Sensory and motor cortical excitability changes induced by rTMS and sensory stimulation in stroke: a Randomized Clinical Trial

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

AFZ and KM-S designed the study and wrote the manuscript. AFZ, ACRS an ABS collected data. ABRM and LSGN analyzed and interpreted the data, performed statistical analysis, KM-S supervised the study. DP helped with data interpretation and manuscript drafting. All authors approved the final version of the manuscript. All authors read and approved the final manuscript.

Keywords

Transcranial Magnetic Stimulation, Stroke, Somatosensory Cortex, Occupational Therapy, Physical Therapy

Abstract

Word count: 309

Background: The ability to produce coordinated movement is dependent on dynamic interactions through transcallosal fibers between the two cerebral hemispheres of the brain. Although typically unilateral, stroke induces changes in functional and effective connectivity across hemispheres, which are related to sensorimotor impairment and stroke recovery. Previous studies have focused almost exclusively on interhemispheric interactions in M1. Objective: To identify the presence of interhemispheric asymmetry (ASY) of somatosensory cortex (S1) excitability and to investigate whether S1 repetitive transcranial magnetic stimulation (rTMS) combined with sensory stimulation (SS) changes excitability in S1 and the motor cortex (M1), as well as S1 ASY, in individuals with subacute stroke. Methods: A randomized Clinical Trial. Participants with a single episode of stroke, in the subacute phase, between 35 and 75 years old, were allocated, randomly and equally balanced, to four groups: rTMS/sham SS, sham rTMS/SS, rTMS/SS, and control groups. Participants underwent 10 sessions of S1 rTMS of the lesioned hemisphere (10 Hz, 1500 pulses) followed by SS. SS was applied to the paretic upper limb (UL) (active SS) or non-paretic UL (sham SS). TMS-induced motor evoked potentials (MEPs) of the paretic UL and somatosensory evoked potential (SSEP) of both ULs assessed M1 and S1 cortical excitability, respectively. The S1 ASY index was measured before and after intervention. Evaluator, participants and the statistician were blinded. Results: Thirty-six participants divided equally into groups (nine participants per group). Seven patients were excluded from MEP analysis because of failure to produce consistent MEP. One participant was excluded in the SSEP analysis because no SSEP was detected. All somatosensory stimulation groups had decreased S1 ASY except for the control group. When compared with baseline, M1 excitability increased only in the rTMS/SS group. Conclusion: S1 rTMS and SS alone or in combination changed S1 excitability and decreased ASY, but it was only their combination that increased M1 excitability.
Clinical Trials (NCT03329807).

Contribution to the field

This study aimed to identify the presence of inter-hemispheric asymmetry of somatosensory cortical excitability and to investigate whether the application of repetitive transcranial magnetic stimulation (rTMS) over the primary somatosensory cortex (S1) associated with sensory stimulation led to cortical excitability changes in S1 and in the motor cortex (M1) as well as changes in S1 inter-hemispheric asymmetry in subacute stroke survivors. The results indicated that rTMS over S1 and SS, alone or associated, change S1 cortical excitability and decrease S1 interhemispheric asymmetry, but only the combination of these therapies led to increased M1 excitability. Furthermore, we shwon, for the first time, that there is an imbalance of interhemispheric excitability in S1. The data highlights the importance of further studies to understand the physiological behavior of upper extremity n stroke survivors.

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Ethics statements

Studies involving animal subjects

Generated Statement: No animal studies are presented in this manuscript.

Studies involving human subjects

Generated Statement: The studies involving human participants were reviewed and approved by Ethics and Research Universidade Federal de Pernambuco Committee (69908217.7.0000.5208).. The patients/participants provided their written informed consent to participate in this study.

Inclusion of identifiable human data

Generated Statement: No potentially identifiable human images or data is presented in this study.

Data availability statement

Generated Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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ABSTRACT

Background: The ability to produce coordinated movement is dependent on dynamic interactions through transcallosal fibers between the two cerebral hemispheres of the brain. Although typically unilateral, stroke induces changes in functional and effective connectivity across hemispheres, which are related to sensorimotor impairment and stroke recovery**.** Previous studies have focused almost exclusively on interhemispheric interactions in M1. **Objective**: To identify the presence of interhemispheric asymmetry (ASY) of somatosensory cortex (S1) excitability and to investigate whether S1 repetitive transcranial magnetic stimulation (rTMS) combined with sensory stimulation (SS) changes excitability in S1 and the motor cortex (M1), as well as S1 ASY, in individuals with subacute stroke. **Methods**: A randomized Clinical Trial. Participants with a single episode of stroke, in the subacute phase, between 35 and 75 years old, were allocated, randomly and equally balanced, to four groups: rTMS/sham SS, sham rTMS/SS, rTMS/SS, and control groups. Participants underwent 10 sessions of S1 rTMS of the lesioned hemisphere (10 Hz, 1500 pulses) followed by SS. SS was applied to the paretic upper limb (UL) (active SS) or non-paretic UL (sham SS). TMS-induced motor evoked potentials (MEPs) of the paretic UL and somatosensory evoked potential (SSEP) of both ULs assessed M1 and S1 cortical excitability, respectively. The S1 ASY index was measured before and after intervention. Evaluator, participants and the statistician were blinded. **Results:** Thirty-six participants divided equally into groups (nine participants per group). Seven patients were excluded from MEP analysis because of failure to produce consistent MEP. One participant was excluded in the SSEP analysis because no SSEP was detected. All somatosensory stimulation groups had decreased S1 ASY except for the control group. When compared with baseline, M1 excitability increased only in the rTMS/SS group. **Conclusion**: S1 rTMS and SS alone or in

combination changed S1 excitability and decreased ASY, but it was only their combination that increased M1 excitability.

Clinical Trials (NCT03329807)

Key words: Transcranial Magnetic Stimulation; Stroke; Somatosensory Cortex; Occupational Therapy; Physical Therapy

BACKGROUND

The ability to produce coordinated movement is dependent on dynamic interactions through transcallosal fibers between the two cerebral hemispheres of the brain (Gerloff and Andres, 2002). Although typically unilateral, stroke induces changes in functional and effective connectivity across hemispheres, which are related to sensorimotor impairment and stroke recovery (Rehme and Grefkes, 2013).

Interhemispheric interaction in the primary motor cortex (M1) has been widely studied (Jang, 2010; Lindenberg et al., 2012). Following a stroke, an imbalance of interhemispheric interaction between motor areas has been observed because of decreased excitability in M1 of the lesioned hemisphere and increased excitability in the non-lesioned hemisphere (Nowak et al., 2009) (reviewed in Rossini et al. (2003)). This functional organization is the underlined hypothetical model that supports the use of non-invasive brain stimulation therapies for increasing M1 excitability of the lesioned hemisphere and decreasing M1 excitability of the non-lesioned hemisphere (Du et al., 2019). Within this model, the rebalance of interhemispheric interactions may enhance motor function recovery in post-stroke individuals (Cramer and Crafton, 2006; Rossini et al., 2007). Previous studies have focused almost exclusively on interhemispheric interactions in M1 (Murase et al., 2004), while the imbalance of interaction between the sensory areas remains unknown (Calautti et al., 2007).

The primary somatosensory cortex (S1) is involved in the integration of multimodal information through connections with M1. Changes in S1 activity and sensory networks are known to be involved in motor learning; therefore, the integrity of sensory cortex connectivity may be an essential marker of post-stroke motor function (Frias et al., 2018).

Several clinical trials have suggested that sensory stimulation (SS) facilitates functional reorganization of M1 (Hamdy et al., 1998; Garry et al., 2005; Ridding and Ziemann, 2010) and promotes motor recovery (Brodie et al., 2014a). Similarly, the application of repetitive transcranial magnetic stimulation (rTMS) over S1 enhances motor learning in patients with chronic stroke individuals (Brodie et al., 2014a). Previous studies using high-frequency rTMS over S1 demonstrated increased M1 excitability in healthy individuals (Rizzo et al., 2004; Boros et al., 2008), suggesting that S1 stimuli may change M1 excitability. However, the neurophysiological mechanisms underlying the effects of somatosensory stimulation are still not fully understood. Insights into how rTMS (i.e., central somatosensory stimulation) and SS (i.e., peripheral stimulation), when applied alone or in combination, acts on cortical excitability of M1 and S1 might help in the development of more effective and efficient therapies.

Thus, this study aimed to investigate the presence of interhemispheric asymmetry (ASY) in S1 and to observe whether the application of rTMS over S1 alone or in combination with SS, modulates M1 and S1 excitability and S1 ASY in subacute post-stroke individuals. While the majority of previous studies were conducted in acute and chronic stroke, the present study addresses the subacute stroke phase. Such a time window represents an appropriate time for rehabilitation to enhance and guide optimal spontaneous reorganization of motor networks, thus facilitating the functional recovery process.

Similar to M1, we hypothesized that interhemispheric asymmetry could also occur in S1 and that the combination of central and peripheral stimulation is superior to monotherapy. We expected that combining rTMS with other therapies, such as SS, could optimize the plastic effects induced by multisensory stimulation and lead to more significant changes in sensory and motor cortical excitability.

MATERIALS AND METHODS

Study design

This research is part of a randomized and triple-blind clinical trial duly registered in the Clinical Trials (NCT03329807) and approved by the local research ethics committee (69908217.7.0000.5208) The research was conducted at the Laboratory of Applied Neuroscience of the Federal University of Pernambuco, Recife, Brazil.

Participants

The inclusion criteria were as follows: participants aged between 30 and 75 years with a diagnosis of ischemic or hemorrhagic stroke in the subacute phase (3–24 weeks, see Bernhardt et al. (2017)) and an upper limb Fugl-Meyer Assessment (FMA) motor score between 10 and 62. Participants were excluded if they had cognitive deficits (Mini Mental State Examination - MMSE score <18 – (Folstein et al., 1975)) or a history of multiple brain lesions, other associated neurological diseases, peripheral sensory disorders, or a history of psychiatric disorders, including drug and alcohol abuse. Participants who were unable to perceive transcutaneous electrical neurostimulation (TENS) at the hand and forearm, were undergoing concurrent treatment for the upper limb, had rTMS contraindications, and were using medication likely to influence cortical excitability were also excluded (Rossi et al., 2009). The sample was selected for convenience.

Randomization and Blinding

The participants were randomized and allocated to four groups of equal size: (i) active rTMS and sham SS (rTMS/sham SS), (ii) sham rTMS and active SS (sham rTMS/SS), (iii) active rTMS and active SS (rTMS/SS), and (iv) sham rTMS and sham SS (control group). A stratified block allocation based on stroke onset and age was generated at www.randomization.com by an independent researcher and packed into sequentially

numbered, opaque sealed envelopes. A researcher who did not participate in the evaluations or interventions generated the random allocation sequence, enrolled participants, and assigned participants to the interventions. Those evaluating and analyzing the outcomes and participants were blinded to the treatment arm. We stratified the sample by age and stroke time because they could interfere with the interpretation of the effects of treatment outcomes.

Therapeutic Interventions

Each therapeutic session lasted 60 min. During the first 20 min, participants were subjected to rTMS (active or sham) followed by SS (active or sham). Daily sessions were held over 2 weeks (10 sessions). The intervention was conducted by a researcher not involved in any of the evaluation or randomization procedures, thus ensuring the blinding of study allocation (see Fig.1 – Flowchart of the trial design).

Repetitive Transcranial Magnetic Stimulation (rTMS)

The rTMS pulses were delivered through a 70 mm 8-shaped coil connected to a Magstim super rapid stimulator (Magstim Co., United Kingdom) over S1. S1 was delimited at a point 3 cm posterior to the hotspot of the first dorsal interosseous muscle (FDI) of the paretic hand. The hotspot was defined as the location where the largest and most consistent visual responses were elicited by single pulse TMS for the FDI muscle. If an FDI hotspot was not found, S1 was delimited at a point 3 cm posterior to M1 and localized by C3/C4 of the 10–20 EEG system. Previous studies had shown changes in sensory function when non-invasive brain stimulation was applied 3 cm posteriorly from the hand area of the primary motor cortex (Fiorio and Haggard, 2005; Koch et al., 2006). Repetitive TMS (10 Hz, 1500 pulses; 120% of the resting motor threshold (RMT) for FDI of the non-lesioned hemisphere) was applied over the lesioned hemisphere.

RMT was defined as the lowest magnetic pulse stimulus intensity required to elicit a visual twitch in five out of ten trials in at least one of contralateral hand resting muscles. This method is a safe, accurate, and reliable technique for obtaining RMT (Varnava et al., 2011) and was adopted because the repetitive-pulse magnetic stimulator (Magstim super rapid) was not connected to an EMG System. RMT was assessed on each stimulation day. As previously done by other authors (Wupuer et al., 2013; Milot et al., 2019), the non-lesioned hemisphere RMT was used to determine rTMS intensity. A substantial portion of M1 or corticospinal tract is usually damaged after a stroke and make RMT of lesioned hemisphere increases substantially, being probable that the stimulator output would not have reached 120% RMT for all subjects.

For sham-rTMS, two coils were used. One coil disconnected from the stimulator was placed over S1. The TMS stimulator was discharged through another coil connected to the stimulator and positioned behind the participant's head (out of his/her view). Thus, while no magnetic pulses were delivered to the participant, they were exposed to acoustic stimulation of the active protocol(Barros Galvao et al., 2014; Albuquerque et al., 2018).

Sensory Stimulation (SS)

Participants in the active SS group underwent a sensory therapy protocol that consisted of 20–25 min of active sensory training and 40 min of mirror therapy concomitant with 45 min of peripheral nerve sensory stimulation (TENS).

The active sensory training protocol was adapted from Carey et al. (2011) and divided into (i) texture discrimination task, employed graded stimuli with various sensory features (texture, shape, size, weight, and hardness), (ii) graphesthesia, (iii) limb position sense task, and (iv) tactile object recognition.

Four different textures and types of objects were used for active sensory training (i). Initially (first stage), the participants were asked, with eyes open and using the non-paretic hand, to identify four characteristics (palpable) of each object (e.g., size, shape, temperature, details, and length of each object). Then, in the second stage, they were encouraged to notice the same four characteristics using the paretic hand. In the third and fourth stages, the tasks of the first and second stages were repeated with the eyes closed. In addition, the participants discriminated textures through a tactile memory game. For graphesthesia exercises (ii), the occupational therapist (OT) asked patients to identify a series of numbers, letters, and geometric shapes that were drawn on the palmar and dorsal surfaces of the hand using a pencil. For the limb position sense task (iii), the paretic upper limb was moved to a position in the flexion-extension, abduction-adduction, and pronation-supination ranges, and the participants indicated the perceived limb position. For tactile object recognition (iv), participants wearing a blindfold had to recognize objects chosen by the OT from a basket placed in front of them. Active sensory training was performed in the non-paretic hand for the sham SS groups.

For the mirror therapy, the paretic upper limb was hidden behind a mirror (50 × 50 cm), and the non-paretic upper limb was placed in front of the mirror. Participants were asked to look at the non-paretic upper limb reflected in the mirror and observe its movements (flexion-extension of the wrist, elbow, and fingers; and pronation-supination of the forearm) (Cho and Cha, 2015). In the sham SS group, non-paretic upper limb movements were performed without a mirror.

TENS was applied to the median nerve at the wrist of the paretic hand. The cathode was placed 20 mm proximal to the anode. Five electrical pulses (1ms duration for each) at 10 Hz were delivered every second over 45 min (Conforto et al., 2010) by an electrical

stimulator (Model Quark – Dualpex 961). Stimulus intensity was set at the level at which individuals reported mild paresthesia in the nerve territories without pain or visible muscle contraction. The participants were instructed not to perform active muscle contractions during the intervention. For the sham stimulation, the device was turned off 60 s after stimulation onset. TENS was administered concurrently with mirror therapy. The use of different sensory strategies has been encouraged in recent studies to treat sensorimotor deficits. Indeed, multisensory stimulation through exposure to an enriched environment increases brain plasticity and recovery of function after stroke (Bolognini et al., 2015; Tinga et al., 2016; Hakon et al., 2018; Sathian and Ramachandran, 2020).

Outcome Measures

Motor Evoked Potential - MEP

The single-pulse TMS-induced MEP of the paretic upper limb was used to assess cortical excitability of the lesioned M1. First, an "8" shaped coil connected to a single-pulse magnetic stimulator (NeuroMS, Neurosoft, Russia) was positioned over the non-lesioned M1 to determine the FDI hotspot of the non-paretic hand. The RMT of the non-lesioned M1 was measured using Motor Threshold Assessment Tool, version 2.0. Different from the RMT measurement for rTMS, since the single-pulse magnetic stimulator was connected to a two-channel digital electromyography (EMG; NeuroMep Micro, Neurosoft, Russia), the RMT for the MEP was defined as the minimum stimulus intensity that produced a peak-to-peak amplitude of 50 μV in the FDI muscle during rest, as observed by EMG recordings. For MEP of the lesioned hemisphere, the coil was moved and positioned over lesioned M1 hand representation at the FDI hotspot or at C3/C4 position when the FDI hotspot was not found.

The coil positions were marked on the patient's scalp with washable non-toxic pencils to guarantee identical positions throughout the study. For the analysis, the mean peak-to-peak MEP amplitude of 20 consecutive stimuli at 120% of RMT was used as the motor cortical excitability. Motor cortex excitability was recorded at baseline and after treatment sessions.

Somatosensory Evoked Potential- SSEP

Through the electrical stimulator (Neuro-MEP, Neurosoft), percutaneous stimuli (square pulse, 0.2 ms, 2mA, 1000 pulses in total, 3 Hz, distal cathode, and proximal anode) were applied to the median nerve bilaterally with the patient lying down. The *somatosensory evoked potential* (SSEP) was recorded by EEG surface electrodes positioned at FPz, CP3, and CP4 (electroencephalogram 10-20 marking system). Reference electrodes were placed in the anterior neck, below the prominence of the hyoid bone (PA), and in the posterior neck, as well as above the C7 vertebra (PP). We used neck assembly but only as a reference. Only the amplitudes of N20 and P23 were analyzed to investigate the primary somatosensory thalamocortical excitability. The analysis was performed only on pre- and post-intervention amplitudes. The electrode impedance was maintained below 7 KOhms. A time window of 50 ms with 25 and 3000 Hz filters was used. NEUROMEP software was used to test for short-latency somatosensory evoked potentials (version 3.7.3.8) to capture and analyze SSEP waves (Cruccu et al., 2008). For the analysis, 1000 responses were averaged.

The components examined were the SSEP peak-to-peak amplitudes (μV) of components N20 and P23. N20 originated from the somatosensory thalamus-cortical radiation, and P23 originated from potential postsynaptic graduates, both generated within the primary somatosensory cortex. For brain injuries, a decrease in the N20/P23 amplitude is expected (Luccas et al., 1990). The results of the amplitudes of the patients who did not

manifest responses capable of being captured by the software algorithm were not subjected to SSEP analysis.

Data Processing and Analysis

Statistical analysis was performed using non-parametric tests since the variables were non-normally distributed (Shapiro-Wilk test, $p>0.05$). A Kruskal-Wallis test for continuous variables and a Fisher's exact test for categorical variables were used to analyze differences in baseline characteristics among the groups. The data are presented as median [interquartile range] unless otherwise specified.

For SSEP, an index of asymmetry between the non-lesioned and lesioned hemispheres was calculated for each group. Interhemispheric asymmetry (ASY) was calculated using the ratio between the non-lesioned/lesioned hemispheres. Thus, an ASY greater than 1 indicates increased non-lesioned sensory cortical excitability relative to lesioned excitability.

For MEP, the baseline and post-treatment MEPs were normalized intra-individually and were given as baseline ratios. Thus, a ratio greater than 1 indicates increased cortical excitability, whereas a ratio less than 1 indicates decreased excitability.

For all measurements, the Kruskal-Wallis test was used to assess differences among groups. Mann-Whitney and Wilcoxon tests were used for between-group and within-group comparisons.

For MEPs, the sample size was calculated based on findings of a pilot study on MEPs amplitude (effect size = 1.7) with an $\alpha = 0.05$ and power (β) of 0.85. Therefore, an estimated total sample size of 32 subjects, with a minimum of 8 subjects for each group, was considered sufficient. For SSPE, based on a $\beta = 0.85$, with $\alpha = 0.05$ and an effect size = 1.9, a total sample

size of 28 subjects, with a minimum of 7 subjects per group, was required. Sample size was computed in G*Power software (Faul et al., 2007).

To investigate the robustness of overall finding, the effect size (d) using Cohen's d was calculated. According to Cohen (1992) d=0.2 is a small treatment effect, d=0.5 represents a moderate effect, and d=0.8 is a large effect. All analyses were performed using Statistical Package for Social Sciences (SPSS) version 18 software. A significance level of $p \leq 0.05$ was adopted.

RESULTS

The descriptive demographic and clinical data of participants are presented in Table 1. No significant differences among the groups were found at baseline.

INSERT TABLE 1

Seven patients (three from rTMS/sham SS, one from sham rTMS/SS, one from rTMS/SS, and two from sham rTMS/sham SS group) were excluded from MEP analysis because of failure to produce consistent MEP at 120% of RMT. One participant was excluded from the rTMS/sham SS group in the SSEP analysis because no SSEP was detected. All participants completed 10 sessions (Figure 1).

INSERT FIGURE 1

As detailed in Table 2, at baseline, an ASY in S1 (SSEP of non-paretic UL *vs.* paretic UL) was observed for all groups (Wilcoxon test, $p<0.05$). Baseline SSEP amplitudes did not differ among the groups (Kruskal-Wallis test, $x2=2.56$, $p=0.464$).

INSERT TABLE 2

Figure 2 shows the SSEP and MEP for the paretic UL at baseline and post-intervention. Compared with baseline, the paretic UL SSEP amplitudes increased in all somatosensory stimulation groups: Sham rTMS/SS: 0.89[0.3 to 3.5] *Vs* 1.21 [0.6 to 6.7],

Wilcoxon test p=0.046, d=0.6; rTMS/SS: 0.39 [0.1 to 4.9] *Vs* 1.12 [0.2 to 6.8], Wilcoxon test p=0.025, d=0.4, except for the rTMS/sham SS group 0.89 [0.7 to 5.5] *Vs* 1.79 [0.7 to 6.1] Wilcoxon test p=0.036, d=0.3 (Table 2). In the control group, the SSEP amplitudes decreased when compared with baseline: 3.03 [0.5 to 4.4] *Vs* 2.40 [0.1 to 3.4] Wilcoxon test p=0.075, d=0.3. No differences were found between the groups (Kruskal-Wallis test, p >0.05). No significant changes were observed in the SSEP of non-paretic UL post-treatment when compared to baseline (Wilcoxon test, p>0.05)

A significant increase in MEP amplitudes in M1 was found after interventions only for the rTMS/SS group: 1 *Vs* 1.58 [1.1 to 7.2], Wilcoxon test p=0.028. No differences were found in the rTMS/sham SS: 1 *Vs* 1.79 [0.9 to 5.1], Wilcoxon test p=0.116; sham rTMS/SS: 1 *Vs* 1.02 [1.0 to 1.4], Wilcoxon test p=0.138), and control groups: 1 *Vs* 0.72 [0.4 to 5.3], Wilcoxon test p=0.866. No differences in the between-groups analysis were observed at baseline and after the intervention (Kruskal-Wallis test, p> 0.05) (Figure 2). The RMT values of non-lesioned hemisphere remained stable before and after the intervention (see Additional file 1, Bland-Altman plots).

INSERT FIGURE 2

DISCUSSION

In summary, our findings indicated interhemispheric asymmetry of the primary somatosensory cortex after stroke at baseline, which was normalized after somatosensory stimulation. All somatosensory stimulation modalities increased S1 excitability, but only combined therapy (rTMS/SS) modulated M1 excitability.

S1 interhemispheric asymmetry

Similar to the motor system, previous studies have pointed out that activation of S1 in one hemisphere increased inhibition from activated sensory areas toward homologous areas of the contralateral hemisphere, suggesting the existence of interhemispheric inhibitory interactions between S1 in human participants (Hlushchuk and Hari, 2006; Blankenburg et al., 2008; Eickhoff et al., 2008; Kastrup et al., 2008; Klingner et al., 2011). Maladaptive functioning of interhemispheric inhibition in M1 has been described in patients with stroke and probably influences functional recovery in these patients (Murase et al., 2004). Assuming that interhemispheric inhibition also occurs between S1 (Brodie et al., 2014b), we expected the existence of an S1 interhemispheric asymmetry after stroke. Indeed, our findings demonstrated S1-S1 asymmetry in all groups before the intervention. The transcallosal disinhibition hypothesis described in M1 could also explain the S1 interhemispheric asymmetry. Following a stroke in the primary somatosensory cortex, S1 cortical excitability of the lesioned hemisphere would be decreased due to the infarct and S1 of the contralesional hemisphere is disinhibited, thus leading to enhanced inhibition towards S1 of the lesioned hemisphere.

Future studies may verify the relationship between S1 interhemispheric asymmetry and motor and sensory impairments in this population.

S1 and M1 cortical excitability changes after central and peripheral somatosensory stimulation

In line with previous studies (Brodie et al., 2014a; Rocchi et al., 2017), we also found that peripheral (SS) or central (S1-rTMS) somatosensory stimulation resulted in normalization of S1-S1 asymmetry. No significant changes were found. While the SSEP tended to decrease in the non-lesioned hemisphere, these changes were not statistically significant. The increased excitability in S1 of the lesioned hemisphere after somatosensory

329 stimulation may have contributed to improvement in the symmetry between excitability and
330 functioning of both cortices. Normalization of hemispheric excitability after stroke has been
331 associated with functional sensorimotor recovery (Cramer and Crafton, 2006; Rossini et al.,
332 2007). However, faced with the opposite theories of central nervous system reorganization
333 after stroke (Di Pino et al., 2014), the normalization of the interhemispheric imbalance may
334 be a too simplified approach to fit for all stroke patients with different levels of sensorimotor
335 severity.

336 The 'status' of the sensorimotor brain areas should vary as a consequence of the input.
337 There is a relatively general agreement that median nerve stimulation is reflected by the peak
338 of the initial negative deflection of the somatosensory evoked potentials (Mariorenzi et al.,
339 1991).

340 SS activates afferent tracts terminating in the contralateral thalamus, which in turn
341 mainly forwards the SI on the postcentral gyrus of the contralateral hemisphere, thus
342 resulting in increased S1 excitability (Kaas, 2004). Indeed, as revealed by functional
343 magnetic resonance imaging (fMRI) and electroencephalography (EEG) studies, peripheral
344 stimulation in one hand is associated with enhanced neural activity in predominantly
345 contralateral somatosensory areas in healthy individuals (Nihashi et al., 2005; Sutherland
346 and Tang, 2006). In addition, observations of movement induced by mirror therapy activate
347 the somatosensory system (Dohle et al., 2009; Fritzsch et al., 2014; McGregor et al., 2018).

348 Proprioceptive afferents reach the sensorimotor cortex and influence the excitability
349 threshold of pyramidal neurons that generate cortico-spinal discharges. Proprioceptive
350 afferents arrive in parietal primary sensory and frontal primary and non-primary motor areas
351 (supplementary motor area, SMA, premotor cortex, PMC) either via direct thalamo-cortical
352 inputs or indirectly (Mariorenzi et al., 1991).

Most previous rTMS studies focused on the modulation of M1 excitability. Few studies have focused on modulating S1 excitability by rTMS. In line with our results, Ragert et al. (2004) induced sustained increases in S1 cortical excitability, indicated by larger SEEPs, after 5 Hz rTMS in healthy individuals. A similar effect was also observed when intermittent theta burst stimulation (iTBS), an excitatory form of patterned rTMS, was applied over S1 (Katayama and Rothwell, 2007; Premji et al., 2010). While the precise mechanisms mediating the effects of rTMS on cortical excitability are not known, it has been proposed that rTMS influences Na^{+} and Ca^{++} channels and NMDA-receptor activity (Valero-Cabre et al., 2017).

Given the somatosensory system has strong structural and functional connections with the motor system (Petreanu et al., 2009; Xu et al., 2012; Borich et al., 2015) as well as the current study's findings of increased S1 excitability of the lesioned hemisphere after the interventions, we expected that rTMS and SS, either alone or combined, would also increase M1 excitability. In contrast to previous studies using a 2-h period of somatosensory stimulation (Kaelin-Lang et al., 2002; Ridding and Ziemann, 2010) and using 30 Hz-rTMS (Jacobs et al., 2014), SS and rTMS over S1 alone did not increase MEP amplitudes in our study. The longer duration of peripheral stimulation and higher rTMS frequency in previous studies could explain these distinct neural responses. Indeed, evidence suggests that rTMS can modulate cortical excitability in a frequency-and intensity-dependent manner (Siebner and Rothwell, 2003; Ridding and Ziemann, 2010). Additional experiments are required to gain additional insights into this issue.

The synchronous application of both forms of stimulation (peripheral and central) could potentially enhance the effect of each therapy alone. This finding is in agreement with previous studies that supported the advantages of associating motor therapies with rTMS

over M1 (Rose et al., 2014; Hosomi et al., 2016; Tosun et al., 2017; Du et al., 2019). The combination of cortical stimulation over M1 with peripheral sensory stimulation was also found to be a promising strategy in facilitating motor function after stroke better than performing each technique in isolation (Celnik et al., 2009) [68]. rTMS-induced increased M1 excitability in the lesioned hemisphere has been associated with sensorimotor functional recovery (Kim et al., 2006), and a stimulus to the motor areas previously conditioned by an adequate input from peripheral nerves could change the excitability of the central motor tract. Proprioceptive inputs have short latency relay in the motor cortex (Mariorenzi et al., 1991), which has important implications for rehabilitation.

Implications for rehabilitation

Stroke might significantly modify the interhemispheric symmetry of the sensorimotor cortex (Nowak et al., 2009). Studies suggest that an interhemispheric imbalance of motor cortices post-stroke is positively associated with the severity of paretic hand impairment (Murase et al., 2004) and likely interferes with recovery (Calautti et al., 2007; Tang et al., 2015). Considering that impairment in somatosensory structures and function may also contribute to motor disability (Borich et al., 2015), we expected S1 excitability asymmetry to be associated with motor impairment. Indeed, previous studies have demonstrated that abnormal interhemispheric connectivity between the primary sensory cortices is associated with motor impairment after stroke (Frias et al., 2018). This leads to the speculation that "rebalancing'' of interhemispheric symmetry of the sensorimotor cortex in patients with stroke by rTMS might promote improvement of upper limb function. Further studies are necessary to demonstrate the efficacy of S1 asymmetry-based rTMS intervention for stroke motor recovery.

Limitations

Due to the small sample size, our study is considered a pilot study. Studies with larger sample sizes are needed to replicate our results and validate our conclusions. We also understand that the patients who were excluded from the MEP and SSEP analyses and other control variables that may influence cortical excitability were limitations of this study, however all the statistical tests shown enough statistical power (i.e., $\beta > 0.08$). Another limitation of the current study is that we did not directly assess M1 cortical excitability in the non-lesioned hemisphere to verify the relationship between M1-M1 and S1-S1 asymmetry. Finally, although implications for clinical practice have been discussed, a relevant limitation of this study was the absence of functional outcomes (sensory or motor); The absence of functional outcomes (sensory and motor) also limited our ability to make clinical inferences of our results. Future studies should consider investigating the relationship between impairments in somatosensory areas and functional outcomes

CONCLUSION

Our results demonstrated that somatosensory stimulation (rTMS and SS alone or in combination) reduces S1 interhemispheric asymmetry in patients with subacute stroke. This reduction in S1-S1 asymmetry is concurrent with enhanced S1 excitability. In addition, based on the findings of cortical M1 excitability, we found that rTMS may enhance the effects of SS. Further research is needed to investigate the effects of combined therapies on stroke rehabilitation.

LIST OF ABBREVIATIONS:

rTMS: repetitive transcranial magnetic stimulation

ASY: interhemispheric asymmetry

S1: somatosensory cortex

SS: sensory stimulation

M1: motor cortex

Hz: Hertz

UL: upper limb

MEP: motor evoked potentials

SSEP: somatosensory evoked potential

FMA: Fugl-Meyer Assessment

MMSE: Mini Mental State Examination

TENS: transcutaneous electrical neurostimulation

FDI: first dorsal interosseous muscle

EEG: electroencephalography

RMT: resting motor threshold

EMG: electromyography

TMS: transcranial magnetic stimulation

OT: occupational therapist

fMRI: functional magnetic resonance imaging

SMA: supplementary motor area

PMC: premotor cortex

iTBS: intermittent theta burst stimulation

NMDA: N-metil D-Aspartato

DECLARATIONS

Ethical Approval and Consent to participate

This study was approved by the Ethics and Research Universidade Federal de Pernambuco Committee (69908217.7.0000.5208). Each participant provided written, informed consent prior to the experiments.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author [DP] on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AFZ and KM-S designed the study and wrote the manuscript. AFZ, ACRS an ABS collected data. ABRM and LSGN analyzed and interpreted the data, performed statistical analysis, KM-S supervised the study. DP helped with data interpretation and manuscript drafting. All authors approved the final version of the manuscript. All authors read and approved the final manuscript.

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TABLES

Table 1 - Demographic and clinical characteristics for each group at baseline

	rTMS/sham SS (n=9)	sham rTMS/SS (n=9)	rTMS/SS (n=9)	Control (n=9)	p value
Age median [IQR]	63[59.7 to 75]	66.5[57.7 to 72]	63.6[60 to 72]	63[59 to 75]	0.998[2]
Gender, male n (%)	4 (44.4)	5 (55.5)	4 (44.4)	3 (33.3)	0.708[1]
Type stroke, ischemic n (%)	9 (100)	9 (100)	9 (100)	9(100)	0.526[1]
Stroke time (weeks) median [IQR]	8.5[5 to 22]	8[4.7 to 20]	11[8 to 22]	11.5[6.2 to 22]	0.601[2]
Hemiparesis, right n(%)	4 (44.4)	3 (33.3)	5 (55.5)	6 (66.6)	0.424[1]
Dominance, right n(%)	9 (100)	8 (88.8)	9 (100)	9 (100)	0.428[1]
MMSE median [IQR]	25.5[24 to 30]	25.5[24 to 30]	24[21.2 to 29]	19.5[18 to 30]	0.109[2]
FM-Motor median [IQR]	53.5[40.2 to 62]	37.5[19.5 to 60]	48.5[39.2 to 60]	36.5[29.7 to 61]	0.422[2]
FM-Sensory median [IQR]	9.5 [7.5 to 10]	7 [5.2 to 10]	8 [7 to 10]	8 [6 to 8.7]	0.514[2]

Legend: FM-M: Fugl-Meyer. MMSE Mini Mental State examination score SS: Peripheral somatosensory stimulation rTMS: repetitive transcranial magnetic stimulation. Data are presented as median, interquartile range, absolute frequency and relative frequency. *$p<0.05$, [1]Chi-square test and [2] Kruskal-Wallis.

Table 2 - Somatosensory Evoked Potential of both upper limbs (non-paretic and paretic) before (baseline) and after treatment (post-treatment) for each group.

	Baseline			Post-treatment		
Groups	**Non-paretic UL (μV)**	**Paretic UL (μV)**	ASY	**Non-paretic UL (μV)**	**Paretic UL (μV)**	ASY
rTMS/sham SS	3.84 [1.1 to 7.9]	0.89 [0.7 to 5.5]*	3.04	2.46 [0.9 to 7.9]	1.79 [0.7 to 6.1]	0.76
sham rTMS/SS	4.36 [1.8 to 8.4]	0.89 [0.3 to 3.5]*	3.56	2.88 [1.7 to 8.4]	1.21 [0.6 to 6.7]	1.67
rTMS/SS	2.88 [1.5 to 7.8]	0.39 [0.1 to 4.9]*	2.58	2.16 [0.8 to 6.1]	1.12 [0.2 to 6.8]	1.04
Control	5.53 [4 to 7.6]	3.03 [0.5 to 4.4]*	2.53	4.53 [3.5 to 7.2]	2.40 [0.1 to 3.4]*	2.13

Legend: ASY, interhemispheric asymmetry; SS, peripheral somatosensory stimulation; rTMS, repetitive transcranial magnetic stimulation; UL, upper limb. Data are presented median and interquartile range. *$p<0.05$,Wilcoxon tests.

FIGURES Captions

Figure 1. CONSORT flowchart of the study

MEP: Motor Evoked Potential, SSEP: Somatosensory Evoked Potential, SS: sensory stimulation, rTMS: repetitive Transcranial Magnetic Stimulation.

Figure 2. Motor evoked potential (MEP) of lesioned hemisphere before (baseline) and after treatment for each group and Somatosensory evoked potential (SSEP) of paretic upper limbs.

In **A**, MEP data were normalized intraindividually and were given as baseline ratios. μV: Microvolts, SS: peripheral somatosensory stimulation, rTMS: repetitive transcranial magnetic stimulation. Data are presented as median and interquartile range. * $p<0.05$. Wilcoxon test (baseline *Vs*. post-treatment).

Additional file 1. Bland-Altman plots of resting motor threshold (RMT) values of non-lesioned hemisphere before and after treatment.

SS: sensory stimulation, rTMS-repetitive Transcranial Magnetic Stimulation.

Figure 1.TIFF

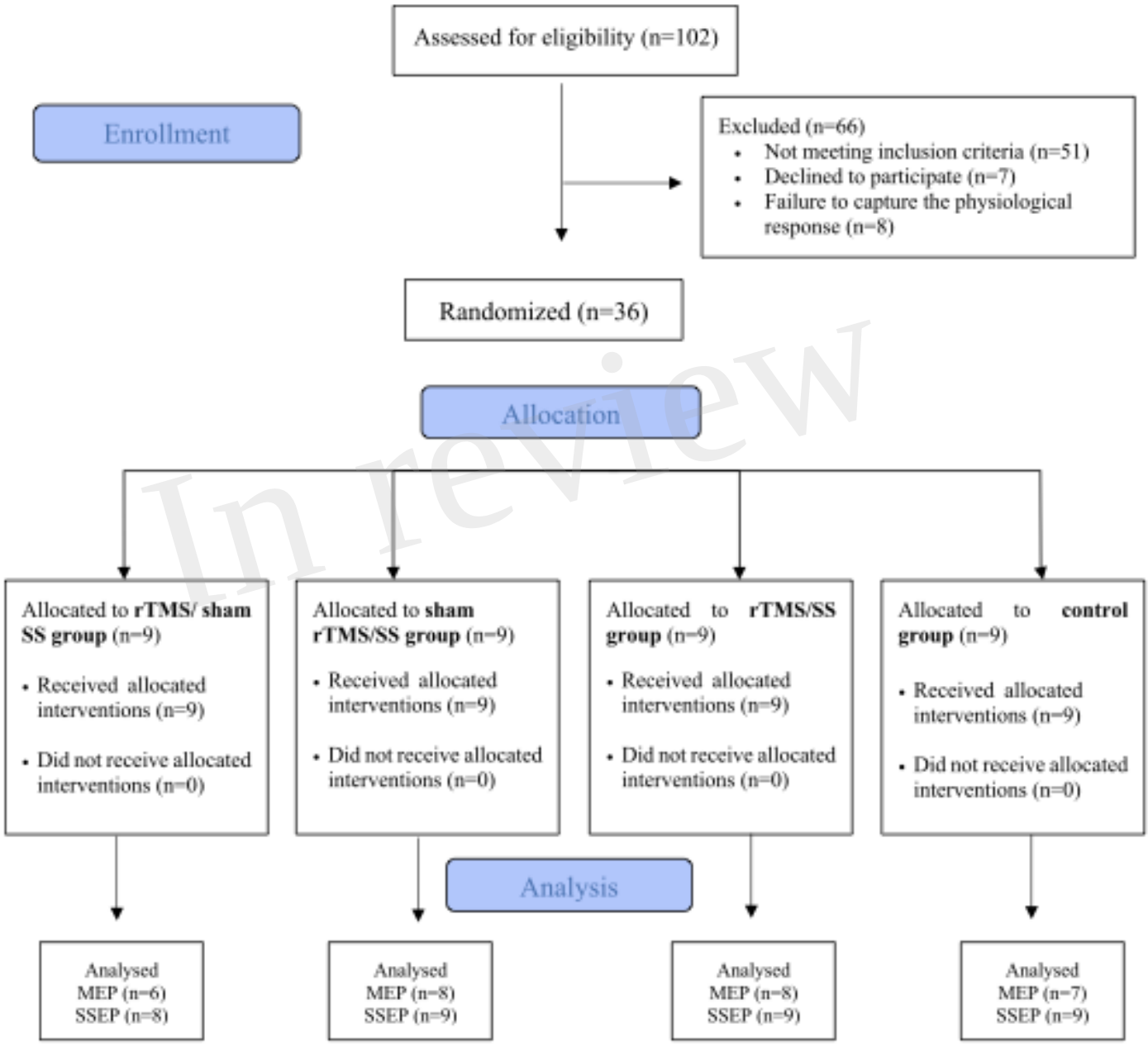

Figure 2.TIFF