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NEUROPHYSIOLOGICAL CORRELATES OF LANGUAGE RECOVERY AFTER TDCS IN APHASIC PATIENTS

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ABSTRACT

In the context of increasing incidence of stroke (but also an increasing rate of survival), non-invasive brain stimulation techniques (NIBS) are more frequently used for patients with post-stroke aphasia (PWA) and post-stroke depression (PSD). NIBS techniques, modulating brain plasticity, might offer valid, alternative therapeutic strategies. The aim is to reach a better outcome because treatment of aphasia can also improve post-stroke depression and vice versa.

Based on two literature reviews on NIBS effects on PSD and post stroke aphasia the conclusion is that, although the field is relatively new, and many more investigations with larger samples of patients are required, transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) clinical application is well tolerated, safe, and feasible.

Starting from these encouraging data, we used a combination of TMS and electroencephalography (EEG) to explore the excitability modulation before and after active (20 sessions) and sham (20 sessions) tDCS in a double-blind crossover experiment. Four chronic non fluent PWA underwent 8 weeks of verbal exercises coupled with tDCS over the perilesional areas close to the left inferior frontal gyrus. To evaluate changes induced by tDCS, TMS-EEG responses over Brodmann area 6 (BA6) were computed using five different parameters. In addition, these data were compared with those recorded from a matched control group.

The results indicated a slight improvement after tDCS stimulation (as compared to sham) for patients with Broca's aphasia, but not for those with global aphasia. Also, TMS-evoked EEG responses recorded from the ipsilesional hemisphere were abnormal in individuals with chronic post-stroke aphasia (slower and simple responses with higher amplitudes) when compared to responses from the contralesional hemisphere and from the control group. Critically, the Global Mean Field Power (GMFP), Local Mean Field Power (LMFP) and Natural Frequency values were modulated by anodal tDCS. Despite these interesting results, further data are needed in order the obtain more direct, stronger evidence linking behavioral tDCS effects and neurophysiological data.

ACKNOWLEDGMENTS

"The human brain has 100 billion neurons,

each neuron connected to 10 thousand other neurons.

Sitting on your shoulders is the most complicated object in the known universe."

Michio Kaku

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General introduction

One of the patients that participated in this project once said, *"After my stroke, I started to avoid my neighbors, my friends… If they speak to me, I feel bad for not being able to answer, and it is hard to explain them what aphasia is…"* This thesis is about post-stroke aphasia and noninvasive brain stimulation techniques (NIBS). The aim of this thesis is to better understand how to use them more efficiently in rehabilitation settings.

This thesis is divided into 4 chapters, the first two being theoretical, while the following two are focused on NIBS clinical applications.

Chapter 1 is an introduction to aphasia, concerning two main topics: a general presentation of the "classic" post-stroke aphasia syndromes, including a short historical overview, and available treatments. Bringing together knowledge from previous studies, the efficacy of these treatments and potential developments are then discussed, arguing that, although (for now) aphasia has no definite treatment, much can be done for language rehabilitation even years after the stroke event.

In Chapter 2, an overview of the most-used NIBS will be presented with particular attention to their action mechanisms and clinical applications. The focus will be on transcranial direct current stimulation (tDCS) mode of action (what is known and which type of further knowledge is required), application, and future research directions; despite effectiveness and working mechanism concerns, tDCS remains a relatively inexpensive and well-tolerated tool that could be used in everyday rehabilitation. The second part of Chapter 2 will discuss the concurrent transcranial magnetic stimulation and electroencephalography (TMS-EEG), namely, the basic principles of TMS and EEG and how the integration of the two instruments offers a unique methodology which can be used to measure different brain states (excitability of the targeted brain area and effective connectivity to distant sites in healthy and in pathological conditions).

Chapter 3 will address NIBS efficacy in post-stroke rehabilitation. We will present the results of two meta-analysis that investigated tDCS and TMS effects on two frequent and highly invalidating post stroke disorders: post-stroke depression (PSD) and post-stroke aphasia. The aim is to offer a quantitative and qualitative

summary of the available literature comparing TMS and tDCS long-term efficacy. The most promising stimulation parameters to facilitate language recovery will be discussed.

In Chapter 4, the results of a feasibility, crossover, repeated-measure study that investigated the long-term effects of tDCS coupled with linguistic exercises in PWA are reported. TMS-EEG co-registration data were analyzed in the time and time-frequency domains in order to assess whether tDCS can modulate brain excitability and connectivity in an effective way and whether these changes can be related to performance in linguistic production.

Finally, this work will conclude with a general discussion, stressing open questions and future directions.

Chapter 1: Post-stroke aphasia — a brief overview

One of the humankind's most distinctive features, disputably considered specific to this species, is the capacity to communicate through language (Graffi, Scalise, Donati, Cappa, & Moro, 2013). Language and communication can be described from different perspectives and can be analyzed at different levels: (i) in terms of modality (auditory, oral, visual, gestural, and orthographic), (ii) domain of linguistic processing (phonology, morphology, syntax, semantics), (iii) cognitive skills relevant to functional performance beyond pure linguistic abilities (emotional communication, pragmatics, self-monitoring, theory of mind or perspective taking, aesthetics, and humor). When some of these components or the general ability to talk and understand (verbal communication capacity) fades away, the impact on everyday life is overwhelming. Neurologic damage, for instance, a middle cerebral artery (MCA) occlusion affecting the left cerebral hemisphere, leads to impairments in comprehension and production of language in the oral modality (aphasia) or in written form (dyslexia and dysgraphia).

There are numerous accepted definitions for **aphasia** but the shared elements are: (i) **the complete or partial loss of verbal language** (i.e., the inability to communicate or to understand such communication), (ii) **as a result of some brain condition** (meaning that previously the language was properly acquired and due to damage to specific areas of the brain that are responsible for language this capacity is now impaired) (iii) **with preservation of the primary inputs** (e.g., auditory, visual) **and outputs** (e.g., motor projections) (Vallar & Papagno, 2018).

Aphasia has different etiologies, such as stroke (ischemic and/ or hemorrhagic), trauma (e.g., head injury), tumors, surgical resection, epileptic foci, brain infections, and neurodegenerative disease (e.g., primary progressive aphasia) (Crinion et al., 2013).

Post-stroke aphasia is the most frequent type of aphasia and is usually the result of left hemisphere lesions in the MCA territory since right language dominance is less frequent regardless of handedness. Crossed aphasia, namely aphasia caused by a lesion in the right hemisphere in right-handed patients is estimated to account for only 0.38–3% of all aphasic syndromes (Marien et al., 2004; Vassal et al., 2010). Ischemic infarctions account for approximately 80% of cases, whereas hemorrhagic damage is less frequent (Tippett & Hillis, 2016).

1.1. A short historical perspective:

Despite decades of research, the functional neuroanatomy of language processing has been difficult to pin down. The modern study of people with aphasia (PWA) exceeds 160 years of research and encompasses different perspectives from the medical, neuropsychological, and linguistic views to the social and welfare approaches. Historically, three critical discoveries regarding the language-brain relationship and language disorders were made between 1860 and 1875 (Heilman, 2015). In 1861 Pierre Paul Broca, after autopsying the brain of his famous patient "Mr. Tan" (Louis Victor Leborgne) reported that loss of articulated speech follows the lesion of the foot of the third frontal convolution (Basso, 2003). He considered that a lesion of the inferior portion of the left frontal lobe (including the pars opercularis and pars triangularis), an area later named after him Broca's area, would cause loss of speech without impairing comprehension. For Broca, this area was the repository of "motor word images." Successively, he examined eight patients with nonfluent aphasia due to anterior perisylvian lesions and concluded that language impairment follows a left (not right) hemisphere lesion in the majority of right-handed people (Berker et al., 1986).

Soon after Broca's publications, Karl Wernicke (1848-1905) reported patients who had fluent speech but made frequent errors and had impaired naming, auditory comprehension, and repetition. This aphasia syndrome is now known as Wernicke's aphasia. Those patients had lesions in the posterior portion of the left superior temporal lobe. Wernicke hypothesized that the affected brain area contained the memories of how words sound, i.e., he indicated this posterior temporal region as the ''area of (auditory) word images." He suggested a second speech–language module, now considered as the phonological input lexicon, and he introduced the concept of an information-processing network. Wernicke, and, later, Lichtheim (1884), considered that a disconnection between the ''area of (auditory) word images'' and the anterior regions, involved in motor programming of speech sounds ("the motor word images"), would produce a fluent speech disorder, called conduction aphasia, and defined by good comprehension, but impaired spontaneous speech, naming, and repetition (Pizzamiglio, Cappa, & Luzzatti, 2006).

Broca is considered a localizationist because he believed that speech could be localized in a specific brain region. Wernicke had a different theoretical position; he thought that language is the result of the

association/connection of various brain areas, each of which has a specialized function. He believed that complex functions result from associating simpler components, and he was the first to describe an associationist model of language functioning. In this historical model, Wernicke's and Broca's areas are connected by the arcuate fasciculus.

Ludwig Lichtheim (1845-1928) further developed the anatomo-functional model of language, the so-called Wernicke-Lichtheim model. Specifically, he introduced the *concept center*, diffusely represented in the brain. He argued that information flows from the auditory center to the concept area and from this to the motor center and that the concept center is connected to both Wernicke's and Broca's areas. In addition to the three forms of aphasia initially hypothesized by Wernicke (motor or Broca's aphasia, sensory or Wernicke's aphasia, and conduction aphasia), the new model added four aphasic syndromes. Those syndromes were: (i) subcortical sensory aphasia, caused by damage to the pathway that links the primary auditory area with Wernicke's area; (ii) subcortical motor aphasia, resulted from the disconnection between Broca's area and the articulatory musculature; (3) transcortical sensory aphasia, due to the interruption of the pathway from Wernicke's area to the concept center; and (4) transcortical motor aphasia, induced by the disconnection of the concept center from Broca's area. Lichtheim's model has been criticized for the lack of relationships between the various aphasic syndromes and specific brain regions (Basso, 2003).

Subsequently, Kussmaul (1877) considered that language could not be dissociated from thought and proposed a schema with four centers of images of words (acoustic, optic, phonic, and graphic) under the concept center control. He described six aphasic syndromes and individuated amnesia for words, but this model also lacks an anatomical basis (Basso, 2003).

Divergently, Joseph Jules Dejerine (1849–1917) and his wife, Augusta Dejerine-Klumpke (1859–1927), postulated a neuroanatomically founded concept of a language zone and clarified the neuroanatomical basis of alexia with agraphia and pure alexia. They considered the arcuate fasciculus as an association fiber tract connecting Broca's area, Wernicke's area, and a visual word image center in the angular gyrus. They were among the first to attribute language-related functions to the fasciculi occipitofrontal and the inferior

longitudinal fasciculus. These fasciculi belong to a functional network known as the Dejerines' language zone, which goes beyond the borders of the classically defined cortical language centers (Krestel et al., 2013).

During the 20th century, Norman Geschwind (1926–1984) and colleagues revived the Wernicke-Lichtheim model, proposing a new neurological theory of language known as the "Wernicke-Geschwind" model. According to Geschwind, sounds of words are conveyed through the auditory pathways to the primary auditory cortex and then to Wernicke's area. For speech production, the meanings of words are sent from Wernicke's area via the arcuate fasciculus to Broca's area, where morphemes are formed and then passed on to the motor cortex. For written words, the information is transferred through the primary visual cortex to the angular gyrus and forward to Wernicke's area (Basso, 2003).

The opposite theoretical perspective proposed **a holistic view** and argued that aphasia is a unitary disorder without any specific brain localization, varying only in severity. For example, John Hughlings Jackson (1878) believed that behavior originates from the superimposition of increasingly complex functions, i.e., from more automatic and involuntary ones, performed by more "primitive" structures, to the complex, intentional functions processed in "advanced" brain areas (Basso, 2003). From his point of view, the right hemisphere supports the automatic use of language while the left hemisphere processes the intentional components of language. Another representative of the holistic school was Pierre Marie (1853-1940). He denied (1906) Broca's area's role in processing expressive speech and cited cases of patients with Broca's area lesions without Broca's aphasia and vice versa. He considered aphasia a single disorder, and from his perspective, "true" aphasia was only Wernicke's aphasia (Basso, 2003).

In the 1960s, Alexander Romanovich Luria, based on the observation of traumatic patients, argued that the brain-language relation is an indirect one; namely, pathological conditions of the brain cannot produce a direct and specific disintegration of a linguistic function. He ignored both the associationist and the holistic approaches and postulated a functional anatomic framework for language, distributed over widespread areas (Akhutina, 2016). From this theoretical perspective, a network of neurological structures, linked to execute a common task, supports functional systems. Brain damage could impair the functional system but being characterized by a certain degree of plasticity, the damaged link could be reorganized and the lost ability, to a certain degree,

recovered. He described six aphasic syndromes: dynamic aphasia, efferent motor aphasia, afferent motor aphasia, sensory aphasia, acoustic amnestic aphasia, and semantic aphasia (Akhutina, 2016).

Finally, one of the most influential contemporary neuropsychological models of language organization is the dual-stream model (Hickok & Poeppel, 2004, 2007; Iyer et al., 2020).

This model describes two language processing routes:

(i) a dorsal stream, strongly left-hemisphere dominant, responsible of phonological processing, namely auditory-to-articulation; the main hubs and white matter connections of the dorsal pathway are: the inferior frontal gyrus, ventral portions of the precentral gyrus, anterior and posterior portions of the insula, ventral portions of the supramarginal gyrus, the arcuate fasciculus, the third branch of the superior longitudinal fasciculus;

(ii) a ventral stream, which is largely bilaterally distributed, supporting semantic processing, i.e., of auditory-to-meaning information; the main hubs and white matter connections of the ventral pathway are: superior temporal gyrus, superior temporal sulcus, middle and inferior temporal gyri, anterior temporal lobe, external capsule, inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, uncinate fasciculus (Hickok & Poeppel, 2004, 2007, 2016; Fridriksson et al., 2016).

Using fMRI data and diffusion tensor imaging (DTI) based tractography, Saur et al. (2008) indicated white matter fibre tracts connecting cortical regions during sublexical pseudoword repetition and sentences comprehension, two language tasks preferentially involving either the dorsal (repetition) or ventral (comprehension) language processing stream.

Studies in post-stroke aphasia have identified distinct neural responses that arise from impaired semantic and phonological processes, namely lexical-semantic processes are more likely to be impaired after a ventral pathway damage, while speech production is more often impaired as a consequence of dorsal stream damage (Hickok & Poeppel, 2004; Saur et al., 2008; Kümmerer et al, 2013; Fridriksson et al., 2018). It is important to notice that many aphasia batteries involve multiple processes that rely on both the dorsal and the ventral streams.

1.2. Aphasia Syndromes

Post-stroke aphasic syndromes are very complex in their clinical phenomenology and classification because the language processing system is extremely elaborate, involving multiple linguistic mechanisms such as phonological, lexical, syntactic, semantic, and pragmatic, which are subsequently connected to other cognitive systems (e.g., working memory, attention, executive function, etc.).

Both traditional and modern classifications of aphasias are based on elementary clinical characteristics of dichotomies (motor-sensory, expressive-receptive, fluent or nonfluent). From a clinical perspective, impairment patterns may take various forms, differing in **perception**, **recognition**, **auditory comprehension, repetition, production** of speech, and **the fluency** with which individuals form an utterance (Goodglass et al., 2001). Aphasia severity could range from a complete inability to produce and understand language to mild problems in word finding. Concomitant nonlinguistic deficits, such as executive functioning or depression may complicate the clinical picture and may represent a barrier for an adequate interpretation of the test results and successful treatment.

Each aphasia subtype is defined by a specific profile of symptoms based on verbal expression fluency, language comprehension abilities, and repetition skills. Some individuals with aphasia are **fluent** in their verbal expression, as they produce many words in the shape of sentence-like utterances. Nevertheless, they use wrong words, disrupting the meaning of communication. Another linguistic ability often impaired among people with **fluent aphasia** is auditory comprehension. Some PWA have considerable difficulty understanding simple words and messages spoken to them; this impairment is usually associated with left superior temporal gyrus damage, i.e., Wernicke's area (Hillis et al., 2001). These patients usually show a damage that affects left postcentral regions of the parietal or temporal cortex, often sparing left frontal regions (Damasio, 2001).

In contrast, **nonfluent aphasia** refers to limited spoken production capabilities. These PWA also lack the grammatical component and struggle to produce prosodic, articulate utterances, which leads to nonfluent verbal expression referred to as agrammatism, and telegraphic speech (Goodglass et al., 2001). These patients have relatively good abilities in understanding simple, direct sentences, yet could experience considerable difficulties when asked to process grammatically complex sentences, such as passive voice formulations, e.g.,

"The boy is chased by the girl" (Caramazza & Zurif, 1976). Instead, they may interpret grammatically complex sentences according to the order of the main content words or the context information, thereby misunderstanding the message. This nonfluent verbal expression tends to occur in people with lesions that encompass the left inferior frontal regions, often extending deeply into the subcortical white matter (Chapey, 2020).

This distinction between fluent and non-fluent aphasia refers to the Boston classification system, which was proposed in the 1960s by Norman Geschwind, Frank Benson, Harold Goodglass, and Edith Kaplan (Basso, 2003). Considering (i) verbal expression (fluent or nonfluent), (ii) words and sentences auditory comprehension, (iii) repetition, and (iv) naming abilities (word retrieval), it is possible to distinguish several language patterns. As previously mentioned, the classification scheme includes 4 fluent and 4 non-fluent aphasia syndromes.

Wernicke's, conduction, transcortical sensory, and anomic aphasia are considered fluent aphasias.

Though typically able to produce speech with normal rate and articulation (fluent speech), the person with **Wernicke's aphasia** has difficulty understanding verbal information (poor auditory comprehension) and produces speech that is often nonsensical (empty content, poor repetition, and poor naming ability). Comprehension disorders in Wernicke's aphasia could arise at any stage of speech perception, recognition, and/or comprehension (Slevc et al., 2011). The verbal output is often abundant; articulation, prosody, and phrase length are preserved. It is common for people with Wernicke's aphasia to be anosognosic (unaware of their language deficit). The caregivers may initially misunderstand these symptoms as reflecting a psychiatric disorder. Wernicke's aphasia may be associated with other cognitive deficits such as impaired error detection, self-monitoring, attention, and ideomotor apraxia.

Referring to the neuroanatomical correlates, Wernicke (1874) indicated the first temporal gyrus on the left hemisphere the center of acoustic images, while Kertesz and Benson (1970) reported that persisting jargon aphasia involves both the left superior posterior temporal gyrus and the supramarginal gyrus (Greenwald, 2015). Poor recovery from Wernicke's aphasia has been correlated with damage to the left superior temporal lobe and the supramarginal and angular gyri (Kertesz et al., 1993).

Conduction aphasia is a syndrome associated with damage in the posterior territory of the left MCA, which may include the posterior superior temporal gyrus, the inferior parietal lobule, and the insula. This syndrome is characterized by impaired word and/or sentence repetition due to difficulties in phonological planning for speech production or, in some cases, reduced short-term memory span. Speech and comprehension are relatively spared (Basso, 2003). The repetition disorder is the primary feature that distinguishes conduction aphasia from (fluent) anomic aphasia, in which repetition is preserved. Patients with conduction aphasia are usually aware of their errors, and often attempt to correct themselves by producing successive phonemic variations of the target word, a strategy known as conduite d'approche (Wilshire, 2015; Wilshire & McCarthy, 1996).

Transcortical sensory aphasia is a rare syndrome, characterized by a severe comprehension deficit, intact repetition, and fluent speech with semantic paraphasias. Language is often unintelligible and lacks communicative value because of the presence of verbal jargon. Transcortical sensory aphasia is associated with multiple posterior cortical sites, including the left temporoparietal junction, and with the posterior superior, and middle temporal gyri (Heilman, 2015).

In **anomic aphasia,** the main deficit is in word retrieval, in both picture naming and spontaneous speech, with relatively spared performance in other language domains, such as auditory comprehension, repetition, and sentence production. Word-finding difficulties can be equally present for all grammatical classes of words or can be more pronounced for nouns (Miceli et al., 1988). Rarely observed are phonemic errors and paraphasias. Damage to a variety of brain areas can produce word retrieval deficits, including regions in the left frontal, temporal, and parietal cortex (Mesulam, 2008).

As previously mentioned, the main characteristics of **nonfluent aphasia** are difficulties of articulation, with short nongrammatical sentences, and prosody disorders.

Broca's aphasia, transcortical motor, mixed transcortical, and global aphasia are within the nonfluent category.

In **Broca's aphasia**, spontaneous speech is markedly reduced, limited in vocabulary, and characterized by the use of more nouns than verbs (Miceli et al., 1984). In contrast with intentional speech, automatisms (e.g., days of the week) or stereotyped speech might be spared. There is also a loss of the grammatical structure

(agrammatism), and speech is often characterized as telegraphic, effortful, and apraxic (Goodglass et al., 2001). Specifically, patients may have long latencies between words. Grammatical markers, inflections, articles, pronouns, adjectives, conjunctions, and prepositions are lacking altogether. Repetition might also be impaired in patients with Broca's aphasia. Despite these difficulties, words that they produce are often intelligible and contextually correct. In pure Broca's aphasia, word comprehension is spared, even if these patients may not be able to interpret correctly reversible, passive constructions and/or complex sentences (Caramazza & Zurif, 1976). People with Broca's aphasia are often very frustrated about their communication difficulty. Besides aphasia, these patients might also have oral apraxia and right hemiplegia or hemiparesis. Usually, the core lesion involved in this aphasic syndrome is in the left frontal lobe, including the Broca's area and the immediate subcortical structures (Brodmann area 45 and Brodmann area 44), but might extend to the insula and the periventricular white matter fibers (Maher, 2016).

The characteristic of **transcortical motor aphasia** is a non-fluent spontaneous speech with preserved repetition and comprehension. Since verbal production is almost absent, it is considered a disorder of initiation and continuation of internally motivated spoken language (Basso, 2003). Naming difficulties, agrammatic output, or even some phonemic paraphasias may rarely be present but are not necessary for the diagnosis. At an anatomical level, the lesion involves the medial frontal cortex, especially the left presupplementary motor area (pre-SMA) and adjacent Brodmann's area 32, or the pathways between these frontal structures. Occasionally the left basal ganglia, the left thalamus, or the ascending dopaminergic pathways' damage can also cause transcortical motor aphasia (Crosson et al., 2015).

Mixed transcortical aphasia or "isolation of the speech area" is a rare syndrome, characterized by reduced or absent spontaneous speech except for echolalic responses, severely impaired comprehension ability, and preserved repetition; in other words, this aphasia type is considered equivalent to global aphasia with preserved repetition. The lesions underlying mixed transcortical aphasia tend to be multifocal or diffused and include left fronto-temporo-parietal, cortico-subcortical areas, sparing the perisylvian speech areas. This syndrome is often targeted in research because its existence may be seen as congruent with the assumption of an independently operating "dorsal pathway" for language (Baumgaertner, 2015).

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Global aphasia is defined by the breakdown of all aspects of oral and written language, and it is the consequence of extensive left hemisphere lesions, encompassing the MCA territory and involving cortical and subcortical areas as well as white matter tracts. The defining symptoms include severe auditory comprehension and oral expression deficits with some spared conceptual knowledge and preserved comprehension of emotional prosody. Global aphasia can evolve into Broca's aphasia even after years.

This scheme does not include all possibilities, and other patterns of aphasia can be observed (Basso, 2003; Kuljic-Obradovic, 2003).

1.3. Treatment

1.3.1 Aphasia Assessment

Assessment and aphasia diagnostics have moved beyond simply classifying patients by aphasia syndromes, and efforts are made to determine which specific linguistic and cognitive processes are impaired and/or preserved. Depending on the objectives, it is necessary to concomitantly and properly evaluate:

(1) **language abilities** - aphasia accurate diagnosis; strengths, weaknesses, and aphasia severity evaluation; screening of specific linguistic domains; language progress evaluation.

(2) **functional communication** (the ability to convey information).

(3) **related disorders** such as motor speech disorders or post-stroke depression.

(4) **other cognitive functions** essential for communication like executive function, attention, and working memory (based on neuropsychological tests and information-processing models of language).

(5) **psychosocial consequences** of aphasia (quality of life, caregiver burden) (Patterson, 2015).

A wide range of tools are available and include informal assessments, language tests developed by healthcare institutions, and commercially published tests available for purchase in pre-packaged kits. Many comprehensive aphasia assessment batteries have been published based on different approaches. For example, the Boston Diagnostic Aphasia Examination – BDAE (Goodglass et al., 2001) and the Aachen Aphasia Test – AAT (Miller et al., 2000) are based on a neurolinguistic view. The Battery for the Analysis of the Aphasic Deficits – B.A.D.A. (Miceli et al., 1994) and the Psycholinguistic Assessment of Language Processing in Aphasia (PALPA) (Kay et al., 1996) offer a psycholinguistic approach to guide speech therapy, while a more practical evaluation is obtained

with instruments such as The Communication Activities of Daily Living- Third Edition – CADL (Holland et al., 2017) and the Functional Assessment of Communication Skills for adults (I-ASHA-FACS) (Muò et al., 2015). Standardized aphasia test batteries have the advantage of quantitation and standardization, permitting accurate follow-up examinations.

There are also tests and scales specifically designed for aphasia screening, particularly in an acute care setting. These tools vary in design, intended outcome, and examiner training requirements. For instance, the Frenchay Aphasia Screen Test (FAST) (Enderby et al., 1986), valid for both acute and chronic aphasia, allows for a fast administration, less than 10 minutes, by a clinician without training as a speech-language pathologist. The Stroke and Aphasia Quality of Life Scale (SAQOL) is an interview-based self-report scale that can be used to assess the quality of life in aphasia (Hilari et al., 2003).

The clinician could also choose tests specific for a particular language domain and related cognitive functions depending on the assessment purpose: (1) to supplement a comprehensive aphasia battery for a more thorough evaluation, (2) to examine functions that could be at ceiling on standard aphasia batteries, (3) to present a greater range of items in an area that is typically included in a comprehensive aphasia battery, and (4) to examine a coincident communication behavior.

Besides the linguistic tests, a variety of neuropsychological tasks can be administered to verify the integrity of other processes, such as general nonverbal intelligence, memory, visual recognition, etc.

1.3.2 Treatment options for post-stroke aphasia

Language and related cognitive deficits in post-stroke aphasia tend to improve spontaneously in the weeks and months that follow the stroke onset, but *speech-language therapy (SLT)* is required in both subacute and chronic phases to improve outcomes.

The main goal of aphasia rehabilitation is to support each person in achieving the highest level of functional and social communication possible, in the shortest time, given his or her degree and type of impairment. Often, treatment encompasses both restitutive approaches, by restoring cognitive-linguistic functions that are impaired, and/or compensation strategies, by encouraging the use of alternative cognitive-linguistic processes that are spared and could convey the linguistic message (Raymer & Gonzalez Rothi, 2017).

The rehabilitation clinician must inform and educate the patient, and his caregivers, in order to identify effective communication strategies. Counseling the caregivers may include providing information about aphasia, the possible evolution, the goals of aphasia rehabilitation, and home training exercises. The training of caregivers as conversational partners can be efficient in improving communicative interactions in aphasia (Galletta et al., 2019).

Current SLT methods are based on neuroscientific principles and are selected depending on the language-processing system(s) that need to be repaired or recruited to improve language deficit. Ten principles of experience-dependent plasticity that could support aphasia rehabilitation have been described by Kleim and Jones (Kleim & Jones, 2008). These are: use it or lose it (lack of activation of specific brain functions can lead to functional degradation), use it and improve it (active engagement of a specific linguistic area can enhance that function), specificity (targeting a specific ability such as naming will promote a gain in that specific area), repetition matters (repetition of a newly relearned behavior may be required to induce lasting neural changes), intensity matters (plasticity requires sufficient training intensity), time matters (training during the acute period could interact with spontaneous recovery), salience matters (the language materials must be interesting and relevant to the patient), age matters (plasticity occurs with ease in younger brains), transference (plasticity in response to one training experience can enhance the acquisition of similar experiences), interference (plasticity in response to one experience can interfere with the acquisition of other behaviors) (Kleim & Jones, 2008).

Identifying the optimal treatment and its dosage is highly complex given the numerous variables that may influence the effectiveness of treatment (Gerstenecker & Lazar, 2019). Important components of rehabilitation may include dosage, therapeutic relationship, medium of therapy delivery, patient motivation, cognitive ability, neurological stability, social support, as well as task-specific practice.

Speech and Language Therapy (SLT)

SLT is the most common approach to aphasia rehabilitation and is considered the standard treatment for PWA in many western countries (Fridriksson & Hillis, 2021). Since neural plasticity is believed to be the basis for both learning in the healthy brain and relearning in the damaged one, through rehabilitation, SLT main aim is to

maximize the capacity of the damaged networks to re-learn with experience and, in doing so, to modify the neural functioning at a synaptic level (Basso et al., 2013).

There is a wide range of SLT approaches depending on which mechanism they tackle or what aspects of language they are expected to improve, based on the patient's clinical profile (e.g., aphasia type, associated disorders, social support, motivation, etc.).

Aphasia therapy changed over time, from methods similar to the ones used for children with delayed speech to specific research-based rehabilitation techniques (Basso, 2003). Usually, SLT is delivered in a one-on-one setting, where a single clinician treats a single patient, but sometimes SLT can be applied also as group therapy (Fridriksson and Hillis 2021).

There are several approaches, based on different concepts of aphasia, that continue to contribute in a more or less direct way to the present rehabilitation process; for example, the behavior modification approach, the stimulation and facilitation approach, the Luria approach, the pragmatic approach, augmentative and alternative communication intervention (AAC), and the cognitive neuropsychological approach (Basso, 2003).

The behavior modification approach uses operant conditioning principles, programmed instruction, and learning strategies, applying Skinner's work to aphasia therapy without considering the damaged function specificity. Since the verbal behavior is not qualitatively different from other behaviors, it can be modulated by external stimuli. To a certain degree, this approach is applied by all treatments because they all aim at modifying the patient's verbal behavior. The most common techniques are shaping (the produced response is manipulated until the desired response is obtained) and fading (progressively reducing the level of assistance needed to complete a linguistic task). Both techniques assume that the required behavior or a similar one exists in the patient's repertoire of responses (Basso et al., 2013).

The Constraint-Induced Language Therapy (CILT) uses a treatment approach based on neuroplasticity principles by incorporating treatment intensity and shaping of verbal responses while preventing the use of nonverbal communication modalities (Difrancesco et al., 2012; Mozeiko et al., 2016).

The **classic school or the stimulation and facilitation approach** encompass many different and heterogeneous interventions, but the main shared assumptions are that: (1) as proposed by the holistic school, aphasia can be

considered a unitary disorder with different degrees of severity, and (2) the capacity to communicate through language is not lost, but the lesion has impaired only the ability to access it. This conviction is based on the presence of the automatic—voluntary dissociation; specifically, in certain facilitation conditions, patients can produce words, sentences, or grammatical structures that they cannot produce during spontaneous speech (Howard, Patterson, Franklin, Orchard-Lisle, & Morton, 1985). The facilitation, motivation, and stimulation with increasingly difficult tasks are the most used strategies in this approach.

One example of SLT developed from the stimulation and facilitation approach is the Melodic Intonation Therapy (MIT). MIT is used for patients with non-fluent aphasia (Broca's and Global aphasias). Given that often patients with non-fluent aphasia are capable of singing words that they cannot produce in normal language, the MIT main goal is to engage the intact right hemisphere in language production by utilizing rhythmic tapping and intoned speech (speech that is musically stylized by exaggerating the prosody of normal speech) (Norton et al., 2009). Naturally accented syllables are sung using the high pitch, with treatment progressing hierarchically through three levels of difficulty. Ultimately, the level of exaggeration of natural prosody is faded until speech resembles normal production.

Luria's Functional approach combines theory and practice in a coherent therapeutic system. As previously mentioned, according to Luria, a network of neural structures, each playing a specific role but all contributing to correct processing, sustains language functions. Aphasia syndromes differ according to the site of lesion, which interferes with a specific linguistic function. Luria (1970) distinguished functional disturbances due to the temporary loss of activity in some brain areas that are characterized by spontaneous recovery (i.e., without the need of treatment) from the functional disturbances resulting from brain tissue death (Pizzamiglio et al., 2006). Since the brain tissue is irreversibly damaged, the impaired function cannot be restored to its previous form and the therapy must be directed towards the reorganization of the function by transferring it to other functional systems or brain areas. The therapist, applying this approach, makes a detailed analysis of the linguistic function, including the level of impairment; then he/she individuates the intact functions that could support the impaired one through neural plasticity mechanisms (Basso, 2013).

The pragmatic approach to the treatment of aphasia is based on the methods and techniques of applied linguistics (the use of language in context). Often the PWA's communication abilities are better preserved than their ability to express themselves through language (Foldi et al., 1983); consequently, this approach aims at improving the capacity to communicate by whatever means, e.g., gestures, mimic, drawing, etc., not only through spoken language. To this purpose, different evaluation instruments, such as CADL (Holland et al., 2017), and therapeutic implementation, like Promoting Aphasics' Communicative Effectiveness (PACE) (Davis, 1985) were developed over time.

Augmentative and alternative communication intervention (AAC) refers to a collection of techniques and strategies designed to help people with severe aphasia to communicate basic needs, deliver information, and maintain social closeness, by using alternative communication systems such as communication books or high-tech computer devices. Although positive AAC treatment effects have been observed, they have been registered in controlled environments, and the treatment has a limited generalization to everyday life (Jacobs, Drew, Ogletree, & Pierce, 2004).

The cognitive neuropsychological approach suggests lexical and sublexical treatment procedures that are based on cognitive models of language processing (Pizzamiglio et al., 2006). Cognitive neuropsychology aims to provide a model of cognitive processing and to explain impaired performance in terms of damage to one or more components of the cognitive function. Cognitive impairments are described concerning explicit neuropsychological and neurolinguistic models and the treatment targets the impaired sub-component(s) of language, as inferred from a model of (normal) language processing. To set a therapy based on the cognitive neuropsychology principles the clinician needs: a) to accurately identify the damaged and the spared linguistic subunit(s); b) to determine if it is an access deficit (the unit is spared but the access is not possible) or a unit impairment (the unit itself is damaged). The treatment plan, including both restoration and compensation techniques, should be tailored to the patient's needs and the clinical characteristics previously individuated (Pizzamiglio et al., 2006, Basso et al., 2013). At the sentence level, the treatment involves meta-linguistic knowledge of verb properties and takes into consideration both the lexical and syntactic properties of sentences (Basso et al., 2013).

An alternative to individualized therapies is the **community support approach** whose main goal is to help PWA successfully participate in their community. An example is the Life Participation Approach to Aphasia (LPAA), which focuses on re-engagement in life by encouraging and supporting the PWA to participate in their recovery and fully engage in daily activities of their choice (Chapey et al., 2000). Community aphasia groups can provide social support, help with social isolation, and offer speech-language therapy services. Unlike impairment-based or functional therapy approaches, which focus mainly on reestablishing the communication capacities, LPAA emphasizes the reintegration of persons with aphasia into the community by focusing on residual personal strengths despite the communication disabilities.

In a 2016 Cochrane review by Brady and colleagues, 27 studies (1620 PWA) were analyzed (Brady et al., 2016). The authors concluded that, compared with no therapy, SLT improves functional use of language, and speech production, while the long-term effects were uncertain. High-intensity speech therapy (up to 15 hours a week) was found to aid in functional language use and to reduce aphasia severity compared to low-intensity speech therapy. Furthermore, social support and stimulation were beneficial to some aspects of participants' language performance, but the results were uncertain due to a higher dropout percentage from social support interventions than from SLT (Brady et al., 2016).

Post-stroke aphasia might improve over many years with SLT, but therapy is often available only a few months after stroke. Therefore, innovative telecommunication technologies seem to be the solution to address this issue. **Telerehabilitation** refers to the delivery of rehabilitation services via information and communication technologies and can include different services, like assessment, monitoring, prevention, intervention, supervision, education, consultation, and counseling (Cacciante et al., 2021). This approach was initially developed to address issues related to rural residence and continuous increases in the cost of care while maintaining the recommended frequency, duration, intensity, and dose treatment. SLT telerehabilitation can offer access to rehabilitation services and can reduce treatment costs and travel expenses (Jacobs et al., 2021) but can also be a useful tool in the unusual circumstance of COVID-19 pandemic lockdown. In a telerehabilitation setting, via videoconference, participants can see, on the computer screen, simultaneously the treatment stimuli and the clinician. The treatment can be delivered while maintaining a one-to-or a group

setting. Recent meta-analysis results indicate that speech and language treatment provided via videoconference could bring similar benefits as those obtained from conventional face-to-face treatment (Cacciante et al., 2021).

A fast-emerging approach to aphasia treatment is **computerized SLT**, i.e., the rehabilitation protocol can be self-managed by using computer speech and language therapy, videogames, virtual reality, or social media. Rebecca Palmer and colleagues analyzed the effectiveness of self-managed computer-based word-finding speech therapy and reported a clinically significant improvement in trained words but did not observe self-perceived improvements in everyday communication (Palmer et al., 2019).

Pharmacotherapy and NIBS

To date, SLT remains the treatment of choice for post-stroke aphasia in subacute and chronic periods, yet the beneficial effects of SLT can be increased and/or accelerated when combined with pharmacological therapies (Berthier, 2021), and with non-invasive brain stimulation techniques (NIBS – that will be discussed in the second chapter) (Bucur & Papagno, 2019). In an acute ischemic stroke, the primary mechanism of recovery is the restoration of blood flow in the penumbral tissue surrounding the core infarct, i.e., the aim is to restore as much as possible the tissue.

Many neurotransmitter systems are used by the brain in distinct functional tasks, and some of these systems can be manipulated pharmacologically to produce therapeutic benefits (Small, 1994). Pharmacotherapeutic studies of aphasia have employed a variety of substances. The theoretical motivations for their use are various and range from clinical rationales (e.g., people who are depressed do not engage in conversations) to clinically correlated neurobiological facts (e.g., catecholamines are decreased after infarction) (Llano & Small, 2015). Pharmacotherapy's main goal is to promote adaptive neuroplasticity and network remodeling by manipulating the activity of neurotransmitters. For example, D-amphetamine, a noradrenergic agent, coupled with SLT, over 5 weeks, facilitated recovery from aphasia in a small group of patients in the subacute period after a stroke (Walker-Batson et al., 2001). Several studies have investigated the role of dopamine therapy coupled with SLT in post-stroke recovery by providing bromocriptine (a D2 agonist) or levodopa (a dopamine precursor). Results were promising, i.e., performance slightly improved on several language metrics over SLT alone (Bragoni et al.,

2000). Other pharmacological agents used in the research were cholinergics and anticholinergics (based on neurophysiological data on the role of cholinergic projections in modulating neural plasticity)(Pashek & Bachman, 2003), piracetam (a derivative of GABA that facilitates cholinergic and excitatory amine neurotransmission and alters neuronal membrane properties), zolpidem (a short-acting nonbenzodiazepine hypnotic that potentiates GABA), memantine (a noncompetitive NMDA-receptor antagonist, currently approved for the treatment of Alzheimer's disease, that might improve the ability of naming, spontaneous speech and repetition), and vasopressin (thought to be important in mediating social behavior). Against expectations, no significant language improvement was reported after pharmaceutical interventions alone (Berthier, 2021), and despite a small number of "proof of concept" studies that found a marginal positive effect when pharmaceutical interventions were coupled with SLT (Fridriksson & Hillis, 2021), there is no consistent evidence that any medication has a substantial long-term impact on boosting aphasia recovery (Berthier, 2021; Llano & Small, 2016).

1.4. Markers for post-stroke aphasia recovery

The most difficult clinical questions in aphasia rehabilitation are ''what is the patient's potential for recovery?'' and ''what is the best rehabilitation strategy given a specific clinical profile?''.

The course of recovery of aphasia after stroke is highly variable. Even with severe aphasia, some patients recover rapidly over the first days after onset. There is little evidence that a single patient-related factor (e.g., age, gender, handedness, cognitive ability at the time of stroke, general health, education, social status, and intelligence) has relevant predictive value for speech recovery. The stroke-related variables seem more reliable predictors (Hillis & Heidler, 2002) and the factors often correlated with post-stroke aphasia recovery are the initial aphasia severity (Pedersen et al., 2004), aphasia type, and lesion size and location. Occasionally, a small lesion in a language critical area will lead to important language deficits and a slower overall recovery of aphasia than a large lesion in areas less strongly associated with language ability (Gerstenecker & Lazar, 2019). Superficial lesions of the cerebral cortex mainly damage specific processing units, while lesions of the subcortical white matter pathways might lead to disconnection symptoms (Pizzamiglio et al., 2006)

Plausible early and late recovery mechanisms include reperfusion of ischemic tissue surrounding the stroke, rapid reorganization of structure/function relationships, regression from diaschisis, and functional reorganization of the language network. Relearning and compensatory strategies can also be relevant for recovery (Hillis & Heidler, 2002). For instance, earlier intervention (commencing at one-month post-stroke) for poststroke aphasia seems crucial to maximize language recovery although recovery continues beyond the chronic period (after 6 months post-stroke) (Ali et al., 2021).

A post-stroke aphasia recovery biomarker can be defined as an indicator of disease that can be used as a measure of underlying processes (e.g., molecular/ cellular) and could be utilized to understand outcome or predict recovery and treatment response. Brain structure and function can be assessed with neurophysiological techniques such as electroencephalography (EEG), diffusion tensor imaging (DTI), magnetoencephalography (MEG), or blood flow-based techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). These instruments allow the in vivo evaluation of brain functions during rest or task performance in healthy and brain-damaged individuals. For example, in the acute period, perfusion-weighted MRI shows that word comprehension deficits are strongly correlated with blood flow within Wernicke's area (Hillis et al., 2001) while lexical processing is more strongly related to the volume of hypo-perfused tissue than to the volume of the lesion (Hillis et al., 2000).

MRI studies compared brain activity patterns of patients with good versus less positive language recovery and most studies found a bihemispheric activation during language processing, even for tasks that, in healthy participants, are correlated with left hemisphere activation only, e.g., the right inferior frontal gyrus (IFG) was more reliably recruited when the left inferior frontal cortex was lesioned (Turkeltaub et al., 2011).

A recent meta-analysis, based on 86 studies, confirmed that the left hemisphere language areas are less activated in PWA as compared to matched controls, and that these regions are more activated in those patients with less language impairment (Wilson & Schneck, 2021). The authors found limited evidence for differential recruitment of additional left hemisphere areas or domain-general networks, while the current evidence regarding the right hemisphere increased activation appears modest and equivocal. Furthermore, the current

fMRI evidence does not support the hypothesis of a dynamic reorganization of the language network over time (Wilson & Schneck, 2021).

The distinction between neuronal reorganization that does and does not sustain recovery in the chronic phase after stroke, either spontaneous or in response to treatment, remains controversial. For instance, sustained right hemisphere activity may contribute to treatment success in individuals with larger lesions and severe aphasia, as explained by the bimodal balance–recovery model that links interhemispheric balancing and functional recovery to the structural reserve spared by the lesion (Di Pino et al., 2014).

In summary, the use of neuroimaging techniques can provide insights into the neural basis of language deficits and improve recovery prediction. For example, Saur et al. (2010) based on early fMRI data, identified good versus poor aphasia recovery, with 86% accuracy. Nevertheless, due to elevated costs and scarce availability, these methods are rarely applied in clinical settings outside research centers.

Chapter 2: Noninvasive brain stimulation (NIBS)

Brains work using electricity, and information is transferred along the axon by electrical currents in the form of action potentials. When the electric potential between the intra- and extracellular space changes, reaching a certain threshold, action potentials are elicited (Engbert, 2021). A normal electrical processing is essential for the functioning of the brain and many neurological and psychiatric disorders are thought to develop following pathological processes concerning the electrical signal propagation between neurons (Gazzaniga, Ivry, & Mangun, 2002; Newson & Thiagarajan, 2019). Consequently, we can explore the effect of externally applied electric current and magnetic field at different levels, by means of tools that can allow us to interfere with the central nervous system (CNS) electrical activity.

Brain stimulation can be categorized as invasive [e.g., direct electrical stimulation (DES) and deep brain stimulation (DBS)] and noninvasive brain stimulation (NIBS) techniques. DES is used during awake surgery for the removal of brain tumors (Papagno 2017) and has provided information concerning a number of cognitive functions. DBS has been confirmed as a long-term effective treatment for patients with certain psychiatric or neurologic disorders, such as Parkinson's disease (PD), but it has been shown that can produce a decline in specific cognitive domains (Bucur & Papagno, 2022); these techniques can be used only for clinical purposes (Leimbach, Atkinson-Clement, Wilkinson, Cheung, & Jahanshahi, 2020).

On the other hand, NIBS encompasses a set of techniques used to modulate brain activity in a non-invasive manner. In this context, "non-invasive" brain stimulation is a procedure where no direct contact with the brain occurs.

The breakthrough for NIBS in humans was in 1980, when it was proved that short-lasting, strong electrical pulses delivered over the motor cortex (M1) can induce hand muscle twitches, and applied over the visual cortex can elicit phosphenes (Merton & Morton, 1980). NIBS has been widely used in both clinical (potential therapeutic modalities) and basic research (neurophysiology) due to its ability to modulate behavior. The brain has the innate ability to undergo neuronal plasticity, i.e., to change in response to external and internal stimuli. NIBS leverages this capacity to induce short and/or long-term beneficial effects both in clinical populations, treating

a variety of physiological conditions and psychiatric disorders, and in healthy individuals, enhancing different skills. NIBS modes of stimulation can be broadly classified as electrical (transcranial electrical stimulation - tES) (Nitsche & Paulus, 2000), magnetic (transcranial magnetic stimulation - TMS) (Barker, Jalinous, & Freeston, 1985), and sonographic (transcranial ultrasound - TUS) (Fomenko et al., 2020).

This chapter will focus on electrical and magnetic stimulations, specifically the transcranial direct current stimulation (tDCS) and the combination of TMS with electroencephalography (TMS-EEG).

2.1. Transcranial electrical stimulation (tES)

Transcranial electrical stimulation includes different neuromodulatory paradigms and tools: transcranial direct current stimulation (tDCS), high definition transcranial direct current stimulation (HD-tDCS); transcranial alternating current stimulation (tACS), transcranial random noise stimulation (tRNS), transcranial pulsed current stimulation (tPCS) (Jaberzadeh, Bastani, & Zoghi, 2014), and in the nerve stimulation category are galvanic vestibular stimulation (GVS) and vagal nerve stimulation (VNS) (Bhattacharya et al., 2021).

The essence of this method has been around in different forms for the last 2000 years. In antiquity, the electric torpedo fish, which can deliver from 8 to 220 volts, was used in an attempt to alleviate the pain of childbirth and headaches, basically numbing the patients. Today's tES uses a weak current intensity (1–4 mV) that feels more like a tingling or an itchy sensation (Sudbrack-Oliveira, Razza, & Brunoni, 2021).

From all the tES paradigms, disputably, tDCS is the most common one. This technique was first used in animal models and human trials in the 1950s, abandoned for a period of time, and reintroduced in human studies approximately 20 years ago, when Michael Nitsche and Walter Paulus showed that a weak electrical current through the human skull can improve task performance (Nitsche & Paulus, 2000, 2001; Priori, Berardelli, Rona, Accornero, & Manfredi, 1998). The first studies find evidence of physiological effects emerging during stimulation and, critically, the maintenance of the after-effects, i.e., the effects outlasted the stimulation time (Nitsche & Paulus, 2001; Priori et al., 1998). The neuromodulatory capabilities and relatively long-term after-effects coupled with other advantages such as being relatively safe (Antal et al., 2017), low cost, easy application, portability, tolerability, home-usable, and the possibility of combination with other therapies,

sparked the interest in cognitive neuroscientists, and a huge number of studies probing cognitive, behavioral, and clinical effects of tDCS (or the lack of such effects) have been published in academic journals.

2.1.1 Transcranial Direct Current Stimulation tDCS

The tDCS device is relatively simple; there is a continuous current-providing component (a battery) and wires that carry the current to the electrodes. Low-intensity electric currents, typically <4 Milliamperes (mA) (Antal et al., 2017), are delivered for 5 up to 30 min through carbon rubber or silver chloride electrodes and an interfacing conductive liquid, gel, or paste placed over the scalp (e.g., bi-cephalic montages) and/or upper body (e.g., monocephalic montages), depending on the targeted area(s) of stimulation [for a review of electrodes characteristics see (Solomons & Shanmugasundaram, 2020)]. Conventional tDCS is usually performed with two electrodes, and when the electrical circuit closes, current flows from one electrode, the anode (positive pole), to the other electrode, the cathode (negative terminal), presumably passing through the brain. When the anodal electrode is placed over a specific brain area, electric current is thought to flow into that region and stimulate neuronal activity. The cathodal electrode is considered the "exit" point for current, and it seems to inhibit neuronal activity (Stagg, Antal, & Nitsche, 2018), i.e., tDCS produces polarity-dependent changes of cortical excitability.

As previously mentioned, neurons are electrically excitable cells, and their function is based on the generation of action potentials, which depend on the resting membrane depolarization (Gazzaniga et al., 2002). Different from other NIBS, like TMS, that often apply a suprathreshold stimulation and directly elicit action potentials, tDCS involves the subthreshold modulation of neuronal populations, shifting the resting membrane potential toward depolarization or hyperpolarization, depending on stimulation parameters and the orientation of the neurons relative to the induced electric field (EF) (Bhattacharya et al., 2021). The tDCS stimulation is too weak to induce action potentials independent from other afferent stimuli inputs but sufficient to alter the excitability and the neuron spontaneous activity (Nitsche & Paulus, 2000). When using this method, the main goal is to modulate the neuronal resting potentials and to alter the state of excitability in the desired direction.

It was estimated that at an intensity between 1-2 mA, tDCS shifts the neuronal membrane potential by approximately 0.2 to 0.5 mV, which may seem to be a trivial change, knowing that the resting membrane

potential is approximately –70mV, and the threshold for action potential firing is approximately –50 mV (Opitz, Paulus, Will, Antunes, & Thielscher, 2015). However, at the whole-brain level, research data indicate that tDCS modulates the cortical excitability and activity by alterations to the action potential generation in extended neuronal networks, by changing the action potential timing, or both (Stagg et al., 2018). For instance, anodal tDCS stimulation of the M1 area enhances spontaneous activity and excitability, and vice versa, after cathodal stimulation, the spontaneous activity and excitability observed in M1 are reduced (Stagg et al., 2018). Usually, the directionality of the tDCS after-effects matches the one observed during stimulation. It is important to note that, being a continuous stimulation over a certain period, tDCS anodal and cathodal stimulation does not always lead to a net depolarization and net hyperpolarization respectively. Due to homeostatic regulatory mechanisms (the ability of the brain to dynamically stabilize its activity around a physiologically reasonable range in order to prevent itself from hyper-/hypo-excitability), the effects may fade away or reverse depending on the history of neural activity (Karabanov et al., 2015).

The efficacy of the resting membrane potential depolarization (increases neuronal excitability) or hyperpolarization (spontaneous activity is reduced) and, consequently, the tDCS effects, depend on many variables (brain, device, and montage related). The commonly considered factors that by themselves or in combination with each other influence in a nonlinear way the degree and the directionality of tDCS induced effects are: (i) age (Habich et al., 2020); (ii) orientation and integrity of neurons, (iii) neuroanatomy (location and size of anatomical targets); (iv) the baseline neuronal state and features (as all the NIBS, tDCS is state-dependent) (Li et al., 2015; Shahbabaie et al., 2014); (v) geometric montage of electrodes (e.g., the distance between the electrodes) and electrode characteristics (e.g., dimension, position); (vi) current density (i.e., current intensity/electrode surface area), polarity, and stimulation duration; (vii) co-administered drugs/treatments, etc. (Das, Holland, Frens, & Donchin, 2016; Woods et al., 2016). For instance, was estimated that up to 75% of the electrical current could be shunted through the human scalp or attenuated by soft tissue (Vöröslakos et al., 2018), especially when the inter-electrode distance is below 7 cm as often happens in bilateral montages (Fregni et al., 2021). Furthermore, modeling research showed that tDCS delivered electrical currents

could spread far away from the stimulation site and that microanatomical differences may vary its path (Datta et al., 2010; Opitz et al., 2015).

TDCS stimulation can induce both acute and neuroplastic alterations of cortical excitability. Acute effects of anodal tDCS appear to primarily depend on changes in membrane potential, while long-lasting aftereffects on cortical activity and excitability were linked to the glutamatergic process, involving NMDA receptors (Yavari, Jamil, Mosayebi Samani, Vidor, & Nitsche, 2018).

Animal model studies demonstrated that a few minutes of direct current (DC) stimulation of the sensorimotor cortex (S1) generate a prolonged change in neuronal activities for 1 up to 5 hours (Bindman, Lippold, & Redfearn, 1964). As already mentioned, human research on the motor cortex indicates that glutamatergic synapses are involved in tDCS induced plasticity (Stagg et al., 2018), especially with the involvement of NMDA receptors, as proven by the suppression of tDCS induced effects on M1 when NMDA-receptor were blockaded (Liebetanz, Nitsche, Tergau, & Paulus, 2002; Nitsche et al., 2003). A direct relation between DC and synaptic plasticity was proposed by several experimental studies that reported tDCS induced long-term potentiation (LTP) in the mouse motor cortex and, a polarity-specific modulation of LTP in rat hippocampal CA3-CA1 synapses (Fritsch et al., 2011; Ranieri et al., 2012). Synapses can strengthen (LTP) or weaken (long-term depression - LTD) their efficacy in response to increases or decreases in their activity and synchronicity, in accordance with Hebb's principle of cell assembly ("*When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased*", Hebb, 1949 p. 62) and tDCS effects seem supported by these mechanisms. Different neurotransmitters and their receptors are considered to be involved in these LTP and LTD processes: glutamate acts through N-methyl D-aspartate, dopamine through D1, adenosine through A2A, and acetylcholine through the muscarinic receptor (Bhattacharya et al., 2021). The nonlinear effects (opposite to the expected ones) reported in tDCS (LTD-like effects of anodal and LTP-like effects of cathodal tDCS, which appear to depend on the specific stimulation protocols) (Batsikadze, Moliadze, Paulus, Kuo, & Nitsche, 2013; Jamil et al., 2017) were explained based on the dependency of the direction of plasticity from the amount of intracellular calcium concentration modulated by the particular stimulation protocol. Ca^{2+} triggers both LTP and

LTD, and what determines whether LTP or LTD occurs is the Ca^{2+} level. Experiments indicate that blocking NA^+ or Ca^{2+} voltage-dependent ion channels activation prevents the excitability enhancement caused by anodal tDCS (Lisman, 2001; Nitsche et al., 2003). Nevertheless, a recent review on neurobiological findings concluded that LTP and LTD phenomena by itself might not be enough to explain the tDCS after-effects and that the modulation of the homeostatic cellular background controlling the strength and direction of synaptic plasticity might better account for the long-term changes induced by tDCS (Cirillo et al., 2017). An activity-selectivity hypothesis for tDCS enhancement of human behavior has been repeatedly proposed but not directly tested (Bikson, Name, & Rahman, 2013).

In conclusion, knowledge about the physiological foundation of the tDCS mechanisms is crucial for properly designed experimental studies, but despite research efforts, the underlying neurobiological mechanisms of tDCS immediate and long-term effects are still debatable and poorly comprehended. According to the current findings, tDCS may induce calcium-dependent plasticity at glutamatergic synapses, which is presumably gated by the reduction of GABA activity (Stagg et al., 2018).

Taking into consideration the complex interactions between single neurons and neural networks within the human brain, no sufficiently comprehensive information is available to properly model the effect of a specific stimulation protocol concerning the resulting functional state of neural networks, especially outside the motor cortex.

2.1.2 tDCS application, limits, and future directions

Despite increasing recognition of the high variability and the recent questions about effectiveness, tDCS remains a popular research/treatment technique in the field of neuromodulation, and two main investigation domains can be individuated.

One focuses on studies in clinical populations that assess tDCS effects on a range of diseases such as stroke (e.g., aphasia, hemiplegia), Parkinson's disease, epilepsy, attention deficit hyperactivity disorder, psychiatric disorders (e.g., schizophrenia, depression), chronic pain (fibromyalgia), eating disorders, drug addiction, etc. (Bhattacharya et al., 2021; Brunoni et al., 2012; Bueno et al., 2011; Fregni et al., 2021).

For example, investigating tDCS effects on depression, Brunoni and colleagues showed that they are similar to conventional pharmacological treatment, and, in a different study, that tDCS combined with sertraline (an antidepressant drug) leads to better results than treatment with sertraline alone (Brunoni et al., 2017; Brunoni et al., 2016). By contrast, in a recent trial examining tDCS efficacy in unipolar and bipolar depression, Loo et al. found no difference between active and sham stimulation (Loo et al., 2018). Despite heterogeneous effects, a recent RCTs meta-analysis on depressive episodes, including more than 1,000 participants, showed that active tDCS is clinically superior and as tolerable as the sham condition (Razza et al., 2020).

Another research direction investigates tDCS role in enhancing different cognitive functions in healthy population, such as learning and cognition (e.g., implicit and working memory, attention, motor skills, mathematical ability, perception, and creative problem solving) (Coffman, Clark, & Parasuraman, 2014).

For example, a meta-analysis based on 61 single-session, sham-controlled studies compared tDCS effects on cognitive functioning when the dorsolateral prefrontal cortex (DLPFC), an often-targeted area, was stimulated (Dedoncker, Brunoni, Baeken, & Vanderhasselt, 2016). The authors reported that anodal tDCS (over DLPFC), on healthy participants, significantly speed-up response time on cognitive tasks but not accuracy. Furthermore, univariate meta-regressions showed that current density (i.e. current/electrode surface area) and density charge [i.e. (current density)*(session duration)] influenced the observed effects; specifically, higher current densities/charges lead to stronger anodal tDCS effects on accuracy. Additional multivariate meta-regression revealed that gender modulated the effects of stimulation dose, namely, the higher the percentage of females included in the trials, the stronger the effect sizes. This finding was explained by sex differences in the anatomical location of the DLPFC and/or by hormonal differences affecting brain stimulation-induced changes in cortical excitability between women and men. In line with previous reviews, this meta-analysis indicated no effects on cognition in healthy samples after cathodal tDCS (Dedoncker et al., 2016).

Another recent meta-analysis that investigated tDCS effects on cognitive functions in healthy elderly people suggests that online, but not offline, tDCS protocols may effectively improve reaction times during the performance of various cognitive tasks (Lee, Lee, & Kang, 2021). Unfortunately, as it often happens, most of the included studies (24 out of 31) used a single session of tDCS and heterogeneous tDCS parameter setups

(different current intensity, different targeted areas) which prevent the authors from indicating efficient stimulation guidelines (i.e., which tDCS parameters could guarantee a positive effect).

As it can be observed from the previous examples, in both clinical and healthy populations' studies, sham stimulation, a reliable blinding method, is generally applied. The setting is identical to that of a real tDCS session, but no current is passed through the device except for up to 10–30 s, with a fade in/out of the current, at the beginning and at the end of the session to mimic the sensation of real stimulation (Ambrus et al., 2012).

Some research protocols are "single-blind," where only the subjects are unaware of the stimulation type (real or sham), whereas others are "double-blind," meaning that both the subject and the experimenter administering tDCS are not informed about the stimulation parameters. It is also possible to use a treatment group instead of a sham group and compare the results of standard treatment to the results of tDCS. The research designs may vary from within-subject designs (often crossover), where, in different sessions, a single participant receives both sham and tDCS stimulation to between-subjects designs where different groups receive different stimulations. The main goal is to obtain a valid experimental control in order to exclude all the confounding variables that may invalidate the registered data.

Several clinical trials and systematic reviews investigated the adverse events and limitations of tDCS. Brunoni et al., 2011, after analyzing 172 reports, found that the main adverse events were: itching (39.3% tDCS vs. 32.9% sham), tingling (22.2% tDCS vs. 18.3% sham), headache (14.8% tDCS vs. 16.2%sham), discomfort (10.4% tDCS vs. 13.4% sham), and burning sensation (8.7% tDCS vs. 10% sham), with no statistically significant difference between the two conditions (Brunoni et al., 2011). Overall, most reported adverse effects are mild and disappear soon after stimulation. Recently, the same research group conducted a systematic review to investigate the tolerability (rate of adverse events) and acceptability (rate of dropouts) of tDCS stimulation for different neuropsychiatric conditions. The authors concluded that the overall dropout rate was low and similar in active and sham groups, and the most commonly reported side effects were tingling, skin redness, itching, fatigue and headache, burning sensation, and sleepiness. Although rarely, more severe negative side effects have been reported: skin burns due to suboptimal electrode skin contact, mania or hypomania in patients with

depression, and mild pain. To date, no conclusive evidence that tDCS can damage brain tissue or impair cognitive functions has been produced (Matsumoto & Ugawa, 2017; Zhao et al., 2017).

Despite tDCS popularity, many challenges need to be overcome in order to use tDCS as a viable instrument for basic and clinical research. Regarding the tDCS limitations, the most critical ones concern the low spatial resolution and difficulty in precisely localizing the electric field current, especially in a lesioned brain (Datta et al., 2010). Another important issue regards the mechanisms of action of tDCS, which continues to elude the scientific community. Namely, as previously briefly described, the mechanisms possibly involve various short– and long–term synaptic and non-synaptic effects on neurons and non-neuronal cells of the CNS. Deep comprehension of these tDCS underlining mechanisms in humans, especially outside the motor cortex, may shed light on the efficacy of combining tDCS with other therapeutic approaches, such as drugs, TMS, and behavioral therapy. A better understanding of the tDCS mechanisms of action is also important for improving the tDCS clinical safety and efficacy (the optimal current intensity and montage calibration). Further development of tDCS protocols should also tackle the ongoing issues of high interindividual variability of stimulation effects (Brunoni et al., 2012). This goal might be achieved, at least partially, by studying the brain mechanisms through combined techniques such as TMS, EEG, and fMRI, but for this, important technical challenges must be overcome (it is not trivial to insert electrical wires in an MRI machine). Another limitation that most NIBS have (except for the TUS) is the inability to target deeper structures, all the effects being limited to the cortical areas.

In conclusion, tDCS has a wide range of potential applications and many advantages that make it appealing especially for clinical research. TDCS can be used to explore the basic aspects of neural functions as well as for the treatment of neuropsychiatric disorders. Research data suggests that tDCS has unique characteristics, such as the ability to induce antagonistic effects in cortical excitability according to the parameters of stimulation, concomitant (''online'') use with other therapies, and noninvasiveness (Brunoni et al., 2012). Unfortunately, tDCS also brings important challenges regarding clinical design, stimulation parameters, and, above all, efficacy issues. In this chapter, we aimed to provide an overview of tDCS role in research as a promising approach that needs further investigation.

2.2. The simultaneous combination of TMS and EEG (TMS-EEG)

2.2.1 Transcranial magnetic stimulation (TMS)

TMS is an indirect and non-invasive method used to induce excitability changes in the cortex via a magnetic field that passes through the scalp. The TMS machine is made of one or more capacitors, where the energy is stored, connected to a stimulation coil through which the electrical currents are driven. TMS consists of the passage of a brief current of very high intensity through a copper wire coil, which in turn produces a magnetic field of several Teslas. The magnetic field, which is parallel but opposite to the direction of the electric field generated in the stimulating coil, induces an electric field in a secondary circuit (in this case, the brain) based on the principle of electromagnetic induction proposed in 1831 by Faraday (Lefaucheur, 2019). If a circular coil is positioned flat on the scalp, currents flow in a plane parallel to both the coil and the scalp. The magnetic field can be reduced by extracerebral tissues (e.g., scalp, bone), but the induced electrical field is enough to depolarize superficial neurons, especially the ones oriented horizontally in a plane that is parallel to both the coil and the brain surface, and generate action potentials (Tofts, 1990). In general, the penetrating depth of the magnetic pulse is relatively short; therefore, TMS stimulates mainly the upper cortical layers rather than subcortical white matter tracts. By briefly interfering with a specific cortical region, TMS can be applied to localize the neural correlates of cerebral processes in time and space (with a focality of 1 to 2 square centimeters) or, for clinical purposes, to obtain persistent effects that outlast stimulation.

As previously discussed for the tDCS, different effects (e.g., inhibitory, excitatory, short- or long-lasting) can be obtained by selecting the right TMS protocols and parameters, such as coil configuration and optimal coil orientation (from the classical figure-of-eight coil to the more recent multi-locus TMS coil) (Koponen, Nieminen, & Ilmoniemi, 2018), intensity, frequency, waveform (monophasic and biphasic), and duration. For example, there are various modes of TMS stimulation that can be applied offline (trains of stimuli before task execution) or online (trains of pulses during the task execution synchronized with specific stimuli): single-pulse TMS, paired-pulse TMS (cortico-cortical or intra-cortical), low (1 Hz - inhibitory) and high frequency (> 5 HZ - excitatory) repetitive (rTMS), and, depending on the stimulation patterns, continuous (cTBS - inhibitory) and intermittent

(iTBS - excitatory) theta-burst stimulation (three 50 Hz pulse trains are applied every 200 milliseconds, i.e. at theta frequency) (Bhattacharya et al., 2021).

Because of natural anatomical variation of the brain size, targeting a specific area with TMS can be inaccurate. To improve this aspect, but also to maintain the exact stimulation parameters in multiple sessions (even months apart), TMS can be combined with an online magnetic resonance imaging (MRI) -guided neuronavigation system that uses the subject's own anatomical MRI or a probabilistic head model to perform accurate navigation. The subject wears a head tracker with spherical reflective parts monitored by an infrared camera. The participant's head is co-registered with the MRI (real or estimated) and, subsequently, the coregistered MRI can be used to localize a specific stimulation target (based on a 3-dimensional coordinate system). The infrared camera, registering the location, orientation, tilting, and induced electric field direction, also monitors the TMS coil and stimulation parameters (Lefaucheur, 2019).

Despite limited knowledge about how TMS affects the neural structure (Klomjai, Katz, & Lackmy-Vallée, 2015), since the Merton and Morton experiment (1980) TMS has been widely used in brain research. Originally applied over the motor cortex, now sensory and associative areas are stimulated with the aim to vary cortical excitability associated with specific sensory and cognitive functions as well as those associated with neurological and psychiatric disorders (Klomjai et al., 2015). To date, TMS has the largest number of studies for clinical applications. For example, a recent meta-analysis investigating the rTMS effectiveness on mild cognitive impairment (MCI) and Alzheimer's disease (AD) found an overall medium-to-large effect size favoring active rTMS (high-frequency on the left DLPFC and low-frequency on the right DLPFC) compared to sham rTMS (Chou, Ton That, & Sundman, 2020). Furthermore, the United States Food and Drug Administration (FDA) approved TMS as a therapeutic clinical treatment for major depressive disorders in 2008 (Bhattacharya et al., 2021).

2.2.2 The electroencephalogram (EEG)

EEG is an electrophysiological technique. It was devised almost 100 years ago when Hans Berger discovered alpha oscillations. Today, it is considered the most common noninvasive method for measuring electrical brain activity, especially in clinical settings and in infants (St. Louis et al., 2016). Despite progresses in structural and functional neuroimaging techniques, EEG remains a method of choice for many research questions and

applications, like brain-computer interfacing (Feyissa & Tatum, 2019). The EEG main advantage over other methods such as fMRI is the optimal temporal resolution, and therefore, one of the main usages is the evaluation of the dynamic neural activity.

Basically, EEG-recorded oscillatory activities are thought to arise from a combination of factors: the intrinsic properties of neurons, structural properties (e.g., propagation speed), the functional properties of neurotransmitter systems, and the network interactions and feedback loops (e.g., thalamocortical pathways) (Farzan et al., 2016). EEG recorded brainwaves are primarily generated through spatial and temporal summation of excitatory and inhibitory postsynaptic potentials generated by cortical pyramidal neurons (mostly in layers 3, 5, and 6) (St. Louis et al., 2016). Brain oscillation detection is maximal when generated dipoles of electrical current are oriented perpendicular to the cortical gyri surface (Feyissa & Tatum, 2019). Due to the EEG electrode dimension, each electrode records a pool of approximately 250,000 neurons synchronously. Before the EEG is recorded from the scalp, the signals travel through multiple biological elements (meninges, skull, skin) that attenuate the signal amplitude and spread the neural activity to a wider area than the source, making the source reconstruction a difficult task.

An EEG system consists of multiple sensors (electrodes), amplifiers, and an analog-to-digital converter for data digitization. In classical EEG recordings, Ag/AgCl-coated electrodes (and more recently TMS- and MRI-compatible Teflon-coated plastic MRI- electrodes) are placed on the scalp, often using the 10-20 system. EEG uses the principle of differential amplification, i.e., the raw EEG signal is the difference between the potential at a recording electrode and another neighboring or distant reference electrode (Acharya & Acharya, 2019). Electrodes are connected with a jack-box, which converts the analog EEG signal into digital code. The jack-box interfaces with the computer system of the EEG machine, storing each bit of information. The EEG amplifiers amplify low-amplitude EEG signals from the brain, providing a near real-time display of ongoing cerebral activity, with the y-axis showing the channel names and/or the voltage in units of μV and the x-axis showing the epoch numbers and/or the time. There are two major types of brain activity captured by the EEG: (i) spontaneous EEG, which is based on internally induced processes and mental tasks that mainly generate changes in the ongoing EEG, and (ii) Event-related potentials (ERPs), which are based on events and external stimuli. ERPs can also be

elicited by actions generated by a person's internal decisions, e.g., starting a movement. The main portion of the signal power originates from rhythmic oscillations in a frequency bandwidth from below 1Hz to approximately 45Hz, even though higher frequencies are also measurable. The frequency range is subdivided into smaller ranges with specific names that are associated with different main functions, like the Delta waves defined by very-low-frequency ($\delta\approx$1-4 Hz) and usually correlated to deep and unconscious sleep or, at the opposite pole, the very high frequencies named Gamma oscillations ($\gamma\approx$30–100 Hz), often associated with arousal (Müller-Putz, 2020; Peng, 2019). Furthermore, spontaneous waking EEG indicates that different areas of the human brain tend to engage in electrical oscillations at different frequencies (Niedermeyer, 1999), for instance, when a person lays eyes closed in a state of relaxation, occipital areas typically oscillate at a frequency of$\approx$10 Hz (αrhythm, 8–12Hz), whereas occipital and sensory-motor cortex frequently displays faster rhythms, at $\approx$20 Hz (β rhythm, 13–20 Hz) (Rosanova et al., 2009).

2.2.3 Transcranial magnetic stimulation and electroencephalography (TMS–EEG)

The principles governing interactions within and between functional neural networks have captured the interest of modern neurosciences, yet many remain poorly comprehended. Likewise, the clinical applications of NIBS require patient-tailored solutions to optimize treatment efficacy; still, specific parameters at the single-subject level are difficult to set up due to the intricacies of the interactions between the externally applied stimulation and ongoing brain dynamics.

The popularity of TMS as a mapping tool for studying motor, perceptual, and cognitive functions is due to the possibility it offers to investigate the causal implication of an area, or of a network, in a specific task (Lefaucheur, 2019). To better understand the neural activity elicited by TMS, fMRI was often used because it shows the activity in the entire brain, thus providing a broader picture of the cortical responses to a TMS pulse. However, fMRI does not have sufficient temporal sensitivity to allow an investigation into the immediate effects of TMS as blood flow responses are slower (Miniussi & Thut, 2010). For this reason, TMS coupled with EEG coregistration was developed, as EEG captures the cortical activity with high temporal resolution (Fitzgerald, 2010). The first TMS-evoked EEG recordings were conducted in the late 1980s (Cracco, Amassian, Maccabee, & Cracco, 1989), but several technical problems occurred, such as major TMS artifacts (the TMS magnetic field

also induces an undesired electric field in nearby conductors such as EEG electrodes, skin, nerves, muscles, etc.) and safety issues, e.g. excessive heating in the electrodes. Since then, progresses have been made, and recent developments aim to create an automated closed-loop system that optimizes online the TMS stimulation parameters based on single-trial EEG responses (Tervo et al., 2021).

The TMS-EEG co-registration offers an in-vivo method to study the neurophysiological characteristics and brain dynamics of any neocortical brain area or network across developmental, behavioral, and disease states (Fitzgerald, 2010; Ilmoniemi & Kičić, 2010; Kallioniemi, Saari, Ferreri, & Määttä, 2022).

As already explained, TMS can depolarize cell membranes and initiate action potentials. Subsequently, the action potentials from under the coil area propagate to the interconnected brain regions through short- or long-range cortico-cortical, thalamocortical, or cerebello-cortical pathways. EEG sensors record the spatial and temporal summations of the excitatory and inhibitory postsynaptic potentials influenced by this cascade of events. In other words, TMS is applied over a focal region (1 to 2 square centimeters), and this activates the cortical neurons and instantaneously creates TMS-evoked EEG potentials (TEPs). The waveform of TEPs is, to a certain degree, influenced by the stimulation site (Kähkönen, Komssi, Wilenius, & Ilmoniemi, 2005). For example, single-pulse TMS on the motor cortex elicits a typical waveform that consists of positive and negative peaks labeled after their polarity and latency (e.g., P30, N45, P60); on the frontal cortex, TMS elicits a similar sequel of deflections but with a more frontal distribution (Kallioniemi et al., 2018). TEPs can be recorded from electrodes near the stimulation site [region-of-interest (ROI) analyses], as well as from the entire cortex. The ability to monitor cortical reactivity across diverse neuronal populations allows for the investigation of widespread changes in cortical dynamics. TEPs are considered highly reproducible, demonstrating consistency over time and they have also been shown to be sensitive to changes in brain state (Casarotto et al., 2010; Massimini et al., 2005).

From a technical point of view, the combination of TMS and EEG requires the compatibility of TMS and EEG equipment, but there are also integrated TMS-EEG systems that include optimized amplifiers and electrodes. For a good signal-to-noise ratio, the recording system must include appropriate technology to avoid amplifier saturation, minimize artifacts during data acquisition, and have appropriate electrode types to avoid electrode

movement or overheating (Kallioniemi et al., 2018). The main challenges related to TMS-EEG recordings are the artifacts in the EEG data that can cover the genuine brain activity. The electromagnetic fields generated within the TMS coil can create a substantial spike artifact; the capacitors need to recharge after each TMS pulse, creating a recharging artifact; the activation of the peripheral nerves evokes muscle artifacts; and the TMS pulse produces auditory and somatosensory-evoked potentials (Conde et al., 2019; Siebner et al., 2019). Artifacts can also appear from the pressure exercised by the TMS coil on the EEG electrodes underneath it. Other common EEG artifacts are also frequently observed, such as: eye movements and blinks (time-locked to the stimulation), facial muscle activity (e.g., jaw clenching), and electrical line noise (e.g., 50 Hz in Europe). Even when taking the proper precautions (e.g., suitable auditory noise masking) with the best equipment, some artifacts are hard to avoid. In this case, before the actual analysis, data needs to be properly preprocessed offline. Often the preprocessing steps include data filtering (removing the non-neural artifactual frequencies from the signal), artifact removal (sometimes by discarding the trials containing an artifact), and segmentation of the continuous signal into trials, baseline correction, and averaging. To date, there are no standardized pipelines for the TMS-EEG data preprocessing, but various toolboxes, with different levels of automaticity (manual, semi-automatic, or automatic bad trials/channels and ICA-component rejection) are available (Bertazzoli et al., 2021). The TMS-EEG connectivity methods, generally, include time (when and where do the neural events occur) and frequency domain analysis (properties of the EEG signal such as amplitude and phase) (Amico, Van Mierlo, Marinazzo, & Laureys, 2015). These approaches can be applied to evaluate functional and effective TMS-EEG connectivity. In *functional connectivity*, the main purpose is to detect brain areas with similar EEG amplitude, frequency, or phase; *effective connectivity analysis* aims at determining the direct and indirect effects of a TMS pulse on a neuronal population (Kallioniemi et al., 2018).

The TMS-EEG methodology is currently used in basic research, cognitive neuroscience, and clinical research. For example, investigating the neurophysiological correlates of behavioral states, Massimini et al. (2005) found evidence for a breakdown of transcallosal and long-range effective connectivity during NREM sleep (Massimini et al., 2005). Another TMS-EEG application is the analysis of the causal relationship between the brain spatiotemporal dynamics (interactions within and between multiple brain networks) and behavior in offline and

online experimental designs. For instance, in a recent publication, TMS-EEG was applied over the right occipital face area (rOFA), to investigate changes in cortical excitability related to conscious and unconscious face perception (Mattavelli et al., 2019). In the clinical area, identifying TMS-EEG neurophysiological indices that underlie a pathological condition could help design targeted and individualized therapies, as Tscherpel et al. did in their study investigating the neural correlates of stroke-induced motor deficits and recovery (Tscherpel et al., 2020). Specifically, TMS-EEG was used to measure the brain responses over the ipsilesional M1 in 25 ischemic stroke patients with mild-to-severe motor impairment in the subacute phase (52 weeks) and after 3 and 6 months post-stroke. As hypothesized, ipsilesional M1 TMS stimulation differentiated between healthy and stroke participants in the time and time-frequency domain. The results indicated that the significant TMS-EEG alterations (e.g., low-frequency oscillations of ipsilesional M1) recorded in the subacute period were correlated to the initial motor impairment severity and the amount of clinical recovery after 3 months, but not with the lesion volume.

Future research would benefit from improvements in both acquisition devices and signal processing methods, e.g., the analysis of factors influencing the TMS-EEG data variability such as inter-individual differences in the EEG properties. Another research direction could be the combination of TMS-EEG with neuroimaging techniques and genetic studies to further advance the study of causal relations governing the gene-brain-behavior relationship (Farzan et al., 2016).

Chapter 3: NIBS and post-stroke rehabilitation

Stroke is defined as a sudden loss of blood supply to the brain leading to permanent tissue damage caused by thrombotic, embolic, or hemorrhagic events. Stroke is the second leading cause of death and a major cause of disability worldwide, affecting around 13.7 million people, provoking death in approximately 5.9 million annually, with a projected increase to 23 million cases in 2030, correlating with the growth of the aging population (Villa, Ferrari, & Moretti, 2018). This cerebrovascular disease is considered the leading risk factor for severe physical disability and cognitive impairment, disproportionately affecting the aging population. Although the majority of stroke victims achieve at least some spontaneous recovery, it remains one of the main causes of permanent disability, requiring a very long-term rehabilitation approach (Benjamin et al., 2017).

As specified in the previous chapters, NIBS can modulate the excitability of targeted brain areas and facilitate neuroplasticity by inducing cortical currents of short duration through magnetic fields (TMS) or by applying weak currents (tES).

This chapter includes two published reviews investigating the NIBS effects on post-stroke rehabilitation, specifically on post-stroke depression (Bucur & Papagno, 2018) and aphasia (Bucur & Papagno, 2019).

3.1 A systematic review of noninvasive brain stimulation for post-stroke depression (PSD)

3.1.1. Introduction

The association of neuropsychiatric disorders with cerebrovascular diseases includes depression, anxiety disorder, apathy, cognitive disorder, mania, psychosis, pathological affective display, catastrophic reactions, fatigue, and anosognosia. Post-stroke depression (PSD) is the most frequent and burdensome neuropsychiatric post-stroke complication. PSD is associated with higher mortality, poorer recovery, more pronounced cognitive deficits, and lower quality of life than in stroke without depression (Medeiros, Roy, Kontos, & Beach, 2020). PSD is described as a depressive syndrome that emerges in the chronological context of stroke (Aben et al., 2001) and is usually noticed immediately following the acute vascular event; nevertheless, occasionally there might be a late onset, even more than 6 months later.

According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), PSD is a *depressive disorder due to another medical condition* (stroke) (American Psychiatric Association, 2013). The diagnostic criteria are: A) presence of depressed mood or anhedonia; B) symptoms are pathophysiologically related to the stroke; C) symptoms are not better explained by other psychiatric disorders; D) disturbance does not occur exclusively in the presence of delirium, and E) symptoms cause significant distress or impairment (American Psychiatric Association, 2013).

The PSD prevalence is estimated to be between 18 and 33%, the discrepancies being explained by the high heterogeneity of the assessment tools, setting, time after stroke, lesion characteristics, etc. (Aben et al., 2001; Jørgensen et al., 2016; Shi, Yang, Zeng, & Wu, 2017). A meta-analysis evaluating mood disorders after stroke reported a 33.5% prevalence of all depressive disorders, but specifically, major depression accounted for 17.7%, minor depression for 13.1%, and dysthymia for 3.1% (Mitchell et al., 2017).

Data indicate that the main risk factor for PSD is the stroke severity while a valid PSD predictor might be the patients' mental history (Shi et al., 2017). Depression is more frequent in stroke patients than in the general population and the main risk factors are female sex, history of psychiatric illness, large or multiple strokes, injuries in frontal/anterior areas or in the basal ganglia, stroke occurrence within the past year, poor social support, and pronounced disability (Grajny et al., 2016; Medeiros et al., 2020). As in the majority of the mood disorders in psychiatry, the mechanism of PSD has a multifactorial nature, being better explained by a mix of psychological (reduced quality of life), social (psychosocial distress), and biological factors (ischemia induced neurobiological dysfunctions) (Villa et al., 2018).

The complexity of PSD mechanisms makes its biologically-based prevention and treatment a difficult task. Despite several similarities between PSD and major depressive disorder (MDD) there are some relevant differences: (i) the pathophysiology of PSD is linked to the vascular injury; (ii) it often coexists with other cognitive and physical impairments (PSD has a disproportionally high prevalence of physical disability such as aphasia); (iii) PSD tends to have more severe depressive symptoms than MDD (De Man-Van Ginkel et al., 2015). From a clinical perspective, all of these coexisting variables require a different treatment approach for PSD, i.e., it cannot be identical with the one for MDD.

The PSD pharmacological treatment efficacy is debatable, with some publications indicating an enhanced recovery (Robinson & Jorge, 2016) while others demonstrating limited efficacy and important side effects (Baker et al., 2018; Paolucci, 2017). The most used antidepressants are paroxetine and escitalopram, but Selective Serotonin Reuptake Inhibitors (SSRIs) usage has been correlated with an increased risk of brain hemorrhage. A more recent alternative strategy for anti-depressants are the anti-inflammatory and the anti-cytokine modulators therapies, including monoclonal antibodies and cytokine inhibitors, but to date the results are uncertain (Villa et al., 2018).

Other studies report some positive impact on depressive symptoms after interventions like ecosystem-focused therapy, life review and problem-solving therapy, music therapy, exercise, behavioral therapy and robotic-assisted neuro-rehabilitation (Baker et al., 2018; Hadidi, Wagner, & Lindquist et al., 2017).

Thus, it seems that identifying additional therapeutic intervention, like NIBS, may lead to a more effective rehabilitation approach. As detailed in the second chapter, TMS and tDCS have been found to be promising noninvasive treatments for a variety of neuropsychiatric conditions.

Neuronal reorganization and plasticity that follow stroke may be beneficial or maladaptive, and NIBS can be used to monitor and modulate this mechanism, facilitating or disrupting the neuronal activity, creating temporary or long-lasting desirable brain changes (Miniussi, 2016) that could improve the PSD treatment by making it more effective and less expensive in terms of money and time. For these reasons, rTMS and tDCS have attracted interest as novel methods to treat neurological disorders, including PSD, but their application protocols (e.g., stimulation area, frequency, intensity, polarity, duration) and consequently their effects are characterized by incertitude and great variability. To our knowledge, the only published meta-analysis investigating neuro-stimulation effects in the treatment of PSD is the one by Shen and colleagues (Shen et al., 2017). The authors concluded that rTMS might have beneficial effects on PSD but they could not make specific recommendations concerning stimulation parameters or target sites.

The aim of this systematic review was to analyze and synthesize the evidence of tDCS and TMS interventions on the treatment of PSD with tDCS and TMS and to discuss their efficacy. For this purpose, we selected and analyzed all the published studies, double-blind, randomized placebo-controlled trials (RCTs) as well as open-

label studies and case reports, paying particular attention to the methods applied, the participants' characteristics, and the results.

3.1.2. Methods

The present systematic review was conducted under the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Liberati et al., 2009).

Search strategy and selection criteria

The following electronic databases were used for papers identification up to December 2017: MEDLINE (accessed by PubMed, https://www.ncbi.nlm.nih.gov/pubmed), PsycARTICLES (via EBSCOHost, https://search.ebscohost.com), PsycINFO (via EBSCOHost) and Web of Science (https://webofknowledge.com/). Keywords were: (1) "tDCS", "transcranial direct current stimulation"; "brain stimulation"; "transcranial magnetic stimulation", "TMS" AND (2) "post-stroke depression", "depression after stroke". All articles retrieved by these search terms were screened for inclusion criteria and, additionally, the reference list of each paper was checked for new, relevant ones.

Titles, abstracts, and full-text articles were screened independently by the authors and evaluated for eligibility based on the following inclusion /exclusion criteria: (1) interventions designed for adults with post-stroke depression, (2) non-invasive brain stimulation methods (TMS or tDCS) specified as the main intervention, (3) peer-reviewed, and (4) published in English. Only patient's studies with an explicit PSD diagnosis, i.e., mood disorders due to stroke with depressive features, major depressive-like episode, or mixed-mood features were analyzed. All publications that investigated vascular depression and in which a specific PSD diagnosis was ambiguous because history of stroke was not a compulsory inclusion criterion were excluded.

Data extraction and Study quality assessment

For each included paper, the relevant information to be extracted concerned: study characteristics and outcomes, participants' characteristics (sample size, diagnosis and diagnosis instruments, gender, age, time since stroke, pharmacological treatment, lesion type and stroke area), study design, stimulation parameters and brain areas of interest, statistical analysis, and reported findings (immediately after treatment and at follow-up); then, we analyzed similarities and differences between the studies with the aim of verifying which

parameters are preferable and in which conditions. Our initial goal was to compare the TMS and tDCS efficacy and to conduct a meta-analysis using the standardized main differences for the main outcomes. Due to the limited number of studies and their high heterogeneity we could not compute a quantitative synthesis of the data in order to create an evidence profile of the estimated effect and offer specific recommendations.

Each study research design was evaluated, and a grade for the level of evidence was assigned according to the modified Sackett Scale (Sackett, Straus, Richardson, Rosenberg, 2000) based on the Physiotherapy Evidence Database (PEDro) scores (see Tables 1, 2 and 3).

PEDro is an eleven yes/no item scale assessing the quality of clinical trials and is considered a valid and comprehensive instrument previously applied in systematic reviews (McIntyre, Thompson, Burhan, Mehta, & Teasell, 2016). Items can be scored as either present (1) or absent (0) and the total score is obtained by summation, with higher values indicating greater quality: 9–10: excellent; 6–8: very good; 4–5: good; <4: poor (Foley, Teasell, Bhogal, & Speechley, 2003).

The Sackett Scale includes five levels of evidence: Level 1 is comprised of high quality RCTs (PEDro ≥6) and meta-analysis, being divided into level 1a and level 1b based on the number of RCTs. Level 2 evidence is also derived from RCTs but with a PEDro score < 6. Level 3 evidence refers to non-randomized clinical trials and case control designs (retrospective studies comparing conditions including historical controls). Levels 4 and 5 refer to case series, uncontrolled pre- post-treatment tests, observational studies, case report designs.

3.1.3. Results

We retrieved 329 citations; duplicates and studies that did not satisfy the inclusion criteria as revealed by the title or abstract were excluded, and 49 papers underwent full review (Figure.1.), resulting in 7 accepted articles.

Figure 1. Flow Diagram of study selection and inclusion.
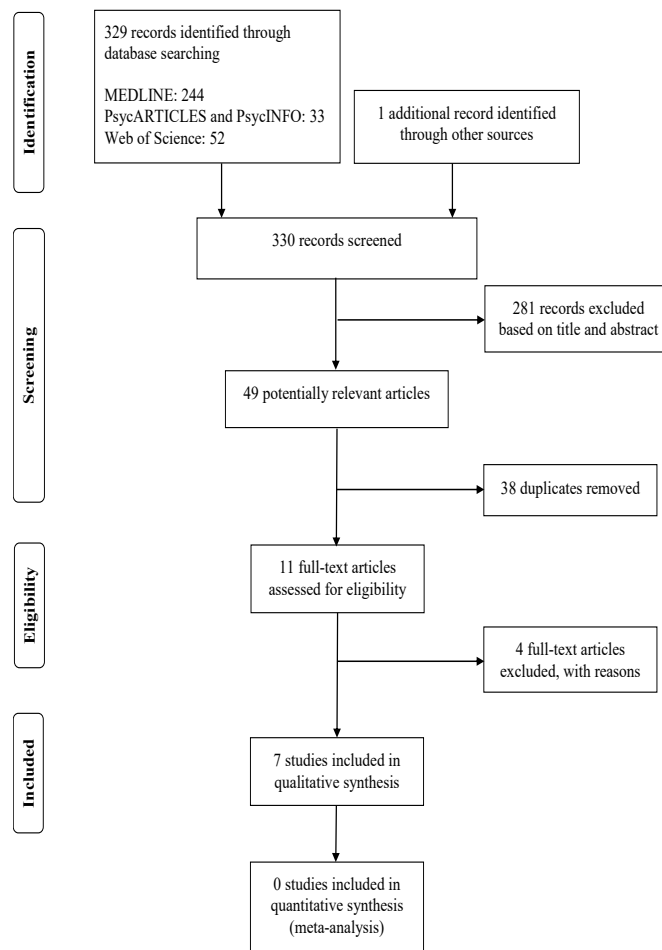
Characteristics of studies

Four experimental studies were therapeutic applications of tDCS (An et al., 2017; Bueno et al., 2011; Valiengo et al., 2016; Valiengo et al., 2017) for PSD treatment: two experimental research, one case series, and one case report (see Table 1). The other three included publications which investigated the effects of rTMS (Etribi & Nahas, 2010; Gu & Chang, 2017; Jorge et al., 2004) on depressed patients after stroke (two were RCTs, see Table 2).

The methodological quality of the 3 RCTs (Gu & Chang, 2017; Jorge et al., 2004; Valiengo et al., 2017) was consistently high, with a mean PEDro score of 8 out of 10 (level 1b evidence). All RCT studies (tDCS and TMS) used randomization; however, the concealed allocation was not specified. Each study used participant blinding and had excellent retention rates. Two studies were non-randomized experimental designs: the El Etribi et al. (2010) uncontrolled pre-post study was rated as level 4 evidence while An et al (2017) having a control group was rated as level 3 on the modified Sackett Scale (Sackett et al., 2000). The case series study (Valiengo et al., 2016) and the case report (Bueno et al., 2011) were considered as level 4 and 5 evidence, respectively (see Table 1, 2 and 3). Overall, the level of evidence can be considered low, with only 3 studies out of 7 having a 1b evidence level, while all the others were below level 3.

Participants' characteristics

The review included seven studies involving 157 patients diagnosed with PSD. Regarding the participants' characteristics, there was heterogeneity among studies (Table 4) especially concerning:

(1)   the time interval from stroke –not always specified and ranging between 2 years and 6 months;

(2)   the PSD diagnosis criteria and the instruments used to assess depressive symptoms - patients were selected on the basis of their scoring above arbitrary cutoff points on depression rating scales (for example: Beck Depression Inventory scores > 12, Hamilton Depression Rating Scale scores > 6, Present State Examination modified, Mini-International Neuropsychiatry Interview); it is important to notice that the effectiveness of these instruments as diagnostic tools for PSD was rarely validated;

(3)   participants' age ranging between 38 and 71 years,

(4)   lesion - the majority of the studies offered only general information about lesion site (left or right hemisphere) and etiology (ischemic or hemorrhagic);

Two studies (Valiengo et al., 2017; El Etribi et al., 2010) report other relevant neurological and psychiatric information like depression type, associated medical conditions, and drugs use. With two exceptions (An et al., 2017; Valiengo et al., 2016), in all studies poor cognitive assessment was performed, e.g., the Mini-Mental State Examination (MMSE), which is totally inappropriate in the case of focal lesions. Only in the case series reported by Valiengo et al. (2016) were aphasic patients with PSD included, while in three papers aphasia was an

exclusion criterion (Gu and Chang, 2017; Jorge et al., 2004; Valiengo et al., 2017), and in the other three (An et al., 2017; Bueno et al., 2011; El Etribi et al., 2010), no explicit information was offered about aphasic participants' inclusion or exclusion.

Intervention

Target area. In all studies (rTMS and tDCS), the target area was identified in the left DLPFC (F3 according to the International 10-20 EEG System).

Stimulation protocol: rTMS. Two studies (Gu and Chang, 2017; Jorge et al., 2004) used the same stimulation protocol: rTMS was delivered at 110% of the resting motor threshold at a frequency of 10 Hz, 20 trains of 5 seconds duration for 10 sessions (2 consecutive weeks). Alternatively, in El Etribi et al.'s (2010) study, an inhibitory rTMS was applied at 100% of the resting motor threshold at a frequency of 1 Hz for 10 sessions. The magnetic pulses were delivered only with figure of eight shaped coils.

Stimulation protocol: tDCS. Electrode montage, intensity, and duration of tDCS stimulation are important variables that influence the outcome of tDCS studies on PSD recovery, among other factors. In all included studies, tDCS was bi-hemispheric and two equal sized electrodes were used. Each electrode had an area of 25 cm2 (5 cm×5 cm) or in some cases 35 cm2 (5 cm×7cm), with the anode invariably placed over the left and the cathode over the right DLPFC, i.e., located in F3 and F4, respectively, according to the International 10–20 electroencephalography system.

The intensity and duration of the stimulus were homogeneous across studies with tDCS being delivered at an intensity of 2 mA for 30 minutes. The only relevant variation concerned the number of sessions, which ranged between 10 (Bueno et al., 2011) and 20 (An et al., 2017) while in Valiengo's studies (2016, 2017) tDCS was delivered for 10 consecutive workdays, with two additional sessions after two and four weeks, with a total of 12 sessions.

Associated therapies. No other intervention or pharmacological treatment were associated with the stimulation protocol except for three studies. More precisely, tDCS was delivered together with conventional occupational therapy (An et al., 2017), while rTMS was coupled with movement therapy (Gu and Chang, 2017). Only in

Bueno's case report (2011) did the patient continue a pharmacological treatment; in all the other studies, participants were antidepressant-free.

Placebo. Three studies (Gu and Chang, 2017; Jorge et al., 2004; Valiengo et al., 2017) were double-blind sham-controlled which means that investigators and patients were blinded to the treatment allocations, i.e., the person who performed the stimulation was not informed about the study protocol and someone else did the pre- and post-treatment evaluation. In the TMS experiments, the sham condition was performed with the angle of the coil positioned at 90° perpendicular to the skull (Gu and Chang, 2017; Jorge et al., 2004), while in the tDCS experiments the sham condition, which was the same in all studies, implied stopping stimulation after 30 seconds.

Duration. In all the rTMS studies, the treatment lasted for 2 weeks (10 sessions) and focused on relatively short-term follow-up periods ranging between 1 week (Jorge et al., 2004) and 4 weeks (Gu and Chang, 2017). In the tDCS protocols, the duration varied between 4 weeks (20 sessions or 12 sessions) and 2 weeks with 6 months of maintenance (Bueno et al., 2011). With the exception of Valiengo et al.'s (2017) study that included a 2 weeks follow-up period, none of the tDCS treatment evaluated participants in a follow-up.

Outcome measures. Outcome assessment was relatively consistent across studies. Hamilton Depression Rating Scale (HAM-D), the 17 or the 21-item version, was applied in five studies: in one being the only evaluation instrument (El Etribi et al., 2010), in two studies being used together with the Montgomery–Åsberg Depression Rating Scale (MADRS), and in the other two with the Beck Depression Inventory (BDI). BDI alone was applied in one research paper (An et al., 2017). Occasionally, these three instruments were used together (Bueno et al., 2011). Other primary efficacy parameters were the response and the remission rates. A good response was defined as a decrease in the Inventory total score so that the patient no longer met the DSM criteria for a depression diagnosis. Remission was defined as a reduction of the scores below a certain point, for example in Jorge et al. (2004) a reduction of Hamilton Depression Rating Scale scores of at least 50% with final HAM-D scores below 8.

Summing up, all the instruments used for the assessment, with the exception of the Aphasic Depression Rating Scale (ADRS) and the Stroke Aphasic Depression Questionnaire (SADQ), in Valiengo et al. (2016), were not

specific for the PSD but were rather designed to measure the severity of depression in patients who are otherwise healthy, not taking into consideration many factors that are highly relevant for PSD. Consequently, the assessment based on such instruments was considered inaccurate as extensively discussed in Aben and colleagues' review (2001). To overcome these problematics, new diagnostic tools have been developed, for example the Structured Assessment of Depression in Brain Damaged Individuals (SADBD) proposed by Gordon et al. in 1991 (Monaco et al., 2005) and the self-rating post stroke depression scale (PSDS) (Yue et al., 2022). The authors aimed to design an instrument sensitive to depression in a brain-damaged population and, for this purpose, they adapted the items, making them easy to comprehend and simple to answer.

Adverse Effects. Overall, rTMS was well tolerated and only common, mild side effects were reported such as local discomfort at the stimulation site or headache. Severe adverse effects like seizures, hearing impairment, or mania were absent. Jorge et al., 2004, described transient headache in six of the 20 participants included in the study, local discomfort at the site of the stimulation in five patients, and an exacerbation of initial insomnia in one patient. Interestingly, they found no significant differences in the adverse events frequency between the active and the sham rTMS groups. El Etribi et al. (2010) reported that 40% of patients complained of headache but were easily relieved with paracetamol, while Gu et al., 2017 found no side effects. As concerns tDCS, Bueno et al. (2011) and Valiengo et al. (2016) describe the treatment as well tolerated by all the participants, with the latter study (2017) reporting no significant difference between the active and the control group.

Results. The first study to explore the effects of NIBS on post-stroke depression was conducted by Jorge et al. (2004). Using a double-blind protocol of active (10 Hz) versus sham rTMS over the left DLPFC in patients with refractory PSD (depression was unresponsive to at least two antidepressant treatments); they reported significant improvement of depressive symptoms after 10 sessions (a reduction of 7.3 points in HAM-D scores). Furthermore, three patients (30%) met the criteria for a clinical response and 1 patient (10%) for remission of depression, all from the active rTMS group. Thirteen years later, another double-blind, sham-controlled study, using an identical protocol, confirmed that excitatory rTMS over the left DLPFC could be used to improve PSD symptoms and that this result was maintained for 4 weeks after the end of the treatment (Gu and Chang, 2017). Crucially, in this case, PSD was not refractory (intended as a failure to demonstrate an "adequate" response to

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an "adequate" treatment) as in the previous research. Another rTMS study (El Etribi et al., 2010) also reported changes in the Ham-D scores compared to the baseline, more precisely a drop of 41.3% that was maintained for over one month in about 60% of the cases. In this study, the authors applied a completely different protocol, namely 10 sessions of inhibitory (1Hz) rTMS at 100% of the rest motor threshold over the left DLPFC. In other words, even if the same brain region was stimulated with inhibitory (in one research) and excitatory (in the other two) rTMS, all studies reported positive findings and a reduction of depressive symptoms. At present, the literature does not provide a proper explanation for these incongruent data, but it is generally accepted that the effects of rTMS may vary depending on many variables like the brain state at time of stimulation (Perini et al., 2012) or the lesion site being on the left or right hemisphere.

Three of the four tDCS studies were conducted at the University of São Paulo between 2011 and 2017 (Bueno et al., 2011; Valiengo et al., 2016; Valiengo et al., 2017). Bueno and colleagues (2011) described a 48-year-old patient who despite being on Fluoxetine for 6 months, was suffering a severe PSD episode. They are the first to report a study in which tDCS is tested as treatment for PSD. After 10 days of 30 minutes anodal tDCS over the left DLPFC and cathodal over the right DLPFC stimulation at a current intensity of 2 mA, the patient showed marked mood and cognitive improvement. A maintenance treatment continued for 6 months (twice a month) and no relapse of symptoms was reported. In 2016, Valiengo et al. also published an open case series (4 cases) that aimed to explore the safety and efficacy of tDCS for the treatment of PSD but on aphasic patients. The stimulation protocol was very similar to the one applied by Bueno et al. (2011) and all patients exhibited improvement in depression after tDCS. The only RCT study with a tDCS stimulation also belongs to Valiengo and colleagues (2017) and the experimental protocol was similar to the previous one. As hypothesized, the response rates revealed a higher response level in the active tDCS than in the sham group (37.5% vs 4.1%), while remission was achieved only in the active tDCS group by 5 (20.8%) patients. Additionally, a significant interaction between the variables time and group was observed: the mean difference at HAM-D 17 scores between active and sham tDCS condition was significant at the end point (6 weeks, i.e., 12 tDCS sessions) but not after 2 weeks (10 tDCS sessions) nor after 4 weeks (12 tDCS sessions). The authors explain these results by hypothesizing that tDCS

antidepressant effect, similar to the pharmacological treatment, might need more time for maximum manifestation.

An et al. (2017) basically used the same stimulation protocol as previous studies, but with a different duration (20 sessions over a period of 4 weeks), and combined conventional occupational therapy with active or sham tDCS. The conclusion was in line with the other studies, confirming that tDCS intervention significantly decreased depression in the experimental group (tDCS and conventional occupational therapy) compared to the control group (sham tDCS and conventional occupational therapy). For instance, the BDI final score change was from 38.8 ± 4.7 (before active tDCS) to 16.8 ± 4.6 (after tDCS), but in this case, the authors did not compare the results from sham vs tDCS. The variable time was also relevant since the depression level of the control group (sham tDCS) also decreased from 39.0 ± 4.6 to 37.8 ± 6.1 but without reaching statistical significance.

3.1.4. Discussion

PSD is a neuropsychiatric manifestation of high clinical importance afflicting many (around 30%) stroke survivors (de Bekker, Geerlings, Uitewaal-Poslawsky, & de Man-van Ginkel, 2022; Robinson & Jorge, 2016). Its main clinical manifestations are profound feelings of helplessness and hopeless, loss of interest, loss of appetite, sleep disorders, pessimism, unworthiness, and even suicidal tendencies. Literature data indicate that rTMS and tDCS may constitute an effective treatment for depressive symptoms that bypasses the risks associated with antidepressant exposure (Lefaucheur et al., 2014; Shiozawa et al., 2014).

There are two main reasons for the TMS and tDCS PSD treatment protocols. The first rationale regards the previous positive results of studies that applied these techniques in depressed patients (without stroke); for instance, the U.S. Food and Drug Administration (FDA) has approved of rTMS for the treatment of resistant depression since 2008 (Lefaucheur et al., 2014; Padberg et al., 1999; Palm et al. 2016; Pascual-Leone et al., 1996). The second argument is based on the evidence that PSD patients show functional and also structural changes in several cortical regions, for example disruption of neural connections among regions regulating mood and cognition, like abnormalities of the cingulate cortex (Ye et al., 2016). In the absence of a pathophysiological hypothesis for PSD and without profound knowledge of the mechanisms that characterize the human brain after lesion, it is not clear if the lessons and results from the depression studies, as for example

the fact that TMS has been shown to be safe and effective for resistant depression in the general population (Conelea et al., 2017), should be extended to the PSD treatment.

Summing up, all of the included studies reported that, following the intervention, the experimental group showed a statistically significant decrease in depression level, but it is hard to make a comparative analysis between the different studies since nonequivalent parameters were used to establish rTMS and tDCS efficacy. With the exception of Bueno et al. (2011) tDCS for PSD study, which mentioned an important improvement in the daily life activity, as reported by the patient's husband (more active and with less "catastrophic reactions"), none of the other papers provide ecological assessment, limiting the data to standardized scales results and, in some cases (e.g., Jorge et al., 2004; Valiengo et al., 2016), including response and remission rates. Therefore, we cannot say how the change observed in the rating scale translates into the patient's everyday life. Even if the results seem encouraging, it is recommended to be very cautious speculating on these findings given the high heterogeneity among studies, the small number of patients, and, especially, the likelihood of publication bias toward positive findings. For example, in a study not included in the review because participants were not explicitly diagnosed with PSD, the authors found that the delivery of bilateral rTMS at 10 Hz for 20 minutes did not produce statistically significant changes in the Quick Inventory of Depressive Symptomatology (QIDS) scores (Sasaki et al., 2017). Crucially, the stimulated area was not the DLPFC but a more extended one, the upper-middle of the forehead extending from the external auditory meatus to 30° above the orbitomeatal line.

As previously mentioned, most intervention descriptions were adequate, but details on treatment procedures and participants' characteristics were missing (e.g., lesion information, the time interval between stroke, depression diagnosis, and treatment onset).

Another problem that makes the comparison of different studies difficult was represented by the PSD diagnosis criteria. None of the included studies distinguished between major depression and other forms of depressive disorders occurring after stroke. In addition, in order to assess mood disorders and the degree of depressive symptoms, different depression scales were used, with various and arbitrary cut-off points for the same scale. Another problem concerns the aphasic population that sometimes is explicitly excluded from the studies (e.g., Valiengo et al., 2016) or, in other cases, the cognitive evaluation of language abilities is missing (e.g., El Etribi et

al., 2010), even if aphasia is a common stroke consequence, ranging up to one third of the stroke population during the acute phase (Kauhanen et al., 2000). People with aphasia diagnosis or cognitive deficit (Kauhanen et al., 1999) should be included in stroke studies investigating depression and mood disorders and the instruments to evaluate PDS should be adequate. For instance, there are specific scales to assess PSD, which differ from the traditional ones. Recommended instruments are the Stroke Aphasic Depression Questionnaire-10 (Sutcliffe and Lincoln, 1998), the Stroke Aphasic Depression Questionnaire-H10, the Signs of Depression Scale (van Dijk et al., 2016) and the Structured Assessment of Depression in Brain-Damaged Individuals (SADBD) by Hibbard et al. (1993).

The main limitation of the present review concerns the low number of studies and selection biases. For instance, the search strategy was restricted to articles published in English, excluding potentially high-quality research data that were published in other languages or belong to the "gray literature", i.e., literature that is not formally published in sources such as books or journal articles (Higgins et al., 2005, p. 106). An example is the previously mentioned meta-analysis exclusively based on Chinese papers (Shen et al., 2017) that found support for the rTMS efficiency in PSD treatment. We could not critically evaluate those studies or challenge the reported data because we had no access to the Chinese journals, but we underline the authors' concern about the heterogeneity, the study's quality, and the potential biases. The same problematic elements were reported in another review, by McIntyre et al. (2016), investigating rTMS for treatment of depression among individuals with cerebrovascular disease (including both PSD and vascular depression). In spite of encouraging results that seemed to indicate some effectiveness of rTMS, they could not formulate a univocal conclusion based on the available data.

Considering the current findings, we cannot support the efficacy of a specific stimulation protocol, or indicate which stimulation technique is recommended, and for what type of patients. Many unanswered questions persist regarding how the differences in parameters of rTMS and tDCS protocols might impact treatment efficacy and what are the possible ways forward to reduce such variations for maximal stimulation benefits.

It can be speculated that rTMS could be more effective for refractory PSD (Jorge et al., 2004), while tDCS, being considered "easy" to apply, more flexible, and less expensive, could be a better choice in other cases.

There seem to be two main theoretical hypotheses from which to choose a certain stimulation protocol for PSD treatment. rTMS studies rather assume the so-called hypofrontality hypothesis (Galynker et al., 1998) targeting only the left DLPFC, but many parameters, such as the stimulation frequency and duration, are still unclear (Singh et al., 2020). Conversely, in the tDCS studies, a bihemispheric montage was used, anodal over the left and cathodal over the right DLPFC, a choice that might be based on an interhemispheric frontal imbalance hypothesis (Debener et al., 2000; Reid et al., 1998). Concerning the positioning of the reference electrode, there is no current evidence that F4 positioning is preferable to the supraorbital positioning. For instance, in the evidence-based guidelines on the therapeutic use of tDCS, Lefaucheur and colleagues' data indicate that anodal stimulation of the left DLPFC with right orbitofrontal cathode is "probably effective in patients with no drug-resistant major depressive episode (Level B) and probably ineffective in patients with drug-resistant major depressive episode (Level B)" (Lefaucheur et al., 2017, p 75). However, these are recommendations for depressed persons without stroke. We consider that attempting to reduce variability in factors such as target location and stimulation protocols, which contribute to the differences in response to brain stimulation, is the way forward for increasing the clinical effectiveness of NIBS.

With reference to safety, both rTMS and tDCS are generally considered safe if the appropriate guidelines and recommendations are followed, e.g., Rossi and colleagues (2009) for TMS, and Nitsche et al. (2003), Woods et al. (2016) for tDCS. The COVID-19 pandemic has disrupted biomedical treatment and research, including NIBS, and new recommendations for risk management have been recently published (Bikson et al., 2020).

Even though none of the considered studies reported any adverse events, more research is needed to verify the safety parameters in specific subgroups like post-stroke patients because the intensity and location of current flow might differ due to changes in the local anatomy and lesion time evolution (Datta et al., 2010). An important challenge for future research will be to collect unequivocal data about tolerability, stimulation parameters, and neuroplasticity changes of tDCS and rTMS in larger RCTs involving PSD patients.

Except for a case study report (Bueno et al., 2011), all studies investigated tDCS and TMS as a mono-therapy in medication-free patients. Concomitant pharmacotherapy can influence the neuroplasticity effects of rTMS and tDCS; for example, mood stabilizers seem to nullify tDCS-elicited excitability changes (Palm et al., 2016), while

in a study by Brunoni and colleagues (2013) on depressed patients, the combined sertraline and tDCS treatment was more effective than sertraline or tDCS alone. Following this tendency, possibly the best intervention for PSD will be to incorporate different approaches in order to maximize the neuronal plasticity.

The future important challenges will be to collect clear evidence for the long-term efficacy of these methods and to investigate the potentiation of TMS and tDCS-elicited neuroplasticity changes by pharmacological therapies. The combined therapy could be a promising asset towards a quicker and more sustained improvement of depressive disorders (Palm et al., 2016). Important returns could be expected. First, the positive cost-benefit ratio of tDCS could reduce treatment costs. Patients or relatives could learn to apply stimulation at home with personalized protocols (Palm et al., 2016), but the objective should be related to the neuropsychological profile and the individual goals.

Table 1 Summary of tDCS study characteristics

Study	Study design	tDCS montage		Current intensity	Number of stimulations Duration	Analysis	Sham	Primary Outcome	Results / Authors conclusions	Follow-up
		Electrode position	Reference electrode							
An et al., 2017	Experimental research, sham-controlled	Anode left DLPFC	Cathode right DLPFC	2 mA	20 sessions of 30 min. (4 weeks)	*pre VS post tDCS *sham VS active	the stimulation was stopped 30 seconds after the application	BDI	TDCS intervention caused a significant decrease in depression levels in the experimental group.	No Follow-up.
Valiengo et al., 2017	Experimental research, (RCT) Double-blind, sham-controlled	Anode left DLPFC	Cathode right DLPFC	2 mA	12 sessions of 30 min. 2 weeks x 10sessions, 2 weeks x 2 sessions	*baseline 2nd, 4th,6th week *sham VS active	the stimulation was stopped 30 seconds after the application	HAM-D17 Response Remission	Active tDCS was significantly superior to sham at end point	2 weeks
Valiengo et al., 2016	Case study Open case series	Anode left DLPFC	Cathode right DLPFC	2 mA	12 sessions of 30 min. 2 weeks x 10sessions, 2 weeks x 2 sessions	*Baseline, 2nd, 4th,6th Week	No sham	ADRS SADQ	All 4 patients exhibited improvement in depression after tDCS	No Follow-up
Bueno. et al., 2011	Case study	Anode left DLPFC	Cathode right DLPFC	2 mA	10 sessions of 30 min. maintenance for 6 months (twice a month)	*Baseline, 2nd Week, 6 Months	No sham	HAM-D BDI MADRS	Marked improvement in the depressive symptoms.	No Follow-up

Abbreviations: tDCS, Transcranial direct current stimulation; DLPFC, dorsolateral prefrontal cortex; RCT, Randomized controlled trial; BDI, Beck Depression Inventory; HAM-D, Hamilton Depression Rating Scale; ADRS, Aphasic Depression Rating Scale; SADQ, Stroke Aphasic Depression Questionnaire; MADRS , Montgomery-Asberg Depression Rating Scale.

Table 2 Summary of TMS study characteristics

Study	Study design	Target area(s)	rTMS Frequency	Intensity (% MT)	Trains of session Number of sessions	Sham	Treatment protocol and Analysis	Outcome measure	Results / Authors conclusions	Follow-up
Gu et al., 2017	Experimental research, (RCT) Double-blind, sham-controlled	left DLPFC	10 HZ	110%	20 trains of 5 seconds, 10 sessions (10000 pulses) (2 weeks)	angle of the coil at 90° perpendicular to the skull	*4 weeks, 1 day pre *1 day, 4 weeks post *sham VS active rTMS	BDI HAM-D17	BDI and HAM-D17were significantly decreased at 1 day and 4 weeks after treatment in the rTMS group compared to the sham	4 weeks
El Etribi et al., 2010	Experimental research	left DLPFC	1 HZ	100%	10 trains of 10 seconds 10 session (1000 pulses) (2 weeks)	No sham	*Baseline, 2nd ,4th, 6th Week	HAM-D21	About 60% of the patients showed drop of at least 41.3 % from the baseline in scores on the HAM-D21 and clinical improvement.	2 and 4 weeks
Jorge et al, 2004	Experimental research, (RCT) Double-blind, sham-controlled	left DLPFC	10 HZ	110%	20 trains of 5 seconds, 10 sessions (10000 pulses) (2 weeks)	angle of the coil at 90° perpendicular to the skull	*Baseline, 2nd and 3rd Week *sham VS active rTMS	HAM-D17 Response Remission	Was found a significant difference between the active and the sham rTMS, active group showed a mean reduction of 7.3 points in HAM-D17 scores.	1 week

Abbreviations: rTMS, repetitive transcranial magnetic stimulation; MT, the motor threshold; DLPFC, dorsolateral prefrontal cortex; RCT, Randomized controlled trial; BDI, Beck Depression Inventory; HAM-D17, 17-item version of the Hamilton Depression Rating Scale; HAM-D21, 21-item version of the Hamilton Depression Rating Scale

Table 3. Review authors´ judgements about each risk of bias domain and Levels of Evidence

Study

The RoB 2.0 tool (individually randomized, parallel group trials): Bias domain

	Bias arising from the Randomization process	Bias due to deviations from intended interventions	Bias due to missing outcome data	Bias in measurement of the outcome	Bias in selection of the reported result	Overall	Levels of evidence
Valiengo et al., 2017	Low	Low	Low	Low	Low	Low	Good
Gu et al., 2017	Some concerns	Low	Low	Low	Low	Some concerns	Good
Jorge et al., 2004	Some concerns	Low	Low	Low	Low	Some concerns	Good

Study

The ROBINS-I tool (Risk Of Bias In Non-randomized Studies - of Interventions): Bias domain

	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall	Levels of evidence
An et al., 2017	Moderate risk of bias	Moderate risk of bias	Low risk of bias	Low risk of bias	Moderate risk of bias	Moderate risk of bias	Moderate risk of bias	Moderate risk of bias	Fair
Valiengo et al., 2016	Serious risk of bias	Moderate risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Serious risk of bias	Poor
Bueno et al., 2011	Serious risk of bias	Moderate risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Serious risk of bias	Poor
El Etribi et al., 2010	Moderate risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Low risk of bias	Moderate risk of bias	Moderate risk of bias	Fair

ROBINS-I tool:

Low risk of bias - the study is comparable to a well-performed randomized trial with regard to this domain

Moderate risk of bias - the study is sound for a nonrandomized study with regard to this domain but cannot be considered comparable to a well-performed randomized trial

Serious risk of bias - the study has some important problems

No information - on which to base a judgement about risk of bias for this domain

Overall risk of bias judgement Criteria:

Low risk of bias: The study is judged to be at low risk of bias for all domains for this result.

Some concerns: The study is judged to be at some concerns in at least one domain for this result.

High risk of bias: The study is judged to be at high risk of bias in at least one domain for this result – or - The study

Table 4. Summary of the participant's characteristics.

Study	Sample	Diagnosis and diagnosis instruments	Experimental group characteristics				Treatment	Lesion type and Hemisphere	Medication
			Male	Female	Mean Age (SD)	Mean Stroke Interval (months)			
An et al., 2017	40 20 control group 20 experimental	PSD * BDI scores >16	17	3	51.0±11.7	14.6±6.3	conventional occupational therapy+tDCS	11 cerebral infarction 9 hemorrhage 7 right hemisphere 13 left hemisphere	not specified
Valiengo et al., 2017	48 (5 drop-outs) 24 control group 24 experimental	PSD * MINI	12	12	51.0 ± 11.7	14.6 ± 6.3	conventional occupational therapy + tDCS	11 cerebral infarction 9 hemorrhage 7 right hemisphere 13 left hemisphere	not specified
Valiengo et al., 2016	4	PSD (confirmed by a psychiatrist) with Broca's aphasia diagnosis	0	4	62.2 ± 12.3	11.1 ± 2	tDCS	11 right side (stroke) 10 subcortical structures (stroke) 8 frontal injury (stroke)	antidepressant-free
Bueno et al., 2011	a 48-year-old woman	PSD * MINI	0	1	48.2 ± 11.6	6 ± 4.08	tDCS	stroke type: 3 ischemic 1 hemorrhage Hemisphere not specified	antidepressant-free
Gu et al., 2017	24 12 control group 12 experimental	PSD * BDI scores >12 * HAM-D17 scores >6	6	6	48	not specified	tDCS	ischemic stroke: left basal ganglia and left insula	Fluoxetine 40 mg/day for 6 months
El Etribi et al., 2010	20 experimental	PSD * K-SADS * SCID for DSM-IV	12		58.1±8.7	10.3 ± 2.7	movement therapy + rTMS	stroke type: 11 infarct 9 hemorrhage Hemisphere not specified	not specified
Jorge, et al., 2004	20 10 control group 10 experimental	PSD * PSE according to DSM- VI *unresponsive to at least two treatments with antidepressants	6	4	63.1±8.1	not specified	rTMS	Stroke 10 left hemisphere 10 right hemisphere	antidepressant-free

Abbreviations: SD, standard deviation; PSD, post-stroke depression; BDI, Beck Depression Inventory; tDCS, Transcranial direct current stimulation; MINI, Mini-International Neuropsychiatry Interview; HAM-D17, 17-item version of the Hamilton Depression Rating Scale; rTMS, repetitive transcranial magnetic stimulation, K-SADS, Schedule for Affective Disorders and Schizophrenia; SCID, Structured Clinical Interview for DSM-IV; DSM-IV, Diagnostic and Statistical Manual Fourth Edition; PSE, Present State Examination

3.2 A systematic review of noninvasive brain stimulation for post-stroke aphasia

3.2.1. Introduction

It was estimated that one third of people who suffered a stroke will be diagnosed with aphasia (Kuriakose & Xiao, 2020). Post-stroke aphasia is an impairment of language comprehension and/or production caused by focal brain damage to regions in the language-dominant hemisphere. It is one of the most debilitating outcomes of stroke, and the symptoms can range from mild impairment to complete loss of the fundamental components of language such as phonological, morphological, semantic, syntactic, and/or pragmatic. Even mild forms of aphasia can negatively impact mood (often patients also have PSD or anxiety), quality of life, social participation, and the ability to work (Basso et al., 2013). Aphasia is associated with higher mortality, morbidity, and utilization of healthcare resources (Berthier, 2005). These patients experience longer hospitalization periods, need more intensive health service utilization, participate in fewer activities and report high distress. The cost of care in one year for PWA was approximately $1700 more than the cost of caring for stroke patients without aphasia (Ellis, Simpson, Bonilha, Mauldin, & Simpson, 2012).

Due to early physiological repair mechanisms involving axon growth, and synaptic modulation, the majority of stroke patients achieve some spontaneous recovery even in the absence of rehabilitative treatment. Nevertheless, around 40% of these patients still have significant aphasia one year after stroke, and residual symptoms may persist for many years (Lazar, Speizer, Festa, Krakauer, & Marshall, 2008; Pedersen, Stig Jørgensen, Nakayama, Raaschou, & Olsen, 1995).

As described in chapter one, the most common causes are the left middle cerebral artery infarct with damage of the cortical and subcortical regions in the left hemisphere; rarely, it is due to a right hemisphere lesion. Neuronal death is the key manifestation of stroke, and the following linguistic impairment takes various forms, differing in the fluency/ non-fluency of verbal output and integrity of comprehension, repetition, and word retrieval competencies, depending on the damage characteristics. The symptoms are heterogeneous, individualized, and can change over time, particularly within the first few weeks and months following a stroke; for instance, patients having one type of aphasia in the acute phase may present a different clinical profile in the chronic phase (Raymer & Gonzalez Rothi, 2017).

From a medical perspective, stroke therapy primarily focuses on restoring blood flow to the brain and minimizing neuronal deficits after ischemic insult (Kuriakose & Xiao, 2020). Research data indicated that aphasia recovery is modulated by different factors like lesion size and site, aphasia type and severity, and, to some extent, the nature of the early hemodynamic response, type of treatment, interval between onset and beginning of speech therapy, the environmental support, etc. (Watila & Balarabe, 2015). After the initial poststroke spontaneous recovery (e.g., blood flow stabilization, resolution of brain swelling), recovery relies on the reorganization of brain networks, which occurs both spontaneously and in response to behavioral training (e.g., speech-language therapy) (Fama & Turkeltaub, 2014).

Current practice standards indicate speech and language therapy (SLT) as the main therapy (Raymer & Gonzalez Rothi, 2017). SLT has been proved effective at alleviating language impairment; nevertheless, the treatment cost is elevated, the progress is often slow, and the effect sizes are sometimes limited (Brady, Kelly, Godwin, Enderby, & Campbell, 2016).

Principles of neuroplasticity support early and intense therapy (the degree of spontaneous recovery gradually flattens during the first 6–12 months); however, at least partial recovery can be made at any point after stroke but requires a huge amount of time and therapy sessions. Research data indicated (Stahl et al., 2017) no added value from more than two hours of daily SLT therapy but instead showed that an increase in treatment duration (even only 2 weeks) contributes significantly to recovery (it is still considered that "more is better"). In reality, due to limited clinical resources, many patients do not receive the 98.4 hours of therapy, which is the literature recommended dose of rehabilitative training (Bhogal, Teasell, & Speechley, 2003; Code & Heron, 2003; Saxena & Hillis, 2017).

In an attempt to manipulate the neurotransmitter systems and subsequently facilitate language recovery, different drugs such as memantine, vasopressin, dextroamphetamine, and piracetam have been tried (Keser et al., 2017; Wortman-Jutt & Edwards, 2017). To date, the findings supporting pharmacological therapy are debatable (Berthier, Pulvermüller, Dávila, Casares, & Gutiérrez, 2011).

Aphasia treatment is progressively more informed by advances in understanding of the neurobiology of recovery and learning, and alternative methods for boosting aphasia treatment effectiveness, by either

increasing the total amount of learning achieved (better results) or by accelerating the learning process (faster results), have been studied.

There is evidence for structural and functional reorganization of language networks after stroke that mediates recovery, and recent research on brain plasticity (Hoogendam, Ramakers, & Di Lazzaro, 2010; Stagg & Nitsche, 2011) has led to new approaches in stroke rehabilitation. The improvement is associated with the reorganization of the balance between the perilesional ipsilateral and contralateral hemispheric activation (Abel, Weiller, Huber, Willmes, & Specht, 2015; Turkeltaub et al., 2012). Additional approaches, with or without SLT, like NIBS interventions, have been evaluated in order to modify cortical excitability and therefore promote post-stroke reorganization of the language networks (Turkeltaub, 2015).

The most-used NIBS protocols are based on tDCS and rTMS. Although both rTMS and tDCS are non-invasive stimulation techniques, their functioning principles and, consequently, their effects differ. As mentioned in the previous chapter, while rTMS induces action potentials in neurons axons via electromagnetic current, tDCS modulates neural firing rates through current-induced changes in resting membrane potentials (Bolognini & Miniussi, 2018; Fertonani & Miniussi, 2017; Giordano et al., 2017; Nevler & Ash, 2015; Woods et al., 2016). Both rTMS and tDCS can induce localized excitation or inhibition of neuronal populations, depending on the stimulation parameters, that can last for many minutes after a short session (Giordano et al., 2017; Klomjai et al., 2015; Nitsche & Paulus, 2000; Stagg & Nitsche, 2011; Terao & Ugawa, 2002). The post-stroke neuronal reorganization may be beneficial or maladaptive (Di Pino et al., 2014), and tDCS and rTMS could have a therapeutic role by potentially reversing an eventual maladaptive pattern of activation and by creating long-lasting desirable brain changes.

Modulating brain plasticity by means of rTMS and tDCS, and consequently affecting behavior, creates new rehabilitation possibilities, but given the heterogeneity of the stimulation protocols and the uncertainty of the NIBS effectiveness, more research data are necessary in order to choose the most useful brain stimulation therapy.

Reviews of tDCS, rTMS, and aphasia literature are disproportionately numerous when compared with the number of original experimental studies (ALHarbi, Armijo-Olivo, & Kim, 2017; de Aguiar, Paolazzi, & Miceli,

2015; Elsner, Kugler, Pohl, & Mehrholz, 2015; Galletta, Conner, Vogel-Eyny, & Marangolo, 2016; Holland & Crinion, 2012; Kapoor, 2017; Li, Qu, Yuan, & Du, 2015; Mendoza et al., 2016; Otal, Olma, Flöel, & Wellwood, 2015; Ren et al., 2014; Rosso, Arbizu, Dhennain, Lamy, & Samson, 2018; Sandars, Cloutman, & Woollams, 2016; Sebastianelli et al., 2017; Shah-Basak, Wurzman, Purcell, Gervits, & Hamilton, 2016; Wong & Tsang, 2013). Wortman-Jutt and Edwards (Wortman-Jutt & Edwards, 2017) showed that between 2008 and 2015, 48% of the publications regarding tDCS and aphasia rehabilitation were review papers. This indicates the relevance of the topic but also the difficulty to conduct experimental research (i) on a clinical population characterized by heterogeneity (especially regarding lesion type and clinical manifestation), (ii) without solid knowledge about the underling mechanism of these techniques, i.e., unable to make predictions on their clinical efficacy and (iii) without guidelines on the parameters to use (e.g., stimulation area, frequency, intensity, polarity, duration, as monotherapy or coupled with SLT, etc.), and (iv) taking into consideration the enormous amount of time and energy that researchers, patients, and caregivers have to invest in this kind of experimental protocols. For these reasons, the published reviews offer conflicting evidence and little information on the real utility of NIBS for aphasia, all concluding that more multicenter RCTs, with larger populations and homogenous intervention protocols, are needed.

In contrast with the previous systematic reviews on this argument, this meta-analysis aim was to systematically synthesize and compare the NIBS long-term efficacy in language recovery in order to verify whether there are new and valid alternatives to improve aphasia treatment, eventually by coupling SLT with rTMS or tDCS. Secondly, because previous studies (Otal et al., 2015; Shah-Basak et al., 2016) revealed a greater therapeutic benefit of rTMS compared to tDCS, we assessed whether the two NIBS effect sizes still significantly differ from each other at follow-up. Furthermore, the immediate post-treatment effects were compared with those reported at the follow-up to evaluate changes over time. By grouping and contrasting subsets of studies according to different methodological aspects, further sub-analyses investigated potential influences of the study design (including in the analysis only RCT studies), post-stroke interval: chronic vs subacute, and different aphasia types: all types vs non-fluent. Finally, the GRADE Pro GDT online software was used to qualify the evidence (https://gradepro.org).

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3.2.2. Methods

The present systematic review was conducted under the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2015).

Literature search and study selection

Our meta-analysis is based on 20 studies investigating post-stroke aphasia treatment using either rTMS or tDCS stimulation. The considered papers were published between August 2004 and February 2019. Studies were selected using four electronic databases: - MEDLINE (accessed by PubMed, https://www.ncbi. nlm.nih.gov/pubmed), PsycARTICLES (via EBSCOHost, https://search. ebscohost.com), PsycINFO (via EBSCOHost) and Web of Science (https://webofknowledge.com/)

The search terms used were: (1) "tDCS", "transcranial direct current stimulation"; "transcranial magnetic stimulation", "TMS" AND (2) "aphasia", "language disorder".

Studies were selected according to the following inclusion /exclusion criteria:

- adults with post-stroke aphasia, excluding other types of post-stroke disorders or aphasia not due to stroke
- rTMS or tDCS stimulation studies (alone or combined with other therapies),
- rTMS or tDCS were specified as the main intervention/ treatment,
- cephalic stimulation designs only,
- minimum 4 weeks (1 month) follow-up period,
- at least four aphasic participants,
- peer-reviewed publications,
- published in English,
- if the same data were reported in different publications, we chose the most recent one and with the highest number of participants
- studies that did not provide adequate information to analyze the treatment effects and the authors did not respond to our e-mails were also excluded

After removing duplicates, research papers which did not satisfy the above criteria were excluded. For example, several studies included patients with primary progressive aphasia or had a short follow-up period (1-3 weeks). Uncertainties regarding some inclusions were solved by the authors through discussion.

Data extraction

From the selected papers, the following data were extracted:

- the patients' characteristics: the sample size (treatment and control group), gender, age, time since stroke (chronic/ subacute), handedness, dropouts (Table 1);

- disorders' characteristics: lesion area, lesion type, aphasia diagnosis and instruments used for the disorder evaluation, aphasia severity, outcome evaluation (Table 2);

- rTMS stimulation protocol: target area(s), rTMS frequency and intensity, number of pulses and duration, number of sessions, online or offline stimulation and the associated therapy (if any), sham, adverse effects (Table 3);

- tDCS stimulation protocol: montage type (unipolar or bipolar, cathodic or anodic), electrode dimension, stimulation area, electrode position, stimulation hemisphere, reference electrode, current intensity, number of stimulations and duration, online vs offline stimulation and associated therapy (if any), sham, tDCS adverse effects (Table 4);

- characteristics of the study: main objective, study design, language of the study, study arms, follow-up results and authors conclusion, levels of evidence on PEDro's scale (Table 5.1 & Table 5.2).

Means, standard deviations, and sample size for the experimental and control conditions were extracted. Across studies, aphasia assessment and outcome measures were heterogeneous, but for the purpose of our review, the primary outcome was based on picture naming accuracy or, if not specified, the explicitly declared primary outcome (e.g., aphasia battery results, reaction times, speech content units). When adequate information was not provided in the results description, means and standard deviations or standard errors were extracted from published figures using the WebPlotDigitizer (https://automeris.io/WebPlotDigitizer/).

The standardized mean difference (SMD) computed as Hedges' *g*, sampling variance for each included study, summary analyses, the likelihood of publication bias, and heterogeneity tests were computed using the

"*metafor package*" for R (version 3.4.3) (Viechtbauer, 2010). Hedges' *g* (computed as the difference between the mean of the experimental condition and the mean of the control condition, divided by the pooled standard deviation) was chosen for the effect size, instead of *Cohen's d*, because, due to often low sample size, we considered it more adequate to the present study: indeed, many researches had seven/ twelve participants (DeVellis, 1991; Faraone, 2008). If only standard errors were reported, we converted them into standard deviations (SD) using the formula $SD = SE\sqrt{n}$; *SE*= standard errors, *n*= number of participants (DeVellis, 1991); if this method of data extraction could not be applied, the missing information was requested from the corresponding author.

For each effect, we included moderator variables related to: i) stimulation type (rTMS or tDCS), ii) time points (after treatment or at follow-up), iii) study design (crossover & open label or RCTs), iv) aphasia type (fluent or non-fluent), and v) time from the symptom onset (chronic or subacute).

In order to avoid entering multiple data points from the same study into the meta-analysis, results from a single experiment were aggregated to obtain one measure per experiment. If several time points or control conditions were reported (e.g., comparing cathodal and anodal stimulation, or different brain regions stimulation within the same patients), the data were extracted using the following criteria:

- from each study, we chose one follow-up time point only, specifically the one that represented the longest follow-up period matching the follow-up time points reported by the other studies included in the meta-analysis;

- only data regarding the treatment effects on language recovery were extracted (e.g., aphasia battery scores, speech, etc.); fMRI data, blood flow, or other parameters used as indicators of the treatment effects were excluded;

- from each study, since often different stimulation protocols were tested, we chose one type only (e.g. montage, stimulation intensity), namely the one that the authors considered as potentially effective, or, in the absence of such information, the one most frequently applied in the literature. This was done in order to reduce variability among the included studies;

- the preferred outcome was naming accuracy; in the absence of this measure, we used a different outcome, e.g., the total score from the aphasia battery, speech content units, etc.;

- if a study reported different outcome measures for the same treatment (e.g., accuracy but also reaction times), we chose the outcome more frequently reported in the included studies.

Study Quality Assessment

Methodological quality of the included studies was assessed using the Physiotherapy Evidence Database (PEDro) tool, and a grade for the level of evidence was assigned to each study according to the modified Sackett Scale (Tables 5.1, 5.2, 6.1 and 6.2 for the level of evidence) (David L. Sackett, Sharon E. Straus, W. Scott Richardson, William Rosenberg, 2000; Moseley, Herbert, Sherrington, & Maher, 2002).

Overall evidence was qualified using the grading of recommendations, assessment, development, and evaluations (GRADEpro GDT, https://gradepro.org) (Meader et al., 2014) GRADE assessment checklist. GRADE provides a transparent approach and guidance on rating the overall quality of research evidence indicating four levels of evidence along a continuum (high, moderate, low, and very low) based on five factors: 1. risk of bias, 2. inconsistency, 3. indirectness, 4. imprecision, 5. publication bias. The risk of bias for each included study was evaluated using the simplified GRADE checklist proposed by Meader et al.(Meader et al., 2014), based on the Cochrane risk of bias tool items (Table 7.1 and 7.2).

For each meta-analysis, the pooled effect and the level of heterogeneity, by means of the Q and I^2 statistics, were calculated (Higgins & Thompson, 2002). Q estimates the amount of variation due to the sampling error while I^2 evaluates which proportion of the observed variance reflects a real difference in effect sizes. I^2 values of 25%, 50%, and 75% have been interpreted as representing small, moderate, and high levels of heterogeneity, respectively. Influential cases were identified using the "inf" function from the "metafor package". We also applied a set of diagnostics derived from standard linear regression to spot potential outliers which could influence the observed heterogeneity. The likelihood of publication bias was assessed graphically by using the funnel plot tool together with Egger's regression test (Egger, Smith, Schneider, & Minder, 1997) and the rank correlation test (Begg & Mazumdar, 1994). The trim and fill method (which imputes "missing" studies to create

a more symmetrical funnel plot) (Duval & Tweedie, 2000) was used for bias correction only if the previously mentioned tests were significant, since a p value < 0.05 is consistent with a non-symmetrical funnel plot.

3.2.3. Results

We retrieved 2497 citations; duplicates and studies that did not satisfy the inclusion criteria were excluded. 125 papers underwent full review (Figure 1.), resulting in 20 accepted articles.

Figure 1. Flow Diagram of study selection and inclusion.
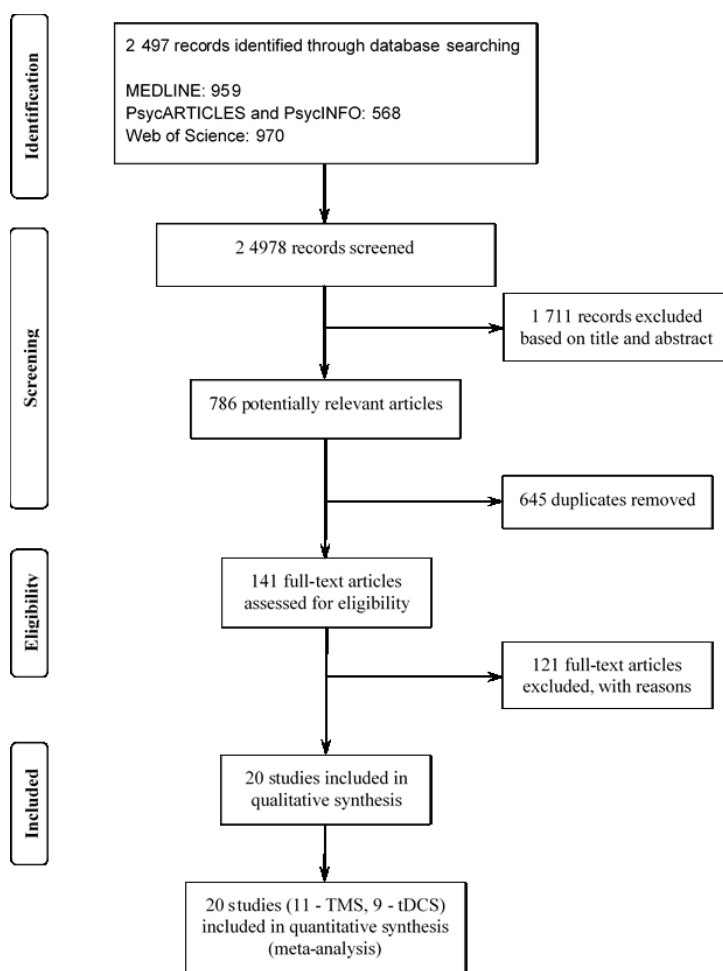
Studies characteristics

Nine studies were tDCS therapeutic applications for aphasia treatment: four randomized controlled trials (RCT) (Fridriksson et al., 2018; Meinzer et al., 2016; Polanowska et al., 2013; Spielmann et al., 2018), three randomized crossover studies (Fiori et al., 2013; Marangolo, Fiori, Calpagnano, et al., 2013; Marangolo, Fiori, Di Paola, et al.,

2013), one partial randomized crossover research (Shah-Basak et al., 2015), and one non-randomized crossover (Ben Basat et al., 2016) (Table 5.2). Eleven studies investigated the effects of rTMS on aphasic patients after stroke: seven were RCTs (Barwood et al., 2013; Hu et al., 2018; Khedr et al., 2014; NAESER et al., 2005; Seniów et al., 2013; Tsai et al., 2014; Waldowski, Seniów, Leśniak, Iwański, & Członkowska, 2012; Wang et al., 2014), one was a randomized partial crossover (Medina et al., 2012), and three were open label studies (Hara et al., 2015; Harvey et al., 2017; Naeser et al., 2005) (Table 5.1). The majority of these studies used a combined therapy, namely noninvasive brain stimulation alongside language training online (behavioral treatment) or offline (taking advantage of the stimulation after-effects, the linguistic training was delivered after the neurostimulation); only five studies, one tDCS (Lifshitz Ben Basat et al., 2016) and four rTMS (Barwood et al., 2013; Harvey et al., 2017; Medina et al., 2012; NAESER et al., 2005), used neurostimulation as a stand-alone therapy.

The methodological quality of the eleven RCTs (Table 6.1 & 6.2) according to the modified Sackett Scale was good, scoring between 6 and 10 on the PEDro scale (level 1b evidence). Five studies had randomized crossover designs, while one had a partial crossover design (Shah-Basak et al., 2015), and their scores ranged between 4 and 9 (evidence level 1b and 2). The main risk of bias was the lack of allocation concealment and the carryover effect due to a very short washout period before the groups were switched to the other experimental arm; for instance, in the Fiori et al. (Fiori et al., 2013) study, the intersession interval (between treatment and sham) was only 6 days. The three open-label studies (Hara et al., 2015; Harvey et al., 2017; NAESER et al., 2005) and the non-randomized crossover study (Lifshitz Ben Basat et al., 2016) were considered as level 4 evidence. Overall, the level of evidence is ranging from poor (level 4 – 4 studies) to good (level 1b – 16 studies).

Participant characteristics

The review included twenty studies involving 509 stroke patients diagnosed with aphasia (271 in the experimental condition, 202 in the sham condition, and 36 in both experimental arms as part of crossover designs); 280 received rTMS stimulation (181 real rTMS and 99 sham), and 229 participated in tDCS experiments (90 real tDCS, 103 sham, and 36 both conditions).

Participants' characteristics were heterogeneous among studies (Table 1), especially regarding:

- time interval from stroke – five papers focused on subacute patients (*N*= 184) ranging from 28 to 56 days post-stroke (Khedr et al., 2014; Polanowska et al., 2013; Seniów et al., 2013; Spielmann et al., 2018; Waldowski et al., 2012), while all the others included only participants with chronic aphasia (*N*= 325); the post-stroke interval ranged from 7 months to 11 years;

- aphasia diagnosis – this was obtained by means of different tests and aphasia batteries based on different approaches (neurolinguistics vs. psycholinguistic), the most frequent ones being: the Boston Diagnostic Aphasia Examination (BDAE), the Boston Naming Test (BNT), Psycholinguistic Assessment of Language Processing in Aphasia (PALPA), Aachen Aphasia Test (AAT), Aphasia Severity Rating Scale (ASRS), and the Western Aphasia Battery (WAB); thirteen studies explicitly selected participants with non-fluent aphasia while 7 studies included all types of aphasia (Table 2);

- participants' age ranged between 37 and 77 years (Table 1),

- lesion – only left hemisphere stroke patients were selected and the majority of the studies provided only general information about lesions (site, extension, etiology), but in some cases more details were reported, including MRI scans (Fiori et al., 2013; NAESER et al., 2005).

Intervention

Target area. The target area was identified in different brain regions depending on the experimental hypothesis and the stimulation type.

All rTMS studies, with two exceptions [Khedr et al. (Khedr et al., 2014) that stimulated both hemispheres and Hara et al. (Hara et al., 2015) that stimulated the right or the left hemisphere based on fMRI results], stimulated anterior language areas (pars triangularis or pars orbitalis) on the contralesional (right) hemisphere. The most often targeted brain region in the tDCS experiments was left Broca's area, but in two papers (Lifshitz Ben Basat et al., 2016; Shah-Basak et al., 2015) a personalized approach was used and, based on pre-treatment tDCS assessment sessions, targeted anterior or posterior, contralesional or ipsilesional language areas. Fridriksson et al. (Fridriksson et al., 2018) chose the temporal lobe region with the highest naming related activation during fMRI, while Meinzer et al. (2016) tested the effectiveness of M1 (primary motor cortex) tDCS stimulation on language rehabilitation.

Stimulation protocol: rTMS. All of the included studies, with the exception of Khedr et al. (Khedr et al., 2014) which applied a dual-hemisphere stimulation (excitatory, 20 Hz over left Broca's area and inhibitory over the right, unaffected Broca's area), used an inhibitory stimulation protocol. rTMS was delivered at an intensity between 110% and 80% of the resting motor threshold (9 out of 11 studies used a 90% RMT intensity) at a frequency of 1 Hz for a duration ranging between 10 minutes and 40 minutes for a total of 10/15 sessions (Table 3). The magnetic pulses were delivered only with figure of eight shaped coils.

Stimulation protocol: tDCS. In all of the included studies, tDCS was unipolar and two equal sized electrodes were used in the majority of the cases; Meinzer et al. (Marcus Meinzer et al., 2016) preferred different dimensions: anode 5x7 cm, cathode 10x10 cm. Each electrode had an area of 25 cm^2 (5 cm×5 cm) or in some cases 35 cm^2 (5 cm×7cm), and with a few exceptions [that chose the best response area (Fridriksson et al., 2018; Lifshitz Ben Basat et al., 2016; Shah-Basak et al., 2015)] the anode was placed over the left, anterior language areas and the cathode over the right supraorbital region.

The ***intensity and duration*** of the stimulus were heterogeneous across studies, tDCS being delivered at an intensity of 1 mA or 2 mA (7 out of 9 publications used 1 mA, current density = 0.028) for 10/20 minutes. Another relevant variation concerned the number of sessions, which ranged between 5 (Fiori et al., 2013; Marangolo, Fiori, Di Paola, et al., 2013) and 16 (Meinzer et al., 2016). The follow-up period was very broad, varying from one/two weeks (NAESER et al., 2005) up to 12 months, and some studies had many follow-up time points. For example, Barwood et al. (2013) evaluated the rTMS effects at 1 week, 2 months, 8 months, and 12 months after stimulation, but in this meta-analysis only a period between one and six months was taken into consideration.

Associated therapies. One tDCS (Lifshitz Ben Basat et al., 2016) and four rTMS studies (Barwood et al., 2013; Harvey et al., 2017; Medina et al., 2012; NAESER et al., 2005) had a treatment intervention based exclusively on neurostimulation; in all the other cases, rTMS and tDCS were delivered together (during the stimulation or immediately after) with a broad range of aphasia classical therapies like speech and language training for 30 – 60 minutes two to five times per week, picture-naming activity during every stimulation session, conversational therapy, word-finding therapy, and computerized anomia treatment (Table 3 and 4).

Placebo: All of the RCTs and randomized crossover studies, apart from Marangolo et al. (Marangolo, Fiori, Di Paola, et al., 2013) and Xue-Yan Hu et al. (Hu et al., 2018), were double-blind sham-controlled i.e., investigators and patients were blinded to the treatment allocations. The majority of the studies reported that randomization was used to assign patients to the treatment or to the control group, but the method of randomization was not always reported (Table 7.1 and 7.2). In the rTMS experiments, the sham condition was performed with the angle of the coil positioned at 90° perpendicular to the skull (Khedr et al., 2014; Medina et al., 2012; Seniów et al., 2013) or with a sham coil (Barwood et al., 2013; Tsai et al., 2014; Waldowski et al., 2012; Wang et al., 2014), while the rTMS open label studies (Hara et al., 2015; Harvey et al., 2017; NAESER et al., 2005) had no sham/control condition, the data being analyzed confronting the pre and post stimulation results. In the tDCS experiments, the sham condition implied turning off the stimulation after a very brief period, 15 - 60 seconds.

Outcome measures. Outcome assessment varied largely across studies and included: picture naming, naming reaction times, comprehension, fluency (discourse productivity), grammatical accuracy, lexical selection, general language scores on aphasia batteries (e.g., Western Aphasia Battery Aphasia Quotient), single photon emission-computed tomography (SPECT) scans, the relation between cerebral blood flow (CBF), and language recovery (Hara et al., 2015), etc.

Adverse Effects. Overall, both rTMS and tDCS were well tolerated and only common, mild side effects, such as local discomfort at the stimulation site (itching, tingling) or dull headache (Hara et al., 2015) and dizziness (Fridriksson et al., 2018), were registered. Importantly no study reported significant differences between the active and the control group regarding the unpleasant effects of the stimulation, but unfortunately almost half of the included papers did not provide information regarding adverse effects (Fiori et al., 2013; Marangolo, Fiori, Calpagnano, et al., 2013; Marangolo, Fiori, Di Paola, et al., 2013).

Table 1. Summary of patients' characteristics

ID	Study	NIBS	Overall sample size	Experimental group characteristics							Control group	Dropout
				Sample Size	Male	Female	Mean Age (SD)	Stroke Interval Chronic/Subacute	Mean Stroke Interval	Handedness	Sample Size	
1	Naeser et al., 2005	TMS	4	**4**	3	1	55 ± 2.9, range 52-58	chronic	99 ± 33.04, range 60-132 months	right	**4**	0
2	Waldowski et al., 2012	TMS	26	13	6	7	62.31 ± 11.03, range 38 - 77	subacute	28.92 ± 19.39 days	right	13	0
3	Medina et al., 2012	TMS	10	5	4	1	60.60 ± 7.1, range 51- 71	chronic	49.8 ± 29.6, range 6-87 months	right	5	0
4	Barwood et al., 2013	TMS	12	6	4	2	61 ± 7.5, range 54- 66	chronic	44.4 ± 15.08, range 31-69 months	right	6	0
5	Seniów et al., 2013	TMS	38	19	8	11	61,65 ± 11.7, range 38-73	subacute	33.4 ± 24.18, range 11-106 days	right	19	2
6	Khedr et al., 2014	TMS	29	19	8	11	61 ± 9.8	subacute	40.6 ± 28.50 days	right	10	1
7	Tsai et al., 2014	TMS	53	31	22	9	62.3 ± 12.1	chronic	17.8 ± 7.2 months	right	22	3
8	Wang et al., 2014	TMS	29	15	14	1	61.3 ± 13.2	chronic	16.8 ± 6.4 months	right	14	0
9	Hara et al., 2015	TMS	50	**50**	40	10	60.3 ± 12.1	chronic	55.9 ± 30 months	right	**50**	0
10	Harvey et al., 2017	TMS	9	**9**	7	2	61± 8, range 47 - 75	chronic	55 ± 33, range 6 - 102 months	right	**9**	0
11	Xue-yan Hu et al., 2018	TMS	20	10	6	4	48.5± 11.2	chronic	7.5 ± 3.2 months	right	10	0
12	Fiori et al., 2013	tDCS	7	**7**	5	2	58.4 ± 9.5, range 44-71	chronic	33.8 ± 27.9, range 7-84 months	right	**7**	0
13	Marangolo et al. 2013 a	tDCS	7	**7**	5	2	62.4 ± 9.5, range 46-77	chronic	40.8 ± 26.7, range 7 - 84 months	right	**7**	1
14	Marangolo et al. 2013 b	tDCS	12	**12**	8	4	59.5 ± 8.14, range 44-71	chronic	37.25 ± 22.15, range 7 - 84 months	right	**12**	0
15	Polanowska et al., 2013	tDCS	37	18	11	7	57.6 ± 9.6, range 34-75	subacute	55.7 ± 44.8, range 10-187 days	right	19	4
16	Shah-Basak et al., 2015	tDCS	5	**5**	ns	ns	63.6 ± 8.6, range 53-78	chronic	31 ± 29.7, range 7-101 months	right	**3**	2
17	Ben Basat et al., 2016	tDCS	7	**7**	5	2	67± 14.74, range 43 - 84	chronic	57.42±55.41 range: 12-168 months	right	**7**	0
18	Meinzer et al., 2016	tDCS	26	13	7	6	59.9± 11.8, range 38 - 77	chronic	46±24.5, range: 15-108 months	right	13	0
19	Spielmann et al., 2018	tDCS	54	23	18	5	57.9± 9.6	subacute	46.9±16.1 days	right	31	4
20	Fridriksson et al., 2018	tDCS	74	34	24	10	60± 11	chronic	44± 45 months	right	40	8

Abbreviations: NIBS, noninvasive brain stimulation methods; SD, standard deviation; tDCS, Transcranial direct current stimulation; TMS, transcranial magnetic stimulation
* NOTE: the bolded numbers indicate that the same patients were in the treatment and control group, as part of open label or crossover designs.

Table 2. Summary of disorders' characteristics

	Study	NIBS	Lesion area	Lesion Type	Aphasia Type	Diagnosis criteria and instruments	Aphasia Severity	Outcome	Outcome Evaluation
1	Naeser et al., 2005	TMS	left hemisphe	stroke	non fluent	BDAE, BNT	mild to severe	Naming	BNT scores
2	Waldowski et al., 2012	TMS	left hemisphe	ischemic stroke	all types	BDAE, ASRS	moderate to severe	Naming	Computerized Picture Naming Test (Accuracy)
3	Medina et al., 2012	TMS	left hemisphe	ischemic stroke	non fluent	BDAE, BNT, Snodgrass & Vanderwart picture inventory	mild to moderate	Speech	Cookie Theft description: Correct Information Units (Discourse Productivity)
4	Barwood et al., 2013	TMS	left hemisphe	ischemic stroke	non fluent	BDAE, BNT, Snodgrass & Vanderwart picture inventory	mild to severe	Naming	BNT mean scores
5	Seniów et al., 2013	TMS	left hemisphe	ischemic stroke	all types	BDAE, ASRS	moderate to severe	Naming	BDAE (Naming subtest)
6	Khedr et al., 2014	TMS	left hemisphe	ischemic stroke	non fluent	BDAE, ASRS	mild to severe	Naming	HSS naming score (changes in mean)
7	Tsai et al., 2014	TMS	left hemisphe	ischemic stroke	non fluent	Picture Naming Test, Concise Chinese Aphasia Test	mild to severe	Naming	Object and Action Naming accuracy (%)
8	Wang et al., 2014	TMS	left hemisphe	ischemic stroke	non fluent	Picture Naming Test, Concise Chinese Aphasia Test	NS	Naming	Object and Action Naming accuracy (%)
9	Hara et al., 2015	TMS	left hemisphe	ischemic or hemorrhagic stroke	all types	SLTA	mild to severe	SLTA scores	The change of the total SLTA scores
10	Harvey et al., 2017	TMS	left hemisphe	ischemic stroke	non fluent	BDAE	mild to moderate	Naming	Follow-up: BDAE Naming (Categories subtest), percent accuracy; After: Naming Performance for Repeated and Novel Stimuli
11	Xue-yan Hu et al., 2018	TMS	left hemisphe	ischemic or hemorrhagic stroke	non fluent	WAB	NS	Naming	WAB- Chinese version (Naming subtest)
12	Fiori et al., 2013	tDCS	left hemisphe	ischemic stroke	non fluent	B.A.D.A. test; Token test	NS	Naming	Mean percentage of response accuracy for nouns and verbs
13	Marangolo et al. 2013 a	tDCS	left hemisphe	ischemic stroke	non fluent	B.A.D.A. test	NS	Naming	Mean percentage of correct responses
14	Marangolo et al. 2013 b	tDCS	left hemisphe	ischemic stroke	non fluent	B.A.D.A. test; Token test	NS	Speech	Mean percentage of correct Content Units
15	Polanowska et al., 2013	tDCS	left hemisphe	ischemic stroke	all types	BDAE, ASRS	moderate to severe	Naming	BDAE Naming
16	Shah-Basak et al., 2015	tDCS	left hemisphe	stroke	non fluent	WAB	mild to severe	WAB-AQ	Mean WAB-AQ scores
17	Ben Basat et al., 2016	tDCS	left hemisphe	stroke	non fluent	PALPA 47, Written Word Association test, Picture Association test.	NS	Naming	Picture naming
18	Meinzer et al., 2016	tDCS	left hemisphe	ischemic or hemorrhage stroke	all types	AAT	NS	Naming	Naming untrained items

19	Spielmann et al., 2018	tDCS	left hemisphe	ischemic or hemorrhage stroke	all types	Shortened token test	mild to severe	Naming	Boston Naming Test
20	Fridriksson et al., 2018	tDCS	left hemisphe	ischemic or hemorrhage stroke	all types	WAB-R	mild to severe	Naming	Change in Correct Naming

Abbreviations: AAT, Aachener Aphasie Test; ASRS, Apraxia of Speech Rating Scale; B.A.D.A., Battery for the Analysis of the Aphasic Deficit; BDAE, Boston Diagnostic Aphasia Examination; BNT, Boston Naming Test; HSS, Hemispheric Stroke Scale; NS, not specified; PALPA, Psycholinguistic assessments of language processing in aphasia; SLTA, Standard Language Test of Aphasia; tDCS, Transcranial direct current stimulation; TMS, transcranial magnetic stimulation; WAB, Western Aphasia Battery; WAB-AQ, WAB Aphasia Quotient; WAB-R, Western Aphasia Battery – Revised;

Table 3 Summary of TMS study characteristics

	Study	Target area(s) Anterior/Posterior areas	RH/LH (contralateral /ipsilateral)	rTMS Frequency	Intensity (% RMT)	Number of pulses and duration	Number of sessions	Online/Offline Stimulation & Therapy	Sham	Adverse Effects
				TMS Stimulation Parameters						
1	Naeser et al., 2005	Anterior: Broca's homologue (anterior part)	RH (contralateral)	1 HZ inhibitory	90% RMT	1200 pulses, 20 min	10 sessions (5 days x 2 weeks)	only TMS	no sham	none of patients had any undesirable side-effectss
2	Waldowski et al., 2012	Anterior: Broca's homologue	RH (contralateral)	1 HZ inhibitory	90% RMT	1800 pulses (900 PTr, 900 POp), 30 min (15 min PTr , 15 min POp)	15 sessions (5 days x 3 weeks)	offline with speech and language therapy	air-cooled sham coil	no adverse effect related to the application of rTMS.
3	Medina et al., 2012	Anterior: pars triangularis (n=9) or pars orbitalis (n=1); (best reponse area between BA 44, BA 45, BA 47)	RH (contralateral)	1 HZ inhibitory	90% RMT	1200 pulses, 20 min	10 sessions (5 days x 2 weeks)	only TMS	the coil perpendicular to head	All subjects tolerated stimulation without complaint of physical discomfort or other adverse effects
4	Barwood et al., 2013	Anterior: Broca's homologue (rPTr)	RH (contralateral)	1 HZ inhibitory	90% RMT	1200 pulses, 20 min	10 sessions (5 days x 2 weeks)	only TMS	sham coil	ns
5	Seniów et al., 2013	Anterior: Broca's homologue	RH (contralateral)	1 HZ inhibitory	90% RMT	1800 pulses, 30 min	15 sessions (5 days x 3 weeks)	offline with speech and language therapy	air-cooled sham coil	patients tolerated rTMS well, and no adverse effects were observed
6	Khedr et al., 2014	Anterior: PTr, POp	both *RH (contralateral) and * *LH (ipsilateral)	*1 HZ inhibitory **20 HZ excitatory	*110% RMT, **80% RMT	* 1000 pulses (500 PTr, 500 POp), **10 trains (5 PTr, 5 POp) of 5 seconds each with an intertrain interval of 30 seconds	10 sessions (5 days x 2 weeks)	offline with language therapy	coil rotated at 90° away from the scalp	No patient developed seizures during the study or on follow-up
7	Tsai et al., 2014	Anterior: rPTr	RH (contralateral)	1 HZ inhibitory	90% RMT	600 pulses, 10 min	10 sessions (5 days x 2 weeks)	offline with speech therapy	sham coil	No subjects reported any adverse effects during the course of the study or after the 3-month follow-up
8	Wang et al., 2014	Anterior: rPTr	RH (contralateral)	1 HZ inhibitory	90% RMT	1200 pulses, 20 min	10 sessions (5 days x 2 weeks)	online with picture-naming training	sham coil	One patient in the TMS group reported a dull pain at first when placed under the activated coil, but the discomfort subsided once the stimulation intensity was reduced by 5%
9	Hara et al., 2015	Anterior or Posterior: rIFG - nonfluent aphasia, rSTG - fluent aphasia	based on fMRI results *RH (contralateral, n=29) or * *LH (ipsilateral, n= 21)	1 HZ inhibitory	90% RMT	2400 pulses, 40 min	10 sessions (6 days x week)	online with intensive speech therapy	no sham	no adverse events were noted during admission and no adverse events were reported during the 3 months following discharge
10	Harvey et al., 2017	Anterior, best response area: rPTr (n=9) or rPOrb (n=1)	RH (contralateral)	1 HZ inhibitory	90% RMT	1200 pulses, 20 min	10 sessions (5 days x 2 weeks)	TMS only	no sham	NS
11	Xue-yan Hu et al., 2018 (1 Hz)	Anterior: Broca's homologue	RH (contralateral)	1 HZ inhibitory	80% RMT	600 pulses, 10 min	10 sessions	offline with language therapy	coil rotated at 90° away from the scalp	NS

Abbreviations: BA, Brodmann area; LH, left hemisphere; min, minutes; NS, not specified; POp, pars opercularis; PTr, pars triangularis; rPTr, right pars triangularis; rIFG, right inferior frontal gyrus; RH, right hemisphere; rPOrb, right pars orbitalis; RMT, resting motor threshold; rTMS, repetitive transcranial magnetic stimulation; rSTG, right superior temporal gyrus.

Table 4 Summary of tDCS study characteristics

ID	Study	tDCS Stimulation Parameters							Sham	Adverse Effects.
		Montage Unipolar /Bipolar (A-tDCS, C-tDCS)	Stimulation Area, Electrode Position	Reference Electrode	Electrode dimension	Current Intensity	Number of stimulations Duration	Online/Offline Stimulation & Therapy		
1	Fiori et al., 2013	Unipolar (A-tDCS)	Anterior: anodic (F5-A) left Broca's area	LH (ipsilateral)	cathode: contralateral frontopolar cortex	5×7cm	1 mA, current density = 0.028	20min, 5 daily sessions	online with naming task	turned off after 30 s	NS
2	Marangolo et al. 2013 a	Unipolar (A-tDCS)	Anterior: anodic (F5-A) left Broca's area	LH (ipsilateral)	cathode: contralateral frontopolar cortex	5×7cm	1 mA, current density = 0.028	20min, 5 daily sessions	online with naming task	turned off after 30 s	NS
3	Marangolo et al. 2013 b	Unipolar (A-tDCS)	Anterior: anodic (F5-A) left Broca's area	LH (ipsilateral)	cathode: contralateral frontopolar cortex	5×7cm	1 mA, current density = 0.028	20min, 10 daily sessions	online with Conversational Therapy	turned off after 30 s	NS
4	Polanowska et al., 2013	Unipolar (A-tDCS)	Anterior: anodic left Broca's area	LH (ipsilateral)	cathode: right supraorbital area	5×7cm	1 mA, current density = 0.028	10 min, 15 daily sessions	offline, language therapy sessions after tDCS	turned off after 15 s	NS
5	Shah-Basak et al., 2015	Unipolar (A-tDCS or C-tDC to the right or left hemisphere): best response at one of the four active montages (Phase 1)	Anterior: n=3: left-frontal anode, n=3 left-frontal cathode, n=1 right-frontal cathode	RH or LH (contralateral or ipsilateral)	anode ot cathode: contralateral mastoid	5×5 cm	2 mA, current density = 0.08	20 min, 10 days sessions	online, picture-naming task	turned off after 1 minute	NS
6	Ben Basat et al., 2016	Unipolar (A-tDCS or C-tDC): best response (anodal and cathodal over left IFG, right IFG, left STG, right STG)	Anodal: R-IFG (n=1), L-IFG (n=1), R-STG (n=1), Cathodal: R-STG (n=2), L-STG (n=2)	RH or LH (contralateral or ipsilateral)	anode ot cathode: contralateral supraorbital area	5×7cm	2 mA, current density = 0.057	10 min, 6 sessions (3 x week)	only tDCS	turned off after 30 s	All participants tolerated tDCS well and no adverse effects related to the application of tDCS were demonstrated.
7	Meinzer et al., 2016	Unipolar (A-tDCS)	Anterior: anodic left M1	LH (ipsilateral)	cathode: right supraorbital region	anode 5 x7 cm, cathode 10 x10 cm	1 mA, current density = 0.028	20 min, 16 sessions in 8 days (2 x 1.5 h/day)	online, computer-assisted naming treatment	turned off after 30 s	Only mild sensations (e.g. itching, tingling, slight burning feeling) during the initial ramping up were reported
8	Spielmann et al., 2018	Unipolar (A-tDCS)	Anterior: anodic left -IFG	LH (ipsilateral)	cathode: right supraorbital region	5 x 7 cm	1 mA, current density = 0.028	20 min, 10 sessions (2 weeks x 5 days)	online, word-finding therapy	turned off after 30 s	Sixty-nine percent reported no pain (score=0); the rest reported very little pain/ little pain (score=1–2), 56% of these participants received sham- tDCS. There was no significant difference in pain rating between groups. Reported side effects were headache and skin irritability; no adverse events were reported during treatment.
9	Fridriksson et al., 2018	Unipolar (A-tDCS)	Posterior: anodic temporal lobe region with the highest naming related activation on the fMRI	LH (ipsilateral)	cathode: right supraorbital region	5×5cm	1 mA, current density = 0.04	20min, 15 sessions (3 weeks x 5 days)	online with naming task	turned off after 30 s	Headache - 2 (S-tDCS), Dizziness - 1 (A-tDCS), 2 (S-tDCS), Erythema - 2 (A-tDCS), Convulsion (S-tDCS), Hypertension - 1 (S-tDCS)

Abbreviations: A-tDCS, anodal transcranial direct current stimulation; C-tDCS, cathodal tDCS; S-tDCS, Sham tDCS; IFG, inferior frontal gyrus; L-IFG, left IFG; R-IFG, right IFG; STG, superior temporal gyrus; R-STG, right STG; L-STG, left STG; M1, primary motor cortex; fMRI, Functional Magnetic Resonance Imaging; LH, left hemisphere; RH, right hemisphere; NS, not specified.

Table 5.1 Summary of the TMS studies' characteristics:

N	Study	Study main objective	Sudy language	Study Arms for the meta-analysis	Follow-up	Results/ Author's Conslusion	Study design	Levels of evidence modified Sackett Scale
1	Naeser et al., 2005	Improved picture naming in chronic aphasia after TMS to part of right Broca's area: An open-protocol study	if 1Hz rTMS to right pars triangularisis is safe and associated with significantly picture naming improvement in chronic aphasia	English	pre VS post TMS	2 weeks, **2months**, 8 months	significant improvement in picture naming at 2 months post-rTMS	open-label	Level 4
2	Waldowski et al., 2012	Effect of Low-Frequency Repetitive Transcranial Magnetic Stimulation onNaming Abilities in Early-Stroke Aphasic Patients: A Prospective, Randomized, Double-Blind Sham-Controlled Study	whether low-frequency rTMS over the Broca's homologues in combination with speech/language therapy improves naming in early-stroke aphasia patients	Polish	TMS VS sham	**15 weeks**	all groups improved but there was no significant difference in average test scores between groups at any time point	RCT	Level 1b
3	Medina et al., 2012	Finding the Right Words: Transcranial Magnetic Stimulation Improves Discourse Productivity in Non-fluent Aphasia After Stroke	whether rTMS improves fluency in individuals with chronic nonfluent aphasia, and to identify aspects of fluency that are modulated in persons who respond to rTMS	English	TMS VS sham	**2 months**	there were trends toward greater change from baseline in subjects receiving rTMS compared to sham (low statistical power)	Randomized, Partial Crossover (2 months of intersession interval)	Level 1b
4	Barwood et al., 2013	Long term language recovery subsequent to low frequency rTMS in chronic non-fluent aphasia	to assess the efficacy of inhibitory rTMS to modulate language performance in non-fluent aphasia	English	TMS VS sham	1 week, **2 months**, 8 months, 12 months	rTMS > Sham for naming, expressive language and auditory comprehension	RCT	Level 1b
5	Seniów et al., 2013	Transcranial Magnetic Stimulation Combined with Speech and Language Training in Early Aphasia Rehabilitation: A Randomized Double-Blind Controlled Pilot Study	whether rTMS inhibiting the right-hemisphere Broca's area homologue improves language restitution if combined with speech/language therapy	Polish	TMS VS sham	**15 weeks**	rTMS was not effective for all poststroke aphasia patients, although it might benefit selected patients	RCT	Level 1b
6	Khedr et al., 2014	Dual-Hemisphere Repetitive Transcranial Magnetic Stimulation for Rehabilitation of Poststroke Aphasia: A Randomized, Double- Blind Clinical Trial	to evaluate the long-term efficacy of dual hemisphere rTMS on poststroke aphasia	Egyptian	TMS VS sham	1 month, **2 months**	greater improvement in the language scores after real rTMS compared with sham rTMS	RCT	Level 1b
7	Tsai et al., 2014	The Persistent and Broadly Modulating Effect of Inhibitory rTMS in Nonfluent Aphasic Patients: A Sham-Controlled, Double-Blind Study	if 1Hz rTMS improves language performance and to identify characteristics of patients predisposed to benefit most from this treatment	Mandarin Chinese	TMS VS sham	**3 months**	inhibition of the contralesional pars triangularis enhances the language recovery	RCT	Level 1b
8	Wang et al., 2014	Efficacy of Synchronous Verbal Training During Repetitive Transcranial Magnetic Stimulation in Patients With Chronic Aphasia	the efficacy of synchronous speech therapy integrated with an rTMS protocol	Chinese	TMS VS sham	**3 months**	rTMS and language training can be combined to achieve outcomes superior to those obtained when used separately	RCT	Level 1b
9	Hara et al., 2015 (LH)	Effects of Low-Frequency Repetitive Transcranial Magnetic Stimulation Combined with Intensive Speech Therapy on Cerebral Blood Flow in Post-Stroke Aphasia	the effects of low frequency rTMS (guided by fMRI data) combined with intensive speech therapy on Cerebral Blood Flow in post-stroke aphasia	Japanese	pre-VS post TMS	**3 months**	significant improvement in speaking at 3 months post-rTMS; more effective rTMS intervention needs to be explored for patients who show right hemisphere language activation in an fMRI language evaluation	open label	Level 4
10	Harvey et al., 2017	Functional Reorganization of Right Prefrontal Cortex Underlies Sustained Naming Improvements in Chronic Aphasia via Repetitive Transcranial Magnetic Stimulation	the effects of inhibitory rTMS (to regions of interest in the right inferior frontal gyrus) on picture naming performance and cortical activation in chronic post stroke aphasia	English	pre-VS post TMS	**2 months** 6 months	naming accuracy increased from pre- to post-rTMS	open label	Level 4
11	Xue-yan Hu et al., 2018 (1 HZ **)**	Effects of different frequencies of repetitive transcranial magnetic stimulation in stroke patients with non-fluent aphasia: a randomized, sham-controlled study	to compare the efficacy of rTMS applied at different frequencies to the contra-lesional hemisphere, to optimize the treatment of post-stroke non-fluent aphasia	Chinese	LF-TMS VS sham	**2 months**	LF-rTMS and HF-rTMS are both beneficial to the recovery of linguistic function in patients with post-stroke non-fluent aphasia	RCT	Level 1b

Table 5.2 Summary of the tDCS studies' characteristics:

N	Study		Study main objective	Study language	Study Arms for the meta-analysis	Follow-up	Results/ Author's Conclusions	Study design	Levels of evidence (PEDro)
12	Fiori et al., 2013 (Broca)	tDCS stimulation segregates words in the brain: evidence from aphasia	whether tDCS, over the left frontal and the temporal regions coupled with an intensive language treatment, would differently improve noun and verb recovery in chronic aphasia	Italian	Broca tDCS VS sham	1 week, **4weeks**	greater improvement in noun naming after tDCS	Randomized Crossover (6 days of intersession interval)	Level 2
13	Marangolo et al. 2013 a (Broca)	Differential involvement of the left frontal and temporal regions in verb naming: A tDCS treatment study	to determine whether coupling tDCS with an intensive language treatment would improve verb retrieval chronic aphasic	Italian	Broca tDCS VS sham	2 weeks, **4weeks**	better response accuracy during the anodic tDCS over Broca's area VS tDCS over Wernicke or VS Sham	Randomized Crossover (6 days of intersession interval)	Level 2
14	Marangolo et al. 2013 b (Broca)	tDCS over the left inferior frontal cortex improves speech production in aphasia	investigated the combined effect of tDCS and an intensive Conversational therapy treatment on discourse skills in chronic aphasia	Italian	Broca tDCS VS sham	**4weeks**	anodic tDCS over the left Broca's area together with intensive "Conversational Therapy" improves informative speech	Randomized Crossover (14 days of intersession interval)	Level 2
15	Polanowska et al., 2013	No effects of anodal transcranial direct stimulation on language abilities in early rehabilitation of post-stroke aphasic patients	the effectiveness of A-tDCS over Broca area to enhance aphasia recovery during early post-stroke rehabilitation	Polish	Broca tDCS VS sham	**3 months**	the results did not confirm a positive impact of repeated A-tDCS, preceding language therapy, on language abilities	RCT	Level 1b
16	Shah-Basak et al., 2015	Individualized treatment with transcranial direct current stimulation in patients with chronic non-fluent aphasia due to stroke	whether tDCS with an individualized optimal montage could lead to persistent reduction of aphasia severity	English	Broca tDCS VS sham	2 weeks, **2 months**	individualized tDCS treatment enhance aphasia recovery	Randomized Partial Crossover (2 months of intersession interval)	Level 2
17	Ben Basat et al., 2016	Transcranial direct current stimulation to improve naming abilities of persons with chronic aphasia: A preliminary study using individualized based protocol	to develop an individually tailored tDCS protocol for naming deficits within chronic aphasia patients	Hebrew	pre-VS post, pre VS 3 Months	1 month, **3 months**	tDCS led to significant improvement (percentage of correct responses) compared to baseline, sham led to no equivalent improvement	Crossover (Sham - three months after the end of treatment sessions)	Level 4
18	Meinzer et al., 2016	Electrical stimulation of the motor cortex enhances treatment outcome in post-stroke aphasia	the effects of intensive language training in combination with M1-tDCS on aphasia impairment and disability parameters	German	M1 A-tDCS VS sham	**6 months**	better results for untrained items in the A-tDCS group	RCT	Level 1b
19	Spielmann et al., 2018	Transcranial Direct Current Stimulation Does Not Improve Language Outcome in Subacute Poststroke Aphasia	to investigate the effect of tDCS on word-finding treatment outcome in subacute post stroke aphasia	Dutch	left IFG tDCS VS sham	10 weeks, **6 months**	results do not support an effect of tDCS as an adjuvant treatment in subacute post stroke aphasia	RCT	Level 1b
20	Fridriksson et al., 2018	Transcranial Direct Current Stimulation vs Sham Stimulation to Treat Aphasia After Stroke A Randomized Clinical Trial	to examine the futility of studying A-tDCS as an adjunctive intervention during speech therapy to improve speech production (naming) for chronic post stroke aphasia	English	A-tDCS VS Sham	**4 weeks**, 24 weeks	anodal tDCS during speech therapy is feasible and potentially transformative for aphasia treatment	RCT	Level 1b

Abbreviations: M1, primary motor cortex; A-tDCS, anodal transcranial direct current stimulation; rTMS, repetitive transcranial magnetic stimulation; IFG, inferior frontal gyrus; LF, low frequency; HF, high frequency; RCT, Randomized controlled trial; PEDro, the Physiotherapy Evidence Database tool.
NOTE: in bold the follow up period that was included into the meta-analysis

Table 6.1. Summary of the Physiotherapy Evidence Database (PEDro) TMS studies

PEDro	Naeser et al., 2005	Waldowski et al., 2012	Medina et al., 2012	Barwood et al., 2013	Seniów et al., 2013	Khedr et al., 2014	Tsai et al., 2014	Wang et al., 2014	Hara et al., 2015	Harvey et al., 2017	Xue-yan Hu et al., 2018 (1 Hz)
Publication	open label	RCT	Randomised Crossover	RCT	RCT	RCT	RCT	RCT	open label	open label	RCT
NO=0, Yes=1											
1. eligibility criteria were specified no yes where:	1	1	1	1	1	1	1	1	1	1	1
2. subjects were randomly allocated to groups (in a crossover study, subjects were randomly allocated an order in which treatments were received) no yes where:	0	1	1	1	1	1	1	1	0	0	1
3. allocation was concealed no yes where:	0	0	0	0	1	1	1	1	0	0	0
4. the groups were similar at baseline regarding the most important prognostic indicators no yes where:	0	1	1	0	1	1	1	1	1	1	1
5. there was blinding of all subjects no yes where:	0	1	1	1	1	1	1	1	0	0	0
6. there was blinding of all therapists who administered the therapy no yes where:	0	1	1	1	1	1	1	1	0	0	0
7. there was blinding of all assessors who measured at least one key outcome no yes where:	0	1	1	0	1	1	1	1	0	0	0
8. measures of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups no yes where:	0	1	1	1	1	1	1	1	1	1	1
9. all subjects for whom outcome measures were available received the treatment or control condition as allocated or, where this was not the case, data for at least one key outcome was analyzed by "intention to treat" no yes where:	0	1	1	1	1	1	1	1	0	0	1
10. the results of between-group statistical comparisons are reported for at least one key outcome no yes where:	0	1	1	1	1	1	1	1	0	0	1
11. the study provides both point measures and measures of variability for at least one key outcome no yes where:	1	1	1	1	1	1	1	1	1	1	1
Total	1	9	9	7	10	10	10	10	3	3	6

Table 6.2. Summary of the Physiotherapy Evidence Database (PEDro) tDCS studies

PEDro	Publication								
	Fiori et al., 2013 (Broca)	Marangolo et al. 2013 a (Broca)	Marangolo et al. 2013 b (Broca)	Polanowska et al., 2013	Shah-Basak et al., 2015	Ben Basat et al., 2016	Meinzer et al., 2016	Spielmann et al., 2018	Fridriksson et al., 2018
	Randomised Crossover	Randomised Crossover	Randomised Crossover	RCT	Randomised Partial Crossover	Crossover	RCT	RCT	RCT
NO=0, Yes=1									
1. eligibility criteria were specified no yes where:	1	1	1	1	1	1	1	1	1
2. subjects were randomly allocated to groups (in a crossover study, subjects were randomly allocated an order in which treatments were received) no yes where:	1	1	1	1	1	0	1	1	1
3. allocation was concealed no yes where:	0	0	0	0	0	0	0	0	1
4. the groups were similar at baseline regarding the most important prognostic indicators no yes where:	1	1	1	1	1	1	1	1	1
5. there was blinding of all subjects no yes where:	1	1	1	1	1	0	1	1	1
6. there was blinding of all therapists who administered the therapy no yes where:	1	0	1	1	1	0	1	1	1
7. there was blinding of all assessors who measured at least one key outcome no yes where:	0	0	0	1	0	0	1	1	0
8. measures of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups no yes where:	1	1	1	0	1	1	1	1	1
9. all subjects for whom outcome measures were available received the treatment or control condition as allocated or, where this was not the case, data for at least one key outcome was analysed by "intention to treat" no yes where:	1	1	1	1	1	1	1	1	1
10. the results of between-group statistical comparisons are reported for at least one key outcome no yes where:	0	0	0	1	1	0	1	1	1
11. the study provides both point measures and measures of variability for at least one key outcome no yes where:	0	0	0	1	1	1	1	1	1
Total	6	5	6	8	8	4	9	9	9

Table 7.1. Risk of bias, TMS studies

Yes = 1 No = 0	Naeser et al., 2005	Waldowski et al., 2012	Medina et al., 2012	Barwood et al., 2013	Seniów et al., 2013	Khedr et al., 2014	Tsai et al., 2014	Wang et al., 2014	Hara et al., 2015	Harvey et al., 2017	Xue-yan Hu et al., 2018 (1 Hz)
	open-label	RCT	Randomized Crossover	RCT	RCT	RCT	RCT	RCT	open-label	open-label	RCT
Was random sequence generation used (i.e. no potential for selection bias)?	0	1	1 NS[a]	1	1	1	1	1	0	0	1 NS[a]
Was allocation concealment used (i.e. no potential for selection bias)?	0	0	0	0	1	1	1	1	0	0	0
Was there blinding of participants and personnel (i.e. no potential for performance bias)?	0	1	1	1	1	1	1	1	0	0	0
Was there blinding of outcome assessment (i.e. no potential for detection bias)?	0	1	1	1	1	1	1	1	0	0	0
Was an objective outcome used?	1	1	1	1	1	1	1	1	1	1	1
Were more than (80%)a of participants enrolled in trials included in the analysis? (i.e. no potential attrition bias)	1	1	1	1	1	1	1	1	1	1	1
Were data reported consistently for the outcome of interest (i.e. no potential selective reporting)? (no potential reporting bias)	1	1	1	1	1	1	1	1	1	1	1
No other biases reported? (no potential of other bias)	0	1	1	1	1	1	1	1	0	0	0
Did the trials end as scheduled (i.e. not stopped early)?	1	1	1	1	1	1	1	1	0	0	1
TOTAL	4	8	8	8	9	9	9	9	3	3	5

NOTE: [a] reported that randomization was used but the method of randomization was not specified

Table 7.2. Risk of bias, tDCS studies

Yes = 1 No = 0	Fiori et al., 2013 (Broca) Randomised Crossover	Marangolo et al. 2013 a (Broca) Randomised Crossover	Marangolo et al. 2013 b (Broca) Randomised Crossover	Polanowska et al., 2013 RCT	Shah-Basak et al., 2015 Randomised Partial Crossover	Ben Basat et al., 2016 Crossover	Meinzer et al., 2016 RCT	Spielmann et al., 2018 RCT	Fridriksson et al., 2018 RCT
Was random sequence generation used (i.e. no potential for selection bias)?	1 NS[b]	1 NS[b]	1	1 NS[b]	1 NS[b]	0	1	1 NS[b]	1
Was allocation concealment used (i.e. no potential for selection bias)?	0	0	0	1	0	0	0	0	0
Was there blinding of participants and personnel (i.e. no potential for performance bias)?	1	0	1	1	0	0	1	1	1
Was there blinding of outcome assessment (i.e. no potential for detection bias)?	0	0	0	1	0	0	0	1	0
Was an objective outcome used?	1	1	1	1	1	1	1	1	1
Were more than (80%)a of participants enrolled in trials included in the analysis? (i.e. no potential attrition bias)	1	1	1	1	0	1	1	1	1
Were data reported consistently for the outcome of interest (i.e. no potential selective reporting)? (no potential reporting bias)	1	1	1	1	1	1	1	1	1
No other biases reported? (no potential of other bias) [a]	0	0	0	1	0	0	0	1	1
Did the trials end as scheduled (i.e. not stopped early)?	1	1	1	1	1	1	1	1	1
TOTAL	6	5	6	9	4	4	6	8	7

NOTE: [a]. carry over effect in the crossover studies; [b] reported that randomization was used but the method of randomization was not specified

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Table 8. Summary of the meta-analysis results

Comparison	Number of Studies	Effect Size Summary	95% CI	Z	p-value for Z	Q - Test for Heterogeneity	p- value for Q	I²: total heterogeneity	Influence Test	Regression Test for Funnel Plot Asymmetry	Rank Correlation Test for Funnel Plot Asymmetry (Kendall's tau)
tDCS vs. TMS at follow-up		Test of Moderators: QM(1) = 1.335, p = 0.248									
Overall NIBS Follow up	20	0.45	0.2555 , 0.6512	4.49	<.0001	20.9	0.34	23.57%	none	z = 1.8118, p = 0.0700	tau = 0.1474, p = 0.3859
TMS only Follow-up	11	0.56	0.2780 , 0.8427	3.89	0	12.7	0.24	31.15%	Khedr et al., 2014 Hara et al., 2015	z = 1.5154, p = 0.1297	tau = 0.0909, p = 0.7612
tDCS only Follow-up	9	0.33	0.0500 0.6041	2.31	0.02	7.17	0.52	13.21%	Spielmann et al., 2018	z = 0.9747, p = 0.3297	tau = 0.2778, p = 0.3585
After vs. Follow-up (Overall NIBS)		paired t–test: t (16)= -0.61, p = 0.54; 99% CI = [-0.20 , 0.13]									
Overall NIBS After	17	0.48	0.2774 , 0.6863	4.62	<.0001	15.3	0.5	15.21%	Spielmann et al., 2018	z = 1.0786, p = 0.2807	tau = 0.1324, p = 0.4896
TMS at follow-up vs. TMS immediately after		paired t–test: t (7)= -0.73, p = 0.48; 99% CI =[-0.48, 0.31]									
TMS only After	8	0.66	0.3850 , 0.9322	4.72	<.0001	3.16	0.87	0.00%	none	z = -0.2039, p = 0.8385	tau = 0.0714, p = 0.9049
tDCS at follow-up vs. tDCS immediately after		paired t–test: t (8)= 0.17, p = 0.86; 99% CI = [-0.13 , 0.14]									
tDCS only After	9	0.33	0.0436, 0.6250	2.25	0.02	8.74	0.36	19.78%	Spielmann et al., 2018	z = 1.0837, p = 0.2785	tau = 0.2778, p = 0.3585
tDCS vs. TMS RCTs follow-up		Test of Moderators: QM(1) = 5.616, p = 0.018									
RCTs Overall	12	0.5	0.2407 , 0.7512	3.81	p=0.0001	15.6	0.16	33.14%	none	z = 1.3544, p = 0.1756	tau = 0.2424, p = 0.3108
RCT TMS	8	0.71	0.4295 , 0.9966	4.93	<.0001	5.94	0.55	3.54%	Tsai et al., 2014	z = 0.6874, p = 0.4918	tau = 0.00, p = 1.0
RCT tDCS	4	0.18	-0.1556 , 0.5175	1.05	0.29	2.64	0.45	21.70%	Spielmann et al., 2018 Fridriksson et al., 2018	z = -0.1602, p = 0.8728	tau = 0.0000, p = 1.0000
Chronic: TMS vs. tDCS		Test of Moderators: QM(1) = 0.203, p= 0.652									
Chronic Overall	15	0.44	0.2335 , 0.6506	4.15	<.0001	9.2	0.82	6.54%	Hara et al., 2015	z = 1.3454, p = 0.1785	tau = 0.0857, p = 0.6972
Chronic TMS	8	0.42	0.1131 , 0.7277	2.68	0.01	6.61	0.47	20.61%	Wang et al., 2014 and Hara et al., 2015	z = 1.4090, p = 0.1588	tau = 0.1429, p = 0.7195
Chronic tDCS	7	0.52	0.2090 , 0.8334	3.27	0	2.01	0.92	0.00%	Fridriksson et al., 2018	z = 0.1887, p = 0.8503	tau = 0.2381, p = 0.5619
Subacute: TMS vs. tDCS		Test of Moderators: QM(1) = 7.801, p = 0.005									

Subacute Overall	5	0.48	-0.0465 , 0.9976	1.79	0.07	11.6	0.02	65.82%	Khedr et al., 2014 and Spielmann et al., 2018	z = 3.0079, p = 0.0026	tau = 0.8000, p = 0.0833
Subacute TMS	3	0.85	0.3773 , 1.3215	3.53	0	2.62	0.27	15.19%	Waldowski et al., 2012 Khedr et al., 2014	z = 0.7712, p = 0.4406	tau = 0.3333, p = 1.0000
Subacute tDCS	2	-0.0458	-0.4601 0.3686	-0.2164	0.83	0.57	0.45	0.00%	Spielmann et al., 2018	not applicable	not applicable
Non-fluent vs. All types aphasia		Test of Moderators: QM(1) = 5.732, p = 0.017									
All types aphasia	7	0.25	0.0121 , 0.4780	0.04	0.03	6.44	0.38	13.58%	none	z = 0.7929, p = 0.4278	tau = 0.2381, p = 0.5619
Non-fluent	13	0.67	0.4117 , 0.9263	5.1	<.0001	8.03	0.78	0.00%	Tsai et al., 2014	z = -0.1756, p = 0.8606	tau = -0.1026, p = 0.6754

3.2.4. Meta-analysis results

Hedges' *g*, a measure of effect size, was computed for each study and pooled into the meta-analysis in an effort to obtain a better understanding of how well post-stroke aphasia NIBS treatment works. Hedges' *g* interpretation is very similar to Cohen's *d* (a *g* of 1 indicates the two groups differ by one standard deviation, a *g* of 2 indicates they differ by two standard deviations). Cohen suggested a rule of thumb for interpreting results: small effect = 0.2, medium effect = 0.5, and large effect = 0.8 (DeVellis, 1991).

In order to calculate the treatment effect, in all analyses, the standardized mean differences (SMD) were pooled using the random-effects model regardless of the heterogeneity test results (Q or I^2) since there is a certain amount of variance between studies due to their particular characteristics (e.g., stimulation parameters, associated therapies, patients' characteristics). Table 8 contains all the test results from all the analyses.

rTMS vs. tDCS follow-up efficacy was the main question of the present study, and the meta-analysis of 11 RCTs, 6 crossover and 3 open label studies showed a significant medium effect of noninvasive brain stimulation for post-stroke aphasia rehabilitation (overall SMD of 0.45; 95% CI = [0.26, 0.65], *p* <.0001; I^2 =20.90%), see Figure 2. Investigating separately the effects of rTMS and tDCS, even though both techniques produced a statistically significant effect, rTMS pooled analysis revealed a moderate effect size (rTMS: SMD = 0.56; 95% CI = [0.28, 0.84], *p* =0.0001; I^2 =31.15%; *N*= 11), while tDCS produced a significant but small effect size (tDCS: SMD= 0.33; 95% CI = [0.05, 0.60], *p* =0.02; I^2 =13.2%; *N*= 9); nevertheless, the test of moderators comparing the two techniques did not reveal a statistical significant difference between the two (QM(1) = 1.335, *p* = 0.248).

Figure 2: Forest plot of effect sizes, broken down by NIBS (TMS vs. tDCS)

Study	Weights	ES	[95% CI]
Fridriksson et al., 2018	9.92%	0.47	[-0.00, 0.94]
Spielmann et al., 2018	8.45%	-0.18	[-0.72, 0.36]
Meinzer et al., 2016	4.47%	0.25	[-0.59, 1.09]
Ben Basat et al., 2016	3.02%	0.37	[-0.69, 1.43]
Shah-Basak et al., 2015	1.73%	0.41	[-1.04, 1.85]
Polanowska et al., 2013	6.66%	0.14	[-0.50, 0.79]
Marangolo et al. 2013 b	4.42%	0.97	[0.12, 1.81]
Marangolo et al. 2013 a	2.48%	0.89	[-0.30, 2.07]
Fiori et al., 2013	3.03%	0.42	[-0.64, 1.48]
tDCS Subgroup (Q = 7.17, df = 8, p = 0.52; I^2 = 13.2%)		0.33	[0.05, 0.60]
Xue-yan Hu et al., 2018	3.89%	0.83	[-0.08, 1.74]
Harvey et al., 2017	3.79%	0.26	[-0.67, 1.19]
Hara et al., 2015	12.05%	0.08	[-0.31, 0.48]
Wang et al., 2014	5.01%	1.10	[0.32, 1.88]
Tsai et al., 2014	8.53%	0.36	[-0.17, 0.90]
Khedr et al., 2014	4.38%	1.43	[0.58, 2.28]
Seniów et al., 2013	6.51%	0.71	[0.06, 1.37]
Barwood et al., 2013	2.59%	0.61	[-0.55, 1.77]
Medina et al., 2012	2.26%	0.36	[-0.89, 1.61]
Waldowski et al., 2012	5.00%	0.53	[-0.25, 1.31]
Naeser et al., 2005	1.81%	0.39	[-1.02, 1.80]
TMS Subgroup (Q = 12.72, df = 10, p = 0.24; I^2 = 31.2%)		0.56	[0.28, 0.84]
Overall Heterogeneity Test (Q = 20.90, df = 19, p = 0.34; I^2 = 23.6%)	100.00%	0.45	[0.26, 0.65]

Standardized Mean Difference

Although Q-statistic and I^2 tests provide evidence for heterogeneity, they do not offer indication about which studies may disproportionately influence heterogeneity. Baujat et al. (Baujat, Mahé, Pignon, & Hill, 2002) proposed a plot to reveal those papers that have a huge contribution to the heterogeneity and the overall outcome (Figure 2.1.0). Studies that are represented in the top right quadrant of the Figure 2.1.2 for tDCS

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(Spielmann et al., 2018) and Figure 2.1.1 for rTMS (Hara et al., 2015; Khedr et al., 2014) mostly contributed to the heterogeneity and the final result. Nevertheless, the visual inspection of a plot might not be conclusive, and to identify potential outliers and influential studies, Viechtbauer and Cheung (Viechtbauer & Cheung, 2010) proposed an influential test derived from standard linear regression. The results indicated that Khedr et al. (Khedr et al., 2014) and Hara et al. (Hara et al., 2015) (studies 6 and 9 on the Baujat plot) for the rTMS subgroup, and Spielmann et al. (Spielmann et al., 2018) (study 8 on the Baujat plot) for the tDCS subgroup, yield observed effects that were well separated from the rest of the data. Looking at the forest plot, it can be easily seen that the Khedr et al. (Khedr et al., 2014) paper reported a very big effect size (1.46) compared to the other papers, and Hara et al.(Hara et al., 2015) crucially contributed to the final result (weights = 12.5%). Spielmann's et al. (Spielmann et al., 2018) study can be considered an outlier since the reported effect size is negative (-0.18), i.e., contrary to all the other studies; indeed, in this study, it seems that tDCS on subacute patients had a slightly negative effect on language recovery.

Figure 2.1.0: Baujat plot at follow-up (overall) — Figure 2.1.1: Baujat plot at follow-up (TMS only) — Figure 2.1.2: Baujat plot at follow-up (tDCS only)
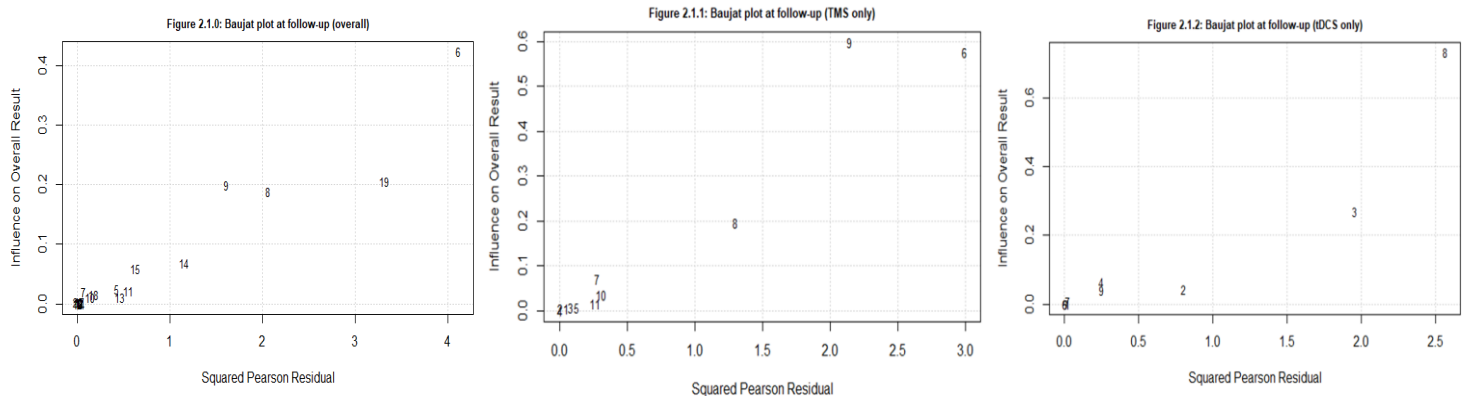
Publication Bias: The funnel plot for rTMS, tDCS and the overall publication bias (Figure 220, 2.2.1 and 2.2.2) was considered symmetrical given the fact that neither the rank correlation nor Egger's regression test were statistically significant (Table 8), so the conclusions regarding the rTMS and tDCS follow-up efficacy, taken alone and pooled together, remained unchanged.

Figure 2.2.0: Publication bias assessed by the funnel plot at follow-up (overall)

Figure 2.2.1: Publication bias assessed by trim and fill funnel plot at follow-up (TMS only)

Figure 2.2.2: Publication bias at follow-up assessed by funnel plot (tDCS only)
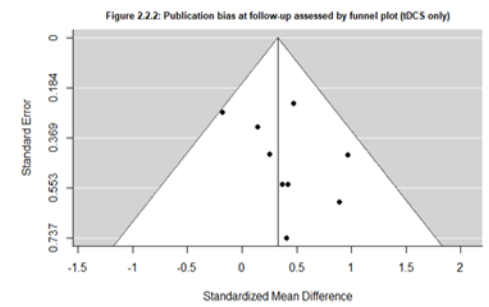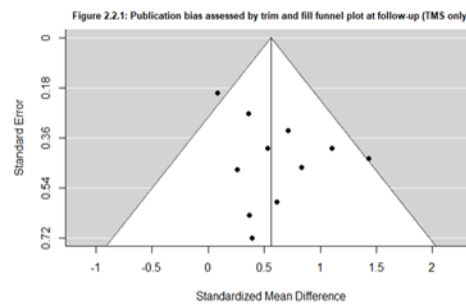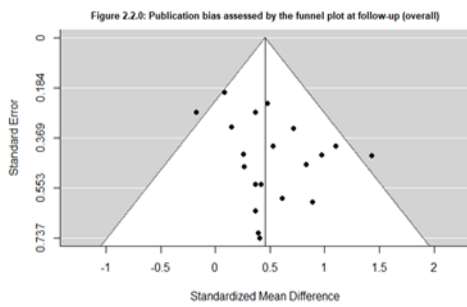
After vs. Follow-up: This study focus was the NIBS follow-up efficacy, but we also collected and analyzed the SMD immediately after the treatment in order explore the effect of time on brain **Stimulation efficacy.** The separate analysis of tDCS and rTMS publications immediately after treatment (no more than one week later) revealed a statistically significant but small SMD of 0.34 (95% CI = [0.05, 0.62]; p = 0.019; N=9) for tDCS, while for rTMS studies, the SMD of 0.66 (95% CI = [0.38, 0.93]; p = 0.31; N= 8) can be considered medium (Figure 3). Furthermore, the paired t-Test comparing the before vs after effect sizes for each technique yielded no significant difference between the two time points, indicating that the effect size observed in the rTMS (t (7)= -0.73, p = 0.48; 99%CI =[-0.48 , 0.31]) and tDCS (t (8)= 0.17, p = 0.86; 99%CI = [-0.13 , 0.14]) studies immediately after treatment did not change significantly when measured at follow-up (an interval extending from one to six months after the last stimulation session).

Figure 3: Forest plot of effect sizes immediately after treatment, broken down by NIBS (TMS vs. tDCS)

Study	Weights	ES [95% CI]
Fridriksson et al., 2018	12.97%	0.42 [-0.04, 0.89]
Spielmann et al., 2018	10.90%	-0.15 [-0.68, 0.38]
Meinzer et al., 2016	5.18%	0.21 [-0.63, 1.05]
Ben Basat et al., 2016	3.40%	0.38 [-0.68, 1.44]
Shah-Basak et al., 2015	1.93%	0.18 [-1.26, 1.61]
Polanowska et al., 2013	8.02%	0.10 [-0.55, 0.74]
Marangolo et al. 2013 b	4.88%	1.18 [0.31, 2.04]
Marangolo et al. 2013 a	2.75%	0.91 [-0.28, 2.10]
Fiori et al., 2013	3.35%	0.58 [-0.49, 1.65]
		0.33 [0.04, 0.62]
tDCS Subgroup (Q = 8.74, df = 8, p = 0.36; I^2 = 19.8%)		
Xue-yan Hu et al., 2018	4.22%	1.11 [0.17, 2.05]
Harvey et al., 2017	4.12%	0.65 [-0.30, 1.60]
Wang et al., 2014	7.85%	0.95 [0.29, 1.60]
Tsai et al., 2014	8.32%	0.40 [-0.23, 1.03]
Khedr et al., 2014	8.32%	0.79 [0.16, 1.42]
Seniów et al., 2013	5.92%	0.47 [-0.30, 1.25]
Barwood et al., 2013	4.35%	0.42 [-0.50, 1.35]
Waldowski et al., 2012	3.53%	0.40 [-0.64, 1.43]
TMS Subgroup (Q = 3.16, df = 7, p = 0.87; I^2 = 0.0%)		
		0.66 [0.38, 0.93]
Overall Heterogeneity Test (Q = 15.29, df = 16, p = 0.50; I^2 = 15.2%)	100.00%	0.48 [0.28, 0.69]

Standardized Mean Difference

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The Viechtbauer and Cheung (Viechtbauer & Cheung, 2010) influential test as the Baujat plot (Figure 3.1.0, 3.1.1, 3.1.2) showed no potential outliers for the rTMS alone, while Spielmann et al. (Spielmann et al., 2018) experiment was identified as a potential outlier for tDCS effects. The tests for **publication bias** indicated no need for bias correction (Figure 3.2.0, 3.2.1, 3.2.2).

Figure 3.1.0: Baujat plot immediately after treatment (overall)

Figure 3.1.1: Baujat plot of TMS studies immediately after treatment

Figure 3.1.2: Baujat plot of tDCS studies immediately after treatment

Figure 3.2.0: Funnel plot immediately after treatment (overall)

Figure 3.2.1: Trim and Fill Funnel plot of TMS After

Figure 3.2.2: Trim and Fill Funnel plot of tDCS After
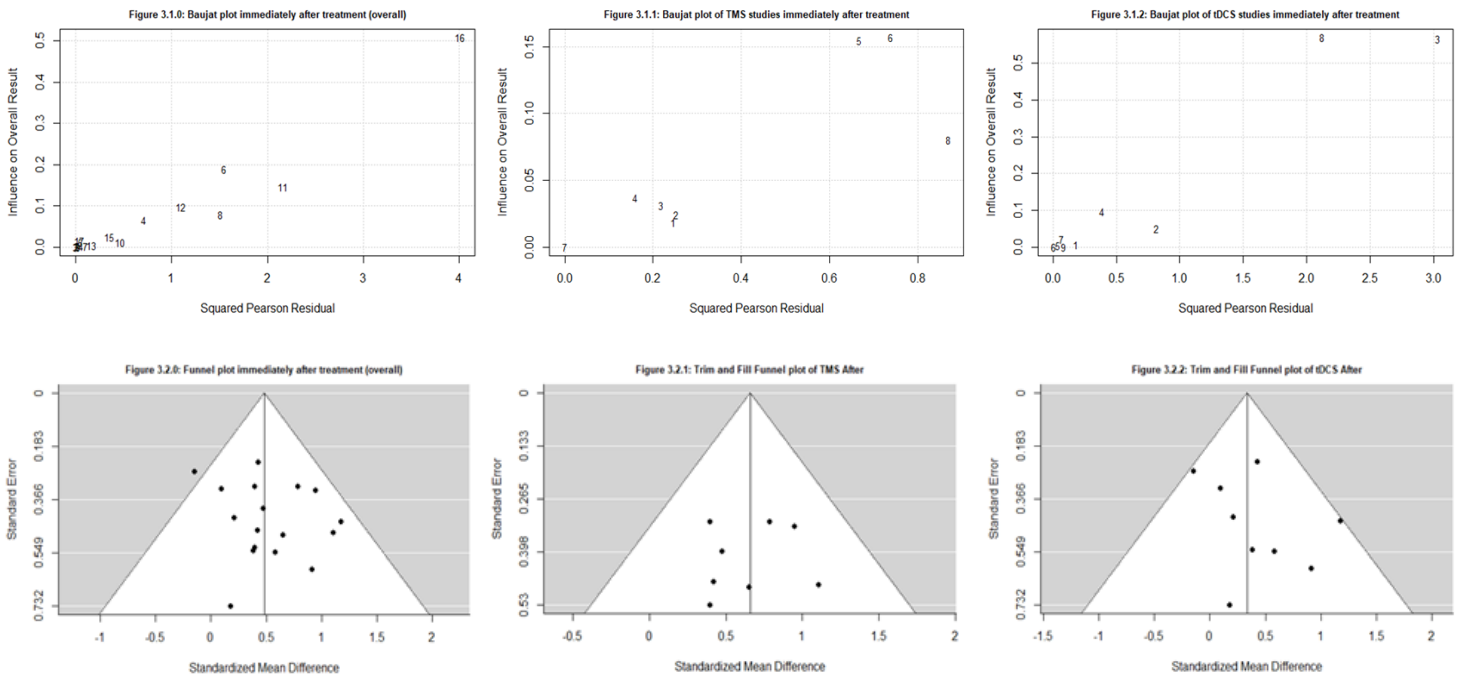
Only RCTs were analyzed separately (*N*=12) in order to exclude possible confounding factors like the carryover effect, which was present in some of the crossover trials, and the placebo effect from the open labels. The only randomized crossover trial that was included was Medina et al. (Medina et al., 2012) because they provided the follow-up results for treatment and control before the sham participants crossover into the real treatment group. The overall weighted mean effect size for rTMS and tDCS was 0.49 (*p* =.0001; 95% CI: [0.24, 0.75]; *N*=12). The test for heterogeneity was not significant (Q= 15.57, p=.16, I^2= 33.14%), indicating that the variance between studies was not larger than is to be expected when including random sample error (Figure 4). Taken separately, rTMS RCTs weighted mean effect size was SMD = 0.71 (*p* <.0001; 95% CI: [0.42, 0.99]; *N*=8), while for the tDCS RCTs the effect size was small and non-significant SMD = 0.18 (*p* = 0.36; 95% CI: [-0.16 , 0.52]; *N*=4), and as expected the test of moderators showed that the difference between estimates was statistically significant (QM (1) = 5.61, *p* = 0.018).

Figure 4: Forest plot of RCTs at follow-up, broken down by NIBS (TMS vs. tDCS)
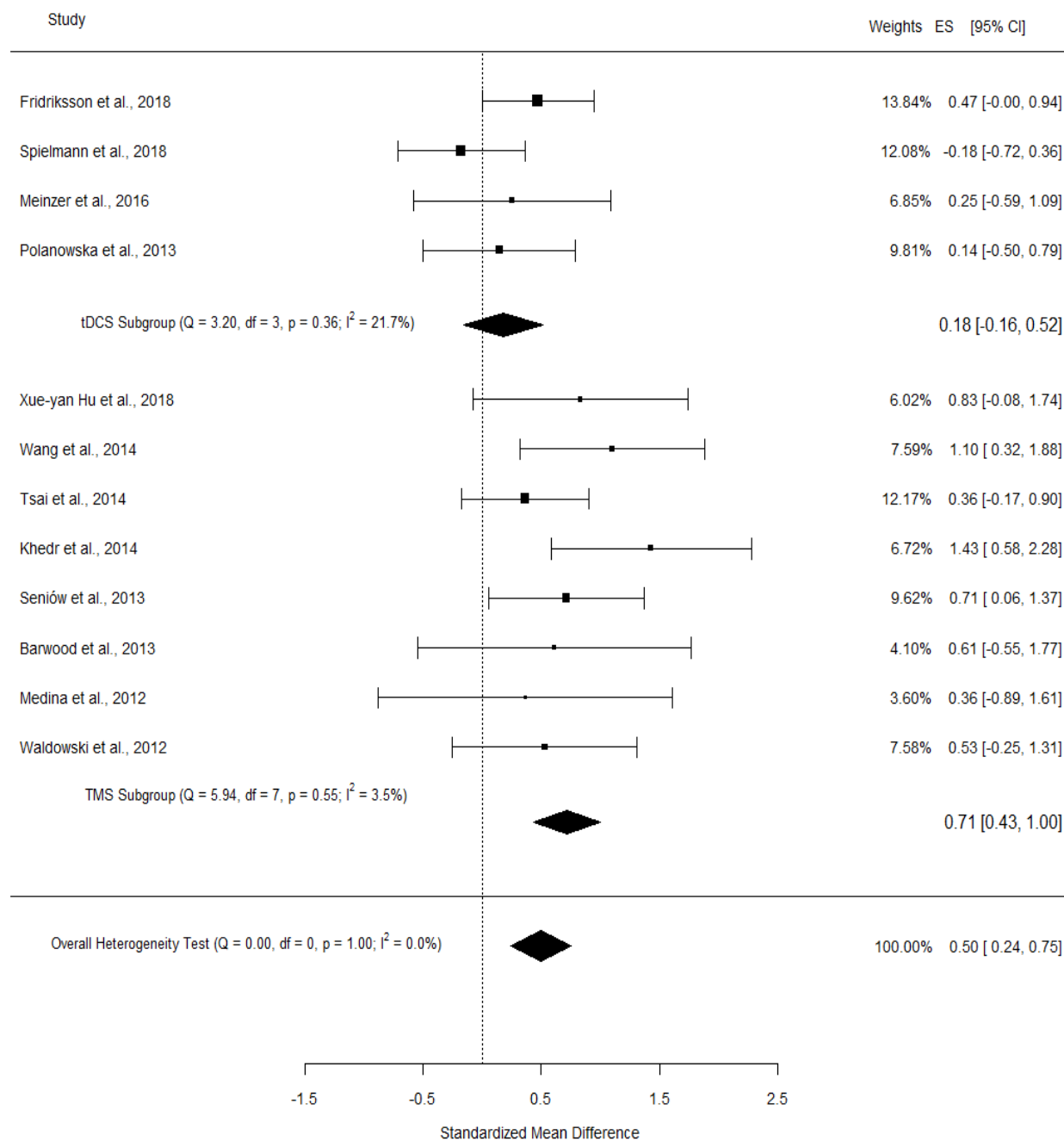
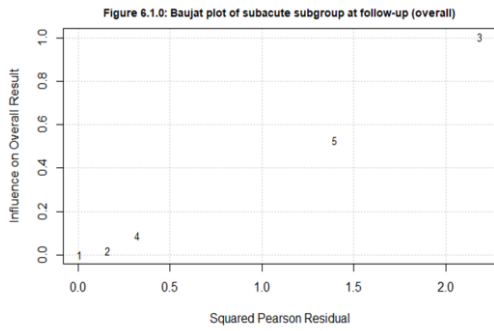The Baujat plot (Figure 4.1.0, 4.1.1, 4.1.2) and the Viechtbauer and Cheung (Viechtbauer & Cheung, 2010) influential test identified three studies as potential outliers: Tsai et al. (Tsai et al., 2014) (study 6) for the rTMS subgroup and Spielmann et al. (2018) and Fridriksson et al.(Fridriksson et al., 2018) (study 3 and 4) for the tDCS subgroup. The *publication bias* tests were not significant, and the funnel plots (Figure 4.2.0, 4.2.1, 4.2.2) can be considered symmetrical.

Figure 4.1.0: Baujat plot at follow-up only RCTs (overall)

Figure 4.1.1: Baujat plot of TMS RCTs at follow-up

Figure 4.1.2: Baujat plot of tDCS RCTs at follow-up

Figure 4.2.0: Trim and fill funnel plot at follow-up (overall RCTs)

Figure 4.2.1: Trim and Fill Funnel plot of TMS RCTs at follow-up

Figure 4.2.2: Trim and Fill Funnel plot of tDCS RCTs at follow-up
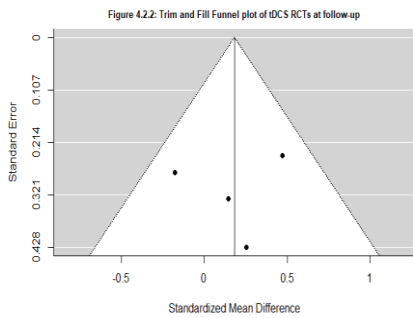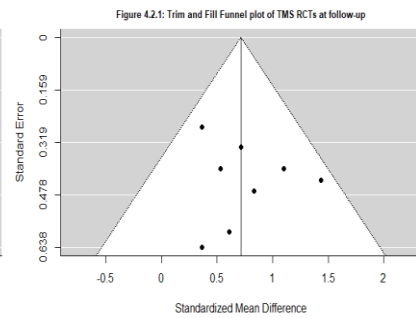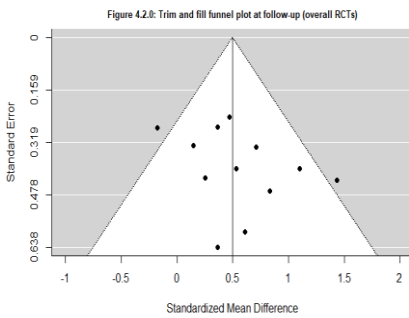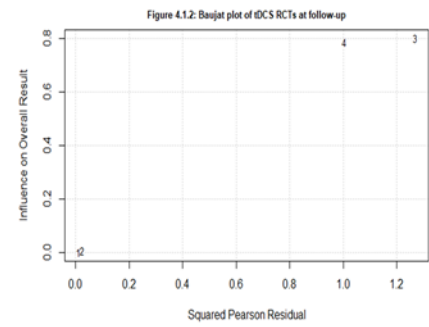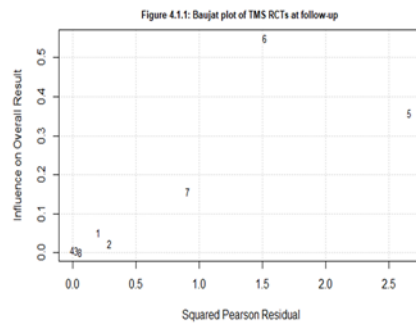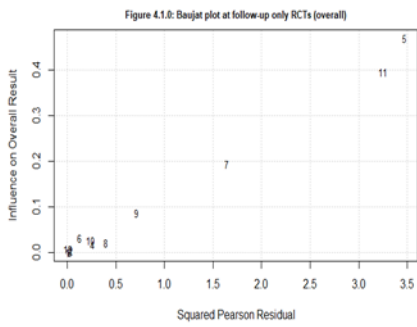
Chronic vs. Subacute: The stroke phase (subacute or chronic) can determine the brain state and the ongoing plastic changes, influencing the treatment effects. To investigate possible differences in NIBS efficacy at follow-up taking into consideration the time passed from the stroke event, studies including only chronic patients (*N*=15, tDCS=7 and rTMS=8) were analyzed separately from those with subacute aphasic participants (*N*=5, rTMS=3, tDCS=2) and the two techniques were compared for each subgroup. This choice was made based on the previous analysis (rTMS vs. tDCS at follow-up) showing that rTMS and tDCS produced very different results especially concerning the subacute population.

rTMS vs. tDCS in chronic aphasia*:* The outcomes revealed significant weighted mean effect sizes of 0.42 (95% CI: [0.11, 0.72], *p*=0.007, *N*=8) for the rTMS studies and 0.52 (95% CI: [0.21, 0.83], *p* = 0.001, *N*=7) for the tDCS studies, while the test of moderators (QM (1) = 0.20, *p* = 0.65) showed no significant difference between the two effect sizes (for details see Figure 5, and funnel plots figures 5.2.0, 5.2.1, 5.2.2).

Figure 5: Forest plot of chronic subgroup effect sizes at follow-up, broken down by NIBS (TMS vs. tDCS)

Study	Weights	ES	[95% CI]
Fridriksson et al., 2018	16.32%	0.47	[-0.00, 0.94]
Meinzer et al., 2016	5.82%	0.25	[-0.59, 1.09]
Ben Basat et al., 2016	3.72%	0.37	[-0.69, 1.43]
Shah-Basak et al., 2015	2.04%	0.41	[-1.04, 1.85]
Marangolo et al. 2013 b	5.73%	0.97	[0.12, 1.81]
Marangolo et al. 2013 a	3.00%	0.89	[-0.30, 2.07]
Fiori et al., 2013	3.73%	0.42	[-0.64, 1.48]
tDCS Subgroup (Q = 2.01, df = 6, p = 0.92; I^2 = 0.0%)		0.52	[0.21, 0.83]
Xue-yan Hu et al., 2018	4.95%	0.83	[-0.08, 1.74]
Harvey et al., 2017	4.80%	0.26	[-0.67, 1.19]
Hara et al., 2015	22.10%	0.08	[-0.31, 0.48]
Wang et al., 2014	6.65%	1.10	[0.32, 1.88]
Tsai et al., 2014	13.14%	0.36	[-0.17, 0.90]
Barwood et al., 2013	3.14%	0.61	[-0.55, 1.77]
Medina et al., 2012	2.71%	0.36	[-0.89, 1.61]
Naeser et al., 2005	2.14%	0.39	[-1.02, 1.80]
TMS Subgroup (Q = 6.61, df = 7, p = 0.47; I^2 = 20.6%)		0.42	[0.11, 0.73]
Overall Heterogeneity Test (Q = 9.20, df = 14, p = 0.82; I^2 = 6.5%)	100.00%	0.44	[0.23, 0.65]

Standardized Mean Difference

Taking a close look to the influential studies, the test revealed three potential outliers: Hara et al. (Hara et al., 2015) for the overall effect (weights 22.10%), Wang et al.(Wang et al., 2014) and Hara et al.(Hara et al., 2015) for the rTMS subgroup, and Fridriksson et al. (Fridriksson et al., 2018) for the tDCS subgroup (Baujat plot figures: 5.1.0, 5.1.1, 5.1.2).

Figure 5.1.0: Baujat plot of chronic subgroup at follow-up (overall)

Figure 5.1.1: Baujat plot of chronic subgroup at follow-up (TMS only)

Figure 5.1.2: Baujat plot of chronic subgroup at follow-up (tDCS only)

Figure 5.2.0: Publication bias assessed by funnel plot of chronic subgroup (overall)

Figure 5.2.1: Funnel plot of chronic subgroup at follow-up (TMS only)

Figure 5.2.2: Funnel plot of chronic subgroup at follow-up (tDCS only)

rTMS vs. tDCS in subacute aphasia: The number of studies investigating the effects of NIBS exclusively on subacute population was particularly small (rTMS=3 and tDCS=2), and consequently the analysis results were neither solid nor reliable but offer a general idea on the main effect differences between rTMS and tDCS applied in the first three months after stroke. The test of moderators yielded a significant difference between the two NIBS effect sizes (QM(1) = 7.8, *p*= 0.005), and the pooled analysis revealed a statistically large and significant SMD of 0.85 (95% CI = [0.30, 1.32]; *p* = 0.0004) for rTMS studies in subacute patients, while for tDCS studies, the SMD of -0.04 (95% CI = [−0.46, 0.36]; *p* = 0.82) was non-significant (Figures 6, 6.1.0., 6.1.1, 6.2.0, 6.2.1).

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Figure 6: Forest plot of subacute subgroup effect sizes at follow-up, broken down by NIBS (TMS vs. tDCS)

Study	Weights	ES [95% CI]
Spielmann et al., 2018	23.16%	-0.18 [-0.72, 0.36]
Polanowska et al., 2013	20.94%	0.14 [-0.50, 0.79]
tDCS Subgroup (Q = 0.56, df = 1, p = 0.45; I² = 0.0%)		-0.05 [-0.46, 0.37]
Khedr et al., 2014	16.96%	1.43 [0.58, 2.28]
Seniów et al., 2013	20.73%	0.71 [0.06, 1.37]
Waldowski et al., 2012	18.21%	0.53 [-0.25, 1.31]
TMS Subgroup (Q = 2.62, df = 2, p = 0.27; I² = 15.2%)		0.85 [0.38, 1.32]
Overall Heterogeneity Test (Q = 11.63, df = 4, p = 0.02; I² = 65.8%)	100.00%	0.48 [-0.05, 1.00]

Figure 6.1.0: Baujat plot of subacute subgroup at follow-up (overall)

Figure 6.1.1: Baujat plot of subacute subgroup at follow-up (TMS only)

Figure 6.2.0: Trim and fill funnel plot of subacute subgroup (overall)

Figure 6.2.1: Funnel plot of subacute subgroup at follow-up (TMS only)

Non-fluent aphasia vs. All types of aphasia: To assess possible variations in NIBS efficacy depending on type of aphasia, the publications that included only non-fluent aphasia participants (*N*=13) were compared to the ones that used no particular criteria regarding the aphasia characteristics (all types of aphasia, *N*=7). Unfortunately, we find no studies investigating the neurostimulation effects exclusively on fluent aphasia; thus, this comparison confronts results obtained when a specific aphasia type is selected (this may imply more similar lesion localization and more precise stimulation area) vs. no specific inclusion criteria for the aphasia subtype (i.e., a more heterogeneous population). The analysis was conducted pooling together tDCS and rTMS effect sizes in order to reach a larger number of studies in each subgroup. The meta-analysis revealed a large effect of NIBS for the non-fluent subgroup (SMD= 0.67, 95% CI = [0.41, 0.93]; *p* <.0001) and a small but still significant effect size for the all-types of aphasia subgroup (SMD= 0.24, 95% CI = [0.01, 0.47]; *p*=0.0392). The data for the all-types of aphasia subgroup should be carefully interpreted due to the small number of publications included and the fact that four out of seven studies were run on a subacute population (2 rTMS and 2 tDCS studies) and, as previously observed, tDCS effect on subacute population is near zero. As expected, the test of moderators yielded a significant difference between the two subgroups (QM(1) = 5.73, *p*= 0.0017). For details on Boujat plots and Funnel plots, check Figures 7, 7.1.1, 7.1.2, 7.2.1, 7.2.2.

Figure 7: Forest plot of effect sizes at follow-up, broken down by types of aphasias (non-fluent vs. all types)

Author(s) and Year	NIBS	Weights	ES [95% CI]
Ben Basat et al., 2016	tDCS	3.02%	0.37 [-0.69, 1.43]
Shah-Basak et al., 2015	tDCS	1.73%	0.41 [-1.04, 1.85]
Marangolo et al. 2013 b	tDCS	4.42%	0.97 [0.12, 1.81]
Marangolo et al. 2013 a	tDCS	2.48%	0.89 [-0.30, 2.07]
Fiori et al., 2013	tDCS	3.03%	0.42 [-0.64, 1.48]
Xue-yan Hu et al., 2018	TMS	3.89%	0.83 [-0.08, 1.74]
Harvey et al., 2017	TMS	3.79%	0.26 [-0.67, 1.19]
Wang et al., 2014	TMS	5.01%	1.10 [0.32, 1.88]
Tsai et al., 2014	TMS	8.53%	0.36 [-0.17, 0.90]
Khedr et al., 2014	TMS	4.38%	1.43 [0.58, 2.28]
Barwood et al., 2013	TMS	2.59%	0.61 [-0.55, 1.77]
Medina et al., 2012	TMS	2.26%	0.36 [-0.89, 1.61]
Naeser et al., 2005	TMS	1.81%	0.39 [-1.02, 1.80]
Non-fluent Subgroup (Q = 8.03, df = 12, p = 0.78; I² = 0.0%)			0.67 [0.41, 0.93]
Fridriksson et al., 2018	tDCS	9.92%	0.47 [-0.00, 0.94]
Spielmann et al., 2018	tDCS	8.45%	-0.18 [-0.72, 0.36]
Meinzer et al., 2016	tDCS	4.47%	0.25 [-0.59, 1.09]
Polanowska et al., 2013	tDCS	6.66%	0.14 [-0.50, 0.79]
Hara et al., 2015	TMS	12.05%	0.08 [-0.31, 0.48]
Seniów et al., 2013	TMS	6.51%	0.71 [0.06, 1.37]
Waldowski et al., 2012	TMS	5.00%	0.53 [-0.25, 1.31]
All types aphasia Subgroup (Q = 6.44, df = 6, p = 0.38; I² = 13.6%)			0.25 [0.01, 0.48]
Overall Heterogeneity Test (Q = 20.90, df = 19, p = 0.34; I² = 23.6%)		100.00%	0.45 [0.26, 0.65]

Standardized Mean Difference

Figure 7.1.1: Baujat plot of non-fluent subgroup at follow-up
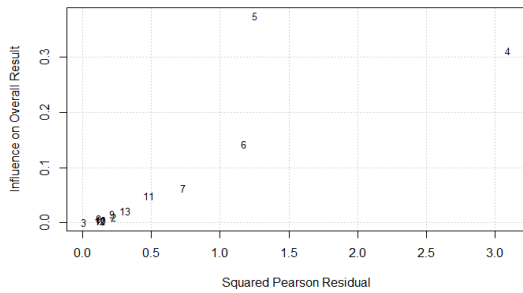
Figure 7.1.2: Baujat plot of all types of aphasias subgroup at follow-up
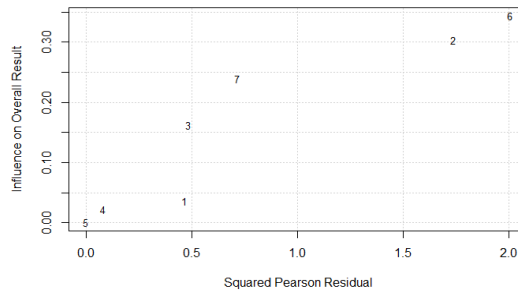
Figure 7.2.1: Funnel plot of non-fluent subgroup at follow-up
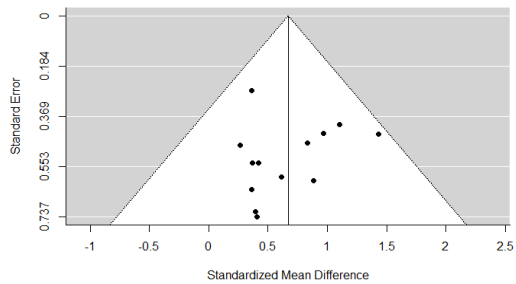
Figure 7.2.2: Funnel plot of all types of aphasias subgroup at follow-up

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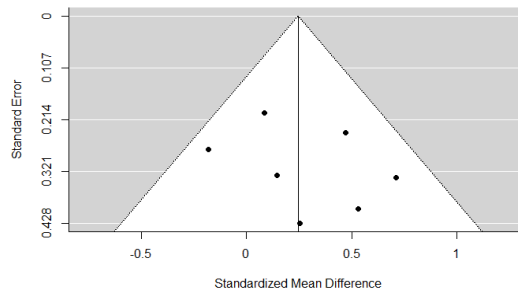
GRADE assessment

Overall evidence was qualified using GRADE (for RCTs, crossover and open-label studies). Low quality of evidence (i.e., the true effect might be markedly different from the estimated effect) shows that people with post-stroke aphasia may have a small to medium long-term benefit from tDCS treatment when compared to the control group, while a medium quality of evidence (i.e., further research is likely to have an important impact on our confidence in the estimated effect and may change the effect size) indicates that aphasic patients might have a medium to large benefit from rTMS treatment. Overall, a moderate level of evidence suggests that the noninvasive brain stimulation treatment (rTMS and tDCS) produces better results (a moderate effect) for the chronic patients with non-fluent aphasia when compared with the control group.

Given the fact that the online GRADEpro software offers only two options for the study designs, namely RCTs or observational studies, we always chose RCTs because the majority of the included studies were randomized designs. The level of evidence for RCTs was downgraded due to the lack of blinding in the open label studies and the carryover effect in the crossover designs and also for the lack of precision due to incongruent effect size direction and the large confidence intervals. The GRADE data are in Table 9.

Table 9. GRADEpro summary

Question: Long-term efficacy of transcranial brain stimulation (TMS & tDCS) vs. Control in post-stroke aphasia treatment.

Certainty assessment						№ of patients		Effect	Certainty
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Transcranial brain stimulation	Control	Absolute (95% CI)	
Long-term efficacy of **tDCS vs. Control** (follow up: range 4 weeks to 24 weeks; Scale from: -1.5 to 2.5)									
9	randomised trials	serious [a]	serious [b]	not serious	not serious	126	103	SMD **0.33** SD **higher** (0.05 higher to 0.6 higher)	⊕⊕◯◯ LOW
Long-term efficacy of **TMS vs. Control** (follow up: range 8 weeks to 15 weeks; Scale from: -1.5 to 2.5)									
11	randomised trials	serious [a]	not serious	not serious	not serious	181	99	SMD **0.56** SD **higher** (0.29 higher to 0.84 higher)	⊕⊕⊕◯ MODERATE
Long-term efficacy of **tDCS** vs. Sham **(RCT only)** (follow up: range 4 weeks to 12 weeks; Scale from: -1.5 to 2.5)									
4	randomised trials	not serious	serious [b]	not serious	serious [c]	119	132	SMD **0.18** SD **higher** (0.15 lower to 0.51 higher)	⊕⊕◯◯ LOW
Long-term efficacy of **TMS** vs. Sham **(RCT only)** (follow up: range 8 weeks to 15 weeks; Scale from: -1.5 to 2.5)									
8	randomised trials	not serious	not serious	not serious	not serious	118	99	SMD **0.71** SD **higher** (0.43 higher to 0.996 higher)	⊕⊕⊕⊕ HIGH
Long-term efficacy of NIBS vs. Control in patients **with chronic aphasia**. (follow up: range 4 weeks to 24 weeks; Scale from: -1.5 to 2.5)									
15	randomised trials	serious [a]	not serious	not serious	not serious	215	209	SMD **0.44** SD **higher** (0.23 higher to 0.65 higher)	⊕⊕⊕◯ MODERATE
Long-term efficacy of NIBS vs. Control in patients with **subacute aphasia**. (follow up: range 4 weeks to 15 weeks; Scale from: -1.5 to 2.5)									
5	randomised trials	not serious	serious [b]	not serious	serious [c]	92	92	SMD **0.47** SD **higher** (0.04 lower to 0.99 higher)	⊕⊕◯◯ LOW
Long-term efficacy of NIBS vs. Control in patients with **non-fluent aphasia**. (follow up: range 4 weeks to 15 weeks; Scale from: -1.5 to 2.5)									
13	randomised trials	serious [a]	not serious	not serious	not serious	118	96	SMD **0.67** SD **higher** (0.41 higher to 0.92 higher)	⊕⊕⊕◯ MODERATE
Long-term efficacy of NIBS vs. Control in patients with **all types of aphasia**. (follow up: range 4 weeks to 6 months; Scale from: -1.5 to 2.5)									
7	randomised trials	serious [a]	not serious	not serious	not serious	170	154	SMD **0.24** SD **higher** (0.01 higher to 0.47 higher)	⊕⊕⊕◯ MODERATE

CI: Confidence interval; **SMD:** Standardized mean difference (Hedges' g)

GRADE Working Group grades of evidence:

High quality: Further research is very unlikely to change our confidence in the estimate of effect.

Moderate quality: The authors believe that the true effect is probably close to the estimated effect

Low quality: Further research is very likely to have an important impact on our confidence in the estimated effect and is possible to change the estimate.

Very low quality: We are very uncertain about the estimate.

Downgraded Explanations

[a.] Downgraded as there were serious limitations identified in the risk of bias, since not all the studies were RCTs, same were crossover or open-label studies

[b.] Point estimates vary widely and the direction of effect was not consistent

[c.] A very small number of included studies

3.2.5. Discussion

SLT represents the standard of care for PWA, but the benefits of these treatments are variable. Moreover, due to financial and/or logistic limitations, the amount of behavioral therapy available to patients is often lower than the level of SLT (frequency, intensity, or duration) that would provide significant and lasting benefit. In an attempt to potentiate the therapy-induced clinical benefits and/or to reduce the rehabilitation time, TMS and tDCS have emerged as promising tools to enhance language abilities for PWA following stroke.

The main objective of this review was to examine the long-term efficacy and reliability of NIBS (tDCS, rTMS) as an intervention for post-stroke aphasia. In other words, we tried to assess to what extent the therapeutic promises are confirmed, and rTMS and/or tDCS (either as an add-on therapy to SLT or as monotherapy) can be considered effective treatment approaches.

The overall analysis, including 20 independent follow-up effect sizes, revealed a medium and significant effect of NIBS (rTMS and tDCS studies pooled together) compared to the control condition (sham or pre-treatment). These data are highly relevant for the rehabilitation research field, suggesting that rTMS and tDCS stimulation may improve aphasia recovery and produce durable results. The effect size hereby found is comparable to those reported by other recent meta-analyses investigating the same topic despite the fact that their analyses were based on data recorded immediately after the treatment ended (Otal et al., 2015; Shah-Basak et al., 2016). Although the magnitude of the treatment effect size was larger in the rTMS than in the tDCS studies, this difference was not statistically significant. Nevertheless, when balancing these results by the GRADE level of evidence it seems that: i) while rTMS coupled with SLT can be considered effective (a medium effect size at follow-up and a moderate level of evidence), ii) there are not enough consistent data to make a strong recommendation regarding the effectiveness of tDCS coupled with SLT in improving post-stroke aphasia (a small to medium effect size at follow up and a low level of evidence, as qualified with GRADE). This means that in some conditions (e.g., anodal stimulation in the subacute population), the tDCS therapy could be ineffective (Spielmann et al., 2018).

Critically, comparing the data immediately after treatment and at follow-up, we found no significant differences between the two time-points. Therefore, we can provide compelling evidence that the treatment effects are maintained in time, i.e., the changes observed at the end of the stimulation intervention tend to remain stable (they insignificantly decrease or do not improve) when measured again after a period extending from one to six months post-treatment.

Considering only the RCT studies in order to exclude eventual confounding variables, as the carryover effects, we observed that the overall (rTMS and tDCS) effect size remained medium and statistically significant, but the sub-analysis indicated an advantage of rTMS over tDCS. More precisely, the rTMS RCTs effect size was large and statistically significant while the tDCS RCTs effect size was small and not statistically significant. This result should be interpreted with caution since the number of selected studies is extremely small. From the four tDCS RCTs, two were conducted on subacute aphasics (Polanowska et al., 2013; Spielmann et al., 2018), and as previously observed, the tDCS effect seems to be sensitive to chronicity. Furthermore, none of the tDCS RCTs, on the chronic population, stimulated the perilesional Broca's area; specifically, Meinzer et al. (Marcus Meinzer et al., 2016) applied anodal tDCS over the left primary motor cortex, while Fridriksson at al. (Fridriksson et al., 2018) choose to stimulate the left temporal lobe, specifically the region with the highest naming related activation during fMRI.

The sub-analysis by stroke chronicity confirmed previous observations (based on data recorded immediately after treatment) (Shah-Basak et al., 2016), namely, that also at follow-up, rTMS was effective in both chronic and subacute aphasic patients while tDCS was effective only in the chronic phase. It has been speculated that an effect of tDCS might be difficult to achieve during the subacute phase, due to spontaneous recovery that could mask the effects of the rehabilitation interventions (Polanowska et al., 2013; Spielmann et al., 2018). Another explanation might be the relation between the stimulation parameters (inhibitory vs. excitatory, ipsilateral vs. contralateral) and the dynamic mechanisms of post-stroke recovery (repair of damaged networks, activation of compensatory areas in the right hemisphere, or activation of previously functionally inactive pathways) and how well each one

supports the other in order to enhance recovery. For example, Polanowska et al. (2013) and Spielmann et al.(2018), using anodal stimulation over the left Broca's area, obtained a null effect, while You et al. (You, Kim, Chun, Jung, & Park, 2011) showed that auditory verbal comprehension improved significantly in subacute patients treated with cathodal tDCS over the right superior temporal gyrus as compared to patients in the other groups (anodal tDCS applied to the left superior temporal gyrus and sham). In 2006, Saur et al. reported that brain reorganization during language recovery might proceed in phases. The right hemisphere could have a supporting and adaptive role in the acute and subacute phases but be maladaptive at a chronic stage since it would prevent the perilesional spared tissue from activating and contributing to a major recovery (Saur & Hartwigsen, 2012). Considering this observation, and knowing that tDCS has a modulation effect, i.e., it only modulates those neurons that are potentially engaged in the execution of a given task (Fertonani & Miniussi, 2017), it might be preferable to activate the perilesional regions during the chronic phase (eventually by using a bi-hemispheric stimulation: cathode over the right and anode over the left language areas). However, the choice of the stimulation type (inhibitory or excitatory) and the stimulation area (right or left hemisphere) depends also on the adopted recovery model: (i) the "vicariation model" - right areas help recovery, (ii) the "interhemispheric competition model" - the right hemisphere excessively inhibits the left one, or (iii) the "bimodal balance-recovery model" - the quantity of spared neuronal structures determines the network reorganization (Di Pino et al., 2014).

Regarding the aphasia type, the results indicate that the NIBS effect is stronger, especially in the case of tDCS, when applied to non-fluent aphasic patients compared to participants with different aphasia types receiving the same stimulation protocol. The smaller effect size might be due to the heterogeneity among patients (different aphasia types often imply different lesion sites)(Raymer & Gonzalez Rothi, 2017a), so future research should try to include a more homogeneous population in order to obtain more powerful results (Fridriksson et al., 2018; Meinzer et al., 2016; Seniów et al., 2013; Waldowski et al., 2012)

There are, however, several limitations in the present meta-analysis such as, for instance, the low number of studies (especially because many papers did not include a follow-up period), the risk of bias (present in the open-label and crossover designs), and the selection biases. The between-study heterogeneity and the risk of publication bias were low. Nevertheless, even if the funnel plot and the specific asymmetry tests did not indicate a significant publication bias, we restricted our search strategy to articles published in English, potentially excluding high-quality research data that were published in other languages or belonging to the "gray literature". Crucially, all of the comments and recommendations were based on publications reporting follow-up results; this means that other relevant information about efficacy, stimulation protocol, and methodology might have been missed (for reviews see Crinion, 2016; de Aguiar et al., 2015; Kapoor, 2017; Otal et al., 2015; Ren et al., 2014; Sebastianelli et al., 2017; Shah-Basak et al., 2016). However, we believe that the relevant outcome is a permanent improvement, not just a limited one.

Summing up, our findings add new data to the existing literature by showing that, with the current stimulation procedures, the rTMS long-term effect size is moderate but more reliable independently of the patients' characteristics, while tDCS appears moderately effective for the chronic population and ineffective for the subacute one. Importantly, the studies' quality was lower in the tDCS subgroup (Table 9, GRADEpro evaluation). Many studies used crossover designs with a very small period between the sessions; therefore, the final result (especially at follow-up) was probably a mix between the different experimental conditions. Another important problem observed in the tDCS studies was the lack of details regarding lesion size, and given the dimension of the tDCS electrodes (often 5x7 cm), it is not always obvious that all patients actually had structurally intact cortex underneath the active electrode. Based on the current data, we can speculate that tDCS and rTMS are more effective in boosting the recovery process not as a monotherapy but, rather, as a complementary instrument coupled with SLT (only five included studies used rTMS and tDCS alone).

In conclusion, each technique has advantages and disadvantages: rTMS produces a medium to large effect but is also more expensive and carries a higher safety risk, while tDCS effect size ranges from

small to medium, but is user-friendly and could be applied at home with a relatively small cost. For these reasons, further evaluation of the utility of these methods for aphasia rehabilitation should combine efficacy and feasibility data, making a cost-benefit analysis. Still, the important future challenge will be to collect clear evidence of the long-term efficacy in the everyday life of these methods.

Chapter 4: Neurophysiological Correlates of Language Recovery after tDCS

4.1. Introduction

As largely covered in the first and third chapters, post-stroke aphasia is an acquired language disorder, occurring in approximately 30%-40% of all stroke cases often leading to chronic disability, because of a permanent injury to the language networks of the brain (Flowers et al., 2016; Kuriakose & Xiao, 2020; Pedersen et al., 2004). Language is central for human communication, and its impairment has dramatic consequences for the patients and their caregivers at many levels: emotional, social, economic, etc. (Flowers et al., 2016; Gerstenecker & Lazar, 2019).

Post-stroke language impairments are considered as the consequence of direct ischemic death of neurons combined with maladaptive connectivity patterns among surviving neurons. (Hartwigsen & Saur, 2019; Sato et al., 2015; Taub et al., 1994). Stroke triggers changes in neuronal excitability concomitant with structural and functional reorganization; post-stroke recovery is enabled at different scales ranging from single cells to whole-brain networks (Sato et al., 2015).

Animal models and human research suggest that during recovery, besides the initial poststroke cascade of cellular and biochemical processes triggering phenomena like ischemic penumbra resolution and axons sprouting, there are also widespread changes in cortical network activity patterns remote to the lesion that extend into the contralesional hemisphere, and which contribute to function restoration (Saur, 2006; Stockert et al., 2020). The cortical long-term reorganization and plastic changes after stroke are (probably) meant to optimize the impaired function; however, the lesion site and extent constraint to a certain degree which kind of recovery processes can be engaged and can influence whether compensatory recruitment of alternate areas, or activation of regions that may hinder recovery, occur (Fridriksson & Smith, 2015; Turkeltaub et al., 2011).

Many different methods have been used to investigate changes in functional activation associated with aphasia treatment, including functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG), and positron emission tomography (PET). Two main patterns of functional brain activation have

been indicated to support post-stroke aphasia recovery. One is based on the functional reactivation of the preserved language areas, including the cortex immediately adjacent to the lesion (Hartwigsen & Saur, 2019; Stockert et al., 2020). A second mechanism involves functional reorganization, i.e., the activation of new areas, either residual left hemisphere structures or right hemisphere homologs (Osa García et al., 2020; Stockert et al., 2020). Several studies have provided evidence indicating better outcomes when left hemisphere perilesional areas are recruited and less favorable outcomes when right hemisphere regions are engaged (Saur, 2006). Nevertheless, there are also studies reporting positive outcomes associated with right hemisphere modulation (Fridriksson & Smith, 2015). Summing up, neuroimaging studies suggest that language recovery (in post-stroke aphasia) takes place in domain-specific areas of a pre-existing language network (often bilateral) with support from the dorsolateral prefrontal cortex (Hartwigsen & Saur, 2019).

Although it has been shown that SLT improves language performance (Raymer & Gonzalez Rothi, 2017), new methods, such as tDCS (combined with language therapy), are being evaluated in an attempt to achieve better and faster results. The main aim is to enhance the potential for training-induced plasticity, either by facilitating activity in language-relevant regions or by suppressing dysfunctional neural processes. For instance, post-stroke tDCS neuromodulation can have a role in rebalancing the activity of both hemispheres by either increasing the output of the perilesional left hemisphere (excitatory stimulation) or by suppressing the activity from the contralateral hemisphere (inhibitory tDCS over the contralesional cortex). Consequently, most of the previous studies focused on behavioral improvements after either anodal or cathodal tDCS over the contralesional or ipsilesional areas (Fiori et al., 2013; Kang et al., 2011; Pestalozzi et al., 2018; Veniero et al., 2019).

Based on the previously described meta-analysis (Chapter 3) it can be summarized that tDCS stimulation protocols often include the following parameters: two equal sized electrodes (5 cm×5 cm or 5 cm×7cm), with the anode frequently placed over the left, anterior language areas and the cathode over the right supraorbital region, at an intensity between 1 mA or 2 mA, and a stimulus duration ranging from 10 to 20 minutes (Bucur & Papagno, 2019; Elsner et al., 2020). Due to the data

heterogeneity, clear indications regarding the most promising stimulation sites and stimulation parameters to facilitate language recovery are difficult to make (Hill et al., 2016).

While the results seem generally promising, the neurobiological mechanisms of the reported beneficial effects remain largely unclear. Currently, much of the available information about the effects of tDCS on cognition and behavior has been obtained within the context of a limited understanding of the neural basis for tDCS effects on brain circuitry. It is known that tDCS induces electric fields in a polarized way (current flows from the anode to the cathode), and that, theoretically, part of the electric field reaches the cortical areas producing polarity-dependent effects (Pestalozzi et al., 2018; Spielmann et al., 2018; Zettin et al., 2021). At a neuronal level, tDCS modulates cortical excitability by shifting the resting membrane potential in a polarity-dependent way: anodal stimulation increases the spontaneous firing rate by slightly inducing a depolarization of the resting membrane potential whereas cathodal stimulation decreases cortical excitability, shifting the resting membrane potential toward hyperpolarization (Liebetanz et al., 2002; Monte-Silva et al., 2013; Nitsche & Paulus, 2000, 2001).

Studies on the motor cortex indicate that the neurophysiological effects induced online by anodal tDCS rely on the subthreshold depolarization of the neuron membrane in the primary motor cortex, mediated by NA^+ voltage-dependent ion channels activation (Liebetanz et al., 2002; Nitsche et al., 2003). Furthermore, in vitro research showed that this modulation of neuron excitability can increase spontaneous cortical activity (Bindman et al., 1964). Concerning the tDCS offline effects, it has been found to be mediated by glutamate N-methyl-d-aspartate receptor activation, which results in a greater CA^{2+} postsynaptic concentration, triggering cortical plasticity (Lang et al., 2005; Nitsche & Paulus, 2000; Stagg & Nitsche, 2011). Additionally, sustained direct current has been shown to produce acute and lasting changes in oscillations in brain slices probably through LTP mechanisms (Reato et al., 2015; Stagg et al., 2018).

Nevertheless, tDCS influences the nervous system at multiple levels and possibly at multiple time points. Consequently, the observed effects do not follow a simple linear relation, i.e., anodal and cathodal stimulation are not synonymous with excitatory and inhibitory stimulation with respect to

their effects on neural function and behavior (Giordano et al., 2017). Research data suggest that tDCS can modulate neural function in the short term through bottom-up effects of neuronal and synaptic activity, as well as by top-down effects of neuronal network dynamics (Fröhlich & McCormick, 2010; Lang et al., 2005). Since such changes in the patterns of neural activity can be self-reinforcing, adaptive processes following an additional disruption to homeostasis are likely to be relevant to both the immediate and short-term responses as well as the long-term responses to tDCS (Giordano et al., 2017). To date, measures of the clinical effects of tDCS are limited, i.e., although there is some information from electrophysiological, imaging, and pharmacologic studies, as already specified, most data describing the effects of the intervention are behavioral.

As detailed in Chapter 2, recording TMS evoked brain responses by EEG is increasingly gaining consideration as a powerful tool to simultaneously assess different neuro- physiological properties of the human cortex and/or of a specific cortical region.

From a clinical perspective, TMS-EEG data could help to characterize the functional abnormalities of certain brain areas in a specific pathology, analyze the evolution in time, and also monitor treatment effectiveness. Therefore, TMS-EEG is seen as a potential tool for differential diagnosis, outcome prediction, and progression monitoring of a pharmacological or neuromodulation (NIBS) treatment. To this aim, TMS–EEG has been recently tested in a wide range of psychiatric (Cheng et al., 2022) and neurological disorders (Tremblay et al., 2019), including stroke (Ding et al., 2022; Tscherpel et al., 2020); epilepsy, autism, dementia, schizophrenia (Frantseva et al., 2014); depression, addiction, autism, dementia (Casarotto et al., 2011); stroke, and disorders of consciousness (Casali et al., 2013; Casarotto et al., 2016; Massimini et al., 2005; Pigorini et al., 2015).

Focusing on stroke and recovery, different publications agree on the conclusion that TMS-evoked EEG responses are abnormal in individuals with acute and chronic stroke (Casula et al., 2021; Pellicciari et al., 2018; Rolle et al., 2021; Sarasso et al., 2020; Tscherpel et al., 2020). For example, TMS-induced silent periods were associated with hand dysfunction (Gray et al., 2017), while patients with a better recovery of the affected hand strength were the ones with a more stable interhemispheric balance (Casula et al.,

2021). Tscherpel et al. (2020) found that TMS-evoked EEG responses recorded over ipsilesional M1 in the early post-stroke phase (6.7± 2.5 days) featured two markedly different response morphologies depending on the impairment severity. Specifically, TMS evoked a slow and simplified local response in severely affected patients, while in less impaired participants, TMS elicited a differentiated and sustained EEG response with a series of deflections sequentially involving both hemispheres, resembling the patterns of bilateral activation as observed in the healthy comparison group.

Experimental paradigms incorporating TMS-EEG and tDCS allow for further exploration of the brain-behavior interrelationship through the evaluation of TEP amplitude changes, which are likely to reflect the level of excitation/inhibition balance within the cortex and TMS-evoked oscillations. This data can provide important information regarding the natural oscillatory frequencies produced by different thalamocortical circuits (Romero Lauro et al., 2014; Rosanova et al., 2009). Furthermore, the ability to record TEPs over the entire scalp allows at investigating connectivity changes across multiple structurally and functionally connected brain regions, providing important information about the effects of tDCS on distributed cortical networks (Pisoni et al., 2018; Romero Lauro et al., 2014). Additionally, it has been shown that EEG oscillations recorded immediately following a TMS pulse also provide an indication of the natural oscillatory frequency (Rosanova et al., 2009), while the Lempel-Ziv Perturbational Complexity Index (PCI^{LZ}) (Casali et al., 2013; Comolatti et al., 2019) and the PCIST [based on dimensionality reduction and state transitions (ST) quantification] (Comolatti et al., 2019) were recently introduced to assess the capacity of thalamocortical circuits to engage in complex patterns of causal interactions. The authors maintained that PCIST could represent a fast clinical aid for the bedside assessment of consciousness as well as serve as a general measure to explore the neuronal mechanisms of loss/recovery of brain complexity across scales and models (Comolatti et al., 2019). As such, analysis of the natural frequencies and PCIST in addition to TEP amplitude could offer relevant information regarding tDCS-induced changes in post-stroke aphasia. Furthermore, considering that the current understanding of tDCS-induced current flow in the brain is based largely on evidence from computational models, the brain reactivity profiles captured by TMS-EEG could provide important

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additional physiological evidence of the excitability changes produced throughout the cortex in response to different tDCS stimulation parameters (Hill et al., 2016). In fact, there are previous studies supporting the EEG data sensitivity to post-stroke network changes and interactions relevant to plasticity and repair. For example, Nicolo et al. (2015), calculating a global index of functional connectivity between critical brain areas and the rest of the brain (based on high-density EEG data of subacute aphasia patients), found that post-stroke language and motor improvement was associated with synchronization of spontaneous neural oscillations between brain areas.

Presently, the number of experiments using TMS-EEG co-registration, during or immediately after tDCS, to gather evidence concerning tDCS-induced regional and global cerebral activations or deactivations effects is fairly limited. One of the first publications on this topic showed that anodal tDCS over the posterior parietal cortex increased cortical excitability as indicated by global and local mean-field power (Global Mean Field Power, GMFP and Local Mean Field Power, LMFP) (Romero Lauro et al., 2014).

Another publication, which inspired this experiment, showed, by means of TMS-EEG, that there is a direct correlation between verbal fluency performance, modulated by anodal tDCS (recorded in healthy, young participants), and increased cortical excitability in relevant cortical sites (Pisoni, et al., 2018). Namely, TMS-EEG recordings were conducted before and after anodal tDCS coupled with semantic and phonemic verbal fluency tasks. The researchers assessed three different conditions: anodal or sham tDCS over the left premotor cortex and anodal tDCS over the left posterior parietal cortex. Modulation of cortical excitability was observed only after anodal tDCS and only in left Brodmann's areas 6, 44, and 45, i.e., key regions of the language production network.

Cipollari et al. (2015) used TMS–EEG to assess the effects of right anodal vs. sham tDCS, coupled with concurrent melodic intonation therapy (MIT), on cortical reactivity. Both MIT (plus sham tDCS) and anodal tDCS plus MIT increased the TEPs amplitude (peaks were registered at approximately 87 ms.) Furthermore, anodal tDCS combined with MIT led to an increase of TEPs (peaks were registered around 118 ms). Behavioral data showed that the language treatment combined with both sham and anodal tDCS led to functional improvements but were significantly greater following anodal tDCS. Their findings

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suggest that the underlying mechanisms of functional improvements can be related to changes in cortical reactivity close to the stimulated region and that anodal tDCS can increase the effect of SLT.

Summing up, in the first part of this introduction, we argued that post-stroke language network reorganization can be both adaptive and maladaptive, and better recovery is expected when left areas are reactivated. Subsequently, we showed that tDCS is often used in post-stroke aphasia rehabilitation in order to promote plastic changes that could boost the recovery of the impaired linguistic function, the most applied protocol being anodal tDCS over anterior language areas. Furthermore, TMS- EEG extends the methodological repertoire in stroke research by allowing: (i) the assessment of individual response profiles to NIBS stimulation, (ii) the measurement of cortical excitability during and after tDCS brain stimulation (or other therapies), and (iii) to directly link behavioral data to plastic changes observed in task-related areas or functional networks. Recent studies have proven that tDCS modulates the language network (in heathy and aphasia patients), and these changes in cortical excitability are correlated with performance enhancement in linguistic tasks (Pisoni et al., 2018) and aphasia recovery (Cipollari et al., 2015).

Starting from this scenario, the main question is: which brain parameters must be modulated by tDCS, and in which direction, to promote post-stroke aphasia recovery? Cipollari et al. (2015) found that stimulation of the contralesional areas increases the TMS-EEG response in specific ROI (F8, right hemisphere). Nevertheless, the stimulation protocol used in their research is an infrequent one (specifically designed to support MIT therapy), with the focus on only the healthy hemispheres and only concerning local changes (underneath the stimulation electrode). Unfortunately, in Cipollari et al. (2015) no correlation analysis was performed to directly link the electrophysiological and behavioral data.

Therefore, we propose to use TMS-EEG in order to investigate the specific effects of anodal tDCS applied over the perilesional anterior areas (as closest to Broca's area as possible given the lesion extension) on brain connectivity and cortical excitability in chronic non-fluent aphasic patients and its potential link to aphasia recovery. Different TMS-EEG parameters (GMFP, LMFP, Natural frequency,

PCIST, and PCI^{LZ}) and behavior data (a complete neuropsychological assessment of the linguistic functions) will be concomitantly used in order to provide a detailed picture of tDCS long-term effects. Furthermore, we will have the opportunity to explore the previously published observation on interhemispheric imbalance and abnormal TME-EEG responses after stroke, both local and global, but in this case on chronic PWA. To this aim, a group of PWA will undergo tDCS coupled with linguistic exercises in a crossover experiment (anodal and sham stimulation). TMS-EEG data from both hemispheres will be compared between the different experimental conditions and with data recorded from a healthy, matched control group.

The hypothesis was that TMS-evoked EEG responses will differ between the ipsilesional and contralesional hemisphere and between healthy participants and PWA in the time and time-frequency domain. We furthermore assumed a close link between tDCS induced plastic changes and behavior data. Specifically, we hypothesized that:

(1) GMFP, LMFP, PCIST, and natural frequency, but not PCI^{LZ}, will reflect a disequilibrium between the two hemispheres in PWA as compared with a matched control group;

(2) tDCS will modulate these parameters to approach the values observed in the control group (pre vs post real and sham tDCS);

(3) real tDCS (as compared to sham) will improve the linguistic production performance as suggested by the literature (Bucur & Papagno, 2019; Shah-Basak et al., 2016);

(4) the neurophysiological changes would have been correlated with behavioral data; but due to the small number of participants, in this thesis, only descriptive data will be provided.

The final aim of this project is to better understand how the functioning of areas involved in language production in chronic PWA changes after tDCS. Obtained information can enhance our understanding of the plastic mechanisms underlying language recovery after stroke. Knowledge on how recovery mechanisms act can provide increased flexibility and control over future tDCS protocols for aphasia rehabilitation.

4.2. Materials and methods:

A feasibility, crossover, repeated-measure study was conducted. This study was approved by the local ethical committee of the University of Trento (protocol number 2017-028). The study was run in accordance with the Declaration of Helsinki.

Written informed consent was obtained from all participants.

4.2.1. Participants

Due to the COVID – 19 pandemic, only *four left hemisphere post-stroke PWA* were included in the study (see Table 1). Selection criteria were: i) presence of chronic (more than 6 months) single vascular left hemisphere lesion; ii) age between 50 and 80 years; iii) at least 5 years of formal education; iv) non-fluent aphasia; v) relatively preserved comprehension (in order to sign the informed consent and to follow the instructions); vi) absence of evident sensory deficits (deafness, cataracts, etc.) or cognitive deficits (dementia, dysexecutive syndrome) that could affect the performance independently from language deficits; absence of psychiatric diseases; vii) absence of contraindications to the TMS and tDCS according to safety guidelines (Antal et al., 2017; Rossi et al., 2021); (viii) Italian native-speakers.

The presence of a single stroke was confirmed by brain neuroimaging, clinical history, and physical examination. A certified speech-language pathologist or neuropsychologist confirmed the diagnosis of non-fluent aphasia in all patients through an extensive language examination.

One participant (P 3) was excluded from the TMS-EEG analysis since it was not possible to register adequate EEG data due to the patient continuously falling asleep during the TMS-EEG recordings.

TABLE 1 | SUMMARY OF THE PWA

subject no.	age/ gender	education (years)	handedness	stroke etiology	lesion side/location	Lesion approximated* volume cm³	post-stroke (months)	aphasia	medication	comprehension Token Test	other problematic areas
P 1	62 / M	11	R (corrected)	MCA ischemia with thrombolysis	L/ frontotemporal, internal capsule	35.88	59	non-fluent	None relevant	11/36	- verbal short-term memory - ideomotor apraxia
P 2	59 / F	8	R	MCA ischemia	L/ frontotemporal, insular cortex	12.67	49	non-fluent anomia	None relevant	30/36	- verbal short-term memory
P 3	67 / M	18	R	MCA ischemia	L/ frontotemporal	33.82	61	non-fluent	mirtazapina (antidepressant) 1cp / h.20	7/36	- executive functions - attention - apraxia
P 4	69 / M	13	R	MCA ischemia	L/ frontotemporal, insular, parietal cortex	168	132	global aphasia	citalopram (antidepressant) 1cp / h.8	N.S.	- buccofacial apraxia - ideomotor apraxia - executive functions - attention

M = male, F = female; L = left hemisphere; R = right, MCA = middle cerebral artery; h. =hour; N.S. = not specified; * given the fact that the MRI and TC scans were from previous years, we can only approximate the lesion volume, since anatomical changes might have occurred from the last scan

Control group: Fourteen healthy participants, matching the PWA group in terms of age and education, took part in the study (being submitted to the TMS–EEG coregistration) after being screened to exclude any contraindication to the use of TMS (Antal et al., 2017; Rossi et al., 2009, 2021). Due to personal problems, one participant had to leave during the TMS-EEG recording. This resulted in thirteen participants being included in the control group (9 males; mean age 68 years, standard deviation [SD] 5.2, range 58–74; mean years of formal education 12, SD 4.8). All participants were right-handed (as assessed by the Edinburgh Handedness Inventory: mean 86, DS 14, range 67 -100; Oldfield, 1971).

4.2.2. Experimental procedures

We applied a double-blind (PWA and neuropsychologist), sham-controlled, crossover design in which each patient underwent the following procedure (Figure1):

(i) recruitment and randomization (to start with the real or the sham stimulation);

(ii) pre-Stimulation 1 TMS-EEG co-registration and neuropsychological evaluation;

(iii) 20 sessions (4 weeks) of tDCS or sham coupled with language production exercises, approximatively 40 minutes a day;

(iv) post-Stimulation 1 TMS-EEG co-registration and neuropsychological evaluation;

(v) 3 months washout; then, PWA who started with real tDCS received sham stimulation and vice versa

(vi) pre-Stimulation 2 TMS-EEG co-registration and neuropsychological evaluation;

(vii) 20 sessions (4 weeks) of real tDCS or sham coupled with language production exercises;

(viii) post-Stimulation 2 TMS-EEG co-registration and neuropsychological evaluation (Figure 1);

(ix) feedback to the patients and caregivers.

All participants were naïve to the purpose of the study and tDCS stimulation. They were informed from the beginning that two tDCS stimulation intensities would be applied and that one of the intensities would be barely perceivable. At the end of the experiment, when asked, none of them suspected a sham condition. The experimenter carried out the tDCS stimulation and the TMS-EEG co-registration, while an expert neuropsychologist carried out the neuropsychological evaluation.

Figure 1. Cross-over, repeated measures, experimental design (inspired by the Sills and Brodie, 2009 paper)
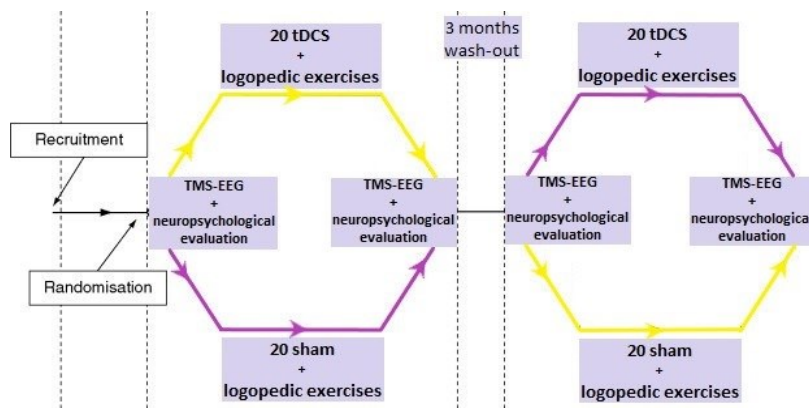
(i) PWA were recruited at CeRiN (Centro di Riabilitazione Neurocognitiva) of Trento University between 2018 and 2021.

(iia) Neuropsychological assessment

• **Language evaluation:**

- Battery for the *Evaluation of Aphasic Disorders (B.A.D.A.),* the short versions A and B were alternated in order to avoid learning (Miceli, Laudanna, Burani, & Capasso, 1994) and included the following subtests: nonword repetition (0-18), nonword reading (0-23/22), auditory lexical decision (0-40), visual lexical decision (0-40), word repetition (0-22/23), word reading (0-46), auditory comprehension of nouns (0-20), visual comprehension of nouns (0-20), auditory comprehension of actions (0-20), visual comprehension of actions (0-20), oral object picture naming (0-15), oral action picture naming (0-14), naming by definition (0-8), phrase and sentence repetition (0-10), sentence reading (0-3), auditory sentence comprehension (0-30), visual sentence comprehension (0-23/22).

- *Phonemic* (F, L, P) and *semantic verbal fluency* (car brands, fruits, and animals) (Novelli et al., 1986),

- *Spontaneous speech* - based on the Cookie Theft picture (Goodglass, Kaplan, & Weintraub, 2001) and L'esame del Linguaggio II (Ciurli, Marangolo, & Basso, 1996) picture description, see Figure 2 (Berndt, 2000; Saffran, Berndt, & Schwartz, 1989; Wilson et al., 2010).

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Figure 2. Picture used for the description task, from the Boston Diagnostic Aphasia Examination (Goodglass and Kaplan, 1983) and L'esame del Linguaggio II (Ciurli et al., 1996).

- Memory, attention, and reasoning evaluation: Attentive Matrices (Spinnler & Tognoni, 1987), Raven's Coloured Progressive Matrices (Measso et al., 1993), WEIGL' sorting test (Weigl, 1941), Forward and backward digit span (M. Monaco, Costa, Caltagirone, & Carlesimo, 2013). These tests were used in order to assess whether the observed changes were specific. Crucially, given that anodal stimulation was close to Broca's area, a frontal region, we aimed at verifying that the observed results were not better explained by executive functions improvement.

- General health perception, emotional states: In order to evaluate the perceived improvement, the following questionnaires were used before and after real tDCS and sham stimulation: Beck depression inventory (BDI-II) (Beck, Steer, & Brown, 1996), The Short-Form Health Survey (SF-36) (Ware, 2000), Structured assessment of depression in brain-damaged individuals (SADBD) (Hibbard, Stein, Gordon, & Sliwinski, 1992).

(iib) TMS-EEG data recording:

Each PWA underwent four experimental TMS/EEG measurements (before and after Stimulation 1, and before and after Stimulation 2), while the control group underwent a single experimental session. Participants were seated in an armchair, relaxed, with eyes open looking at a fixation cross on a screen to minimize eye movements. During all TMS-EEG recordings, a masking sound (based on an early version of the TMS Adaptable Auditory Control -TAAC) was played via earphones in order to avoid auditory EEG responses evoked by the TMS coil discharge (Russo et al., 2022).

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Single-pulse TMS was delivered through a Magstim Rapid[2] magnetic stimulator (Magstim, Whitland, UK) and a 70-mm figure-of-eight coil. TMS targeted intact cortical portions of both hemispheres. More specifically, the TMS target area was located over the left (damaged hemisphere) and right premotor cortex (BA6), specifically between the electrodes FC1 and F1 on the left, and FC2 and F2 on the right hemisphere (Scrivener & Reader, 2022). This area was selected as a TMS hotspot for the following reasons: (i) this brain region is usually characterized by fewer muscular artifacts, and (ii) according to previous studies, a greater activation of BA6 was reported for verbal fluency with respect to word repetition (Meinzer et al., 2012) and BA 6 stimulation increased the number of item produced in a verbal fluency task (Pisoni et al., 2018). The SofTaxic Optic - neuronavigation system (E.M.S., Bologna, Italy; http://www.softaxic.com) was used to guide an accurate and consistent positioning of the TMS coil across the repeated registrations. As a side note, during neuronavigation, for ethical reasons, we used the patient's MRIs or CT scans. This caused some coregistration problems due to the very low MRI and CT scan quality but was considered the best option available. For the control group, an individualized probabilistic head model was used.

TMS protocol and targeting: In the first session, the stimulation hot spot, coil orientation, and TMS intensity were decided using an early version of the rt-TEP software (Casarotto et al., 2022). Specifically, individual stimulation sites (over BA6) were determined based on the assessed amplitude, morphology, and topography of the average EEG response, following the indication reported in the paper (based on 20-trial average EEG responses to TMS, we looked for a peak-to-peak amplitude near Vpp > 10 μV, in the early 10–50 ms components in the EEG channels located underneath the TMS coil, avoiding the muscle artifacts as much as possible by rotating the coil). If after six attempts we were unable to obtain a satisfying TMS-EEG response, we chose the parameters with the best registration. Critically, the TMS intensity was identical for both hemispheres, and once the parameters were selected, they remained unchanged during all following registrations. We inserted 20-minute breaks between left and right TMS-EEG registrations, and the order of stimulation targets was randomized across sessions and between participants in order to avoid confounding variables (the stimulation of one hemisphere might

impact the TEPs registered, in a second moment, from the opposite side). Approximately 180 biphasic TMS pulses were delivered on each hemisphere with a random inter-stimulus interval of 2–2.3 s. The TMS intensity was for the control group: mean 88% of the TMS machine, SD 0.7, range 70%–95%; for PWAs: mean 83% of the TMS machine, SD 0.1, range 70%–95%.

As it can be observed from the Figure 3, there was no positive correlation between the stimulation intensity and the registered GMFP (first 250ms), i.e., increasing the stimulation intensity did not directly increase the registered values.

Figure 3. Correlation between the TMS intensity and the GMFP (on the control group) in the first 250 ms over the left and right hemispheres
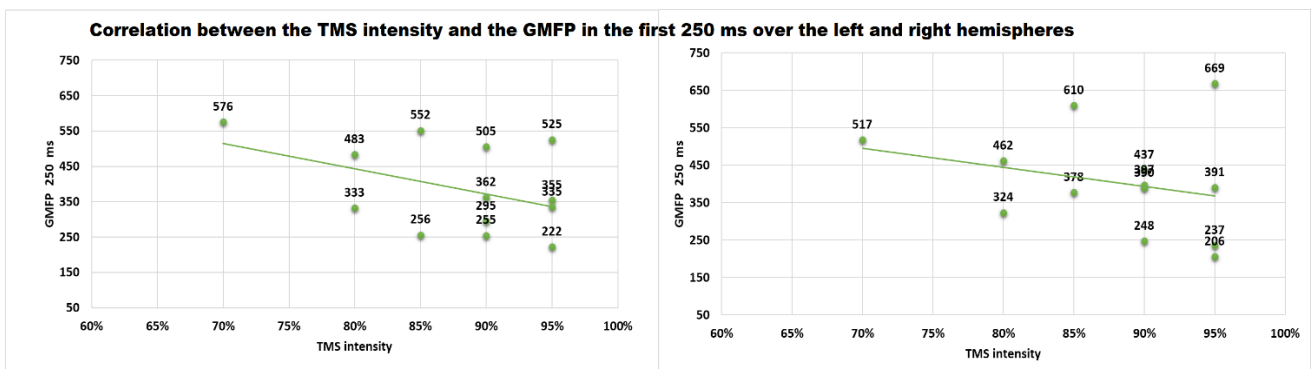
EEG Recording During TMS: EEG signals were acquired with a TMS-compatible EEG amplifier (BrainAmp DC, BrainProducts GmbH, Munich, Germany) with 62 high-density TMS-compatible C-ring AG-AgCl EEG electrodes (EASYCAP, Germany) arranged in the International 10–20 EEG montage. To monitor eye movements and blinking, two additional electrodes were used. AF7 was used as online reference, and AF8 was the ground electrode. Signals from all channels were hardware-filtered (lower pass-band edge to critical frequency (DC), high cut-off 1000 Hz) and digitized with a sampling rate of 5 kHz. All electrode impedances were maintained at < 5 kΩ throughout the sessions. Trigger marks at the TMS pulse delivery were sent to the EEG system using a customized MATLAB script (MathWorks®, Inc., Massachusetts, USA).

Data preprocessing was carried out using Matlab R2020b (The MathWorks, Massachusetts, USA) and in-house scripts based on functions of the open-source toolbox EEGLAB

(https://sccn.ucsd.edu/eeglab/). After EOGs removal, the first step was to cut out the TMS artifacts, namely, TMS/EEG data from 2 ms before to 5 ms after the TMS pulse (containing the TMS artifact) was removed and replaced with the data from 4 to 2 ms before the TMS pulse. The triggers too close to the borders were removed from the continuous recording. The next step, after detrending, was to segment the continuous signal using a time window of ± 800 ms around the TMS stimulus. Bad channels (≤ 5% of channels per recording) were interpolated using the spherical splines interpolation function of EEGLAB (Fecchio et al., 2017). Trials with excessive artifacts were removed by visual inspection. TEPs were then average referenced and baseline corrected (baseline was the entire pre-stimulus period). After reducing the number of independent components to the number of good, non-interpolated channels by performing singular value decomposition (SVD), independent component analysis (ICA) was applied (Sarasso et al.,2020. Rosanova et al.,2018) in order to remove residual muscular and magnetic artefacts. Data were also down-sampled to 1000 Hz, and the signal was band-pass filtered between 1 and 45 Hz (Casarotto et al., 2016).

(iii) Real or sham tDCS stimulation coupled with logopedic exercises:

TDCS online stimulation was performed with a standard tDCS device (BrainSTIM stimulator, http://www.emsmedical.net/prodotti/tdcs/942-brainstim). Based on previous studies (Bucur and Papagno, 2018), we used a bihemispheric montage. The anode was placed over the left perilesional area as closest as possible to Broca's area (F7 and F5 areas, according to the international 10–20 EEG system) and the cathode was over the contralateral supraorbital area. Rubber electrodes (5 x 5 cm^2) were positioned using the Ten20 conductive EEG paste and fixed with a headband. A constant current of 2 mA intensity was applied for 20 min. The same electrode position was used for sham stimulation. The sham condition consisted of a brief stimulation period in the beginning (30 s) and at the end (30 s) of each session to mimic common skin sensations, with no stimulation during the between period (19 min off).

Stimuli during real or sham stimulation: In each session, during tDCS (sham or real), all participants performed the following tasks: (i) object and action picture naming, (ii) phonemic and semantic fluency,

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(iii) picture description. Depending on the participant's verbal speed, session duration varied between 35 and 45 minutes.

Materials: The experimenter and a speech and language therapist chose all the stimuli. The same stimuli and the same setting were used for all PWA participants. Critically, none of the stimuli presented during the tDCS sessions were used during the pre/ post evaluations. The stimuli were different for each day of the week (from Monday to Friday), but they received the same stimuli on the same day (four Mondays, four Tuesdays, etc.); therefore, each group of stimuli was presented four times during the complete treatment.

For the picture naming tasks five different lists of 20 objects and 11 actions (155 pictures) were used. The items consisted of objects belonging to living and non-living semantic categories with different levels of frequency (e.g., house, fennel, flute, to slide, to feed, to jump) and were chosen using the Banca Dati dell'Iitaliano Parlato (BADIP); http://badip.uni-graz.at/it/. Pictures were presented on a computer screen using a customized MATLAB script (MathWorks®, Inc., Massachusetts, USA). The experimenter was able to choose when to present the following picture, allowing the participants the necessary time to answer. If the patient was unable to name a specific picture, phonological and semantic cues were given; if no correct answer was produced, the examiner named the picture, and the participants were encouraged to repeat it. The aim was to make the participants produce as many linguistic elements during the stimulation as possible.

In each session, participants also performed a fluency task with one semantic (e.g., clothing, sweets, jobs, vehicles, drinks) and one phonemic (e.g., T, N, S, D, C) cue for each weekday. Specifically, they were asked to produce in 1 min as many words as they could beginning with a given letter or belonging to a specific semantic category. Subjects were also instructed that a word could not be produced twice. For spontaneous speech, five black and white pictures (one for each day of the week) were used, and the patient was asked to describe it (Figure 4). If the description was poor, the patient was given two prompts: (1) who is in the picture and what are they doing?, (2) which objects are in the room, park, on

the beach, etc.? If the subject remained silent for 30 s or showed that this was the end of his/her response, some examples were provided, and the participant was invited to repeat.

The language exercises used during the tDCS were decided based on (i) the literature review data (what is commonly used), and (ii) on the language therapist's clinical expertise, namely what type of exercises could be effective and properly performed by the majority of the PWA given the fact that we cannot predict the level of aphasic impairment of our future participants. In addition, we preferred to maintain the same exercises for everyone. Indeed, considering that all patients suffered lexical impairments, and our aim was to improve production, it was decided to train this component.

Figure 4. Complex pictures (not at scale) for the spontaneous speech during the tDCS stimulation. The images were taken from the following tests: the Neuropsychological Examination for Aphasia Battery (ENPA) (Capasso & Miceli, 2001), the Comprehensive Aphasia Test (CAT) (Swinburn et al., 2005), SAND: the Screening for Aphasia in Neuro Degeneration (SAND) (Catricalà, et al., 2017), the Western Aphasia Battery (Kertesz, 1982), The Picture Description Task, designed by Sasanuma et al. (1985).

After the neuropsychological and TMS-EEG evaluations (at the beginning and at the end of each experimental session), participants were treated individually during 40 sessions (20 real and 20 sham tDCS, with a 3 month wash-out period). They were seated at a distance of approximately 60 cm from the monitor in a quiet room and, after the initial tDCS preparation, they completed the three different tasks previously described.

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4.2.3. Planned analysis

Given the complexity of the experiment and the limited number of PWA, the following variables were considered for analysis:

i) **Behavioral data**, when possible, adjusted (for age and educational level) scores were used, with the advantage of controlling for variance associated with premorbid factors rather than changes in neurological functioning.

- in the case of **B.A.D.A.**, the total score (for each participant) was computed as the mean of percentage of correct responses, pre/post tDCS real and sham;

- **Verbal Fluency** (phonemic and semantic) scores corresponded to the total number of correct words produced in a minute for each given cue (excluding repetitions and derivations from the same word);

- **Spontaneous Speech** was scored as the sum of the number of words and sentences correctly produced in the first 2 minutes (without any help from the examiner).

- Attentive Matrices (Spinnler & Tognoni, 1987), Raven's Progressive Matrices (Measso et al., 1993), WEIGL test (Weigl, 1941), Forward and backward digit span (Monaco et al., 2013): means and standard deviations of participants were computed following the Italian normative data.

- for SF-36, the score difference between the pre- and post-stimulation evaluations (e.g., post-pre tDCS real or sham) was calculated;

ii) for the **TMS-EEG** data, the following parameters were considered (the procedure was identical for PWA and controls):

- *Global Mean Field Power (GMFP)* reflects the level of global neuronal activity and is a reliable measure of cortical excitability and connectivity of the targeted area and of the related functional networks (Massimini et al., 2005). The GMFP is the standard deviation of the potentials across all the EEG electrodes as a function of time (Lehmann & Skrandies, 1980) and is calculated using the following formula [copied from (Esser et al., 2006)].

$$GMFP(t) = \sqrt{\frac{\left[\sum_i^k (V_i(t) - V_{\text{mean}}(t))^2\right]}{K}}$$

K defines the number of channels, V_i defines the voltage measured with channel i, and V_{mean} represents the average of the voltages across all included channels. GMFP belongs to the time domain analyses. TEPs can be evaluated by their latency and amplitude, and time points corresponding to TEP peaks produce high GMFP peaks, whereas flatter TEP components produce low GMFP peaks. GMFP is usually calculated as the average of all subjects; as we only have the data of three participants, we will calculate the GMFP within each PWA (and of course for the control group). The GMFP was calculated for five temporal windows: $0 - 30$, $31 - 60$, $61 - 100$, $101 - 150$, $151 - 250$ msec, and used as a random factor in the analysis.

- The *Local Mean Field Power (LMFP)*, computed similarly to the GMFP, was used to specifically assess cortical excitability near the stimulation area on the left and right hemispheres.

$$LMFP(t) = \sqrt{\frac{\left[\sum_i^k (V_i(t) - V_{mean}(t))^2\right]}{K}}$$

Where t is time, K is the number of channels, V_i is the voltage in channel i averaged across subjects, and V_{mean} is the averaged voltage in the channels of interest (Casula et al., 2016).

We chose 4 channels on each hemisphere using the topography plot of each participant (once chosen, the channels were maintained during all of the local analysis). Specifically, we visually inspected the topography corresponding to the first GMFP peak and chose four channels with higher activation closer to the stimulation area. For example, in Figure 5, the first GMFP peak was after 30 ms from the TMS pulse, and we chose the electrodes encompassed by the red hotspot, namely FCz, FC1, Fz and F1. The LMFP was calculated for five temporal windows: $0 - 30$, $31 - 60$, $61 - 100$, $101 - 150$, $151 - 250$ msec, and used as a random factor in the analysis.

Figure 5. Example of butterfly plot and topography after left TMS stimulation (PWA).
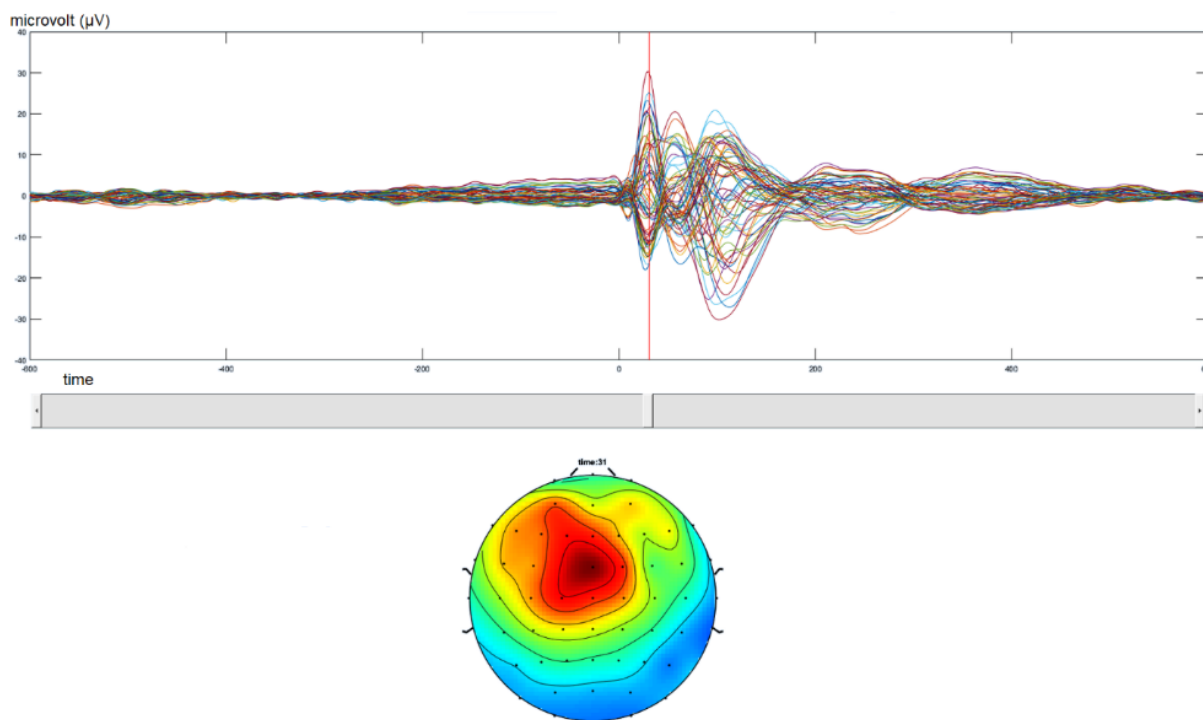
- PCI^{LZ}, *Lempel-Ziv Perturbational Complexity Index* was introduced by (Casali et al., 2013) and provides a data-driven metric that can discern the state of consciousness in single subjects under different conditions, e.g., wakefulness, dreaming, sleep, anesthesia. PCI^{LZ} was calculated by first perturbing the cortex with TMS to engage distributed interactions in the brain (integration) and afterwards compressing the spatiotemporal pattern of these electrocortical responses to measure their algorithmic complexity (information). "PCI is defined as the normalized Lempel-Ziv complexity of the spatiotemporal pattern of cortical activation triggered by a direct TMS perturbation" (p.2, Casali et al., 2013). PCI^{LZ} was calculated over all 62 electrodes.

Given the assumption that all participants, PWA and controls, shared a similar level of consciousness, i.e., they were all awake, no difference between the two groups were expected, despite the presence of cortical lesions in PWA patients. Nevertheless, PCI^{LZ} was used as a checking measure for data quality.

- PCI^{ST}, *Perturbational Complexity Index* based on dimensionality reduction and *state transitions (ST)* quantification of TEPs, was recently proposed by Comolatti et al. (2019). This index reflects the overall

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capacity of thalamocortical circuits to engage in complex patterns of causal interactions, typically present in conscious awake individuals. Similar to Sarasso et al. (2020), a generalization of the original method was applied and, for each stimulation session, PCIST restricted to 19 channels on each hemisphere was calculated, specifically: F5, F3, F1, Fz, FC5, FC3, FC1, FCz, C5, C3, C1, Cz, CP5, CP3, CP1, CPz, P3, P1, Pz on the left and F6, F4, F2, Fz, FC6, FC4, FC2, FCz, C6, C4, C2, Cz, CP6, CP4, CP2, CPz, P4, P2, Pz on the right hemisphere. The aim was to assess the impact of sleep-like off-periods on signal complexity between the two hemispheres.

- ***Natural frequency Peaks***: Cortical regions tended to preserve a "preferred" natural frequency (each corticothalamic module is normally tuned to oscillate at a characteristic rate) also when indirectly engaged by TMS (Rosanova et al., 2009). Following Rosanova et al. (2009), for the responses in the time-frequency domain analysis the event-related spectral perturbation (ERSP) based on Morlet wavelets was calculated:

$$ERSP(f, t) = \frac{1}{n} \sum_{k=1}^{n} \left| F_k\left(f,\ t\right) \right|^2$$

where for n trials, the spectral estimate F was computed at trial k, at frequency f and time t.

The local ERSP was computed by averaging the ERSPs across the 4 channels previously mentioned for the LMFP, in a given session. Then, the local ERSPs were cumulated over time between 20 and 200 ms to obtain the power spectrum. The natural frequency was computed as the normalized average frequency of the power spectrum by applying the "meanfreq" function (MatLab 2020b).

4.3. Results

In the present study, we used concurrent TMS and EEG to investigate the long-term effects of tDCS coupled with linguistic exercises in PWA.

To this aim, each aphasic patient's data were compared with the data recorded in the control group using Bayesian multilevel single case models in an R statistical computing software environment (R Core Team, 2014) with the "bmscstan" package (Scandola & Romano, 2021). The Bayesian approach estimates the posterior probability (PP) distribution of effect θ given the data using the likelihood and prior knowledge (Ortega & Navarrete, 2017). The main reason for choosing Bayesian analysis was the very small number of PWA included in the experiment; the second reason was that it allowed to better depict to what extent the investigated phenomena may occur by directly testing the plausibility of both the null and the alternative hypothesis (Ortega & Navarrete, 2017). As a rule of thumb, a Bayes factor greater than 3 is considered good enough evidence for H_1 over H_0, and a Bayes factor inferior to 1/3 as good enough evidence for H_0 over H_1, while a Bayes factor between those values is interpreted as being nonevidential (Dienes, 2019).

Aphasic participants were registered four times (pre and post each stimulation type) while controls only once. Since the "bmscstan" package requires the same data points from both the single case and the control participants, we basically compared each time point from the PWA with the control group. In particular, GMFP, LMFP, PCI^{ST}, PCI^{LZ} and natural frequencies were considered as continuous dependent variables, while the stimulation hemisphere (left or right) was tested as fixed factor. The by-subject intercept was included as a random factor. The Time Window (factorial, 5 levels: 0–30; 31–60; 61–100, 101–150, and 151–200 ms) was also used as a random factor for the GMFP and LMFP.

To determine whether the difference between the data points (in this case the stimulation effect) is statistically significant, analysts often compare the credible intervals (CI) for those groups. If those intervals overlap, they conclude that the difference between groups is not statistically significant; conversely, if there is no overlap, the difference is considered significant. Due to the small sample size, it was not possible to conduct direct pre vs. post tDCS statistical analysis

TDCS and TMS stimulations were well tolerated by all patients and no adverse effects were observed or reported by patients and/or caregivers. For privacy reasons, we will use P1, P2, P3, and P4 instead of the patients' initials. P1 and P2 started with real tDCS; P3 and P4 started with sham stimulation and crossed over to the other condition after more than 3 months. The main reason we were unable to maintain a constant washout period was the lockdown period due to COVID-19.

For clarity, each patient will be described as a single case; Attentive Matrices (Spinnler & Tognoni, 1987), Raven's Coloured Progressive Matrices (Measso et al., 1993), WEIGL' sorting test (Weigl, 1941), Forward and backward digit span (Monaco et al., 2013), BDI-II (Beck et al., 1996), and SF-36 (Ware, 2000), will be discussed at the end.

4.3.1. P1.

Patient P1 is a 62-year-old man. Before his stroke, he had an active professional life as the owner of a small mechanical shop and was a volunteer firefighter (now early retired due to the stroke). Fifty-nine months before the experiment, he suffered from chronic non-fluent aphasia due to an ischemic stroke with a hemorrhagic complication that required decompressive craniotomy (Figure 6). The lesion involved left frontotemporal and internal capsule regions. P1 also showed right hemiplegia, namely a severe paralysis of the right arm and hand and very reduced mobility of the right leg. The patient underwent different cycles of motor and speech therapy rehabilitation before this study. After intense physiotherapy, he managed to regain partial walking abilities. The initial neuropsychological evaluation revealed the presence of non-fluent aphasia characterized by greatly reduced, slow, and effortful speech. Naming, due to verbal anomias and paraphasias, was the most impaired ability. Comprehension and repetition (for single words or short sentences) were less impaired. Written language and auditory-verbal memory were also significantly impaired. Additionally, he presented with ideomotor apraxia, but preserved logical-deductive reasoning skills for non-verbal stimuli. He retained awareness of his language impairments and his motivation to communicate was well preserved. The patient had suffered from depression immediately after stroke, but, at the moment of examination, according to the SADBD and BDI-II scores, he had totally recovered.

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The patient stimulation started with active tDCS, and the TMS intensity was 80% of the Magstim Rapid[2]

(on both hemispheres).

Figure 6. P1 MRI (T1 from 29/05/2017), lesion in the MCA territory
(MRIcron and MRIcroGL visualization Software, https://www.nitrc.org/projects/mricron).
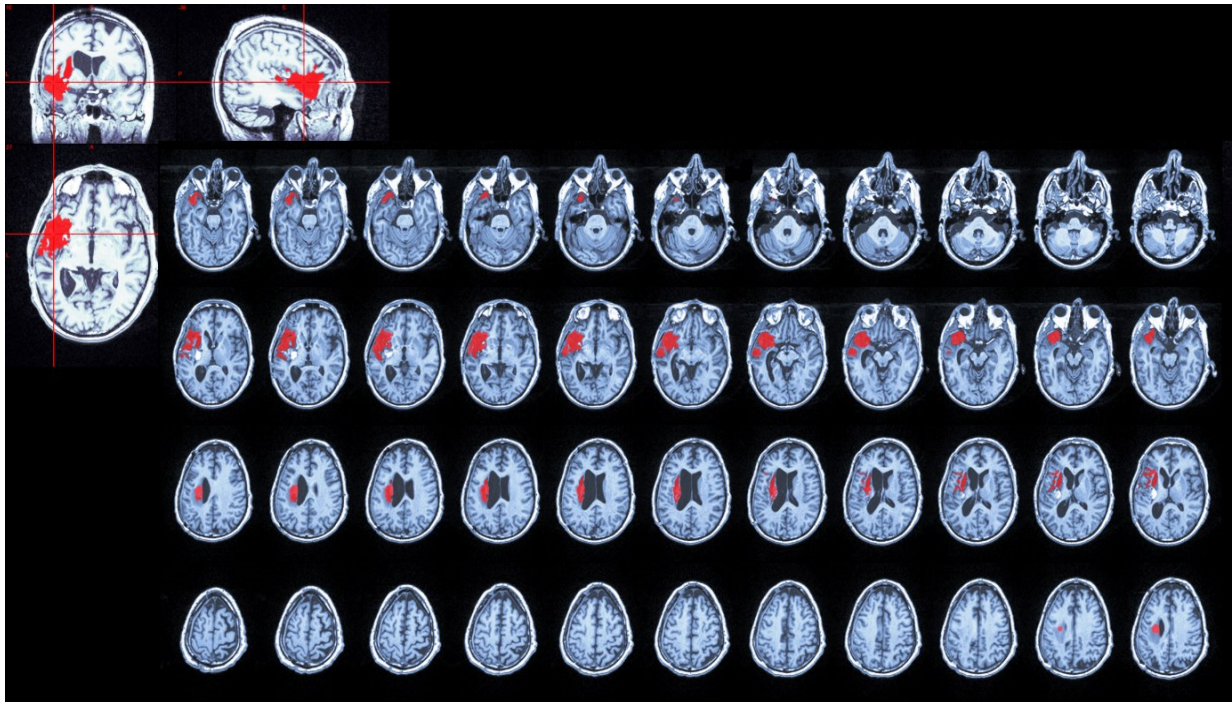
i) Behavioral data:

B.A.D.A.'s total score was computed as the mean of the percentage of correct responses of all the

subtests. As can be observed in the graphic below (Figure 7), P1 improved to some degree after both

real (from 63% to 67% correct answers) and sham stimulations (from 69% to 70%). Even if the

improvement was slightly greater after real tDCS, a confounding factor can be the treatment order; as

this patient started with real stimulation, the margins for improvement could be reduced in the second

part of the experiment (in this case the sham condition).

Figure 7. P1 B.A.D.A. results, pre/ post tDCS and sham (error bars are SD).
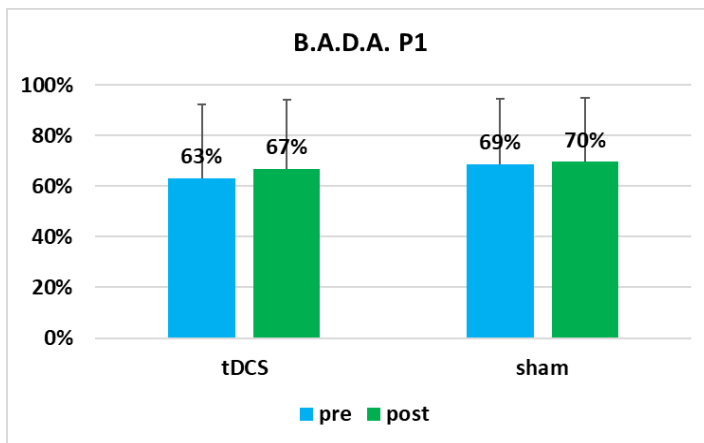
Verbal Fluency: Phonemic fluency improved after tDCS (from 11 to 21, adjusted score), but not after sham, while semantic fluency improved in both conditions (Figure 8).

Figure 8. P1 Verbal Fluency, phonemic and semantic, results: pre/ post tDCS and sham
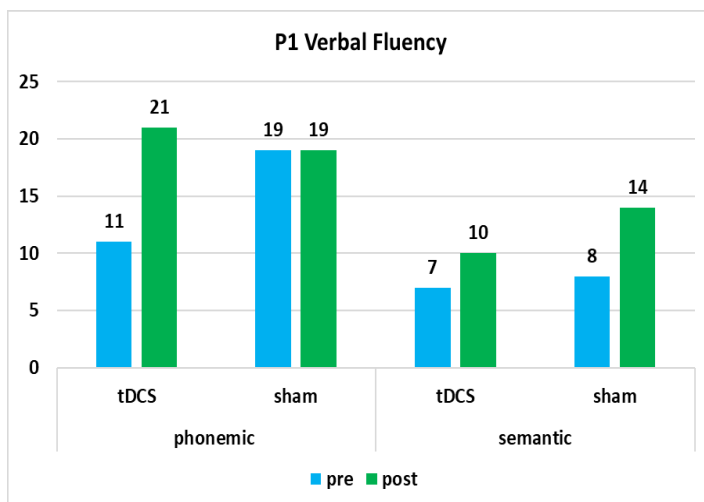
Spontaneous speech revealed an increase in the number of total words, in particular nouns, and verbs in both conditions (Figure 9). Concerning the total number of words, the pre vs post difference was slightly higher after real stimulation (increasing from 37 to 53 after tDCS and from 42 to 53 after sham). The total number of sentences, as the average sentence length increased only after real stimulation (the number of sentences incremented from 3 to 4 and the average sentence length from 4 to 7).

Figure 9. P1 Spontaneous speech results: pre/ post tDCS and sham
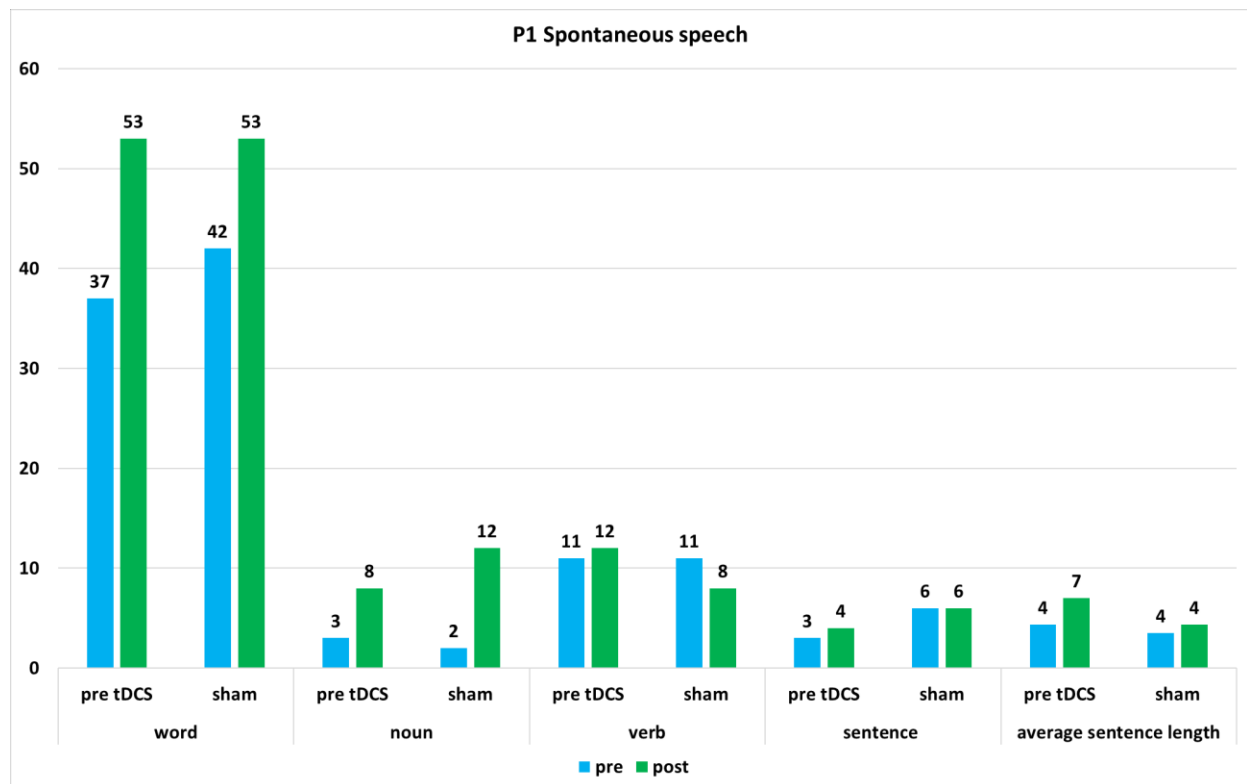
ii) TMS-EEG data:

GMFP: The Bayesian analysis for P1, compared with the control group, provided moderate support in favor of the alternative hypothesis, mainly that the left-right GMFP difference was larger for the clinical case (although the stimulation intensity was constant) when compared to the control group (Figure 10a, 10b, 10c): pre tDCS Bayes factor (BF_{10} = 5.14; CI: -17.4 / 48.6); post tDCS (BF_{10} = 5.34; CI: -21.6 / 55.1); pre sham (BF_{10} = 4.98; CI: -23 / 27); post sham(BF_{10} = 5.55; CI: -21 / 58.7). In other words, while in the control group, the majority of participants had very similar GMFP responses in both hemispheres, for P1 the GMFP, recorded from one hemisphere (during the same sessions) was quantitatively and qualitatively different from the one recorded on the opposite hemisphere.

It is possible to notice from the CI that there were no significant changes between the stimulation conditions since all CI overlap greatly. One can see from Figures 10a that no changes are observed after sham, while a very small increase of the GMFP is recorded after active tDCS on the left hemisphere, enlarging the GMFP interhemispheric difference.

Figure 10a. P1 GMFP, left hemisphere stimulation, pre/ post tDCS, and sham.
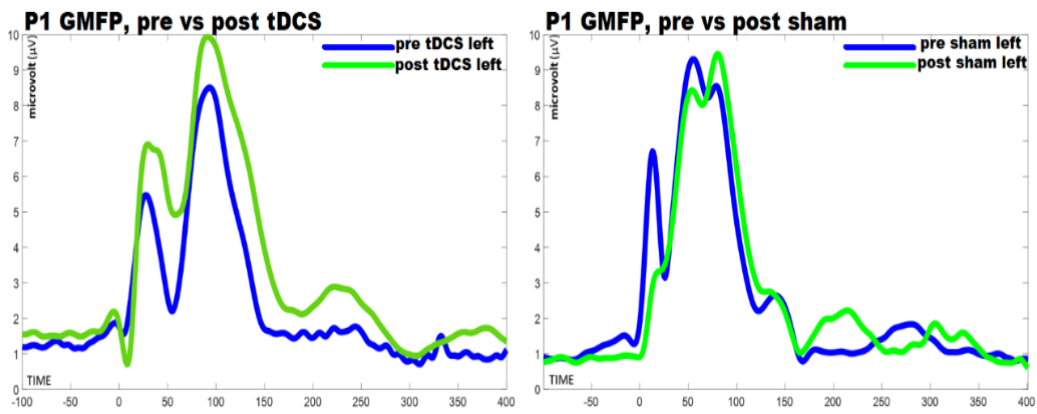
Figure 10b. Control group GMFP (overlap of 13 elderly, healthy participants), and P1 GMFP, left vs right hemisphere stimulation.
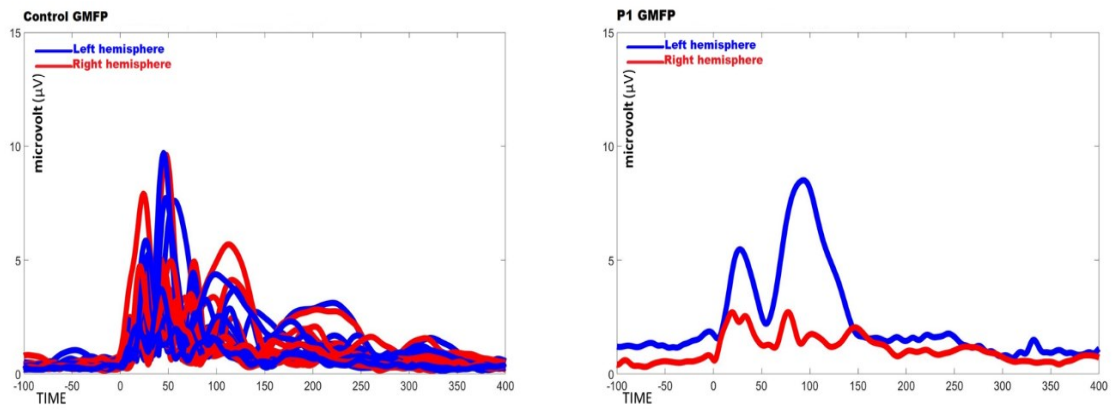
Figure 10c. GMFP P1 left vs right pre/post tDCS and sham stimulation; GMFP mean and SD of the control group.

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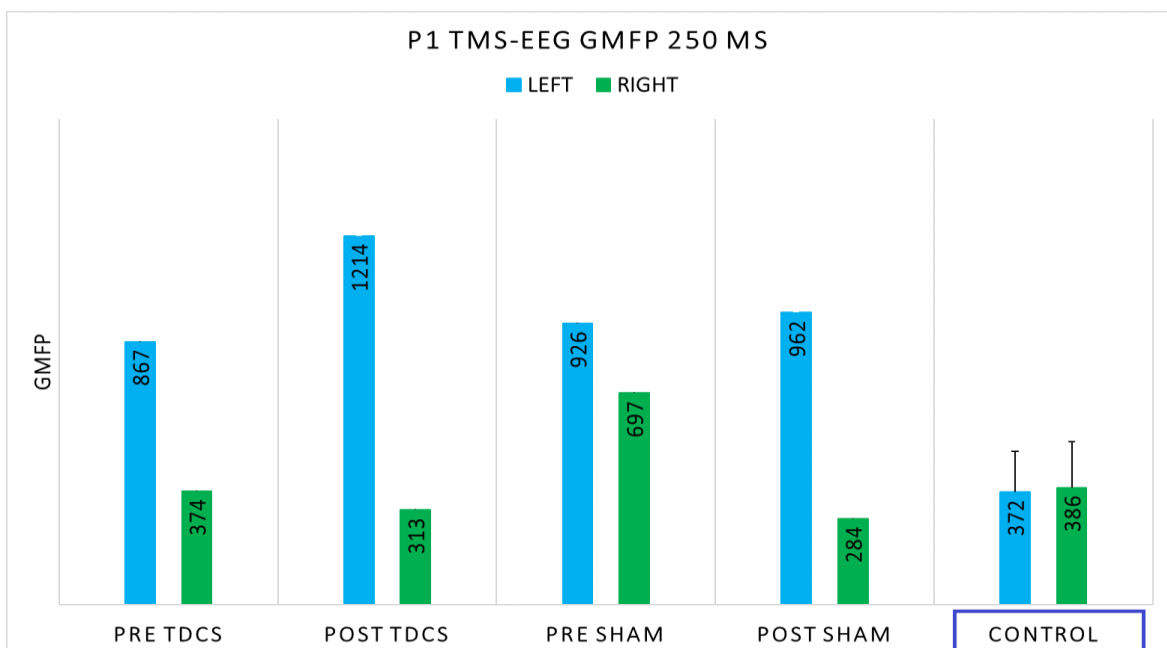
The Bayesian analysis of the **LMFP** (channels: left Fz, FCz, F1, FC1; right Fz, FCz, F2, FC2) continued to provide moderate support in favor of the alternative hypothesis. The left vs right LMFP difference was larger for P1 than observed in the control group (Figure 11): pre tDCS (BF_{10} = 4.83; CI: -27 / 40); post tDCS (BF_{10} = 4.93; CI: -30 / 51); pre sham (BF_{10} = 4.87; CI: -26 / 20); post sham (BF_{10} = 5.68; CI: -23 / 70).

Figure 11. Butterfly plot of the TEPs recorded at all 62 electrodes for the perilesional stimulation site (in blue) and contralesional (red). The four EEG electrodes closest to TMS, and used to calculate the LMFP, are displayed in color. The two crosses indicate the position of the coil focus over the scalp. Note that the y-axis of the left (lesioned hemisphere) is double (from 0 to 40 microvolt) as compared to the right one (from 0 to 20 microvolt). It can be observed that the left hemisphere is characterized by fewer peaks (the signal oscillates less) as compared to the right hemisphere.
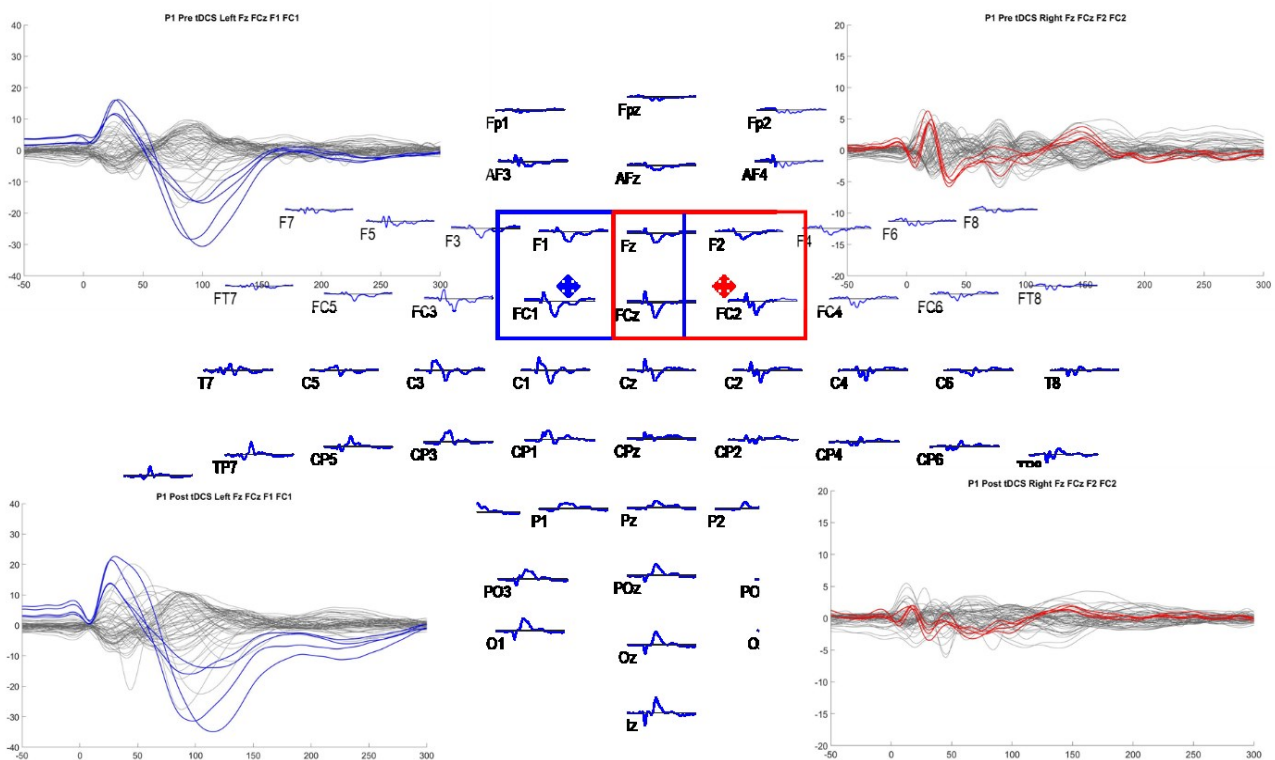
Bayesian analyses run on *PCI^ST* (F5, F3, F1, Fz, FC5, FC3, FC1, FCz, C5, C3, C1, Cz, CP5, CP3, CP1, CPz, P3, P1, Pz on the left and F6, F4, F2, Fz, FC6, FC4, FC2, FCz, C6, C4, C2, Cz, CP6, CP4, CP2, CPz, P4, P2, Pz on the right hemisphere) *and Natural frequency* (left hemisphere Fz FCz F1 FC1 and right Fz FCz C2 FC2), contrary to our hypothesis, indicated inconclusive results. TDCS did not significantly modulate these parameters. Specifically, when comparing P1 with the control

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group, the PCI^{ST} interhemispheric difference was: pre tDCS - BF_{10} = 1.9; post tDCS - BF_{10} = 1.19; pre sham − BF_{10} = 1.18; post sham − BF_{10}= 1.21; and *Natural frequency* was: pre tDCS − BF_{10} − 2.77; post tDCS − BF_{10} = 2.89; pre sham − BF_{10} = 2.12; post sham - BF_{10}= 2.6.

Figure 12. PCI^{ST} and Natural frequency: P1 left vs right pre/post tDCS and sham stimulation; PCI^{ST} and Natural frequency mean and SD of the control group.
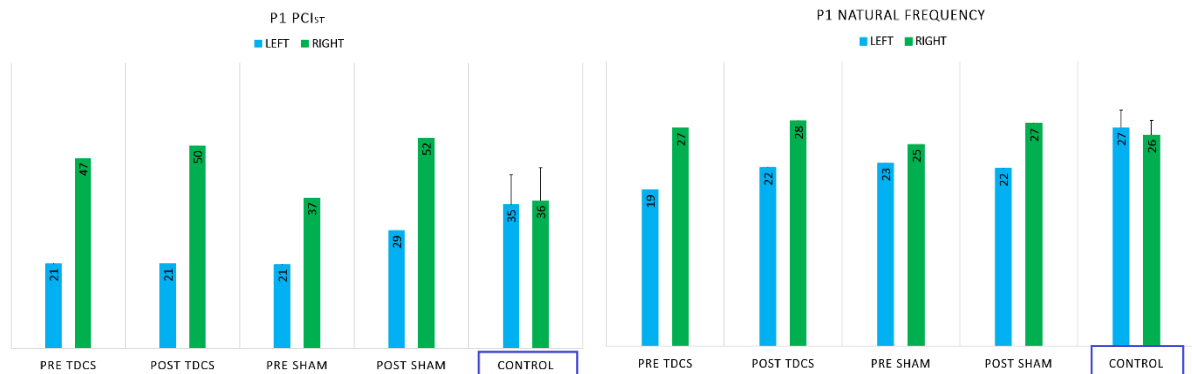
PCI^{LZ} was used to control the data quality, and indeed the Bayesian analysis, although inconclusive, was near the threshold usually indicated as supporting the null hypothesis. Namely, no significant difference between the left vs right hemisphere response (between the P1 and the control group) was revealed: pre tDCS - BF_{10} = 0.52; post tDCS - BF_{10} = 0.44; pre sham − BF_{10} = 0.45; post sham - BF_{10}=0.42.

In conclusion, P1 was engaged and enthusiastic during the entire experiment. No complaints were made by him or his caregiver.

Regarding the behavioral data, a very small variation was observed in the B.A.D.A. scores, while the phonemic fluency and the number of total words and sentences during the spontaneous speech increased slightly after real tDCS as compared to sham.

When TMS was applied using the same stimulation parameters over both hemispheres, an important interhemispheric difference was observed, especially for the GMFP and the LMFP. TEPs obtained from the stimulation of the contralesional site appear to be composed by a similar amplitude and complexity as the data recorded from the control group, while TEPs from the perilesional site were characterized by slow EEG response and lower PCI^{ST} values. The tDCS stimulation, in this patient, produced an

unexpected effect; specifically, after real tDCS, the GMFP and the LMFP difference between the two hemispheres slightly increased, while no changes were observed after sham stimulation (Figure 10 a, 11). One possible explanation might be related the fact that P1 was originally left-handed, and he was corrected in childhood, to use the right hand. This means that although he became aphasic after a left-hemisphere lesion, his language representation could be less lateralized. We observed that the PCI[ST] values and the Natural frequencies values, although slightly different between the two hemispheres, did not differ from the control group.

4.3.2. P2.

Patient P2 is a 60-year-old woman, with a good support network. She suffered a left subcortical frontotemporal ischemic stroke six years ago (2016), involving also the insular cortex (Figure 13). She went through six cycles of speech and language therapy improving from an initial global aphasia to nonfluent Broca aphasia.

The last neuropsychological evaluation confirmed the presence of a non-fluent aphasia and orofacial apraxia. Specifically, spontaneous speech was very reduced and characterized by anomia, conduites d'approche, latencies, and paraphasias. Her comprehension was preserved for single words and contextual information, while syntactic comprehension was impaired, regardless of the stimulus presentation modality (auditory / visual). Naming, reading, and repetition of words and non-words were impaired. Having a good awareness of the linguistic errors, P2 was able to effectively self-correct most of the time. A verbal short-term memory deficit and a decrease in visual selective attention were also observed.

Although not clinically depressed according to the SADBD and BDI-II scores, P2 often showed a pessimistic, hopeless, and self-devaluating mood during the experiment.

P2 stimulation started with active tDCS, and the TMS intensity was 70 % of the Magstim Rapid[2] (on both hemispheres).

Figure 13. P2 MRI (T1 from 28/09/2017), lesion in the MCA territory.
(MRIcron and MRIcroGL visualization Software, https://www.nitrc.org/projects/mricron).
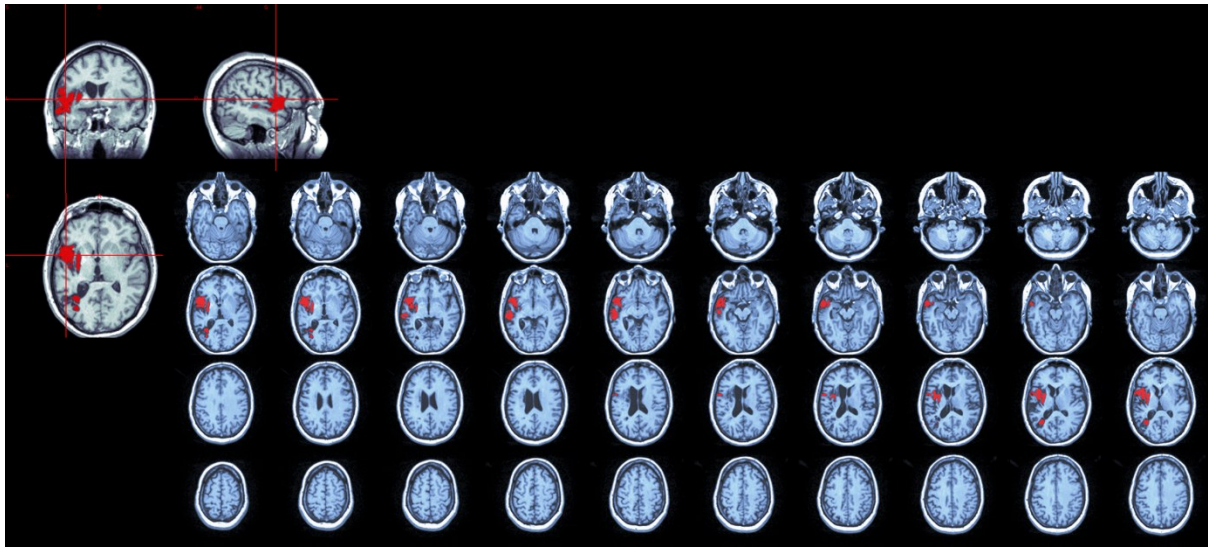
i) Behavioral data:

B.A.D.A.: P2 improved to some degree after real (from 73 % to 77 % of correct answers) but not after sham stimulation (from 80 % to 75 %), see Figure 14. Although after sham stimulation, her performance decreased, the end score was still two points above the starting value (pre tDCS 73%, post sham 75%).

Figure 14. P2 B.A.D.A. results, pre/ post tDCS, and sham (error bars are SD).
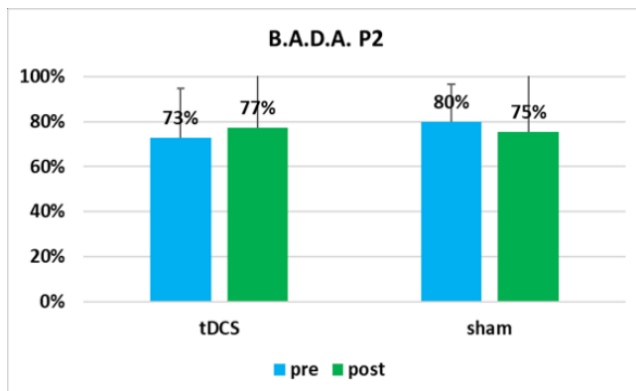
Verbal Fluency: Both phonemic (from 6 to 10) and semantic (from 9 to 11) fluency improved after real tDCS but not after sham (Figure 15).

Figure 15. P2 Verbal Fluency, phonemic and semantic, results: pre/ post tDCS and sham.
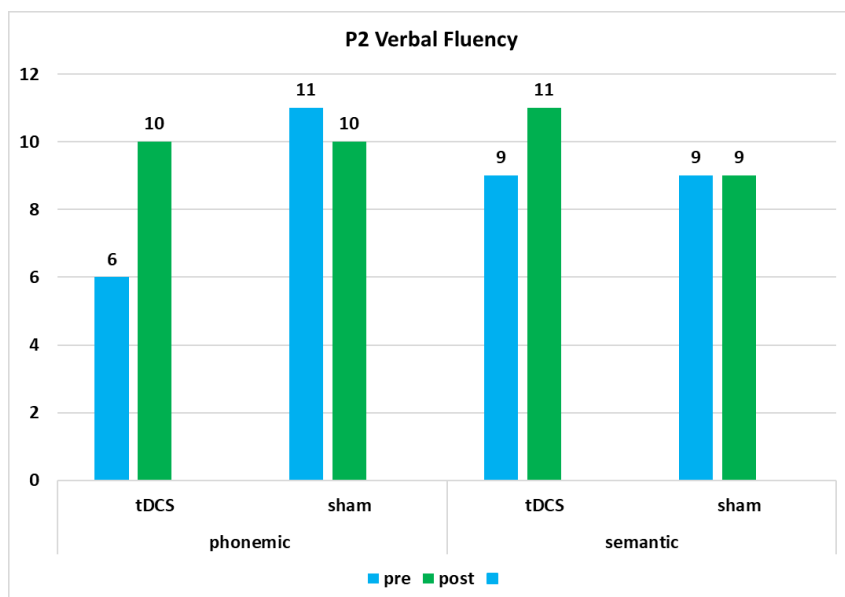
Spontaneous speech scores revealed an increase in the number of total words, in particular nouns and verbs in both conditions (Figure 16). Concerning the total number of words, the pre vs post difference was greater after real stimulation (increasing from 7 to 19 after tDCS and from 17 to 21 after sham). The number of sentences increased after both tDCS (from 0 to 1) and sham (from 2 to 3), while the average sentence length improved only after tDCS (she managed to produce a four words sentence) and remained constant in the sham condition (2/3 sentences with five words average length).

Figure 16. P2 Spontaneous speech results: pre/ post tDCS and sham
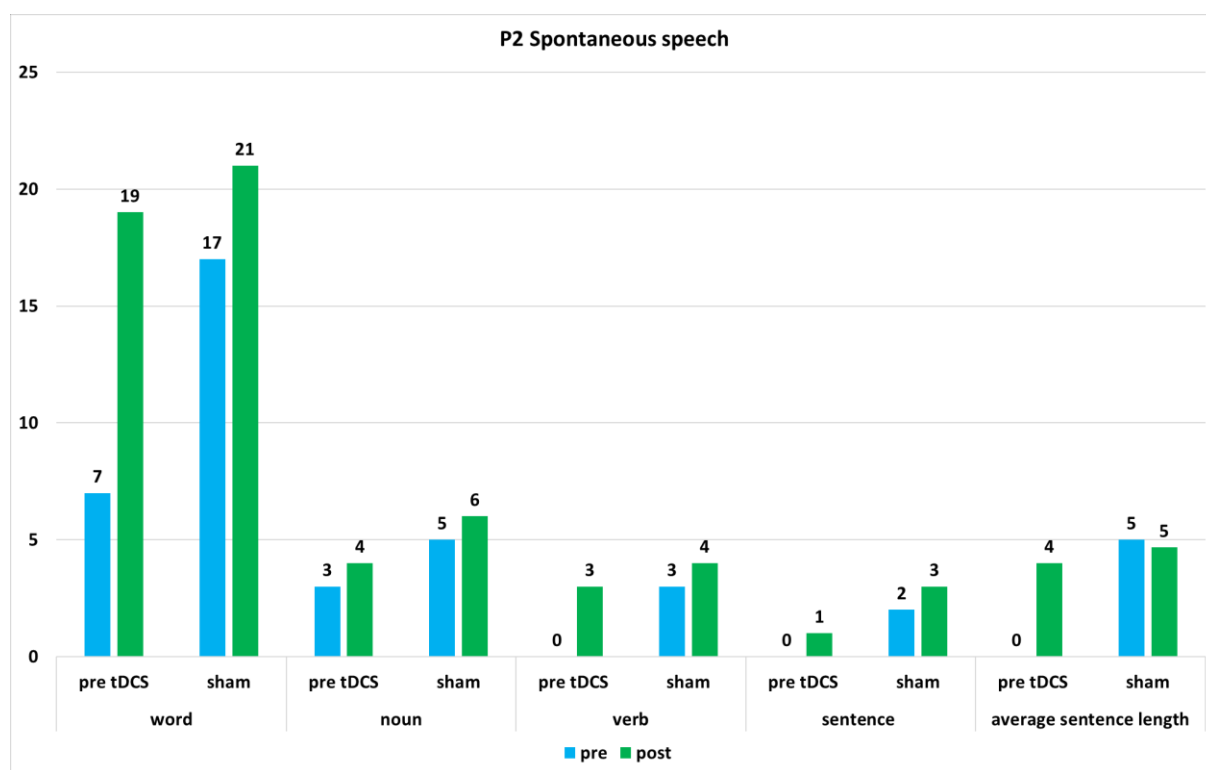
ii) TMS-EEG data:

Bayesian analysis for the *GMFP* (P2 compared with the control group) provided moderate support in favor of the alternative hypothesis, essentially the left-right GMFP difference was larger for the patient as compared to the control group (Figure 17a, 17b, 17c), pre tDCS (BF_{10} = 5.07; CI: -31 / 139); post tDCS (BF_{10} = 5.12; CI: -33 / 21); pre sham (BF_{10} = 5.35; CI: -3 / 52); post sham(BF_{10} = 6.88; CI: -3 / 242).

There were no significant changes between the stimulation conditions as all CI overlap to some degree. Figure 10a indicates that no changes are observed after sham, while a decrease of the GMFP is recorded after active tDCS on the left hemisphere, diminishing the GMFP interhemispheric difference (as it can be observed from Figure 17b).

Figure 17a. P2 GMFP, confronting pre vs post tDCS and sham stimulation (on each hemisphere). It can be noticed that only after real tDCS on the left hemisphere there is a slightly decrease in the GMFP (x-axis = time, y-axis =microvolt). Note that the right hemisphere y-axis is half (10 microvolt) of the left hemisphere y-axis (20 microvolt).
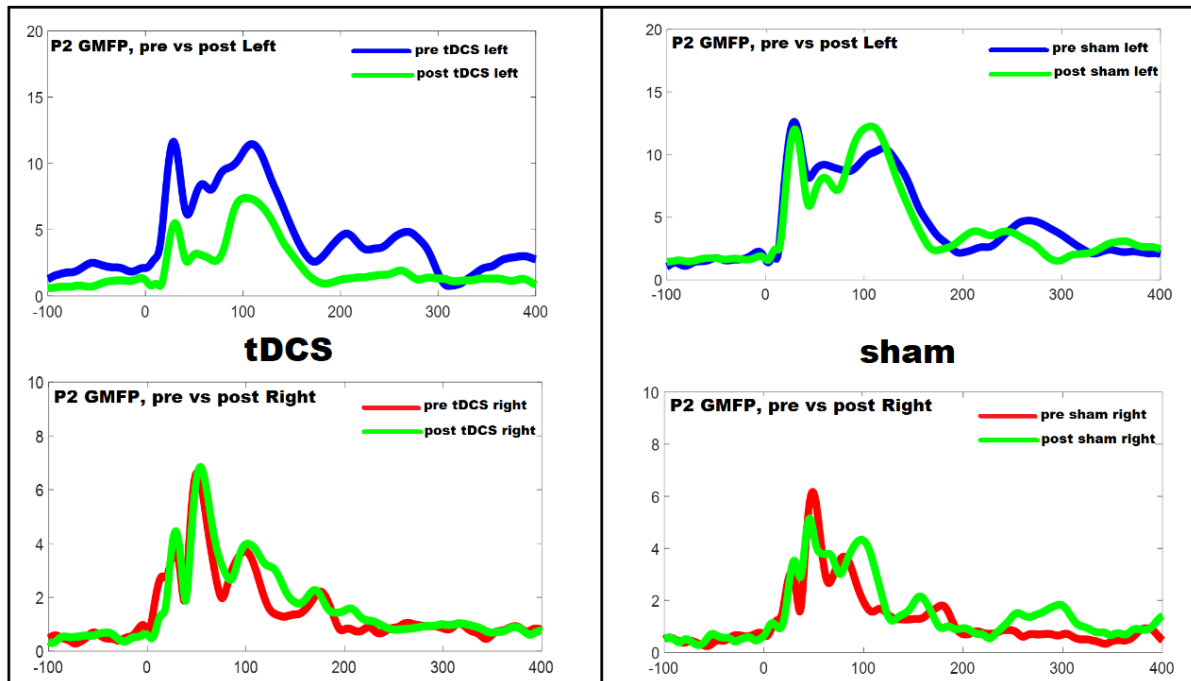
Figure 17b. P2 GMFP, comparing left vs right hemispheres, during the four evaluation time points (pre/post, tDCS sham). It can be notice that especially after real tDCS, on the left hemisphere, the difference between the two hemispheres was reduced (the blue and red lines almost overlap).
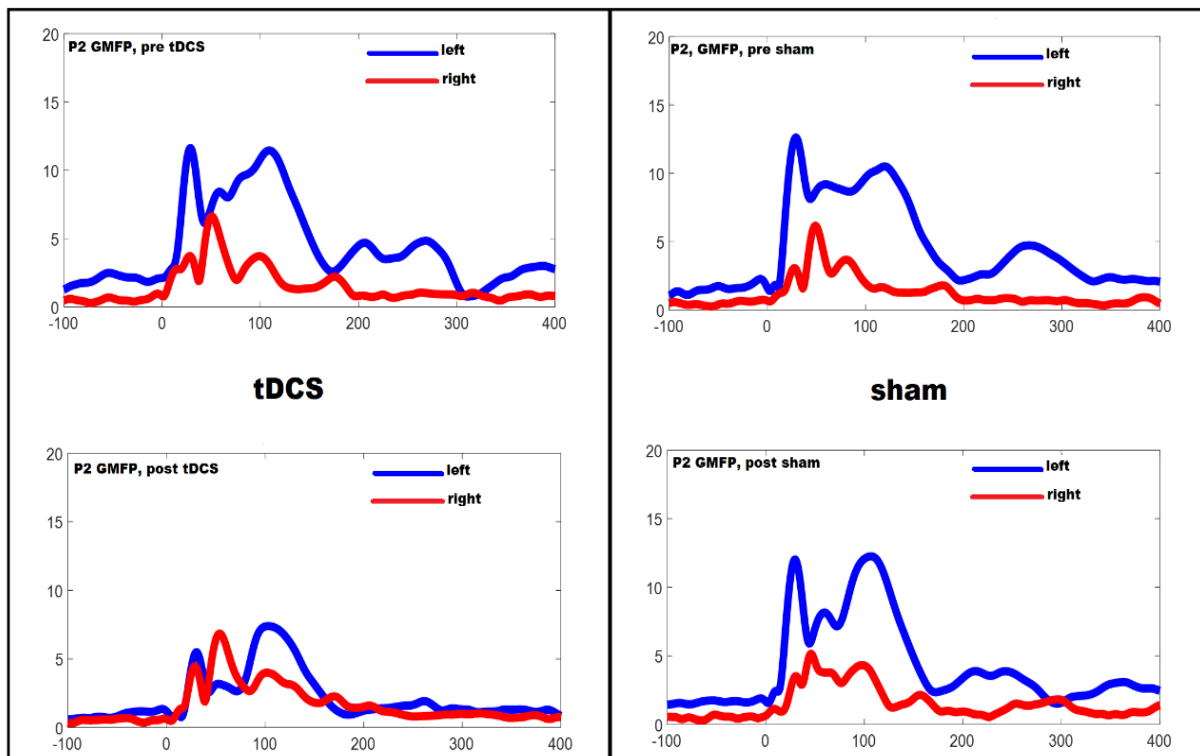
Figure 17c. GMFP P2 left vs right pre/post tDCS and sham stimulation; GMFP mean and SD of the control group
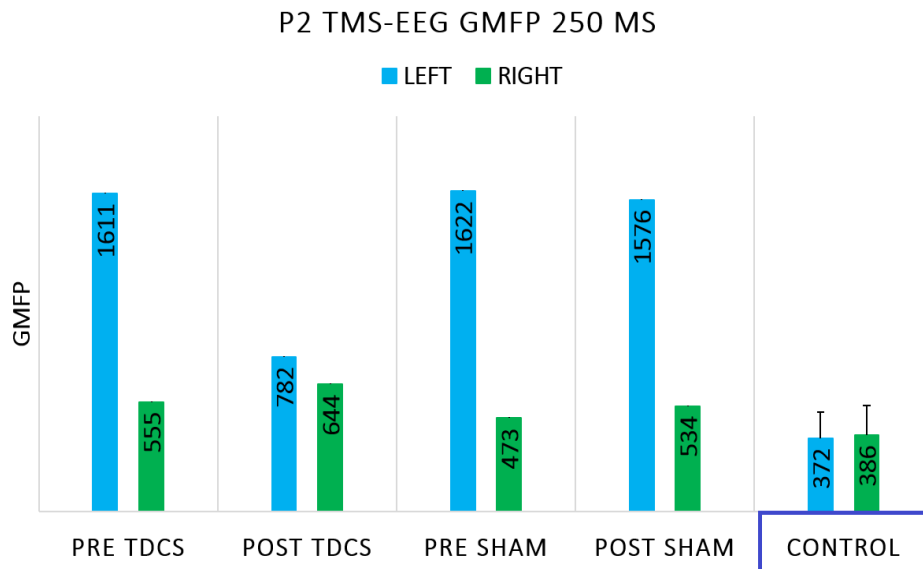
For the **LMFP** (channels: left Cz, FCz, C1, FC1; right Cz, FCz, C2, FC2), the Bayesian analysis also provided moderate support in favor of the alternative hypothesis, mirroring the results observed for the GMFP. Namely, the left vs right LMFP difference was larger for P2 than for the control group, and it decreased after real tDCS stimulation, (Figures 18a and 18b): pre tDCS (BF_{10} = 5.09; CI: -45 / 41); post tDCS (BF_{10} = 4.96; CI: -40 / 20); pre sham (BF_{10} = 4.63; CI: -36 / 30); post sham (BF_{10} = 5.36; CI: -32 / 40).

Figure 18a and Figure 18b. P2 butterfly plot of the TEPs recorded at all 62 electrodes for the perilesional stimulation site (in blue) and contralesional (red), after real tDCS (18 a) and after sham (18b). The four EEG electrodes closest to TMS, and used to calculate the LMFP, are displayed in color. The two crosses indicate the position of the coil focus over the scalp.

Figure 18a tDCS stimulation
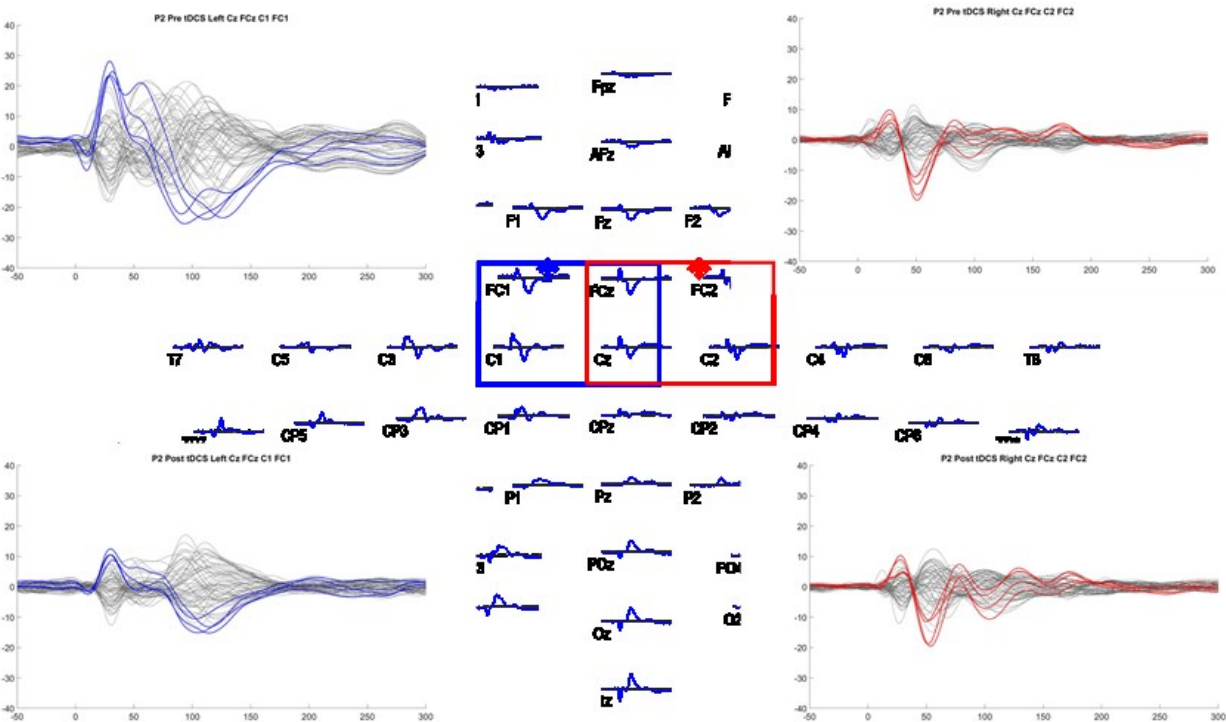
Figure 18b. LMFP sham stimulation
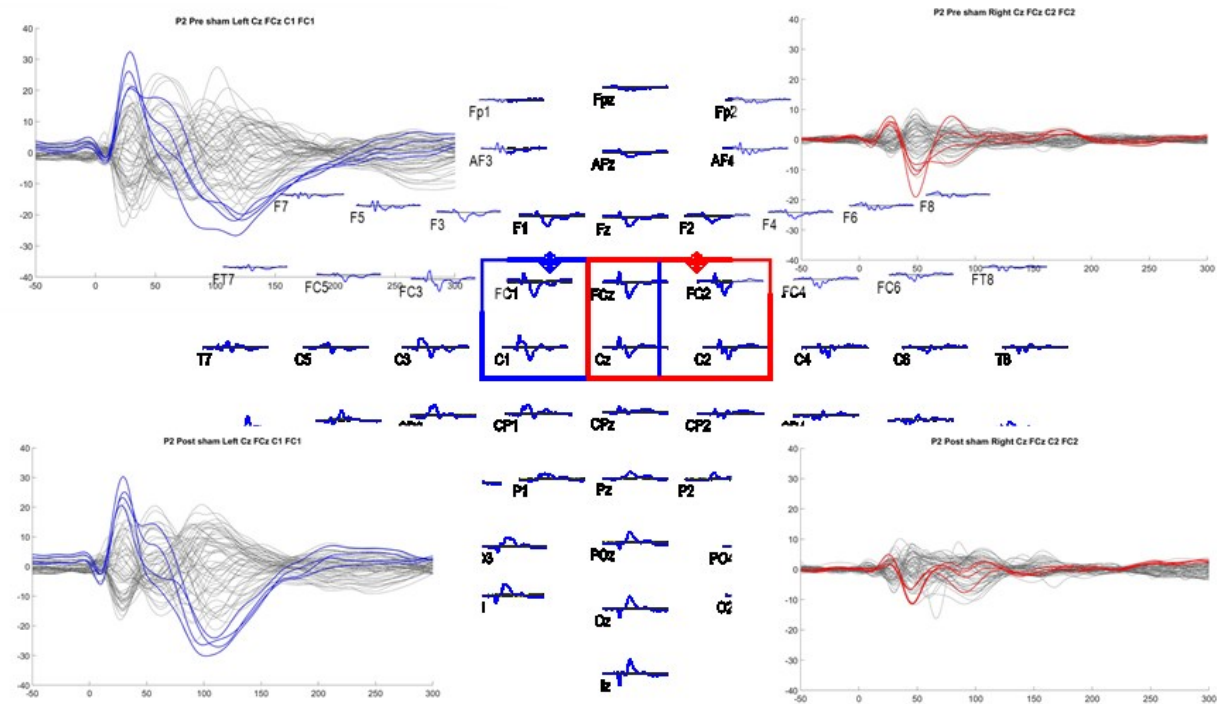
Bayesian analyses run on PCI^{ST} (F5, F3, F1, Fz, FC5, FC3, FC1, FCz, C5, C3, C1, Cz, CP5, CP3, CP1, CPz, P3, P1, Pz on the left and F6, F4, F2, Fz, FC6, FC4, FC2, FCz, C6, C4, C2, Cz, CP6, CP4, CP2, CPz, P4, P2, Pz on the right hemisphere) *and Natural frequency* (channels: left Cz, FCz, C1, FC1; right Cz, FCz, C2, FC2) indicated inconclusive results (Figure 19). TDCS did not significantly modulate these parameters. Specifically, when comparing P2 with the control group, the PCI^{ST} interhemispheric difference was: pre tDCS - BF_{10} = 1.08; post tDCS - BF_{10} = 0.91; pre sham - BF_{10} = 0.85; post sham - BF_{10}= 1.28; and *Natural frequency* was: pre tDCS - BF_{10} = 2.08; post tDCS - BF_{10} = 2.79; pre sham - BF_{10} = 2.51; post sham - BF_{10}=2.73.

Nevertheless, it should be noted for this patient that PCI^{ST} values after the real tDCS were very similar between the left and right stimulation, while in all other conditions it remained fairly unbalanced.

Figure 19. PCI^{ST} and Natural frequency: P2 left vs right pre/post tDCS and sham stimulation; PCI^{ST} and Natural frequency mean and SD of the control group
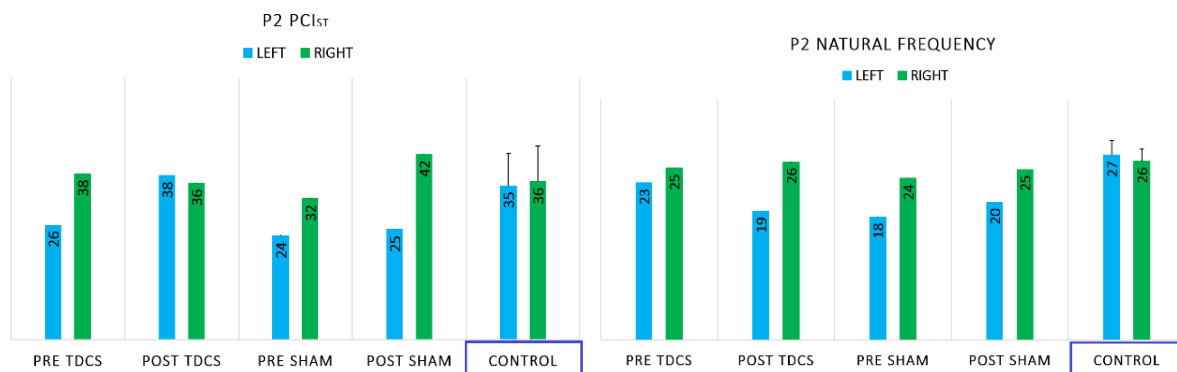
PCI^{LZ} Bayesian analysis, although inconclusive, was near the threshold supporting the null hypothesis as no significant difference between the left vs right hemisphere response (between P2 and the control group) was revealed: pre tDCS - BF_{10} = 0.52; post tDCS - BF_{10} = 0.56; pre sham - BF_{10} = 0.39; post sham – BF_{10}=0.38.

Overall, the behavioral data indicate a slight improvement after real stimulation for the B.A.D.A., phonemic and semantic verbal fluency, and the number of words produced during spontaneous speech.

The neuropsychological data are consistent with this patient's TMS-EEG data. Firstly, we observed an important interhemispheric difference, especially for the GMFP and the LMFP. Similar to P1 data, TEPs

obtained from the stimulation of the perilesional site were characterized by slow EEG response and lower PCIST values. The tDCS stimulation effects on P2 support our hypothesis; after real tDCS, the GMFP and the LMFP difference between the two hemispheres were slightly diminished, while no changes were observed after sham stimulation. In addition, the PCIST values also increased after real tDCS.

4.3.3. P3.

P3 was a 68-year-old man with global aphasia. Now retired, he formerly worked as a high school teacher. He has the support of his extended family (a nephew) as he does not have a spouse nor children, and he spends the majority of his days alone, working in the vineyard. He suffered a left temporal ischemic stroke 73 months prior to the experiment (Figure 20). Since then, he has received two cycles of speech and language therapy, and although his linguistic capacity remains generally stable (minimally improved), his non-verbal communication skills have improved significantly. Spontaneous speech is absent, but contextual comprehension was largely preserved. Besides aphasia, the patient was also diagnosed with apraxia (ideative, ideomotor and buccofacial apraxia) and executive functions difficulties. Attention, visual memory, and logic-deductive reasoning on non-verbal material were preserved. The SADBD and BDI-II scores were in the normal range.

P3 stimulation started with sham, and the TMS intensity was 85 % of the Magstim Rapid[2]. As already explained, the TMS-EEG data of P3 did not have the necessary quality to be analyzed.

Figure 20. P3 MRI, lesion in the MCA territory.
(MRIcron and MRIcroGL visualization Software, https://www.nitrc.org/projects/mricron).
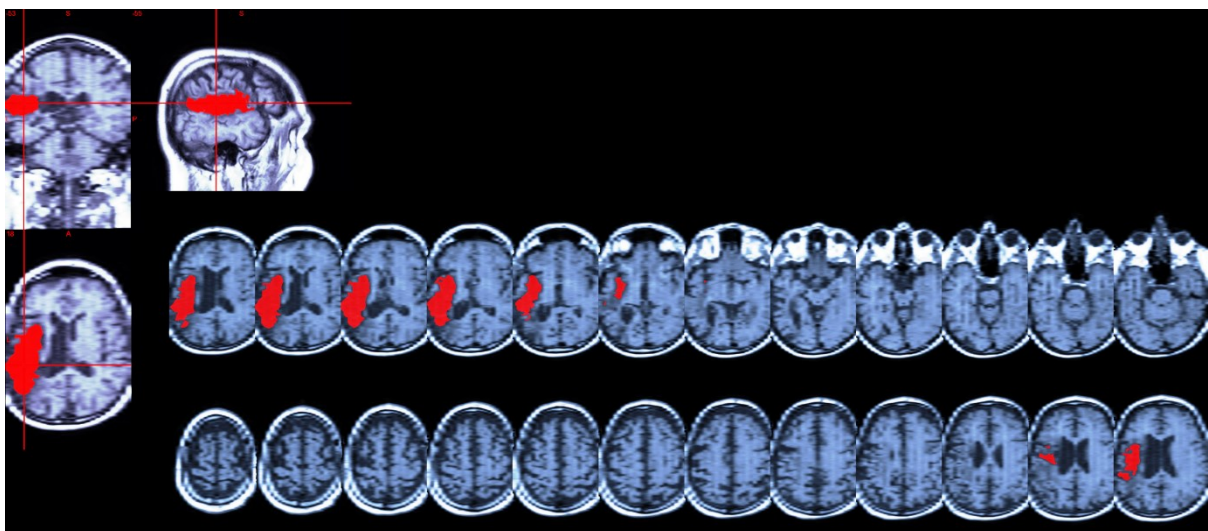
i) Behavioral data:

B.A.D.A.: P3 did not improve after tDCS stimulation, and actually, a slight decrease in performance can be observed. A hypothesis could be that he might have been too severely impaired to obtain any improvement (Figure 21).

Figure 21. P3 B.A.D.A. results, pre/ post tDCS, and sham (error bars are SD).
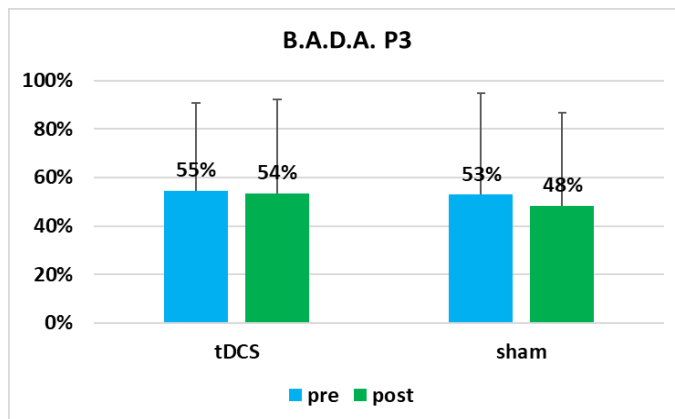
Verbal Fluency: P3 produced just one word after real tDCS and after sham during the semantic fluency; no words were produced on phonological cue.

Spontaneous speech revealed an increase in the number of total words and nouns in both conditions (Figure 22) but especially after real tDCS (11 words). He did not produced sentences. A learning effect could have contributed to this result (this patient started with sham stimulation); however, different pictures were used.

Figure 22. P3 Spontaneous speech results: pre/ post tDCS and sham
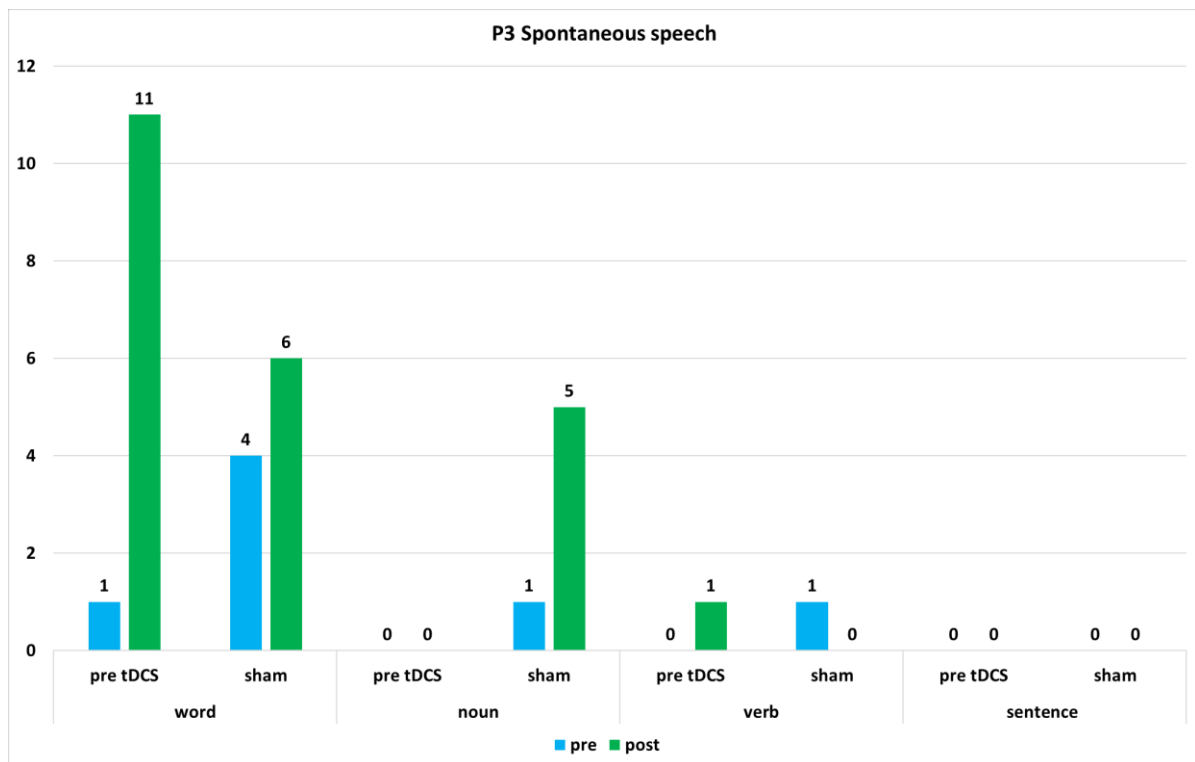
Overall, the patient produced more words during spontaneous speech, while the B.A.D.A. and the verbal fluency remained unchanged.

4.3.4. P4.

Patient P4 was a 70-year-old man. He suffered from global aphasia and right hemiplegia due to an ischemic stroke (June 2009) that affected extensive areas of the left hemisphere (fronto-temporo-parietal cortico-subcortical regions) (Figure 23). He was also diagnosed with apraxia (ideative, ideomotor, and buccofacial apraxia). His behavior is characterized by impulsiveness, perseveration, and rigidity. The patient underwent different cycles of motor and speech therapy rehabilitation before this study. The language improvement was limited. Spontaneous speech is limited almost exclusively to yes / no answers, neologisms, and onomatopoeic sounds. He usually fails to provide a verbal answer in naming tasks but mimics the target, showing a correct comprehension of the stimuli. The patient is unable to repeat words presented by the experimenter (he is also unable to produce or repeat individual phonemes). However, non-verbal communication is preserved, thanks to facial expressions

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and gestures. The comprehension of simple stimuli (in familiar contexts) is preserved. He lives in a

protected residence and is supported by an assistant. He remains autonomous and keeps himself busy

by going on short trips on his motorbike for disabled people.

P4 started with sham stimulation, and the TMS intensity was 95% of the Magstim Rapid[2].

Figure 23. P4 TC scan (22/07/2010), lesion in the MCA territory.
(MRIcron and MRIcroGL visualization Software, https://www.nitrc.org/projects/mricron).
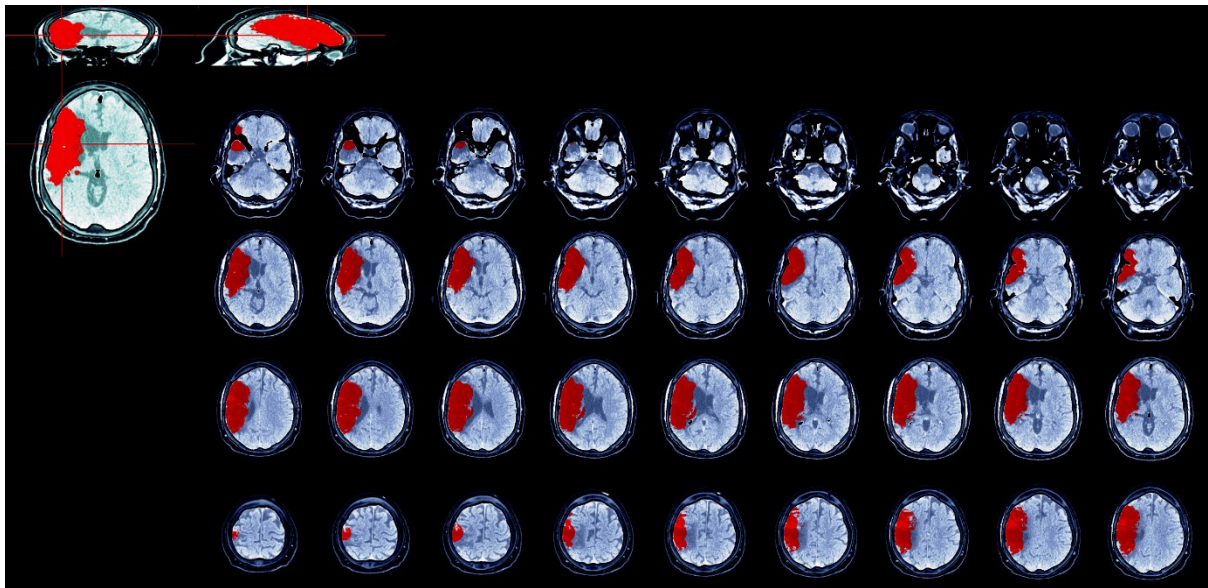
i) Behavioral data:

B.A.D.A.: P4 improved to some degree after sham stimulation, the first phase of his treatment, from

35% to 37%, but remained stable afterward (Figure 24).

Figure 24. P4 B.A.D.A. results, pre/ post tDCS, and sham (error bars are SD).
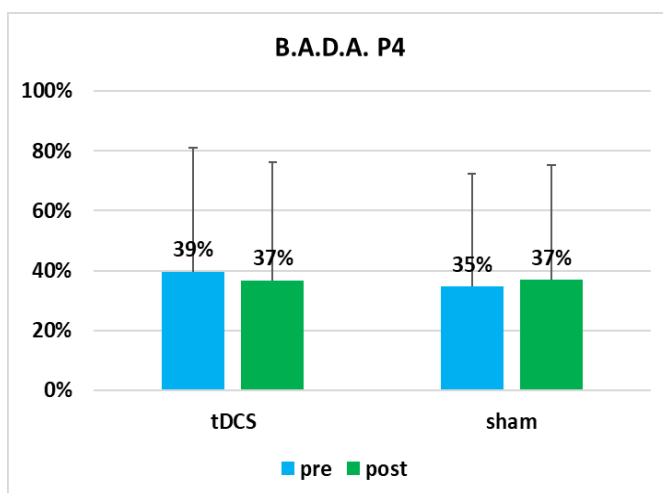
No changes were observed in the **verbal fluency** ability and **spontaneous speech**. During the four evaluation time points, the patient produced one word during the semantic (post sham) and one word during the phonemic fluency (pre tDCS). He described the picture using gestures and onomatopoeic sounds but without using actual words.

ii) TMS-EEG data:

GMFP: The Bayes factor, as for the previously described patients, provided moderate support in favor of the alternative hypothesis. The left-right GMFP difference was larger for the clinical case, as compared to the control group (Figure 25a, 25b), pre tDCS (BF_{10} = 5.54; CI: -28 / 37); post tDCS (BF_{10} = 5.07; CI: -20/ 30); pre sham (BF_{10} = 4.89; CI: -36 / 53); post sham (BF_{10} = 4.82; CI: -52 / 64).

We can observe from the CIs that there were no significant changes between the stimulation conditions since all CIs overlap to a certain degree. In Figure 25a, there is a visible decrease of the GMFP after active tDCS on the left hemisphere, reducing the GMFP interhemispheric difference. We also noticed a relatively small decrease in the GMFP post sham on the right hemisphere, but it is not clear why this change occurred.

Figure 25a. P2 GMFP, confronting pre vs post tDCS and sham stimulation (on each hemisphere).
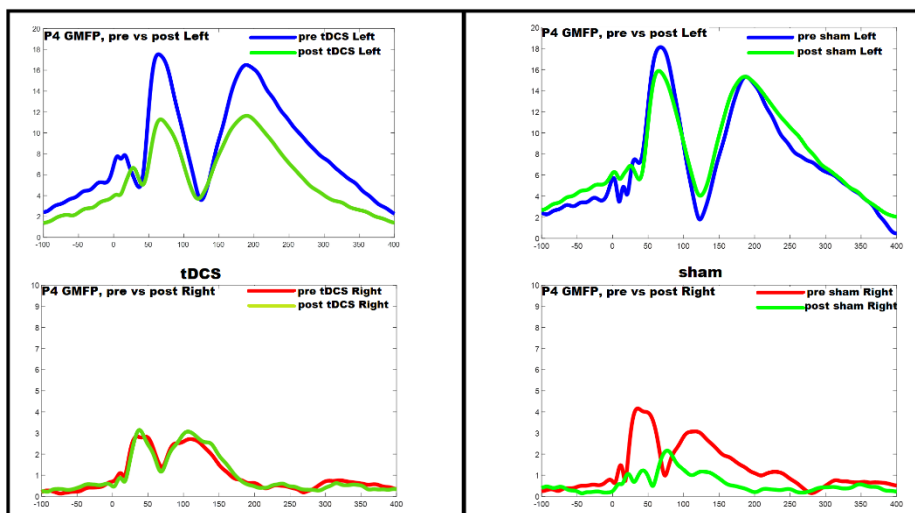
Figure 25b. GMFP P4 left vs right pre/post tDCS and sham stimulation; GMFP mean and SD of the control group.
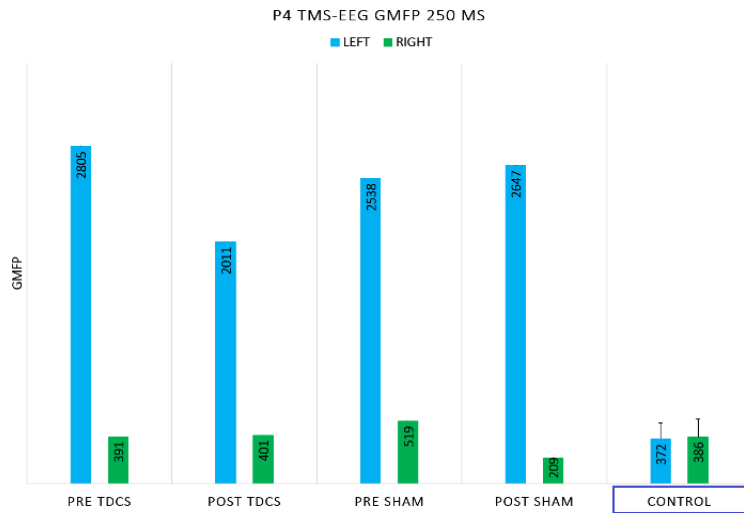
The **LMFP** (channels: left Fz, FCz, F1, FC1; right Fz, FCz, F2, FC2) Bayesian analysis indicated that the left vs right LMFP difference was larger for P4 than recorded in the control group (Figures 26a and 26b); pre tDCS (BF_{10} = 5.23; CI: -40 / 39); post tDCS (BF_{10} = 4.76; CI: -99 / 28); pre sham (BF_{10} = 5.43; CI: -47 / 44.9); post sham (BF_{10} = 4.80; CI: -26 / 23).

Figure 26a – tDCS stimulation and Figure 26b – sham stimulation. Butterfly plot of the TEPs recorded at all 62 electrodes for the perilesional stimulation site (in blue) and contralesional (red). The four EEG electrodes closest to TMS (and used to calculate the LMFP) are displayed in color. The two crosses indicate the position of the coil focus over the scalp. Note that the y-axis of the left (lesioned hemisphere) is double (from 0 to 40 microvolt) as compared to the right one (from 0 to 20 microvolt).

Figure 26a – tDCS stimulation
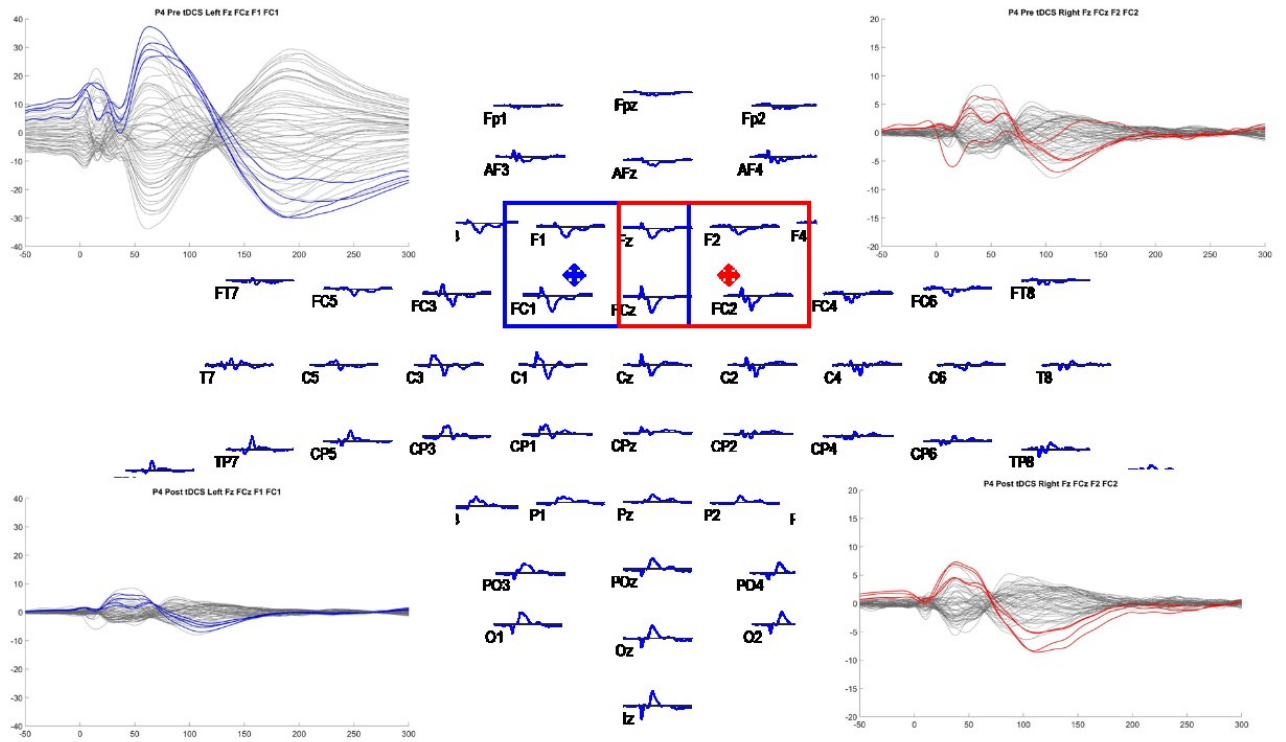
Figure 26b – sham stimulation
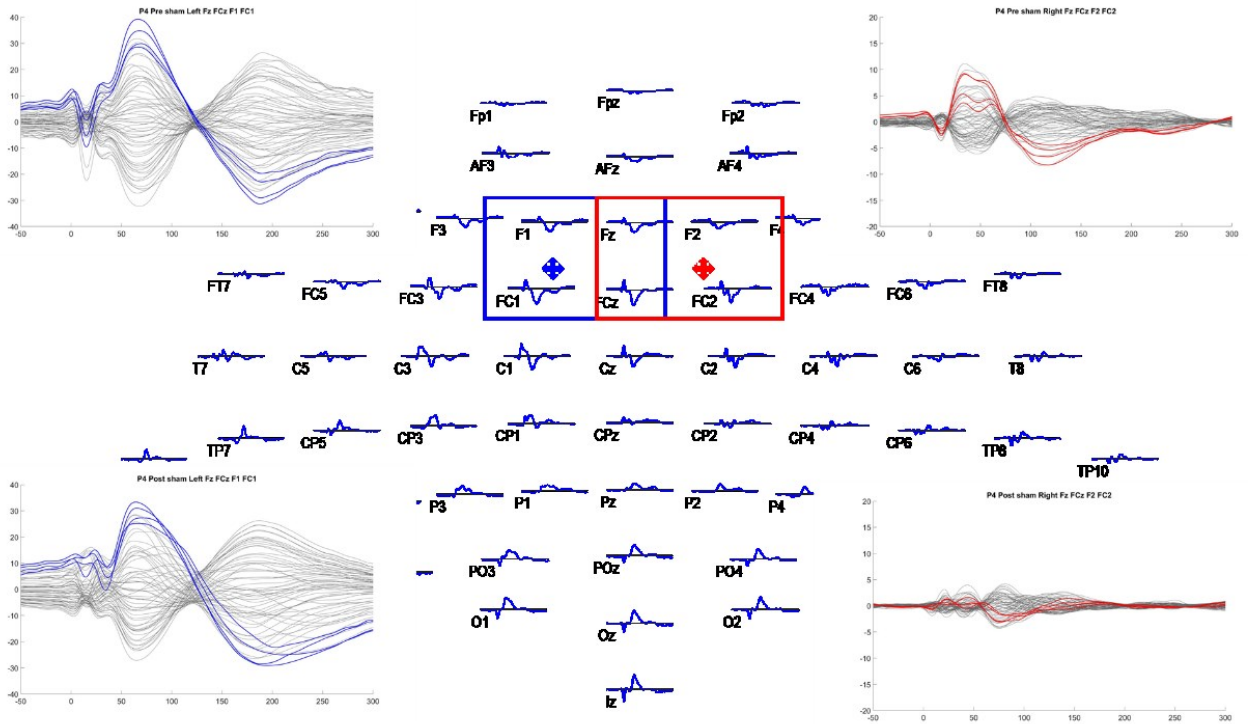
Bayesian analyses run on *PCIST* (F5, F3, F1, Fz, FC5, FC3, FC1, FCz, C5, C3, C1, Cz, CP5, CP3, CP1, CPz, P3, P1, Pz on the left and F6, F4, F2, Fz, FC6, FC4, FC2, FCz, C6, C4, C2, Cz, CP6, CP4, CP2, CPz, P4, P2, Pz on the right hemisphere) *and **Natural frequency*** (left hemisphere Fz FCz F1 FC1 and right Fz FCz F2 FC2) showed inconclusive results. TDCS did not significantly modulate these parameters. Specifically, the observed interhemispheric differences between P4 and the control group were: ***PCIST*** pre tDCS - BF$_{10}$ = 1.14; post tDCS - BF$_{10}$ = 0.96; pre sham - BF$_{10}$ = 0.61; post sham - BF$_{10}$= 1.19; and ***Natural frequency***: pre tDCS - BF$_{10}$ = 2.64; post tDCS - BF$_{10}$ = 2.66; pre sham - BF$_{10}$ = 2.73; post sham - BF$_{10}$=2.11.

While the PCIST values reflected the interhemispheric imbalance, the Natural frequency values were quite balanced between the two hemispheres despite the lesion, but inferior to those observed in the control group.

Figure 27. PCIST and Natural frequency: P4 left vs right pre/post tDCS and sham stimulation; PCIST and Natural frequency mean and SD of the control group.
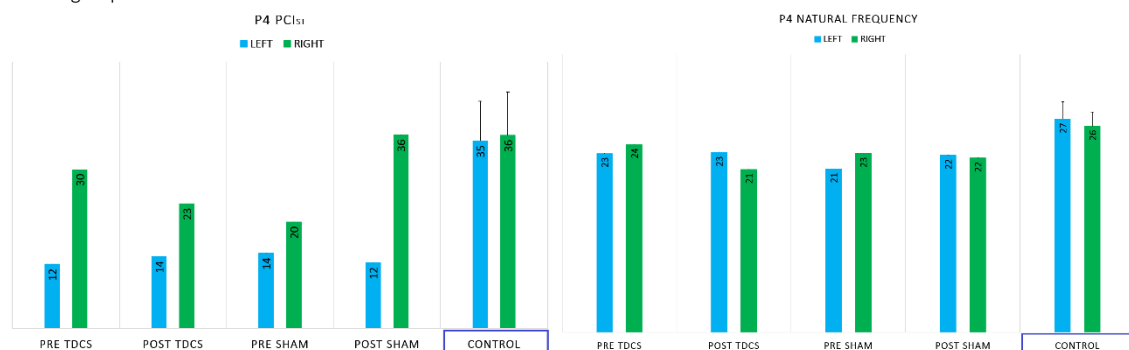
PCI^{LZ} Bayesian analysis, also inconclusive, was in line with the data observed in the previously described patients, i.e., no significant difference between the left vs right hemisphere responses (between P4 and the control group): pre tDCS - BF$_{10}$ = 0.46; post tDCS - BF$_{10}$ = 0.49; pre sham - BF$_{10}$ = 0.57; post sham - BF$_{10}$=0.26.

Summing up, despite the aphasia severity, P4 was engaged and cooperative during the entire experiment. No complaints were made by him or by his caregiver.

Regarding the behavioral data, an extremely small variation in the B.A.D.A. was observed after sham, while all other parameters remained unchanged. Of all participants, P4 least benefited from tDCS.

As hypothesized, an important interhemispheric difference was recorded, especially for the GMFP and the LMFP. TEPs obtained from the stimulation of the ipsilesional site showed abnormally large values, while TEPs from the contralesional hemisphere were characterized by values closer to the ones observed in the control group. The tDCS stimulation effect was similar to that observed in P2; namely, after real tDCS, the GMFP and the LMFP difference between the two hemispheres was slightly diminished. The Natural frequencies and PCI[LZ] values did not significantly differ from the control group.

As previously detailed, other neuropsychological tests were used in this project as possible covariates, i.e., although not part of the main experimental manipulation, they could have had an effect on the dependent variable. For example, the increase in language performance might have correlated with attention or executive function improvement given that the tDCS area was fairly anterior. To this aim, means, and standard deviations for Attentive Matrices (Spinnler & Tognoni, 1987), Raven's Coloured Progressive Matrices (Measso et al., 1993), WEIGL' sorting test (Weigl, 1941), Forward and backward digit span (Monaco et al., 2013), were computed.

Forward and backward digit span could not be administered to P3 and P4, because of aphasia severity, as well as backward digit span to P1 because he failed to understand the task. As can be observed from Figure 28, no detectable differences could be found between real and sham stimulation in the previously mentioned tests.

Regarding SF-36 and tests used for depression, no relevant changes were recorded during the four evaluation moments.

Figure 28: Neuropsychological tests, mean and standard deviations during the four testing points.
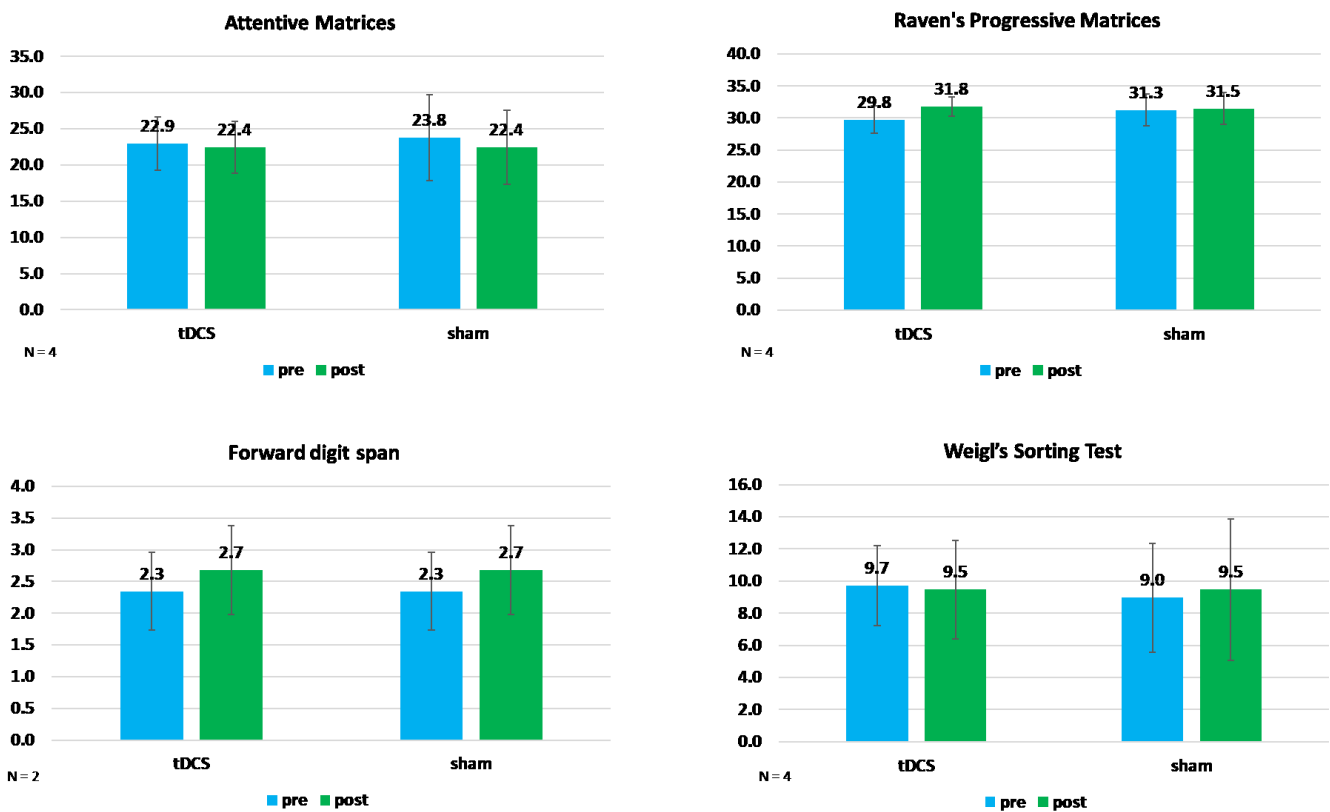
4.4. Discussion

The current perspectives on the loss and recovery of language consider that language is organized in complex networks, extending beyond the area of structural damage (Fridriksson et al., 2018; Hickok & Poeppel, 2007). Promoting the recovery of linguistic function and optimizing rehabilitation strategies for stroke patients should take into account also individual differences (Iorga et al., 2021; Stockert et al., 2020). Due to the heterogeneity of results on language recovery after tDCS coupled with SLT, discussed in chapters two and three, clear predictions, with respect to the most promising stimulation parameters to facilitate language recovery, are difficult to state at present. As tDCS continues to be employed in cognitive and clinical neuroscience, there is an increasing need to (i) provide objective and quantifiable evidence of its effects within interconnected brain areas and (ii) detect indices that could suggest how to effectively promote recovery in individual patients.

TMS-EEG technique has a demonstrated capacity to assess the neurophysiological state of the cortex, providing all at once information about various processes including cortical reactivity, local excitation

and inhibition, oscillatory activity, effective connectivity, and neural plasticity (Kallioniemi et al., 2018, 2022). It is generally accepted that TEPs provide a read-out of the neuronal properties of the damaged area while simultaneously assessing the integrity of the entire functional network (Sato et al., 2015; Tscherpel et al., 2020). As mentioned in the introduction, different studies, quantifying TMS-EEG responses in the time and time-frequency domain, found that compared to healthy controls or to data recorded from the unimpaired hemisphere, stroke patients exhibited perilesional sleep-like responses, i.e., slower and simpler TEPs, suppression of high frequencies, and off-periods (Gray et al., 2017). This simple and slow TMS-evoked response with high amplitudes was also associated with a local disruption of signal complexity (PCI^{ST}) (Casarotto et al., 2016; Gray et al., 2017; Rosanova et al., 2018; Sarasso et al., 2020; Tscherpel et al., 2020). However, the majority of publications focused on the motor area (Pellicciari et al., 2018; Tscherpel et al., 2020), therefore, less is known about post-stroke language impairment.

In this pilot crossover study, patients underwent 20 sessions of 20-min anodal tDCS and 20 sessions of 20-min sham stimulation, coupled with linguistic tasks, over the perilesional Broca's area. This stimulation protocol is considered the most promising and often used (Elsner et al., 2020; Küçükdeveci, 2020).

Although the meta-analysis results (Chapter 3.2: A systematic review of noninvasive brain stimulation for post-stroke aphasia) indicated a medium to large rTMS effect and a small to medium tDCS effect, we decided to apply the tDCS stimulation (coupled with language training) for two reasons:

(i)   The first one is related to the TES characteristics, specifically by having a relatively low cost and by being easily operated, if we can gain considerable knowledge on the instrument neurophysiological effects and identify effective stimulation protocols, tDCS could be used by speech therapists during SLT sessions or by patients at home. This direct and fast applications is less feasible for TMS stimulation due to the machine characteristics and economical cost.

(ii)   The second reason refers to the previous publications which investigated the tDCS effects on the language network using TMS-EEG coregistration on healthy and aphasic participants (e.g., Pisoni et

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al., 2018; Cipollari et al., 1015) which offered a theoretical background and a starting point for this project, helping us to decide the TMS-EEG stimulation parameters and setting.

We conducted concurrent TMS and EEG measurements and language assessment at four time points (pre/post anodal tDCS and sham coupled with linguistic tasks). TMS was applied over the ipsilesional and contralesional BA6, as previously performed by Pisoni et al. (2018) on healthy participants.

The nature of this study should be considered explorative since the pandemic prevented testing of additional patients. The aim was to assess:

(i) whether the abnormal TMS-EEG responses reported in the literature on stroke are also reflected in chronic PWA as measured by GMFP, LMFP, PCIST, and natural frequency;

(ii) whether tDCS can modulate the mentioned parameters in an effective way and whether these changes can be related to the performance in linguistic production.

The ultimate goal, which goes beyond the scope of the present work but toward which this research may contribute, is to detect novel pathophysiological markers with practical implications concerning tDCS treatment indications and the prediction of possible outcomes.

The results of the neuropsychological assessment revealed a complex pattern; specifically, out of four patients, two improved to a certain degree in all the evaluated language production tasks while two showed a relatively stable condition regardless of the stimulation type. Based on the previous literature (Bucur & Papagno, 2019; Shah-Basak et al., 2016), our hypothesis was that there would be an improvement in linguistic production after anodal stimulation as compared with sham, but this hypothesis was only partially confirmed.

While **P1** and **P2** performance on the B.A.D.A. increased after sham, tDCS had a stronger effect. Instead, **P3** and **P4 showed** a slight decrease, which can be related to aphasia severity as they were both diagnosed with global aphasia. This mild negative change was also noticed after sham.

Considering **semantic fluency**, an increase in the number of words was recorded independently from the experimental condition, while **phonemic fluency** was further boosted by the real tDCS stimulation in P1 and P2. An increase in verbal fluency after anodal tDCS stimulation was previously obtained in

healthy participants (Pisoni et al., 2018), and the behavior data were related to an increase of both LMFP and GMFP (triggered from left BA6, the same area used in this experiment).

Of all the measures, **spontaneous speech**, based on the correct number of words and sentences (number of sentences and average length) used to describe a picture, can be considered the most ecological form of evaluation (among the tests that were employed in this study). Except for P4, who used gestures to describe pictures, all other participants improved and performed better in this task after real tDCS, as indicated by the number of words, and for P1 and P2 also by the number of sentences. This is true even for P3 whose performance was not enhanced in the other tests, and who started with sham stimulation.

By chance, the less impaired participants, P1 and P2, started with real stimulation, and this complicates the interpretation of the results; we cannot claim that a similar amount of change would have been obtained if they had started with sham stimulation. Nevertheless, in my opinion, the order and the learning effect should not be relevant variables in this specific study knowing that this research included patients with chronic aphasia (years have passed since the stroke and before the experiment, the PWA underwent multiple speech rehabilitation interventions). Specifically, if the aphasic patients would learn so fast (one repetition) and retained so well the information, a month or three months later (our test-retest interval), the speech and language therapy would have been more effective for the PWA, which is not the case for our participants

Given the duration of this experiment (over 5 months for each participant), it is not possible to control additional variables: for example, how much support does each participant get from family members, or was perhaps a "bad day" the testing day? For this reason, I decided to avoid any speculation on other possible variables related to an increase/decrease in performance since it is impossible to correctly identify and control them.

We noticed that the results recorded after tDCS were maintained after 3 months (when we started the second part of the experiment). This trend was observed for the B.A.D.A., verbal fluency, and spontaneous speech although greater variability can be noticed. These results corroborate the data

from our meta-analysis, namely that the observed results after tDCS, even if sometimes characterized by a small effect size, are maintained over time. Although from a quantitative point of view changes observed in linguistic production might seem modest, we would like to point out that these results were obtained from chronic patients (years having passed since the stroke) that before the experiment underwent multiple speech rehabilitation interventions.

It is important to note that the two patients who did not improve had a more severe type of aphasia, namely global aphasia with only a spared contextual comprehension at baseline and they had not improved even after previous speech and language therapy sessions. Aphasia severity, often correlated with overall lesion volume, is one of the few variables that has been identified as a reliable predictor of aphasia recovery; specifically, more severe patients benefit less from SLT (Breitenstein et al., 2017; Fridriksson & Hillis, 2021). Taking into consideration that P3 and P4's lesions involved not only extended left frontotemporal cortex areas but also subcortical white matter tracts, we hypothesize that the linguistic network was too damaged in order to obtain an efficient tDCS modulation of the extended linguistic network.

Another possible explanation might be related to the stimulation parameters. The interhemispheric competition (the affected hemisphere suffers from the undamaged hemisphere abnormal inhibition) and vicariation models (the unaffected hemisphere has a compensatory function) of stroke recovery lead to contrasting conclusions about the best tDCS stimulation parameters. Should one inhibit the right hemisphere while stimulating the left one or, conversely, should one further increase the right hemisphere activity to better compensate for the left areas damage? Di Pino et al. (2014) integrated the interhemispheric competition and vicariation models of recovery, which they consider oversimplified and not adequate for all stroke patients, in a more elaborated model named the "bimodal balance–recovery model." This theoretical perspective takes into account the individual residual network. Extrapolating the authors' claims from the motor to the language functions, although a better recovery is correlated with left area MRI activations (Saur, 2006), when the damage is extremely extensive and the remaining structural reserve is insufficient, the only alternative to support

aphasia recovery might be the homologue right areas. Therefore, it makes sense to assume that for patients with global aphasia and extensively damaged areas, a different montage approach, stimulating the contralesional site with anodal tDCS, could be more effective (Cipollari et al., 2015). It is also necessary to underline the fact that the linguistic exercises during tDCS were identical for all participants, i.e., no individual speech therapy was performed. Although, at the behavioral level, results suggest a null effect of anodal tDCS in two cases (out of four), further corroborating evidence from different approaches is necessary to support such a negative conclusion. In addition, even when no linguistic improvement was revealed by our tests, a cortical excitability modulation was present.

The TMS-evoked EEG responses were quantified in the time and time-frequency domain. Specifically, five different parameters were analyzed: GMFP, LMFP, PCI^{ST}, PCI^{LZ}, and Natural Frequency (computed as the normalized average frequency of the power spectrum). As specified in chapter two, cortical response to TMS depends on the neural activation state as well as on the synchronous activation of the neural populations (Lehmann & Skrandies, 1980). Each of these measures, recorded from the patients, was compared with the data recorded on the control group using Bayesian multilevel single case models (Scandola & Romano, 2021).

We found evidence for substantial alterations of the TMS-evoked EEG response over the ipsilesional hemisphere for both local (LMFP) and diffused effects (GMFP) (Romero Lauro et al., 2014). Despite small variations, all the results of the Bayesian analysis converge to suggest that, compared to the control group, the discrepancy between the left and right hemisphere, especially concerning LMFP and GMFP, was higher in PWA. Furthermore, this disequilibrium was modulated by anodal tDCS, i.e., the GMFP and LMFP shifted after tDCS when the TEPs were recorded from the left hemisphere. No changes were detected when sham tDCS was delivered, indicating that the modification in cortical excitability recorded after real tDCS sessions was due to an interaction between the neurophysiological modulation and cortical activity elicited by the linguistic tasks (and not by the tasks alone). Since the tDCS active electrode was located near F7, F5 electrodes (Broca's area) and TMS pulse was delivered over BA6 (between C1 and FC1, 10-20 EEG system); both global and local indexes of cortical excitability show

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that the effects of tDCS are widespread rather than being restricted to the area underneath the active tDCS electrode. Of course, as previously argued by (Pisoni et al., 2018), although distant, these two areas are functionally connected (especially during verbal fluency tasks). Additionally, Pisoni and colleagues (2018) found an increase of the LMFP and the GMFP after active stimulation, while in our case, the direction of the change is not linear since in P1 there was an increase in the GMFP and LMFP while in P3 and P4, a decrease and a better balance between the two hemispheres. A reasonable explanation could be the fact that P1 was a "corrected left-handed" person, suggesting that language lateralization could differ from the other two patients. It is important to notice that in Pisoni et al.'s (2018) experiment, TMS-EEG data were recorded immediately after tDCS while in the present study recording took place at least 48 hours after the last tDCS or sham session. This implies that, in this case, neurophysiological data are based on long-term tDCS mechanisms.

For each patient, the PCIST was then estimated, i.e., the complexity of the principal components of the EEG response to TMS, as described in Comolatti et al. (2019). PCIST values were lower on the damaged hemisphere without reaching a critical level, namely without being statistically different from the ones observed in the control group, as revealed by the Bayes factor that, remaining under the value of 3, was not informative. PCIST was modulated by anodal tDCS only in one case (P2); specifically, the two hemispheres had similar values after real tDCS. P2 was the patient with the less severe aphasia and she improved after real stimulation in all the behavioral tests. In a recent publication, Sarasso et al. (2020) reported that TMS triggered, in stroke patients, perilesional sleep-like responses that were associated with a local disruption of signal complexity. This experiment data do not show similar results, but it is important to note that in Sarasso's experiment, TMS was applied over the perilesional areas, i.e., the authors reported a local disruption of signal complexity, while in the present case the stimulation was more distant from the lesion site.

Following stroke, the natural frequency (which reflects the brains electrical oscillations at different frequencies) was found to be significantly slower in the ipsilesional hemisphere as compared to the contralateral areas or to healthy control participants (Ding et al., 2022; Tscherpel et al., 2020). For

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instance, Ding et al. (2022) investigated the aftereffects of intermittent theta burst stimulation (iTBS) on patients with upper limb motor deficit due to stroke. They reported that at baseline, the natural frequency was slower in the ipsilesional compared to the contralesional hemisphere and provided evidence that iTBS increases natural frequency. Although the mechanisms underlying the post stroke natural frequency alterations remain undetermined, it is hypothesized to be the result of a stroke-related disruption of thalamocortical connections, i.e., a structural disconnection (as thalamocortical neurons are considered to be involved in generating fast oscillations) (Ferrarelli et al., 2012; Tscherpel et al., 2020). A different theory links the natural frequencies post stroke changes to a reduction in GABA-mediated intracortical inhibition, that although initially might promote neural plasticity, in the long run, may hamper stroke recovery (at least of the motor functions) by slowing the natural frequency (Ding et al., 2019; Marconi et al., 2011).

Our current data do not suggest such a disequilibrium between the two hemispheres. More precisely, qualitatively, the natural frequencies are always lower in the damaged hemisphere as compared to the contralateral areas and to the control group, but the Bayes factor was inconclusive although the results are very close to the threshold for significance. Nevertheless, it is important to notice that in this study, unlike other ones, the natural frequency was computed as the normalized average frequency of the power spectrum. The main reason for trying a new explorative analysis method was to get a better representation of the data, since; occasionally we observed more than one activation peak. In the future, the Natural frequency analysis will be done maintaining the formula used in the previously cited papers.

A further assumption was that PCI[LZ] (Casali et al., 2013) would have similar values for PWA and controls due to the fact that they shared a similar level of consciousness, all being awake during the experiment (Casarotto et al., 2016), and the recorded data confirmed this hypothesis.

It is obvious that the small number of subjects prevents us from providing a definite conclusion about the relation between tDCS effects and behavioral data, e.g., it is not possible to decide which information from the TMS-EEG analysis can be considered a better predictor of aphasia recovery and

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tDCS effectiveness. This limitation could be overcome by including a larger sample of subjects. However, in aphasia research, it is very difficult to find a large sample of participants, when strict recruitment criteria are decided and especially so during the COVID-19 pandemic. Despite the small number of PWA, this study can offer important information for future research.

From a technical perspective, it is essential to have the adequate data acquisition instruments because repeated measure registrations after a prolonged period of time (months) might be problematic. Many settings and patient-related variables can change, and it is essential to be able to accurately discriminate brain changes from setting variations. For instance, the simple co-registration of the MRI to the participant's real head can produce a small shift in the TMS position, and, consequently, one can end up stimulating a different neuronal population that, although very close to the initial one, might elicit a different cortical response (Casarotto et al., 2010). In this study, we cannot be 100% certain whether the small decrease observed in the GMFP after sham on the right hemisphere of P4 is related to changes in the patient's cortical excitability, or if during stimulation a small technical parameter shifted without being aware of it, producing the observed result.

A novelty of this study is the use of two new tools: the rt-TEP software that helped us to assess online the initial stimulation parameters and coil position (Casarotto et al., 2022) and the TMS Adaptable Auditory Control –TAAC (Russo et al., 2022). Using TAAC we managed to avoid auditory artifacts except for one participant in the control group that complained of an increased sensitivity to the white noise, so we had to lower the volume under the necessary threshold to mask the TMS click. The auditory artifacts, as shown by Bertazzoli et al. (2021) are time-locked with the TMS pulse and are therefore almost impossible to remove (at least with the present algorithms).

Based on this research experience and previous literature data, some considerations are:

(i) it would be useful to obtain a recent fMRI that could offer information about language reorganization after stroke in order to potentiate the area naturally involved in language recovery after stroke; this way, instead of determining the stimulation area (i.e., the electrode position) a priori, the treatment could be tailored with consideration to the patient's clinical profile (Di Pino et al., 2014);

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(ii) it is necessary to have a computational model of the electrical field for the electrodes montage based on the patient's individual MRI data (Bikson et al., 2012; Datta et al., 2010) in order to avoid a blind stimulation as much as possible, i.e., no information on the current flow.

In conclusion, by combining TMS and EEG, we hereby present a different approach to assess the functional properties of the language network in chronic aphasic patients after tDCS coupled with linguistic exercises, which provides a novel insight into the nature of the electrophysiological consequences of tDCS stimulation in a clinical setting. To our knowledge, this is the first study that, analyzing five different TMS-EEG parameters, investigates at a functional level tDCS effects when applied over perilesional areas in chronic, post-stroke non-fluent aphasia. As mentioned in the introduction, Cipollari et al. (2015) assessed the effect of contralesional (right hemisphere) anodal tDCS with simultaneous MIT training and focused their analysis exclusively on ROI TEPs, near the tDCS stimulation area (F8).

Summing up, although supported by limited data (4 PWA), our results suggest that:

(i) TMS-evoked EEG responses recorded from the ipsilesional hemisphere tend to be abnormal in individuals with chronic post-stroke aphasia as indicated by GMFP and LMFP analysis (the Bayes factor revealed moderate evidence for the alternative hypothesis). Specifically, we observed significant alterations in TEP amplitude and complexity, a pattern very similar to the one reported by Sarasso et al. (2020) on patients with chronic focal and multifocal brain injuries of ischaemic, hemorrhagic, and traumatic etiology, and by Tscherpel et al.(2020) on patients with mild to severe motor deficits at different intervals from the stroke and with different lesion locations. For instance, they showed that TEPs were absent when a TMS pulse targeted the lesion directly; TEPs were simple, similar to those reported in healthy sleeping subjects when recorded over the perilesional areas (especially for severely affected patients) (Tscherpel et al., 2020); and complex, like those found in lesion free awake subjects, when they stimulated the contralesional hemisphere (Sarasso et al., 2020).

(ii) anodal tDCS modulates cortical reactivity recorded from the electrodes close to the TMS hot spot (LMFP, Natural Frequency), from each hemisphere (PCIST), as well as from all 62 electrodes (GMFP).

Although the GMFL, LMFP, and natural frequency post tDCS changes, did not reach a statistically significant threshold, these first observations are promising results, worthy of future investigations.

We also noticed an improvement in the linguistic production performance (in P1 and P2), but further data are needed in order the obtain more direct, strong evidence linking behavioral tDCS effects and neurophysiological data.

We also observed that:

(i) Different activation patterns of the GMFP and LMFP (but not for natural frequency) were registered after real tDCS in P1, namely a slight increase in the local and global excitability. Given that, at a behavioral level, the improvement of P1 was greater after real tDCS, we can only speculate that this result might reflect a different language lateralization pattern (knowing that this patient was a corrected right-hander). Language is one of the most clearly lateralized functions in the human brain. The left hemisphere controls this capacity in 88% to 96% of right-handers, although cases of atypical speech lateralization in the right hemisphere (mainly found in left-handers, between 6.5% to 27%) or bilateral patterns can be found (Van der Haegen & Brysbaert, 2018). In participants with typical language development, early imaging data indicated no relationship between the strength of laterality and language abilities. (Bradshaw et al., 2020). Nevertheless, more recent fMRI results suggest that weak lateralization for language was associated with reduced performance on both verbal and non-verbal tasks relative to individuals showing strong lateralization (Mellet et al., 2014). Therefore, there might also be a relationship between weak language lateralization, language recovery, and tDCS effects on the cortical excitability, which might differ from the one observed in people with strong language lateralization.

It is difficult to find a compelling explanation based on the current literature since tDCS studies specifically target only right-handed participants (to avoid ambiguous results that are hard to pin down) and because left-handed patients with aphasia due to left hemisphere stroke are less common in general. We cannot claim that the change we observed is clinically relevant (given the fact that there was no statistically significant change neither for the GMFP nor for the LMFP), yet further research on

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patients with weak language lateralization or crossed aphasia could still be an important source of information about post-stroke language recovery.

(ii) As previously specified, the two patients with the most severe form of aphasia, global aphasia, did not show improved linguistic performance after 40 days of anodal tDCS and sham coupled with linguistic exercises, as they had not benefit from previous SLT interventions. In this case, the lack of tDCS efficacy is mostly explained by aphasia severity. It is well accepted that the location and degree of damage to language-related brain structures and the stroke impact on the language network will place limits on the neuroplasticity necessary for a successful recovery (Osa García et al., 2020). As previously mentioned, the preferred pathway for aphasia rehabilitation is the recruitment of primarily perilesional areas (in the left hemisphere). This recovery path is possible if patients have enough spared areas to support language rehabilitation (Vines et al., 2011). Jung et al (2011) investigated the factors associated with tDCS efficacy for treatment of aphasia and found that lower initial severity was associated with better responses. This makes sense if we take into consideration that tDCS does not generate action potentials in neurons but bi-directionally modulates their spontaneous firing activity via sub- threshold alterations of resting membrane potentials (it influences the rates at which neurons discharge), but if the neurons affected by the tDCS current-flow are not near the activation threshold then no action potentials will be triggered, and consequently no plasticity mechanism will develop (Stagg et al., 2018). The fact that tDCS has only a modulatory effect is the reason many studies paired tDCS stimulation with speech therapy (to try to activate the language network in order to modulate its activity) (Volpato et al., 2013). In this case, maybe a form of TMS stimulation (that induced local action potentials), or the stimulation of the spared contralesional areas, might be a more effective rehabilitation strategy for the PWA suffering from severe impairment (Coslett, 2015; Giordano et al., 2017).

Critically, despite the experiment duration and pandemic conditions, there were no drop-outs, and the patients, as well as their caregivers, collaborated for the entire sessions, showing a genuine interest toward the project and research in general.

General Conclusions

With an aging population worldwide, ischemic stroke has emerged as a leading cause of disability and a clinical research priority. Among the most devastating consequences of stroke are post-stroke depression and post-stroke aphasia. Given the deep impact of these two disorders after stroke (aphasia and PSD), neuroplasticity research has been receiving considerable attention for its potential benefits for use in future post-stroke rehabilitation therapies (as standalone treatment or combined with standard intervention).

In this thesis, two meta-analyses were performed, one focused on tDCS and TMS effects on PSD and the other on post-stroke aphasia.

The main conclusions of the PSD review were the following: due to the small sample sizes, heterogeneous methodologies, and lack of uniform diagnostic criteria, the reported positive data suggesting that these methods can be considered effective therapeutic options are preliminary and do not offer conclusive results. We proposed a new experiment for PSD, with the aim to evaluate the effectiveness of tDCS in a double blind, sham controlled protocol. Unfortunately, due to healthcare insurance divergences between the University and the local healthcare company (Azienda Sanitaria), this project remained in stand-by. The depression levels in the four PWA included in the experiment were evaluated using the BDI-II and SADBD. None of them were clinically depressed, and the profile remained stable for the entire duration of the experiment.

Based on the results of the second meta-analysis (focused on NIBS long term effects in language recovery), we conclude that a medium to large positive effect for rTMS (in subacute and chronic patients) and a small to medium positive effect for tDCS (only in chronic patients) can be expected at a 6-month follow-up.

The experimental study is limited to four patients (and 13 heathy participants in the control group) partly because of the pandemic: during more than six months, access to the TMS lab was forbidden, and, after that, many people were still afraid to take part in an experiment requiring daily treatment

that would involve a long interval in an environment with unknown persons. Additionally, the inclusion criteria were as strict as possible, and this prevented the recruitment of any type of PWA who accessed the Center (besides non-fluent aphasia).

Future research would benefit from the inclusion of a large sample of patients (eventually a multicenter study) and the inclusion of patients with moderate aphasia severity (given that the tDCS parameters used in our experimental setting seem ineffective for global aphasia). From a technical point of view, having good quality MRI data would also facilitate this study. For a more targeted and effective tDCS protocol, it would be helpful to use pre-treatment fMRI data and to take into account different metrics of individual differences like the genetic polymorphisms (e.g. Fridriksson et al., 2018).

Given the complexity of the language function, another possible research line could be focused on assessing by means of NIBS and TMS-EEG online coregistration (Mattavelli et al., 2019) only one specific domain of the language functions at a time (e.g., naming or verbal fluency) in both healthy and clinically impaired participants. The final goal would be to define the most effective stimulation protocol taking into account: (i) how a healthy network activates during a specific task (e.g. verbal fluency), (ii) which changes could be expected after inhibitory or excitatory stimulation in terms of cortical excitability and connectivity in both healthy and clinical populations, (iii) which parameters can be used as markers to suggest an effective rehabilitation protocol with regard to the network changes recorded in a specific patient after stroke.

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