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Evaluation of a combined physical and cognitive training protocol in people with Down syndrome via graph theory

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Αξιολόγηση ενός πρωτοκόλλου νοητικής και σωματικής ενδυνάμωσης σε ανθρώπους με σύνδρομο Down μέσω της θεωρίας γράφων

Evaluation of a combined physical and cognitive training protocol in people with Down syndrome via graph theory

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Οι απόψεις και τα συμπεράσματα που περιέχονται σε αυτό το έγγραφο εκφράζουν τον συγγραφέα και δεν πρέπει να ερμηνευτεί ότι εκφράζουν τις επίσημες θέσεις του Α.Π.Θ.



Abstract

Trisomy of human chromosome 21 is the most common genetic cause of mental retardation and leads to Down syndrome (DS). The existing treatments (pharmaceutical and non-pharmaceutical) of the DS deficiencies focus mainly on ameliorating only a number of symptoms. Our electroencephalography (EEG) study examined the effects of combined physical and cognitive training on DS adults implementing functional cortical network mapping and graph analysis. We investigated whether the cortical alterations could reflect the modification of the cognitive and physical capacity. A three-month intervention protocol of LLM Care was performed to twelve adults with DS. The results were estimated via the comparison of pre- and post-training 5-minute long resting-state EEG recordings, 15-minute long MMN EEG recordings, as well as somatometric, and cognitive assessments. EEG analysis indicated the strengthening of cortical network connectivity in resting-state, as well as the statistically significant increase of both network's global efficiency and transitivity and the statistically significant decrease of the network's characteristic path length. The cortical alterations due to the intervention triggers the network's reorganization. The induced complexity is plausibly a positive result, given the simplified organization of DS functional network. Also, the cognitive and physical capacity of DS people were enhanced after the intervention. To the best of our knowledge, this is the first computerized study that points out amelioration to the cognitive and physical skills of people with DS combined with EEG evidence of neuroplastic utility. These results are promising for the improvement of daily life and a higher level of independence that can be induced by training to the DS population.

Keywords: EEG, resting-state, Down Syndrome, combined physical and cognitive intervention, graph theory



Η τρισωμία του ανθρώπινου χρωμοσώματος 21 είναι η πιο κοινή γενετική αιτία νοητικής υστέρησης και προκαλεί Σύνδρομο Down (DS). Οι υπάρχουσες θεραπείες που αφορούν το DS εστιάζουν κυρίως στην αντιμετώπιση κάποιων συγκεκριμένων συμπτωμάτων. Στην παρούσα εργασία εξετάστηκε η επίδραση της νοητικής και σωματικής ενδυνάμωσης σε ενήλικες με DS μέσω χαρτογράφησης του λειτουργικού φλοιϊκού δικτύου με Ηλεκτροεγκεφαλογραφία (EEG) και της θεωρίας των γράφων. Για το σκοπό αυτό εφαρμόστηκε ένα τρίμηνο πρωτόκολλο παρέμβασης του συστήματος Φροντίδας της Υγείας LLM Care σε δώδεκα ενήλικες με DS. Τα αποτελέσματα της παρέμβασης προέκυψαν μέσω της σύγκρισης των σωματομετρικών, των γνωστικών εκτιμήσεων, των πεντάλεπτων EEG καταγραφών σε κατάσταση ηρεμίας και των δεκαπενταλεπτών EEG καταγραφών με το παράδειγμα της μουσικής αναντιστοιχίας που λήφθηκαν πριν και μετά την παρέμβαση. Από τις αναλύσεις προέκυψαν τόσο η βελτίωση των γνωστικών και σωματικών ικανοτήτων των ατόμων με Σύνδρομο Down όσο και EEG στοιχεία που υποστηρίζουν την αξιοποίηση της εγκεφαλικής νευροπλαστικότητας. Συγκεκριμένα, οι αναλύσεις των EEG έδειξαν την ενίσχυση της συνδεσιμότητας του φλοιϊκού δικτύου σε κατάσταση ηρεμίας, όπως επίσης τη στατιστικά σημαντική αύξηση της αποτελεσματικότητας (global efficiency) και της μεταβατικότητας (transitivity) του δικτύου καθώς και της στατιστικά σημαντικής μείωσης του μήκους μονοπατιού (characteristic path length) του δικτύου. Οι φλοιϊκές αλλαγές των ατόμων με DS φαίνεται να οδήγησαν στην αναδιοργάνωσή του και στην απόκτηση μιας πιο πολύπλοκης δομής, γεγονός που μπορεί να θεωρηθεί θετικό δεδομένης της απλοϊκής οργάνωσης του φλοιϊκού δικτύου των ατόμων με DS. Οι αλλαγές αυτές, πιθανώς, αντικατοπτρίζουν τη βελτίωση των γνωστικών και των σωματικών ικανοτήτων, καθιστώντας τη παρέμβαση μια πολλά υποσχόμενη μέθοδο στον τομέα της υγειονομικής περίθαλψης του πληθυσμού με DS.

Λέξεις κλειδιά: EEG, κατάσταση ηρεμίας, Σύνδρομο Down, συνδυασμός φυσικής και σωματικής εκπαίδευσης, θεωρία γράφων



1	Introduction	8
1.1	Genotype and phenotype	8
1.1.1	Deficits and Health issues	8
1.2	Therapeutic approaches	8
1.2.1	Pharmaceutical interventions	9
1.2.2	Non-pharmaceutical interventions	9
1.3	Resting-state networks (RSNs)	10
1.4	EEG recordings, functional connectivity, and cortical graph	11
1.5	Event-related potential (ERP) – Mismatch Negativity (MMN)	13
1.6	Findings of brain functional organization in population with DS	14
2	Methods	15
2.1	Participants	15
2.2	Intervention Protocol	16
2.2.1	Physical training	16
2.2.2	Cognitive training	17
2.3	Psychometric and somatometric assessment	17
2.4	EEG Recordings	18
2.4.1	Resting-state EEG data analysis	18
2.4.2	MMN EEG data analysis	20
2.5	Statistical analysis	21
3	Results	22
3.1	Graph analysis	22
3.1.1	Resting-state	22
3.1.2	MMN	24
3.2	Somatometric and cognitive assessments' analysis	25
4	Discussion	26
4.1	Strengthened connectivity of the post-resting-state network	26
4.2	Strengthened connectivity as result of the oddball paradigm after the intervention	27
4.3	Neuroplastic capacity of DS brain	28
4.4	Graph measures	28
4.5	Improvement of physical and cognitive abilities	30
4.6	Limitations	31
4.7	Conclusion	31
5	Bibliography	32

1 Introduction

1.1 Genotype and phenotype

1.1.1 Deficits and Health issues

Down syndrome (DS) (or trisomy 21) occurs about one in 650-1000 live births worldwide and is considered to be the most prominent cause of intellectual disability (Alan H. Bittles et al. 2007) (Table 1). The extra copy of chromosome 21 leads to protein overexpression, which, associated with epigenetic and environmental factors, influences the organ systems and results in impaired health. People with DS develop various pathogenic phenotypes such as mental retardation, seizures, early aging and mortality, heart deficiency, muscle degeneration, weak immune and endocrine systems, leukemia, and other systemic or anatomical abnormalities (Hassold and Hunt 2001; Conrad et al. 2006). The mental retardation is manifested mainly as deficits in memory, learning, and language production, whereas the visuospatial abilities are relatively preserved (Dierssen, Herault, and Estivill 2009; Laws and Bishop 2003; Chapman and Hesketh 2000). The accelerated or premature aging leads to the early development of dementia with neuropathological symptoms similar to those of Alzheimer's disease (AD) (Zigman 2013).

Table 1. *Genotype and Phenotype of people with Down Syndrome (DS)*

Genotype
Trisomy of chromosome 21
Phenotype
Muscle degeneration
Heart deficits
Weak immune and endocrine systems
Leukemia
Seizure
Altered brain structure
• Reduced volume of the hippocampi, cerebellum, frontal and temporal lobes • 20-50% fewer neurons than typically developed (TD) brains • Decreased growth of left hemisphere
Cognitive functions
• Intelligence quotient (IQ) less than average: 25-75 • Compromised motor skills, language, cognition, learning, and memory • Relatively preserved spatial memory and visuospatial perception • High risk of dementia and Alzheimer's Disease in DS adults over the age of 40
Early aging and mortality

1.2 Therapeutic approaches

The therapeutic interventions (pharmaceutical and non-pharmaceutical) in people with DS aim to target not only the health problems but also the limited independence in performing daily activities (Table 2). The interventions improve the quality of life of both people with DS and their family members, in addition to having positive effects on the psychological distress and economic burden of this condition (Fonseca et al. 2015).

1.2.1 Pharmaceutical interventions

The use of drugs as a treatment or preventive measure in people with DS resulted in raising their life expectancy from 12 to almost 60 years (A. H. Bittles and Glasson 2004; Penrose 1949). Drugs have been used in clinical trials to decrease the cognitive deficits of people with DS, included mainly drugs that were previously proved beneficial in the case of AD. The drugs were tested not only on demented DS patients but also on non-demented DS patients. This was because of the high similarity in neuropathology among DS and AD, the high incidence of dementia in people with DS, as well as the common brain modifications that DS and AD share. Nevertheless, clear benefits are yet to be confirmed due to the high heterogeneity of people with DS (Torre and Dierssen 2012; Fonseca et al. 2015).

1.2.2 Non-pharmaceutical interventions

The primary forms of non-pharmaceutical interventions are physical training, cognitive training, and the combination of both pieces of training. These have already been proved useful for the delay, or even prevention of age-related cognitive and physical deterioration, considering the environmental influence in gene expression and brain plasticity (Lott and Dierssen 2010; Supekar, Musen, and Menon 2009).

1.2.2.1 Physical interventions

Physical training is conducive to physical health (e.g. decreases the risk of cardiovascular disease, obesity, diabetes, cancer), mental health (e.g. reduces depression, anxiety) and cognitive health (e.g. delays cognitive decline, promotes neurogenesis during adulthood) of healthy people and people with several diseases as well (Rooney 1993; Penedo and Dahn 2005; Pérez and Cancela Carral 2008; Cotman, Berchtold, and Christie 2007). Mainly, physical interventions in people with DS are advantageous for attenuating some of their health problems like being overweight and obese. Thus, physical training makes them more functional to perform some of their daily life activities independently and increases their social skills on condition that the physical training includes social physical exercises (Hardee and Fетters 2017; Bertapelli et al. 2016).

1.2.2.2 Cognitive interventions

Cognitive training enhances cognitive functions (i.e. memory) of both people with normal cognition and people with cognitive deficits (e.g., AD patients), as well as prevents or slows down mental or cognitive deterioration (i.e. development of age-relating dementia or depression) (Acevedo and Loewenstein 2007; Clarke et al. 2003). Cognitive interventions specifically in people with DS were limited and were focused mainly on behavioral and memory training as well on the rehabilitation of dementia, one of the most severe problems of elders with DS (40-60 years old). However, there is a clear need for further implementation and evaluation of cognitive intervention in people with DS and dementia symptoms (Fonseca et al. 2015).

1.2.2.3 Combined physical and cognitive interventions

There is evidence that the combination of physical and cognitive training is more efficient than each intervention on its own (Oswald et al. 2006). Combined training in older people had long-term positive results with considerable improvement in everyday functioning and health status (Oswald et al. 2006). Furthermore, combined training in elders with mild cognitive

impairment (MCI) and its neurophysiological evaluation for the LLM project (<http://www.longlastingmemories.eu/>) indicated the beneficial neuroplastic effects that can be achieved due to the training (Styliadis et al. 2015). The combination of physical and cognitive training has not been applied to people with DS so far, although it is considered to be a suitable approach of training for them due to the physical difficulties caused both by orthopedic limitations and cognitive impairment (Rigoldi 2009).

Though, the considerable effects of combined intervention in the MCI population have been examined with the use of electroencephalographic (EEG) recordings and neurophysiological tests, to the best of our knowledge a similar neurophysiological approach has not been evaluated on people with DS, leaving in this way a significant knowledge gap. Given the necessity to ameliorate the daily difficulties of people with DS and their family members, our study aims to fill this knowledge gap.

Table 2. *Therapeutic approaches to people with DS*

Pharmaceutical interventions	Non-pharmaceutical interventions
Increase in life expectancy	<u>Physical training</u>
Not distinctly beneficial for impaired cognition	Improvement of physical health
	More effective and independent performance of daily activities
	Augmentation in social participation
	<u>Cognitive training</u>
	Focused effectively on behavior and memory
	Lack of interventions in DS people with AD
	<u>Combined physical and cognitive training</u>
	<i>No evidence in the literature</i>

1.3 Resting-state networks (RSNs)

Functional magnetic resonance imaging (fMRI) studies have indicated that the cortical brain at a resting state (i.e., no external stimuli) is organized in distinct subsystems with neuroanatomical and functional specialization (Beckmann et al. 2005; M. D. Fox et al. 2006; Damoiseaux et al. 2006). RSNs are suggested to be the passive processing mode of the brain between the active tasks, that secure the consolidation of the past and the preparation of the future (Raichle and Snyder 2007; Buckner and Vincent 2007). The default mode network (DMN) is the RNS that has received most attention and investigation, and its activation is observed mainly in resting state and less in the mode of task performance (Raichle et al. 2001). The cortical regions that mainly constitute the DMN are precuneus, posterior cingulate cortex (PCC), inferior parietal cortex, medial temporal lobes, medial frontal cortex, and anterior cingulate cortex (Raichle and Snyder 2007; Greicius and Menon 2004; Greicius et al. 2003).

The investigation of resting-state networks is an effective way for detecting differences in brain functionality of people with intellectual retardation (Vega et al. 2015; Anderson, Cox, et al. 2013; Supekar, Musen, and Menon 2009). There is evidence that aging and possible neurodegeneration are associated directly with alterations in the RSNs and their functionality. Cognitive decline is the entailed result of these modifications not only in people with dementia

but also in people who undergo a normal aging process (Damoiseaux, Beckmann, and Arigita 2008). Thus, RSNs can give insightful details about the cortical functioning of demented and non-demented people with DS, as well as about the potential outcomes of the intervention to people with DS.

1.4 EEG recordings, functional connectivity, and cortical graph

The understanding of both typical and pathological brain function has been at the center of neuroscience and has been based on the definition of the activation and the interaction of brain regions correspondingly (Sakkalis 2011). The interaction among the cortical areas is estimated by the identification of cerebral connectivity which classified into the neuroanatomical (structural), functional and effective connectivity.

The structural connectivity is innately hard to be estimated due to the microscopic nature of neurons, as well as their dynamic synaptic connections which frequent alterations are in conjunction with the cognitive processes (Ooyen 2005). The existence of particular fiber pathways among extended brain areas, which are aligned with the common cerebral anatomical definitions, simplified the complex issue of structural connectivity (Koch, Norris, and Hund-Georgiadis 2002). The remoted brain areas, that their activation is correlated temporally under the terms of statistical significance, assemble the functional connectivity (Fingelkurts, Fingelkurts, and Kähkönen 2005). Several brain neuroimaging techniques can be used for the estimation of functional connectivity, namely EEG, Magnetoencephalography (MEG), Positron Emission Tomography (PET) and fMRI (Sakkalis 2011). Effective connectivity is regarded as the indirect or direct effect that one neuronal circuit cause to another (Friston, Frith, and Frackowiak 1993) and its estimation is data-driven. Due to the blurring concepts of functional and effective connectivity, clarifying definitions have been given in terms of the model that characterizes the respective connectivity. Particularly, functional connectivity is "model free", while effective connectivity is featured by a causal model that describes the interaction among the neurons assembles (Lee, Harrison, and Mechelli 2003) (Table 3). Notably, Horwitz et al. alleged that every member of the neuroscience community that tries to evaluate these concepts gives her or his own operational definitions. This is why they use different quantities as measures of functional or effective connectivity and thus they describe different aspects of neuronal interactions (Horwitz 2003).

Table 3. *The brain connectivity classification*

Anatomical connectivity	Fiber pathways among distant regions
Functional connectivity	Model free
Effective connectivity	Causal model

The most prominent technique used for cerebral connectivity's estimation is fMRI due to the plenty of scanners that are available (Sakkalis 2011). FMRI measures the hemodynamic activity of the brain, namely the spontaneous fluctuations of Blood Oxygenative Level-Dependence (BOLD) that originate not only from the cerebral electric activity but also from other non-neuronal physiological processes of metabolism, like respiratory and cardiac activities (Liu et al. 2010) which can result in spurious fMRI signal (Brookes, Hale, et al. 2011).

Though fMRI preserves the high spatial accuracy of neuronal activities, BOLD is the response to the electrical fluctuations and reacts slowly to the rapid and transient electrical activities caused by the cognitive processes. Therefore, fMRI has neither temporal accuracy (Liu et al. 2010), nor sensitivity to Event-related potentials (ERPs) (Debener et al. 2006). The indirect recording of the neuronal activities via fMRI is considered to be an important pitfall in the precise understanding of the brain's functional mechanism (Brookes, Hale, et al. 2011).

EEG/ MEG are noninvasive electrophysiological measurements that record directly the oscillations of the brain's electrical activity and thus are characterized by high temporal resolution. However, the diffusion of the cerebral electrical potentials into the scalp sets the conductivity of the brain inhomogeneous. Consequently, the possible distortion of electrical activity implies that the EEG signal of one electrode measures the average activity of a broad cerebral area. Given these facts, EEG can not define precisely the position of each electrical oscillation. The possible spatial inaccuracy of EEG makes hard the definition of the real source of a particular oscillation because there are more than one sources that can influence one sensor (Brookes, Hale, et al. 2011). As fMRI does, the EEG signal is affected by non-neuronal physiological signals like eye movements and muscle or cardiac activities, too (Ille, Berg, and Scherg 2002).

In accordance with Brookes et al., the key for the clarification of cerebral functional connectivity is the use of non-hemodynamic measurements due to the indirect nature. But, to overcome the pitfalls of electrophysiological techniques, advanced source reconstruction methods are necessary (Brookes, Woolrich, et al. 2011) (Table 4).

Table 4. Features of fMRI and EEG measurements

	Temporal accuracy	Spatial accuracy	Analyzed regions	Affected by non-neuronal physiological activities	Disadvantage
fMRI	Low	High	Regions of interest	Respiratory, cardiac or vascular activity	Indirect response
EEG/MEG	High	Low	Whole head	Eye movement, blink, cardiac or muscle activity	Inhomogeneous conductivity

The study of functional connectivity of the RSNs has been based upon fMRI, MEG and EEG (Michael D Fox and Raichle 2007; Stam and Reijneveld 2007). The fluctuation of electrical activity over time permits the definition of correlation among cortical regions with high functional association. The functional association and thus the correlation among cortical regions is defined by the exchanging amount of information, building in this way the functional connectivity network of the cortex. Under the graph theory, this network could be depicted as a graph whose nodes are the cortical regions and the edges are the connections between the nodes (Stam and Reijneveld 2007). The information flow in this graph reflects the activation of cortical areas and, consequently, the performing of cognitive processing. Substantial evidence supports that the cortex of typical developing populations is organized as a small-world network with balanced integration and segregation of its subregions, in order to optimize both the synchronization and the efficiency of information transference and

minimize the wiring cost and the energy needs. Nevertheless, this kind of network organization is disrupted in cases of people with intellectual disabilities and deficient functional connectivity (Bassett and Bullmore 2006).

1.5 Event-related potential (ERP) – Mismatch Negativity (MMN)

Event-related potentials (ERPs) constitute the fluctuations of cerebral activity that emerged from the reception and the processing of sensory and non-sensory information that pertain to higher-level cognitive processes. ERPs are associated in time with a cognitive or physical event, and are typically measured from the scalp-recorded EEG as the average of the signal. ERP is a dynamic technic of neuroimaging with high temporal accuracy of the information processing and thus is one of the most informative methods assessing both the typical and the pathological brain activity (Duncan et al. 2009).

Mismatch Negativity is an ERP component, that is elicited by the detection of differences between an auditory stimulus and the sensory memory of previous auditory stimuli (Figure 1). This process is considered to be automatic and can be elicited either without the participants' attention. Thus, it is a suitable monitoring method in the assessments that participate very young or impaired people. MMN is located in the auditory cortices bilaterally, and secondarily in the right frontal cortex (Giard et al. 1990). The subcomponent of MMN located in supratemporal regions is a cortical marker of a perceptual auditory change. The subcomponent of MMN in frontal regions signifies that the frontal cortex participates activating the involuntary attention as a response to modification of auditory stimulation (Näätänen and Michie 1979; Giard et al. 1990). The perception of the deviant auditory stimuli and consequently MMN has been indicated to be decreased or absent in many disorders. So, MMN is a useful cognitive marker that can be used for the evaluation of treatment protocols and interventions (Näätänen et al. 2014).

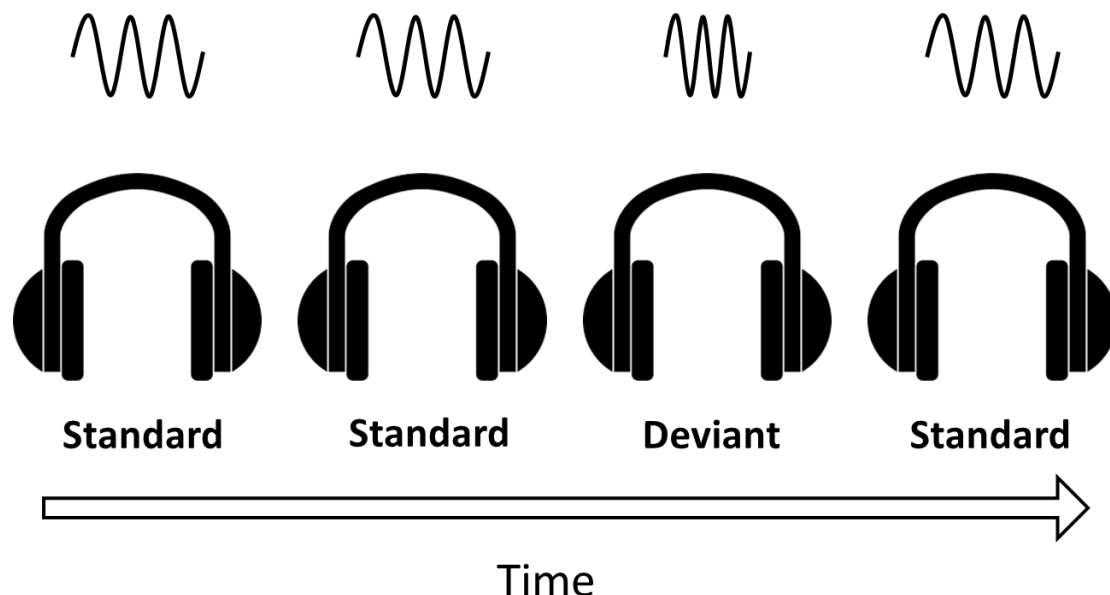


Figure 1. The experimental procedure to elicit a MMN response.

1.6 Findings of brain functional organization in population with DS

EEG and fMRI studies in individuals with DS have previously offered valuable insight into the functional architecture of the DS brain at rest. The earlier studies, after the comparison with typically developing people, demonstrated that people with DS have a slow wave EEG signal, with alpha rhythms (8-12Hz) to oscillate, especially slowly (Politoff et al. 1996; Katada et al. 2000). Slow-wave brain activity (higher activity in lower frequencies and lower activity in higher frequencies) was defined to have impaired cortical neural synchronization. These alterations were localized mainly within the left posterior areas (Babiloni et al. 2010, 2009). Following that, the correlation between the impaired cognition and the abnormal EEG signal of individuals with DS could suggest that the 3D neuroimaging, particularly the Low-Resolution Electromagnetic Tomography (LORETA) method, is a suitable and sufficient analysis of DS EEG signals (Velikova et al. 2011). Later studies indicated that non-linear analysis is more appropriate, as it is resulting in better comprehension of DS brain complexity (Hemmati et al. 2013; Ahmadlou et al. 2013). Notably, Ahmadlou et al. suggested that the complexity of the functional cortex should be examined not only locally but also globally. This study demonstrated via graph analysis that the functional cortical network of DS children and adolescents is organized randomly with decreased long-range connections (Ahmadlou et al. 2013). Furthermore, fMRI studies provided significant evidence that the DS functional cortex is organized in a simplified network with impaired differentiation of the cortical areas and increased global synchrony (Anderson, Nielsen, et al. 2013; Pujol et al. 2015; Vega et al. 2015).

The investigation of RSNs connectivity correlated with physical and neurophysiological assessments is a reliable biomarker that can be further employed to evaluate the outcome of combined physical and cognitive training to people with DS. This is because the combined training can influence not only the behavior and the daily functionality of people with DS but also their brain organization. To the best of our knowledge, this is the first study that implements combined physical and cognitive intervention to DS adults and makes the attempt to evaluate it via the simultaneous assessments of EEG, somatomotor and cognitive indices. To fill this knowledge gap, we hypothesized that combined physical and cognitive training tailored to the capacity of DS individuals could potentially induce the reorganization of the brain networks. Particularly, the functional connectivity mapping of EEG resting state indicates how the functional topography of the brain is altered by training. Thus, our analysis can provide insights into the training-induced plasticity of large-scale functional networks. Such insights are foundational to the type of training and the design of interventions that will best facilitate neuroplastic effects. Our hypothesis was tested via resting-state eyes-open EEG analysis with the use of Low-resolution electromagnetic tomography (LORETA) (Michel and Lehmann 1994) for defining the sources of electrical activity taking into account a general head model limited to the cortical regions. The functional connectivity cortical network was calculated by Phase Transfer Entropy (PTE) (Lobier et al. 2014). Statistical analysis was performed in order to determine whether the intervention led to significant changes in cortex organization and whether these changes reflected possible alterations in the somatometric and cognitive tests.



Figure 2. The experiment procedure

2 Methods

2.1 Participants

23 individuals with Down Syndrome and 18 typically developed participants as control subjects were recruited (Table 5). The DS participants attended a training intervention consisted of physical and cognitive exercises (LLM Care, <https://www.llmcare.gr/en/home/>). Carers supervised the 3-month intervention in the Thessaloniki Active and Healthy Ageing Living Lab (Thess-AHALL), and the premises of the Greek Association of Down Syndrome. Before and after the training, EEG recordings, somatometric and cognitive assessments were performed for every subject (Table 6). The protocol of this study (Figure 2), which was transacted under the Declaration of Helsinki, was validated by the Bioethics Committee of the Medical School of the Aristotle University of Thessaloniki.

Table 5. Demographic information of the participants separately for each analysis

		Resting-state	MMN
Adolescence	Subjects	12 (6 females)	14 (7 females)
	Mean	28,66667	28,78571
	Standard deviation	10,78158	9,625116
Adolescence + Children	Subjects	15 (6 females)	18 (7 females)
	Mean	25,06667	24,88889
	Standard deviation	12,14476	11,30793
Control	Subjects	18 (10 females)	
	Mean	24,11111	
	Standard deviation	3,348115	

Table 6. *Somatometric and cognitive assessments.*

Somatometric	Psychometric
Short physical performance battery - Balance test - Gait speed test - Chair stand test - total score	WISC-III[3] - Block design score - Picture arrangement score - Mazes score - Digit Span - Digits forward span - Digits backward span
10-meters walk test - Self-selected (sec) - Fast velocity (m/sec)	Digit - Digit forward - Digit backward
Body mass index (BMI)	Raven - Ravens A - Ravens AB - Ravens B - Ravens total
Back scratch	Face total
Arm curl	Eyes total
Sit and reach	
Single leg left (sec)	
Single left-right (sec)	
Time up and go (sec)	

2.2 Intervention Protocol

LLM Care is an integrated ICT platform that combines modern mental exercises with physical activity through an advanced environment, aiming at independent living and at developing brain functionality for the elderly, but also vulnerable groups. It includes hardware and software of new technology (Bamidis et al. 2015) and is designed as a social ecosystem providing health care specialized for elderly and vulnerable populations (Romanopoulou et al. 2018).

The intervention of 8-10 weeks duration consisted of hourly sessions per day (30 minutes for physical training and 30 minutes for cognitive training), with a frequency of two to three days per week during. The intervention took place at the Thessaloniki Active and Healthy Ageing Living Lab (Thess-AHALL) (Konstantinidis, Billis, Bratsas, et al. 2016), at the Greek Association of Down Syndrome, as well as at corresponding associations located in other cities of the country.

2.2.1 Physical training

The physical training program webFitForAll (Zilidou et al. 2016; Billis et al. 2010; Konstantinidis, Billis, Mouzakidis, et al. 2016) is an exergaming platform that uses a motion sensor device, (i.e., Kinect), allowing the users to train and maintain their physical status and well-being. The protocol included aerobic exercises (hiking on the spot and cycling), exercises for flexibility (stretching and warm-up), strength (weightlifting and resistance), and balance (static and dynamic), as well as brain exercises that enhance attention, memory and brain speed.

Sports specialists / physical educators and physiotherapists have defined a 50-70% intensity level of the maximum heart rate (HRmax). The 5-minute warm-up phase precedes the main body of training (20 minutes) that are followed by 5 minutes of full recovery. In aerobic exercises, the users were transferred to a virtual environment through Google maps and

waled through cities or landscapes. When the patients completed the exercises of strength and flexibility, enjoyable images were revealed as rewards. The exercises of balance required movement of the body in a horizontal or vertical axis.

2.2.2 Cognitive training

The cognitive training program BrainHQ is an online interactive environment that includes cognitive empowerment techniques. It accelerates and improves both visual and auditory processing. Notably, BrainHQ exercises are organized into six categories with 29 practical cognitive training exercises of hundreds of levels, focusing on attention, memory, brain processing speed, people skills, navigation, and intelligence. Each exercise is automatically adjusted to the level of the trainee's skills to produce brain improvements.

2.3 Psychometric and somatometric assessment

Before and after the intervention, all participants were subjected to a battery of tests to assess their cognitive and physical capacity. The psychometric assessment conducted by M. Karagianni and consisted of a set of neurocognitive tests that measure memory, attention, concentration, verbal and non-verbal mental capabilities, processing speed, problem-solving, visuospatial processing, organization skills, social intelligence, and identification of emotions. These tests included: WISC-III (Block Design, Picture Arrangement, Digit Span, Mazes) (Woolger 2001), Raven (John and Raven 2003), Reading the mind in the eyes (Baron-Cohen et al. 1997, 2001) and a variation: Reading the mind in the face (emotion recognition from video).

Somatometric evaluation was fulfilled by V. Zilidou and E. Romanopoulou through the Short Physical Performance Battery (SPPB) (Guralnik et al. 2012), 10 Meter Walk (Bohannon 1997), Back Scratch (Jones and Rikli 2002), Sit and Reach (Wells and Dillon 1952), Arm Curl (Jones and Rikli 2002), Four Square Step (FSST) (Whitney et al. 2007), Stork Balance (for both legs) (Johnson and Jack K. Nelson 1979) Timed Up and Go (Shumway-Cook, Brauer, and Woollacott 2000) tests and Body Mass Index (BMI). These appraise functional mobility, flexibility (in specific areas), dynamic stability, and static and dynamic balance.

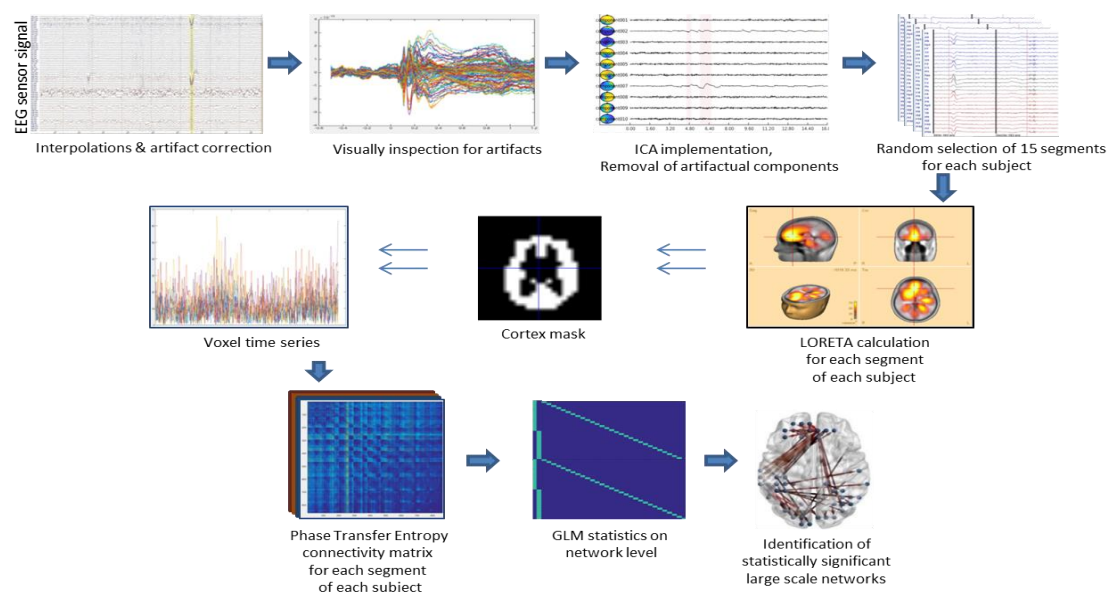


Figure 3. EEG signal analysis

2.4 EEG Recordings

2.4.1 Resting-state EEG data analysis

Resting-state (eyes open for 5 minutes) EEG recordings of DS participants before and after the intervention were performed by C. Karali and other members of the Medical Physics Laboratory at AUTH, with a Nihon Kohden EEG system of 122 active electrodes at a sampling rate of 1000 Hz, inside an electromagnetically shielded and sound-attenuated room in the AUTH Medical Physics Laboratory. Resting-state EEG recordings were implemented also for TD participants. The electrode impedance was kept below five k Ω . Eyes-closed EEGs were not recorded because the majority of the DS patients were unable to remain relaxed while following our instructions to maintain their eyes closed.

2.4.1.1 Preprocessing and resting-state Neuroimaging

A. Anagnostopoulou and I preprocessed the resting-state EEG data with the Brain Electrical Source Analysis software (BESA research, version 6, Megis Software) and the Matlab-based FieldTrip toolbox (Oostenveld et al. 2011). Using BESA, bad channels were interpolated accordingly to the signal of the neighboring channels and artifacts due to blinks and eye movements (vertical and horizontal EOG) were corrected with a spatial filtering method which is taking into account artifacts topography and brain signal topography (Ille, Berg, and Scherg 2002). Using FieldTrip we followed the methodology described by Styliadis and his colleagues to filter the artifact-corrected EEG recordings with a low-pass IIR filter at 97 Hz, a notch IIR filter at 48–52Hz, and a high-pass IIR filter at 0.53 Hz (Styliadis et al. 2015). The filtered EEG recordings were inspected visually, and any observed artifacts were removed. Independent Component Analysis (ICA) was performed to segregate the EEG data to temporally and functionally independent components (Jung et al. 2000). The artifactual ICA components with electrooculogram (EOG) and electrocardiogram (ECG) were detected visually and were removed. A final visual inspection checked the success of the previous steps. Fifteen segments of four minutes total duration were selected randomly from every EEG recording.

Source imaging analysis of the segments was performed with BESA at 0.5-35 Hz. The current density reconstructions (CDR) of each segment and each time point were estimated with low-resolution electromagnetic tomography (LORETA), producing 10mm voxels for each one of 4000-timepoints (Michel and Lehmann 1994). We used LORETA, because it does not need *a priori* definition of the number of sources, so we were able to analyze the cortical network globally. Fifteen 4D volumetric images were exported and were processed via a mask of gray matter in order to restrict the source space. The source space consisted of 863 voxels, and every voxel was defined as a node of the cortical network (15 subjects, 15 segments, 4000 time-samples, 863 voxel time series).

2.4.1.2 Connectomics analysis

The calculation of the cortical network required the estimation of the 863 $\times$ 863 adjacency matrix from the voxel time series. The connectivity of voxel nodes was estimated via phase transfer entropy (PTE) (Lobier et al. 2014), a method that predicts the directed information flow between neurons and the strength of their interaction. Matteo Fraschini and Arjan Hillebrand compiled the MATLAB algorithm for this method. The probability distribution of neurons causal relationships were extracted from the phase synchronization of time series

(Hlaváček, Paluš, and Vejmelka 2007), a method being based on the information theory metric of transfer entropy (TE) (Schreiber 2000). The advantage of this metric is the absence of both the linear (or other particular model parameters) constraints, due to nonlinear probability distribution, and the sensitivity to EEG signal nuisances. In addition, by choosing PTE as a metric we were able to implement a large-scale connectivity analysis to the whole cortical network and to compute which region's signal predicts and modulates the signal of other regions. Directed and weighted connectivity matrices based on PTE were calculated for each segment, and the 15 connectivity matrices of every EEG recording were averaged into one.

2.4.1.3 Graph measures computation

The Brain Connectivity Toolbox (Rubinov and Sporns 2010) was used to compute the graph measures of global clustering coefficient (transitivity (TS)), global efficiency (GE), characteristic length path (CPL), local clustering coefficient (LCC), local efficiency (LE) and node betweenness centrality (BC) for each participant. We computed the node degree centrality (DC) with the use of a Matlab function (Table 7). We calculated the density of each graph by summarizing all the weights of the graph. Notably, the proportion of the triangles (three nodes that are connected by three links) to the triplets (three nodes that are connected by three or two links) is called TS. CPL is the minimum number of connections that link one node to another. GE indicates the efficacy of information transfer between the neurons and associates inversely with the CPL (Bullmore and Sporns 2009). BC measures the importance of a node in the communication of the network's other nodes and corresponds to the fraction of all shortest paths that pass through the node (Rubinov and Sporns 2010). The DC refers to the inward and outward edges that link to each node summing of the weights of the edges.

We wanted to examine whether our intervention to people with DS alters the cortical organization and thus, we computed the specific graph measures which can inform us about the architecture and the complexity of the brain network before and after the intervention. Brain networks, as physically expensive systems, have to control the trade-off between wiring cost and functional efficiency (Bullmore and Sporns 2012). This is why brain networks have small-world architecture and consequently, their information processing is both segregated and integrated among the neuronal regions (Danielle Smith Bassett and Bullmore 2006; Bullmore and Sporns 2012). CLP, GE and LE are considered to be the main measures for the level of integration into a network, while TS and LCC are connected with the level of segregation that characterizes a network (Rubinov and Sporns 2010). Another feature of brain topology is the hierarchical structure of which has a central role to the complexity and the critical dynamics that neural networks have due to the cognitive processing' needs (Robinson 2009; Kaiser and Hilgetag 2010; Danielle S Bassett et al. 2008). Hierarchical topology can be quantified by the estimation of the network's centralities, like DC and BC (Danielle S Bassett et al. 2008).

Table 7. *The estimated graph measures and their role in the brain network's architecture.*

Graph measures	Neurobiological interpretation
Global efficiency (GE)	
Characteristic length path (CLP)	Integration
Local efficiency (LE)	
Transitivity (TS)	Segregation
Local clustering coefficient (LCC)	
Betweenness centrality (BC)	Hierarchy
Degree centrality (DC)	

2.4.2 MMN EEG data analysis

15 minutes of MMN paradigm with 1000 Hz sampling rate was conducted in DS participants before and after the training as performed in resting-state EEG recordings. The paradigm was designed by P. Kartsidis via the presentation software of Neurobehavioral systems with standard and deviant tones as shown in Table 8. The DS participants watched videos with animals in order not to get tired or bored during the MMN recordings.

Table 8. *MMN paradigm design*

Tone	Frequency	Amplitude	Duration	Repetitions
Standard tone	540 Hz	Normal	300 ms	450 epochs
Frequency Up	590 Hz	Normal	300 ms	90 epochs
Frequency Down	490 Hz	Normal	300 ms	90 epochs
Intensity Up	540 Hz	+50%	300 ms	90 epochs
Intensity Down	540 Hz	-50%	300 ms	90 epochs
Shorter duration	540HZ	Normal	100ms	90 epochs

2.4.2.1 Preprocessing and MMN Neuroimaging

P. Kartsidis preprocessed the MMN EEG data via BESA (BESA research, version 6, Megis Software). The EEG data were inspected visually. The detected bad channels were interpolated and the artifactual channels were corrected as performed in resting-state EEG data. The data were divided into epochs with duration -200 ms to 800 ms after the stimulus onset. The artifactual epochs were rejected. The other epochs divided into epochs with standard stimuli and epochs with deviant stimuli, and were averaged.

Source imaging analysis of the averaged epochs was performed by C. Karali with BESA at 2-35 Hz. The current density reconstructions (CDR) of each averaged epoch were estimated for 0ms to 800 ms after stimulus onset with low-resolution electromagnetic tomography (LORETA), producing 10mm voxels for each one of 800-timepoints (Michel and Lehmann 1994). 2 4D volumetric images were exported for every subject (one with standard stimulus and one with deviant stimulus) and were processed via a mask of gray matter in order to restrict the source space. The source space consisted of 863 voxels, and every voxel was defined as a node of the cortical network (18 subjects, 801 time-samples, 863 voxel time series).

The calculation of the cortical network required the estimation of the 863×863 adjacency matrix from the voxel time series. I estimated the connectivity of voxel nodes via transfer entropy (TE) (Schreiber 2000), a method that predicts the directed information flow between neurons and the strength of their interaction. The calculation of TE connectivity matrices were conducted by C. Karali via Hermes Matlab toolbox (Niso et al. 2013). TE was used for the same reasons that PTE used in resting-state. However ERP data are identified by high synchronization of oscillations, so phase metrics are not suitable for the calculation of ERP connectivity matrices. Directed and weighted connectivity matrices based on PTE were calculated for each subject and each tone.

2.5 Statistical analysis

P. Kartsidis conducted the comparison of the pre- and post-intervention somatometric and psychometric assessment scores with the use of a non-parametric Wilcoxon test and a paired t-test. The statistical comparisons were completed in the IBM SPSS 25.0 software. Wilcoxon test was performed for the psychometric battery tests and the ones of the somatometric ones with discrete-values scores, while the t-test was executed for the rest somatometric assessment tests.

The Network-Based Statistics (NBS) (Zalesky, Fornito, and Bullmore 2010) toolbox was employed for the estimation of the statistically significant differences between the whole-head connectivity networks. The comparisons that were conducted via NBS referred analytically in Table 9. A. Anagnostopoulou and I performed a paired samples t-test corrected for 10000 random comparisons via False Discovery Rate (FDR) correction. This methodology evaluates the significance of each edge independently, providing an independent p-value for each connection. The significant differences between the two time points were visualized as weighted graphs through the BrainNet Viewer (Xia, Wang, and He 2013) toolbox (Figure 3.A). We estimated DC from the outcome of this comparison and the results were depicted in the same graph.

Table 9. *Comparisons via NBS.*

Resting-state		MMN	
Pre-intervention <i>DS adolescences</i>	Post-intervention <i>DS adolescences</i>	Pre-intervention Standard-Deviant tone <i>DS adolescence</i>	Post-intervention Standard-Deviant tone <i>DS adolescence</i>
Pre-intervention <i>DS adolescences</i>	<i>TD participants</i>		
Post-intervention <i>DS adolescences</i>	<i>TD participants</i>		
Pre-intervention <i>DS adolescences and children</i>	<i>TD participants</i>	Pre-intervention Standard-Deviant tone <i>DS adolescence and children</i>	Post-intervention Standard-Deviant tone <i>DS adolescence and children</i>
Post-intervention <i>DS adolescences and children</i>	<i>TD participants</i>		

For the graph measures, M. Klados suggested and performed a more suitable method than t-test, which we performed firstly. So, he used Analysis of Covariance (ANCOVA), where each measure was the dependent variable and density served as the covariate because density affects significantly the values of the other measures. He performed ANCOVA for local measures (i.e., CC and BC) for each of the 863 nodes and FDR correction on the p-values for Type I errors. Results below the 5 percent threshold were considered to be significant.

The relationship between graph measure and physical or cognitive assessments was defined by C. Karali with the use of linear regression model. The model is a linear approach that determines the possible prediction of the dependent variable via the explanatory variable (independent variable). Physical or cognitive assessments were identified as the explanatory variable and graph measures as the dependent variable. The linear regression model was performed to the difference between pre- and post-training measurements. Thus, the implementation of linear regression investigated whether the difference between pre- and post-intervention graph measures could be predicted by the difference of pre- and post-training physical or cognitive assessments. The statistical significance of the prediction was tested by an F-test and the significance level was set at $p < 0.05$.

3 Results

3.1 Graph analysis

3.1.1 Resting-state

The resting-state networks of the participants were compared in order to determine any statistically significant differences before and after the training. The two-time points of the group had significantly different connectomics at resting-state, with the cortical network exhibiting amplified strength in connections after the intervention ($p < 0.05$) (Figure 4). The network from the pre-post comparison constituted 19 nodes and 19 edges, with the most strengthened connectivity to be observed at the left hemisphere. The cortical reorganization in the DS brain characterized by strengthening of connections within: i) left parietal lobe (paracentral, postcentral, precuneus, superior and inferior parietal), and ii) left occipital gyrus, and between: i) left superior/inferior parietal nodes to precentral and middle temporal nodes in the right hemisphere, and ii) left superior/inferior parietal node to left superior frontal nodes (Figure 3). In reference to Yeo's parcellation of core resting-state networks (Yeo et al. 2011), we reported connections between i) visual, dorsal attention (DAN) and default mode (DMN) network, ii) frontoparietal network (FPN) and DMN, iii) DAN and the somatomotor network (SMN), iv) DMN and DAN, and v) DMN and the SMN.

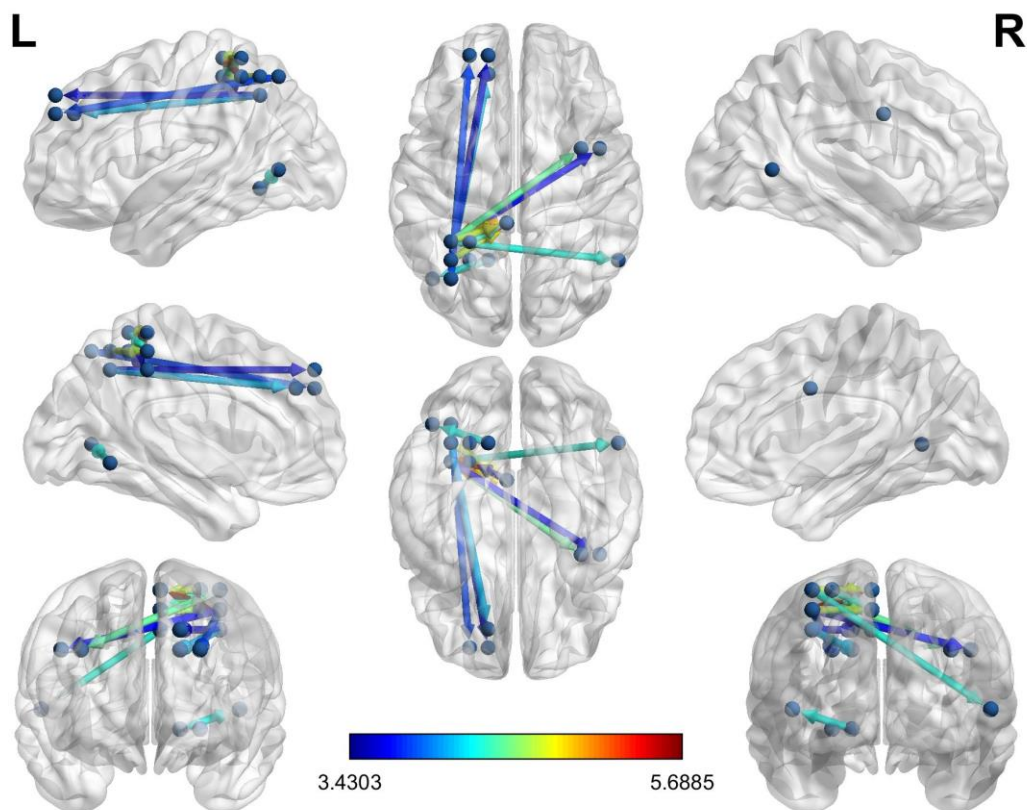


Figure 4. The resting-state functional network of the DS cortex indicating the changes that emerged from combined intervention. Statistical comparison of post- to pre-training network's difference. The significant level was defined at $p < 0,05$, with FDR correction.

The comparison among TD resting-state networks and DS resting-state networks before and after the intervention showed more organized differences between TD networks and DS post-intervention networks in both comparisons with and without children (Figures 5,6).

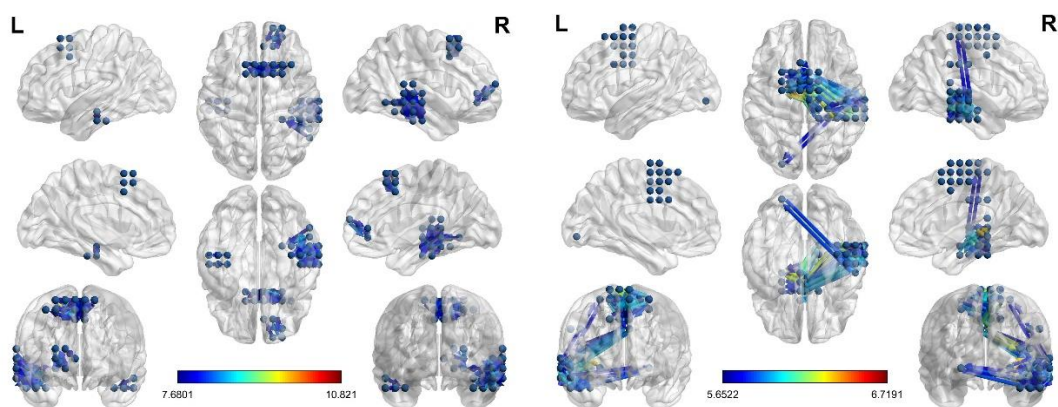


Figure 5. The resting-state functional network of TD and DS cortex indicating the changes between TD and DS that emerged from combined intervention. (Left) Statistical comparison between TD networks and DS pre-training network's difference. (Right) Statistical comparison between TD networks and DS post-training network's difference. The significant level was defined at $p < 0,05$, with FDR correction. DS participants were only adolescents.

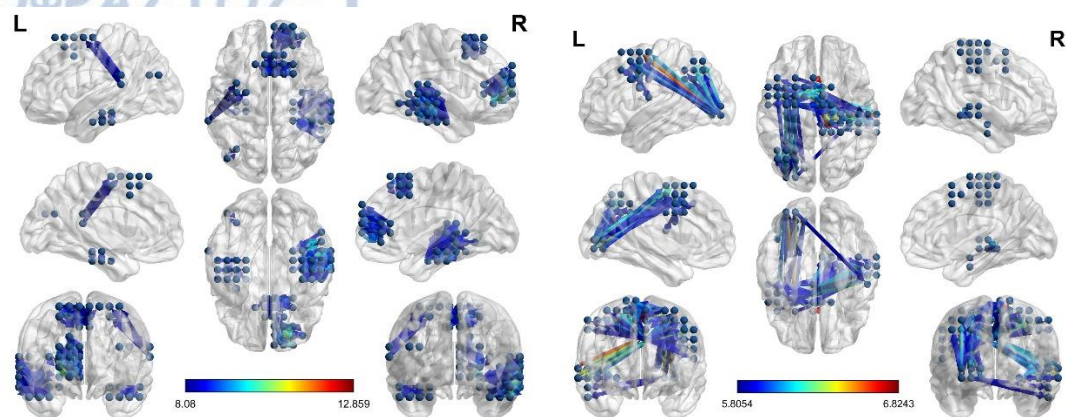


Figure 6. The resting-state functional network of TD and DS cortex indicating the changes between TD and DS that emerged from combined intervention. (Left) Statistical comparison between TD networks and DS pre-training network's difference. (Right) Statistical comparison between TD networks and DS post-training network's difference. The significant level was defined at $p < 0.05$, with FDR correction. DS participants were both adolescents and children.

The graph measures' statistical comparison showed that the post-training resting-state network compared with the pre-training network had decreased TS and CLP, and increased GE ($p < 0.05$). In respect to local measures, LCC and LE comparisons resulted in a significant difference with 124 nodes showcasing an increase in value. These nodes pertain to the left and right frontal lobe, left pre- and postcentral lobe, left superior parietal lobe, left superior temporal lobe, and lingual gyrus, as well as the right cuneus, precuneus, right pre- and postcentral lobe, right middle and inferior temporal lobe, right fusiform gyrus and lingual gyrus. The rest 739 nodes exhibited a decrease. BC showed an increase in one node of the left fusiform gyrus and a decrease in the value of four nodes situated in the left superior and inferior frontal gyrus (Table 10).

Table 10. The comparison of pre- and post-training graph measures with a significant level defined at $p < 0.5$.

Graph Measure	p-value	Effect
Transitivity (Global Clustering Coefficient)	0.00	Increase
Global Efficiency	0.00	Increase
Characteristic Path Length	0.00	Decrease
Local Clustering Coefficient	1.63e-07/8.59e-07	Increase/Decrease
Local efficiency	1.63e-07/8.59e-07	Increase/Decrease
Node Betweenness Centrality	0.0221/0.0487	Increase/Decrease

The calculation of linear regression among graph measure and physical or cognitive assessments had not statistically significant results.

3.1.2 MMN

The comparison of MMN EEG data was indented to identify whether the intervention influenced the cortical connectivity as was configured during the standard and the deviant stimuli. The comparison only with adult participants has not statistically significant results, while the comparison with both adults and children participants showed strengthened

connectivity in both hemispheres (Figure 7). However, the nodes that modulate the activation of many other nodes more intensively are located in the left hemisphere. Specifically, the nodes in Brodmann areas 39 and 40 regulate the activation of the inferior prefrontal cortex (IPC).

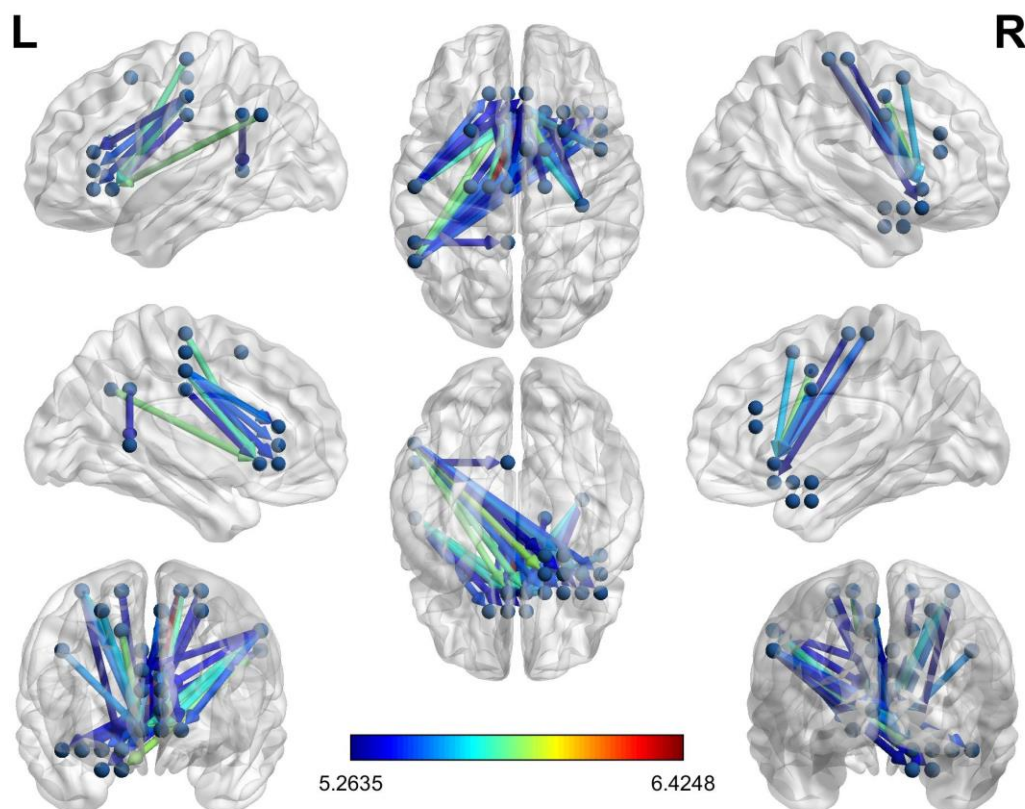


Figure 7. The MMN functional network of the DS cortex indicating the changes that emerged in MMN perception from combined intervention. Statistical comparison among post-training deviant stimuli, post-training standard stimuli, pre-training deviant stimuli and pre-training standard stimuli network's difference. The significant level was defined at $p < 0.05$, with FDR correction. The DS participants were both adolescence and children.

3.2 Somatometric and cognitive assessments' analysis

The somatometric assessments that changed significantly after a one-tailed, paired t-test determined after the intervention. The results displayed significant improvement in the test of Arm Curl ($t = 1.75$, $p = 0.017$) due to the increase, and in the test Time up and go ($t = -1.67$, $p = 0.017$) because of the decreased performing time. Statistically significant changes were not observed for the other physical assessments (Table 5).

The one-tailed, paired t-test on the measurements of the participants' cognitive capacity indicated that the cognitive measures of Digits Forward Score ($t = 1.52$, $p = 0.032$), Mazes ($t = 0.97$, $p = 0.032$) and Raven AB and total score ($t = 1.12$, $p = 0.017$, $t = 3.13$, $p = 0.015$) had significant improvement after the training. The rest of the cognitive measures did not point out a significant difference between the pre- and post-training measurements (Table 11).

Table 11. The comparison of pre- and post-training somatometric or cognitive assessments with one-tailed, paired t-test, and significant level defined at $p < 0.05$.

Somatometric measures	Mean difference	p-value
Arm curl	1.75	0.017
Time up and go	-1.67	0.007
Cognitive measures		
Digits forward	1.52	0.032
Mazes score	0.97	0.032
Ravens AB	2.12	0.017
Ravens total score	3.13	0.015

4 Discussion

Our resting-state EEG study investigates the results of the combined physical and cognitive intervention on DS adults. The intervention triggered changes to the DS cortical function network for the resting state, as well as the physical and the cognitive capacity of the participants. Notably, the combined intervention influenced both cognitive and physical abilities positively. Furthermore, the intervention led to the statistically significant strengthening of resting-state cortical connectivity. The increased connectivity associated with the significant decrease in CPL and the significant increase of both GE and TS, as well as the significant changes to the LCC, LE and the BC, demonstrate the reorganization of the resting state network.

4.1 Strengthened connectivity of the post-resting-state network

Our results of the strengthened resting-state connectomics indicated not only that people with DS retain the brain neuroplastic capacity but also that the combined physical and cognitive intervention can tap into neuroplasticity and utilize it beneficially. These neuroplastic effects as revealed by the EEG connectivity are in line with the results of previous studies performed in MCI and AD patients indicating that the combination of both physical and cognitive training can promote brain flexibility (Herholz et al. 2013; Styliadis et al. 2015). The cortical regions that demonstrated reorganization were located mainly in the left cortical hemisphere. This hemisphere of the DS brain is characterized by the most abnormal organization and is associated with language deficits (Ahmadlou et al. 2013). The DS atypical laterality has been strongly connected with mental retardation and poor linguistic ability. The limited education and motor training, that people with DS usually receive, contribute to the conservation of the DS intellectual disability caused by the atypical laterality (Grouios, Ypsilanti, and Koidou 2013). This is maybe one of the main reasons that the training of LLM project resulted in the influence of the more abnormal hemisphere.

An MRI study associated the abnormalities of DS grey matter density within the brain domains with the deficient DS cognitive phenotypes via neurophysiological measures (Menghini, Costanzo, and Vicari 2011). The authors specified that abnormalities in inferior parietal lobule, insula, superior temporal gyrus, medial occipital lobe, and cerebellum were correlated with the deficient short-term memory, while the orbitofrontal cortex, lateral and medial temporal lobe were correlated with the deficient long-term memory. Malfunctions in verbal abilities

were connected with aberrant cerebellum, middle, and inferior temporal gyrus. Also, visuo-perceptual deficiencies were ascribed to abnormal left middle frontal gyrus (Menghini, Costanzo, and Vicari 2011). Most of these regions were influenced by our intervention and showed amplified connections which may eliminate to a certain extent the abnormalities of DS grey matter density. Consequently, we could expect effects in the corresponding cognitive abilities.

The RSNs' study of Anderson et al. indicated that the functional subnetworks of DS brain communicate with greater synchrony compared with typical brain regions' synchrony (Anderson, Nielsen, et al. 2013). The prevalence of short-range connections over the long-range connections between the DS cortical regions contributes to the deficient segregation of DS brain into functional subnetworks. The increased global synchrony and the decreased differentiation of brain regions imply a simplified network organization exhibiting immature cortical network connectivity. Hence, the information flow is limited mainly to long distances, and the creation of coherent networks between remote brain regions is insufficient due to impaired communication (Anderson, Nielsen, et al. 2013; Vega et al. 2015). The amplified connectivity of our post-intervention network was located to most of the RSNs, as defined by Yeo and his colleagues (Yeo et al. 2011), and the strengthened connections were observed not only within the RSNs but also between the RSNs. The between RSNs enhanced connections may imply that the combined training induced better communication among remote regions and, thus, a more complex organization of DS resting-state network.

Taking into account i) the immature connectivity of the RSNs associated with distinct functions, as suggested by Anderson et al. and Vega et al., ii) the abnormal density of cortical regions, as connected with deficient functionality by Meghini et al. and iii) the abnormal EEG rhythms, as correlated with the DS cognitive capacity by Velikova et al., combined with iv) the suggestion of Anderson et al. and Grouios et al. that the symptomatology of people with DS is the downstream consequences of the genetic brain malfunction as expressed by the impaired cognition, in conjunction with our results indicate the utility of neuroplasticity and the amplified connectivity at resting state we can suggest that the post-intervention reorganization of participants' brain reflects the improvement of cognition as indicated by the cognitive assessments.

4.2 Amplified connectivity as a result of the oddball paradigm after the intervention

In addition to the resting state connectivity outcomes, the results of MMN connectivity confirmed that DS brain retains the neuroplastic capacity. The combined training tapped into the cortical neuroplasticity in a way that the deviant stimuli among standard stimuli implied the strengthening of specific connections. One of the prominent strengthened connection circuits originates from the left angular and supramarginal gyrus (Brodmann 39,40 – Superior temporal gyrus) and ends up in dorsal prefrontal cortex. This pathway has been connected with the process of attention shifting (Pammer et al. 2006). Specifically, the left superior temporal gyrus (Wernicke's area) is involved in the process of nonverbal sounds mainly and verbal sounds secondarily (Saygin et al. 2003). The inferior prefrontal cortex participates in attentional control (Hampshire et al. 2010) and in the definition of auditory pattern violation

regions but also sets the information flow vulnerable to disturbances and to complete network's disconnection in the case of few connections' loss (Ahmadlou et al. 2013).

The small-world topology of brain connectivity is considered to be a critical transition state that allows the brain to be adapted in the environment's demands. A small-world network can be easily reconfigured toward a random network in the case of broad or general functions and toward a regular network in the case of a specific cognitive operation (Barbey 2018; Beggs 2008). This network's flexibility is more substantial when the task demands change. However, a stable network in a segregated state seems to be more effective in the instances of both the same retained task and a resting-state condition (Fong et al. 2019; Hilger et al. 2019). This ability of the brain has been connected with a higher level of cognitive functioning (Hilger et al. 2019).

Due to the aforementioned studies, we suggest that our combined intervention to people with DS triggered the reorganization of cortical topology to a transitional state towards a less-random and more healthy architecture (Figure 4). Our results showed that the CLP was decreased, and both the GE and TS were increased after the training. Thus, the simultaneous increase of both GE and TS and decrease of CPL may imply that the network became more organized with better segregation and integration concurrently. This enhanced trade-off between segregation and integration makes the network more flexible in the case that task-demands change and the cortical network has to be reconfigured instantly in a task-specific state (Barbey 2018). This organization sets the network less-random and with features that can eventually compose a small-world network (Danielle Smith Bassett and Bullmore 2006; Bullmore and Sporns 2012). Furthermore, the augmentation of the network's segregation may suggest that DS resting-state network operates at a higher level of cognitive performance after the intervention (Hilger et al. 2019; Fong et al. 2019) Except for the global graph measures, alterations were also observed in the local graph measures of the cortical network. Notably, the LCC and the LE were increased in 739 nodes of the frontal cortex and the occipital lobe bilaterally, the right parietal, and the right temporal lobe. The BC was augmented in one node of the fusiform gyrus and was reduced in the left frontal gyrus. These modifications of the local centralities can suggest that the intervention triggered the reorganization of the network, increasing and decreasing the importance of the nodes' role in the transmission of information. The nodes with similar connection patterns tend to exhibit similar functionality (White et al. 1986). Hence, the differentiation of the local connectivity patterns may suggest the shift of the functionality between nodes to be accomplished the greater segregation of the network.

The strengthened connections may be proved beneficial for the robustness of the cortical network in order not to be disconnected in the case of disturbances. Also, the better trade-off between segregation and integration and the alterations in centralities may turn the initial simplified functional network into a more complex brain network, better organized that would be more efficient and adaptive in the environments' demands. In accordance with this evidence, our suggestion, that after the intervention DS brain was induced in a healthier transitional state between random and small-world networks, proved to be plausible and consistent with the existing literature.

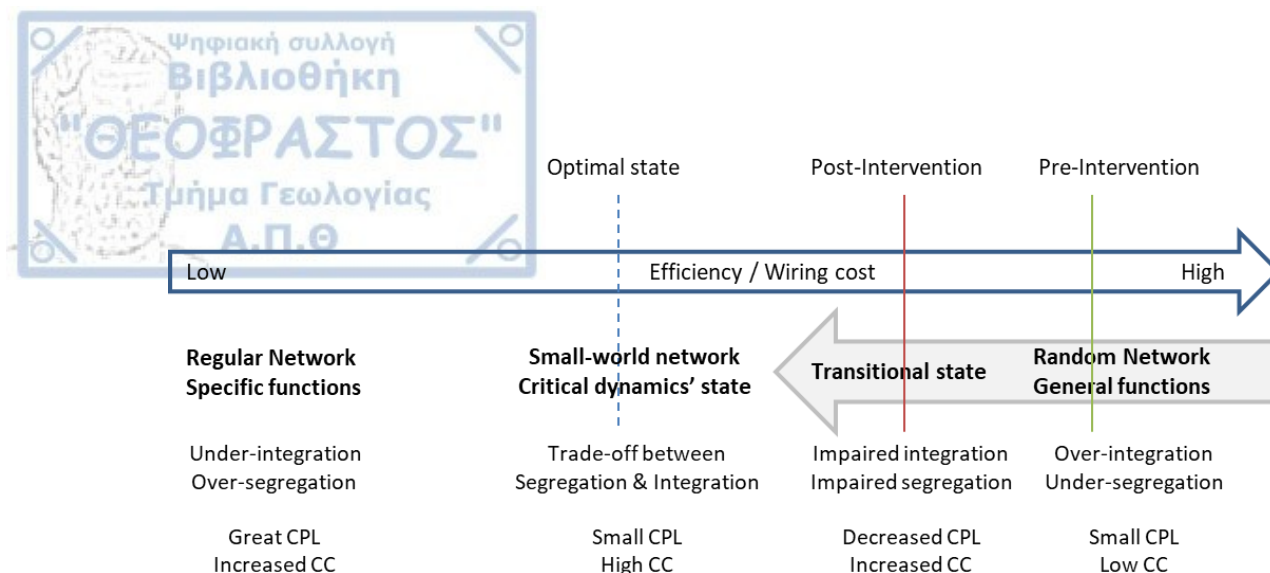


Figure 4. The transitional state from a random network toward a healthier functional architecture of DS brain after the intervention.

4.5 Improvement of physical and cognitive abilities

The combination of physical (balance, stamina, flexibility, and strength exercises) and cognitive (memory, attention, orientation, and brain processing speed exercises) intervention affected positively the pre-intervention inabilities of the participants, as emerged from the cognitive and somatometric assessments.

The results of the physical training are in agreement with the results of previous studies that regarded populations with DS and examining physical interventions with similar activities (Hardee and Fetters 2017). Nonetheless, our study included a physical training program with more types of trained domains, as well as a combination of some domains. The measurable enhancement of the participants' physical fitness can index the potential to reach a higher level of independence in the daily activities performance and a lower risk of health complications, like cardiovascular disease, as well (Rimmer et al. 2004; Brill et al. 2000).

The cognitive training protocol contained activities intending to improve the overall cognition of the people with DS. The intervention influenced beneficially not only the memory, as is mainly evidenced in the existing literature, but also various multiple cognitive skills and specifically attention, orientation, and brain processing speed (Fonseca et al. 2015). Most of the previous cognitive interventions in people with DS contained principally behavioral or memory training and their impact were not clearly defined (Fonseca et al. 2015). However, McGlinchey and her colleagues performed an intervention protocol that indented to train many cognitive domains simultaneously, in a similar fashion to our own protocol (McGlinchey et al. 2019). Our study resulted in cognitive assessments' improvement, as McGlinchey et. al' s study did. The assessments focused on the examination of the executive functions' performance to thoroughly investigate the intervention's effects in the daily life of people with DS (Kelly et al. 2014). Hence, the strengthening of the cognition assessments indicates not only the decrease probability of appearing cognitive impairment or dementia, but also the increase of functional dependence in everyday living (Bennett 2001; Fonseca et al. 2015). Further studies had shown that the improvement of cognition can be triggered by physical training as well (Gerontol 1996; Pérez and Cancela Carral 2008). Thus, the enhanced cognitive

skills induced by our combined intervention may be the outcome of both cognitive and physical training.

4.6 Limitations

The study sample was low so we were not able to identify specific phenotypes of the DS participants that responded better to our intervention. The simultaneous physical and cognitive training of our intervention protocol, despite providing fertile knowledge, did not permit us to define the exact effects of each type of training. Further, future comparison of age- and gender-matched typically developed group with our DS group would inform us whether the cortical network was re-organized into a more typical pattern or a compensatory pattern. A temporal examination of network organization during resting-state is necessary for testing the dynamics and stability of the network's segregation induced by the intervention, in order to define more accurately the amelioration of DS cognitive processing. The calculation of graph measures of MMN functional networks would be necessary so as to validate the reorganization of MMN functional network, also towards a healthier architecture. More experiments with measures across the time (follow up) would be insightful for the stability of intervention's effects, the progress of the possible cortical network's maturation and the gaps of the existing literature.

4.7 Conclusion

Conclusively, our study provided useful and innovative insight on our original hypothesis, that the three-month combined physical and cognitive training to DS people could be proved beneficial as evidenced by the conjunction of EEG indexes and both somatometric and cognitive assessments. The EEG connectivity showed that the DS brain retains the neuroplastic capacity which can be triggered, under specific circumstances, as our combined intervention did. DS cortex reorganized and shifted to a more complex network due to the amplified connectivity and the alterations of the graph measures that indicated better trade-off between segregation and integration. Thus, it is plausible that after the intervention DS brain revealed a transitional state of less-random and more small-world architecture, but not entirely healthy yet. These cortical alterations may reflect the improvement of cognitive and physical capacity. These pieces of evidence prepare the ground for further research and are promising for the challenging domain of health care generally and to DS specifically.

5 Bibliography

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