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New techniques for neuro-rehabilitation:
Transcranial Electric Stimulation and Virtual Reality

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CHAPTER 1

1.1 INTRODUCTION

Recovery of motor and cognitive performances after a neurological illness (e.g., stroke, Multiple Sclerosis, Parkinson's disease, etc.) remains a significant challenge for rehabilitation specialists. Rehabilitation is prescribed to reduce the impact of a disease involving the central and/or peripheral nervous system and to ultimately enhance the patient’s quality of life. For acute and chronic patients with neurological disorders, early access to rehabilitation is crucial for symptom recovery and long-term continuity of care (*Gittler et al.,2018*). Effective and efficient rehabilitation programs could increase functional recovery, mitigate activity limitation, prevent secondary impairments and reduce subsequent costs.

The traditional rehabilitative interventions are usually delivered using a multidisciplinary approach (*Robinson et al.,2016*):

- exercise
- multi-task training
- occupational therapy

These approaches have generally been shown to induce a clinical improvement which is, however, not always satisfactory. These limitations in functional recovery have led researchers to consider alternative approaches. The hypothesis of providing new therapeutic possibilities in the different patients treated is, as a rehabilitator, very rewarding and represents a challenge for the future. The application of simple and low-cost techniques, defined by the literature as "unconventional" or “novel”, can provide new ideas not only in the field of research but above all of application in clinical reality.

A suitable approach to improve the rehabilitation outcome is to utilize these novel rehabilitation techniques that act as a substitute or an addition to the traditional ones. In this context, some recent approaches have been proposed that might increase the effectiveness of a traditional treatment. Among them, two techniques have been demonstrated to be very promising, namely non-invasive brain stimulation (NIBS) and Virtual Reality (VR) (*Massetti et al., 2017*).

1.1.1 NON-INVASIVE BRAIN STIMULATION

Non-invasive brain stimulation (NIBS) techniques have recently emerged in restorative neurology due to their hypothetical advantage in enhancing the efficacy of traditional therapeutic intervention (*Holland et al.,2012*). The most promising of them are considered repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) (Fig.1a). The application of rTMS to a target brain area for several minutes induces after-effects that outlast the period of stimulation in a frequency-dependent manner. Low frequency (≤1 Hz) rTMS reduces cortical excitability whereas high-frequency (5–20 Hz) rTMS does the opposite (*Chen et al.,1997*). The application of the magnetic fields is carried out by a magnetic coil that is placed near the scalp over the cortical region of interest. TDCS treatment consists of applying a direct current flow of low intensity (1-2 mA) over the scalp to modulate cortical excitability, which occurs in an opposite direction, depending on the polarity (anodal vs. cathodal) of the electrodes placed on the chosen areas. It is commonly stated that cathodal stimulation (C-tDCS) decreases cortical excitability due to neural hyperpolarization, while anodal stimulation (A-tDCS) reaches the opposite effect by a subthreshold depolarization. The main advantages of tDCS include low cost, ease of application, safety and transportability. The mechanisms of action underlying the modulation of neuronal activity induced by tDCS are still not completely understood. However, studies have demonstrated that the electric current generated by tDCS interferes with the resting membrane potential of neuronal cells, which modulates the spontaneous brain circuits activity. Some studies have suggested that tDCS could have an effect on neuronal synapsis strength, changing the activity of NMDA and GABA receptors. This in turn triggers the plasticity processes, such as long-term potentiation (LTP) and long-term depression (LTD) (*Nitsche et al.,2000*). The long-term effects of tDCS are also thought to be associated to changes in protein synthesis and gene expression. Neuroimaging studies have also shown significant blood flow changes following stimulation, which may be related to a direct effect of tDCS over blood flow, with an increase in oxygen supply on cortical areas and subsequent enhancement of neuronal excitability (*Cirillo et al.,2017*). Given these mechanisms, tDCS seems to act as a potential valuable means to stimulate brain activity and plasticity following a brain damage. Further, the effects of tDCS can last up to an hour after a single stimulation session and may persist for days or even months in case of multiples days of stimulation (*Stagg et al.,2018*). Of note, almost all tissues and cells are sensitive to electric fields and, therefore, tDCS might also elicit changes in non-neuronal tissues in the brain, including endothelial cells, lymphocytes, or glial cells (*Jarmo Ruohonene et al.,2012*) In addition, DC fields can enhance axonal regeneration and neurite outgrowth (*Wood et al.,2006; Pelletier et al.,2014*) and therefore hypothetically improve functional recovery. Finally, tDCS could also be useful to limit the vicious circle of auto-destructive events due to the increased Ca2+ influx resulting from excessive membrane depolarization or intra-axonal Na+ overload in the context of ischemia or energetic resource failure (*Lefaucheur,2009*). In other words, tDCS might be able to influence several pathological processes and pathogenetic cascades in the central nervous system, well beyond the mere changes of neuronal excitability.

Technical notes. The constant electric current of tDCS is produced by a battery-operated generator connected to at least 2 electrodes of 25 squared cm (anode and cathode): the active electrode is placed on the site overlying the cortical target, while the reference electrode is usually placed over the contralateral supraorbital area or in a non-cephalic region (Figure 1b). Given that the size and shape of the head vary among individuals, the use of a common method for choosing the electrode location is of crucial importance. Several methods are available in order to address this issue: 1) The International 10–20 (or 10-5) electrode placement system (*Oostensveld et al.,2001*), or another gross anatomical coordinate system (*Seibt et al.,2015*); 2) the neuro-navigation systems (e.g., guided MRI) (*Santarnecchi et al.,2014*); 3) the physiology-based positioning (e.g., TMS-generated MEPs). The current is of low-intensity, ranging from 0,5 to 2 mA, being applied for a period of time varying between 10 and 20 minutes. The procedure is well-tolerated, with no evidence of risk for serious adverse effects and no associated risk of seizures. Some side effects as tingling, itching, burning, and pain are seldom reported. These effects are typically mild and fade within 30 to 60 seconds of stimulation. In addition, the potential to include a “placebo” (or sham) condition (subjects cannot reliably distinguish between real and sham stimulation) makes tDCS the ideal method for reliable double-blind or single-blind investigations. Noteworthy, tDCS-induced plasticity differs whether tDCS is applied in a passive, relaxed subject (*off-line* approach) or in a subject performing cognitive or motor task, with the aim of generating synergistic effects (*on-line* approach). By targeting specific pools of neurons, the *on-line* approach is generally considered the better choice to activate the brain neuroplasticity (*Woods et al.,2016*). Most of the studies conducted on humans, therefore, utilized the *on-line* approach.

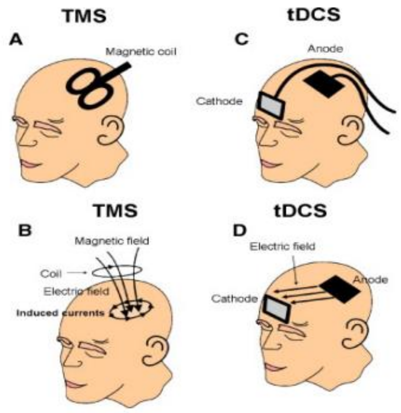

Figure 3. Comparisson of the coil (A) and electrodes (C) and the respective currents generated (B & D) in TMS and tDCS (figure from *Floel & Cohen, 2017*).

Fig.1a

Fig.1b

1.1.2 VIRTUAL REALITY

Virtual Reality (VR) is a trending, widely accessible, contemporary technology of increasing utility to biomedical and health applications (*Mazurek et al.,2019*). In the last years, VR has emerged as a supplementary approach to address some of the limitations associated with traditional therapy (*Laver et al.,2015*). VR therapies or interventions are based on real-time motion tracking and computer graphic technologies and can display the patient's performance during the required task. Through visual perception and proprioception, on-line sensory feedback is integrated into the patient's mental representation so providing a multimodal representation. VR can create an enriched environment, facilitate task-specific practice and provide multimodal feedback to enhance functional recovery. Systematic reviews on VR in rehabilitation across different neurological conditions (*Nascimento et al.,2021; Laver et al.,2017)* suggest that VR serves as a motivating and engaging method of rehabilitation, thus potentially increasing compliance and expanding the therapeutic experience, in which patients have the opportunities to perform novel tasks which they haven't done before. In addition, VR has the potential to provide the key elements of neuroplasticity (i.e., feedback, repetition, intensity, and task-specific training), which are not usually possible in conventional physical therapies (*Massetti et al.,2018*). Concerning the degree of immersion, VR systems range from immersive to semi-immersive, or non-immersive depending on the level of perceived isolation that a patient feels from the real environment when interacting with the virtual environment. Non-immersive VR allows for interacting with the environment through a controller mouse or joystick, while immersive VR tools allow a total immersion in the virtual word (*Sherman et al.,2018*). Semi-immersive VR uses computer automatic virtual environments without losing contact with the real world.

VR can provide customized high intensity training, relay back information on patients' performance via multimodal feedback, and improve motivation. Further, the "Challenge Point Hypothesis" (*Guadagnoli et al.,2004*) tells us that there is an optimal level of difficulty to keep the patient engaged without inducing boredom or fatigue, which can result in frustration and abandonment of the therapy. VR lends itself to including automatic changes in difficulty level of the rehabilitative task, and thus adapting to the patient's ability level at any given point in the training. It has, therefore, the ability to bring diverse benefits to rehabilitation programs: a) more ecologically valid training environments; b) interactive and progressive learning with immediate feedback, customized tasks and task parameters; and c) safe and secure environment, minimizing fear or hesitation as errors are without physical consequences. VR is well-suited to the use of exergames. The term exergame refers to video games that impart physical exercise/support rehabilitation practice (in the context of their clinical application) in which the repetitive and task-oriented components of rehabilitation activities are reformulated in terms of video game tasks. Their purpose is, therefore, far beyond the simple entertainment. They are very promising for teaching new motor and/or cognitive behaviors and are being incorporated into several rehabilitation therapies with encouraging results (*Cassani et al., 2020*). Utility for both cognitive and motor rehabilitation relies on their beneficial effects on attention, visuo-spatial function, executive control, strategic planning, and processing speed.

The following paragraph describes in details the VR tool used for this thesis (ReMOVES)

1.1.3 REMOVES

ReMOVES is an IoMT (Internet of Medical Things) system (*Trombini et al.,2021*) developed by the Department of Electrical, Electronic and Telecommunications Engineering and Naval Architecture (DITEN) of the University of Genova. The ReMOVES system is a user-friendly platform that can be considered as a semi-immersive VR tool, providing activities based on exergames to support motor and cognitive assessment and rehabilitation. The therapies or interventions are based on real-time motion tracking and computer graphic technologies displaying the patient performance during the required task in a virtual environment. ReMOVES interface is very simple and intuitive even for people with impairments and the delivery of the exercises is personalized. Based on various devices and motion sensors (Touchscreen, Kinect, Leap Motion), ReMOVES Patient Client performs calibration on the individual user to provide “tailored” results and allows the therapist to monitor tests and activities through a Therapist Client that can be consulted from different devices (PCs, phones, tablets). On the server side, patient data recorded by motion sensors for each activity is processed through signal pre-processing techniques. At the same time, the system favors the practice of task-oriented exercises, promoting the improvement of the patient’s functionality. To take into account the wide variability of patients’ conditions and disease progression, the most appropriate exercises has to be assigned, and the complexity of the required task can be adapted by defining a range of difficulty levels. Thanks to the collection, integration and remote analysis of patient signals and data, the solution allows continuous monitoring of the activities on which the therapist constantly updates the personalized exercise plan. ReMOVES differentiates from other existing solutions by being an auxiliary tool to provide therapists with objective rehabilitation data even when they cannot directly supervise their patients, such as during unattended use at their home, including automated data processing techniques (*Morando et al.,2019*).

Device and technologies

Microsoft Kinect V2 is a motion-sensing input device based a Time-of-Flight camera to build a depth map of the environment. It can simultaneously 3D-track up to 25 fundamental joints of the framed human body. It offers a wide field of view (70 ⇥ 60 degrees) and recognition up to 4.5m from the device (Fig.2). Several studies have demonstrated that Microsoft Kinect V2 can validly obtain spatiotemporal parameters (*Geerse et al.,2015*). The interface configuration between Microsoft Kinect V2 and the Unity engine is straight forward since the manufacturer provides the Software Development Kit (SDK) and an add-on for Unity. Developers can easily access the positions and orientations of body joints for direct use in the virtual environment of exergame.

Touchscreen for interacting with the subset of exergames for cognitive rehabilitation. The monitor is positioned on a table with an angle to the plane of a few degrees (10 degrees is the minimum tilt of the product used). The cognitive exergames present in the ReMOVES platform are a digital reinterpretation of some gold-standard tests administered on paper to patients. The interaction through a touchscreen monitor allows a more complete collection of data. The normal test procedure involves the administrator taking notes on a limited set of information. The software support, on the other hand, collects auxiliary data such as interaction speed and methods / strategies used by the patient to complete the test. The touchscreen is not required for exergames for motor rehabilitation and is therefore optional.

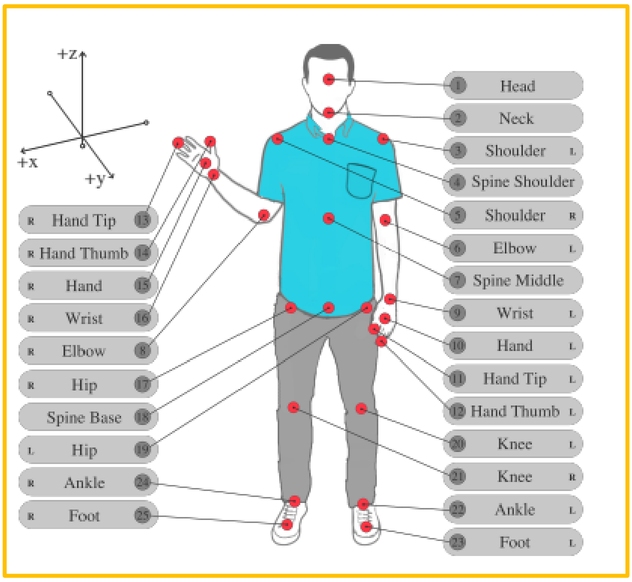

Fig.2

<u>*Exergame*</u>*:* As stated before, the term “exergame” refers to video games that impart physical exercise/support rehabilitation practice (in the context of their clinical application) in which the repetitive and task-oriented components of rehabilitation activities are reformulated in terms of video game tasks. The exergames are complementary to traditional rehabilitation with similar movements, but the purpose with which they were developed is to entertain the patient and facilitate data collection. Ideation and design of the ReMOVES exergames for this thesis involved the DITEN engineers, who created the process aimed at the mere entertainment of the patient, including the graphic aspect and the methods of interaction, and the physicians and therapists, who dealt with the features directly related to the movement and the difficulty of the exercises. ReMOVES includes 12 different exergames that can be chosen by the therapist according to the subtype of neurological disorder. They can also be modified according to level of disability and other parameters, such as duration, range of motion, speed, or others (*Morando et al.,2019; Ponte et al.,2015*) (Fig.3). The activities are automatically customed to the patient abilities thanks to a calibration phase and a real-time tuning: for example, the rate increases progressively until the patient experience a critical difficulty. Three other activities, representing the digital version of the paper and pencil test for visuo-spatial mapping of neglect, have been added to ReMOVES: the Albert test, the Bisection test and the Apple test (Fig.4)

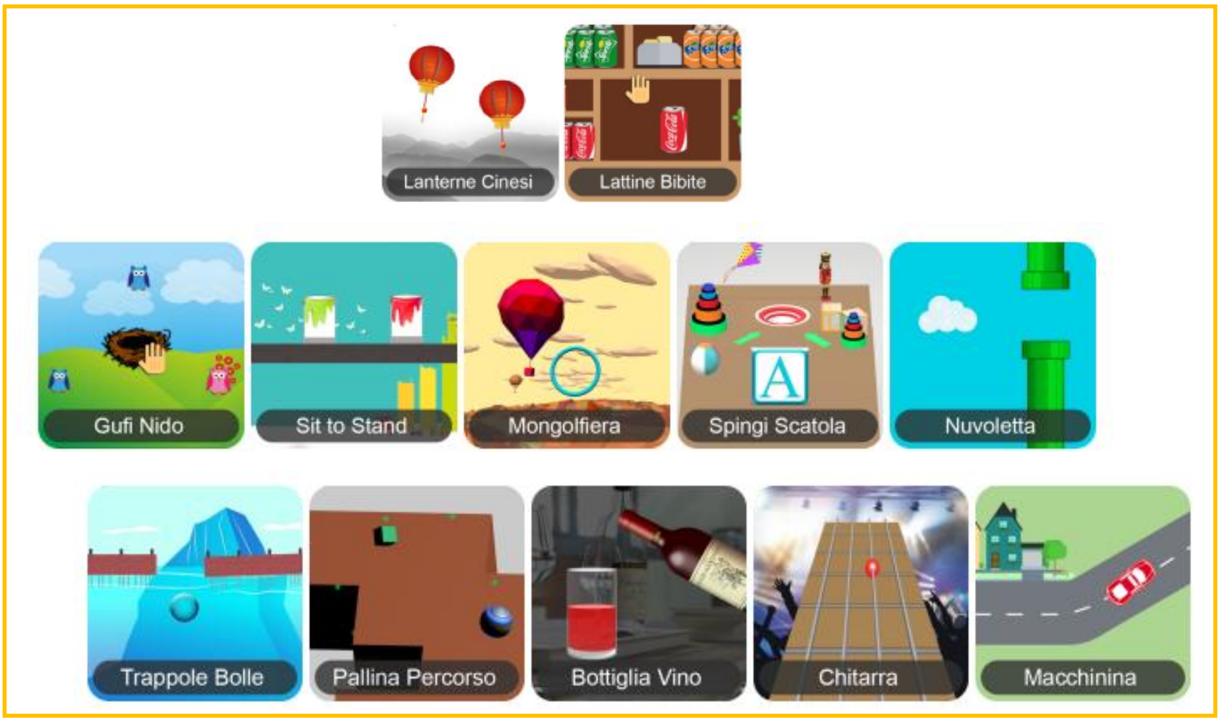

Fig.3

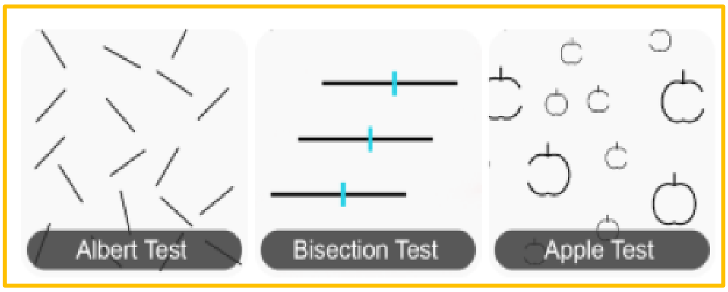

Fig.4

1.2 AIMS OF THE THESIS

In light of the foregoing, my thesis has been divided into two main lines of research, namely: a) the study of the effects of transcranial direct current stimulation (tDCS) in different neurological conditions. (Chapters 2,3,4,5,6 and 7). In particular, the application of tDCS has been studied in:

- post-thalamic stroke aphasia,
- post-stroke spatial neglect
- one case of Posterior Cortical Atrophy (PCA)
- post COVID-19 fatigue
- post COVID-19 chronic hypo/anosmia.

b) the application of VR (used alone or combined with tDCS) in the improvement of some neurocognitive disorders. A semi-immersive VR tool (ReMOVES system) has been used as a user-friendly platform providing activities based on exergames. This section also includes the description of new exergames designed with the aim of improving cognitive and/or motor disorders in Multiple Sclerosis patients (Chapter 8).

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Chapter 2

Impact of tDCS in Persistent COVID-19 Olfactory Dysfunction: a Double-blind, Sham-Controlled Study.

2.1 Introduction

In December 2019, the COVID-19 outbreak originated in China and rapidly spread across the world, causing a global pandemic. To date, there have been more than 600 million confirmed cases of COVID-19 and 7 million reported deaths as result of the SARS-CoV-2 virus infection (*Krishnakumar et al.,2022).* These data make us understand the huge health emergency that the entire planet has had to face in the last 3 years (*Guo et al.,2022*).

The commonest clinical manifestations due to COVID-19 are those of respiratory tract infections and included dyspnea, dry cough, fever, myalgia, headache, dysgeusia and anosmia (*Huang et al.,2020*). (Fig.1)

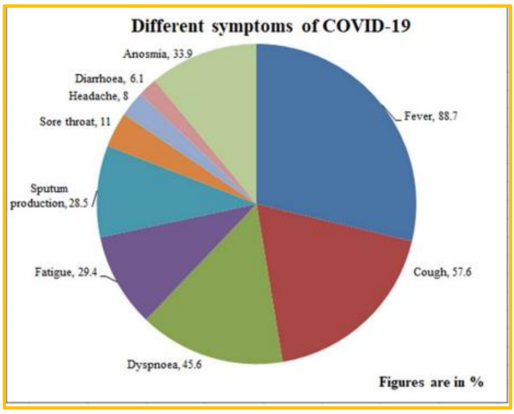

Fig.1

Reduction of smell and taste is recognized as one of the cardinal symptoms of COVID-19. It can also occur independently or represents the initial symptom in more than 27% of patients. (*Haldrup et al.,2020*). The evolution and prognosis of smell loss is favorable: the median duration has been reported to be around 10 days, with a complete resolution after 4 weeks from diagnosis. However, 5% of people reported no improvement (*Karamali et al.,2022*), meaning that as many people as 20 million worldwide will suffer from chronic post-COVID-19 anosmia.

Generally speaking, the olfaction disturbances consist of a decrease or loss of smell, named hysmosmia or anosmia, and/or a variation in the quality of the chemosensory perception, termed phantosmia (odor perception in the absence of an odor source) and parosmia (distorted odor perception in the presence of an odor). Most importantly, olfaction is essential for detecting environmental hazards and for enjoying food. Anosmia is thus a condition that can have a significant impact on a person's quality of life. People with smell dysfunction may lose interest in eating and socializing. As a result, weight loss or malnutrition may occur, together with significant mood disorders and even suicidal ideation (*Yom-Tov et al.,2021*).

According to a recent metanalysis, the olfactory dysfunction related to COVID-19 has a higher prevalence in Europe, The Middle East and North America compared with Asian countries (*Butowt et al.,2021*) (Fig.2).

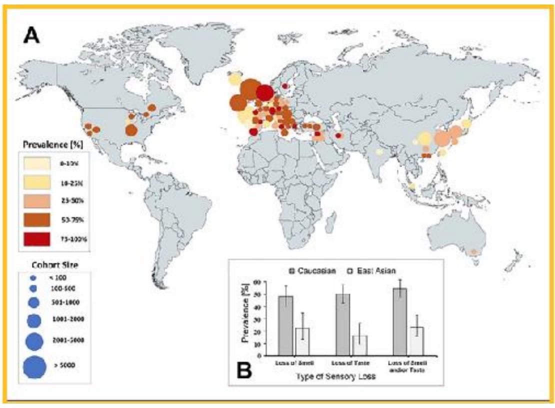

Fig.2

Coelho e coauthors (2022) found that, compared with those who had been infected with the original virus, people who had contracted the Alpha variant were 50% as likely to have chemosensory disruption. This probability fell to 44% for the Delta variant and to 17% for the Omicron one. The properties of the SARS-CoV-2 virus variants, indeed, differ in ways that likely determine to what extent the olfactory epithelium become infected and whether their loss will lead to different amounts of olfactory dysfunction. Further, epidemiological studies related to COVID-19 anosmia demonstrated a less olfactory involvement in patients with a moderate infection and that females are more likely to suffer than men. (*Lechien et al.,2020)*.

2.1.1. PATHOGENETIC MECHANISMS OF COVID-19 ANOSMIA.

The timing and duration of the sensory deficits are so unique in COVID-19 that they can provide important clues about the potential underlying mechanisms. Three different main hypotheses have been considered to explain olfactory dysfunction in COVID-19 patients:

1. nasal obstruction/congestion and rhinorrhea.
2. loss of olfactory receptor neurons.
3. damage of the support cells in the olfactory epithelium.

1. The possibility of a physical obstruction (conductive olfactory loss) was initially considered a likely explanation of the anosmia in COVID-19. Olfactory obstruction may be a plausible mechanism causing hyposmia or anosmia in viral infections. Olfactory cleft obstruction affects air flow and prevents odors from traveling to the intact olfactory epithelium, hence causing conductive loss. This hypothesis in COVID-19, however, has been now ruled out by several studies, primarily demonstrating that a large fraction (nearly 60%) of patients with anosmia do not have nasal congestion, obstruction or rhinorrhea (*Kaye et al.,2020; Tong et al.,2020; Vaira et al.,2020; Xydakis et al.,2020*).

2. A sensorineural olfactory loss has been considered a conceivable explanation of anosmia. However, at a closer look, there are three major inconsistencies with this scenario: the time course of cellular regeneration versus clinical recovery, the lack of expression of viral entry proteins and the absence of the virus within the olfactory neurons. When olfactory receptor neurons die, their replacement requires 8 to 10 days plus about 5 days for cilia maturation, but the time course of smell recovery in COVID-19 often is less than one week (*Bryche et al.,2020; Liang,2020*). Regarding expression of the virus entry proteins, mature olfactory receptor neurons do not express ACE2, and therefore are not likely to be infected by SARS-CoV-2 (*Baxter et al.,2020; Bilinska et al.,2020*).

3. The virus produces a damage to the support cells in the olfactory epithelium and thereby diminish rapidly, but transiently, the sense of smell. This mechanism is supported by the abundant expression of the two entry proteins, ACE2 and TMPRSS2, in the sustentacular cells of the olfactory epithelium. Interestingly, death of sustentacular cells does not seem to necessarily cause death of olfactory receptor neurons; the study by Bryche and others (2020) showed that the neurons cilia (the dendritic extensions of the olfactory receptor neurons that bind the odorant molecules) can transiently retract or lose protein expression, implying temporary neuronal dysfunction despite the persistence of the olfactory nerve axons. Death and regeneration of sustentacular cells occurs much faster than death and regeneration of olfactory

neurons. Rapid replenishment of sustentacular cells is thus consistent with the rapid recovery of the sense of smell that is clinically observed in most cases. The damage or inactivation of sustentacular cells in the olfactory epithelium is sufficient to cause functional deficits of smell sensation and it is consistent with the time course and the peculiarities of the impairment reported by COVID-19 patients.

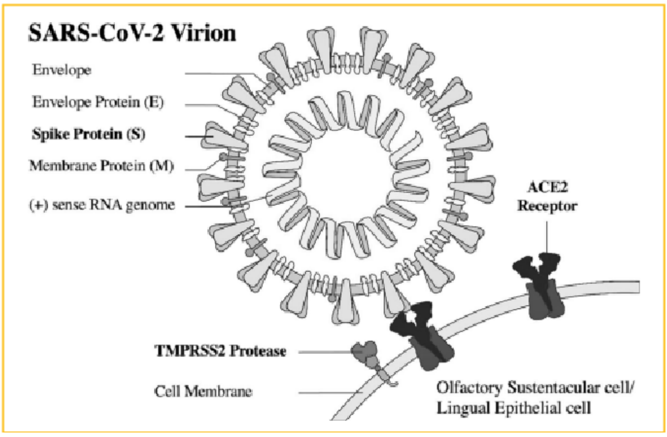

Fig.3

The reliable hypothesis of the death of the sustentacular cells infected by SARS-CoV-2 can explain COVID-19-related anosmia in patients who spontaneously recover olfactory function. The same hypothesis, however, cannot justify the persistent post-COVID-19 anosmia, for which other assumptions must be advocated. At this regard, a number of studies have demonstrated the structural and/or functional involvement of the Central Nervous System (CNS) in COVID-19 anosmia, with reduced metabolic activity in several brain areas, particularly the orbitofrontal cortex (OFC), the neural substrate for conscious olfactory perception (*Thunell et al.,2022; Donegani et al.,2021; Yousefi-Koma et al.,2021; Voruz et al.,2022; Douaud et al.,2022*). Interestingly, Karimi-Galougahi et al. (2020) demonstrated a significant reduction of metabolic activity in the left orbitofrontal cortex in COVID-19 anosmia, suggesting that an impairment of neural function in this region could act as the underlying cause of persistent anosmia (Fig.4)

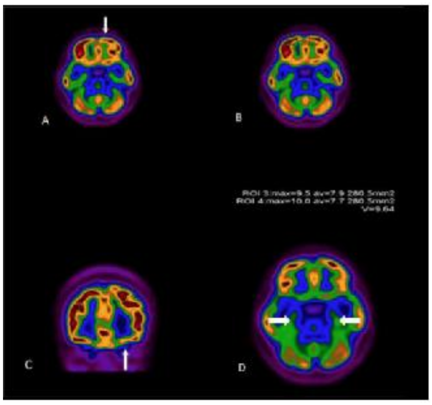

Fig.4

2.1.2 TREATMENT

Treating olfactory dysfunction is of paramount importance, whatever the cause. Different pharmacological and non-pharmacological interventions have been tried in order to alleviate COVID-19 hyposmia, but with limited efficacy (*O'Byrneet al.,2021, Neta et al.,2021*).

-*Corticosteroids*: no significant effect on recovery of anosmia in patients with COVID-19 was demonstrated.

-*Intranasal insulin films*: insulin appear to improve odor discrimination in treated patients. Additional analyses are necessary, however, to confirm the data.

-*Olfactory training (OT):* it consists in a persistent and intended sniffing of odorants like eucalyptus oil, cloves, lemon and rose. When begun early and with good compliance, OT was reported to be most beneficial in enhancing olfactory function and was linked with fewer negative side effects (*Whitcroft et al.,2020*).

2.2 OUR STUDY: MATHERIAL AND METHODS

Non-invasive brain stimulation (NIBS) techniques can be deemed as a promising alternative (or add-on) to traditional rehabilitative approaches in several neurological disorders, including smell disturbances (*Henkin et al.,2011; Kullaci et al.,2021*) and chronic COVID-19 symptoms as well. In this doubles-blind, sham-controlled study, we implemented a two-week session (5 days for week) of combined OT and anodal transcranial direct current stimulation (A-tDCS) in seven patients with persistent post COVID-19 hypo/anosmia. The aim of the study was to investigate the effectiveness of tDCS in improving olfactory functioning and to verify the potential duration of effectiveness.

2.2.1 PARTICIPANTS

Seven consecutive patients (5 males and 2 females) were enrolled in the study from June to September 2021, according to the following inclusion criteria: 1) persistent hypo/anosmia due to COVID-19 (at least six months after the second negative nasopharyngeal swab); 2) a score below 12 on the “Sniffin’ Sticks” identification subtest (see below). The exclusion criteria were: 1) severe mood disorder; 2) nasal endoscopy showing presence of rhinological disease; 3) history of epilepsy; 4) sensitive scalp. Ruling out mood disorders is of importance since olfaction and depression can share common underlying biological mechanisms (*Kullakci et al.,2021*) and tDCS can ameliorate depression (*Saiote et al.,2014*). The principles of the Declaration of Helsinki were followed and patients provided signed informed consent at the beginning of the procedures. The study protocol was approved by the Institutional Review Board.

Baseline demographic and clinical characteristics are described in Table 1. No medications for alleviating olfactory symptoms were allowed during the study.

Pts	**Sex**	**COVID-19 diagnosis**	**COVID-19 clinical features**	**Duration of loss smell (months)**	**VAS-smell 0-10**	**Sniffin' Sticks odor identification**
#1	M	11-6-2020	Body ache, loss of smell, fever and headache	7	2	7/16
#2	M	11-3-2020	Respiratory symptoms requiring mechanical ventilation, fever, loss of smell and taste, parosmia and phantosmia.	7	2	6/16
#3	M	10-15-2020	Dyspnea requiring oxygen supplementation, loss of smell, fever and cough.	8	1	5/16
#4	M	10-6-2020	Fever, cough, dyspnea requiring mechanical ventilation, loss of smell and taste, body ache.	8	1	8/16
#5	M	12-20-2020	Fever, loss of smell and taste	6	1	1/16
#6	F	3-2-2021	Fever, loss of smell, headache	7	2	6/16
#7	F	2-26-2020	Loss of smell and taste, parosmia	6	3	8/16

Tab.1

2.2.2 OLFACTION ASSESSMENT

Patients' smell performances were first assessed immediately preceding the brain stimulation (t0 or baseline). A visual analog scale, VAS smell 0-10, was employed as a subjective measure. Patients indicated the line on the scale that represented the level of smell perception (0 = complete loss of smell, 10 = full sense of smell). The "Sniffin' Sticks" (*Hummel et al.,1997*) is a validated objective test consisting of three subtests (smell threshold, discrimination and identification). Individual subtests, such as smell identification, can be used separately to assess olfactory function, allowing a shorter test time, while maintaining high test-retest reliability (*Haehner et al.,2009*). The identification subtest consists of 16 reusable penlike odor-dispensing devices that the subject is asked to identify and characterize by choosing 1 of 4 options. The test is scored out of a total of 16 points (12-16 normosmia, 9-11 hyposmia and 8 or below anosmia). All the patients were classified as anosmic at baseline.

2.2.3 PROCEDURE

For each OT session, patients had to carefully sniff ten odors provided in a smell kit (i.e. rose, eucalyptus, lemon, star anise, rosemary) for approximately 10 seconds each, over a 20-minute session. On-line tDCS stimulation was concurrently applied with a battery-driven stimulator (Newronika s.r.l, Italy) with 1.5 mA intensity. The contact impedance was kept below 5 kΩ and the current density (0,06 mA/cm2) was maintained below the safety limits. The anode was placed over the left prefrontal cortex (PFC), since the OFC is not directly accessible by superficial stimulation techniques such as tDCS. The PFC was chosen due to its close anatomical and functional connection with the OFC (*Howard et al.,2020*). The cathode was applied to the contralateral shoulder. Repeated sessions of combined OT and A-tDCS were carried out for five consecutive days, for two weeks (10 sessions). The same protocol was first utilized for the sham stimulation (S-tDCS). In this case, the current was ramped up for 30 sec until reaching 1,5 mA and ramped down for 30 sec at the end of the stimulation period, making this condition indistinguishable from A-tDCS and thus proving effective blinding. The order of S-tDCS and A-tDCS across subjects was not counterbalanced because we intended to avoid potential carry-over effects if A-tDCS had been applied first. The patients and the assessors collecting the data were blinded as to the design of the study. The smell assessment was then repeated immediately after S-tDCS (t1), A-tDCS (t2) and three months from the end of stimulation (t3).

2.2.4 STATISTICAL ANALYSIS

For both the subjective and objective measurements, the Wilcoxon test was used to compare each assessment (t1, t2 and t3) to baseline. A two-sided α less than 0.05 was considered statistically significant. Statistical analyses were performed using Stata version 16.0 (Stata Corporation, College Station, TX, USA). At each time-point, results are summarized as mean ± standard deviation. Individual measures of the seven patients are reported.

2.2.5 RESULTS

The stimulation procedure was well tolerated. Both the Sniffin' Sticks identification and the VAS smell 0-10 data showed a statistically significant improvement following A-tDCS (t2) and after three months (t3), with average measurements doubled or even tripled compared to baseline (t0) and S- tDCS (t1). Each individual demonstrated notable improvement in smell performance. All patients, except for #5, reached a score of at least 12, corresponding to normosmia. Patient #5 was still anosmic at t2 (Sniffin' Sticks score 7), though achieving a remarkable improvement as compared to both t0 and t1 (score 1).

Figure 1 provides a summary of the effects in each patient.

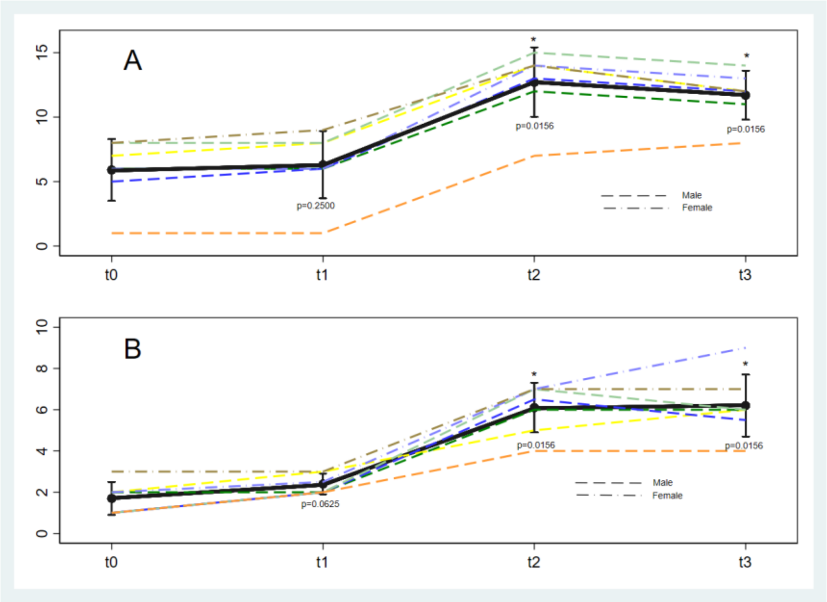

Figure 1. Mean ± Standard Deviation of the Sniffin' Sticks test (A) and VAS smell 0-10 (B) measurements at baseline (t0), after S-tDCS (t1), A-tDCS (t2) and three months later (t3). Time-points marked with * showed significantly different ($p<0.05$) results compared to baseline based on the Wilcoxon test. Dashed lines show the individual results of the seven patients (patient #1: yellow; patient #2: green; patient #3: purple; patient #4: light green; patient #5: orange; patient #6: light purple; patient #7: brown.

2.2.6 DISCUSSION

This double-blind, sham-controlled study is the first attempt to treat persistent olfactory symptoms in patients who recovered from COVID-19 by means of repeated sessions of simultaneous OT and A-tDCS over the left PFC. The statistical analyses showed a significant improvement in both subjective and objective measures of hypo/anosmia in all treated patients, compared to baseline and sham stimulation. Importantly, the beneficial effect of A-tDCS was maintained over a period of up to 3 months following the end of stimulation. The implementation of stimulation at least at six months after a negative SARS-CoV-2 swab allowed us to generally rule out the possibility of spontaneous recovery. Although placebo effects may play a nontrivial role in any trial, the data indicate that OT coupled with sham stimulation did not achieve any benefit. It is therefore tempting to speculate that the amelioration of smell in the current sample may be due to the effect of anodal tDCS on the neuroplasticity of the OFC, or, alternatively, the PFC (the stimulated area), which is extensively connected to the planned OFC target. We cannot exclude that the effect of tDCS on the left PFC could have contributed to the observed results, consistent with other reports (*Kullakci et al.,2021*). However, given the essential role of OFC in conscious olfactory perception (*Villafuerte et al,2019; Bérard et* *al.,2021*), the results suggest that modulation of OFC connectivity induced by tDCS may be of foremost importance.

2.2.7 CONCLUSIONS

The current study is the first attempt to use NIBS techniques in the treatment of individuals with persistent hyposmia due to COVID-19. Caution is needed in drawing any definitive conclusions based on a study on a small group of patients. However, the significant and long-lasting improvement of olfactory function observed in the current sample suggest that this safe, non-invasive and inexpensive treatment could alleviate chronic, debilitating post-COVID-19 hypo/anosmia. In addition, the results may support the hypothesis that the cortical areas involved in olfaction may have a non-negligible role in the development of chronic smell loss due to COVID-19. Larger studies are necessary to extend and confirm this intriguing hypothesis.

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CHAPTER 3

FUTURE DEVELOPMENTS

3.1 PHASE 2: EXPANSION OF THE SAMPLE AND ANALYSIS OF THE NEW RESULTS

The publication of our preliminary results, together with the considerable media coverage in Italy (newspapers, Radio) and abroad (Medscape Medical News), has facilitated an important number of requests for treatment by post-COVID-19 anosmic patients. To date we have been contacted by about 140 people suffering from persistent olfactory disorder, coming from all the Italian regions.

Since July 2022, 27 new patients (aged 22 to 72 years) have been enrolled in the study, following the same criteria of inclusion/exclusion provided for in the pilot study. The duration of smell loss ranged from 7 to 31 months.

This second sample of patients confirms the following data from the literature:

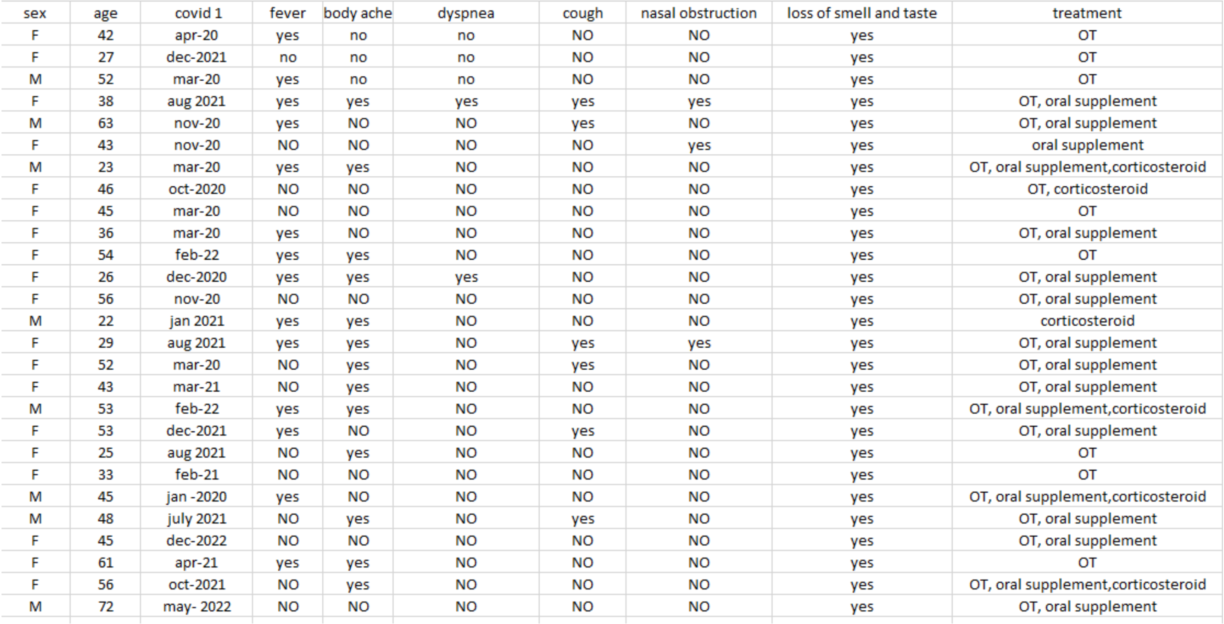
1. Higher proportion of female subjects than men (19 F, 8 M).
2. Only 3/27 patients reported nasal congestion and rhinorrhea during COVID-19 infection
3. No patients required hospitalization, so that anosmia seems unrelated to the entity of the SARS-CoV-2 infection

After a careful analysis of the anamnestic data, we observed that all the patients had undergone pharmacologic therapies (systemic or local corticosteroids and / or oral supplements) and olfactory training (OT) for more than 3 months, without significant results.

We expanded our preliminary study into a confirmatory, randomized, double-blind, placebo-controlled investigation to assess the efficacy of concurrent A-tDCS and OT in patients diagnosed with persistent hypo/anosmia due to COVID-19. Eligible patients were randomized (1:2 ratio, on the basis of a predetermined computer-generated randomization list) to receive either daily sham tDCS (S-tDCS) or A-tDCS over the left PFC, concurrently with OT, for 10 days (5 days for two weeks). Neither patients nor odor investigators knew whether assignment would have been to the S-tDCS or A-tDCS. To allow that patients assigned to the placebo group would also receive the active treatment, those who agreed to participate in the long-term follow-up study will be subsequently treated with A-tDCS. Both the group of patients will be followed for 6 months to evaluate the long-term efficacy. The outcome measures employed are the same as those used in the preliminary study: the primary efficacy measures are the change in the

Sniffin’ Sticks test and VAS scores over the duration of the study. The smell assessment will be done at the baseline (t0), immediately after S-tDCS or A-tDCS (t1), one month (t2) and six months from the end of stimulation (t3).

Baseline demographic and clinical characteristics are described in Table 1



sex	age	covid 1	fever	body ache	dyspnea	cough	nasal obstruction	loss of smell and taste	treatment
F	42	apr-20	yes	no	no	NO	NO	yes	OT
F	27	dec-2021	no	no	no	NO	NO	yes	OT
M	52	mar-20	yes	no	no	NO	NO	yes	OT
F	38	aug 2021	yes	yes	yes	yes	yes	yes	OT, oral supplement
M	63	nov-20	yes	NO	NO	yes	NO	yes	OT, oral supplement
F	43	nov-20	NO	NO	NO	NO	yes	yes	oral supplement
M	23	mar-20	yes	yes	NO	NO	NO	yes	OT, oral supplement,corticosteroid
F	46	oct-2020	NO	NO	NO	NO	NO	yes	OT, corticosteroid
F	45	mar-20	NO	NO	NO	NO	NO	yes	OT
F	36	mar-20	yes	NO	NO	NO	NO	yes	OT, oral supplement
F	54	feb-22	yes	yes	NO	NO	NO	yes	OT
F	26	dec-2020	yes	yes	yes	NO	NO	yes	OT, oral supplement
F	56	nov-20	NO	NO	NO	NO	NO	yes	OT, oral supplement
M	22	jan 2021	yes	yes	NO	NO	NO	yes	corticosteroid
F	29	aug 2021	yes	yes	NO	yes	yes	yes	OT, oral supplement
F	52	mar-20	NO	yes	NO	yes	NO	yes	OT, oral supplement
F	43	mar-21	NO	yes	NO	NO	NO	yes	OT, oral supplement
M	53	feb-22	yes	yes	NO	NO	NO	yes	OT, oral supplement,corticosteroid
F	53	dec-2021	yes	NO	NO	yes	NO	yes	OT, oral supplement
F	25	aug 2021	NO	yes	NO	NO	NO	yes	OT
F	33	feb-21	NO	NO	NO	NO	NO	yes	OT
M	45	jan -2020	yes	NO	NO	NO	NO	yes	OT, oral supplement,corticosteroid
M	48	july 2021	NO	yes	NO	yes	NO	yes	OT, oral supplement
F	45	dec-2022	NO	NO	NO	NO	NO	yes	OT, oral supplement
F	61	apr-21	yes	yes	NO	NO	NO	yes	OT
F	56	oct-2021	NO	yes	NO	NO	NO	yes	OT, oral supplement,corticosteroid
M	72	may- 2022	NO	NO	NO	NO	NO	yes	OT, oral supplement

tab.1

3.2 STATISTICAL ANALYSIS

For both the subjective and objective measurements, the Wilcoxon test was used to compare each assessment (at the time of this thesis only t1) to baseline for both groups (S-tDCS and A-tDCS). A two-sided α less than 0.05 was considered statistically significant. Statistical analyses were performed using Stata version 16.0 (Stata Corporation, College Station, TX, USA). At each time-point, results are summarized as mean ± standard deviation.

3.3 RESULTS

The stimulation procedure was well tolerated. Both Mean ± Standard Deviation of the Sniffin’ Sticks test and VAS smell 0-10 measurements at baseline (t0) and after S-tDCS or A-tDCS (t1) showed a significant improvement as compared to baseline, only for the A-tDCS group (p <

0.0003 and p < 0.0002, respectively). The S-tDCS group did not demonstrate any smell improvement.

Figure 1(a-b) and 2 (a-b) show the Box and Spaghetti plots of the VAS and Sniffin' Sticks measures in the A-tDCS group.

	VAS T0	**VAS T1**	**p-value (Wilcoxon Test)**
Median(IQR)	3(1;4)	5(4; 6.5)	**0.0002**

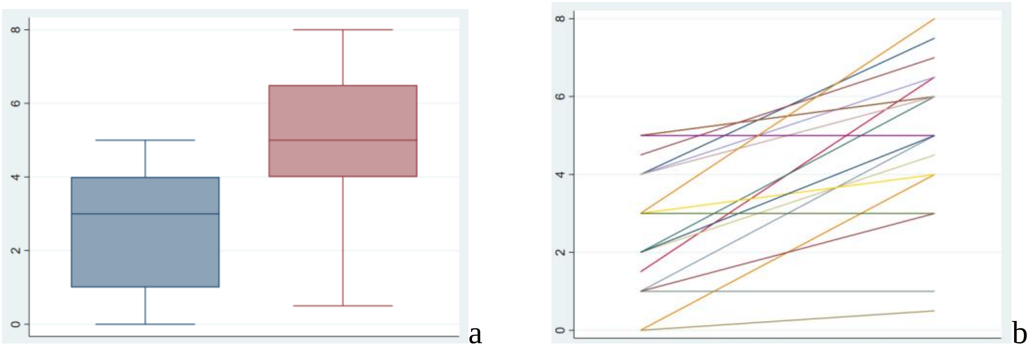


Fig1: (a) Box plots for VAS at t0 (blue) and at t1 (red). (b) Spaghetti plot for VAS, each line refers to a patient.

	Sniff T0	**Sniff T1**	**p-value (Wilcoxon Test)**
Median(IQR)	9(6; 11)	12(9; 14)	**0.0003**

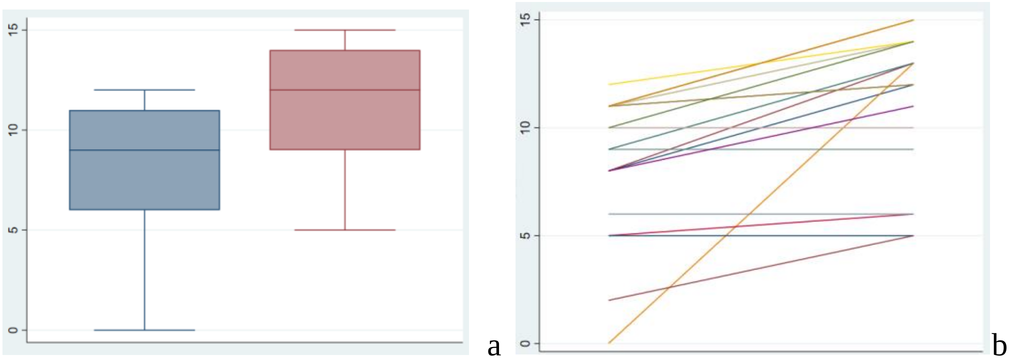


Fig 2: (a) Box plots for Sniff at t0 (blue) and at t1 (red). (b) Spaghetti plot for Sniff, each line refers to a patient.

Of note, 70% of the A-tDCS patients showed a significant improvement in objective smell outcomes (Tabel 2: Simmetry Test and Stuart-Maxwell test). Specifically, of 8 patients who were anosmic on the Sniffin' Sticks identification test at the baseline, 4 (50%) did not improve, 1 (12%) turned from anosmia to hyposmia and 3 (38%) improved to normosmia. Importantly, more than 80% of the patients with hyposmia (9 out of 11) reached a complete recover of their olfactory function.

	Anosmia t1	**Hyposmia t1**	**Normosmia t1**
Anosmia (0-8) t0	4(50%)	1(13%)	3(38%)
Hyposmia (9-11) t0	0(0%)	2(20%)	8(80%)
Normosmia (12-16) t0	0(0%)	0(0%)	1(100%)

Tab. 2 Simmetry Test: p=0.0074; Marginal homogeneity Stuart-Maxwell test: p=0.035

3.4 CONCLUSIONS

In conclusion, this randomized, double blind, placebo-controlled study that follows a preliminary investigation on a limited number of patients has shown significant, clinically relevant, and persistent benefit of A-tDCS (plus OT) in patients with chronic olfactory disturbances following SARS-CoV-2 infection. The long-term efficacy has to be determined at the time of this thesis. However, the short-term results provide evidence that the use of anodic tDCS over the left PFC induces a significant improvement of smell function in a disease affecting millions of people around the world.

Our aim in the next future will be to investigate the possible correlation between clinical and instrumental data, thus formulating hypotheses on the mechanisms of efficacy / non-efficacy of the treatment and implementing possible national and international diagnostic and therapeutic guidelines on the treatment of anosmia / hyposmia post COVID-19.

In order to reach these aims we will implement both structural and functional neuroimages of the brain by means of Magnetic Resonance Imaging (MRI). MRI will be acquired on a magnet operating at 3 Tesla (PRISMA, Siemens Healthineers) at the baseline and after A-tDCS treatment.

The MRI protocol will include structural MRI sequences (i.e. 3DT1 MPRAGE, 3D T2 fluid attenuation inversion recovery (FLAIR) and susceptibility weighted (SWI) MRI), multishell diffusion MRI, and resting state MRI. Post processing of T1 scans will include measures of volume and cortical thickness, including the assessment of regions previously connected to anosmia in COVID patients (*Douaud et al.,2022*) such as in the orbitofrontal cortex and parahippocampal gyrus. By inspecting T2 FLAIR we will assess the presence of signal intensity alterations which might reflect inflammation or tissue damage. SWI will define the presence of microbleeds, which have been described in COVID encephalopathy (*Napolitano et al.,2022*). Resting-state functional MRI will be analyzed to assess the neural connectivity of the central olfactory system in patients before and after treatment (*Espositoet al., 2022*). Diffusion MRI measurements will be used to gauge white matter microstructure and to assess integrity in areas providing anatomical connectivity between areas related to the olfactory process.

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CHAPTER 4

THE EFFECT OF TRANSCRANIAL DIRECT CURRENT STIMULATION ON POST COVID-19 FATIGUE: A DOUBLE-BLIND, SHAM-CONTROLLED EXPLORATORY STUDY.

4.1 INTRODUCTION

The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused a human infection (COVID-19) which originated in China, in December 2019 and rapidly became a public and international health emergency. The clinical spectrum of COVID-19 ranges from asymptomatic or paucisymptomatic patients to septic shock and multiorgan failure. The most common symptoms include fever, dry cough, fatigue, dyspnea and myalgia (*Ge et al.,2020*). Fatigue has been observed in about 70% of patients with COVID-19, having a detrimental impact on quality of life and deeply limiting the recovery phase of the disease (*Nicola et al,2020; Tian et al.,2020; Wang,2020*). Recent studies have estimated that fatigue may persist in patients who recovered from COVID-19 as frequently as 53% and that it may last up to 3 months (and beyond) after the infection is resolved (*Tenforde et al.,2020; Carfi et al.,2020; Ceban et al.,2022*).

Fatigue is defined as a “subjective lack of physical and/or mental energy that is perceived by the individual to interfere with usual and desired activities” (*Lapierre et al.,2007*). It frequently occurs in neurological diseases, such as multiple sclerosis, Parkinson’s disease and neuromuscular disorders. Primary fatigue may be subdivided into peripheral and central fatigue. The former is generally due to changes at or distal to the neuromuscular junction, while the latter is thought to denote the inability of the brain to maintain the drive necessary to produce the desired power output (*Zwarts et al.,2008; Claros-Salinas et al.,2013*).

The pathophysiological mechanisms of fatigue remain only partially understood. Various pharmacological (mainly psychostimulants) and non-pharmacological interventions (aerobic exercises, cooling therapies, psychotherapy, etc.) have been tried in order to alleviate fatigue symptoms but with limited benefits (*Khan et al.,2014; Heine et al.,2016*). Among the non-pharmacological treatments, noninvasive brain stimulation (NIBS) techniques, such as transcranial direct current stimulation (tDCS), have been shown to produce some therapeutic effects on the levels of fatigue both in healthy individuals (*Abdelmoula et al.,2016; Victor-*

Costa et al.,2015; Machado et al.,2019) and in patients with medical (*Avery et al.,2015*) or neurological disorders (*Acler et al.,2013; Forogh et al.,2017; Chalah et al.,2017; Charvet et al.,2018; Dobbs et al.,2018*). TDCS acts by a tonic modulation of the resting membrane potential of the cortical neurons, which occurs in an opposite direction, depending on the polarity (anodal vs. cathodal) of the electrodes placed on the chosen areas. It is commonly stated that cathodal stimulation (C-tDCS) decreases cortical excitability due to neural hyperpolarization, while anodal stimulation (A-tDCS) reaches the opposite effect by a subthreshold depolarization (*Nitsche et al.,2000*). The effects of tDCS on fatigue have been usually investigated by stimulating either the primary motor cortex (M1) or the dorsolateral prefrontal cortex (DLPFC). The rationale behind these targets relies upon the assumption that brain stimulation over M1 can directly modulate the output of the corticospinal tract, while DLPFC stimulation may achieve the same effect by activating cortico-cortical connections (*Rupp et al., 2008*). In addition, it has been shown that fatigue is linked to frontal and/or prefrontal cortices hypoactivation (*Van Duinen et al.,2007; Saiote et al.,2014).*

Recent studies in multiple sclerosis indicated that A-tDCS over the left DLPFC, applied when the patients are at rest (*off-line* approach), significantly reduces fatigue symptoms (*Chalah et al.,2017, Mortezanejad et al.,2020; Chalah et al.,2020*), though others were not able to reproduce these positive findings (*Van Duinen et al.,2007*).

In this double blind, sham-controlled exploratory study, we investigated, in a small cohort of patients who recovered from COVID-19, the effects on fatigue of repetitive sessions of *off-line* A-tDCS over the left DLPFC. Most of the previous studies assessed fatigue symptoms only subjectively, using self-report scales (i.e. Fatigue Severity Scale, Modified Fatigue Impact Scale). Given that the absence of objective evidence could hamper the outcome reliability, the median frequency (MDF) of the power spectrum of the EMG signal and the Root Mean Square (RMS) amplitude of the EMG signal during motor exercise was added as an objective measure of fatigue.

4.2 MATERIALS AND METHODS

4.2.1 PARTICIPANTS

Three male patients were enrolled according to the following inclusion criteria: 1) previous diagnosis of COVID-19; 2) two consecutive negative nasopharyngeal swabs; 3) fatigue after COVID-19, persisting at least 4 weeks after the second negative nasopharyngeal swab; 5) a score equal or greater than 20 at the 21-Modified Fatigue Impact Scale (MFIS) (*Fisk et al.,* *1994*), performed just before the brain stimulation. The exclusion criteria were: 1) depression, as assessed by the Beck's Depression Inventory (BDI) (*Beck et al.,1996*); 2) intracranial metal implants; 3) history of epilepsy; 4) history of fatigue before COVID-19. Ruling out mood disorders is of importance since fatigue and depression can share common underlying biological mechanisms (*Chalah et al.,2019, Workman et al.,2020*) and tDCS can ameliorate depression (*Saiote et al.,2014*). According to the classification of COVID-19 severity presentation (*Cascella et al.,2020*), all the patients had suffered from a severe type of the disease with acute respiratory distress syndrome (ARDS). Patients #1 and #2 (both 56 years-old) required intubation and mechanical ventilation for a period of 2 weeks. For patient #3 (81 years-old) only non-invasive ventilation was necessary. None of the patients had preexisting significant comorbidities. No drugs for alleviating fatigue were used during the experimental period. The principles of the Declaration of Helsinki were followed. An informed consensus was obtained from the patients prior to the beginning of the procedures.

4.2.2 PROCEDURE

Transcranial electric stimulation was applied with a battery-driven stimulator (Newronika s.r.l, Italy) for 20 min with 1.5 mA intensity, using sponges (5 cmx 7 cm) soaked in saline solution. The anode was placed over the left DLPFC (F3 in the 10–20 international EEG system), while the cathode was applied to the contralateral supraorbital area. An electroconductive gel was applied under the electrodes to constantly reduce the contact impedance below 5 kΩ. Current density (0,06 mA/cm2) was maintained below the safety limits (*Poreisz et al.,2007*). During the first week, five consecutive sessions of S-tDCS were carried out. The current was ramped up for 30 sec until reaching 1,5 mA and ramped down for 30 sec at the end of the stimulation period, proving effective blinding. In the second week a real-stimulation (A-tDCS) over the same area was administered for five consecutive day, with a constant stimulation intensity of 1,5 mA for 20 min. During the stimulations, subjects were comfortably seated in a quiet room, with eyes closed and not performing any task (*off-line* condition). We did not counterbalance the order of S-tDCS and A-tDCS across subjects first because the number of patients (3) would have not allowed a complete balance of the stimulation order and, secondly, we intended to avoid potential carry-over effects if A-tDCS has been applied first. The patients and the assessors collecting and analyzing the clinical (questionnaires) and EMG data were blinded as to the design of the study.

Fatigue evaluation. Subjective and objective measures of fatigue were evaluated just before the first session of S-tDCS (t0 or baseline), immediately after the last session of S-tDCS (t1) and immediately after the last session of A-tDCS (t2). The patients were admitted to a neurological rehabilitation program, but no cycling exercises were allowed throughout the period of the study.

<u>Subjective measures</u>. As subjective outcome measures, we used a structured questionnaire (the 21-MFIS) and a global scale, the Visual Analogue Scale for Fatigue or VAS-F (0-100) (Hewlett et al., 2011). The 21-MFIS is divided in subscales related to the physical (9 items), cognitive (10 items) and psychosocial (2 items) impact of fatigue. The total 21-MFIS score ranges from 0 to 84: higher scores indicate a greater impact of fatigue. The VAS-F (0-100) is a visual analogue scale ranging from 0 to 100 mm and consists of 18 items related to the subjective experience of general fatigue, with patient asked to mark a number indicating the level of his/her feeling about each item (i.e. sleepiness, energy, concentration).

<u>Objective measures</u>. As objective measures of fatigue, we utilized the MDF of the power spectrum and the RMS amplitude of the EMG signal. We implemented an increasing intensity-controlled exercise using a cycle ergometer, since dynamic, isotonic and concentric contractions may better reflect functional performance as compared to the isometric ones (*Sliwowski et al.,2018*). The subject was seated on a chair with his feet strapped into the pedals of the cycle ergometer (Motolife EVO model, Chinesport, Italy) and his hands placed on the handlebars. After an adequate warm-up, he had to cycle at his maximum speed for one minute, followed by ten minutes of rest. Then we applied a progressive, power-incremented cycling, with a stepwise protocol of increasing workloads, in order to achieve the maximum exertion within ten minutes: the patient was asked to cycle at 60% of his maximum speed for the first two minutes, then increasing by 10% every 2 minutes, until reaching his maximum speed or until volitional exhaustion. Pedal resistance was identical in each session. Oxygen saturation (SPO2) was measured just before the progressive cycling exercise, after 5 minutes and at the end of the performance period. Patient's visual access to the effort-related parameters (speed, distance covered, time remaining) was allowed.

The surface EMG signals from rectus femoris (RF), vastus medialis (VM) and biceps femoris (BF) of the left leg were measured for the entire progressive cycling exercise (10 minutes) using a wireless EMG system (Cometa srl, Milan, Italy). The signals were filtered with a band-pass Butterworth filter of order 4 in the range of 10-250 Hz. The MDF of the power spectrum and the RMS of the surface EMG signal were calculated with an open-source software distributed by Anaconda (open-source distribution of the Phyton). Since dynamic contractions were used, only the EMG signals from the most biomechanically repeatable portions of the movement were considered (*Bonato et al.,2001*, *Beck et al.,2014*). Fatigue was measured as time variation of MDF and RMS, quantified by the slope of the linear regressions of these parameters versus time. A negative MDF slope means fatigue. The change of RMS is indirect evidence of fatigue, in that a positive RMS slope may be related to recruitment of additional motor units to compensate for fatigue.

4.2.3 STATISTICAL ANALYSIS

Changes in the median values of 21-MFIS and VAS-F (0-100) scales at t0, t1 and t2 were compared by a Wilcoxon signed-rank test for related samples. A two-tailed P-value of 0.05 was used as a threshold for significance. In addition, statistical differences between MDF or RMS values at t0, t1and t2 were estimated using the one-way ANOVA test for repeated measurements.

4.2.4 RESULTS

All the three patients completed the entire study and did not complain with any side effects due to A-tDCS.

Subjective measures. Variations of subjective fatigue parameters following sham and anodic stimulation sessions are shown in Fig.1.The Wilcoxon signed-rank test for related samples did not show a significant effect of A-tDCS on the scales 21-MFIS (both global and subscales) and VAS-F (0-100) (Tab.1). In addition, the single-case analysis showed that such negative results were consistent among the three patients.

Objective measures. Patients #1 and #2 reached the final stage of the progressive cycling exercise both at the baseline and after the sham and anodic stimulations, thus achieving their 100% speed. Patient #3 completed the test only at t2, while interrupting it after 6 minutes at t0 and after 8 minutes at t1. Therefore, to calculate the MDF slopes for this patient, only the first 6 minutes of the progressive cycling exercise were considered both at t0, t1 and t2.

Figure 1 shows the MDF slopes for all the 3 patients. Figure 2 shows the mean MDF slopes across the 3 subjects. One-way ANOVA showed no differences between t0, t1and t2.

Figure 3 shows the mean normalized RMS slopes across the 3 subjects, where normalization is done with respect to the initial RMS value. Also, in this case, one-way ANOVA showed no differences between t0, t1 and t2.

21-MFIS			
	Baseline	**A-tDCS**	**P value**
Overall scores	29 (29-52)	27 (20-75)	1
Physical score	24 (14-26)	18 (15-33)	0.59
Cognitive score	13 (3-22)	10 (0-35)	1
Psychosocial score	2 (2-4)	2 (2-7)	0.31
VAS-F (0-100)			
Overall scores	54 (52-96)	47 (47-91)	0.1

Tab.1 Median Scores of 21-MFIS and VAS-F (0-100) (range values in brackets) were not significantly different at the baseline (t0) and after A-tDCS (t1).

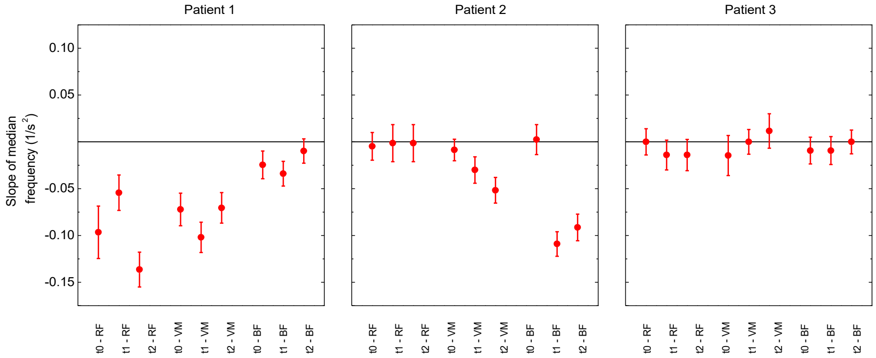

Fig. 1: MDF slopes during the progressive cycling exercise in Rectus Femoris (RF), Vastus Medialis (VM) and Biceps Femoris (BF) in the 3 subjects. Error bars represent standard deviations of linear regressions.

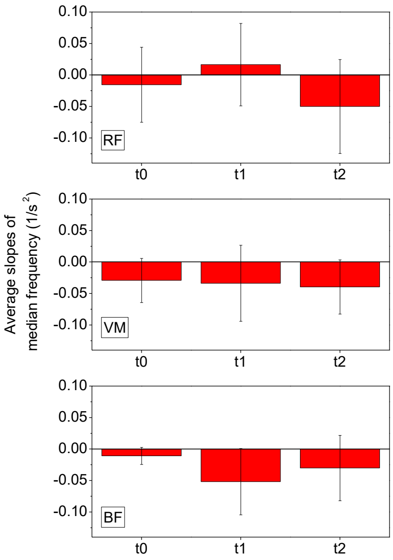

Fig. 2: MDF slopes averaged over the 3 patients, for Rectus Femoris (RF, upper panel), Vastus Medialis (VM, middle panel) and Biceps Femoris (BF, bottom panel). The error bars indicate the standard deviations.

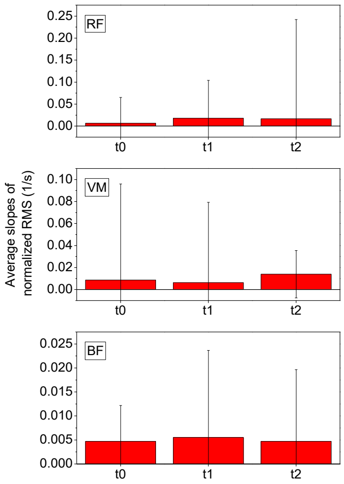

Fig.3: Normalized RMS slopes averaged over the 3 patients, for Rectus Femoris (RF, upper panel), Vastus Medialis (VM, middle panel) and Biceps Femoris (BF, bottom panel). The error bars indicate the standard deviations.

4.2.5 DISCUSSION

This sham-controlled, exploratory study was the first attempt to treat fatigue symptoms in patients who recovered from COVID-19. This was done through repeated sessions of *off-line* A-tDCS over the left DLPFC. The subjective fatigue perception was assessed using two questionnaires, namely the structured 21-MFIS and the global VAS-F (0-100). In order to minimize the intrinsic limitations of the subjective measures of fatigue, we included the increase in RMS amplitude and the shift of the power spectrum of the surface EMG during a progressive cycling test as an objective measure of fatigue.

The statistical analyses showed no significant improvement in both subjective and objective measures of fatigue after DLPFC stimulation in the whole group. The 21-MFIS and VAS-F scores showed a fluctuant behavior throughout the experiment regardless of the type of stimulation (sham or real) and did not significantly differ from baseline. As to the EMG measures, the F_{med} decreased during the tests (negative slopes), thus indicating the occurrence of fatigue for all the muscles and all the patients, both before and after brain stimulation. The results of this study, therefore, suggest that repeated sessions of *off-line* tDCS over the left DLPFC are not beneficial for post-COVID-19 related fatigue.

Some hypotheses can be taken in to account in order to explain these negative results. First, one has to consider that the pathophysiological mechanisms of fatigue are largely undetermined. This uncertainty holds particularly true for COVID-19 related fatigue, a very recently described clinical feature whose nature (central vs peripheral) is actually unknown and only roughly hypothesized (proinflammatory cytokines production, axonal injury, metabolic and respiratory dysfunction). Given this uncertainty, it is possible that the inefficacy of noninvasive brain stimulation could either reflect a not entirely appropriate choice of the assessment instruments (MDF and RMS are reliable measures of “peripheral” myoelectric fatigue, but not of “central” fatigue) or that the prefrontal brain region was an incorrect stimulation target in order to improve post-COVID-19 (“peripheral”) fatigue. Alternatively, it is possible that an *off-line* approach was not sufficient to reduce fatigue by itself and that tDCS could only act by increasing the effects of a simultaneous rehabilitative protocol (i.e. A-tDCS coupled with motor training). Support to this hypothesis comes from studies on healthy individuals (*Fertonani et al.,2010, Angius et al.,2018*) and patients with neurological disorders (*Vestito et al.,2014; Pilloni et al.,2020*) which emphasized the crucial role of synaptic activation induced by behavioral treatments combined with concurrent brain stimulation (i.e. *on-line* approach). On the other hand, the *off-line* approach has been demonstrated to be effective in several neurological disorders such as multiple sclerosis, epilepsy, and Parkinson’s disease (*Rotwell,2012*, *Chalah et al.,2017*). Other methodological issues may explain our negative results. For instance, the stimulation intensity was set at 1.5 mA, in agreement with most of the previous studies (*Bikson et al.,2016*). However, some studies reported that stronger stimulation might achieve larger effects (*Liu et al.,2018; Esmaeilpour et al.,2020*). The placement of the cathode on the right forehead (bi-cephalic montage) could have also influenced the effects of the simulation. Variations in the position of the return electrode may indeed change the current path and density, with a non-cephalic montage producing a higher current density than the bi-cephalic one (*Russell et al.,2017*).

4.2.6 CONCLUSIONS

Although the small number of participants and the uncontrolled nature of the experiment may decrease the generalizability of our results, the present study suggests that repetitive sessions of *off-line* A-tDCS over the left DLPFC does not relief fatigue symptoms in post COVID-19 patients, as assessed by both subjective and objective outcome measures. We discussed some variables that could have contributed to these negative results. Given the high prevalence and the disabling nature of fatigue in post COVID-19 patients, other tDCS protocols need to be tested rapidly. In this context, the present negative findings could be of interest and help for implementing future studies on fatigue in post COVID-19 patients.

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CHAPTER 5

IMPROVED VISUOSPATIAL NEGLECT AFTER TDCS AND COMPUTER-ASSISTED COGNITIVE TRAINING IN POSTERIOR CORTICAL ATROPHY: A SINGLE-CASE STUDY.

5.1 INTRODUCTION

In 1988 Benson and coworkers first introduced the term “Posterior Cortical Atrophy” (PCA) to describe a neurodegenerative condition clinically characterized by a deficit in higher-order visual functions associated with atrophy of the parieto-occipital areas, in the absence of ophthalmologic disorders (*Benson et al.,1988*). PCA is generally considered an atypical form of Alzheimer’s disease (AD), but, at a variance with AD, several cognitive skills, such as speaking, memory, executive functions and insight are typically preserved in the early stages of the disorder (*Suarez-Gonzalez et al.,2015*). According to Crutch et al. (2017), two types of PCA can be described: a ventral type, with visuoperceptive dysfunctions, alexia, visual agnosia and prosopagnosia, and a dorsal type, characterized by complete or incomplete Balint’s syndrome (simultanagnosia, oculomotor apraxia, optic ataxia, environmental agnosia), some features of the Gerstmann’s syndrome (acalculia, agraphia, finger agnosia, left/right disorientation) and visuospatial neglect (VN).

VN is a condition characterized by a reduction or loss of spatial awareness for the contralesional space. It mainly occurs after right hemisphere lesions, usually stroke. In PCA, VN can be overlooked because of the occurrence of additional cognitive impairments, which are more easily detectable, and because it may be unrecognized when brief cognitive screening procedures are used. Andrade et al. (*2010*), however, have demonstrated that VN can occur in up to 66% of the patients with PCA, provided that specific tests are used.

VN is highly disabling and requires specific rehabilitation (*Svaerke et al.,2019; Zebhauser et al.,2019*). Several rehabilitative interventions have been suggested, using either top-down or bottom-up strategies (*Azouvi et al.,2017*). Unfortunately, there is a little evidence about the efficacy of the different treatment options and it is impossible to date to recommend one rehabilitative approach over another. In light of this limited evidence-based treatment opportunities, non-invasive brain stimulation (NIBS) techniques have emerged, such as repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS). NIBS techniques represent a very promising alternative (or add-on) to the traditional rehabilitative approaches (*Zebhauser et al.,2019; Veldema et al.,2019*), particularly for conditions where there are a few effective therapies, if any. NIBS is based on the Kinsbourne's model of "Interhemispheric rivalry" (*Kinsbourne,1987,1993*), where excitatory and/or inhibitory stimulations aim to restore the interhemispheric imbalance caused by a unilateral brain damage. In tDCS, a direct-current flow of low intensity (1–2μA) is delivered via two electrodes placed on the scalp. It acts by a tonic modulation of the resting membrane potential of the cortical neurons, which occurs in an opposite direction, depending on the polarity (anodal vs. cathodal) of the electrodes placed on the chosen areas. It is commonly stated that cathodal stimulation (C-tDCS) decreases cortical excitability due to neural hyperpolarization, while anodal stimulation (A-tDCS) reaches the opposite effect by a subthreshold depolarization. (*Nitsche & Paulus,2000*)

In this single-case study, we implemented a combined rehabilitative treatment on a patient with PCA, whose main clinical feature was represented by left VN. Our aim was to investigate whether or not CCT on its own and/or coupled with concurrent A-tDCS over the right Posterior Parietal Cortex (PPC) has a role in VN rehabilitation. VN was assessed by a digital and web-based version of the standard tests, namely the Remote Monitoring Validation Engineering System (ReMOVES) platform (*Ferrara et al., 2017*). The same system was also used for computer-assisted cognitive training (CCT) by means of several serious games with a top-down approach (i.e. training the direction of gaze by means of visual cues). CCT has recently begun to be used in neurorehabilitation due to its relatively low cost and high flexibility. It is also able to enhance patients' motivation and visual attention by the use of dynamic visual stimuli (*Laver et al., 2015; Svaerke et al., 2019*).

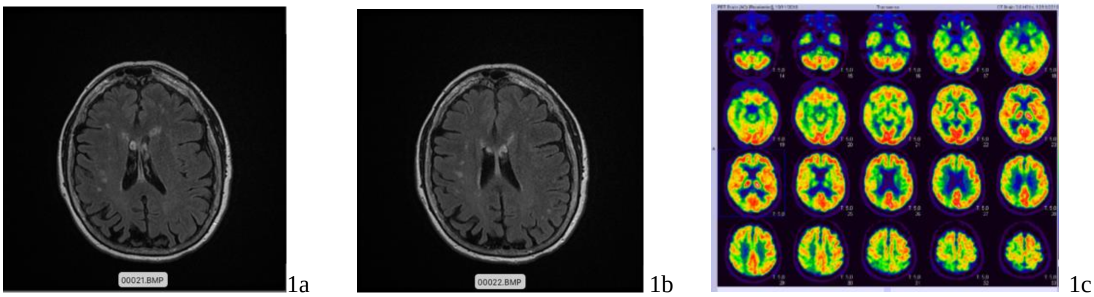
5.2 CASE ANALYSIS

The patient was a 70-year-old right-handed woman, with 17 years of education. She did not have a significant past medical or surgical history and was unmedicated. She presented with a one-year history of gradual, progressive difficulty in reading, writing on the computer, driving and parking her car. She was still able to take care of the basic activities of daily living (ADL score 6/6) (*Katz,1963*), but experienced some problems in the instrumental activities, especially in the presence of some external distractors (i.e. difficulties in locating left-sided objects in the kitchen while cooking) (IADL score 5/8) (*Lawton & Brody,1969*). Entering the car from her left side (as a passenger) was almost impossible, while the same did not happen when doing the same action as a driver (right side). While driving, she experienced difficulties in taking roundabouts, sometimes bumping on the left sidewalks.

She was first seen by an ophthalmologist, whose assessment was unrevealing. The neurological examination did not reveal any sign of pyramidal, sensory, cerebellar and cranial nerves dysfunction. The extinction phenomenon was not observed. On visual confrontation testing no evidence of visual field defects was detected. A computerized perimetry testing was not performed.

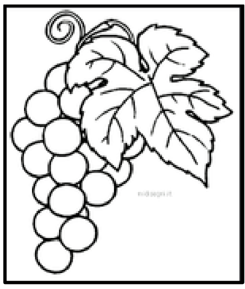
5.2.1 ASSESSMENT

Brain imaging (MRI, Figure 1a-b) revealed atrophy of the posterior parietal and occipital cortex (right worse than left), while the positron emission tomography-scan (FDG-PET) showed a significant hypometabolism in the same areas (Figure 1c). An extensive neuropsychological examination was performed: the patient was alert and cooperative, entirely aware of her visual deficits. She was oriented to person, time and location. Non-visual attention was preserved. The Mini Mental State Examination (MMSE) score was 27/30 (normal value ≥24/30): one point was lost in reading a written command, one point in intersecting pentagons, one point in calculation. Patient's spontaneous speech was fluent and grammatically correct, and the phonemic and semantic fluencies were intact. Episodic memory was not impaired. Executive functions were normal. Color, facial and object recognition was within the normal values. A mild oral dyscalculia was observed, but no left/right discrimination or finger agnosia. Imaginary spatial representation was unimpaired. Signs of simultanagnosia were found when asking the patient to describe overlapping and complex figures (Figure 2a). Drawing a clock was severely impaired (Figure 2b), as well as reading and writing. When required to read a text she constantly ignored the left side of the page and got lost from one line to the next. When asked to write a sentence, she was unable to maintain the horizontal line of writing, moving downward and always started writing from the middle of the paper sheet. Based upon clinical, neuropsychological and imaging features a diagnosis of posterior cortical atrophy (PCA) was made (dorsal type), fulfilling Crutch et al.'s criteria (2017).

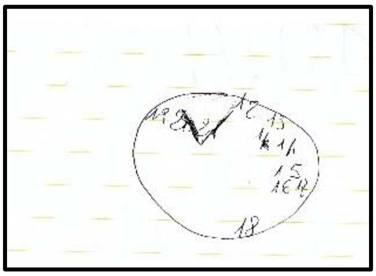


1a 1b 1c

Figure 1a-b-c: Representation of structural (MRI) and metabolic (PET) impairment of the parietal occipital cortices.
a-b) Brain MRI showing atrophy of the posterior parietal and occipital cortex (right worse than left). c) FDG-PET scans revealing a significant hypometabolism in the same areas.



2a



2b

Figure 2a: A grape cluster picture was described as follows: “there are a lot of marbles or balls, and I see a lot of pushpins up higher and down lower, in the top part I see curlicues like a Roman column”.

Figure 2b: When drawing a clock, the patient drew an oval. When inserting the numbers into the circle she wrote them in a digital modality (13, 14, 15 … instead of 1, 2, 3..). She was also inaccurate in spatial positioning of the numbers on the left side; furthermore, numbers 19, 20 and 21 were turned in a clockwise direction (upper left quadrant) and aligned on the same horizontal line.

5.3 METHODS AND PROCEDURE

This single-blind, uncontrolled single-case study consisted of 2 weeks (5 days per week, 20 min) of daily CCT, followed by 2 weeks (5 days per week, 20 min) of CCT combined with repeated sessions of simultaneous A-tDCS (“on-line” approach). VN outcome measure were assessed at the baseline (t0), after CCT (t1) and after CCT coupled with A-tDCS (t2). The patient and the therapists were aware of the type of treatment, while the assessors who analyzed the neuropsychological data were blinded as to the design of the study. In order to control for transfer effect on IADL, a questionnaire was filled out by the husband and the patient herself. An informed consensus was obtained from the patient and from the husband prior to the beginning of the procedures and the principles of the Declaration of Helsinki were followed. The study protocol was approved by the Institutional Review Board.

5.3.1 VN ASSESSMENT.

The digital, web-based ReMOVES platform was utilized for VN assessment. ReMOVES has been developed by the Department of Naval, Electrical, Electronics, and Telecommunications Engineering (DITEN), University of Genoa, Italy. Technical details on this device are reported elsewhere (*Ferrara et al.,2017; Morando, Ponte, et al.,2018*). Briefly, it is a highly flexible platform based on a multi-client/server architecture created for motor/cognitive assessment and training, through serious games and digital versions of standard tests. The patient seats in front of a monitor subtending 15° of visual angle at a viewing distance of 60 cm. The screen is aligned to the midsagittal plane of the patient, who interacts with the activities shown on screen without wearing sensors, markers or controllers in hand. The calculation of correct targets number and their position is automatic, thus making the analysis easier and faster. Differently from other existing solutions, ReMOVES provides objective data, including automated data processing techniques It makes it possible, therefore, to track the trajectories followed by the patient, delivering further information on the adopted strategy and the velocity of execution, as in our patient (see below). According to our preliminary results, ReMOVES shows a good level of test-retest reliability and convergent correlation (unpublished data).

Three specific tests were chosen for VN assessment, which were based upon the conventional paper-pen tests (*Morando, Bonotti, et al.,2018*):

a) *Digital Line bisection test.* The test includes a total of 22 horizontal lines. Two lines are used for a practice run-in task and are not included in the analysis. The remaining 20 lines are shown on the screen, one at a time, in a random fashion regarding position (left, center, right) and length (8, 16 and 24 cm). With respect to the paper-pencil test, the digital version allows customization of the color, width, height, position, and rotation of the line in order to provide multiple variants of the standard test and in-depth analysis. In addition, the digital version displays a new line whenever the patient manages to cross out the line, continuously up to two minutes. This allows repeating the measurements more times than the traditional test that is based on just three lines. The patient is instructed to touch the middle of the line. Invalid and accidental touches are automatically ignored by the system. Deviations in screen pixels from the true midline are averaged and converted into millimeters, thus allowing the automatic computation of the bisection point position.

b) *Digital Albert's test.* Forty lines (2.5 cm) with fixed distribution and pseudorandom orientations are displayed on the screen. The game area can be divided by two different approaches. The first one requires to divide it into seven rows containing six targets each, except for the central one which contains only four targets. The percentage of cancelled out targets in each row is collected into a 1X7 vector. In the second one, which permits a deeper feedback on the explored regions, the game area has to be divided into eight square sub-regions, four in the upper part of the game set, and four in the lower one. Consequently, the size of the vector is 1X8. ReMOVES automatically calculates the percentage of correct responses.

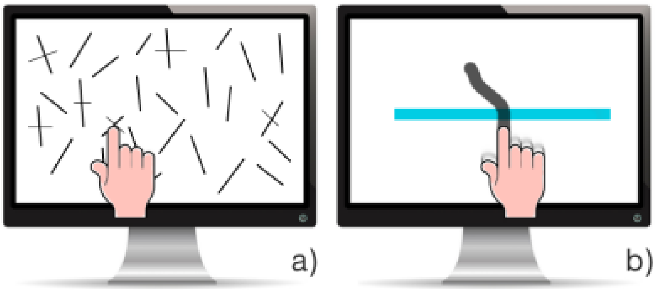


Fig.5 Digital version of Albert Test(a) and Bisection Test (b) included in ReMOVES platform

c) *Digital Apple Test*: This activity is the digital version of the Italian standardization (*Mancuso et al.2015*). Apple test is a cancellation task in which 150 apples are shown on the screen with a pseudorandom distribution. Fifty apples are complete (targets), while 100 are open either on the left or the right side (distractors). The game area is divided into a grid with two rows and five columns to ensure an equal distribution of the apples across the page. Each cell of the grid contains 15 apples: three large ones (one with no opening, one with an opening on the left and one with an opening on the right) and 12 small apples (four without openings, four with openings on the left and four with openings on the right). The subject is asked to touch only the complete apples. ReMOVES automatically computes the percentage of correct responses.

5.3.2 CCT AND SIMULTANEOUS TDCS

For the CCT, the training was implemented by means of ReMOVES exergames. Among the several exergames incorporated in ReMOVES, we chose the “ShelfCans”, the “OwlNest” and the “ChinaLanterns”, which were specifically tailored for CCT (*Ferrara et al.,2017; Morando et al.,2019*). The following is a detailed information about the exergames:

Shelf Cans: This exergame introduces the patient to a virtual environment, i.e. a kitchen. With the arm movement, the patient grabs one of the colorful drink cans showed in the middle of the screen and drags it to the corresponding shelf. This game is appealing because it requires the user to be attentive to drop off the drink can on the correct shelf according to its color.

Owl Nest: The patient is encouraged to reach an on-screen target with the arm motion (Reaching Task) to achieve a high in-game score. Many colorful owls appear randomly in any position of the screen for a given timeframe: the user must grab and put them in the nest (in the middle of the screen) before they disappear. The patient is encouraged to reach the consecutive on-screen targets with the arm motion (reaching task) to get rewarded with a high in-game score. An initial calibration phase adapts the games to the user, which would be able to complete the tasks even with reduced mobility. The game auto-adapts the rate of generation of new targets based on the patient's speed, and at the same time, limits to three the maximum number of owls present on the screen if the patient fails to dispose of the queue quickly enough.

ChinaLanterns: In this exergame, Chinese lanterns appear from the lower side of the screen and rise towards the "sky" (upper part of the screen). The player must act as quickly as possible and touch them using a touchscreen. By comparing the performances relative to the targets shown on the right or left of the screen, this game is very useful for neglect rehabilitation

When coupled with CCT, stimulation with tDCS was delivered by a battery-driven, constant current simulator (Newronika s.r.l, Italy), with two holding bags of plant cellulose (5 cm × 5 cm), and two electrodes of conductive silicone. The active (anodal) electrode was placed on the scalp overlying the right PPC (P4, according to the international 10-20 EEG system). The reference electrode was positioned over the contralateral supraorbital region. Electrodes impedance was kept constantly below 5 kΩ. During the training task, A-tDCS (current of 1.5

mA) was delivered for 20 min. Current density (0.06 mA/cm2) was maintained below the safety limits (*Poreisz et al.,2007*). The "on-line" approach and the multiple stimulation sessions were chosen in order to accomplish a synergistic effect and, therefore, enhance the beneficial effects of the treatment (*Brem et al.,2014; Yang et al.,2017*).

5.3.3 STATISTICAL ANALYSIS

The statistical analysis was carried out on the following measures: the percentage of correct responses in the Digital Albert's test and in the Digital Apples Cancellation test; the deviation of the bisection from the true midline in the Digital Line bisection test. In order to compare the results of the three tests at times t0, t1 and t2, the non-parametric McNemar chi-square test for repeated measures was used. A two-tailed *P*-value of 0.05 was used as a threshold for significance. Data were analyzed with MatLab R2018b software (mathworks.com/products/matlab).

5.4 RESULTS

The patient completed the entire study and did not complain with any side effects related to A-tDCS. At the baseline (t0), she demonstrated a left VN involving all the test modalities employed. After the two-weeks of CCT alone (t1), there were no significant differences from the baseline in the patient's performances. A significant improvement was conversely observed in all the outcome measures after the combined treatment with CCT and A-tDCS (t2): the percentage of correct responses in the Albert's test significantly increased both in the 1X7 and 1X8 sub-regions ($p<0.003$ and $p<0.009$, respectively); in the Line bisection test the midline was deviated rightward (28.06 ± 12.11mm) at t0 and no significant changes were observed at t1 (23.12 ± 11.05 mm); a significant average reduction of the rightward deviation (19.36 ± 9.96 mm) was observed ($p < 0,012$) at t2, with a few leftward deviations; in the Apples cancellation test the percentage of crossed-out targets were 16% at t0; it remained unchanged at t1 (22%, *ns*) and significantly increased up to 60% at t2 ($p < 0.0001$). As to the transfer of the VN symptoms amelioration into the non-trained instrumental activities of daily life, the patient showed an improvement in IADL, which increased from 5/8 to 7/8 (specifically in food preparation and mode of transportation items) as measured by the questionnaire.

5.4.1 ADDITIONAL RESULTS

As mentioned above, ReMOVES offers a series of automated data that allows to investigate a number of additional information. In the Albert's test, for example, the system automatically tracks the path followed by the patient to cancel the targets, thus providing a reliable feedback on the search strategy adopted (Figure 3)

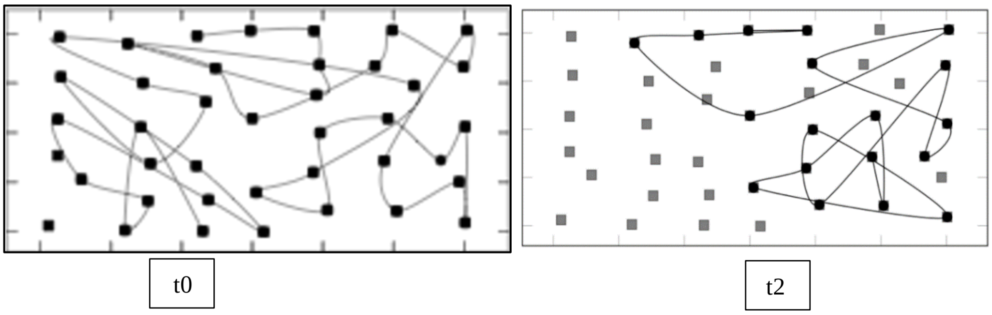


Fig.3 Trajectories reproducing the order of targets cancellation at t0 l(eft) and t2 (right). At t2 the field of visual exploration is markedly increased.

ReMOVES also automatically evaluates the distance between two consecutive cancelled targets: the smaller the distance, the more efficient the visual exploration strategy. In our patient the average distance at t0 was 3.71 ± 1.86 cm, while at t2 it became 3.02 ± 1.82 cm (average distance of a healthy subject 2.38 ± 1.26 cm), thus showing a better exploration strategy. The time taken by the patient between two consecutive cancellations is also computed by the system. Longer time is generally observed at the end of the session, when the subject is looking for the remaining few targets (Figure 4).

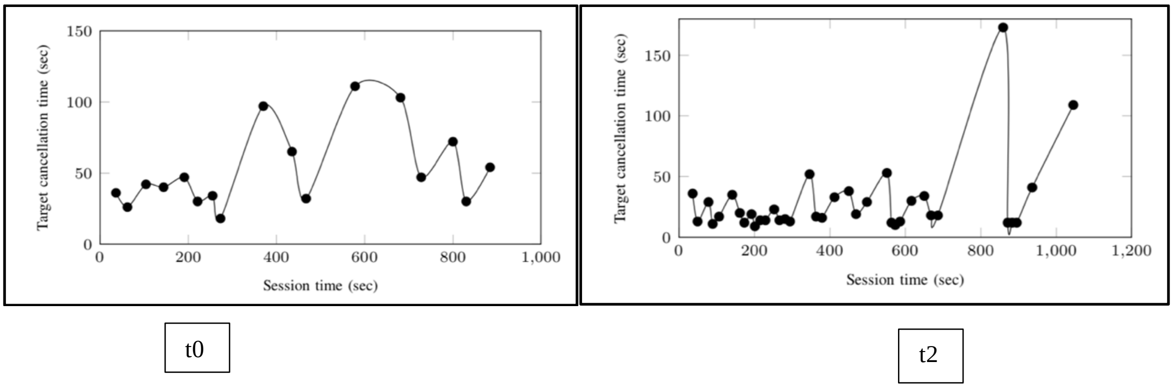


Figure 4: The variation of the time between two consecutive cancellations at t0 and t2 is represented. The average time at t0 was 49.11 ± 29.15 sec, while at t2 it was reduced to 27.50 ± 21.78 sec (average time of a healthy subject 5.95 ± 1.64 sec). A short time denotes a good exploration strategy. The time increases at the end of the session, until the patient notices there are no more targets to be cancelled.

5.5 DISCUSSION

This single-case study was implemented to evaluate the potential therapeutic effect of a computer-assisted rehabilitation, alone or in combination with repeated sessions of simultaneous A-tDCS, on VN symptoms in a patient with PCA. We found a significant improvement of the VN outcomes measures only with the simultaneous approach (CCT plus A-tDCS), while CCT training on its own did not lead to significant changes.

All the VN symptoms ameliorated and, interestingly, the ipsilesional deviation in the line bisection test turned into a contralesional deviation at the end of the treatment. This infrequent phenomenon might suggest for overcompensation. Unfortunately, there was no follow-up and the possible transient nature of overcompensation could not be determined. The beneficial effects also generalized outside the experimental setting, showing some ecological activity: a measurable transfer into the IADL was actually reported (ADL were already unimpaired). Recently, a number of studies have been published regarding the role of tDCS in stroke-related VN rehabilitation (for a review see *Zebhauser et al.,2019*). All of these studies were implemented by unilaterally or bilaterally stimulating the posterior parietal cortex (PPC), which plays a dominant role in visuospatial attention. Overall, a significant improvement of post-stroke VN was observed. To our knowledge, this is the first study aimed to evaluate the impact of CCT in combination with A-tDCS (over the PPC) on PCA-related VN. A significant effect was achieved in a neurodegenerative disease showing notable atrophy and hypometabolism in that region. The posterior parietal area is considered the most critical lesion site for VN and has been selected in all the previous tDCS studies for VN rehabilitation. In another study on a PCA patient, the authors intended to explore the effect of tDCS over the dorsolateral prefrontal cortex (DLPC) on a looser range of cognitive abilities (executive functions, visual memory, visual attention and visuo-spatial short-term memory) (*Gramegna et al.,2018*). Patients with stroke-related VN show a spontaneous recovery in about 60% of the cases (*Nijboer et al.,2013*). This is unlikely in our case because of the intrinsic nature of the degenerative disease. In fact, PCA-related VN is supposed to persist and eventually deteriorate over time due to disease progression. Inconsistency of performances on VN tests could be an alternative reason for our findings, but this hypothesis can be reasonably excluded since unpredictability of results is typical of stroke-related VN (*Hamilton et al.,2008*). The presence of nonlateralized deficits in attention, which are often prominent in patients with unilateral neglect, could have also influenced the outcome of lateralized symptoms (*Buxbaum* *et al.,2004*). This is again unlikely in our patient, since she was always fully alert and performed well to the non-visual attention tests.

Our study has several limitations. First, it was a single-blind, uncontrolled study. Second, we did not perform a sham (placebo) stimulation and, therefore, we cannot exclude the possibility that the beneficial effects were due to CCT alone. However, the VN improvement after the combined CCT and A-tDCS sessions, in spite of the unsuccessful CCT, may support the critical role of A-tDCS. Third, there was no follow-up, so the durability of the effect could not be determined. Finally, the core nature of a single-case study hampers the possibility of generalize the results to a larger population. As such, our results must be interpreted very cautiously.

5.6 CONCLUSIONS

Within the limit of a single-case study, our results might suggest that multiple sessions of A-tDCS over PPC, coupled with cognitive training, can alleviate the symptoms of VN even in a neurodegenerative disorder such as PCA. This should not be of much surprise, since NIBS techniques have already been shown to produce beneficial effects in other neurodegenerative diseases, like AD, Frontotemporal dementia, Primary Progressive Aphasia (*Roncero et al.,2017,2019; Fenner et al.,2019*). In PCA, stimulation of the PPC may facilitate the neuroplasticity of the cortical networks involved in visuospatial attention and awareness specific of this brain region.

As a secondary outcome, our findings also support the use of the ReMOVES digital platform as a reliable and flexible device for computer-assisted assessment and training of VN patients. It allows an automatic computation of the patient’s data and delivers activities using low-cost off-the-shelf components and an easy-to-use interface.

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CHAPTER 6

VISUAL NEGLECT REHABILITATION SUPPORTED BY A DIGITAL SOLUTION AND tDCS: TWO CASE-STUDIES.

6.1 INTRODUCTION

Visual Neglect (VN) is a term which denotes the failure to detect, respond or orient toward stimuli located in the hemi/body and or hemispace contralateral to the lesioned hemisphere (*Buxbaum et al.,2004*). It is a common and severely disabling neurobehavioral disorder induced by discrete cerebral lesions such as stroke, tumors, trauma, and degenerative diseases. It is most frequently associated with damage involving the right parietal and occipital lobes, the basal ganglia and the thalamus (*Ringman et al.,2004*). Patients suffering from VN show slow functional progress during rehabilitation and need long hospitalization times, and are less likely to be able to live independently with deterioration of the quality of life. Three bedside tests can be used to detect the presence of VN: line bisection, target cancellation, and drawing. In line bisection, the patient is asked to mark the middle of twelve lines presented on a paper. The longer are the lines, the easier is to reveal neglect. In target cancellation, the patient is asked to mark out or cancel the targets (i.e., bells, stars or apples) among a variety of distractors. In particular, Apple cancellation test is aimed at differentiating between allocentric (object-centered) and egocentric (stimuli-centered) form of neglect (*Mancuso et.,2015*). Some other cancellation tasks, instead, have only target items (Albert’s task) (*Albert, 1973*). In drawing, the patient is required to copy a figure, or to draw it from memory. Several rehabilitation strategies for VN (TENS, optokinetic stimulation, somatosensory electrostimulation, mirror therapy) have been reported (*Klinke et al.,2015*). Their efficacy, however, is still controversial. A meta-analysis by Pollock et al (2011) states that there is only a limited number of high-quality studies suggesting the efficacy of VN interventions in improving functional outcomes and reducing disability. Azouvi et al (2017), in a recent review, concluded that there still is only a low level of evidence for the different rehabilitation methods and emphasized the need for longer validation trials using innovative techniques such as noninvasive brain stimulation (NIBS). NIBS techniques have recently emerged in restorative neurology due to their hypothetical advantage in enhancing the efficacy of traditional therapeutic intervention. In this view, the re-discovery of the application of a direct-current flow of low intensity (1–2μA) has raised much interest. This technique is known as transcranial direct-current stimulation (tDCS). It acts by a tonic modulation of the resting membrane potential of the cortical neurons, which occurs in an opposite direction, depending on the polarity (anodal vs cathodal) of the electrodes, placed on the chosen areas. It is commonly stated that cathodal stimulation (C-tDCS) decreases cortical excitability due to neural hyperpolarization, while anodal stimulation (A-tDCS) reaches the opposite effect by a subthreshold depolarization (*Nitsche et al.,2000*). The use of tDCS has been shown to be a promising approach in order to improve post-stroke neglect. A-tDCS and bilateral tDCS appears to be more effective than C-tDCS (*Veldema et al.,2019*). A cognitive therapy is usually associated to the tDCS approach, which consists of performing tasks with the aim of improving the patient's capability of investigating the ignored hemispace. This complementary activity can be provided by digital solutions as in (*Morando, Bonotti et al.,2018; Pedroli et al.,2015; D'Amico et al.,2013*). In this study, we used the ReMOVES platform *(Ferarra et al.,2017*). The data-acquisition capabilities of the ReMOVES platform allow therapists to monitor the patient's activity and progress. Specific tests to assess the visuo-spatial disorder severity and appropriate exercises for the training of visual attention in the neglected region have been developed. The tests have been implemented according to the traditional paper-based test versions, and supervised by therapists. The validity of this approach is based on the statistical study in (*Morando, Bonotti et al.,2018).* In this study, we present two patients with neglect who were treated with tDCS. Both patients underwent ten consecutive A-tDCS sessions (five days per week over a two-week period) coupled with a simultaneous cognitive training ("on-line" approach). The innovative aspect of this work consists of presenting the combined use of tDCS and a tool for digital support to assess and treat VN. Indeed, the cognitive therapy was essentially based on computerized neglect test exercises, provided by the ReMOVES system, which had the finality of increasing the patient's attention and the spontaneous exploration of the left hemispace.

6.2 CASE-STUDIES

6.2.1 PATIENT A

Patient A was a 70 year-old right-handed woman, who started complaining with vague difficulty in reading, writing on the computer, driving and parking her car. She also experienced problems in finding objects on the left (e.g., in the kitchen while cooking). Entering the car from her left side (as a passenger) was almost impossible, while the same problem did not happen when getting in the car as a driver (right side). The patient was first seen by an ophthalmologist and then by a neurologist. But for a minimal bilateral myopia, the physical examination was normal. Optical coherence tomography (OCT), fluorescein angiography, visual evoked potentials (VEPs) and electroretinogram (ERG) were unremarkable. Automatic perimetry did not show hemianopia or other visual field defects. The neuropsychological examination showed the following findings: signs of simultanagnosia, writing and reading disturbances, and left neglect. The Mini Mental State Examination (MMSE) was 27/30 (cut-off ≥ 24). The patient showed severe impairment when reading, writing and copying (intersecting pentagons), while the verbal memory, the phonemic and semantic fluencies were intact. Cerebral magnetic resonance imaging (MRI) revealed a bilateral atrophy of the parietal and occipital cortices (right worse than left). The FDG-PET showed a significant hypometabolism in the same areas (Figure 2). A diagnosis of posterior cortical atrophy (PCA) was made. PCA is a neurodegenerative condition characterized by an insidious onset, gradual progression and prominent early disturbance of visual functions, in the absence of ophthalmologic causes (*Crutch et al.,2012*). The most frequently neuropsychological deficits of PCA are alexia and features of Balint's syndrome (simultanagnosia, oculomotor apraxia, optic ataxia, environmental agnosia), Gerstmann's syndrome (acalculia, agraphia, finger agnosia, left/right disorientation) and VN (*Beh et al.,2015).*

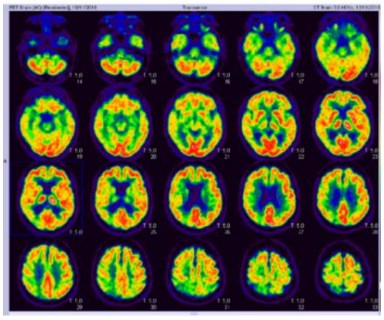


Fig.2 Patient A: cerebral FDG-PET showing a significant hypometabolism in the posterior temporal and occipital cortex and in the bilateral parietal cortex.

6.2.2 PATIENT B

Patient B was a 68 year-old man who was referred to the Neurological Rehabilitation Unit of the Ospedale Policlinico San Martino IRCCS of Genova with sequelae of a right cerebral hemorrhage. CT revealed a large right nucleuscapsular intraparenchymal hematoma extending into the homolateral temporal and fronto-parietal area (Figure 3). The physical examination showed a left hemiparesis, normally fluent speech, impairment of superficial tactile sensitivity and proprioception on the left. In a sitting position, a tendency to retropulsion and left lateropulsion of the trunk was observed. The neuropsychological examination showed a left VN while the other cognitive functions (i.e., orientation, memory, and language) were intact.

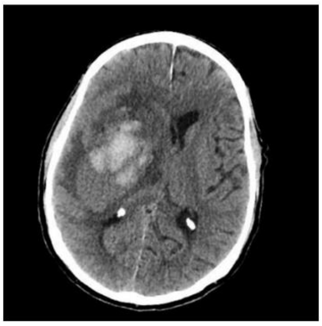


Fig. 3. Patient B: cerebral CT shows a large right intraparenchimal hemorrhage with surrounding edema involving the fronto-temporo-parietal areas.

6.3 VN ASSESSMENT AND REHABILITATION

The ReMOVES platform has been developed by the Department of Naval, Electrical, Electronics, and Telecommunications Engineering (DITEN) of the University of Genova (*Ferarra et al.,2017*). It is a flexible telerehabilitation platform that provides a set of services to support motor and cognitive recovery through exergames and digital versions of standard rehabilitation tests, carried out via Microsoft Kinect, Leap Motion and touchscreen. ReMOVES is based on a multi-client/server architecture which allows for both the collection and access to information from different locations. Indeed, it has been designed for use in rehabilitation centers, with the help of physicians and therapists, or even independently at the patient’s home, thus enabling the continuity of care also after de-hospitalization. Differently from other existing solutions, ReMOVES is an auxiliary tool which provides therapists with objective data even when they cannot directly supervise their patients, such as during unattended use at their home, including automated data processing techniques (*Morando, Ponte et al.,2018; Morando et al.,2019*). Although the ReMOVES platform includes fourteen activities, here only the two tasks used in the assessment and rehabilitation of the two patients are presented. They are the digital version of Albert’s test and Apples test for VN. The proposed system requires the use of a touchscreen as a user interface. The patient interacts with the activity shown on screen without wearing sensors, markers or controllers in hand (Figure 1).

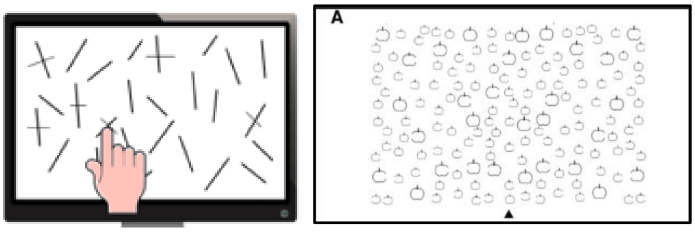


Fig. 1. Setting for the activity via ReMOVES

6.3.1 ALBERT'S TEST

The first description of the Albert's test delivered by ReMOVES regards the similarity between the digital and the paper versions results. In both cases, the game area is divided into seven rows containing six targets each, except for the central one which encloses only four targets (accordingly to *Fullerton et al.,1986*). For each version, the percentage of cancelled out targets in each row is collected into a 1 × 7 vector, called P_d for the digital version and P_p for the paper version. The similarity between digital and paper versions is defined as the correlation coefficient between p_d and p_p, namely

$$Sim = \frac{\sum_{i=1}^{N}(p_{d_i} - \bar{p_d})(p_{p_i} - \bar{p_p})}{\sqrt{\sum_{i=1}^{N}(p_{d_i} - \bar{p_d})^2 \sum_{i=1}^{N}(p_{p_i} - \bar{p_p})^2}}, \qquad (1)$$

where $\bar{p}_d$ and $\bar{p}_p$ are the mean values of p_d and p_p, respectively and N is their length. The resulting coefficients, which are Sim = 0.77 at time T0 and Sim = 0.94 at time T1, show a strong correlation between the two versions, thus denoting a high similarity between the digital and paper approach. High values of the Sim coefficient are obtained also if the game area is divided in an alternative manner. Indeed, in order to have a deeper feedback on the regions that Patient A explored, the game area has been divided into eight square sub-regions, four in the upper part of the game set, and four others in the lower. Consequently, p_d and p_p result to be of size 1×8 and the values of the Sim coefficient, obtained by applying Equation (1), are Sim = 0.71 at time *T0* and Sim = 0.73 at time *T1*, thus confirming the similarity between the two versions. The digital version of the test, when used for cognitive therapy, allows the supervision of the activity along all of the sessions.

6.3.2 APPLES TEST

Apples test is a cancellation test with the task of crossing the entire apples on the game area, without considering the apples open either on the left side or the right side (called distractors). Here, the game area is divided into ten subregions, accordingly to [3]. The average difference between both correctly and erroneously cancelled targets in digital and paper versions is used as similarity metrics at time *T0* and *T1*. The ReMOVES platform provides the automatic calculation of the score indices suggested in (*Mancuso et al.,2015*), namely total number of crossed-out targets, asymmetry score for egocentric neglect, and asymmetry score for allocentric neglect.

6.3.3 PROCEDURE

Both patients underwent ten A-tDCS consecutive sessions (five days per week over two weeks period) coupled with simultaneous digital treatment with ReMOVES. A-tDCS (current of 1.5 mA) was delivered for twenty minutes, delivered by a battery-driven, constant current simulator (Newronika s.r.l, Italy), with two holding bags of plant cellulose (5 cm × 5 cm), and two electrodes of conductive silicone. The active (anodal) electrode was placed on the scalp overlying the right parietal site (P4, according to the international 10–20 EEG system). The reference electrode was located over the contralateral supraorbital region. An electroconductive gel was applied under the electrodes to reduce impedance, which was kept constantly below 5kΩ. The neglect was assessed one week before the stimulation (T0) using the standardized neuropsychological tests both paper-and-pencil and computer-assisted. The same evaluation was performed at the end of the stimulation period (T1) to evaluate the patients' progress. The experimental procedure involving human subjects reported in this paper was approved by the Institutional Review Board, an informed consensus was obtained from participants prior to the beginning of the stimulation, and the principles of the Declaration of Helsinki were followed.

6.4 STATISTICAL ANALYSIS

The statistical analysis was carried out on the following measures: the percentage of correct responses in the Digital Albert's test and in the Digital Apples Cancellation test. In order to compare the results of the tests at times t0 and t1, the Mann-Whitney U test was used. A two-tailed *P*-value of 0.05 was used as a threshold for significance. Data were analyzed with MatLab R2018b software (mathworks.com/products/matlab).

6.5 RESULTS

Both patients tolerated the application of tDCS without any adverse side-effects. After the tDCS treatment, Patient A showed a clear improvement in both tests ($p < 0.003$). Also, Patient B improved up to normal performance in the Albert's test and in the Apples test ($p < 0.02$ and $p < 0.01$ respectively).

6.6 ADDITIONAL RESULTS

In this section, the feedback provided by ReMOVES during the rehabilitation treatment is described. In particular, the activity of Patient A is discussed with respect to the Albert's test, while the activity of Patient B refers to the Apples test.

6.6.1 PATIENT A AND ALBERT'S TEST

The improvement of the exploration capability of Patient A was monitored via the *covered area index*. This is the ratio between the area of the smallest rectangle containing the cancelled out targets and the area of the smallest rectangle containing all the targets. Figure 4 shows the covered area index variation along the sessions.

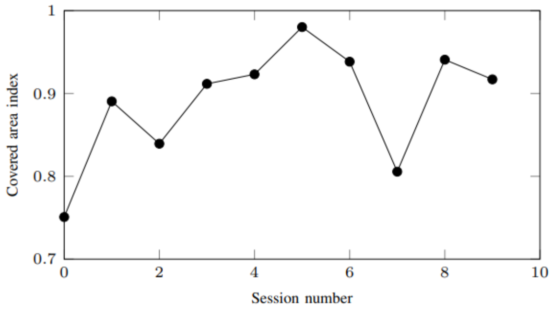
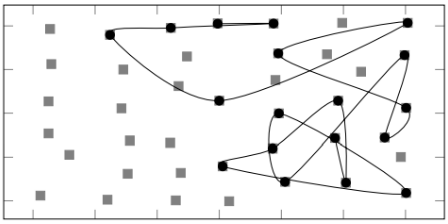
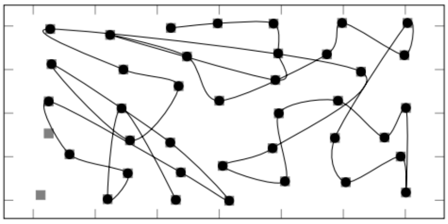


Fig. 4. Patient A: covered area index variation along the sessions. No regular behavior can be noticed, thus proving the absence of learning effect on the task. The path followed by the patient to cancel the targets provides feedback on the strategy adopted to fulfill the task. Figures 5(a) and 5(b) depict the trajectories at time T0 and T1. The more regular path at time T1 shows a clear improvement in the patient's conditions, thus denoting a rehabilitative success.



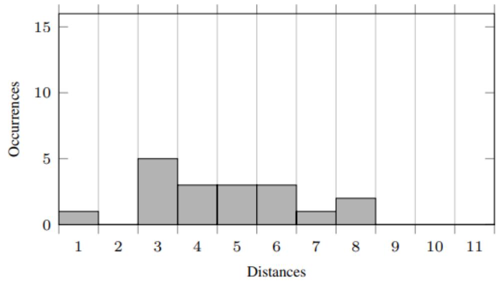
(a) Time $T0$



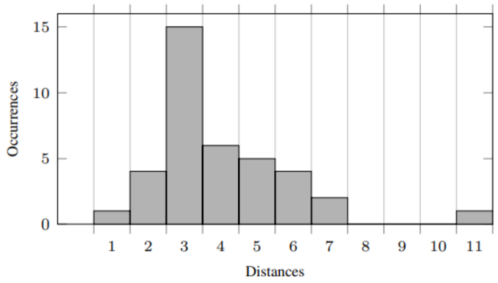
(b) Time $T1$

Fig. 5. Patient A: trajectory reproducing the order of targets cancellation. Note that, at the assessment T0, the majority of cancelled out targets is on the right side, coherently with the left VN diagnosis. At the end of the stimulation T1, the patient managed to enlarge her field of view, thus denoting a rehabilitative success.

The adopted strategy could also be evaluated by calculating the distance between two consecutive cancelled targets: the smaller the distance, the more efficient the strategy. Figures 6(a) and 6(b) show the histogram of approximate distances between consecutive cancelled targets at *T0* and *T1* respectively. The number of occurrences on the bars on the left is larger at *T1* than the one at *T0*, thus showing a better strategy adopted after the rehabilitation treatment, coherently with what can be inferred from the trajectory analysis. The average distance at *T0* is 3.71±1.86 cm, the average distance along the rehabilitation process is 3.17 ± 1.83 cm, and the average distance at *T1* is 3.02 ± 1.82 cm (the average distance of a healthy subject 2.38 ± 1.26 cm).



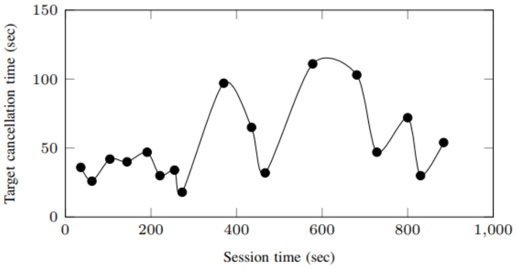
(a) Time $T0$



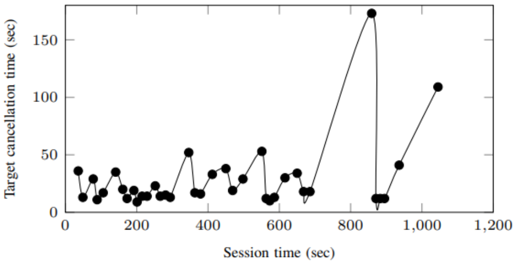
(b) Time $T1$

Fig. 6. Patient A: histograms of approximate distances between a cancelled-out target and the following one. A good strategy is to cancel out all the target in a small neighborhood, thus the more occurrences appear on the left bars, the better strategy has been adopted. Histogram 6(a), which refers to the first session (at T0), has some occurrences for long distances. Histogram 6(b) depicts the good strategy adopted for the session at T1.

ReMOVES platform provides also the waiting time between two consecutive cancellations. A small time between targets cancellations denotes that a good strategy has been adopted. Conversely, a large waiting time means that the patient is exploring a new region of the game area, thus requiring more time to detect a new target. Figure 7(a) and 7(b) show the variation of waiting time between two consecutive cancellation at *T0* and *T1*. The waiting time increases at the end of the session, until the patient states that non more targets are present. The average waiting time at T0 was 49.11±29.15 sec, while the average waiting time during the rehabilitation period was 34.70 ± 27.43 sec. Finally, the average waiting time at T1 was 27.50±21.78 sec (the average waiting time of a healthy subject is 5.95±1.64 sec).



(a) Time $T0$

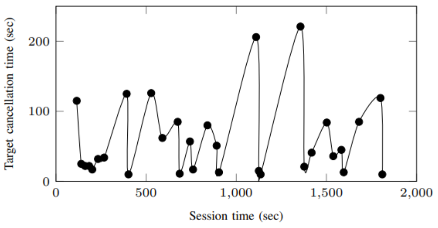


(b) Time $T1$

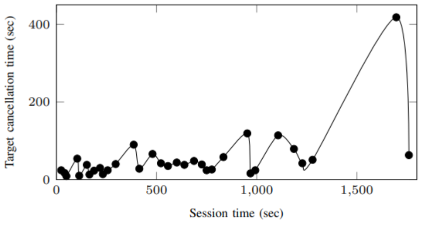
Fig. 7. Patient A: curves depicting the variation of waiting time between a cancellation and the following one. A small time between targets cancellations denotes that a good strategy has been adopted. Long waiting time are supposed to be at the end of the session, namely when the patient is looking for targets that he did not find before. Curve 7(a) shows the variation of the waiting time at the first session, curve 7(b) refers to time T1. Each session ends when the patient believes that there are no more targets to cancel out, and that explains also the different duration of the considered sessions.

6.6.2 PATIENT B AND APPLES TEST

The similarity between the digital and paper versions is discussed also for the Apples test. At time *T0* the average difference between correctly cancelled targets was 1.6 globally, 0.75 in the four left sub-regions, and 1.75 in the four right sub-regions. Furthermore, at time *T1* it was 1.1 globally, 2 in the four left sub-regions, and 0.25 in the four right sub-regions. As for the average difference between the number of cancelled distractors, at time *T0* it was 0.9 globally, 0.25 in the four left sub-regions, and 0.5 in the four right sub-regions, while, at time *T1* it was 0.6 globally, 0.75 in the four left sub-regions, and 0.25 in the four right sub-regions. The average difference between the number of cancelled targets in the two versions was smaller than 2, thus denoting a good similarity between digital and paper versions. The automatic calculation of the total number of crossed-out targets, asymmetry score for egocentric neglect, and asymmetry score for allocentric neglect revealed the following results: at *T0* they were respectively 25, 13, and 17, while at *T1* they were respectively 29, 8, and 5. The positive values of the asymmetry score for both egocentric and allocentric neglect at *T0* are coherent with the diagnosis of left VN. Furthermore, the increase in the total number of crossed out targets and the decrease of the asymmetry score for both egocentric and allocentric neglect at *T1* denote an improvement of the patient condition, and thus a rehabilitative success. As for the Albert's test, the waiting time between two consecutive cancellations is provided. Figure 8(a) and 8(b) show the variation of waiting time between two consecutive cancellations at *T0* and *T1*. The average waiting time at *T0* was 56.56 ± 54.77 sec, the average waiting time during the rehabilitation process was 46.47 ± 44.40 sec. Finally, the average waiting time at *T1* was 40.67±32.59 sec (the average waiting time of a healthy subject is 8.29±6.64 sec).



(a) Time $T0$



(b) Time $T1$

Fig. 8. Patient B: curves depicting the variation of waiting time between the cancellation of a correct target and the following one, similarly to the ones provided for the Albert's test 7. Curve 8(a) shows the variation of the waiting time at the first session, curve 8(b) refers to time T1.

6.7 DISCUSSION

The ReMOVES platform delivers activities for combining cognitive therapy with tDCS, playing a crucial role for both the assessment and the rehabilitation phases of VN patients. By using low-cost off-the-shelf components and an easy-to-use interface, ReMOVES provides the therapists with different indicators which will describe the activity and progress of the patients. In this paper, two case-studies for VN rehabilitation have been presented. Both Patient A and B suffer from such a disease, which however has been originated from a different cause. The same treatment has been used for both the patients. By describing these two particular cases, which are well representative of the disease, a glimpse into a future and deeper study on VN rehabilitation via tDCS and digital support is provided. Such a work will be conducted on a wide population and involving many rehabilitation centers. Test-retest reliability and intra/inter rater reliability will be investigated, in order to make this study statistically relevant.

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CHAPTER 7

TRANSCRANIAL DIRECT CURRENT STIMULATION IN THE TREATMENT OF SUBACUTE POST-STROKE THALAMIC APHASIA

7.1 INTRODUCTION

Thalamic aphasia is a rare language disorder resulting from damage to the thalamus of the dominant hemisphere. The thalamus conducts diffuse afferent and efferent connections from the brainstem to the cortex, by which it is thought to play a vital role in the integration of sensory information (Nadeau et al., 1997). Regarding the properties of language, the left thalamic nucleus serves as a hub connecting various language-related structures, namely the frontal cortex and basal ganglia. The thalamic nucleus receives projections from the basal ganglia (mainly the caudate or putamen) and has bidirectional connections with regions of the prefrontal cortex, including Broca's area (Figure 1). As a consequence, it is suggested that the role of the thalamus is that of a moderator leading the transfer of lexical information to cortical areas (*Fritsch et al.,2022*). Llano (2013) summarized the commonly noted features of thalamic aphasia: 1) deficits in naming, 2) a high frequency of semantic paraphasic errors, comprehension less impaired than spoken output.

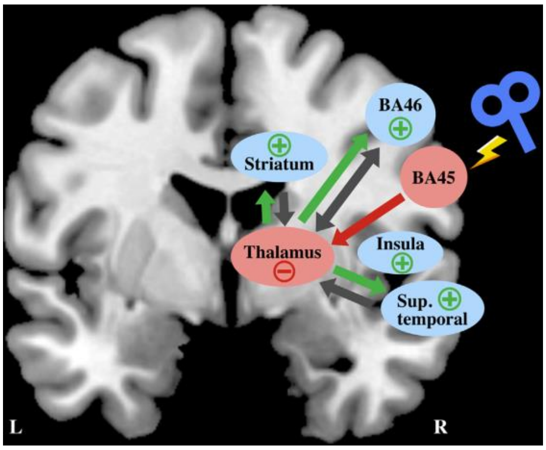


Fig. 1 The striatum provided projections to the thalamus, which then innervated the prefrontal cortex (BA46) and Broca's area (gray line). The mutual connection between the prefrontal cortex and the thalamus can enable NIBS (i.e. rTMS) to exert excitatory (green line) and inhibitory (red line) effects on specific regions.

Recovery from aphasia is usually a long and complex process with an uncertain outcome, so that various interventions have been proposed to augment the opportunities of regaining this important function. Recent evidence suggests that Non-invasive Brain Stimulation (NIBS) techniques may act as a safe and painless tool for manipulating performance in a variety of motor and cognitive domains, and researchers have begun to explore the use of transcranial Direct Current Stimulation (tDCS) as a promising rehabilitation tool for patients with post-stroke, including language impairment. Several studies in the last years have demonstrated the effectiveness of tDCS in improving speech disturbances in post-stroke aphasia (*Vestito et al., 2014; Harvey et al., 2022*). To the best of our knowledge, however, patients affected by thalamic aphasia have never been investigated.

7.2 CASE DESCRIPTION

A 65-year-old, right-handed man, with 8 years of education, presented a hemorrhagic stroke in the left thalamus associated with endoventricular blood outflow and perilesional oedema, as shown by magnetic resonance imaging (Fig. 1).

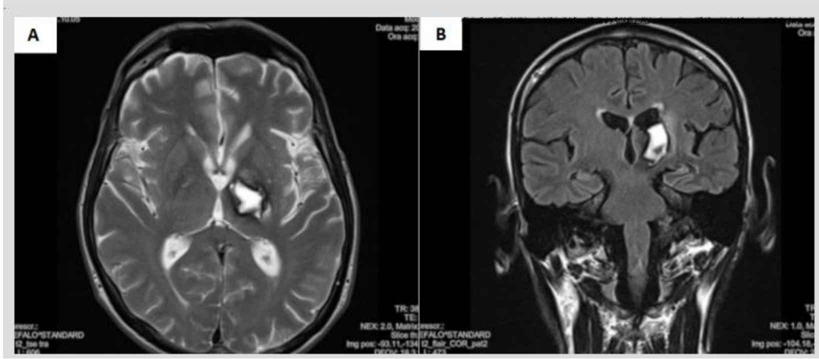
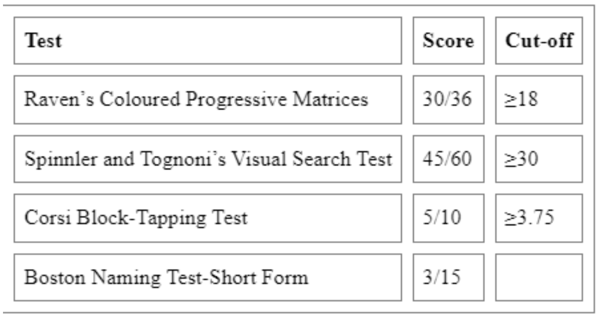


Figure 1. Cerebral magnetic resonance imaging performed in the acute phase. T2-weighted axial (A) and T2-flair sagittal (B) slices disclosed a hyperintense area in the left thalamus.

The patient was admitted to the Neurology department. Clinical examination demonstrated a right hemiplegia associated with severe expressive aphasia (NIHSS 16). He underwent 20 days of daily physiotherapy and speech therapy. He was then transferred to our Neuro-rehabilitation Unit for intensive rehabilitation. At admission (t0), the patient underwent the Raven's Colored Progressive Matrices, the Visual Search Test and the Corsi Block-Tapping Test for the cognitive assessment. He also underwent a language assessment by means of the Neuropsychological Examination for Aphasia (ENPA) and the Boston Naming Test-Short Form (BNT-SF). The patient performed normally on the cognitive tests (Table 1). On the contrary, he showed a very poor performance both in ENPA (Table 2, First two columns) and BNT-SF tests



Test	Score	Cut-off
Raven's Coloured Progressive Matrices	30/36	≥18
Spinnler and Tognoni's Visual Search Test	45/60	≥30
Corsi Block-Tapping Test	5/10	≥3.75
Boston Naming Test-Short Form	3/15	

Tab.1 Neuropsychological assessment at t0

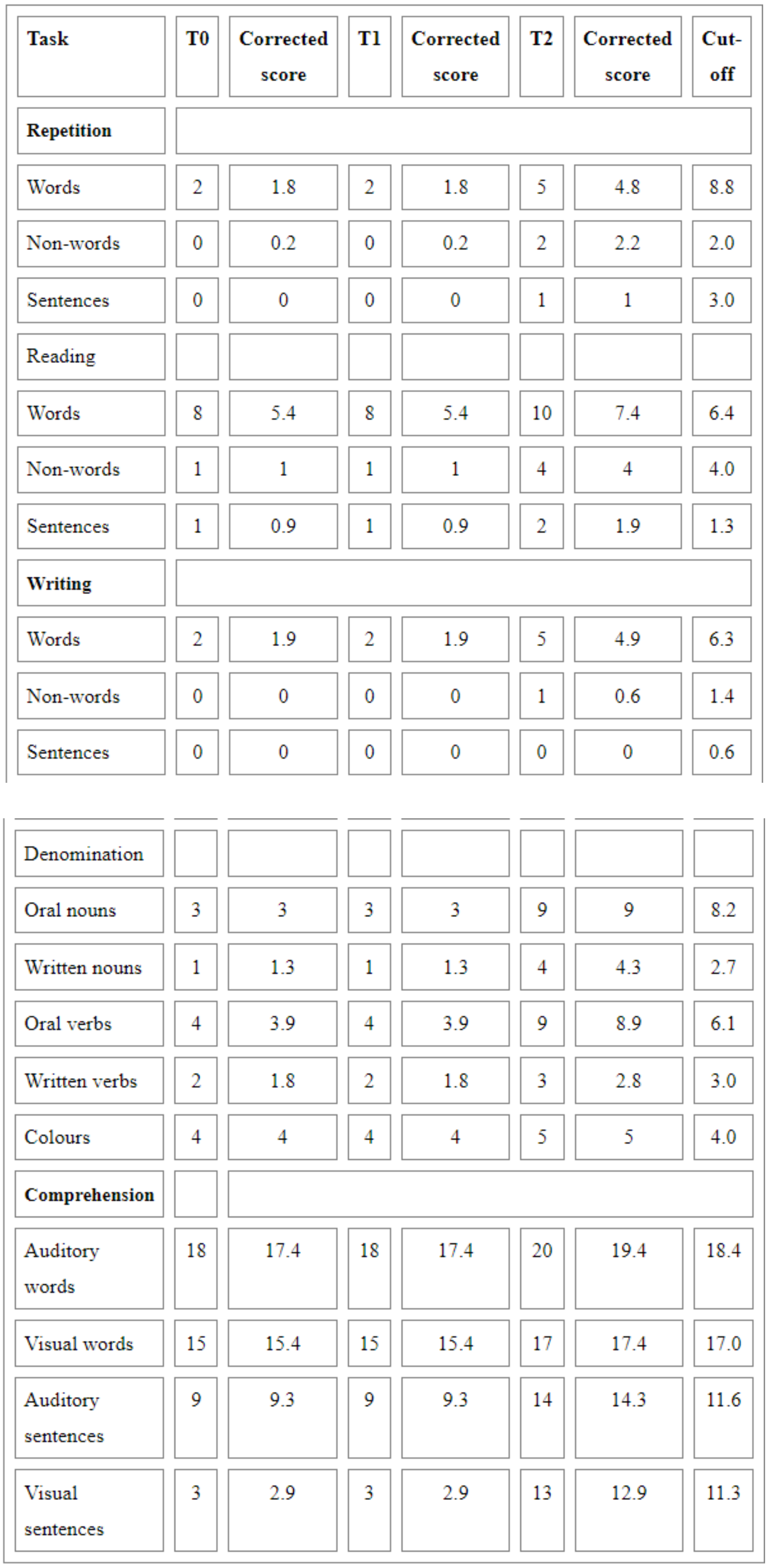
The patient was classified as having a severe non-fluent aphasia. His spontaneous speech was very poor with short sentences and frequent wordfinding difficulties; oral denomination of nouns and verbs was deficient, and anomia and semantic paraphasia were present. Auditory and visual comprehension of words was only slightly impaired, while auditory comprehension of sentences appeared very compromised. Reading aloud was preserved despite some phonetic distortions. Reading and writing non-words and sentences was severely impaired. There was no bucco-linguofacial, ideomotor, ideational or constructive apraxia. The patient also presented with personality changes and behavioral disinhibition.

7.3 METHODS AND PROCEDURE

The patient initially underwent a conventional speech rehabilitation. After 2 weeks (t1), however, his scores demonstrated only a slight improvement (Table 1). In light of his high level of motivation and the substantial integrity of his verbal receptive ability, we decided to treat the patient with transcranial direct current stimulation (tDCS) concurrently with speech and language therapy (SLT). tDCS is a safe and relatively painless technique of application of a low-intensity direct current (1–2 mA) over specific scalp areas in order to modulate cortical excitability. The patient and his caregiver were both informed of treatment details, the possible beneficial effects and an informed consent was obtained. Stimulation electrodes measuring 5 cm×5 cm were placed on the patient's scalp and held in place with a cloth cap. The most common configuration, as supported by functional magnetic resonance imaging results, involves the application of anodal-tDCS (A-tDCS) over the left prefrontal inferior gyrus with the reference electrode positioned over the contralateral supraorbital region (*Polania et al., 2012*). To target the Broca's area, the active electrode was placed between T3-Fz and F7-Cz (according to the EEG 10–20 system), while the reference electrode was over the contralateral supraorbital region. A-tDCS was applied with a constant stimulation intensity of 1.5 mA. We applied an *on-line* approach, meaning that concurrently with A-tDCS a session of SLT lasting 20 minutes was carried out, for 10 consecutive days (5 days a week). SLT consisted of denomination of 40 two-dimensional black and white pictures, divided into 20 higher frequency use stimuli (e.g., plate, flower, etc.), 10 lower frequency use stimuli (skyscraper, seahorse, etc.) and 10 actions (applauding, pouring, etc.). Each stimulus was presented for 25 seconds, with a lag time of 5 seconds between one stimulus and the next. The patient was required to correctly name all the stimuli and, in case of anomia, no help was given by the examiner. To avoid the learning effect, during the second week, the stimuli were modified maintaining the same subdivisions. Further, stimuli presented during the training session were different to those included in the ENPA test or in the BNT-SF.

7.4 RESULTS

The procedure was well tolerated, and the patient was able to complete the protocol. He showed a progressive improvement in his performance. At the end of treatment (t2), the patient was again evaluated with the ENPA test (Table 2) and BNT-SF (13/15), demonstrating a clear improvement in all fields explored, and succeeding in naming 85% of the stimuli (Fig. 2). Formal evaluation of language at t2 showed a patient with good intentional communication. He was able to express his personal needs and manage informative conversations with other subjects. Spontaneous language was characterized by good syntactic structure and rare anomia was overcome with correct circumlocutions. His speech was informative and appropriate to his level of education. The positive results were extended to other aspects of the aphasic syndrome. The oral and written denomination tests were within the normal range, although still with a few errors. Oral and written comprehension of words and sentences was normal, as was reading words, non-words and sentences. The behavioral disinhibition which affected the patient at t0 and t1 were no longer present.



Task	T0	Corrected score	T1	Corrected score	T2	Corrected score	Cut-off
Repetition							
Words	2	1.8	2	1.8	5	4.8	8.8
Non-words	0	0.2	0	0.2	2	2.2	2.0
Sentences	0	0	0	0	1	1	3.0
Reading							
Words	8	5.4	8	5.4	10	7.4	6.4
Non-words	1	1	1	1	4	4	4.0
Sentences	1	0.9	1	0.9	2	1.9	1.3
Writing							
Words	2	1.9	2	1.9	5	4.9	6.3
Non-words	0	0	0	0	1	0.6	1.4
Sentences	0	0	0	0	0	0	0.6
Denomination							
Oral nouns	3	3	3	3	9	9	8.2
Written nouns	1	1.3	1	1.3	4	4.3	2.7
Oral verbs	4	3.9	4	3.9	9	8.9	6.1
Written verbs	2	1.8	2	1.8	3	2.8	3.0
Colours	4	4	4	4	5	5	4.0
Comprehension							
Auditory words	18	17.4	18	17.4	20	19.4	18.4
Visual words	15	15.4	15	15.4	17	17.4	17.0
Auditory sentences	9	9.3	9	9.3	14	14.3	11.6
Visual sentences	3	2.9	3	2.9	13	12.9	11.3

Table 2. Results of the ENPA test at different follow-up times

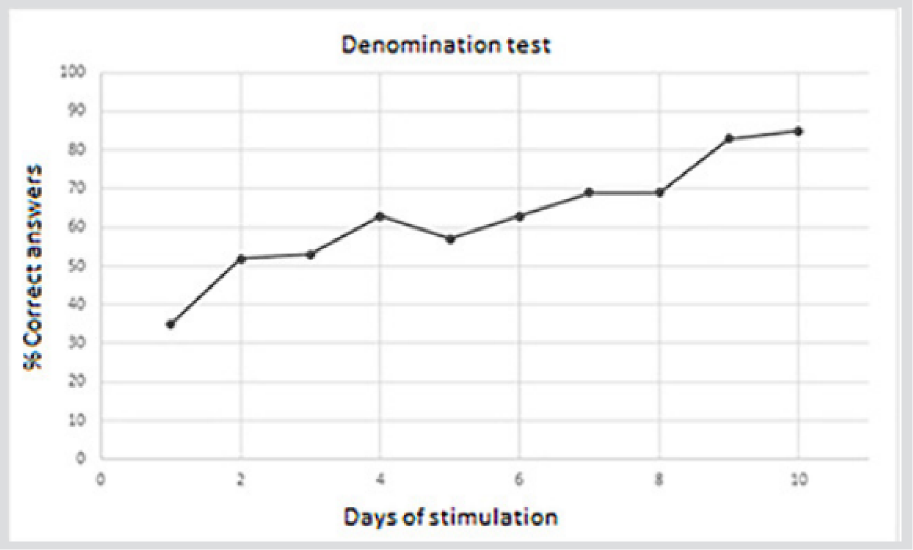


Figure 2. Progressive improvement in denomination test performance during tDCS and speech and language therapy (SLT)

7.5 DISCUSSION

The primary function of the thalamus is to process most of the information reaching the cerebral cortex from the spinal cord and the other parts of the central nervous system. The thalamus also has a central role in the neural network serving the higher functions, such as memory, regulation of behavior and emotions, and language elaboration. The advent of modern techniques in neuroimaging has led to better understanding of the role the thalamus and other subcortical structures in language disturbance. The thalamus projects to all areas of the neocortex including those in the frontal and temporal regions that are associated with language, with direct and reciprocal connections between subregions of Broca's area and subcortical structures (*Ford et al.,2013; Llano,2013; Fritsch et al.,2022; Lee et al.,2022*). A recent review (*Biou et al.,2019*) shows that tDCS is effective for treating post-stroke aphasia when applied in combination with SLT in the chronic post-stroke phase, although the benefits of its application in the subacute phase are more difficult to demonstrate since the real effect of stimulation can be masked by the physiological process of spontaneous recovery. Initially, it was thought that tDCS was capable of modifying only the excitability of the cerebral cortex, but recent functional neuroimaging and behavioral studies have also demonstrated rTMS and tDCS effects on cortical-subcortical functional circuits (*Polania et al.,2012; Lee et al.,2022*). However, to the best of our knowledge, its efficacy specifically in thalamic aphasia has not previously been investigated. In this case of subacute thalamic aphasia, we assessed the effectiveness of combined tDCS and SLT. Since the patient showed no improvement in the first 40-day period after the stroke, which is considered the critical period for spontaneous recovery, we hypothesize that tDCS may have stimulated significant cerebral neuroplasticity and functional reorganization. It is well known that neuroplasticity is greater during the earlier stages of recovery. The period of maximum neuroplasticity in humans is not entirely clear (*Coleman et al.,2017*), but it has been suggested that modulation of brain excitability with tDCS is more efficient during the early stages because of neuroprotective and anti-inflammatory mechanisms, and neural growth promotion (*Pavlova et al.,2020*). Our patient showed improvement in linguistic and behavioral communication. This may be due to the fact that the thalamus has a central role in the development of the higher functions, such as memory, behavioral regulation and emotions. Our results, even though the treatment was applied in the subacute phase, are important for language rehabilitation. The long-term effects of this treatment should be assessed and monitored and our findings investigated further.

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CHAPTER 8

A STUDY PROTOCOL FOR OCCUPATIONAL REHABILITATION IN MULTIPLE SCLEROSIS

8.1 INTRODUCTION

Multiple sclerosis (MS) is a common neurological disease that affects the central nervous system (CNS) (*Keune et al.,2017*) with detrimental effects on several functions including motor skills (*Squillace et al.,2015*), balance (*Burschka et al.,2012*), cognition (*Rao,1990*) and decline in the activities of daily living (ADLs). Cognitive impairment (CI) affects up to 70% of the MS population and refers to domain-specific deficits rather than to a uniform global cognitive decline. The patient with MS (pwMS) may encounter difficulties in information processing speed, sustained and selective attention, visuospatial abilities, learning and episodic memory and executive functions (*Chiaravalloti et al.,2008; Benedict et al.,2020*).

Therapy for MS can be divided into two main categories: disease modifying therapies (DMT) and symptomatic or supportive therapies. In principle, DMTs might improve cognition as their agents are primarily designed to arrest the disease and prevent relapses, but their actual benefit on intellectual performances is still matter of debate (*Yap et al.,2017*). Alternative strategies, such as cognitive and behavioral rehabilitation, are therefore needed in order to improve the cognitive abilities of pwMS and, consequently, their quality of life. The number of studies addressing the need for effective cognitive rehabilitation programs has been substantially increased over the past decade (*Stuifbergen et al.,2011; Munger et al.,2021*) and the Italian National MS Society (*Associazione Italiana Sclerosi Multipla-AISM*) has recently recommended remedial interventions and accommodations to manage cognitive impairment and improve everyday functioning in both adult and pediatric MS populations (*AISM,2021*).

Rehabilitation therapy is considered an interdisciplinary approach in which different professionals carry out a rehabilitation intervention aimed at the individual patient, called “Individual Rehabilitation Project” (PRI), in order to improve his/her physical, psychological, and social functions and maintain his/her autonomy. Novel technologies, such as Virtual Reality (VR) and Exergame, are suggested as a supplement to the rehabilitation therapy of pwMS (*Maggio et al.,2019; Taylor,2015*). Thanks to the impressive development of the last few decades in Information and Communication Technologies (ICT), including Electronics, Telecommunications, and Signal Processing, the Internet of Medical Things (IoMT) is starting to represent a preferred solution with the goal of supporting rehabilitation in a continuous and safe way, guaranteeing both social distancing and the reduction of travel to the rehabilitation site. In this context, the present article describes the "Solution Towards Occupational Rehabilitation in Multiple Sclerosis (STORMS)" project as an example of an IoMT work in progress and study protocol devoted to remote hospital and home monitoring of patients with multiple sclerosis. STORMS is one of the two winners of the "2020 Digital Innovation in Multiple Sclerosis" award, sponsored by Merck (*Merck,2021*), whose goal is to promote adaptation and coexistence with the disease through new digital technologies. STORMS is based on the ReMOVES IoT system developed at the University of Genoa and described in previous works (*Morando, Ponte, et al.,2018*). ReMOVES has been designed to provide remote tests for the assessment of patients' impairments and exercises to support motor/cognitive rehabilitation. It has already been tested on neurological (*Trombini et al.,2020; Ferraro et al.,2021; Morando, Bonotti et al.,2018*) and rheumatic patients (*Ferraro et al.,2021*) as well as on the healthy elderly (*Trombini et al.,2021*). ReMOVES interface is very simple and intuitive even for people with various kinds of impairment; the delivery of the exercises is personalized and follows the individual activity plan defined by the therapist in agreement with a multidimensional team. Based on various devices and motion sensors (Touchscreen, Kinect, Leap Motion) ReMOVES Patient Client performs calibration on the individual user in order to provide "tailored" results and allows the therapist to monitor tests and activities through a Therapist Client that can be consulted from different novel technologies (PCs, phones, tablets). On the server side, all the activity-related data and signals are processed using adaptive approaches, enabling the assessment of performance quality, the detection of the pattern of complex activities actually performed, and the recognition of any compensating movements. The extraction of significant data is performed only through suitable nonlinear filters and adaptive segmentation which allow to extract statistics and key indicators from signal fragments characterized by stationarity properties and related to homogeneous measurement states. The playful component of the exergame helps to increase motivation and adherence to rehabilitation. At the same time, the system facilitates the practice of task-oriented exercises and muscle strengthening, thus favoring patient's functionality. To take into account the wide variability of patients' conditions and disease progression, the most appropriate exercises can be assigned and the complexity of the required task can be adapted by defining a range of difficulty levels. Thanks to the collection, integration and remote analysis of patient signals and data, the ReMOVES solution allows continuous monitoring of the activities allowing the therapist to constantly update the personalized exercise plan. According to the ISO/IEC/IEEE 12207 standard, the development of the ReMOVES IoT system followed the requirements engineering process consisting in defining the scope of the project, the IoT system and its requirements (*Silva et al.,2019*), based on the defined stakeholders and to the destination user group.

Based on the clinical needs of pwMS and the particular types of their CI, new activities delivered in form of exergames have been developed to address functional abilities of attention, memory, executive functions, and information processing speed. The main originalities and strengths of this work are as follows:

- Implementation of an IoMT system for the assessment and support of in-hospital and in-home rehabilitation in pwMS.
- Exploitation and rapid adaptation of the ReMOVES IoT system to the new target user group.
- Ability to exercise, monitor, evaluate and analyze both the motor aspects (upper limbs, lower limbs, trunk movements, and balance control) and cognitive aspects (attention, memory, working memory, etc.).
- Provision of a personalized service tailored to the needs of the individual who has been assigned an individual care plan.
- Ease of use, low cost, and integrability with other systems.
- Robustness and resilience with respect to temporary telecommunication problems.
- Adaptive nonlinear filtering and segmentation of signals for data extraction, analysis, and visualization.

8.1.2 STATE OF THE ART

Signs of cognitive involvement generally occurs in the early stages of the disease already (*Mitolo at al.,2015*), even though severe cognitive impairment is more likely in persons with secondary progressive MS. Indeed, approximately half of the pwMS reports having either minimal or mild cognitive difficulties within the first year of diagnosis, with greater complaints over the first decade. Furthermore, although uncommon, some pwMS present cognitive impairment as their primary symptom; sometimes, cognitive issues may be present pre-clinically (*Moccia et al.,2016*). As a matter of fact, these impairments yield to major consequences for everyday life and CI is the leading cause of occupational disability and of difficulties in ADLs for such patients (*Goverover,2018*). To manage such impairments, some recommendations include more comprehensive assessment for anyone who tests positive for cognitive impairment or demonstrates substantial cognitive decline, as well as neuropsychological evaluation for any unexplained change in academic performance or behavioral functioning in school-aged children with MS. Evidence suggests that cognitive rehabilitation has a long-term impact well beyond the treatment period and might enhance cognition in the face of future brain changes. Such sustained effects have been already documented in aging, with an improvement in everyday life activities and a 29% reduction in dementia risk 10 years after treatment (*Rebok et al.,2014*). PwMS also belong to the so-called “fragile” populations subjects who are at higher risk in relation to pandemic emergencies, such as the current one. ICT and IoT solutions can represent a very important tool for supporting rehabilitation and favoring adaptation and coexistence with disability.

8.1.3 TECHNOLOGIES EMPLOYED IN MULTIPLE SCLEROSIS

The importance of using technology in the treatment of MS has long been acknowledged, so that several solutions addressing diagnosis, monitoring, and rehabilitation can be found in the literature (*Rintala et al.,2018; Rajavenkatanarayanan et al.,2019*). Several studies have shown that patients better perceive their goals and physical and mental well-being, thanks to the improved feedback that technology provides, thus leading to a better practice and to an enhanced engagement in the therapy (*Manuli et al.,2020*). In addition, technology support also favors intense and repetitive training that yields effective results in functional recovery for pwMS (*Maggio et al.,2019; Calabrò et al.,2017*). Of note, the IoMT technologies can support patients in taking control of their own disease and collaborate more effectively with the clinical staff (*Grigoriadis et al.,2016*). Despite the large interest towards assistive technology in MS, solutions are still not as widespread as they may be, because of some barriers for patients in terms of usability, feasibility and the high costs of some devices which hinders the possibility of a large home-based usage (*Burridge et al.,2010*). Further, pwMS can experience difficulties in dealing with technological devices, as well as poor skills in using it (*Goverover et al.,2021*). In addition, technological solutions made up of wearable devices or controllers may require external support from caregivers, limiting their independent use.

8.1.4 EXERGAMES IN THE FIELD OF REHABILITATION

The term exergame refers to video games that impart physical exercise/support rehabilitation practice (in the context of their clinical application) in which the repetitive and task-oriented components of rehabilitation activities are reformulated in terms of video game tasks. In recent times, exergames have gained great popularity and demonstrated scientific reliability, thus going beyond their original goal of mere entertainment. The exergames can also be considered as a VR tool, which can be a safe instrument to access activities otherwise not accessible to an individual with cognitive and motor disabilities in everyday life contexts. Furthermore, gamification (*Johnson et al.,2016*) determines a motivating and engaging environment in order to contrast boredom and fatigue in patients, and thus fostering the continuity of care. Exergames have demonstrated their utility for both cognitive and motor rehabilitation, as they exert beneficial effects on attention, visuo-spatial function, executive control, strategic planning, and processing speed (*Kuhn et al.,2014*). Several studies reported the efficacy in maintenance and improvement of cognitive functions in the elderly population (*Stanmore et al.,2017*) and in post-stroke rehabilitation (*Lanctôt et al.,2020*). Some solutions providing exergames for entertainment have been utilized for rehabilitative purposes, also in pwMS (*Taylor et al.,2015*).

8.1.5 DIFFERENCES WITH REMOVES SYSTEM

Some rehabilitation protocols for multiple sclerosis disease have been presented in the literature. However, assessment and rehabilitation are often either cognitive (*Achiron et al.,2019*) or physical (*Wood et al.,2017*) physical. In the current study, the multidimensional team created a PRI aimed at reintegrating both cognitive and motor dysfunctions that can be studied through the analysis of data obtained from motion sensors such as Microsoft Kinect. The latter has been recognized as a tool with certain validity and efficacy for the study and analysis of movement in humans (*Gholami et al.,2016*), being also ease to use and inexpensive. The particular attention paid to signal processing is another important aspect in the description of the proposed solution. In general, the existing systems are not particularly dedicated to the lack of stationarity and homogeneity of the acquired signal, resulting in erroneous statistical results. To cope with this drawback, adaptive signal segmentation was introduced at each game session in order to separate the complex pattern of the patient's execution task into primitive elements. The published studies provide sections dedicated to the analysis of the results obtained with their systems, even commercial (i.e., Jintronix and Mira), but there is a lack of the preprocessing phase of the raw signal (*Martel et al.,2018; Moldovan et al.,2017*). In addition, ReMOVES is programmed to acquire the positions of all the patient's joints while they are carrying out their rehabilitation program. The performance characteristics such as angles, range of motion, and trajectories will then be identified in order not only to assess the correctness of the movement suggested by the activity in question, but also to analyze any compensations and strategies. Due to the large individual variability among patients and their impairments, and given the relatively small sample of users, a supervised approach or generalized model is not practical in this case. Compared to other systems based on machine learning techniques (such as in *Haghighi Osgouei et al.,2019*), a wide use of unsupervised adaptive analysis methods is used here to derive useful information from the acquired signals.

8.2 MATERIALS AND METHODS

In this section, after a description of the ReMOVES system and the technologies used, the inclusion criteria, operating protocol, and patient–client activation procedure are introduced.

8.2.1 THE REMOVES SYSTEM

ReMOVES is an IoT system for remote rehabilitation developed at the Department of Electrical, Electronic and Telecommunications Engineering and Naval Architecture (DITEN) of the University of Genoa. An ad hoc version of the ReMOVES services was designed for the STORMS project and a series of exergames suitable for pwMS were created. According to the Institute of Electrical and Electronics Engineers (IEEE) Standard for an Architecture Framework for the Internet of Things, Figure 1 shows the architecture of the developer's point of view. The close collaboration between the multidimensional team and the technical research institute allows the definition of targeted activities, as in this case for pwMS, identifying both exercises (cognitive, motor, visual, balance tasks) and their evaluation (values and graphs of the trend of the task sessions). Following the standard engineering process, the Therapist Client is then built according to the target user group requirements and the needs of the clinical staff. Figure 2 shows the patient–viewpoint architecture. On the basis of the patient's observations, the multidimensional team updates the PRI consisting of a suitable program of prescribed activities. Through the cloud, the Patient Client receives the assigned schedules of the exergames, either in the hospital or at home. All data and signals are collected and the clinical staff can acquire feedback on activity performance through the Therapist Client. From the IoT viewpoint, ReMOVES has a classical four-layer architecture, as described in detail in Trombini et al. (2021), which is now briefly summarized: Figure 3 describes the physical layer of the so-called "Things" as defined by IEEE. The STORMS project uses the Microsoft Kinect V2 for motor/cognitive activities. Kinect is a motion-sensitive input device that produces a depth map of the environment using a time-of-flight camera. It offers a 70° × 60° field of view and recognition up to 4.5 m away. The Microsoft Kinect sensor can simultaneously 3D track up to 25 key joints of the framed human body. The 3D position signals are sampled at a frequency of 10 Hz. Several studies have shown that Microsoft Kinect V2 can validly obtain spatio-temporal parameters and also be an acceptable tool for rehabilitation due to its low cost and adequate spatial accuracy (with an order of magnitude of centimeters) (*Otte et al.,2016*).

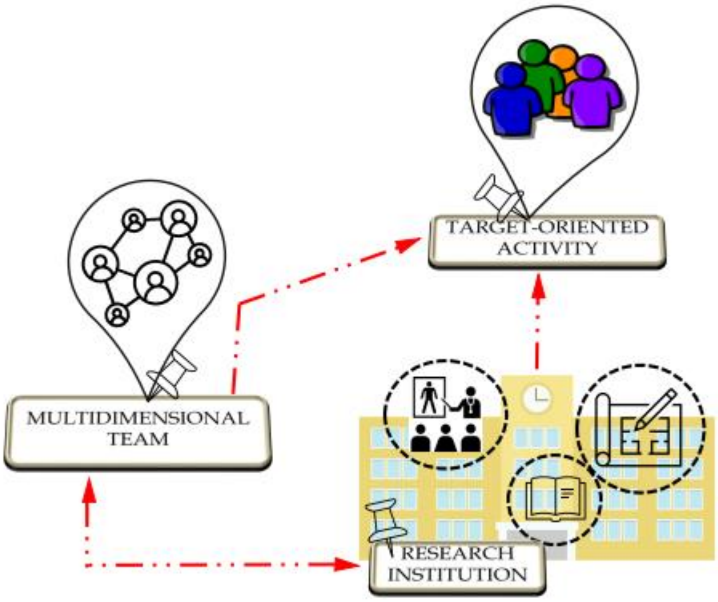


Figure 1. Developers viewpoint architecture.

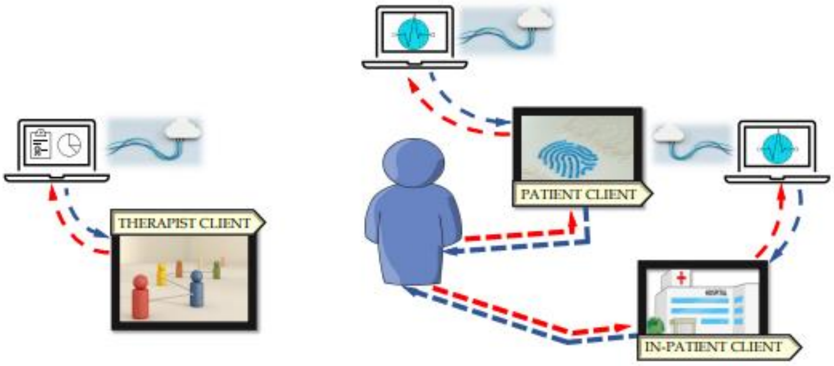


Figure 2. Patient viewpoint architecture.

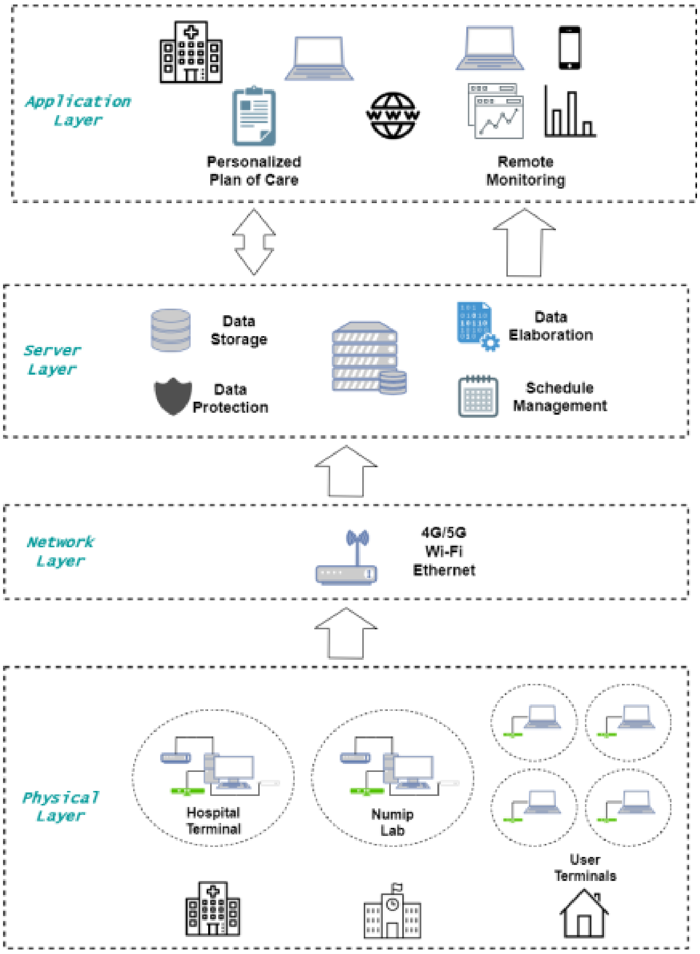


Figure 3. IoT architecture of ReMoVES system.

The automatic execution of the scheduled task, the synchronization with sensors, and the acquisition and storage of the signals and data are performed via a standard PC when patient is in the hospital (where an additional patient identification phase is required) or via an industrial PC without a keyboard when at home. Exergames are digital games that encourage patient to carry out motor/cognitive exercises. A detailed description of the exergames for pwMS is presented in paragraph 8.3.1. Unity refers to a cross-platform game engine used for the development of 3D and 2D games as well as virtual reality applications. Among the programming languages supported by Unity, C# was chosen for developing the ReMOVES exergames. The interface configuration between Microsoft Kinect V2 and the Unity engine is straightforward as the manufacturer provides the Software Development Kit (SDK) and an add-on for Unity. Developers can easily access the positions and the orientations of body joints for direct use in the virtual environment of the game scenes. In the ReMOVES project, all the 2D and 3D graphical resources have been downloaded from different online sources with a Creative Commons license. The network layer represents the link between physical and server layers. To obtain a resilient functionality facing eventual connection disruption, data log-files are temporarily stored in the local unit in JavaScript Object Notation (JSON), and the actual transmission is executed when connection is available via Ethernet or Wi-Fi.

Data communication is in secure mode based on hypertext transfer protocol secure (HTTPS), the communication protocol is encrypted using transport layer security (TLS), and certificates are issued by the Let’s Encrypt authority. The server layer can manage content-independent dataflow to be compliant with software reuse logic. Server software consists of a traditional Linux–Apache–MySQL– PHP (LAMP) stack, and provides data storage solutions, data processing methods, and a web application for clinicians to view information through dedicated graphic interfaces. Only three types of application programming interfaces (APIs) for the management of client/server data synchronization are used. The MySQL relational database contains a structured collection of JSON files, each of them describing an array of temporal events. In each element of the array, there are key-value pairs that provide data. Some keys are common to all exergames, in addition, other keys are provided depending on the game. Finally, the application layer includes the therapist client, who provides the medical staff with all the necessary information on the patients being followed, displaying their performance in graphical mode and verifying their current treatment plan.

8.2.2 INCLUSION AND EXCLUSION CRITERIA

For this study, pwMS from the Neurorehabilitation Unit of the San Martino-IRCCS Hospital in Genoa will be recruited. They must be self-sufficient and can be recruited regardless of disease subtype, gender and age. The inclusion criteria are as follows:

a. Clinically defined MS with Expanded Disability Status Scale (EDSS) < 6.
b. Absence of significant linguistic-communicative deficits (Token test score > 25).
c. Normality of the mental state i.e., Mini-Mental State Examination (MMSE) > 24.
d. Absence of psychiatric pathologies.
e. Bilateral visual acuity not lower than 6/10.

The exclusion criteria are as follows:

a. Inability to maintain adequate visual fixation (for example, in case of nystagmus).
b. Presence of post-chiasmatic perimetric defects.
c. Photosensitive epilepsy.

d. Poor compliance or, more generally, insufficient motivation to follow a continuous daily treatment such as the one in question.

Recruited patients will be evaluated using the following scales: Barthel Index (BI), Sollerman test, Disability of arm, shoulder and hand (DASH), 10-meter walk test (10MWT), 2 minutes walk test (2MWT), Berg Balance scale (BBS), Short Physical Performance Battery (SPPB), and 12-Item Short Form Survey (SF12) for the quality of life.

8.2.3 OPERATIONAL PROTOCOL

The operational procedure will be mostly carried out at home, in order to ensure support in dealing with the disease even in everyday life. Due to the user-friendly features of ReMOVES and the use of off-the-shelf components for the construction of the system, the current protocol could be replicated on a large scale. The proposed treatment begins when patients make a first visit (time T0). Then, after an initial period of training in the hospital, the activity continues at home. The sessions last 30 min and are held daily for five days a week and for three weeks (the end term is defined T1 time). Thereafter, patients are offered to continue the rehabilitation program at home for an additional four weeks, with the same schedule as before. Subjects will be re-evaluated after these additional 4 weeks of home treatment (time T2). Patients are informed about the type of treatment and their written consent is always required.

8.2.4 PATIENT CLIENT ACTIVATION PROTOCOL

In the following, the phases necessary for the activation of the Patient Client are described.

a. The patient is identified through their personal ID.

b. After the calibration phase, a simple video tutorial starts which explains the particular activity to be performed.

c. The patient performs the activity, with a specific level of difficulty, according to the PRI formulated and assigned by the multidimensional team.

d. At the end of the game session, the patient receives a feedback regarding the results of his/her performance.

e. If an Internet connection is available, the client sends the session results to the server in order to process the data.

8.3 ACTIVITIES FOR OCCUPATIONAL REHABILITATION

The current Section introduces the new exergames developed to meet the needs of the STORMS project, designed according to the result of the requirement analysis process. In particular, levels and functionalities are described. Then, the extracted data are described through the definition of the fields that appear in the log-files.

8.3.1 EXERGAMES

The new set of exergames is mainly focused on cognitive recovery rather than motor rehabilitation. ReMOVES for the STORMS project includes six adaptive activities presented in visual format. The patients interact with the console through their body movements using the Microsoft Kinect sensor. Three types of movement, flexion–extension and abduction–adduction of the shoulder joint, and bilateral inclination of the trunk are monitored. An initial calibration phase and real-time adjustment adapt the game to the mobility of the user. Each game is made up of simple 2D elements to prevent distraction due to irrelevant background elements. The performance during each session is recorded. At the end of the game session, the parameters useful for investigating the player's performance are collected in a JSON file and provided for the analysis. Also motion data can be reconstructed, as the joints positions are recorded by the Kinect. The JSON file is composed of an array that collects temporal events. Each element of the array expresses data in key-value form. Some keys such as Time, Score and Kinect (i.e., the position of the joints detected by the sensor) are common to all exergames, while Level refers to all games that have multiple levels. The features CalibSide, RealSide and HandPositionX-Y are present only in games that involve the movement of the arm, and they represent, respectively, the body side detected during calibration phase (0 for none, 1 for right side, 2 for left side), the hand detected during the game phase (0 for none, 1 for right hand, 2 for left hand), and the x and y positions of the hand in the game scene. The main cognitive functions involved in games include attention, memory and executive functions. Among the sub-categories of such abilities, the games will mainly address the following:

- Working memory: describes the processes involved in the control, regulation, and active maintenance of information relevant to the task at the service of complex cognition.
- Inhibition control: an executive function that allows a person to control the impulses and behavioral responses to stimuli in order to choose the most appropriate behavior consistent with achieving the objectives.

- Selective attention: the act of focusing on a single object in the environment for a specific period of time.
- Task switching and cognitive shifting: two executive functions that involve the ability to unconsciously or consciously shift the attention between one task and another.
- Multitasking: the ability to focus on multiple tasks or activities at once.
- Sustained attention: the ability to focus on an action or stimulation for an extended period of time.
- Top-down attention task: this type of attention refers to the ability to focus on specific features, objects or regions in space that are relevant to a goal, filtering out irrelevant stimuli.

The new cognitive games here introduced aim to restore some of the most common symptoms of MS such as coordination disturbances, unbalance and dizziness (Hot Air); vision disturbances which may include impaired color vision (Owl's Nest), cognitive dysfunctions such as memory and learning deficits, difficulties in maintaining concentration and attention, and inability to perform operations of a certain complexity and to correctly perceive the environment (Supermarket, Numbers, Shelf Cans, and Business by Car). It is also possible to obtain indirect measurements of symptoms such as numbness of the body and/or extremities or spasticity which can complicate movement when games are performed with pelvic or limb movement. The targeted cognitive domains of each exercise are summarized in Table 1.

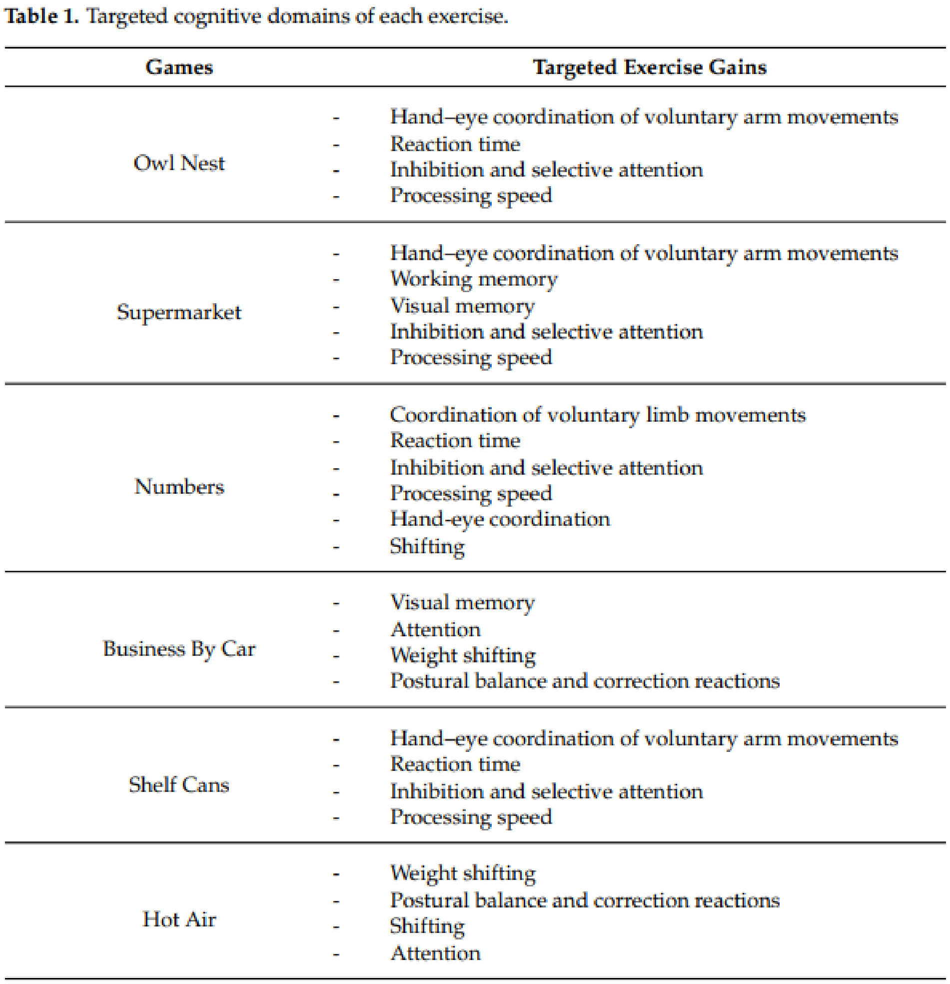


Table 1. Targeted cognitive domains of each exercise.

Games	Targeted Exercise Gains
Owl Nest	- Hand–eye coordination of voluntary arm movements - Reaction time - Inhibition and selective attention - Processing speed
Supermarket	- Hand–eye coordination of voluntary arm movements - Working memory - Visual memory - Inhibition and selective attention - Processing speed
Numbers	- Coordination of voluntary limb movements - Reaction time - Inhibition and selective attention - Processing speed - Hand-eye coordination - Shifting
Business By Car	- Visual memory - Attention - Weight shifting - Postural balance and correction reactions
Shelf Cans	- Hand–eye coordination of voluntary arm movements - Reaction time - Inhibition and selective attention - Processing speed
Hot Air	- Weight shifting - Postural balance and correction reactions - Shifting - Attention

In some exergames, the patient is encouraged to reach consecutive on-screen targets with the arm motion (reaching task). The more targets are taken, the higher the in-game score. Such games aim at improving hand–eye coordination and spatial awareness. Other exergames promote the trunk balance used to guide an object along a path. In the Owl Nest, Supermarket, Numbers, and Business by Car exercises there are different levels, from easy to extremely difficult, while Shelf Cans and Hot Air activities have a single level and can be used by patients to familiarize with the system. The different difficulty levels allow to define the treatment plan based on the patients' disability, aimed at selecting the most appropriate game and level to start and continue the rehabilitative treatment. The total game time is 90 seconds for all the exergames, except Business by Car. The latter has a longer duration (600 seconds) in order to ensure that the patient can reach the end of the game even in case of mistakes along the path.

8.3.1.1 OWL NEST

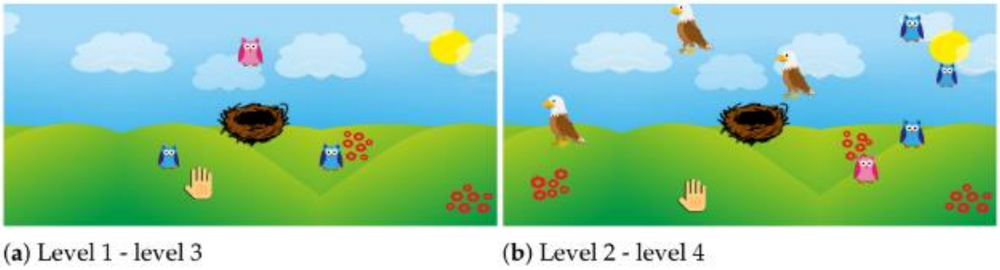
The goal is to grab the owls that randomly appear on the screen with the flexion– extension of the arm and bring them into a nest in the middle of the screen.

First level: no more than three owls can appear simultaneously, with no distracting elements. When the user brings an owl into the nest, another owl appears at a different point and the in-game score increases.

Second level: some eagles appear on the screen as distracting elements. No more than five owls and three eagles can appear simultaneously. The time between the appearance of two consecutive eagles randomly ranges from 0 to 5 s. After 10 s the eagle disappears. Catching an eagle makes the game score decrease.

Third level: the player is required to catch only the pink owls (among the blue ones as distractors) as quick as possible, since after 15 s the pink owls disappear and another one shows up in a different location. A maximum of seven owls can simultaneously appear on the game scene. The score decreases when either a blue owl is grabbed or a pink owl disappears.

Fourth level: this is a combination of all the goals of the previous levels. No more than four owls and three eagles can appear simultaneously. The patient must bring the pink owls into the nest and avoid both eagles and blue owls. The eagles disappear after 10 s, while the owls after 8 s. Figure 4 shows some game levels while Table 2 shows the features extracted. The functions involved are coordination of voluntary limb movements, reaction time, inhibition and selective attention, processing speed, and hand–eye coordination.



(**a**) Level 1 - level 3 (**b**) Level 2 - level 4

Figure 4. Screenshots of some levels of Owl Nest exergame

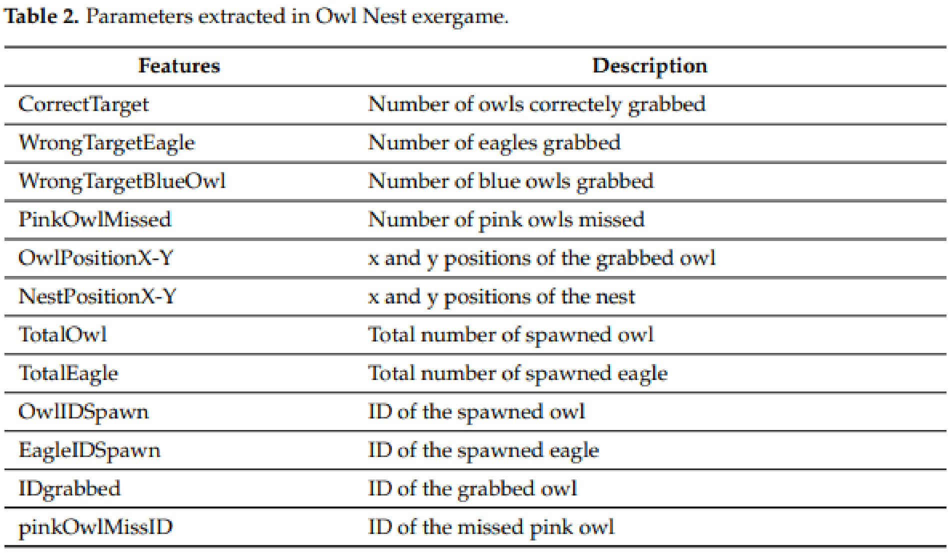


Table 2. Parameters extracted in Owl Nest exergame.

Features	Description
CorrectTarget	Number of owls correctely grabbed
WrongTargetEagle	Number of eagles grabbed
WrongTargetBlueOwl	Number of blue owls grabbed
PinkOwlMissed	Number of pink owls missed
OwlPositionX-Y	x and y positions of the grabbed owl
NestPositionX-Y	x and y positions of the nest
TotalOwl	Total number of spawned owl
TotalEagle	Total number of spawned eagle
OwlIDSpawn	ID of the spawned owl
EagleIDSpawn	ID of the spawned eagle
IDgrabbed	ID of the grabbed owl
pinkOwlMissID	ID of the missed pink owl

8.3.1.2 SUPERMARKET

This exergame is set into a supermarket where the patient is instructed to buy some objects. A list of items to be picked up is displayed at the beginning of the game. A time ranging from 8 and 25 s is allowed to memorize the list, depending on the level, then the patient has to take the correct objects by moving his/her arm. An audio feedback is provided and a positive/negative sound occurs in case of correct/incorrect action.

First level: a list of three food names must be memorized in 8 s. Then the player has to pick up the corresponding objects, moving them from the two lateral shelves into the shopping bag (in the middle of the screen). Some non-food distractors are present on the shelves. The semantic property (food or non-food) of the objects is the crucial correctness factor of the activity. The game score decreases if wrong objects are put into the bag. A visual feedback is also provided (a yellow health bar turns red in case of mistake).

Second level: the player has to memorize four objects in 10 s. These objects must be picked up while sliding on a conveyor belt among some distracting items. The game score decreases in case a wrong object is taken.

Third level: the player must memorize and follow four sequenced instructions in 20 s. Each instruction refers to a different semantic characteristic (shape, color, or material) of the objects to be picked up. Collected items do not re-appear.

Fourth level: the same as the third level, except that a higher number of objects instructions are provided (5 instead of 4, to be recalled in 25 s). In addition, the objects reappear on the screen once collected.

In Figure 5, the screenshots of the four levels are provided, while Table 3 contains the list of features extracted. The aim of this game is to train memory (verbal, non-verbal, and visual), inhibition and selective attention, and hand–eye coordination.

(**a**) List of items in the Level 2. (**b**) Level 1.

(**c**) Level 2. (**d**) Level 3.

Figure 5. Some screenshots of the levels of Supermarket exergame.

Table 3. Parameters extracted in Supermarket exergame.

Features	Description
CorrectTarget	Number of target correctly grabbed
WrongTarget	Number of target erroneously grabbed
CorrectTargetNOList	Number of target not in the list grabbed but semantically correct (only level 1)
ObjOutOfSequence	Number of target grabbed out of sequence (only level 2, 3, 4)
PassedLevel	Number of completed round
TargetPositionX-Y	x and y positions of the grabbed target
BagPositionX-Y	x and y positions of the shopping bag (only level 1)
Hand-Object	if the hand has grabbed an object (0 for no object grabbed, 1 for object grabbed)

8.3.1.3 NUMBERS

The patient has to pop some numbered (from 0 to 99) balloons according to some temporary instructions. Four instructions alternate according to the level difficulty:

- pop the balloons in ascending order

- pop the balloons in descending order
- pop the balloons with even numbers
- pop the balloons with odd numbers

The number of balloons varies depending on the difficulty levels; they also have different colors and sizes to make the game more dynamic and visually appealing. If the user pops the wrong balloon, a red mark appears at the bottom of the screen.

First level: four balloons appear on screen. They must be popped in either ascending or descending order.

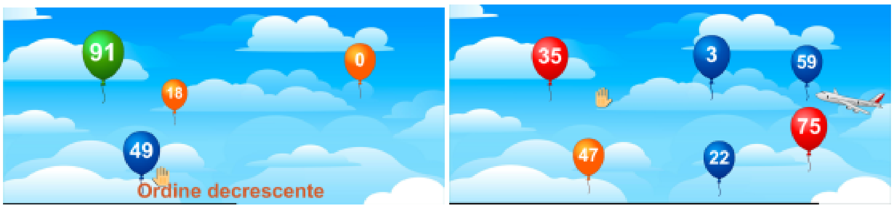
Second level: the same as the first level, except that the patient is also required to pop either the even or the odd balloons and there are five balloons in the game scene. Two visuo-verbal stimuli are added. When the text relating to the assignment "take the odd numbers" shows, a red bird will appear for a few seconds flying from one side of the scene to the other. Conversely, a plane will appear on the screen for a few seconds when the text relating to the task "take the even numbers" appears.

Third level: six balloons are simultaneously displayed. The patient must remember the stimuli association previously described, because in the cases of "take the odd numbers" and "take the even numbers" no sentences appear on the screen.

Fourth level: it is structured like the third level, but also the writings "pop in ascending/descending order" will disappear after a couple of seconds.

Fifth level: the player has to quickly pop as many balloons as possible before they disappear, since all the four balloons flies off and disappear from the screen. Only the tasks about the odd and the even numbers, with their corresponding visual-stimuli, are displayed.

In Figure 6, the screenshots of some levels are provided. In Table 4, a short description of the parameters extracted is showed. The aim of this exergame is to improve the attention, the task and cognitive switching, the processing speed, and the hand–eye coordination.



(a) Level 1.   (b) Level 3.

Figure 6. Screenshots of the level one and level three of Numbers exergame.

Table 4. Parameters extracted in Numbers exergame.

Features	Description
Round	Number of total round
Text	Text visualized on screen for each round
CorrectRound	Number of successfully completed rounds
WrongRound	Number of incorrectly completed rounds
BalloonSpawned	Number of balloons displayed on screen
ColorBalloon	Colors of the current spawned balloons
SpawnPosition	Positions of the current spawned balloons
ScaleBalloon	Sizes of the current spawned balloons

8.3.1.4 Business by Car

The patient drives a car along a randomly generated road. The car turns left or right as the player moves the trunk correspondingly. Car speed increases progressively. The player must maintain the correct carriageway, otherwise a penalty occurs and the car returns to the initial speed. A list of places to be visited appears at the beginning of the game. The patient has to memorize the list in a time ranging from 10 to 20 s, depending on the game level. Then, he/she has to drive and select the correct streets at the crossroads to reach the required places. Once finished, a series of multiple-choice questions appears on the screen, regarding the details of the visited places. The three levels differ as to the number of places to be remembered and the difficulty of the final questions.

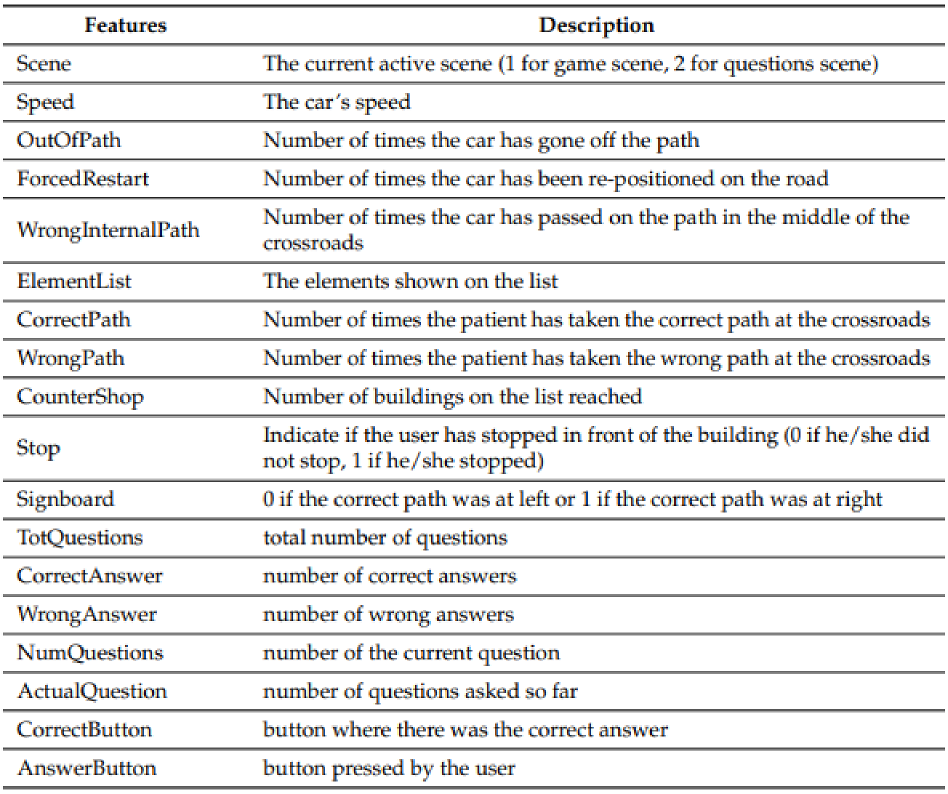
First level: the patient must remember only four places and the buildings are written on a list. At a crossroad, the navigator at the bottom-right indicates the correct way. In case of mistake, a message appears reminding the correct place. In the question scene, there are two simple questions, e.g., "Was the Supermarket the first building to visit?"

Second level: in this level the patient must remember five places. A list of errands to be carried out appears at the beginning of the game (e.g., "Do the grocery shopping at the Supermarket").

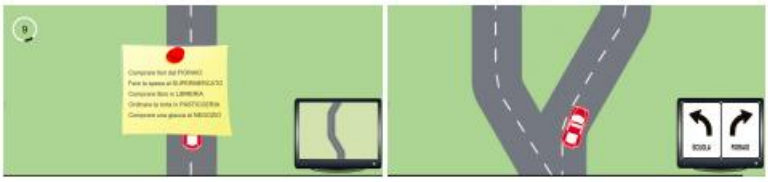
The navigator does not indicate the correct pathway and only a warning message appears on screen if the player takes the wrong direction. The final questions are three.

Third level: the player must keep in mind six places. No warning message appears in case of errors. In the final scene, the patient has to answer four questions, one related to the initial list and three concerning details seen in the visited buildings. Figure 7 shows some screenshots of the exergame and its features are collected in Table 5. The aim is to train visual memory, attention, postural balance, and correction reactions.

Table 5. Parameters extracted in Business By Car exergame.



Features	Description
Scene	The current active scene (1 for game scene, 2 for questions scene)
Speed	The car's speed
OutOfPath	Number of times the car has gone off the path
ForcedRestart	Number of times the car has been re-positioned on the road
WrongInternalPath	Number of times the car has passed on the path in the middle of the crossroads
ElementList	The elements shown on the list
CorrectPath	Number of times the patient has taken the correct path at the crossroads
WrongPath	Number of times the patient has taken the wrong path at the crossroads
CounterShop	Number of buildings on the list reached
Stop	Indicate if the user has stopped in front of the building (0 if he/she did not stop, 1 if he/she stopped)
Signboard	0 if the correct path was at left or 1 if the correct path was at right
TotQuestions	total number of questions
CorrectAnswer	number of correct answers
WrongAnswer	number of wrong answers
NumQuestions	number of the current question
ActualQuestion	number of questions asked so far
CorrectButton	button where there was the correct answer
AnswerButton	button pressed by the user



(**a**) List of errands to do.

(**b**) The crossroad with the directions given by the navigator.

La lista di cose da fare era:
MERCATO, SUPERMERCATO,
FIORAIO, NEGOZIO.

(**c**) The building to visit.

(**d**) The questions' scene.

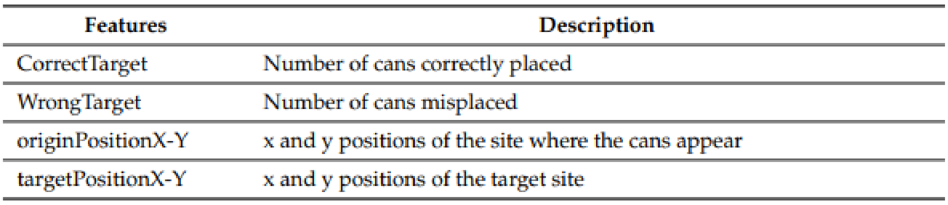
Figure 7. Screenshots of the Business By Car exergame.

8.3.1.5 SHELF CANS

In this exergame, the player is required to store items on a wall unit, moving a colored can of soda towards the corresponding shelf. The up-right, up-left, and bottom-left corners are for orange, green, and red tin cans, respectively. Therefore, Shelf Cans contributes to improve the attentive capacities by correctly matching the color between the can of soda and the shelf. A screenshot of the game is provided in Figure 8 and Table 6 contains the list of the extracted parameters. This game is aimed at stimulating hand-eye coordination of voluntary arm movements, reaction time, inhibition and selective attention, and processing speed.

Figure 8. Screenshot of Shelf Cans exergame.

Table 6. Parameters extracted in Shelf Cans exergame.



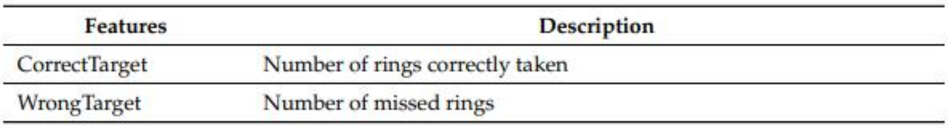
Features	Description
CorrectTarget	Number of cans correctly placed
WrongTarget	Number of cans misplaced
originPositionX-Y	x and y positions of the site where the cans appear
targetPositionX-Y	x and y positions of the target site

8.3.1.6 Hot Air

This exergame is set in a hilly landscape where a hot-air balloon automatically flies. The player can control the lateral displacement of the balloon by a corresponding trunk shifting. The aim of the game is to guide the balloon towards the blue rings that appear along the way. The progressive position of the rings is pseudo-random so that there is a continuous trunk shifting. An in-game scene is depicted in Figure 9 and Table 7 contains the list of the extracted parameters. This activity, like Shelf Cans, has only one level. This game is for postural balance and correction reactions, shifting, and attention.

Figure 9. Screenshot of Hot Air exergame.

Table 7. Parameters extracted in Hot Air exergame.



Features	Description
CorrectTarget	Number of rings correctly taken
WrongTarget	Number of missed rings

8.4 RESULTS

Each patient receives a personalized rehabilitation path. The IoMT ReMOVES system on which the STORMS project is based favors the continuity of care after hospitalization. It includes motor and cognitive exergames that incorporate enjoyment, technology, and health care. Indeed, the platform will process all the data to make them clear and available within the Therapist Client, processed as a set of graphs and values. This type of layout provides analysis from a cognitive and rehabilitation perspective and allows the multidimensional team to more easily interpret the information. The analysis of the indicators concerning attention, working memory, speed of processing information about the environment or situation simulated by the exergames and those concerning the movements performed to reach the goal, defines a complete picture of the physical and mental state of the patient. This in turn allows an accurately monitoring of the symptoms of the disease. As the current study is still ongoing, the game sessions and the data collected refer to a preliminary experimentation phase performed on a group of frail elders, affected by neurological sequelae of stroke, post-surgery recovery following hip and limb fractures, and short-term complications (injuries, traumas). The generic indicators presented in this paragraph prove the feasibility and reliability of the approach on the elderly group and will be extended to the pwMS STORMS target group. A series of test session graphs are provided in order to present the visual approach that makes signal analysis possible. The main indicators and graphs are analyzed from a cognitive and motor point of view. In fact, as already pointed out in the previous sections, although the focal point of the STORMS project is cognitive rehabilitation, it is also necessary to study the movement performed by the patient, predict any error or compensation, as well as analyze the precision and speed of the gesture itself.

8.4.1 Analysis of Cognitive Tasks

Cognitive functions are continually used to accomplish each daily task. A deficit in one or more of these functions results in a range of difficulties in ADLs, work, and social life. The MMSE will be used to assess the state of impairment and/or deterioration of cognitive efficiency. In previous works, the results obtained with MMSE test have been correlated with the data collected by the system and a significant match has been obtained, as shown in Figure 10 with regards to Owl Nest exergame. The average MMSE score of the population was 25.15 ± 1.84. This analysis shows that patients with better cognitive abilities can learn the techniques and strategies to play faster, regardless of their physical conditions: they could benefit from subsequent levels of difficulty in the game. The same test will be used to ascertain the capacity of cognitive functions of pwMS and a positive correlation between the two methods of investigation is expected.

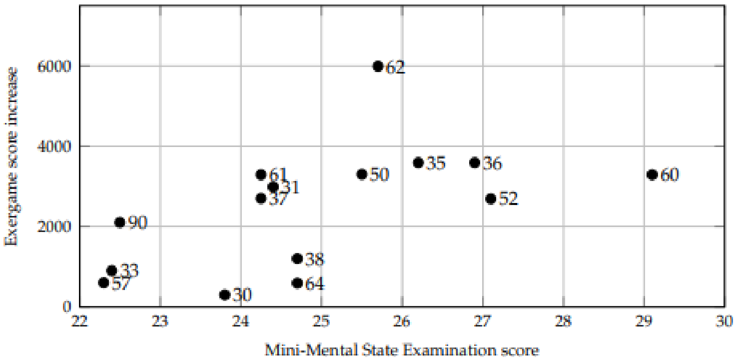


Figure 10. Scatter plot of the MMSE score versus the maximum in-game score increase achieved by the patients, calculated as the difference between their overall best result and their first session. Each point is labeled with patient ID.

Moreover, a preliminary analysis concerns the learning curves of the patients according to the obtained game score. Figure 11 shows an example of the learning curve obtained from previous study with elderly and frail patients when playing Owl Nest exergame. A low score is expected in the initial sessions and a subsequent steady improvement during the final sessions. Similarly, a graph relative to pwMS is expected to have the same trends demonstrating how the execution of exergames involving memory, attention, perception, and reasoning lead to an improvement in performance compared to the initial session highlighting, at the same time, that the various programmed objectives are really treated by the STORMS system.

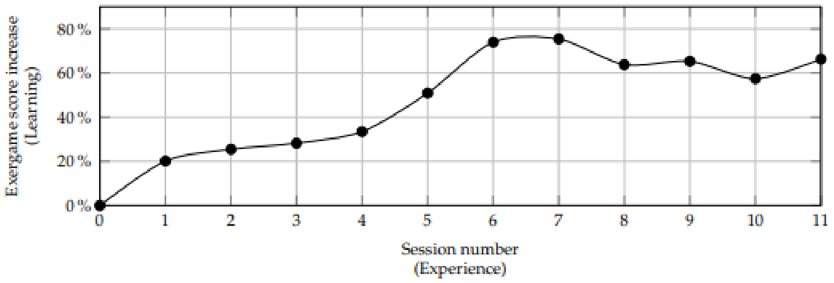


Figure 11. Learning curve of frail elderly population in the context of Owl Nest activity. On the vertical axis, it is evaluated how gaming performance improves with more experience (number of sessions). The game performance is defined as a percentage increase compared to the initial session.

8.4.2 ANALYSIS OF UPPER-LIMB AND TRUNK MOVEMENT

The tool used to measure the disability status of pwMS will be the well-known EDSS. As already mentioned, the inclusion criteria will include patients with a score less than 6. The expected trends will be learning curves that will include information related to the motor performance obtained in multiple game sessions. In particular, for each exergame, the proper joints are tracked in order to detect and evaluate the patient movement patterns. Then, the raw signals are collected, and a series of preprocessing methods, i.e., filtering and segmentation operations (Figure 12), are used to process them and extract the most important characteristics related with the range of motion and the angles.

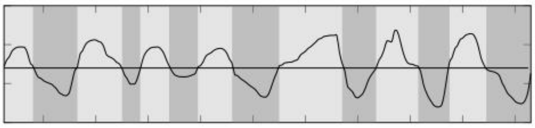


Figure 12. Example of movement segmentation in Hot Air exergame. Dark gray areas are for right displacement, while light gray areas are for the left displacement.

Referring to the upper-limb movement, the possible indicators are the range of motion (ROM) of the shoulder, and elbow defined as

$$ROM(\theta_{shoulder}) = \max\theta_{shoulder} - \min\theta_{shoulder}, \tag{1}$$

$$ROM(\theta_{elbow}) = \max\theta_{elbow} - \min\theta_{elbow}, \tag{2}$$

where the angles are described by the following equations:

$$\theta_{shoulder} = \arctan\frac{z_{elbow} - z_{shoulder}}{x_{elbow} - x_{shoulder}}, \tag{3}$$

$$\theta_{elbow} = \theta_{shoulder} - \arctan\frac{z_{elbow} - z_{wrist}}{x_{elbow} - x_{wrist}}. \tag{4}$$

Possible compensations with the trunk can be observed using the following formula:

$$ROM(\theta_{trunk}) = \max\theta_{trunk} - \min\theta_{trunk}, \tag{5}$$

where

$$\theta_{trunk} = \arctan\frac{z_{spine\_shoulder} - z_{spine\_middle}}{y_{spine\_shoulder} - y_{spine\_middle}}. \tag{6}$$

Last, on the basis of the exergames considered, the precision of the movement and the trajectory will be studied. The optimal trajectory and the trajectory performed by the patient will be computed and compared. A small angle between the two lines will mean the movement has been controlled and precise.

A preliminary study on Shelf Cans will be made calculating the average distance between the position of the hand (N samples over time) and the shortest line passing through the origin (location where the tin cans appear) and the targets (the correct matching shelf):

$$d = \frac{1}{N}\sum_{n=1}^{N} \frac{|(y_t - y_o)x_n - (x_t - x_o)y_n + x_t y_o - y_t x_o|}{\sqrt{(y_t - y_o)^2 + (x_t - x_o)^2}} \tag{7}$$

where $hand_n = (x_n, y_n)$ (x and y are the horizontal/vertical coordinates of the coronal plane); $origin = (x_o, y_o)$; $target = (x_t, y_t)$. Lower values of d are better because they indicate a more precise path.

In previous works, studies relative the evolution over time of the performance of an elderly patient affected from fibula and ischio-pubic fractures is conducted. Figure 13 shows a motor-cognitive analysis when the patient was playing Shelf Cans activity. Despite the trajectories seeming more controlled and precise, the patient hesitates and fails to lead the can of soda towards the shelf of the corresponding color, indicating a cognitive deficit. The average distance d is 1.72 which is a quite large value as compared to a healthy population.

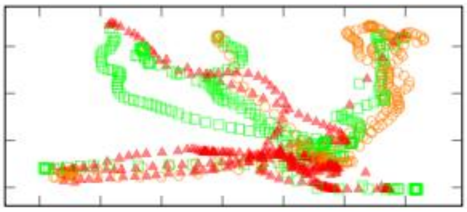


Figure 13. Each point corresponds to the temporal position of the tin can on the screen. Orange circles, red triangles, and green squares refer to orange cans, red cans, and green cans, respectively. In the session, the subject has a controlled movement but sometimes delivers the tin can with hesitation in the wrong shelf as defined by the high value distance d.

More in-depth studies concerning MS patient will be discussed after the collection of their game sessions.

8.5 CONCLUSIONS

In the current study, the use of the IoT system ReMOVES as a support to MS rehabilitative treatment and the related monitoring is described. Patients' activity can be tracked even when performed without the therapist supervision by means of low-cost off-the-shelf components and an easy-to-use interface. This also allows the patients to follow their personal plan of care with continuity also at their home. The current discussion addresses several aspects that are tackled by the presented solution and that derive from its application.

Patient management point of view: The use of IoMT devices favors the intensive and continuous practice of rehabilitation and task-oriented exercises for pwMS. The resulting cognitive improvement may lead to a better adaptation to the intrinsic difficulties of the disease. The playful component of exergame can be helpful in increasing motivation and adherence to rehabilitation. At the same time, the muscle strengthening that derives from the practice of these exercises favors the improvement of the patients' functionality, in order to make them as self-sufficient as possible in carrying out daily activities. Overall, these outcomes can allow a better management of the patients who will not feel abandoned once discharged from the healthcare facilities, but can be continuously monitored and supported by the clinical staff even during home practice.

Social point of view: pwMS, as well as patients suffering from other disabling pathologies, are in need of particular attention and care in order to face the everyday challenges deriving from their conditions. Here, IoMT and Digital Technology are crucial for promoting adaptation and coexistence with the disease. The COVID-19 pandemic has highlighted the huge potential relying in medical technologies and now it is mandatory to leverage on them in the everyday life, to address the several issues of other diseases and improve the lives of patients. It is therefore necessary that institutions promote the realization of strategic plans to bring novel technologies to everyone. In such a context, solutions like the one here presented should be replicated on large scale and forwarded to other applications.

Theoretical point of view: IoMT solutions enable data collection which improves health diagnoses and patients monitoring. To this purpose, the extraction of meaningful features, along with the use of signal and data processing operations can provide the clinical staff with a clear picture of patients' conditions. The main implication in such a context is the development of data mining and artificial intelligence techniques for supporting clinical practice. This is a task that will be tackled in the following phases of the project STORMS, after having visualized and made acquainted with the collected data.

Future work: In the next months, the experimental phase of the project will start. An operational protocol will be defined and tested along with the exergames, first in hospital and then at home. Meanwhile, data analytics will be implemented and strengthened more thoroughly. As a further development of the project, the activity of ReMOVES could be combined with other IoT services or additional devices for the evaluation of brain functions, for example electroencephalography (EEG), or stimulation (for example, transcranial direct current stimulation or tDCS), in order to better monitor the patient's practice. As already suggested in the social-point-of-view section, the application of this project can be exported to other diseases, to design an aid framework based on IoMT devices to address the different needs of different diseases. Indeed, the STORMS project was thought to act as a groundwork for the development of digital telerehabilitation solutions that support MS patients and others in order to improve their quality of life and help them in the activity of daily living. Finally, ReMOVES system is under continuous updating, it can integrate other sensors and IoT systems, and is easily adapted to new technological devices as, for instance, the new Azure Kinect instead of the current Kinect v2.

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CHAPTER 9

CONCLUSIONS

Neurological disorders are a leading cause of adult disability worldwide. Improving the quality of independent life through different treatment protocols is the main goal of rehabilitation. Unfortunately, health care costs have increased significantly over the past two decades, and the amount of time of treatment and its intensity for each patient are scarcely sufficient to achieve the optimal goal in the short timeframe.

Several studies have suggested that combining the conventional rehabilitation protocols with more recent technologies (including Robotics, Brain Computer Interfaces, NIBS) results in an optimal solution for rehabilitation. Combining different techniques may indeed speed-up and maximize the potential benefit of rehabilitation treatments (*Bullock et al, 2020; Calabrò 2021).*

Among the different NIBS techniques, tDCS might be a reliable alternative to TMS, also showing some important advantages. As a matter of fact, TMS might induce seizures, especially in patients who are seizure prone, requires an expensive special equipment not easy to apply concurrently with behavioral tasks and needs trained health professionals (*Rossi et al., 2021*). On the other hand, tDCS has several compelling characteristics for clinical use, since it is quite safe, with mild and transient side effects (*Fertonani et al.,2015*), easy to use (including portability), and less expensive.

Besides NIBS, other recent rehabilitative approaches have gained increasing interest due to their hypothetical advantages in the treatment of patients with neurological disorders. Virtual Reality (VR) technology has attracted attention as a new means of rehabilitation, and in recent years, the literature in this area has greatly expanded. VR can provide patients with more sensory stimulation and a more immersive environment, reflecting motor learning and neuroplasticity. Another important advantage of VR is that the inclusion of a virtual therapist does not require the physical presence of a clinician; thus, it allows the patient to practice at any time. This would further reduce the costs of the therapy and, thus, ensure intensive treatment (*Massetti et al., 2018; Chen et al.,2022*) As a consequence, this modern approach can also be considered as a complement to traditional rehabilitation therapies.

The combination of the newer technologies, such as tDCS and VR, can provide further opportunities in order to optimize the rehabilitative approach to patients with cognitive and/or motor disorders. These combined methodologies have been already shown to provide stronger beneficial effects than each intervention alone (*Viana et al., 2014; Bassolino et al., 2018; Schneider et al., 2021*)

This thesis has the objective of describing the effectiveness of these novel restorative methods, especially tDCS, in a series of neurological disorders. Specifically, we investigated the effects of tDCS in a neurodegenerative disorder such as Posterior Cortical Atrophy (PCA), in post-stroke aphasia and neglect and in some well-known consequences of SARS-CoV-2 pandemia such as long-COVID fatigue and smell loss. We also combined tDCS and VR with the aim to investigate their efficacy in patients with unilateral neglect due to stroke or PCA.

Overall, our studies highlighted some relevant and encouraging findings regarding the beneficial and enduring effects of these techniques on some disabling neurological illnesses and, therefore, on the quality of life of such patients. A limitation of our studies concerns the limited number of patients enrolled. This limitation could hamper the generalization of our findings, though this is a general issue in tDCS studies available from the literature.

Within these limits, the results of our studies are very promising. In addition, the ones conducted within this thesis project in collaboration with DITEN (Chapters 6 and 8) also highlighted the possible development of modern, simple, inexpensive and safe rehabilitative methods that can be used alone or in combination with the aim to achieve the optimal outcome measures for our patients. A realistic scenario in which these solutions can also be adopted in telerehabilitation mode, offers new possibilities for more comfortable, intensive and prolonged rehabilitative treatments.

Finally, we maintain that the study concerning the application of brain stimulation (tDCS) for the treatment of persistent COVID-19 anosmia deserves a specific mention. A significant part of the course of study and, therefore, of this thesis was focused on trying to provide a rehabilitation treatment for COVID anosmia through a safe method such as tDCS at a time when the scientific world had provided therapeutic options that are still insufficient. The exciting and very promising results of our study shed an optimistic light of hope on a disabling condition affecting millions of people around the world.

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CHAPTER 10

PHD ACTIVITIES

FULL PAPERS

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