Computerized physical and cognitive training improves the functional architecture of the brain in adults with Down Syndrome: a longitudinal network science EEG study

Alexandra Anagnostopoulou[1]*, Charis Styliadis[1]*, Panagiotis Kartsidis[1], Evangelia Romanopoulou[1], Vasiliki Zilidou[1], Chrysi Karali[2], Maria Karagianni[1], Manousos Klados[3], Evangelos Paraskevopoulos[1]**, Panagiotis D. Bamidis[1]**

[1] Medical Physics Laboratory, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Greece.

[2] School of Biology, Faculty of Science, Aristotle University of Thessaloniki, Greece.

[3] Department of Psychology, The University of Sheffield International Faculty, City College, Thessaloniki, Greece.

* Joined first authorship

** Joined senior authorship

Corresponding Author: Dr. Charis Styliadis, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece. Aristotle University of Thessaloniki; University Campus; P.C. 54124; Thessaloniki; Greece. Email: cstyliadis@auth.gr

Keywords: Down Syndrome, Physical Training, Cognitive Training, Electroencephalography, Network Science Indices, Adaptive Neuroplasticity

Abstract

Understanding the neuroplastic capacity of people with Down Syndrome (PwDS) can potentially reveal the causal relationship between aberrant brain organization and phenotypic characteristics. We used resting-state EEG recordings to identify how a neuroplasticity-triggering training protocol relates to changes in the functional connectivity of the brain's intrinsic cortical networks. Brain activity of 12 PwDS before and after a ten-week protocol of combined physical and cognitive training was statistically compared to quantify changes in directed functional in conjunction with psycho-somatometric assessments. PwDS showed increased connectivity within the left hemisphere and from left to right hemisphere, as well as increased physical and cognitive performance. Our results reveal a strong adaptive neuroplastic reorganization, as a result of the training that leads to a more complex and less-random network, with a more pronounced hierarchical organization. Our results go beyond previous findings by indicating a transition to a healthier, more efficient, and flexible network architecture, with improved integration and segregation abilities in the brain of PwDS. Resting-state electrophysiological brain activity is used here for the first time to display meaningful longitudinal relationships to underlying DS processes and outcomes of importance in a translational inquiry. This trial is registered with ClinicalTrials.gov Identifier NCT04390321.

Author Summary

The effects of cognitive and physical training on the neuroplasticity attributes of people with and without cognitive impairment have been well documented via neurophysiological evaluations and network science indices. However, there is still insufficient evidence for people with Down Syndrome (PwDS). We investigated the effects of a combinational training protocol on the brain network organization of 12 adult PwDS using EEG and network indices coupled with tests assessing their cognitive and physical capacity. We report evidence of adaptational neuroplastic effects, pointing to a transitional state towards a healthier organization with an increased ability to integrate and segregate information. Our findings underline the ability of the DS brain to respond to the cognitive demands of external stimuli, reflecting the possibility of developing independent-living skills.

Introduction

Neuroplasticity can emerge in both typical and atypical brains and allows for either development (evolutionary plasticity), reaction (reactive plasticity), recovery (reparation plasticity), or adaptation to internal and external stimuli (adaptational plasticity)[1]. The different aspects of plasticity are suggested to have the same molecular basis[1]. In contrast to the brain of typically developed (TD) individuals, the Down Syndrome (DS) brain presents atypical levels of inhibition, due to gene over-expression, which lead to prolonged failure of synaptic plasticity and a reduced capacity for remodeling[2]. These characteristics, along with morphogenetic modifications, have been identified as some of the leading causes of brain disability in people with DS (PwDS)[3].

Children and young adults with DS, in comparison to age-matched individuals of typical development, have smaller brain volumes (i.e., decreased volume in frontal gyrus, amygdala, and cerebellar structures but also increased parahippocampal volume)[4,5], a pattern which over-pronounces after their 50th year of age[6]. The current consensus has associated the cognitive capabilities and deficiencies of PwDS with several distinct brain regions emphasizing an abnormal, and less efficient DS brain organization[7].

Electroencephalography (EEG) and magnetoencephalography (MEG) studies have complemented the notion of this atypical organization; PwDS when compared to TD controls exhibit slow brain wave, especially in left posterior areas[8,9], with higher delta band and lower alpha and beta band activity, a pattern also evident in patients with Alzheimer's Disease (AD)[8–11]. fMRI studies have shown that the DS network has a rather-simplified organization[12], lacking the appropriate efficiency and flexibility[13]. Given the limited capacity of the DS brain to consolidate information due to its disorganized architecture of reduced segregation and impaired integration, diffused connectivity (hyper-synchrony), and decreased long-range connectivity[12,13], the DS brain network's potential for plasticity is in question[13].

Despite these DS-related atypical deviations, the DS brain does possess neuroplastic capabilities, at least in the form of compensatory events[14–16]. The emergence of AD-related characteristics (i.e., altered theta band activity and power) in PwDS, at least a decade before the manifestation of dementia and much earlier than in TD people[11,17], reaffirm the possibility of compensatory mechanisms, before and during the expression of AD[14,16]. Characterizing neuroplasticity in PwDS is vital in understanding causality between aberrant brain circuitry and the cognitive and behavioral phenotype. This understanding would allow the quantification of the remodeling potential of evidence-based interventions that aim to purposely reawake neural plasticity and permit improved cognition.

Longitudinal and interventional studies are required to investigate whether it is possible to overcome, at least partially, the cognitive disability through novel therapies. Such interventional approaches have shifted from a pharmaceutical concept, with, so far, inconclusive results regarding beneficial effects [18–22], to non-pharmaceutical interventions of physical and behavioral components, aiming to trigger neuroplasticity and enhance brain health or to protect against neurodegenerative events[14]. Such interventions have shown promise in healthy aging, and populations with cognitive impairments, and have provided mounting evidence for lifelong brain plasticity[23–31].

Despite evidence supporting the positive impact of exercise-based[32,33], as well as behavioral and cognitive[34,35] interventions for PwDS, DS literature features a substantial knowledge gap, as there are no reports of neurophysiological investigations of the training-induced effects, and a network theory based assessment of the training-induced plasticity. Deviations in the brain functionality of PwDS have been previously addressed with the investigation of resting-state networks (RSNs) [8,9,12,36–38].

We hypothesized that our training protocol of combined physical (PT) and cognitive training (CT), will trigger neuroplasticity and reorganize the DS brain network to adapt to the increased cognitive and physical demands. Resting-state (eyes-open) EEG data were acquired to characterize plasticity in the DS brain through network science and to investigate how the training-induced neuroplasticity affects the state of the DS network, in terms of organization and characteristics, since it is known to maintain a random-like architecture[38] with impaired integration and segregation capabilities. Could the triggering of neuroplasticity bring about the emergence of a more efficient, complex, and specialized network organization?

Source analysis was performed using low resolution electromagnetic tomography (LORETA)[39] and directed functional connectivity was estimated via phase transfer entropy (PTE) in the 0.53-35 Hz frequency band. This measure quantifies the direction of the information flow, allowing for a whole-head analysis without requiring an *a priori* head model definition. An FDR statistical model was applied to extract the significant, within-network differences (post- vs. pre-intervention) in connectivity. A graph-theoretical analysis was performed to index the training's neuroplastic effects through the statistical comparison of graph measures between the two time-points.

Results

Psychometric and somatometric results

Psychosomatometric score comparisons between the two time-points were performed with the use of non-parametric Wilcoxon tests and paired t-tests. Physical assessments exhibited a significant improvement in score for the arm curl test (Table 1). Significant differences in the time of completion (decrease in duration) were evident in the Time Up and Go assessment test (Table 1). The comparison of psychometric assessments revealed an increase in the Digits Forward score (Digits Span test), the Mazes test, as well as the Ravens AB, and Ravens Total Score (Table 1). The rest of the tests showed no significant post-pre score changes.

Table 1. Somatometric and psychometric tests with significant differences post vs. pre, along with the post-pre mean difference. Results were considered significant for $p < 0.05$.

Somatometric	**p-value**	**mean difference**
Arm Curl	0.017	1.75
Time Up and Go	0.007	-1.67
Psychometric		
Digits Span (Digit Forward)	0.032	1.50
Mazes	0.032	0.97
Ravens AB	0.017	2.12
Ravens Total Score	0.015	3.13

PTE results

Statistical comparisons of the PTE matrices between the pre- and post-intervention EEG measurements indicate the reorganization of cortical connections (19 nodes and 19 edges), due to training, in the DS resting-state network ($p < 0.05$, FDR corrected, 10000 permutations) (Figure 1). Specifically, the cortical reorganization in the DS brain is characterized by the strengthening of connections within: i) the left parietal lobe (paracentral, postcentral, precuneus, superior and inferior parietal), with most connections originating from superior parietal nodes, and ii) left occipital gyrus, with a modulating node at the lingual 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 1). In respect to the core

RSNs, as defined by Yeo et al.[40], we have noted increased connectivity within the visual network, dorsal attention network (DAN), and default mode network (DMN), and between the frontoparietal network (FPN) and DMN, DAN and somatosensory network (SMN), as well as DMN and DAN (Table 2).

[place Figure 1 here]

Table 2. Significant increase in connectivity of RSNs as defined by Yeo et al[40]. The black color signifies the within-network connectivity, the green color the bilateral connectivity between-networks, and the blue color the connectivity between-networks from the RSN in the first column to the RSN in the first row.

RSN	VIS	SMN	DAN	VAN	LIM	FPN	DMN
VIS							
SMN							
DAN							
VAN							
LIM							
FPN							
DMN							

Graph measures

The significant changes ($p < 0.05$) in global efficiency (GE), transitivity (TS), characteristic path length (CPL), clustering coefficient (CC), local efficiency (LE), and betweenness centrality (BC) between the pre- and post-networks were investigated using Analysis of Covariance, where graph measures served as the dependent values and density as the covariate (Table 3). Regarding the global measures, we report increases in GE, and TS, and a decrease in CPL. From the comparison of local measures, CC and LE show significant differences in all nodes, with increased values in 739 out of 863 nodes (Figure 2). From these, 374 nodes belong to the left hemisphere (91 in the frontal lobe (FL), 55 in the central midline (CM), 64 in the parietal lobe (PL), 69 in the temporal lobe (TL), 40 in the limbic cortex (LC), 55 in the occipital lobe (OL)) and 365 to the right hemisphere (99 in FL, 50 in CM, 63 in PL, 57 in TL, 36 in LC, 58 in OL, 1 in the uvula, 1 in the insula) (Figure 2). From the remaining 124 nodes (significant decrease in value), 49 nodes are localized in the left hemisphere (37 in FL, 2 in CM, 4 in TL, 2 in LC, 2 in OL) and 75 in the right hemisphere (27 in FL, 15 in CM, 2 in PL, 19 in TL, 11 in LC, 1 in OL). BC increased in one node of the left fusiform gyrus and decreased in three nodes of the left superior frontal and one of the inferior frontal gyrus

Table 3. Significant post-pre changes in global (Global efficiency, Transitivity, Characteristic Path Length) and local (Local Clustering Coefficient, Local Efficiency, Node Betweenness Centrality) graph measures. Results were considered significant for $p < 0.05$. *For the local graph measures the p-value reported is the mean value of the nodes showing a significant increase and a significant decrease, respectively.

Graph Measure	p-value	Direction of Effect
Global Efficiency	0.00	Increase
Transitivity	0.00	Increase
Characteristic Path Length	0.00	Decrease
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

[place Figure 2 here]

Discussion

We present novel evidence, indicating that a ten-week intervention of combined physical and cognitive training, in adults with DS, can trigger neuroplasticity causing a cortical reorganization, quantified via EEG indices and graph measures. Our results reveal that: i) short-term training can modify the physical and cognitive performance of adults with DS, ii) the DS brain can adapt to novel stimulation and challenges by utilizing neuroplasticity and reorganizing itself, and iii) adaptive neuroplasticity may lead to a more complex, flexible and healthier functional network.

Short-term training modifies physical and cognitive performance

The physical training (aerobic, flexibility, strength, and balance exercises) followed here can significantly improve physical capacity for upper body strength and endurance (Arm Curl Test), mobility, static and dynamic balance (Time Up and Go). Our findings for PT are in line with previously reported findings[33,41] on adult PwDS. However, our protocol targets multiple physical domains, while most studies investigating the effect of PT on the somatic capacity of adult PwDS[33,41] have mainly focused on a specific domain, i.e. resistance training or aerobic training and, less often, on a combination of two or more domains. The changes reported here can result in improved health status and decreased risks for several DS related complications, such as cardiovascular disease[42].

The cognitive module of the intervention targets memory, attention, cognitive processing speed, and orientation, as well as social skills. Our participants with DS exhibited an improved general cognitive capacity, i.e., in the level of general intelligence (Raven AB and Raven Total), planning and organization skills (WISC-III, Mazes), and in short-term memory, attention, and concentration (WISC-III, Digit Span, Digit Forward). Previous cognitive interventions on adult PwDS have mainly focused on memory[34] and, less often, on executive functions (i.e., planning, attention, working memory, problem-solving, and processing speed)[35]. In line with the findings of McGlinchey and colleagues [35], we also report that adult PwDS can complete a computerized CT program that targets multiple domains of cognition, and we similarly report significant improvements.

Cortical reorganization in the DS brain and neuroplastic capacity

Our findings on the changes (i.e., increased connectivity) of the eyes-open resting-state network indicate that PwDS present capacity for neuroplasticity. Regarding the type of neuroplasticity that is expressed here, we can rule out the triggering of evolutionary and reactive plasticity, since the first is most active during the development of the brain[1], and the latter is most commonly triggered by a single stimulus[1] (our training utilizes repetitive stimuli). Adaptational and reparation plasticity can both cause cortical reorganization through long-term stimulation.

In the present study, combined physical and cognitive training has increased the engagement of the left hemispheric nodes in information processing and has also increased communication between the nodes of the two hemispheres (Figure 1A&C). The DS cortical networks are characterized by a simplified organization[12] with hyper-synchrony between brain regions[12,13,36]. Therefore, the reported shifts in connectivity indicate the emergence of a more complex network organization which can be the result of either adaptational or reparation plasticity. Our finding regarding the increased left intra-hemispheric information flow can be regarded as the result of adaptational neuroplasticity being triggered in the DS brain given the atypical right-hemispheric functional preference[43]. However, the left-to-right hemisphere directionality is a pattern that has been previously evidenced in young PwDS[8], and its enhancement in adult PwDS may indicate that the training has triggered reparation neuroplasticity.

The increased connectivity within (visual, DAN, DMN) and between (FPN and DMN, DAN and SMN, DMN and DAN, DMN and SMN) the core RSNs[40], further highlights the DS network complexity due to training. Given the evidence pointing towards an inverse relationship between the level of cognitive impairment of PwDS and the within DAN[36,37] connectivity (i.e. the lower the DAN connectivity, the higher the cognitive impairment for DS), we suggest that the increased connectivity within DAN may associate with the increased cognitive capacity due to training in our DS sample. This would rule out the possibility that the type of plasticity represents reparation. In essence, future studies could potentially confirm or rule out this possibility.

The cortical reorganization in conjunction with the increases in general intelligence indicates that the DS brain has entered a more flexible state. The DS network has been previously reported to lack the flexibility that characterizes the development of the TD brain[13] and subsequently higher intelligence[44,45]. Flexibility can reflect the brain's capacity to adapt to novel stimulations[44]. It is plausible that the transition to a healthier organization emerges from the increased DS network flexibility and further points to the triggering of adaptational neuroplasticity.

Graph theory characteristics support the neuroplastic transition towards a healthier DS network organization

We suggest that the network's reorganization, due to training and triggering of neuroplasticity, indexes a transitional state from a random-like network towards a healthier functional architecture (please see Figure 3 for an illustration of our theoretical proposal). Considering the DS brain's simplified architecture, and the lack of functional specialization with impaired integration and segregation[12], the significant changes (i.e., increase) in GE and TS hint that an adult DS functional network, similar to the one here before training, retains features of a random network[38]. This is not surprising given that basic cognitive abilities (low level of general intelligence, which also characterizes PwDS) are exemplified by random networks, while broad cognitive abilities (high level of general intelligence) would require a small-world (SW) network[44,45]. SW networks maintain an optimal balance of integration and segregation (combining characteristics of random and regular networks), to support not only general but also specific abilities[44,45]. In contrast, the absence of SW properties in the DS brain, namely reduced GE, TS, and therefore robustness, indicates the increased risk of communication loss between connected regions and their causality for cognitive decline[38,46]. The simultaneous increment in global efficiency and transitivity denotes the increase of both integration and segregation and the transition to a less-random, healthier network organization. Potentially, the training could further induce cortical reorganization, resulting in a more modular network capable of facilitating higher cognitive abilities and engaging broad and perhaps specific abilities.

The decrease of CPL in TD individuals could be interpreted as a contradicting shift towards a more random network that disturbs the SW balance. In our case, the reduction of CPL further supports our hypothesis of a transitional stage, since the adult DS network deviates from its random-like characteristics by displaying a greater CPL. Previous fMRI studies report that the brain network of PwDS presents increased local connectivity[12,13] indicating increased CPL. Hence, the decrease of CPL indeed indicates the transition to a less-random network and could point to the deceleration or even reversal of degenerative processes in the DS brain.

If the optimal function of the brain is to be attributed to SW network architecture, it is plausible that the adaptational neuroplastic transition has characteristics that would eventually lead to such a network. Here, these characteristics are evident not only on a global level (i.e., changes in CPL and GE) but also from a local network perspective. The increases in CC and LE support our hypothesis, as they indicate an increase in segregation and robustness, less randomness in functional clustering organization, and additionally, an increase of fault tolerance in the network[47]. The post-training network robustness and segregation can potentially serve in neuroprotection for PwDS[38]. Still, the effectiveness of such a mechanism would rely on the trade-off between integration and segregation, mainly maintaining SW network characteristics[48]. These shifts signal the decrease in wiring cost and subsequent increase in cost-efficacy[47,49] (GE-wiring cost), rendering the network more efficient as a whole. Finally, the transition towards SW network characteristics is backed up by the changes in BC (increase and decrease), which point to a more hierarchical and less random organization. Previously, it was reported that the DS brain is characterized by decreased centrality[10].

[place Figure 3 here]

Future directions and limitations

A limitation of the study was the small sample size of the experimental group. Our results could potentially be interpreted differently in reference to a control group (TD or passive DS). Though this study is the first to provide insights into the benefits of combined PT and CT in adults with DS through the use of neuropsychological and neurophysiological assessments, a gap remains in our basic understanding of the sole contributions of each training to DS related cognition and brain function. Further studies are necessary to index the influence of each component on the triggering of neuroplasticity and subsequently network organization and cognition. Similarly, the stability of the reported neuroplastic shifts can only be addressed through additional and more in-depth investigations, including follow-up and further longitudinal studies, to fill the gaps in the existing literature.

Conclusion

The rise of DS prevalence sets a significant challenge in developing innovative health care interventions that provide the best quality of care. Neurocognitive treatments can augment the functional and cognitive abilities of PwDS and thus allow for a more independent, productive, and fulfilling daily life. Our results are based on brain imaging, connectivity, and graph-theory analysis, and emphasize the significance of introducing stimulation and adaptable challenges in the environment of PwDS. We provide evidence that triggering adaptational neuroplasticity in the DS brain provokes the emergence of a less-random, more complex, hierarchical network, accompanied by an increased ability to integrate and segregate information and improved efficiency, robustness and flexibility. Designing focalized neurobehavioral interventions can aid in the development of a balanced and stable functional DS phenotype, in terms of SW characteristics.

Materials and Methods

Subjects

The study's pool consisted of 12 subjects with DS (age: 29±11, 6 females). Participants were recruited from a variety of local organizations (please see Acknowledgements). The training procedure took place in the Thessaloniki Active and Healthy Ageing Living Lab (Thess-AHALL)[50], and the premises of the Greek Association of Down Syndrome. This study is part of the dsLLM clinical trial, registered with ClinicalTrials.gov, with identifier code NCT04390321. The study protocol was approved by the Bioethics Committee of the Medical School of the Aristotle University of Thessaloniki and was conducted per the Helsinki Declaration of Human Rights. The participants' legal guardians signed a written informed consent prior to their inclusion in the study.

[place Figure 4 here]

LLM Care Intervention

The intervention protocol consisted of physical and cognitive training (LLM Care[51], http://www.llmcare.gr/en). The study protocol was further developed to improve the quality of life and aid in the development of independent living skills in PwDS [52,53]. LLM Care also aims to further improve brain functionality[51]. All training sessions were computerized, center-based, and conducted under supervision. The sequence of training methods was pseudo-randomized and counterbalanced. The details of each training intervention have been previously described in detail[54,55] and are summarized in Figure 4.

Cognitive training

The cognitive training (CT) component of LLM Care uses the BrainHQ software (Posit Science Corporation, San Francisco, CA, USA), an online interactive environment in Greek language[56]. It consists of six categories (29 exercises in total), with customizable difficulty levels, utilizing audiovisual stimuli. CT targets memory, attention, cognitive-processing speed, navigation, and people skills. This regime was selected since patients with DS exhibit deficiencies in these processes. CT was conducted for half an hour, with a frequency of 2 days per week for 10 weeks. In every CT session, the participants were required to complete at least one task from each category. During the training, participants were urged to complete as many exercises as they could from each category.

Physical training

The physical training (PT) component of LLM Care is based on the WebFitForAll protocol[55,57], adequately adjusted to the needs and capacity of PwDS. It utilizes motion sensor devices (i.e., Kinect). Games and physical exercise are combined, providing a pleasant experience throughout the training. PT sessions lasted for half an hour and were conducted with the same frequency as CT. The training consists of aerobic (cycling, in-place-hiking), flexibility (stretching), strength (resistance, weightlifting), and balance (static, dynamic) exercises. The warm-up and cool-down routines (5-minutes duration), signify the start and completion of every session, respectively. During aerobic exercises, the participants entered a virtual environment, set up in Google maps, and explored cities and landscapes. Upon correct completion of the flexibility and strength exercises, the trainees were progressively rewarded with an array of pleasing images. The scope of balance exercises was to move their bodies either horizontally or vertically, which was achieved through games.

Psychometric and somatometric assessments

The participants' cognitive and physical capacity was assessed before and after the intervention. The psychometric evaluation consisted of a set of neurocognitive tests that measure memory attention, concentration (WISC-III[58]: Digits Span), verbal and non-verbal mental capabilities (Raven[59]), processing speed (WISC-III: Digits Span and Picture Arrangement), problem-solving, visuospatial processing, organization skills (WISC-III: Block Design, Picture Arrangement, Mazes), social intelligence (WISC-III: Picture Arrangement), and identification of emotions (Reading the mind in the eyes[60,61], and a variation: Reading the mind in the face (emotion recognition from video)).

The somatometric evaluation included the Short Physical Performance Battery (SPPB)[62], 10 Meter Walk[63], Back Scratch[64], Sit and Reach[65], Arm Curl[64], Four Square Step (FSST)[66], Stork Balance (for both legs)[67], Timed Up and Go[68] tests, and Body Mass Index (BMI). These tests appraise functioning mobility, flexibility (in specific areas), dynamic stability, strength, and static and dynamic balance.

EEG recording

Pre- and post-intervention resting-state EEG activity was recorded for 5 minutes, using a high-density Nihon-Kohden EEG device (128 active scalp electrode) at a sampling rate of 1000Hz. The EEG recordings were performed in an electrically shielded, sound, and light attenuated booth. The electrode impedances were lower than 10 kΩ. The participants were instructed to remain in a resting position while keeping their eyes open. Eyes-closed EEGs were not measured due to the limited capacity of the participants with DS to stay relaxed with their eyes closed.

[place Figure 5 here]

EEG data analysis

Pre-processing

The raw EEG data were visually inspected and bad channels were interpolated while any eye-movement related artifacts (blinks and horizontal movement) were corrected, through adaptive artifact correction[70] using the Brain Electrical Source Analysis software (BESA research, version 6, Megis Software, Heidelberg, Germany) (Figure 5, blue section). The artifact-corrected data from each measurement were imported in the Fieldtrip Matlab toolbox[71] for additional processing

(Figure 5, blue section). The signals were denoised by filtering the data (0.53 Hz high-pass IIR filter, 48-52 Hz notch IIR filter, 97 Hz low-pass IIR filter). The filtered signals were analyzed into independent components[72], and the artifactual components were rejected. After signal reconstruction, the data was, once again, visually inspected for any remaining artifacts. Randomly selected fifteen segments (4 seconds each) from each artifact-free EEF recording for additional processing.

Source reconstruction

Each subject's segments (15 segments, 4000 samples, 4 seconds duration each) were imported into BESA (Figure 5, orange section). The current density reconstructions (CDR) were estimated for each sample point, solving the inverse problem using LORETA in the 0.5-35 Hz frequency range. LORETA[39] was utilized, as it does not require the a priori declaration of the number of sources and is suitable for whole cortex analysis. The CDRs were exported as four-dimensional images (4-D) in the Analyze format (keeping all sampling points), which were, in turn, imported into Matlab. A cortex mask was superimposed on the images. The mask includes only grey matter, and excludes the subcortex, the brainstem, and cerebellum, to limit the source space[69]. The source space consisted of 863 voxels.

Functional connectivity

After extracting the time-series of each voxel from the 4-D images, they were used to compute the Phase Transfer Entropy (PTE)[73] (Figure 5, green section). The computation resulted in 863×863 adjacency matrices. The metric is calculated independently for every pair of voxels in each segment. Each voxel represented a node of the brain network, with the node's coordinates corresponding to the center of each voxel. PTE is the estimation of TE between phase time-series, i.e., it evaluates the influence of one signal's phase on another signal's phase[73]. PTE was selected because its results are not based on a specific data model since its computation is reliant on non-linear probability distributions. Therefore, it allows the detection of higher-order relations in the phase information flow and renders the measure resistant to source leakage [73]. The algorithm applies the Hilbert transform to estimate the phases of each signal. It determines the number of bins by utilizing the Scott methodology[74], resulting in 37 bins on average (13 samples per bin). The adjacency matrices of each subject were averaged, resulting in two sets of 12 adjacency matrices (Figure 5, green section).

Graph measures computation

Using the Brain Connectivity Toolbox[75], the graph measures of global efficiency (GE), transitivity (TS, a variation of global clustering coefficient), characteristic path length (CPL), clustering coefficient (CC), local efficiency (LE) and node betweenness centrality (BC) were computed for each participant. In a later step, the centrality Matlab function, which measures node importance, was used to compute the node degree centrality (DC) of the network mapping the pre- and post-intervention differences. The density of each graph was estimated by summarizing the weights of each graph.

These measures were chosen to examine the influence of our intervention on the characteristics of the DS brain and its functional organization. To that aim, GE and CPL were utilized to index the effects on integration[75], TS, CC, and LE to measure the effects on segregation[75] and BC and DC to check for hierarchical changes in the network. GE is the average inverse shortest path length of the network[76] and quantifies the efficacy of information transference and its assimilation in the network[75]. CPL is the average shortest path length of the edges connecting the nodes of the network[77] and characterizes its robustness[75]. TS is the ratio of closed triplets to the maximum number of triplets (open (three nodes with up to two connections between them) and closed (three nodes with three connections between them))[78], while CC of a node is the ratio of its connected neighbors to the maximum number of possible connections[77]. These two measures reflect the clustering organization of the DS brain network on a global and local level, respectively, and also quantify its robustness[75]. LE of a node is the computation of GE on a local level, strongly related to CC[76,79], and measures the efficiency of information transference to the node's neighbors. BC corresponds to the fraction of shortest paths that pass through a node[80] and is a measure of centrality, measuring the importance of a node in the information flow between nodes[75]. So, the higher the value of BC is, the more vital that node is for the transfer of information in the network. Lastly, DC calculates the number of links connected to a specific node, i.e., it measures if a node exhibits higher or lower connectivity to other nodes.

Statistical analysis

The pre- and post-intervention somatometric and psychometric assessment scores, were compared with the use of non-parametric Wilcoxon tests and paired t-tests. The statistical comparisons were performed using the IBM SPSS 25.0 software. Wilcoxon tests were performed on the psychometric battery tests and somatometric tests with discrete-values score, while paired t-tests were applied on the remaining somatometric assessment tests.

The Network Based Statistics (NBS)[81] MATLAB toolbox was employed to estimate the statistically significant differences between the whole-head network of the pre- and post-intervention connectivity networks of our subjects. A paired samples t-test corrected for 10000 random comparisons via False Discovery Rate (FDR) correction[82] was performed. This methodology evaluates the significance of each edge independently, providing an independent p-value for each connection. Significant differences between the two time-points were visualized as weighted graphs through the BrainNet Viewer[83] toolbox (Figure 1). DC was estimated from the outcome of this comparison, and the results were depicted in the same graph.

For the graph measures, Analysis of Covariance (ANCOVA) was used, where each measure serves as the dependent variable and density as the covariate, because density seriously affects the values of the other measures. For local measures (i.e., CC, LE, and BC), ANCOVA and FDR correction were performed for each of the 863 nodes and p-values for Type I errors, respectively. Results below the 5 percent threshold were considered significant.

Acknowledgments

This study is an extension of the European CIP-ICTPSP. 2008.1.4 Long Lasting Memories (LLM) project (Project no. 238904) (http://www.longlastingmemories.eu/). The authors would like to thank all the participants, their families, and caregivers, the Down Syndrome Association of Greece, Spring Children Mixed Living Center – Daily Care Activity & Training for Disabled People, The Down Syndrome Association of Serres, Center for the Rehabilitation of Social Support & Creative Employment of People with Disabilities, Child Care Center in Ptolemaida, Centre For Persons With Learning Difficulties - Zoodohos Pigi, Center of Creative Activities - Municipality of Neapoli and Sykies, Special laboratory for vocational education and training in Alexandria, Center for the Rehabilitation of Social Support & Creative Employment of People with disabilities "O Sotir", Center of Creative Activities for People with Disabilities-Municipality of Neapoli and Sykes. Also, the authors would like to thank a list of people assisting in the trial facilitation, as follows: Dimitris Bamidis, Foteini Dolianiti, Ioanna Dratsiou, Sotiria Gilou, Maria Karagianni, Katerina Katsouli, Eleni Manthou, Maria Metaxa, Annita Varella (in alphabetical order), as well as, students from the Dept of Psychology, The University of Sheffield International Faculty, City College (Greece) who were practicing under the supervision of Dr. Anna Vivas and Dr. Elissavet Chrysochoou who were also advising the authoring team on the psychological aspects of the whole endeavor. Last but not least, the authors would like to thank Emeritus Professor of Pediatrics and Genetics Dr. Charikleia Chatzisevastou-Loukidou for advising the team on all medical aspects of the project and for linking the team with many of the pilot sites.

Conflict of Interest

There are potential conflicts of interest (other, not financial, outside the scope of the submitted work) for the author P. Bamidis in respect of PositScience and the Aristotle University of Thessaloniki. There is a co-marketing agreement between PositScience and the Aristotle University of Thessaloniki to exploit Brain HQ within the LLM Care self-funded initiative that emerged as the non-for-profit business exploitation of the Long Lasting Memories (LLM Project) (www.longlastingmemories.eu) originally funded by the ICT-CIP-PSP Program of the European Commission. Brain HQ now forms part of LLM Care, a technology transfer/self-funded initiative that emerged as the non-for-profit business exploitation of LLM. Additionally, FitForAll (FFA) has been developed in the Aristotle University of Thessaloniki during the Long Lasting Memories (LLM Project) (www.longlastingmemories.eu) originally funded by the ICT-CIP-PSP Program of the European Commission. It now forms part of LLM Care, a technology transfer/self-funded initiative that emerged as the non-for-profit business exploitation of LLM.

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Figure Captions

Figure 1. Cortical connectivity between post- and pre-intervention networks and for each time point. The color scales represent t-values. **A: Post vs. Pre.** Significant post-pre connectivity differences. Information direction is depicted though line arrows. The visualized networks are significant at a level of $p < 0.05$, FDR corrected. The difference in nodal size depicts the increase in the node degree centrality; the nodes with the most increased connectivity are located at the left

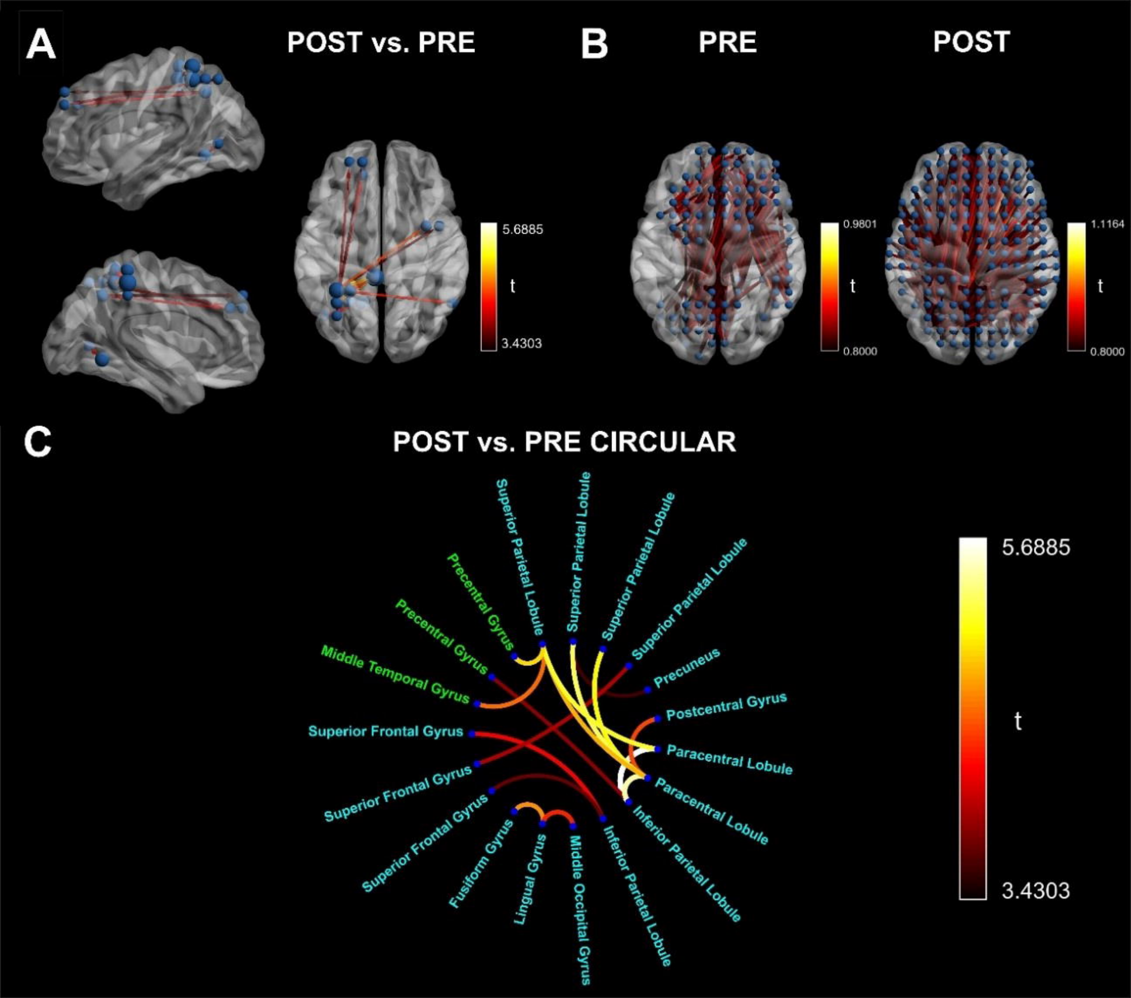
parietal lobe. **B:** Depiction of pre- and post-intervention networks in comparison to the null hypothesis. The t-values indicate that the post resting-state network has significantly stronger connections than the pre network. **C:** Circular graph depicting the cortical reorganization in the DS brain (cyan: left hemisphere, green: right hemisphere). The cortical reorganization is characterized by the strengthening of direct 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 2. Graph depicting nodes with a significant increase in the clustering coefficient (CC). Node size quantifies the increase of the CC value, while the color map indicates the significance of the shift (p-value).

Figure 3. Illustration of our theoretical proposal. Changes in connectivity and graph-theory characteristics, as an outcome of the adaptational neuroplasticity in the DS brain, characterize the post-intervention DS network (**B** → **C**) as a transitional state from the random-like organization of the pre-intervention DS network[38] (**B**) towards a healthier functional structure. Random networks (**A**) are characterized by high global efficiency (over-integration) and low clustering (under-segregation) and exemplify the general abilities of general intelligence (low intelligence level). Small-world networks (**C**) feature the functional organization of TD brain networks, and incorporate characteristics of both random and regular networks, achieving an optimal balance between global and local characteristics. SW networks (**C**), are at large associated with the broad abilities component of general intelligence (higher intelligence level). The pre-intervention DS network (**B**) showed a random-like, simplified architecture, with impaired segregation and integration, as evidenced by the low CC and LE (random network characteristics) and decreased GE and increased CPL, respectively. This is in line with previous literature[12,36–38]. The DS pre-network's (**B**) integration-related characteristics (lower GE, higher CPL), are not common in random networks. Hence, the DS pre-network (**B**) is classified as random-like, and not entirely random, maintaining an equal distance from random (**A**) and SW (**C**) networks. The DS post-network (**B**   **C**), exhibits an increase in integration (random network characteristic), as well as segregation (regular network characteristic), so it is interpreted as a step towards an SW-like architecture that highlights a healthier brain organization.

Figure 4. DS-LLM Care design and flow of participants with DS.

Figure 5. EEG data analysis schematic. **Pre-processing** (blue): EEG data were interpolated and artifact corrected, visually inspected, high-pass, bandpass, and low-pass filtered. Independent component analysis (ICA), as well as visual inspection, were used to reject artifactual data. Following, 15 segments of 4-s were randomly selected. **Source reconstruction** (orange): The data were processed within 0.53-35 Hz frequency range, source reconstructed (4-D LORETA, for all time points), and a previously used 863-node atlas[69] was applied to extract the time-series of every voxel from every segment per subject. **Functional connectivity** (green): Functional connectivity was computed for every segment of every subject, using the phase transfer entropy metric (PTE), and the 15 matrices of every subject were averaged into one. A network science approach was taken for the computation of graph measures per subject. Group average statistics were calculated to identify the statistically significant differences of post- and pre-intervention networks.



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POST vs. PRE CIRCULAR
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Superior Parietal Lobule
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Precuneus
Postcentral Gyrus
Superior Frontal Gyrus
Paracentral Lobule
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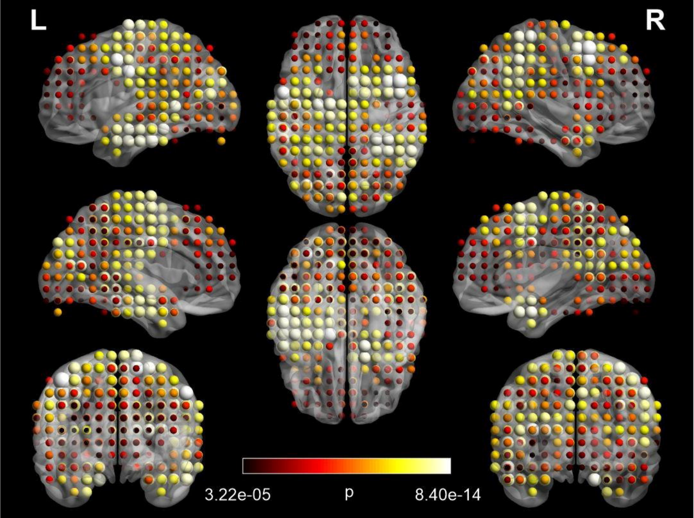

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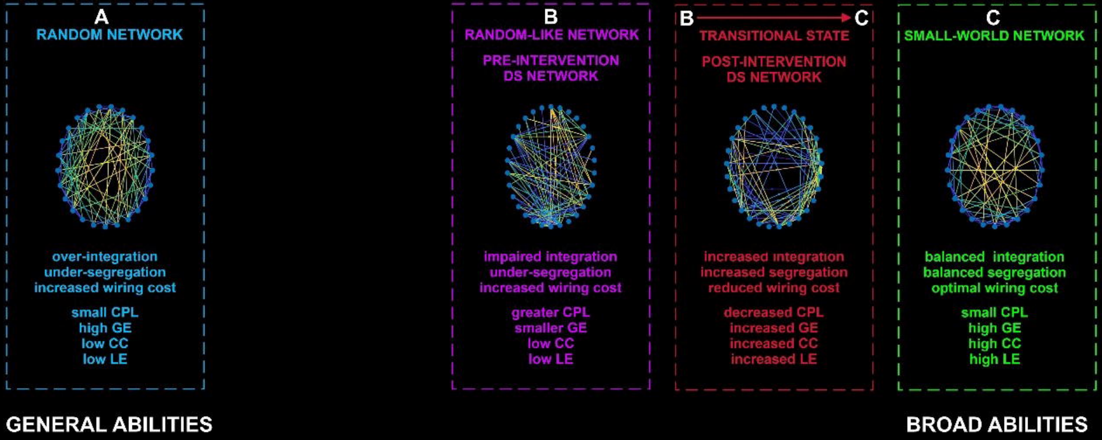
A
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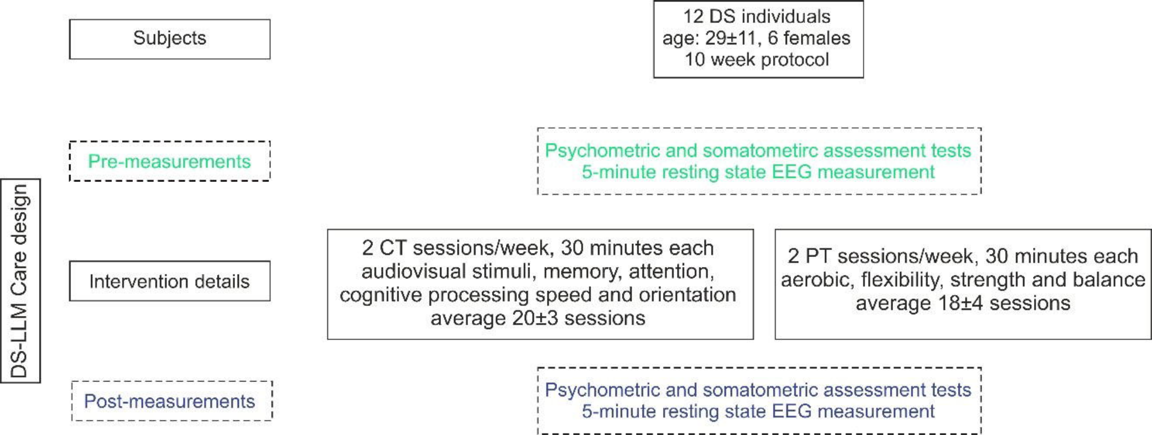
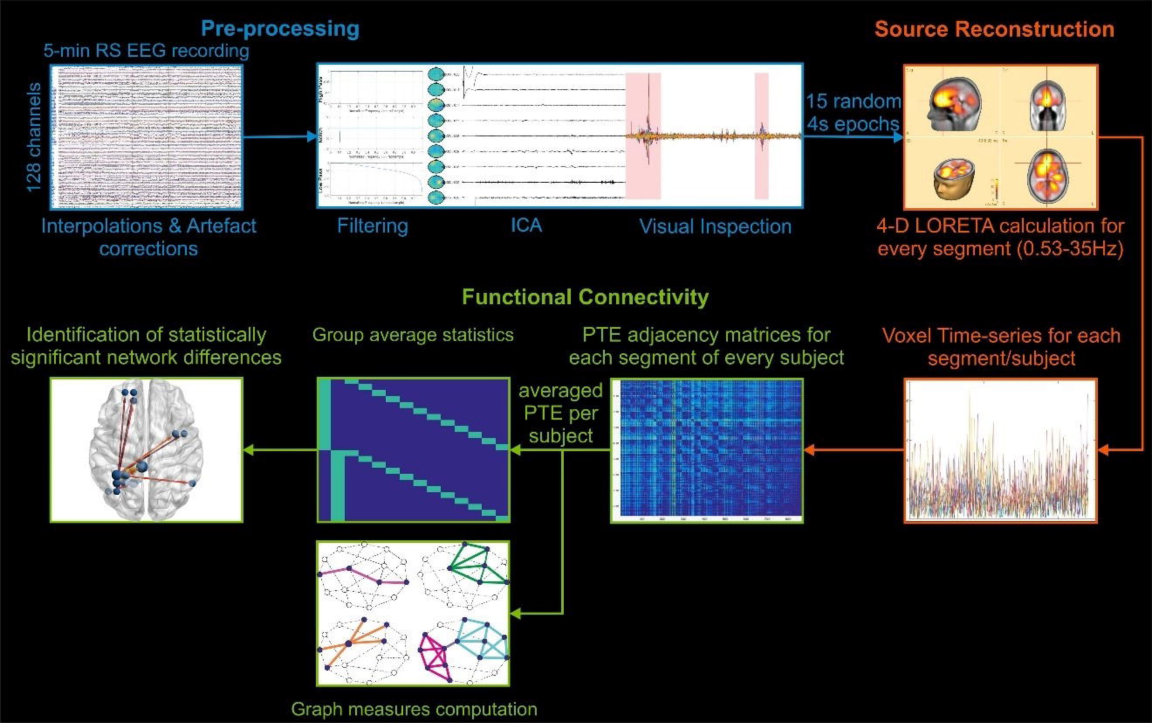
under-segregation
increased wiring cost
small CPL
high GE
low CC
low LE
B
RANDOM-LIKE NETWORK
PRE-INTERVENTION
DS NETWORK
impaired integration
under-segregation
increased wiring cost
greater CPL
smaller GE
low CC
low LE
B
C
TRANSITIONAL STATE
POST-INTERVENTION
DS NETWORK
increased integration
increased segregation
reduced wiring cost
decreased CPL
increased GE
increased CC
increased LE
C
SMALL-WORLD NETWORK
balanced integration
balanced segregation
optimal wiring cost
small CPL
high GE
high CC
high LE
GENERAL ABILITIES
BROAD ABILITIES


DS-LLM Care design
Subjects
12 DS individuals
age: 29±11, 6 females
10 week protocol
Pre-measurements
Psychometric and somatometirc assessment tests
5-minute resting state EEG measurement
Intervention details
2 CT sessions/week, 30 minutes each
audiovisual stimuli, memory, attention,
cognitive processing speed and orientation
average 20±3 sessions
2 PT sessions/week, 30 minutes each
aerobic, flexibility, strength and balance
average 18±4 sessions
Post-measurements
Psychometric and somatometric assessment tests
5-minute resting state EEG measurement


Pre-processing
5-min RS EEG recording
128 channels
Interpolations & Artefact corrections
Filtering
ICA
Visual Inspection
15 random 4s epochs
Source Reconstruction
4-D LORETA calculation for every segment (0.53-35Hz)
Functional Connectivity
Identification of statistically significant network differences
Group average statistics
PTE adjacency matrices for each segment of every subject
Voxel Time-series for each segment/subject
averaged PTE per subject
Graph measures computation