ocular torsion [140] – a finding that is yet to be replicated. Previous attempts investigated the cortical modulation of static ocular torsion used repetitive TMS (rTMS) in healthy subjects [75]. The authors conducted the assessments with participants' heads tilted 20 degrees to the right, revealing that rTMS over the supramarginal gyrus affected VV but not ocular torsion [75]. In contrast, here we will assess VV and ocular torsion with the head in an upright position. Furthermore, Finite Element (FE) modelling of the electric field indicates that the stimulation area of our HD-tDCS protocol over TPJ is broader than that of the previously used rTMS [66]. Therefore, this research breaks new ground in understanding the relationship between VV and ocular torsion in the subacute phase after supratentorial strokes and examining whether cortical neuromodulation via HD-tDCS influences this relationship.

From a neurovascular perspective, the innovation of this study is multifaceted. It represents the pioneering investigation into the effects of HD-tDCS (2 mA for 20 minutes) over the TPJ on cerebrovascular reactivity following a stroke. Distinctive methodological features include the use of sophisticated robotic technology for bilateral data acquisition, the collection of continuous data to examine temporal reactions, and a rigorous safety assessment of the protocol during the acute and subacute stages of recovery. Given the established links between cognitive performance and cerebrovascular reactivity [141], and considering that neuromodulation has been shown to regulate neurovascular coupling—with tDCS specifically associated with enhancements in the breath hold index

[86,142,143]—we hypothesise that improvements in verticality perception (a cognitive task) will correlate with BHI enhancements.

In accordance with standard recommendations, the primary objective of this phase IIa clinical trial is to provide a preliminary evaluation of efficacy—based on our primary outcome measure, VV—and to gather supplementary safety data. These findings will facilitate future power calculations and the determination of the intervention's effect size [144]. As the first study to test HD-tDCS for post-stroke V-GN, this research also aims to address literature gaps by investigating the physiological aspects of the condition and our protocol using rigorous methods. However, we have carefully considered the potential for participant burden associated with extensive data collection across multiple outcomes [145]. To ensure effective recruitment and retention while maintaining ethical and accessible standards, our strategies include transparent communication between the research team and participants, valuing patient perspectives, actively engaging the clinical staff, and implementing flexible scheduling of assessments throughout the day to align with both hospital routines and participant needs.

Upon completion of this study, we will have determined whether focal non-invasive neuromodulation is safe, feasible, and preliminarily effective in treating the widely prevalent population of individuals with graviceptive neglect, providing the effect size and evidence to support a future phase III clinical trial to establish effectiveness. As well, we will confirm the involvement of putative brain regions underlying V-GN, and their responsiveness to neuromodulation treatment. Moreover, since imaging and lesion studies have indicated numerous roles for the temporoparietal junction [53] – including social cognition, attention and neglect, speech, language, and auditory processing – the results of this trial will set directions for future research in several fields of interest.

Ultimately, our goal is to gather critical information to guide the therapeutic use of this method. This will benefit the broader participant community by refining methods to improve physical rehabilitation outcomes in stroke survivors and other participants with graviceptive neglect and postural control imbalance. These findings will support the use of HD-tDCS for other cortical targets as a focal and reliable method of neuromodulation and warrant further studies in clinical populations with this protocol.

While this protocol includes individuals with various stroke severities based on NIHSS and

neuroimaging, the total volume of the infarct and the use of reperfusion therapies, such as thrombolysis and mechanical thrombectomy, remain critical variables for future analysis. Successful reperfusion can salvage penumbral tissue and preserve structural connectivity [111], potentially altering the interhemispheric excitability balance that HD-tDCS aims to modulate. Future analyses should determine if the efficacy of V-GN correction differs significantly between patients with extensive territorial infarcts and those with smaller focal lesions. Understanding how the extent of the lesion and the restoration of blood flow interact with neuromodulation will be essential for identifying which patients are most likely to benefit from this intervention in the acute setting.

Furthermore, the recovery of verticality perception may be moderated by baseline burden of cerebral small-vessel disease. Markers such as lacunes, white matter hyperintensities, and atrophy are consistently linked to poorer functional outcomes [112] and may impede the "network-wide" plastic changes required for recovery. Although this protocol includes an exploratory analysis of leukoaraiosis using the Fazekas scale, it is hypothesized that chronic white matter damage could reduce focal stimulation efficacy at the TPJ by disrupting the structural integrity of the V-GN network and transcallosal pathways. Identifying these imaging biomarkers as potential modifiers is a vital step toward personalized neurorehabilitation in the subacute phase.

Supplementary Information

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Dissemination policy

We plan to disseminate the study findings at national and international conferences and to publish the results in a peer-reviewed journal in the field of neurological rehabilitation. We will also share the results with all relevant stakeholders, including patients, stroke survivors, family members,

caregivers, and healthcare providers. Clinical trial registration and results reporting will be conducted in accordance with applicable policy requirements.

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Declarations

Ethics approval and consent to participate

The Clinics Hospital at Ribeirão Preto Medical School, University of São Paulo Ethics Committee approved the study protocol (CAAE: 74877723.4.0000.5440). Before randomization, all participants will be asked to provide written informed consent, which will be obtained by an investigator. This study was registered on http://www.clinicaltrials.gov (NCT number, NCT06747676). The authors are responsible for the conduct of the study, endpoint adjudication, data management, and the oversight of all trial-related activities carried out by the study team and any other individuals or groups involved in trial supervision.

Competing interests

The authors declared no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors contributions

Conceptualizing the research idea and formulating the overarching research goals: TGSE, DJE. Funding: TGSE, DJE. Development of methodology: all authors. Writing the initial draft: TGSE. Critical review and commentary of the draft: all co-authors. Project administration: TGSE.

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