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NON-VERBAL LEARNING DISABILITY: NEUROBIOLOGICAL AND COGNITIVE CORRELATES

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Abstract

Non-Verbal Learning Disability (NVLD) is a neurodevelopmental disorder characterized by a deficit in processing visuospatial information but with age-level or higher verbal skills. The condition is not recognized by the main diagnostic systems and consequently it does not have appropriate intervention tools enabling children to have a higher quality of life. In fact, the main deficits in NVLD can lead to important consequences in other domains such as academic performance and social functioning. Considering these promises, the present project represents a combined effort by clinicians and researchers to establish the prevalence of NVLD, to characterize its core deficits, to study its brain correlates, and to provide important information for the differential diagnosis.

Contents

Introduction

Non-Verbal Learning disability is a neurodevelopmental disorder characterized by visuospatial deficits but spared verbal abilities. Since its first conceptualization [1], few attempts have been made to characterize NVLD as a discrete disorder but at the present not enough information has been gathered from clinicians to the attention of the main diagnostic systems in order to create a formal protocol for the diagnosis. In order for the NVLD to be recognized as an independent disorder, there are two important steps to be considered.

The first step involves the needs to identify the core deficits in visuospatial processing that better describes NVLD, to estimate the prevalence of the disorder, and to investigate the neural underpinning of the deficit that differentiate the clinical group from typically developing children. In fact, while other developmental disorders are described in terms of specific symptoms, NVLD is instead characterized by a deficit in a cognitive function that is the processing of nonverbal information. This definition poses some difficulties in defining the core deficits and their consequential effects on other domains. In order to explain the core symptoms of NVLD, Rourke [2] proposed the so-called White Matter (WM) model entailing that the core symptoms of NVLD are a direct consequence of damaged, dysfunctional or underdeveloped cerebral WM connections. However, none of the studies investigating NVLD have properly tested this hypothesis, and therefore there is a lack of knowledge about these biological mechanisms and its link to the cognitive profile of NVLD. In addition to the lack of large-scale studies testing the model of Rourke, very few studies have explored neuropsychological measures in relation to structural or functional data.

The second step concerns the need to differentiate the NVLD population from clinical groups that have overlapping symptomatology (i.e., Autism Spectrum Disorder without

Intellectual Disabilities -ASD without ID). The core features of NVLD are characterized by a difficulty in visuospatial processing, with a discrepancy between verbal IQ, being well-preserved, and a below range performance IQ, along with impairments in visual motor integration and accompanying fine motor skills associated to learning difficulties in the areas of mathematics, geometry and drawing [3]. In addition to visuospatial deficits, studies have also reported difficulties in social interaction abilities for individuals with NVLD. The symptomatic proximity between ASD and NVLD poses the greatest diagnostic challenge in case of ASD children without intellectual disability, a condition previously known as Asperger's syndrome. In fact, individuals with NVLD and ASD without ID manifest deficits in social competence and interpersonal awkwardness [4–7], as well as impairments in visuospatial processing [8]. Despite the similarities in the neuropsychological profiles of the two disorders, it is important to point out that social impairments are typically more severe in children with ASD without ID than in NVLD children, while visuospatial deficits are more pronounced among children with NVLD relative to children with ASD without ID [9].

In the present project, we addressed the first step of the process, related to recognizing NVLD as a discrete disorder, by performing two studies. Study one compared three alternative sets of criteria to estimate the prevalence of NVLD in a large, representative sample of US children and characterized the structural cerebral WM correlates that distinguished the NVLD groups from neurotypical children. This part will be reported in chapter one. Study two investigated the link between functional brain connectivity in specialized networks and the visuospatial performance in NVLD compared to typically developing children. This part will be reported in chapter two.

The second step of the process is still ongoing and it will be implemented by analyzing the brain connectivity patterns and their relationship with performance in visuospatial and social domains in three groups: children with NVLD, ASD without ID and typical development. Preliminary results are presented in chapter three.

The present Ph.D. dissertation will have the following structure: The first chapter will introduce the reader to the cognitive profile of NVLD, its estimated prevalence and the brain structural correlates. This chapter is currently being prepared for the submission coauthored

with Banich, M., Mammarella, I., Liotti, M.. The second chapter will describe the neurocognitive marker of the clinical population of interest with an electroencephalographic approach. This chapter, in full, is a reprint of the manuscript currently under review in Brain Sciences coauthored with Di Bono M.G., Maffei, A., Orefice, C., Lievore, R., Mammarella, I., Liotti, M.. The third, and last chapter, will briefly describe our ongoing analysis on the research exploring the different characteristics of NVLD and ASD without ID, and future directions of this project. In all three projects the dissertation author is the primary researcher and the author of the material.

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Chapter 1 Estimating the prevalence of NVLD in the ABCD sample

Nonverbal learning disability (NVLD) is a neurodevelopmental disorder characterized by deficits in visuospatial processing but spared verbal competencies. Only few attempts have been made to estimate the prevalence of this disability including a limited sample size, compared to that of the Adolescent Brain Cognitive Development (ABCD) Study, and populations with other disorders. Therefore, the first objective of the present investigation was to estimate the prevalence of the NVLD profile among 11 876 children and studying the relationship between the cognitive criteria. The second objective was to explore the structural brain correlates to test the neurological model of NVLD indicating that the core deficit is consequent to a dysfunctional white matter, especially on the right hemisphere.

1.1 Introduction

Non-Verbal Learning disability (NVLD) was first described in a work by Johnson and Myklebust in 1967 [1]: they described the clinical symptomatology, which is characterized by difficulties in processing information in the non-verbal domain accompanied by spared verbal abilities. In more recent years, several researchers have studied more in-depth children with visuospatial processing deficits and examined the possible associations with problems in attention, motor, academic and social skills, in the absence of associated frank neurological symptoms or genetic disorders [2–4]. There is evidence showing that the difficulties in visuospatial processing interfere with a child's quality of social, school or life functioning [5]. In fact, although the main problem found is in visuospatial processing, symptoms can often impact the social domain, especially when pertaining to non-verbal processing, such as facial processing, that influences social abilities [6]. In particular, children with NVLD show more severe problems in the visuospatial domain compared to either children with Autism Spectrum Disorder (ASD) or ADHD [2,7]. These include difficulties with visuospatial working memory

[8–10], spatial organizational skills and comprehension of spatial descriptions [11,12], and nonverbal problem-solving abilities [13], all within the context of preserved language abilities.

At the clinical level, despite increased awareness of the characteristics of NVLD derived by research findings, there are currently no "official" diagnostic criteria for NVLD [14,15]. From a review of the literature, Fisher et al. in 2022 [4] highlighted that the most common criteria used in the past to define NVLD was a discrepancy between verbal and visuospatial intelligence (generally 10 to 15 points between verbal and performance IQ) [14]. However, this criterion has been criticized by some researchers [16,17] because it is not rare to find such a discrepancy in neurotypical children [18,19]. Given that NVLD is defined by an impairment in a cognitive process, more specifically in the realm of visuospatial processing, general heterogeneous consequences pertaining to academic achievement and social interactions would be expected. Hence, it could be appropriate not to use them as a diagnostic criterion. Obviously, which criteria are used (a discrepancy score, or just the level of Non-verbal difficulties) will influence estimation of the actual prevalence of NVLD [20]. These considerations have inspired the current investigation with the goal of exploring the prevalence rates of NVLD depending on different criteria for defining NVLD.

At the neurological level, the cognitive profile observed in NVLD has been explained as resulting from a 'white matter' syndrome (term coined by Rourke in 1989) [21], indicating that there are damaged or dysfunctional long myelinated white matter fibers in the brain [22]. Within the context of NVLD it is assumed that these abnormalities are mainly located in the right hemisphere. This strong hemispheric association could have been influenced by the oversimplified dichotomy between the left hemisphere being involved in language [23–25] and the right hemisphere being associated with visuospatial processing [26–28]. Nonetheless, both animal and human studies point toward the importance of intact white matter for spatial processing [29,30], but there are no studies in the literature specifically linking white matter to the cognitive profile of NVLD, probably due to the fact that, since there are no shared diagnostic criteria, it is very difficult to find sufficient sample sizes that are appropriate for

such studies. In this regard, the Adolescent Brain Cognitive Development (ABCD) study allows researchers to investigate the white matter contribution to performance in the visuospatial domain in children with a NVLD profile, and to examine how white matter might differ from a control group of children of the same age and thus at a similar stage of development. Furthermore, the ABCD dataset offers a major opportunity to test which criteria and cut-offs are most suitable for identifying the characteristics of NVLD. In fact, this sample is composed of 11 876 children that at baseline are 9/10 years old and they will be part of the study until 18 years old. The longitudinal nature of the study allows researchers to follow the developmental trajectory of this population which is an unprecedented opportunity and to further confirm or modify the best criteria for identifying NVLD.

Given the above considerations, the first goal of the present research is to estimate the prevalence of the symptomatology associated with a NVLD profile, and to test different criteria in order to investigate which are most informative in describing the population of interest. In fact, even considering that the first conceptualization of NVLD was made over 50 years ago, only a few attempts exist for the estimation of its prevalence. Moreover, they are generally based on small sample sizes [4], on underrepresented samples in terms of demographic characteristics, and often involved populations with Learning Disorders (LD) more generally [4] (but see [31]). A great advantage of the present investigation, compared with the one of Margolis [31], is that the sample size is larger, it has a representative sample of the US in terms of socioeconomic/ethnic backgrounds and it does not involve children/adolescents with selected problems of specific nature, either psychological, neurological, physical and/or social. For these reasons the present research can yield a more accurate estimation of the prevalence of NVLD.

The second goal is to investigate the neurobiological underpinnings of NVLD by examining whether there are differences in the white matter tracts of each hemisphere between NVLD and non-NVLD groups and to understand the relationship between these measures of white matter and visuospatial performance in children with and without an NVLD profile.

The final objective of the current study is to investigate the full range of potentially comorbid conditions that are associated with NVLD. Using the unusually broad set of data

obtained in the ABCD study, it is possible to examine the associations of the NVLD cognitive profile with questionnaires administered to parents regarding psychological/psychiatric diagnoses, as well as psychological well-being and social functioning. This investigation is of importance because identifying unique behavioural and neurobiological features of NVLD may help in early diagnosis and in the implementation of more effective interventions.

1.2 Methods

Overview: First, the present investigation aimed at estimating the prevalence of the cognitive profile associated with NVLD following three different sets of criteria. Within these three identified groups and in the whole ABCD sample, we studied the correlations between the cognitive criteria, and we tested for differences in these links among the studied populations. Next, we investigated if there were differences in the white matter measures and if the link between the brain and the visuospatial processing presented a different relationship in the populations of interest. Finally, we tested whether there were differences in children with and without the NVLD profile in the level and nature of psychological/psychiatric diagnoses as well as measures of social functioning and psychological well-being.

1.2.1 Sample

The ABCD dataset (release 3.0; https://abcdstudy.org/) includes 11 878 children aged 9–10 years. This is a longitudinal dataset being collected at 21 sites across the US. Full recruitment details are described in [32]. Worth to mention here, participants were drawn from a diverse range of geographic, socioeconomic, ethnic, and health backgrounds representative of the US population [33,34].

Institutional review board approval was obtained for each site before data collection and all parents provided written informed consent in addition to assent from the participants.

1.2.2 Behavioural measures for the estimation of the prevalence

Visuospatial processing skills were measured with three tests: Matrix Reasoning, the Little Man and the 0-back task. The Matrix Reasoning Task is taken from the Wechsler Intelligence Scale for Children, fifth edition (WISC-V) and it was administered with automated technology: Q-interactive [35]. It is a measure of visual processing and abstract, spatial perception. The Little Man Task involves visuospatial processing and specifically mental rotation with varying degrees of difficulty (see [36] for a detailed description). Regarding the n-back task, only the 0-back condition was used in order to measure memory/recognition for visuospatial stimuli: participants indicate if the presented stimulus (either a face or a place) matches a target presented at the beginning of the block (See [37] for a detailed description).

A second cognitive measure employed in the present study was intelligence, more specifically a composite score of fluid, crystallized and total intelligence. Total and crystallized intelligence were used as criteria for identifying the NVLD groups instead of the classical total and verbal IQ. Fluid intelligence was used to investigate the relationship between the two components of intelligence in the studied populations and, since it is part of the composite score creating the total intelligence, together with the crystallized component, it will be briefly described below. The ABCD consortium included in the protocol the NIH Toolbox Cognition Battery which has been validated by Heaton et al. [38] and Akshoomoff et al. [39] as a means to measure intelligence: Crystallized, fluid and total components. The crystallized composite is formed by the scores on Picture Vocabulary and Oral Reading Recognition Tasks [40]. While the first test measures language skills and verbal intelligence, the second one is a reading test of single words. In fact, this type of measures reflects the general knowledge, vocabulary and reasoning based on acquired information, and it is contrasted to the fluid intelligence which is described as abstract reasoning and adaptive problem-solving.

Fluid intelligence was measured using scores from: The Pattern Comparison Processing Speed Test [41–43], the List Sorting Working Memory Test, the Picture Sequence Memory Test [44,45], the Flanker Task (a variant of [46]), and the Dimensional Change Card Sort Task [47]. The Toolbox Pattern Comparison Processing Speed Test is a measure of rapid visual processing. The Toolbox List Sorting Working Memory Test assesses working memory for

sequence stimuli. The Toolbox Picture Sequence Memory Test measures episodic memory. The Toolbox Dimensional Change Card Sort Test measures cognitive flexibility while the Toolbox Flanker Task assesses conflict monitoring abilities. These composite scores were calculated by averaging the normalized scaled scores for the relevant test measures (i.e., two for Crystallized, five for Fluid, and seven for Total Intelligence Composites) and they were extracted from the DEAP (Data Exploration and Analysis) portal offered by the ABCD consortium.

Social problems were assessed through the Child Behavior Checklist (CBCL) also known as Achenbach System of Empirically Based Assessment, which is a questionnaire comprising 113 items that measures different behavioural characteristics of the child in the past 6 months, such as acts too young, too dependent, doesn't get along with peers. We focused only on the social problem scale, measuring social competencies of the child in various contexts. Then, a questionnaire about the presence of psychological/psychiatric diagnoses administered to parents was used to exclude a diagnosis of autism spectrum disorder.

We were also interested in exploring the presence of psychological, social problems and comorbidities extracted from the self-report questionnaire administered to the parents in order to further substantiate that the estimated NVLD population presented additional problems beyond visuo-spatial difficulties compared to the general ABCD sample.

1.2.3 Magnetic Resonance Imaging

The structural MRI measures used in the present study were the following: one anatomical MRI metric, that is white matter (WM) volume, and two diffusion MRI metrics, Fractional Anisotropy (FA) and Mean Diffusivity (MD). WM volume represents the volume in $mm^3$of the right/left hemisphere separately. FA measures the directionality of water diffusion within brain tissues that is found to be greater in organized WM tracts: in fact, a decrease in FA is found in the cognitive decline of elderly [48]. MD refers to the rotationally invariant magnitude of diffusion in the brain and its increase is often reported in case of disease, such as in schizophrenia [49], indicating that pathological processes affected the barriers and

in turn the water motion. Both diffusion MRI measures were considered for each hemisphere, separately. For a detailed description of the analytic approaches applied to MRI data see [34].

1.2.4 Data analysis

The study aimed at estimating the prevalence of the symptoms associated with a NVLD profile and to further explore the neurobiological correlates. Firstly, we set the criteria and identified three different NVLD groups by adjusting the cut-offs related to intelligence, reading abilities and social problems, while keeping constant the criteria applied to the visuospatial processing and the exclusion of children with a diagnosis of Autism Spectrum Disorder. Then we performed three sets of bivariate correlations on the various measures: The first within the different behavioural criteria, the second within MRI data, and the last between behavioural performance and white matter data. A comparison was made between each correlation observed for the NVLD group with that of the whole ABCD by applying a z test (equation 1) of the difference of the Fisher's z transformed correlations divided by the standard error of the difference. For sample sizes of n and n2, we found the z of the difference between the z transformed correlations divided by the standard error of the difference of two z scores.

$$\text{Z-Observed} = (z1 - z2) \,/\, (\text{square root of } [\,(1 / n - 3) + (1 / n2 - 3)]) \quad (1)$$

Afterward, in order to test if there were actual differences between the WM measures in the three NVLD groups compared to the whole ABCD sample, t-tests were performed. A Welch t-test was used since it does not rely on the assumption that the two comparison groups have equal variance (or sample size) considering the large difference in the sample size involved in the comparison.

Finally, we applied a test of proportion on the data from a questionnaire administered to the parents regarding clinical diagnosis, mental health, social behaviours in general and specifically at school with the goal of testing if comparable proportions in each population

were found in the whole sample and in the groups with an NVLD profile. All data analyses were performed in R (version 4.1.0).

1.3 Results

1.3.1 Prevalence of NVLD profile in the ABCD sample

The present research used three different sets of criteria to define the group of children presenting the possible symptoms associated with the NVLD profile, as shown in Table 1.

Table 1 – Each column represents a domain involved in the criteria applied to identify each NVLD group.

Group	Visuospatial	Reading	Intelligence	ASD	Social
1st	≤16th percentile	≥25th percentile	≥50th percentile	No diagnosis	≥85th percentile
2nd	≤16th percentile	≥16th percentile	≥25th percentile	No diagnosis	≥85th percentile
3rd	≤16th percentile	≥25th percentile	≥50th percentile	No diagnosis	No

Visuospatial domain measured by Little Man, Matrix reasoning, 0-Back; Reading skills measured by Oral Reading Recognition task; Intelligence: Crystallized Intelligence -measured by Oral Reading Recognition task and Picture Vocabulary task- or Total Intelligence - composite score based on Crystallized (tasks listed above) and Fluid (measured by scores on the Pattern Comparison Processing Speed Test, the List Sorting Working Memory Test, the Picture Sequence Memory Test, the Flanker Task, and the Dimensional Change Card Sort Task) components; ASD -Autism Spectrum Disorder- diagnosis measured by a questionnaire administered to parents about psychological diagnoses; Social domain measured by the Child Behavior Checklist, subscale on social problems.

The first NVLD group was composed by participants showing the following characteristics:

1) *Visuospatial* abilities equal to or lower than the 16th percentile on either of the following tasks: Little Man, Matrix reasoning or 0-Back;

2) Words *reading* abilities, as measured by Oral Reading Recognition task, equal to or higher than the 25th percentile;
3) *Total* or *Crystallized Intelligence* equal to or greater than the 50th percentile. Crystallized Intelligence was measured by scores on the Oral Reading Recognition task and Picture Vocabulary task. Total Intelligence was a composite score based on Crystallized (tasks listed above) and Fluid (including scores on the Pattern Comparison Processing Speed Test, the List Sorting Working Memory Test, the Picture Sequence Memory Test, the Flanker Task, and the Dimensional Change Card Sort Task) components.
4) No *ASD diagnosis* measured by a questionnaire administered to the parents;
5) Scores on the *social* subscale (Child Behavior Checklist, CBCL) equal to or higher than the 85th percentile - the higher the score, the greater the social problems.

For the remainder of this paper, we will refer to this group as NVLD with social problems and average or above intelligence.

The second NVLD group differed from the first in that they had a lower level of intelligence and reading skills, and it was identified as follows:

1) *Visuospatial* abilities equal to or lower than the 16th percentile in one of the following tasks: little man, matrix reasoning or 0-back;
2) Words *reading* abilities equal to or greater than the 16th percentile;
3) *Total* or *Crystallized* Intelligence equal to or higher than the 25th percentile;
4) No *ASD diagnosis*
5) Scores on the *Social* subscale (CBCL) equal to or greater than the 85th percentile - the higher the score, the greater the social problems.

For the remainder of the paper, we refer to this group as NVLD with social problems and lower or above intelligence.

The third NVLD group differed from the preceding group since a criterion in the social domain was not set, and it was defined by the following criteria:

1) *Visuospatial* abilities equal to or lower than the 16th percentile as measured in one of the following tasks: Little man, matrix reasoning or 0-back;
2) Words *reading* abilities equal to or greater than the 25th percentile
3) *Total* or *Crystallized* Intelligence equal to or higher than the 50th percentile;
4) No *ASD diagnosis*;
5) *Social* subscale (CBCL) not used as a criterion.

For the remainder we refer to this group as NVLD without regard to social problems and average or above intelligence.

In the following table, there is a summary of the percentile used in each task and their corresponding value.

Table 2. Cut-offs applied to cognitive tasks used as a criterion for identifying NVLD profiles.

VISUOSPATIAL DOMAIN		
Little Man	16th%ile	0.44
Matrix Reasoning	16th%ile	7
0-Back	16th%ile	0.66
READING SKILLS		
Oral Reading Recognition	16th%ile	85
	25th%ile	90
INTELLIGENCE		
Total Intelligence	25th%ile	88
	50th%ile	100
Crystallized Intelligence	25th%ile	90
	50th%ile	103
SOCIAL DOMAIN		
Child Behavior Checklist	85th%ile	57

Following these different sets of criteria, the estimated sample sizes of the three groups were:

1) 144 children (1.21%),
2) 277 children (2.33%),
3) 977 children (8.23%).

Each group of children identified as having a NVLD profile is a subcategory of the other as presented in Figure 1.

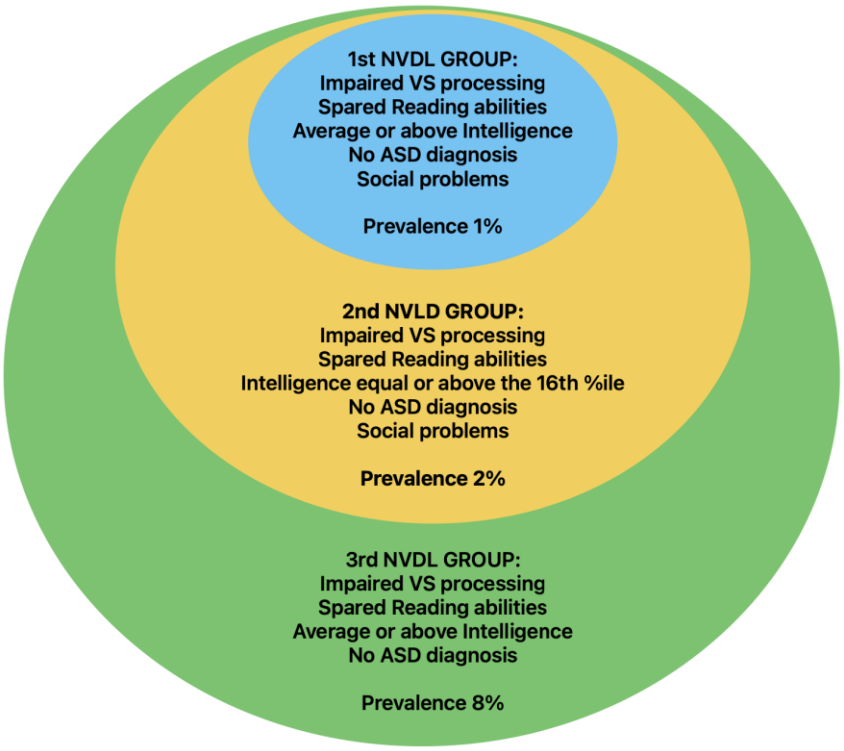


Figure 1. Representation of the NVLD groups and the cognitive criteria with the corresponding estimated prevalence in the ABCD sample [VS: visuospatial; ASD: Autism Spectrum Disorder].

1.3.2 Behavioural data

1.3.2.1 Correlations

After the estimation of the prevalence, the second goal was to investigate how the measures of visuospatial processing correlated to each other and to other criteria used to select the NVLD profile, in the whole population and in the three identified NVLD groups (Fig. 2).

The main characteristic that differentiated NVLD from the whole sample was a negative correlation between the mental rotation task (Little Man Task) and the other two visuospatial tasks, that is matrix reasoning and 0-back. In contrast, in the whole ABCD sample a significant positive correlation was evident between these two tasks and the mental rotation, consistent with the idea that they measure similar underlying processes related to the visuospatial domain. The NVLD group without regard to social problems, with average/above reading skills and intelligence was also characterized by a significant negative correlation between the performance in the 0-back task and the scores on the matrix reasoning test that was not present in the ABCD sample.

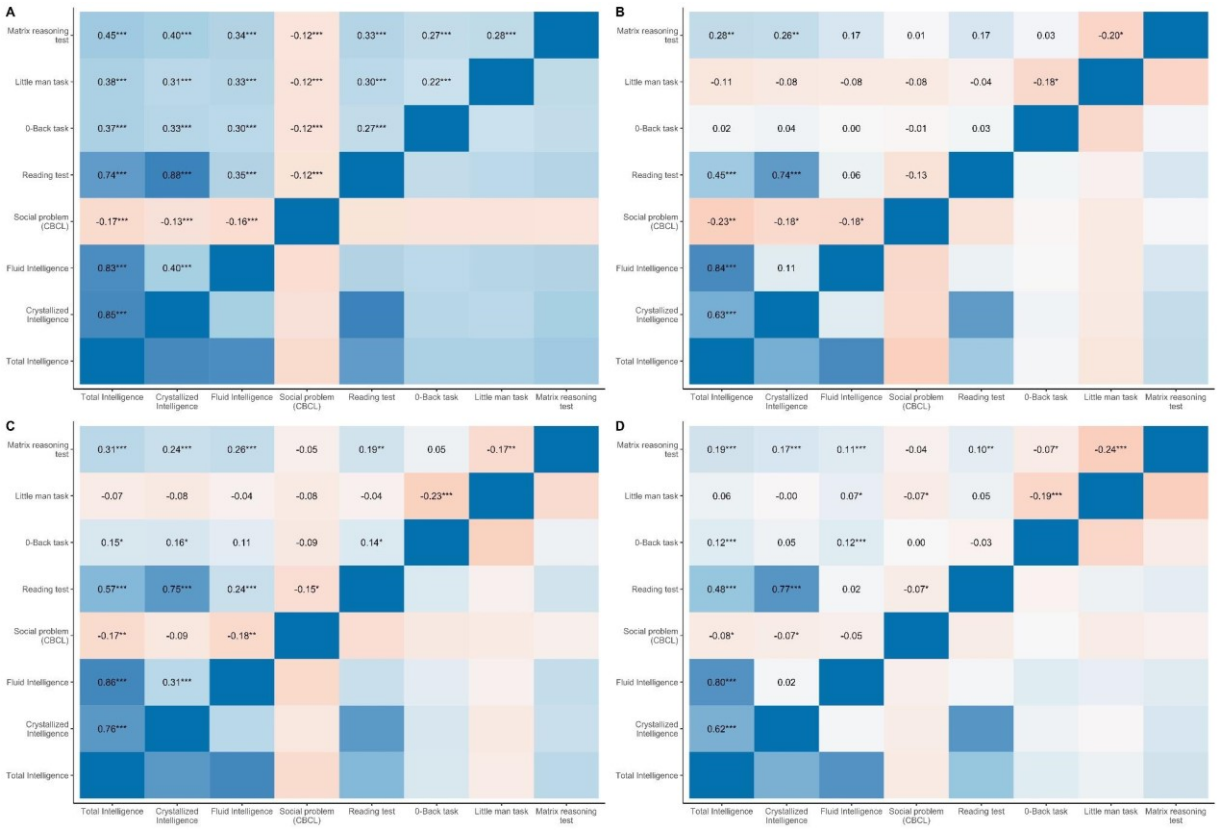


Figure 2. The figure presents the correlation matrices between the criteria used for estimating NVLD symptoms with the addition of the fluid component of intelligence. *Panel A* includes the whole ABCD sample; *Panel B* presents the NVLD profile with social problems and the most restricted criteria for reading skills and intelligence; *Panel C* depicts the NVLD group in which reading skills and intelligence criteria is relaxed, and with social problems; *Panel D* corresponds to the NVLD group without regard to social problems as a criterion but with the strictest cut-off for reading skills and intelligence.

The correlations between reading skills and visuospatial performance were significant for the matrix reasoning test and it was positive in two NVLD groups (mimicking the results in the ABCD sample): the one with average/above reading skills and intelligence without regard to social problems and the one with the least stringent criteria for intelligence/reading skills and social problems. In this last group, we also found a significantly positive correlation between the reading abilities and performance in the 0-back task.

The scale on social problems extracted from the CBCL (the higher the score, the more pronounced the social problems) was the only measure expected to be always negatively correlated with all the other scores: this held true for the whole ABCD sample. In the NVLD group without regard to social problems we found a significant negative correlation between social problems and the scores on the mental rotation task that was not found in the remaining NVLD populations in which we used as criterion the social subscale of the CBCL.

We found a positive correlation between the scores on matrix reasoning and total/crystallized intelligence in all the three NVLD groups. In addition, in the NVLD group with average/above reading skills and intelligence without regard to social problems, and in the one with the least stringent criteria for intelligence and reading skills there was a significant positive correlation between the fluid component and matrix reasoning, and between total intelligence and the performance on the 0-back task. While in the former NVLD group without regard to social problems there was also a positive correlation between 0-back scores and fluid intelligence, in the latter we found that this performance was significantly correlated with crystallized intelligence. Only in the NVLD group without regard to social problems, we found a significant positive correlation between the mental rotation task and the fluid intelligence. Finally, an unexpected result was related to the correlation between the two components of intelligence, within the NVLD groups, it emerged that in the two samples with strictest cut-offs for intelligence and words reading skills either with or without the social criterion, the two components of intelligence do not correlate with each other.

1.3.2.2 Z-Tests

This section is dedicated to the results of the z-tests that allow to highlight the significant differences between the studied populations in the correlations presented in the previous section.

In the visuospatial domain, an interesting relation was found between the little men task and the other two visuospatial tasks. In fact the z-test highlighted that the correlations of the little men task with the matrix reasoning (1st NVLD: $z = 5.85$, $p < .001$; 2nd NVLD: $z = 7.49$, $p < .001$; 3rd NVLD: $z = 16.07$, $p < .001$) and 0-back (1st NVLD: $z = 4.78$, $p < .001$; 2nd

NVLD: z = 7.40, p < .001; 3rd NVLD: z = 12.54, p < .001) were significantly different in the whole sample compared to each NVLD group. Furthermore, the results from the z-tests also confirmed that the correlation between the matrix reasoning scores and the 0-back were significantly different in the whole ABCD sample compared to the NVLD groups (1st NVLD: z = 2.89, p < .01; 2nd NVLD: z = 3.61, p < .001; 3rd NVLD: z = 10.42, p <.001).

On the correlations between reading skills and visuospatial performances, there was a significant difference between the whole sample and the NVLD groups in the little man task (1st NVLD: z = 4.12, p < .001; 2nd NVLD: z = 5.72, p < .001; 3rd NVLD: z = 7.75, p < .001), matrix reasoning (1st NVLD: z = 2.13, p < .05; 2nd NVLD: z = 2.50, p < .05; 3rd NVLD: z = 7.31, p < .001) and 0-back (1st NVLD: z = 2.87, p < .01; 2nd NVLD: z = 2.22, p < .05; 3rd NVLD: z = 9.19, p < .001).

The correlations between intelligence and the performance during the little men task were different in NVLD groups compared to the whole ABCD sample: there was a significantly different relationship of the visuospatial performance with total (1st NVLD: z = 5.97, p < .001; 2nd NVLD: z = 7.72, p < .001; 3rd NVLD: z = 10.34, p < .001), crystallized (1st NVLD: z = 4.72, p < .001; 2nd NVLD: z = 6.55, p < .001; 3rd NVLD: z = 9.66, p < .001) and fluid intelligence (1st NVLD: z = 5.01, p < .001; 2nd NVLD: z = 6.26, p < .001; 3rd NVLD: z = 8.07, p < .001). Furthermore, another striking result was found for the correlation between the two components of intelligence. While the NVLD groups with strictest criteria for intelligence and reading skills showed a significant difference compared to the whole sample (1st NVLD: z = 3.70, p < .001; 3rd NVLD: z = 12.27, p < .001), this was not true for the NVLD population with the least stringent cut-off on intelligence and reading abilities (2nd NVLD: z = 1.72, p = .09). In fact, when we tested for differences within the second NVLD group and the other two, we found a significantly different correlation between the fluid and crystallized intelligence (1st NVLD: z = 2.02, p < .05; 3rd NVLD: z = 4.45, p < .001).

When testing for differences in the link between social problems and visuospatial performances, we found a non-significant difference with the scores in the little man task of children with the NVLD profile compared to the whole ABCD sample (1st NVLD: z = 0.52, p = .60; 2nd NVLD: z = 0.67; p = .50; 3rd NVLD: z = 1.50, p = .13). In relation to the

performance during the matrix reasoning test, only the NVLD group without regard to social problems (3rd NVLD: $z = 2.23$, $p < .05$) showed a significantly different correlation with the subscale on social problems compared to the whole ABCD sample (1st NVLD: $z = 1.46$, $p = .14$; 2nd NVLD: $z = 1.10$, $p = .27$). The same pattern of results was found for the performance in the 0-back task (1st NVLD: $z = 1.25$, $p = .21$; 2nd NVLD: $z = 0.53$; $p = .60$; 3rd NVLD: $z = 3.72$, $p < .001$).

1.3.3 Anatomical data

1.3.3.1 T-Tests

We wanted to test if actual differences could be found in the WM measures between the whole sample and the NVLD groups using a Welch t-test that allows to contrast two samples that have different sample sizes and variability. Therefore, we compared the ABCD sample against each NVLD group for each WM index: Fractional Anisotropy, Mean Diffusivity, and volume of the WM separately for each hemisphere. The only significant differences were found in the comparison between the NVLD group without social problems and the overall ABCD sample: except for Fractional Anisotropy in the left hemisphere, all the other measures were different in the two populations involved. Specifically, we found that Fractional Anisotropy in the right hemisphere was significantly increased in the whole sample compared to NVLD, while Mean Diffusivity and volume of both hemispheres were found to be significantly increased in the NVLD group. Results are shown in Table 3 (See Appendix A for the t-tests between the other NVLD groups and the whole sample - Table A1 and A2).

Table 3. Welch t-test results on WM measures comparing the third NVLD group and the ABCD sample.

Measures	t-value	Degree of Freedom	p-value	CI
Fractional Anisotropy LH	1.81	1106.45	0.071	[-2.248397e-03, 9.190445e-05]
Fractional Anisotropy RH	3.29	1096.01	0.001***	[-0.0032843924, -0.0008318199]
Mean Diffusivity LH	-2.01	1120.13	0.045*	[3.818323e-05, 3.209814e-03]
Mean Diffusivity RH	-2.27	1118.38	0.024*	[0.0002461103, 0.0034103685]
Volume WM LH	-2.73	1173.67	0.006**	[597.0861, 3660.2841]
Volume WM RH	-2.64	1172.30	0.008**	[529.4333, 3592.9414]

1.3.3.2 Correlations

Next, we were interested in comparing the white matter (WM) measurements in the overall ABCD sample and in the three NVLD groups (Fig. 3). In the whole ABCD sample a significant negative correlation was found between Mean Diffusivity and Fractional Anisotropy within each hemisphere and across hemispheres. All the other measures were significantly and positively correlated to each other.

Among all NVLD groups, a significant negative correlation was found between Fractional Anisotropy and Mean Diffusivity within each hemisphere and across hemispheres. Only the NVLD group without regard to social problems presented a significant positive correlation between WM volume and Mean Diffusivity. It should be noted that while the other correlations did not reach significance (perhaps because of the reduced sample size), they were all in the same direction.

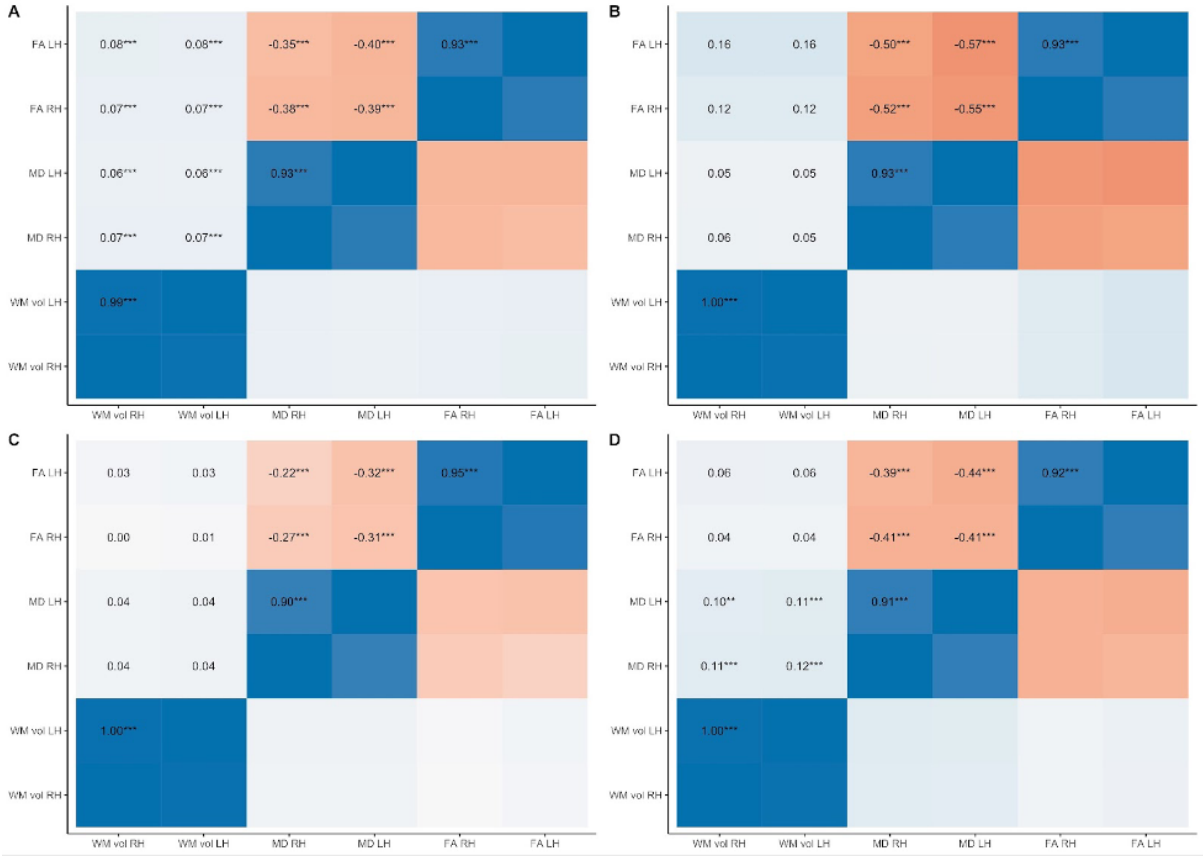


Figure 3. *Panel A* presents the correlation matrix between white matter measures within the whole ABCD sample. *Panels B, C and D* correspond to NVLD group 1, 2 and 3 respectively [WM vol: White Matter Volume; MD: Mean Diffusivity; FA: Fractional Anisotropy; RH: Right Hemisphere; LH: Left Hemisphere].

1.3.3.3 Z-Tests

We made use of the z-test to investigate if within the two hemispheres there were different correlations in relation to the Fractional Anisotropy, the Mean Diffusivity and the Volume of the White Matter in the whole ABCD sample compared to the three NVLD groups.

1.3.3.3.1 Right hemisphere

The correlations between FA and MD were significantly different in the NVLD group with social problems, average/above intelligence and reading skills ($z = 2.1$, $p < .05$), and in the NVLD group with the least strict criteria on intelligence and reading abilities ($z = 1.99$, $p < .05$) compared to the neurotypical sample; while the NVLD population without regard to social problems did not differ from the whole sample ($z = 1.4$, $p = .16$).

The correlations between FA and the WM Volume did not differ between the three NVLD groups and the neurotypical population (1st NVLD: $z = 0.63$, $p = .53$; 2nd NVLD: $z = 1.04$; $p = .30$; 3rd NVLD: $z = 0.83$, $p = .41$). The same results were found for the correlation between MD and the volume of WM (1st NVLD: $z = 0.09$, $p = .93$; 2nd NVLD: $z = 0.43$; $p = .67$; 3rd NVLD: $z = 1.34$, $p = .18$).

1.3.3.3.2 Left hemisphere

A significant difference in the correlations between FA and MD was found only in the NVLD group with social problems and the strictest criteria for intelligence and reading skills ($z = 2.78$, $p < .001$) while the other two NVLD groups presented no differences compared to the neurotypical sample (2nd NVLD: $z = 1.33$; $p = .18$; 3rd NVLD: $z = 1.59$, $p = .11$).

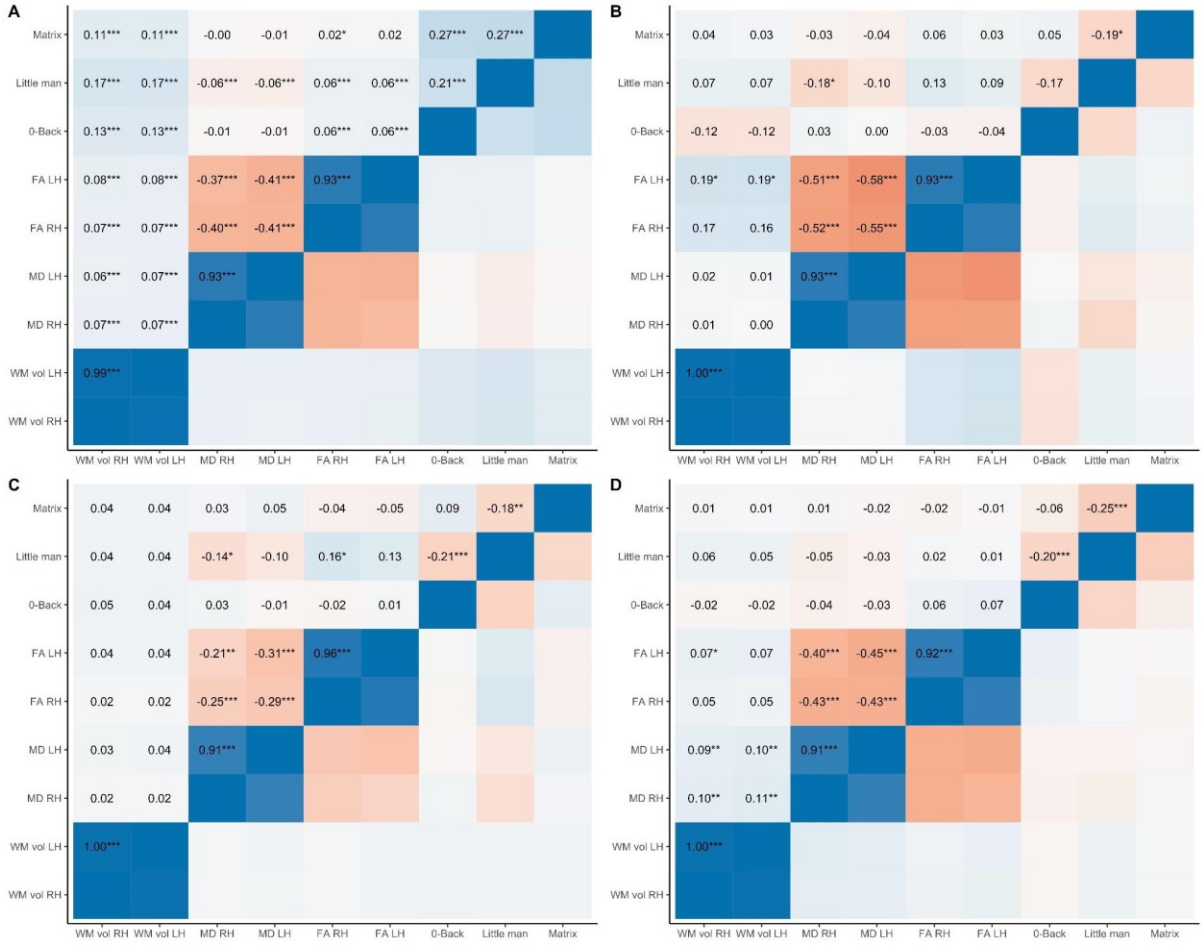
The link between FA and Volume of WM was not different in the whole sample compared to the NVLD groups (1st NVLD: $z = 0.97$, $p = .33$; 2nd NVLD: $z = 0.83$; $p = .41$; 3rd NVLD: $z = 0.58$, $p = .56$). The same pattern of results was found for the correlation between MD and the volume of WM (1st NVLD: $z = 0.16$, $p = .87$; 2nd NVLD: $z = 0.32$; $p = .75$; 3rd NVLD: $z = 1.49$, $p = .14$).

1.3.4 Associations between brain and behaviours

1.3.4.1 Correlations

The last set of correlational analyses was conducted on White Matter measures and the cognitive functions of interest, that is performance on visuospatial tasks (Fig. 4).

In the whole ABCD sample, a significant positive correlation was found between visuospatial processing, as measured by the little man and the 0-back task, and two white matter indices: volume and Fractional Anisotropy of both hemispheres. A negative correlation was, instead, found between MD and the performance in the little man task. The scores on the matrix reasoning test were found to be positively correlated with the volume of white matter on both hemispheres and with the Fractional Anisotropy of the right hemisphere.



* p < .05, ** p < .01, *** p < .001

Figure 4. *Panel A* presents the correlation matrix between cognitive performances and white matter measures within the whole ABCD sample. *Panels B, C and D* correspond to NVLD group 1, 2 and 3 respectively.

In the NVLD group with the strictest criteria on intelligence and reading skills, we found that performance during the mental rotation task was negatively correlated with Mean Diffusivity in the right hemisphere. From the NVLD group with the least stringent cut-offs on intelligence and reading abilities, instead, emerged two significant correlations related to the performance in the mental rotation task: there was a positive correlation with Fractional Anisotropy and a negative one with Mean Diffusivity. Both

the correlations were found in the right hemisphere. No significant correlations were found for the NVLD sample without regard to social problems.

1.3.4.2 Z-Tests

1.3.4.2.1 Right Hemisphere

Significant differences were only found in the correlation between visuospatial performances and WM Volume. Specifically, the correlation between matrix reasoning and WM Volume was found to be different in the whole ABCD sample and the NVLD group with social problems unselected ($z = 2.88$, $p < .01$), whereas this difference was not found in the other two NVLD groups (1st NVLD: $z = 0.75$, $p = .45$; 2nd NVLD: $z = 1.14$, $p = .26$). The correlations between WM Volume and the little man task were found to be different in two NVLD groups compared to the whole sample: in the sample without regard to social problems ($z = 3.56$, $p < .01$) and in the population with the more relaxed criteria on reading skills and intelligence ($z = 2.24$, $p < .05$). The NVLD profile with the strictest cut-offs on intelligence and reading with social problems was not significantly different from the control group ($z = 1.22$, $p = .22$). Consistent with the other results between white matter measures and visuospatial functioning, the correlations between volume of the white matter and scores in the 0-back task were significantly different in the whole population compared to the two NVLD groups with the strictest criteria for intelligence and reading skills, with ($z = 3.08$, $p < .01$) or without regard to social problems ($z = 4.70$, $p < .001$). The remaining NVLD group was not different from the whole sample ($z = 1.49$, $p = .14$). See Table A3 in the Appendix A for complete results.

1.3.4.2.2 Left Hemisphere

The same pattern of results found in the right hemisphere emerged also in the left hemisphere with the NVLD group without regard to social problems having a

significant difference from the neurotypical sample in the correlation between volume and matrix reasoning ($z = 2.96$, $p < .05$), whilst no such a difference was found for the other two NVLD groups (1st NVLD: $z = 0.95$, $p = .34$; 2nd NVLD: $z = 1.14$, $p = .25$).

The correlations between the volume of the WM and the little man task were found to be different in two NVLD groups compared to the neurotypical sample: in the cognitive profile without regard to social problems ($z = 3.65$, $p < .001$) and in the population with the more relaxed criteria in reading skills and intelligence ($z = 2.28$, $p < .05$). The NVLD profile with the strictest cut-offs on intelligence and reading with social problems was not significantly different from the whole ABCD sample ($z = 1.23$, $p = .22$).

In line with the results found in the right hemisphere, only the two NVLD groups with the more stringent criteria for intelligence and reading skills, with ($z = 3.02$, $p < .01$) or without regard to social problems ($z = 4.72$, $p < .001$) showed a difference in the correlations between the 0-back performances and the volume of the white matter compared to the whole population that was not found in the remaining NVLD group ($z = 1.5$, $p = .13$). See Table A4 in the Appendix A for complete results.

1.3.5 Group Differences in Measures associated with Psychological, Social Measures of Health and Well-being

Using a screening questionnaire, we aimed at estimating if the characteristics of the identified groups differed from the whole ABCD sample in various contexts: clinical diagnosis and psychological well-being, school and social behaviours.

1.3.5.1 Clinical diagnosis and psychological well-being

In the following table (Table 4) we present the questions and the percentage of each population answering affirmatively.

Table 4. Percentage of positive responses to questions about diagnosis and mental well-being. 1st NVLD: average and above intelligence/reading criteria with social problems; 2nd NVLD: relaxed cut-offs on intelligence and reading with social problems; 3rd NVLD: average and above intelligence/reading criteria without regard to social problems. [*p < .05; **p < .01; ***p < .001]

Sample	1) Has your child been diagnosed with any psychological or psychiatric diagnoses?	2) Has your child been diagnosed with ADHD, Depression, Bipolar Disorder, Anxiety, Phobias	3) Is too fearful or anxious?	4) Is unhappy, sad, or depressed?
ABCD	0.95%	7.55%	17.93%	8.27%
1st NVLD	4.86% ***	28.47% ***	58.33% ***	34.72% ***
2nd NVLD	5.78% ***	35.74% ***	55.96% ***	36.82% ***
3rd NVLD	1.74% *	12.69% ***	35.21% ***	16.58% ***

In the first question parents were asked if the child was diagnosed with a psychological/psychiatric disease and we found a significantly higher presence of diagnoses in all the three NVLD groups compared to the whole sample (1st NVLD: χ^2 (1) = 18.22, p < .001; 2nd NVLD: χ^2 (1) = 55.49, p < .001; 3rd NVLD: χ^2 (1) = 4.85, p < .05). When asked about specific diagnosis (Q2), we confirmed again that all the three NVLD groups (1st NVLD: χ^2 (1) = 83.65, p < .001; 2nd NVLD: χ^2 (1) = 282.11, p < .001; 3rd NVLD: χ^2 (1) = 31.90, p < .001) had a significantly higher rate of diagnosis

of mental disorders, specifically related to ADHD, depression, bipolar disorder, anxiety and phobias. Furthermore, we found that the NVLD profile was more anxious/fearful (1st NVLD: χ^2 (1) = 151.96, p < .001; 2nd NVLD: χ^2 (1) = 254.03, p < .001; 3rd NVLD: χ^2 (1) = 172.42, p < .001) and sad/depressed compared to the whole ABCD sample (1st NVLD: χ^2 (1) = 123.51, p < .001; 2nd NVLD: χ^2 (1) = 268.17, p < .001; 3rd NVLD: χ^2 (1) = 75.91, p < .001).

1.3.5.2 School and social behaviours

In Table 5 we present the percentage of parents that answered about their children not enjoying going to school (Q5), about the child being disobedient (Q6) and enjoying reading (Q7).

Table 5. Percentage of negative answers to question 5 and positive responses to questions 6 and 7. 1st NVLD: average and above intelligence/reading criteria with social problems; 2nd NVLD: relaxed cut-offs on intelligence and reading with social problems; 3rd NVLD: average and above intelligence/reading criteria without regard to social problems. [*p < .05; **p < .01; ***p < .001]

Sample	5) Enjoy school?	6) Is disobedient at school?	7) Enjoy reading?
ABCD	1.13%	7.74%	49.15%
1st NVLD	6.25% ***	27.08% ***	94.44% ***
2nd NVLD	6.86% ***	30.32% ***	91.33% ***
3rd NVLD	3.07% ***	13.92% ***	96.21% ***

There was a significant difference in the proportion of children not enjoying school between all the three NVLD group and the ABCD sample (1st NVLD: χ^2 (1) = 27.54, p < .001; 2nd NVLD: χ^2 (1) = 66.98, p < .001; 3rd NVLD: χ^2 (1) = 25.51, p < .001). More children in the NVLD group presented disobedience at school compared to the ABCD sample (1st NVLD: χ^2 (1) = 69.98, p < .001; 2nd NVLD: χ^2 (1) = 179.4, p < .001; 3rd NVLD: χ^2 (1) = 44.971, p < .001). Conversely, and quite interestingly, more children in the NVLD groups enjoyed reading compared to the whole ABCD population (1st NVLD: χ^2 (1) = 114.96, p < .001; 2nd NVLD: χ^2 (1) = 191.01, p < .001; 3rd NVLD: χ^2 (1) = 800.31, p < .001).

Finally, table 5 presents the percentage of parents responding about if the child likes helping others and if they are slow at making friends.

Table 6. Percentage of the answer no to question 9 and yes to question 10. 1st NVLD: average and above intelligence/reading criteria with social problems; 2nd NVLD: relaxed cut-offs on intelligence and reading with social problems; 3rd NVLD: average and above intelligence/reading criteria without regard to social problems. [*p < .05; **p < .01; ***p < .001]

Sample	8)Likes helping others?	9)Tends to be slow at making friends?
ABCD	0.45%	13.25%
1st NVLD	2.08% *	45.14% ***
2nd NVLD	1.80% **	49.82% ***
3rd NVLD	0.72%	23.85% ***

We found that for question 8 there was a significant difference between the first two NVLD groups and the ABCD sample (1st NVLD: χ^2 (1) = 5.07, p < .05; 2nd NVLD: χ^2 (1) = 7.85, p = .01) while no difference was found for the third NVLD group (3rd NVLD: χ^2 (1) = 0.90, p < .34). Instead, we found that for the last question all the three NVLD groups are significantly slower at making friend compared to the whole ABCD sample (1st NVLD: χ^2 (1) = 120.14, p < .001; 2nd NVLD: χ^2 (1) = 296.03, p < .001; 3rd NVLD: χ^2 (1) = 82.99, p < .001).

1.4 Discussion

1.4.1 Prevalence of the NVLD profile

The first goal of the present investigation was to estimate the prevalence of the NVLD profile in a large, representative sample of 11 876 North American children aged 9-10 years old. Our multi-pronged approach allowed us to identify a first group composed of 1.21% of the total population, a second group formed by 2.33%, and a third group formed by 8.23% of the total ABCD sample. Since the first group had very strict criteria applied on both intelligence/reading as well as on social problems, it is likely that it did not include all the children presenting the symptomatology associated with NVLD.

For the second group, with more relaxed criteria set for intelligence/reading, there could have also been an underestimation of the clinical population of interest, since, as mentioned earlier, social problems are highly variable and not always present or evident. In fact, while it has been found that NVLD is associated with increased risk for internalizing psychopathology [50], another study found that NVLD was not linked to levels of internalizing psychopathology as rated by the parents [51]. In addition, two comprehensive reviews [52,53] of the literature highlighted that the results on socioemotional functioning in children with NVLD may be inconsistent.

Nonetheless, the results of this estimation approach are comparable to the one of Margolis [31] in terms of the percentage and also for the criteria applied. It should be considered that we were able to compute the prevalence in a dataset that does not specifically include children with psychiatric or psychological pathologies.

Furthermore, the third NVLD group seems to represent the best definition since it is the one better describing the learning disability of interest, that is children with a deficit in visuospatial processing, spared verbal intelligence and specifically reading abilities, and with no diagnosis of ASD. The estimation of the prevalence found to be at about 8% is doubled the one found in Margolis et al. (3-4%) [31] and this difference could be due to various factors.

First, we used consistent criteria throughout the whole sample while Margolis used various measurements due to the involvement of 3 different datasets. Furthermore, in order for our estimation not to be built on the score of single tasks, we have also used composite scores for measuring intelligence which are more comprehensive measures of the underlying cognitive process.

Second, Margolis employed the discrepancy between verbal and visuospatial measures whereas we decided not to include it since this approach has been criticized [16,17] and it could influence the estimation of the prevalence [20].

Third, even considering that Margolis used inflation factors weights to account for overrepresentation of the psychiatric disorder in their sample, our estimation was made on the ABCD data which not only involved mainly neurotypical children but it was composed by a sample that was representative of the whole population in terms of geographic, socioeconomic and ethnic backgrounds.

Finally, the sample size of the ABCD dataset is considerably larger than the one of Margolis (11 878 and 2 596 respectively).

1.4.2 Cognitive profile of NVLD

The most salient aspect shared among the three NVLD groups, remarkably different from the ABCD sample as a whole, was the negative correlation between performance on the mental rotation task and the other two visuospatial tests, that is matrix reasoning and 0-back. A possible explanation is that NVLD children may try to compensate the visuospatial deficits with their intact verbal reasoning abilities: Such an account appears more likely for the matrix reasoning and the 0-back, mainly involving memory and attention, while it is harder to apply to the mental rotation task relying on strategies strictly related to the visuospatial domain. For instance, Mammarella and Cornoldi [9] found that children with visuospatial difficulties were more impaired in actively manipulating items in memory during a backward span test compared to a forward span. Their explanation of such a phenomenon was that the backward task loaded on the visuospatial non-sequential domain while the forward task may have relied on sequential process strategies: nonetheless this resource in children with a profile of NVLD are not enough in order for them to use the sequential strategy to compensate.

Another characteristic feature of the two NVLD profiles with social problems is that there is no relationship between performances in the 0-back task and in the matrix reasoning test, while in the NVLD group without regard to social problems that correlation is significant and in the negative direction. It should be noted that this correlation was positive in the whole sample since the two tasks both involve visuospatial processing.

When looking at the relationship between reading abilities and performance in visuospatial processing, we found that the higher the reading skills, the better the performance in the matrix reasoning task. This positive correlation was significant in the whole sample, in the NVLD group without regard to social problems and in the one with the least stringent criteria for intelligence and reading. The performance during the

little man task was not significantly correlated with the reading scores within the NVLD group, nonetheless there was a significant difference in this link between the profile of interest and the whole ABCD population. Furthermore, the higher reading abilities, the better the performance in the 0-back task in the whole sample and in the NVLD population with the least stringent criteria for intelligence and reading skills. This link was not found for the other two NVLD groups.

Regarding the link between intelligence and visuospatial abilities, the higher the matrix reasoning scores, the higher the total and crystallized intelligence in all three NVLD groups and in the whole population. This relationship was also found for fluid intelligence in the total ABCD sample and in two NVLD groups: the profile with the least stringent criteria on intelligence and reading abilities with social problems, and in the one without regard to social problems and the strictest cut-offs in intelligence and reading scores. For the 0-back task we found that the better the performance, the higher the total intelligence in the NVLD group without regard to social problems and in the one with the least strict criteria on intelligence and reading. Nonetheless, in the former group this result is depending more on the fluid intelligence whereas in the latter group on the crystallized component as shown by the difference in the significant correlations found in the two. On the little man task, the two NVLD groups with social problems presented a nonsignificant correlation with intelligence that was pointing toward a negative relationship between them. While in the case of the NVLD profile without regard to social problems, we found that the higher the fluid intelligence, the better the performance. However, all three NVLD groups had a significantly different relationship between visuospatial processing, as measured by the mental rotation task, and all components of intelligence.

In the two NVLD groups with the strictest criteria on intelligence and reading abilities, with and without regard to social problems, the link that is usually found between crystallized and fluid intelligences was not present any longer. Therefore, children with higher crystallized or total intelligence within these NVLD groups

preliminarily showed that the two components are not linked to each other as expected and found in the whole ABCD population. Therefore, the evidence of uncorrelated intelligence components can be used for experimental researches in place of the discrepancy between verbal and performance IQ in order to differentiate group with and without an NVLD profile, since it does not entail that the two measures have to be discrepant by a certain number of points (generally 10 to 15) [14], but instead give salient information about the relationship that they have with each other.

When looking at the relationship between the social domain and the visuospatial functioning, we found a significant result only in the NVLD group without social problem: Those individuals who exhibited a larger degree of social problems scored more poorly on the little man task.

1.4.3 Brain correlates of NVLD

The third, most inclusive NVLD profile (selected without the criterion of the social problems) was the only one showing significant differences in the white matter measures when compared to the neurotypical sample. Fractional Anisotropy of the right hemisphere was significantly lower in the NVLD group compared to the control group. This result was not found for the left hemisphere. Taken together, these findings support the hypothesis that the cognitive profile of NVLD could be derived from unorganized white matter tracts in the right hemisphere. We also found that the Mean Diffusivity and the volume of the white matter was significantly increased in the NVLD group compared to the control sample in both hemispheres. Research is pointing out that the maturational process during the development is sustained by an increase in FA and a decrease in MD [54,55] and a deviation from this developmental trajectory could be the result of ongoing pathological processes [56]. Furthermore, there is evidence that increased MD is generally connected to disorganization of the WM tracts in disease: For instance, in multiple sclerosis patients, symptom severity was positively correlated with MD while negatively correlated to FA [57]. Conversely, the literature appears to

indicate that a low MD is associated with greater density of synapses and strengthening axons and dendrites [58,59], and in turn such decrease is linked to better cognitive functioning [60]. In relation to the enlarged volume of the WM in the NVLD group, such a phenomenon was found in other developmental disorders such as high-functioning autism and developmental language disorder [61,62].

When looking at the correlation within the considered measures on white matter, we found an interesting pattern of results on the two NVLD populations with social problems. Specifically, in the group with the strictest criteria on intelligence and reading abilities, there was a significant difference in the correlation between Mean Diffusivity and Fractional Anisotropy compared to the whole sample: in fact, the NVLD profile showed a more pronounced negative link between the two white matter metrics in both hemispheres. In the NVLD group with the least stringent criteria on intelligence and reading abilities, we found again a significantly different correlation between the two measures compared to the ABCD sample but in this case the negative correlation was less pronounced and it was significantly different only for the right hemisphere.

1.4.4 Brain-behaviour link

The two NVLD groups with social problems showed that the lower the performance in visuospatial processing, as measured by the little man task, the higher the Mean Diffusivity in the right hemisphere. The opposite relationship was found for Fractional Anisotropy in the right hemisphere only in the NVLD group with the least strict criteria in terms of intelligence and reading abilities.

When analysing the differences between correlation in the NVLD groups compared to the whole ABCD sample, the relationship of the visuospatial processing with the volume of the white matter yielded interesting results. In the NVLD group without regard to social problems, there was no relationship between visuospatial ability, as measured by all the three tasks, and the volume of white matter. This link

was significantly different compared to the whole population, showing that the higher the volume, the better the visuospatial performance. This pattern was also found for the NVLD sample with the strictest criteria on intelligence and reading with social problems but only in the 0-back task. While in the NVLD group with the more relaxed cut-offs on intelligence and reading with social problems, the same result was found for the little man task. All the above findings on the link between visuospatial abilities and the volume of the white matter were consistent across hemispheres. Furthermore, linking these results with the significantly higher volume of the group without regard to social problems compare to neurotypical children, could be evidence that this increase does not have a relationship with visuospatial performance and could be due to a pathological mechanism.

1.4.5 Comorbidities in NVLD

By looking at parent's evaluations of their children's mental and social health, it could be clearly noted that in NVLD groups there was a higher percentage of psychological/psychiatric diagnoses (i.e., ADHD, Depression, Bipolar Disorder, Anxiety, Phobias) as well as lower psychological and social well-being. The NVLD profile was accompanied by more anxious and depressive symptoms compared to the whole ABCD sample, confirming results on increased risk of developing internalizing psychopathology [50]. Moreover, within the school context, NVLD children were enjoying school less and were more disobedient; However remarkably they took significantly more pleasure in reading than the whole sample, perhaps due to the fact that, compared to their peers, they capitalized more on their preserved verbal skills. In fact, while in the whole sample we found that about 50% of the population was enjoying reading, in all the three NVLD groups more than 90% of the children took delight in this activity. These findings are in line with the cognitive profile described for clinically diagnosed NVLD children. Note however that no NVLD diagnosis was made in this

study, yet children were struggling more at school and in the social context, and they had lower psychological health [63] compared to the rest of the ABCD sample.

1.5 Conclusions

Among the identified groups presenting the symptomatology associated with NVLD, we found an interesting result that should be taken into account when measuring the performance in the visuospatial domain in the population of interest. The performance in a strictly visuospatial task involving active information manipulation, as in the mental rotation test, should be employed along with other visuospatial tasks measuring memory and attention. In fact, in NVLD the processes involved in the former are not similar to the ones used for the latter: while in the 0-back and in the matrix reasoning task it was possible for them to utilize verbal or non-visuospatial strategies, the same did not held true for active manipulation of visuospatial information during the little man task.

Furthermore, we believe that the cut-offs of the third group better capture the clinical group of interest, since social problems are not always present and the thresholds imposed to intelligence, reading and visuospatial abilities were likely to reveal the actual difficulties that a child with NVLD has to face. One of them is represented by the fact that the higher the discrepancy between verbal and non-verbal abilities, the more unlikely it was for the child to adaptively use the two components of intelligence, which therefore resulted to be uncorrelated.

We found that only the group without regard to social problems showed unorganized white matter tracts, in line with the WM model of Rourke and the more recent research on pathological mechanisms involved in diseases. In addition, a difference was found in the relation between the volume of the WM and the visuospatial performance, consistently within the three considered tasks, pointing toward a dysfunctional link between the two.

Finally, it will be highly informative to follow the developmental trajectories of children in the NVLD groups to investigate how the coping strategies may evolve as they grow up, and to find out whether the cut-offs and the tasks utilized in the present investigation continue to be the best standards as the NVLD children transition from middle childhood to the pre-adolescent phase.

1.6 Appendix A

Table A1. Results from the Welch t-test comparing the measures of White Matter between the NVLD group (with strictest criteria applied on intelligence/reading with social problems) and in the rest of the ABCD sample.

Measures	t-value	Degree of Freedom	p-value	CI
Fractional Anisotropy LH	-0.05	141	0.96	[-0.003, 0.003]
Fractional Anisotropy RH	0.22	141	0.82	[-0.002, 0.003]
Mean Diffusivity LH	-0.83	142	0.41	[-0.006, 0.002]
Mean Diffusivity RH	-0.57	141	0.57	[-0.005, 0.003]
Volume WM LH	-0.70	145	0.49	[597.086, 3660.284]
Volume WM RH	-0.78	145	0.44	[529.433, 3592.941]

Table A2. Results from the Welch t-test comparing the measures of White Matter between the NVLD group (with more relaxed cut-offs on intelligence/reading with social problem) and in the rest of the ABCD sample.

Measures	t-value	Degree of Freedom	p-value	CI
Fractional Anisotropy LH	0.44	264.95	0.66	[-0.002, 0.003]
Fractional Anisotropy RH	0.37	265.09	0.71	[-0.002, 0.003]
Mean Diffusivity LH	0.17	270.89	0.86	[-0.003 0.003]
Mean Diffusivity RH	0.31	270.30	0.76	[-0.003 0.003]
Volume WM LH	-1.52	286.80	0.13	[-5304.339, 673.616]
Volume WM RH	-1.51	286.71	0.13	[-5285.118 695.813]

Table A3. Results from the Z t-test comparing the correlations between measures of White Matter in the right hemisphere and visuospatial performances in the NVLD groups (with more relaxed cut-offs on intelligence/reading with social problem) and in the whole ABCD sample.

Correlations	1st NVLD	2nd NVLD	3rd NVLD
Matrix and FA	z=0.44 p=.66	z=0.94 p=.35	z=1.24 p=.22
Matrix and MD	z=0.28 p=.78	z=0.50 p=.62	z=0.36 p=.72
Matrix and volume	z=0.75 p=.45	z=1.14 p=.26	z=2.88 p<.01**
Little man and FA	z=0.72 p=.47	z=1.53 p=.13	z=1.47 p=.14
Little man and MD	z=1.49 p=.14	z=1.39 p=.16	z=0.24 p=.81
Little man and volume	z=1.22 p=.22	z=2.24 p<.05*	z=3.56 p<.01**
0-BAck and FA	z=1.02 p=.31	z=1.17 p=.24	z=0.18 p=.86
0-Back and MD	z=0.46 p=.65	z=0.60 p=.55	z=1.10 p=.27
0-Back and volume	z=3.08 p<.01**	z=1.49 p=.14	z=4.70 p<.001***

Table A4. Results from the Z t-test comparing the correlations between measures of White Matter in the left hemisphere and visuospatial performances in the NVLD groups (with more relaxed cut-offs on intelligence/reading with social problem) and in the whole ABCD sample.

Correlations	1st NVLD	2nd NVLD	3rd NVLD
Matrix and FA	z=0.17 p=.87	z=1.02 p=.31	z = 0.67 p=.50
Matrix and MD	z=0.36 p=.72	z=1.04 p=.30	z = 0.23 p=.82
Matrix and volume	z=0.95 p=.34	z=1.14 p=.25	z = 2.96 p<.05*
Little man and FA	z=0.29 p=.77	z=1.98 p=.33	z = 1.71 p=.09
Little man and MD	z=0.53 p=.60	z=0.60 p=.55	z= 0.84 p=.40
Little man and volume	z=1.23 p=.22	z=2.28 p<.05*	z=3.65 p<.001***
0-BAck and FA	z=1.11 p=.27	z=0.71 p=.47	z=0.29 p=.77
0-Back and MD	z=0.17 p=.86	z=0.04 p=.97	z=0.58 p=.56
0-Back and volume	z=3.02 p<.01**	z=1.5 p=.13	z=4.72 p<.001***

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Chapter 2 Resting-State dynamic reconfiguration of Spatial Attention cortical network and Visuospatial functioning in NVLD: a HD-EEG investigation

Nonverbal learning disability (NVLD) is a neurodevelopmental disorder characterized by deficits in visuospatial processing but spared verbal competencies. Neurocognitive markers may provide confirmatory evidence for characterizing NVLD as a separate neurodevelopmental disorder. Visuospatial performance and High-Density electroencephalography (EEG) were measured in 16 NLVD and in 16 typically developing (TD) children. Cortical source modeling was applied to assess resting state functional connectivity (rs-FC) in spatial attention networks (Dorsal -DAN- and Ventral -VAN- Attentional Networks) implicated in visuospatial abilities. A machine-learning approach was applied to investigate whether group membership could be predicted from rs-FC maps and if these connectivity patterns were predictive of visuospatial performance. Graph theoretical measures were applied to nodes inside each network. EEG rs-FC maps in the gamma and beta band differentiated children with and without NVLD, with increased but more diffuse and less efficient functional connections bilaterally in the NVLD group. While rs-FC of the left DAN in the gamma range predicted visuospatial scores for TD children, in the NVLD group rs-FC of the right DAN in the delta range predicted impaired visuospatial performance, confirming that

NVLD is a disorder with a predominant dysfunction in right hemisphere connectivity patterns.

2.1 Introduction

Nonverbal learning disorder (NVLD) is a neurodevelopmental disorder with a neuropsychological profile characterized by visuospatial processing deficits, within a profile of intact verbal abilities [1–4]. Individuals with NVLD show major problems with visuospatial working memory (VSWM) [5–7], visuo-constructive and spatial organizational skills [8–11], comprehension of spatial descriptions [12,13] and nonverbal problem-solving abilities [14]. Such neuropsychological deficits may be associated with learning difficulties in the areas of mathematics, geometry and drawing [7,15–17]. Finally, studies have also reported difficulties in social interaction abilities for individuals with NVLD [18,19] albeit less pronounced relative to Autism Spectrum Disorder (ASD). Although in recent years researchers have collected many data supporting the main characteristics of NVLD, in spite of its growing recognition, NVLD is not yet identified in the current classification systems as a distinct developmental disorder (DSM-5, APA, 2013; ICD-11; World Health Organization [WHO], 2018). In addition, neurobiological markers of brain structure and function may contribute important convergent evidence that NVLD is indeed a distinct disorder.

2.1.1 Neural correlates of NVLD

Based on the neuropsychological profile and the presence of mild left-sided motor and sensory signs [20,21], Rourke [1] postulated that the neurological basis of NVLD is a “white matter” syndrome, with a predominant dysfunction in right hemisphere connectivity patterns, an hypothesis based on the available evidence linking the right hemisphere with specialized visuospatial processing [22–25].

An electroencephalography (EEG) study [26] tested Rourke's right hemisphere hypothesis employing EEG, comparing two groups of children, one with NVLD and the other with verbal learning disorder (dyslexia). They computed EEG coherence, a frequency-specific measure that reflects functional interregional coupling, mainly depending on structural connections [27,28]. Consistent with Rourke's hypothesis, in the NVLD group they found in the resting state a relative decrease in coherence in the gamma band between distant locations restricted to the right hemisphere (long-distance hypoconnectivity), interpreted as reflecting defective neuronal interactions between distant cortical regions in the right hemisphere [26]. Albeit promising, the study had some limitations. First, there was no comparison group of typically developing (TD) children. Second, no behavioral performance data in the visuospatial domain were reported. Third, EEG functional connectivity was calculated only at the scalp level from a sparse sensor array (19 electrodes).

Later advances in imaging techniques based on Magnetic Resonance Imaging (MRI) have addressed brain correlates of visuospatial and social deficits in NVLD. Concerning the former, an anatomical MRI study measured the volume of the splenium of the corpus callosum, connecting temporal, posterior parietal, and occipital cortices across the two hemispheres. Compared to TD children and other clinical groups (Attention Deficit-Hyperactivity Disorder and Autism Spectrum Disorder), the NVLD group showed smaller splenial volumes, which were associated with lower performance IQ but not Verbal IQ scores, suggesting that the visuospatial deficits may derive from the inability to integrate visuoperceptual and visuospatial information across the hemispheres [29]. A second MRI study compared resting state functional connectivity (rs-FC) among children with NVLD, reading disorder (RD) and TD children [30]. They analyzed a broad spatial network including nodes in the Dorsal Attentional Network (DAN) involved in VSWM and spatial attention (see below), as well as other cortical areas involved in topographical memory (retrosplenial cortex, parahippocampal gyrus, and others). Across all groups, global network efficiency was associated to

performance IQ. Within the spatial network, reduced rs-FC in NVLD relative to the other two groups combined was found between the left posterior cingulate cortex (PCC) and the right retrolimbic area (RA), which correlated with differences between groups in performance IQ [30]. Since the splenium contains fibers directly connecting left and right retrosplenial cortices [31] - which include both PCC and RA-, these results could still be accounted for by a white matter abnormality centered in the splenium, as reported in the previous study [29]. Concerning the neural substrates of social abilities in NVLD, a structural MRI study found smaller volume of the Anterior Cingulate Cortex (ACC) in NVLD relative to typically developing (TD) children [32]. A second study reported reduced rs-FC between ACC and the anterior insula (hubs of the Salience Network) in NVLD relative to TD children [33].

In recent years, developments of the EEG technique, including high density sensor arrays and EEG cortical source modeling, allow the study of functional connectivity with much greater detail and spatial resolution then before. 'Dynamic network neuroscience' aims to investigate the interconnected nature of neurophysiological phenomena underlying human cognition in health and disease [34,35]. A flexible dynamic reconfiguration of the modular organization of cortical networks has been related to learning proficiency in healthy individuals [34], to memory and executive functions performances [36], and to social cognitive abilities [37]. This approach has been recently successfully employed in network disorders such as temporal lobe epilepsy [38].

2.1.2 Visuospatial Working Memory and EEG oscillations

In EEG studies with healthy volunteers performing visuospatial processing tasks, findings were reported associating oscillatory patterns to spatial short-term memory, in particular mental rotation, especially in the gamma band (30-80 Hz) [39,40], but also in the beta band (5-30 Hz) [41]. Of relevance was a review by Tallon-Baudry [42], concluding that in working memory tasks gamma and beta bands address different

processing stages. The gamma frequency would be prevalent during the presentation of the item to be held in memory, while the beta band would be prominent during the maintenance period. In fact, Von Stein and Sarnthein [43] proposed that the bottom-up or perceptually driven processes are mediated by local gamma frequency, whereas top-down processes would involve long distant oscillations in the beta, alpha (8-12 Hz) and theta (4-7 Hz) bands. The gamma frequency band has also been linked to perceptual binding, that is the process whereby the sensory stimuli are combined together in order to create a meaningful and unitary percept.

Importantly, Basso Garcia et al. [44] assessed visual short-term memory for shapes and colors and the binding of shapes and colors comparing a group of children at risk of NVLD with a control group. They found that groups did not differ in retention of either shapes or colors, but children at risk of NVLD were poorer than controls in memory for shape-color bindings, exhibiting a binding deficit.

2.1.3 Spatial Attentional Networks

The investigation of brain network dynamics through high-density EEG and cortical source modeling may help to elucidate the neural mechanisms underlying cognitive impairment in NVLD. Particularly relevant would be to address potential abnormalities in the dorsal and ventral attentional networks (DAN and VAN), two anatomically and functionally distinct cortical systems previously identified by fMRI studies of active attentional processing, mainly involved in top-down and bottom-up attentional processes respectively [45].

The DAN supports endogenous attention and comprises the Frontal Eye Fields (FEF) and the Intraparietal Sulcus (IPS). These core regions have retinotopically organized maps of contralateral space [46] which make them particularly suitable for VSWM processes. Activity in these frontal and parietal areas creates maps of prioritized space that rank the importance of locations in the visual field in accordance with their attentional priority [47]. The priority maps are then used in order to select between

competing representations of actions in the motor system or between competing representations of objects in the visual system. In addition, there is a substantial body of research describing the involvement of DAN in two main contexts: The representation of spatial information [47,48], and working memory and sustained attention tasks [49,50].

The VAN supports exogenous attentional processes recruiting areas of the Ventral Prefrontal cortex (VPFC) and the Temporo-Parietal Junction (TPJ). This network has been found to be lateralized to the right [51] but neuroimaging studies have highlighted also a role of the left hemisphere for the TPJ in attentional processes [52], or a bilateral involvement of the same region [53].

2.1.4 Aims

Based upon these premises, the first aim of the present project was to attempt to discriminate between the NVLD and TD groups employing maps of EEG resting state functional connectivity (rs-FC) in the DAN and VAN of the left and right hemisphere by applying a machine learning approach, i.e., a support vector regression (SVR) model. The second aim was to determine whether such rs-FC measures would be predictive of performance in the visuospatial domain. Our final aim was to apply graph theoretical analysis to ascertain whether network topology properties (degree, strength, clustering coefficient, and local efficiency) would be effective measures for discriminating between NVLD and TD groups.

2.1.5 Hypotheses

We hypothesized that the DAN and VAN would show differential resting state connectivity maps between the two groups, and that this information solely would be able to distinguish between children with and without NVLD. Given their relevance for visuospatial and working memory processes, we predicted these changes mostly to affect gamma and beta frequency bands. We further hypothesized that visuospatial

performance in NVLD would more robustly depend on connectivity patterns within the DAN - particularly in the right hemisphere, given its role in active visuospatial processing. Finally, we predicted that, by examining each cortical node falling in the two neural networks in the two groups, the NVLD cohort would show differential connectivity patterns and networks' topology from TD children.

2.2 Materials and Methods

2.2.1 Participants

A total of 32 participants (16 males and 16 females), aged 8 to 16 years old, were selected to take part in the present study. The experimental group included participants diagnosed with NVLD (n = 16, 2 left-handed) and participants without any diagnosis (not diagnosed), and for whom a typical development was assumed (TD, n = 16; 3 left-handed). Only children who achieved a standard score of 80 or above on the full-scale IQ on the Wechsler Intelligence Scale (WISC-IV; [54]) were included in the sample. All participants were native Italian speakers and had normal or corrected-to-normal vision and hearing. None of them had a history of neurological and/or psychiatric disorders, as reported by an anamnestic interview conducted with parents. NVLD and TD groups were not statistically different regarding chronological age [F (1, 30) = 0.049, p = .827, η^2_p = 0.002], gender distribution [$\chi 2$ (1) = 0.183, p = .669] and verbal abilities [F (1, 30) = 0.732, p = .399, η^2_p = 0.024], as measured using the Vocabulary subtest from the WISC-IV (Wechsler, 2003).

Children with NVLD had previously received an independent clinical diagnosis by private psychologists or child psychiatrists at clinical specialized centers, following recommendations from the literature [55], while children in the TD group were recruited via local schools or community contacts. The Developmental Test of Visual-Motor Integration (VMI; [56]) was used as a screening measure to assess visuospatial processing: the two groups' performances resulted statistically different one from the

another [F (1, 30) = 21.550, p < .001, η^2_p = 0.519], highlighting the presence of significant impairments only in the NVLD group. Moreover, aiming to perform a differential diagnosis between NVLD and Autism Spectrum Disorder (ASD) without intellectual disability, the Autism Diagnostic Interview-Revised (ADI-R; [57]) was administered to the participants' parents. All participants, from both the NVLD and the TD groups, scored below the clinical cut-offs in all the assessed areas (*i.e.*, Reciprocal Social Interactions, Language/Communication, and Repetitive Behaviors/Interests). Descriptive statistics concerning inclusion and screening measures are provided in Table 1.

Table 1. Descriptive statistics for the inclusion and screening measures.

Measures	NVLD (N = 16) Mean (SD)	TD (N = 16) Mean (SD)	Group differences
Chronological age (months)	157.19 (21.78)	155.44 (23.09)	NS
Gender (M:F)	12:4	14:2	NS
Vocabulary [1]	11.56 (2.53)	12.25 (1.98)	NS
VMI[2]	77.92 (11.96)	101.60 (11.86)	NVLD < TD
ADI-R[3]: A (Reciprocal Social Interactions)	6.31 (4.99)	2.29 (2.28)	both groups < clinical cut-off
ADI-R[3]: B (Language/Communication)	4.88 (3.65)	2.12 (2.62)	both groups < clinical cut-off
ADI-R[3]: C (Repetitive Behaviors/Interests)	2.64 (2.37)	0.41 (0.62)	both groups < clinical cut-off

[1] Scaled scores on Vocabulary subtest from [54].
[2] Standard scores in the Developmental Test of Visual-Motor Integration (VMI; [56]).
[3] Autism Diagnostic Interview-Revised (ADI-R; [57]), higher raw scores reflect more severe impairments in each domain.

All participants' parents or legal-guardians gave written informed consent before the experiment, and the participants' own agreement to take part in the study was acquired. All experimental procedures were approved by the Ethics Committee of the

School of Psychology at the University of Padua (protocol n◦ 3921) and were conducted according to the principles expressed in the Declaration of Helsinki.

2.2.2 Visuospatial performance

The Rey–Osterrieth complex figure test (ROCFT; [58]) assesses visuo-constructive abilities and visuospatial memory. Participants are asked to copy a complex geometrical figure as accurately as possible. After 3 minutes, they are requested to reproduce it from memory. Accuracy is determined by scoring each element based on its presence, accurate reproduction, positioning and respect for proportions [58]. In the present study, individual scores for the copy and recall portions were averaged together for the behavioral prediction from EEG connectivity data (see below).

2.2.3 EEG Resting-State recording

For each participant, the rs HD-EEG activity was recorded before the active tasks (not reported here) in a 4-minute eyes closed session. We used a Geodesic high-density EEG System (EGI® Net Amp GES-400) with a pre-cabled 256-channels, through NetStation EEG Software. The elastomer structure of the EEG net is formed by polyvinyl alcohol sponges that are housed within the HydroCel Hydrating Skin interface chamber. The sampling rate of the recording was set to 500 Hz with an automatic alignment of real time EEG.

2.2.4 EEG preprocessing

The preprocessing was performed in MATLAB (v2019b) using functions from the EEGLab (v.2020.048) Toolbox. Continuous data were down-sampled to 256 Hz, high-pass filtered at 0.01 Hz, and re-referenced to the average of all channels. Following, the clean_artifacts routine in EEGLab was used with default parameters to detect bad channels and exclude them from further processing. A lowpass filter was applied at 30

Hz and excluded channels were interpolated. Finally, Independent Component Analysis (ICA) was performed, and artifact components were marked with ICLabel and manually discarded.

2.2.5 EEG Source Modeling and Connectivity Analysis

The processing phase was carried out with Brainstorm and Matlab (MathWorks, Inc.). In order to model the source activity, a forward model was calculated with the BEM, a three-layer boundary element method, and the source was estimated with the weighted Minimum Norm Estimation (wMNE) method. This inverse solution was then downsampled to 148 cortical parcels defined by the Destrieux Atlas [59]. The connectivity matrices were calculated with the Magnitude Squared Coherence (MSC), which describes the linear relationship (covariance) between two signals in the frequency domain and it is calculate as follows:

$$| C_{xy}(f) |^2 = \left(\frac{| S_{xy}(\mathrm{f}) |}{\sqrt{S_{xx}(\mathrm{f}) S_{yy}(\mathrm{f})}} \right)^2 \quad (1)$$

$S_{xy}(\mathrm{f})$: Cross-spectrum

$S_{xx}(\mathrm{f})\ and\ S_{yy}(\mathrm{f})$: Auto-spectra or power spectral density

Thus, the MSC (C) between two signals (x and y) is estimated by the square of the coherence value between x and y divided by the square root of the coherence of x with x multiplied by the cohere of y with y.

2.2.6 Discrimination Between NVLD and TD groups: A machine learning approach

After EEG signal preprocessing, source-reconstructed cortical activity and whole-brain resting state functional connectivity (rs-FC) were computed. Subsequently, phase coherence values were extracted from the parceled cortex (Destrieux atlas, 148 ROIs; [59]) to estimate individual rs-FC in the DAN and the VAN. These cortical networks were distinguished by hemisphere, given the recognized role of the right hemisphere for visuospatial processing [23–25]. A machine-learning approach (i.e., support vector machine) was applied in order to investigate whether group membership could be predicted from rs-FC maps in each hemisphere and frequency band (Delta: 2-4 Hz, Theta: 5-7 Hz, Alpha: 8-12 Hz, Beta: 15-29 Hz, Gamma: 30-59 Hz). The objective of this first analysis was to investigate whether: (i) there was such information, within the coherence maps of the selected networks of interest, able to discriminate between the NVLD and TD group; (ii) the hemisphere played a crucial role in differentiating the two groups. In order to test these hypotheses, we used a machine learning approach based on the SVM classifier. The Matlab functions *svmtrain* and *svmclassify*, respectively, were used in order to train a linear SVM model (with default parameters) for discriminating between the clinical and the control group, starting from the functional connectivity matrices. Matlab function *cvpartition* was employed, at each run, for implementing the *leave one subject out* cross-validation scheme. The prediction accuracy was computed at the end of the cross-validation loop on the corrected predicted classes (one per each test subject, at each run).

2.2.7 Behavioural predictions from functional connectivity matrices

In order to understand the characteristics driving the successful classification of FC maps of NVLD with respect to TD children, we applied a series of linear regression

models. The goal of this approach was to link the functional connectivity information, which could discriminate the NVLD group from the control one, to the individual visuospatial performance in the ROCFT. We adopted an approach similar to that used in Duma et al. [60]. A SVR (Support Vector Regression) model can be considered a generalization of the SVM model for regression problems. For each group, network of interest (considered separately in the left and right hemisphere) and frequency band, we created a dataset of connectivity patterns, one per subject. In this case, we have only one sample (i.e., the coherence value) per subject for the entire resting state activity, thus our regression problem was implemented at the population level.

In more details, each subject-connectivity pattern was obtained by extracting the inferior triangular part of the coherence adjacency matrix. As reported above, the target parameter to be predicted from the population-connectivity data was the average performance in the ROCFT across the copy and memory parts. A leave-one-subject-out cross-validation (i.e., a leave-one-out cross validation scheme, implemented across population) was used to estimate the test generalization prediction accuracy. Specifically, for each group separately (i.e., the NVLD and the TD group), the training and test phases were performed a number of times (runs) equal to the population size (i.e., 16 subjects). For each run, we randomly selected and used data from all the subjects but one (i.e., 15) for the training phase, and the data from the discarded subject were used as a test sample. As suggested by Yadav et al. [61], leave-one-out cross validation is preferred with datasets with a sample number less than 100. By contrast, with very large datasets, using this cross-validation scheme could increase the overfitting probability, and therefore other schemes, like-folds, can be used [61,62]. For these reasons, given our sample size dimension (i.e., 16 subjects per group) we selected this cross-validation scheme amongst the other possible ones. The ROCFT target parameter was standardized (z-scored) in order to have zero mean and standard deviation one.

Moreover, the connectivity data matrix for training was also standardized across subjects. We used the Matlab functions *fitrsvm* and *predict*, respectively, to train a linear

SVR model (used with default parameters) and predict the target parameter of the test subject, from functional connectivity matrices. Matlab function *cvpartition* was used, at each run, for implementing the *leave one subject out* cross-validation scheme. The prediction accuracy was computed at the end of the cross-validation loop on the predicted parameter values (one per each test subject, at each run). Specifically, we computed the Bayesian correlation between the vector containing the concatenation of the target value to be predicted at each run (i.e., one for each test subject) and the vector containing the concatenation of the corresponding predicted values. Thus, the prediction accuracy was expressed in terms of a correlation coefficient (see [60,63] for a similar procedure applied respectively to EEG and fMRI data). We used Bayesian correlation since it provides a measure of the likelihood of the alternative hypothesis. Only positive correlations were reported as an index of a good-quality fitting. Note that negative correlations are an index of a very bad fitting and therefore were not considered. Using Bayesian correlation allows us to get a ratio between the null hypothesis vs. the alternative hypothesis, and provides a measure of the strength of evidence of one hypothesis over the other, which is highly valuable in clinical research. In the frequentist approach, instead, the p-value is not informative for the alternate hypothesis, it only computes the likelihood of the null hypothesis and allows researchers to discard it. Moreover, the use of the Bayes Factor Robustness Check, allows us to test the robustness of the obtained results by varying the beta-prior width. This analysis has been performed using the software JASP (https://jasp-stats.org/). We reported the Bayes Factor (BF) in favor of the alternative Hypothesis H1 (positively correlated), according to which there is a positive correlation between the considered variables. Note that the reported BF values correspond to the assumption of a Cauchy prior width equal to 0.5, but contextually, a BF robustness check has been considered in order to estimate the robustness of the results. Considering the scale of interpretation of the BF (Jeffreys, 1998), we adopted a conservative approach, reporting only the correlations with a BFs ≥ 3, which is considered from moderate ($3 <= BF < 10$) to a strong ($10 <= BF < 30$), very

strong (30 <= BF< 100), or extreme (BF >= 100) evidence toward the alternative hypothesis.

2.2.8 Discrimination between NVLD and TD: a graph theory approach

2.2.8.1 Graph Construction

We used a single-subject-connectivity-matrix approach, as suggested by Langer et al. [64]. Thus, for each network of interest, frequency band, and subject, we constructed a graph and then extracted some graph measures (i.e., 3 global measures and 3 local measures) by using the Brain Connectivity Toolbox (BCT). Graphs were constructed starting from the N × N adjacency matrix, where N is the number of ROIs included into the network under examination. Note that graph connections were not binarized, in order to avoid a loss of information. Moreover, we maintained all the weighted connections in graph construction. For reasons related to the numerosity of nodes in our selected networks (i.e., twelve nodes in both hemispheres), we preferred a more conservative approach. Indeed, in this way we preserved complete network topology and retained the maximum information within the graphs, avoiding defragmented structures and its consequent loss of information.

2.2.8.2 Graph measures

All the measures were computed on the weighted graphs normalized by using the function weight conversion (with the parameter option “normalize”), contained in the BCT for normalizing the graph connectivity. This function scales all weight connections to the range [0, 1] by dividing the connection values to the maximal weight and should be done prior to computing some network parameters (e.g., clustering coefficient). Indeed, since network measures strictly depend on the mean of the weighted connections, weighted graphs need to be normalized in order to perform statistical analysis on the extracted measures. We extracted graph measures both at a global and a

local (i.e., nodal) level. For global measures, we tested the hypothesis of a difference between the NVLD and the TD control group, taking into account potential hemispheric asymmetries. Thus, we extracted the global measures on the graphs computed separately for each hemisphere (e.g., Left DAN and Right DAN graphs). At the nodal level, we extracted the graph measures from the complete graphs, considering both the hemispheres in a single graph (i.e., bilateral DAN and VAN).

2.2.8.3 Global measures

In order to characterize both segregation and integration properties of each functional network at rest, we extracted three global measures (i.e., one value per subject for each graph of interest, in each hemisphere - L DAN, R DAN, L VAN, R VAN): (i) *Global efficiency*, which can index both segregation and integration functional properties; (ii) *Assortativity*, which can index the presence of hierarchy in structuring the information flow; (iii) *Modularity*, which can index segregation (i.e., specificity) in information elaboration.

2.2.8.4 Nodal measures

At a nodal level, we extracted two local measures (i.e., one value per subject for each node within the graph of interest - DAN and VAN) from each considered brain network modeled as a graph: (i) *Degree*, which is the number of connections incident to the node, survived after normalization of the adjacency matrix, and could be interpreted as an index of integration (ii) *Strength*, which is computed for each node of the graph as the sum of the weights on its connections, and could be interpreted as an index of integration and synchronization of brain activity; (iii) *Clustering coefficient*, which quantifies, for each node, how close its neighbors are to being a complete graph; it reflects the prevalence of clustered connectivity around individual nodes, and roughly corresponds to an index of segregation and specialization.

2.2.8.5 Statistical analyses between NVLD and TD

We performed Bayesian Independent Sample T-tests using the software JASP (https://jasp-stats.org/), reporting the Bayes Factor (BF) in favor of the alternative Hypothesis H1 (NVLD ≠ TD). As described for the correlation analysis, we used Bayesian independent t-test that provides a measure of the strength of evidence of the alternative hypothesis over the null hypothesis, by considering the previously mentioned parameters.

2.3 Results

2.3.1 Visuospatial performance measures

As expected, the NVLD and the TD control group significantly differed both in the copy and the memory parts of the Rey-Osterrieth Complex Figure Test, with worst performance in the NVLD group. For consistency with the EEG connectivity approach, results in Table 2 employed Bayesian independent sample t-tests. Note that the results held the same employing traditional F-tests for the ROCFT copy [F (1, 30) = 29.67, p < .001, η^2_p = 0.497] and the ROCFT recall [F (1, 30) = 22.19, p < .001, η^2_p = 0.425].

Table 2. Descriptive statistics for the copy and recall trials of the Rey-Osterrieth Complex Figure Test.

Measures	NVLD (N = 16) Mean (SD)	TD (N = 16) Mean (SD)	BF_{10}	Group significance
ROCFT copy trial[1]	-4.93 (2.69)	-0.88 (1.27)	44791.26	NVLD < TD
ROCFT recall trial[1]	-2.52 (1.15)	-0.56 (1.21)	357.90	NVLD < TD

[1] Z-scores on the Rey-Osterrieth Complex Figure Test [58,65].

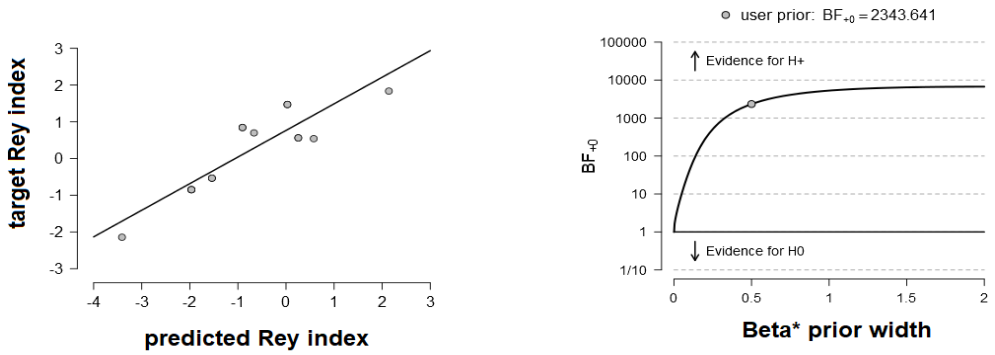
2.3.2 Rs-connectivity differences in the DAN and in the VAN

The support vector machine model highlighted that our selected networks contained information able to discriminate between the NVLD and TD group. Specifically, classification results showed that the DAN in both hemispheres (left DAN: accuracy of 62.5% in the beta band, 62.5% in the delta band, and 84.38% in the gamma band; right DAN: accuracy of 56.29% in the alpha band and 59.38 in the delta band), and the VAN in both hemispheres (left VAN: accuracy of 65.63% for the beta band; right VAN: accuracy of 59.38 % in alpha, beta and gamma bands, and 65.63% in the theta band) contained functional connectivity information able to discriminate (above the chance level of 50%) between the two groups. We focused the analysis on graph-theory measures on those frequency bands that were most informative, on average, across networks and hemispheres, in discriminating between the two groups: i.e., beta (M = 57.03, SE = 2.81) and gamma (M = 56.25, SE = 5.34) bands. For the remaining frequency bands, the mean accuracy across hemispheres and networks did not exceed the chance level of 50% (alpha: M = 49.22, SE = 10,33; delta: M = 50, SE = 15,52; theta: M = 42,19, SE = 20,65). In order to better understand and characterize these findings, we applied successive analyses based on graph theory and regression models, focusing on the beta and gamma frequency bands.

2.3.3 Behavior prediction from functional connectivity matrices

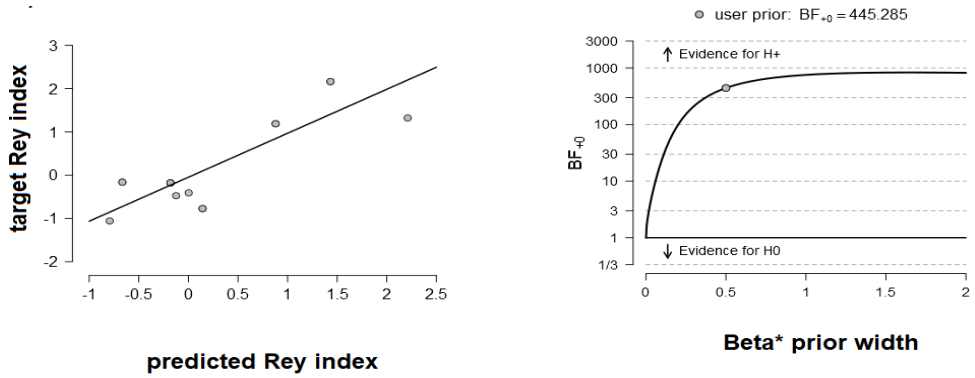
Results showed a differential pattern of predictions in NVLD and TD children. In the TD group, rs-functional connectivity the left DAN in the gamma band (R = 0.89, BF_{10} = 2343.64, extreme evidence) and in the left VAN in the delta band (R = 0.55, BF_{10} = 5.65, moderate evidence) were predictive of visuospatial abilities in the ROFC. In sharp contrast, in the NVLD group, it was rs-functional connectivity in the right DAN in the delta band to be predictive of visuospatial performance level (R = 0.84, BF_{10} = 445.29, extreme evidence). Figure 1 and 2 (panel b), show the BF robustness checks for

the predictions where the evidence was preserved as extreme (BFs_{10} > 100) at varying the Cauchy prior width.



(**a**) (**b**)

Figure 1. Prediction of the visuospatial performance from the connectivity maps in TD children. (**a**): prediction of visuospatial individual performance (as indexed by the Rey index) from rs-functional connectivity within the left Dorsal Attentional Network (DAN; R = 0.89, BF_{10} = 2343.64) in TD children in the gamma frequency band. (**b**): the BF robustness check showed that the evidence was preserved as extreme (BFs_{10} > 1000) at varying the Cauchy prior width.



(**a**) (**b**)

Figure 2. Prediction of the visuospatial performance from the connectivity maps in NVLD children. (**a**): prediction of visuospatial individual performance (as indexed by the Rey index) from rs-functional connectivity

within the right Dorsal Attentional Network (DAN; R = 0.84, BF_{10} = 445.28) in NVLD children in the delta frequency band. (**b**): the BF robustness check showed that the evidence was preserved as extreme (BF_{10} > 100) at varying the Cauchy prior width.

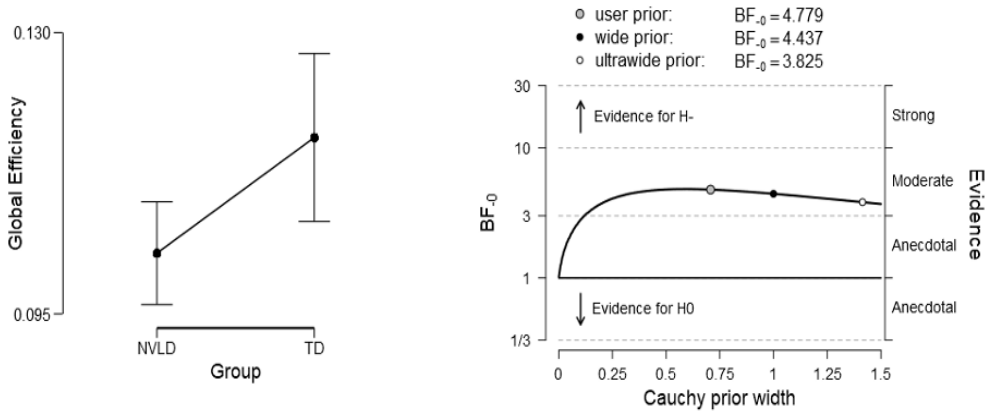
Table 3. Results of the regression analysis including the coherence level in the networks and the visuospatial performance in the two groups separately. The Bayes Factor is related to the robustness check on the analysis [10 > BF > 3 = moderate evidence, 30 > BF > 10 = strong, 100 > BF > 30 = very strong, BF > 100 = extreme].

ROI	Freq-bands	Group	BF_{10}
R-DAN	delta	NVLD	445.285
L-DAN	gamma	TD	2.343.641
L-VAN	delta	TD	5.645

2.3.4 Discrimination between NVLD and TD: a graph theory approach

2.3.4.1 Global measures

At a global level, we tested the directional hypothesis that the *global efficiency* of the NVLD group is lower than that of the TD control group. Bayesian independent sample t-tests showed that in the left VAN (in the beta band), there was moderate evidence of a lower mean global efficiency for the NVLD relative to the control children (BF_{10} = 4.78; NVLD: M = 0.10, SE = 0.003; TD: M = 0.12, SE = 0.005), as displayed in Figure 3. No effect on *global efficiency* emerged in the gamma band (BF_{10} = 1.45), nor other effects emerged when considering *assortativity* (BF_{10}= 0.18) or *modularity* (BF_{10}= 0.2) across networks and hemispheres. As shown in Figure 4 (panel B), the BF robustness check showed that the evidence was preserved as moderate (3 < BF_{10}< 10) at varying the Cauchy prior width.



(**a**) (**b**)

Figure 3. (**a**): Mean global efficiency for the NVLD children was lower than that of the controls (BF_{10}= 4.78) in the left VAN (in beta frequency band). (**b**): BF robustness check showed that the evidence was preserved as moderate ($3 < BFs_{10ß} < 10$) at varying the Cauchy prior width.

2.3.4.2 Nodal measures: Dorsal Attentional Network

Results from Bayesian independent sample t-tests showed an *increased* connectivity in the bilateral DAN regions (i.e., Frontal Eye Fields - FEF and IntraParietal Sulcus – IPS, bilaterally) for NVLD children compared to TD controls, as shown by the *degree* and *strength* measures in both beta and gamma frequency bands (see table A1 and A2 in the Appendix A for statistical information, and Figure 4, 5).

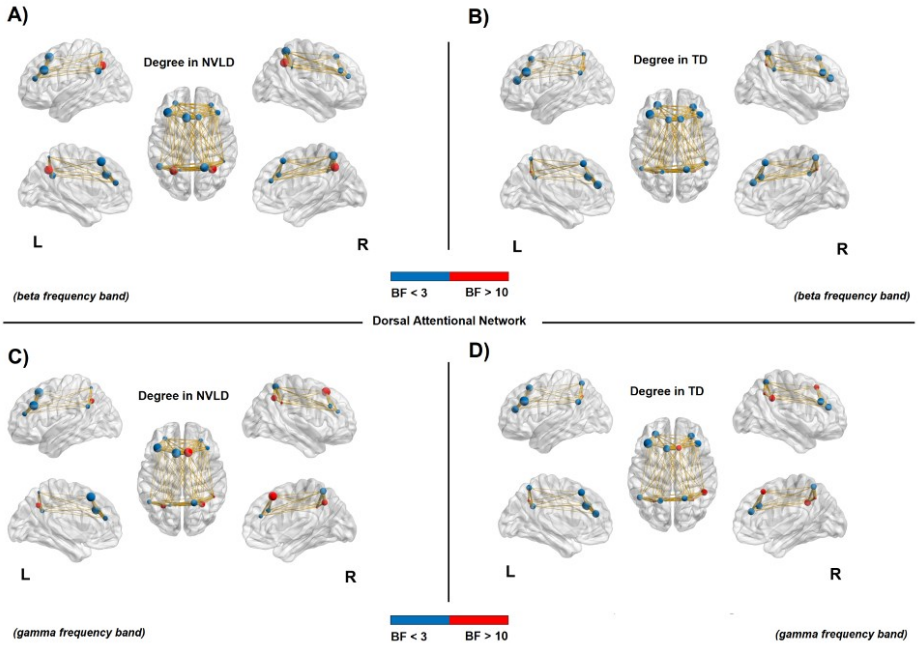


Figure 4. (**a-b**): Increased connectivity degree in the bilateral Intraparietal Sulcus (IPS) within the Dorsal Attentional Network, for NVLD children compared to TD controls beta frequency band. (**c-d**): Increased connectivity degree for NVLD children compared to TD controls, in bilateral IPS and areas of the Frontal Eye Field (FEF) in the right hemisphere, within the Dorsal Attentional Network, in the gamma frequency band (see table A1, for details on statistics).

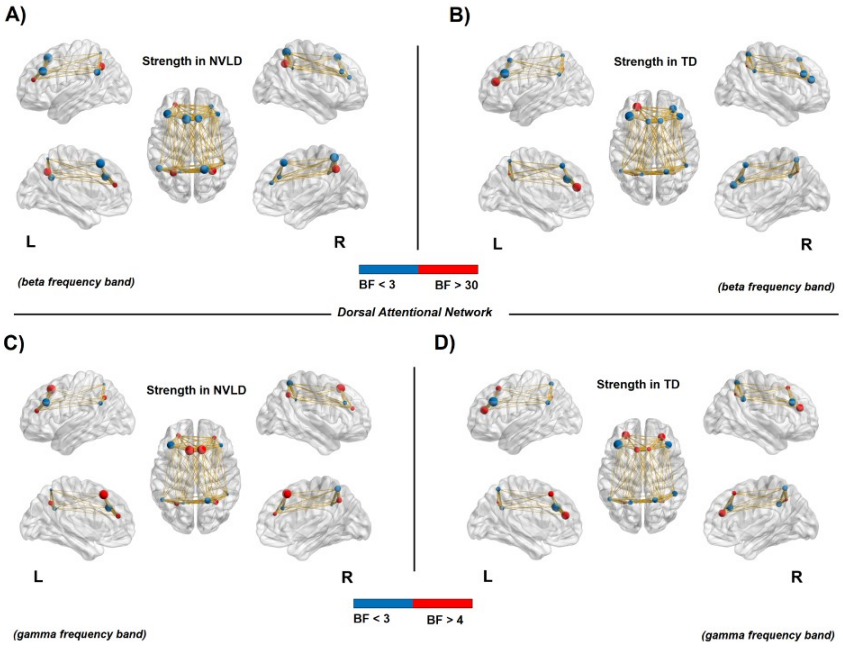


Figure 5. (**a-b**): Increased connectivity strength in the bilateral Frontal Eye Field (FEF) areas within the Dorsal Attentional Network, for NVLD children compared to TD controls beta frequency band. (**c-d**): Increased connectivity strength for NVLD children compared to TD controls, in bilateral IPS and areas of the Frontal Eye Field (FEF) in the right hemisphere, within the Dorsal Attentional Network, in the gamma frequency band (see table A2, for details on statistics).

Instead, bilateral frontal areas of the DAN showed a *decreased* local specificity in NVLD children with respect to the TD controls, as measured by a reduced clustering coefficient in the beta band (see table A3 for statistical information in the Appendix A, and Figure 6).

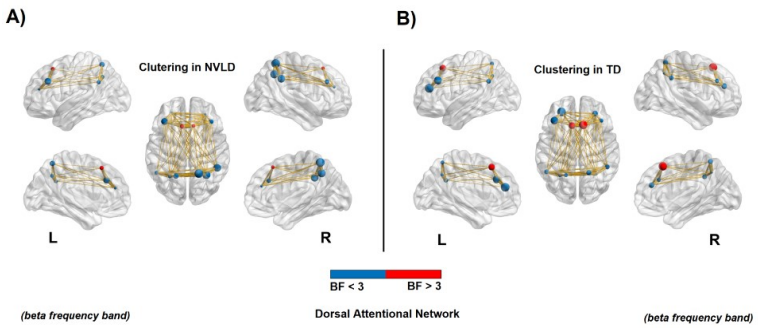


Figure 6. (**a-b**): Decreased clustering coefficient for NVLD children compared to TD controls, in bilateral areas of the Frontal Eye Field (FEF), within the Dorsal Attentional Network, in the beta frequency band (see table A3, for details on statistics).

2.3.4.3 Nodal measures: Ventral Attentional Network

Results from Bayesian independent sample t-tests showed a *decreased* clustering coefficient in the left ventral prefrontal cortex (in the beta band) for the NVLD children, with respect to the controls, as an index of reduced local specificity. The opposite pattern of results emerged in the right Temporo-Parietal junction (in the gamma band), where it was found an *increased* clustering coefficient for the NVLD group with respect to the TD group (see table A4 in Appendix A for statistical information, and Figure 7).

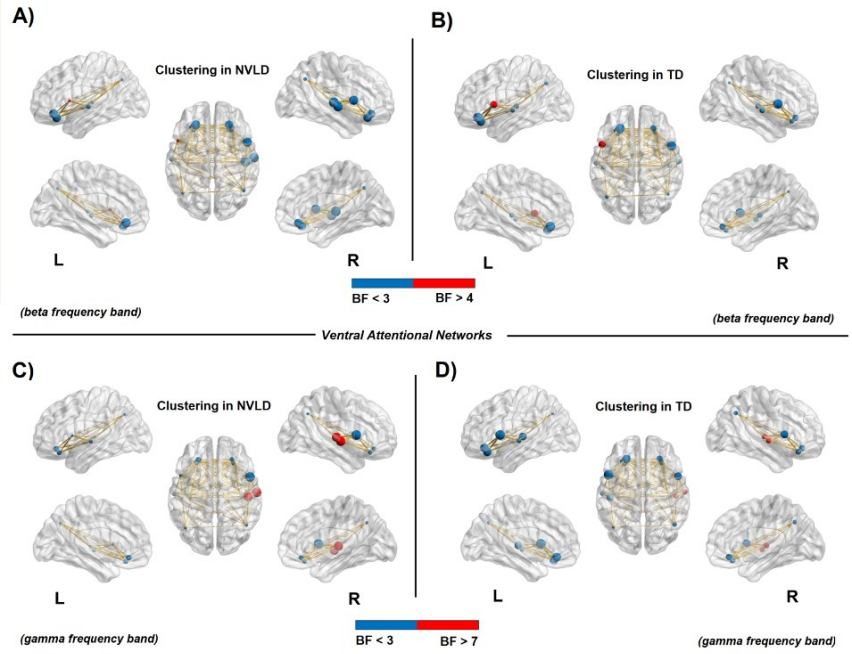


Figure 7. (**a-b**): Decreased clustering coefficient for NVLD children compared to TD controls, in left frontal areas, within the Ventral Attentional Network, in the beta frequency band. (**c-d**): Increased clustering coefficient for NVLD children compared to TD controls, in areas within the right Temporo-Parietal Junction (TPJ), within the Ventral Attentional Network, in the gamma frequency band (see table A4, for details on statistics).

2.4 Discussion

Visuospatial performance levels and EEG resting state functional connectivity in spatial attention networks were investigated in children with nonverbal learning disability (NVLD) and in typically developing (TD) children. A machine learning approach was employed to test whether the two groups could be discriminated by EEG connectivity patterns. The resting state connectivity maps were then employed to predict the individual performance in the visuospatial domain. Finally, a graph theoretical approach was employed to determine whether network topology properties (degree, strength, clustering coefficient, and local efficiency) would be effective measures for discriminating between NVLD and TD groups.

2.4.1 Behavioural measures

As expected, performance in Rey-Osterrieth complex figure was significantly different in children with and without NVLD as previous research reported [8,11,16,66], reflecting the presence of core visuospatial and visuoconstructive processing deficits among the NVLD children.

2.4.2 EEG rs-functional connectivity: discrimination between NVLD and TD

The support vector machine model proved that the selected networks of interest (DAN and VAN) contained information able to reliably discriminate between the NVLD and TD groups, based on resting EEG functional connectivity in the gamma and beta bands which are implicated in visuospatial and working memory processing in healthy participants [39–41]. The centrality of gamma band abnormalities confirms the results of a previous resting EEG study comparing a NVLD group to a group with verbal LD, reporting a decrease in gamma band coherence between distant locations in the right hemisphere [26].

2.4.3 EEG rs-functional connectivity: behaviour prediction

An important and novel finding of this study is that, while for the control TD children, increased resting state connectivity (rs-FC) in the DAN in the gamma band (in the left hemisphere) strongly predicted visuospatial performance, in the NVLD group it was rs-FC in the delta band in the right DAN to strongly predict individual visuospatial performance. The results in the TD children confirm the role of gamma rhythm in spatial and working memory processes in healthy individuals [39,40]. The different prediction profile in NVLD can be explained by previous resting EEG findings in verbal learning disabilities reporting a preponderance of slow frequency activity (“slowing”) [67–69]. In one such study, more delta power in fronto-temporal regions predicted worse

educational evaluations in children with verbal learning disorder, interpreted as a sign of underlying cerebral dysfunction in areas involved in reading and writing processes [70].

Importantly, it has been proposed that the slower EEG activity of children with an LD is akin to that of younger healthy children, since slow EEG activity in the delta range is prevalent in early life, later replaced by faster rhythms [71]. This apparent lag in the brain functional development of children with LDs has led to the hypothesis that a delay in the maturation of brain's electrical activity impairs children's ability to keep up with their academic milestones [69]. In agreement with the above studies and their conclusions, we interpret the NVLD-specific behavioral prediction with rs-FC in the right DAN in the present study as indicating a suboptimally functioning resting-state network that provides a detrimental 'starting point' for task-specific brain activations, pinpointing to an inefficient neural resource control due to a delay in neural maturation.

2.4.4 EEG rs-functional connectivity: discrimination between NVLD and TD with a graph theory approach

In this paper we also report novel evidence of reconfiguration of resting state functional connectivity in spatial attention networks relevant to core symptoms in the visuospatial domain in NVLD. Within the DAN, we found evidence of bilaterally increased functional connectivity at rest for NVLD compared to the TD group, measured through the graph measures of degree and strength. Importantly however, such increased functional connectivity was associated with reduced local specificity in the frontal nodes of the same network for NVLD children (FEF). The specificity was calculated with the clustering coefficient which represents the propensity of the network to segregate in order to execute specialized processes. In other words, in the frontal nodes such connections were more diffuse, less modular: they were not directed within the neural nodes composing the functional network.

Within the VAN, the global efficiency of the left VAN was found to be reduced in the NVLD group. Furthermore, children with NVLD exhibited reduced local specificity (segregation) in the ventral prefrontal (opercular) areas, coupled with an opposite pattern of increased clustering (segregation) in the posterior node of the temporo-parietal junction (TPJ) of the right hemisphere.

2.4.5 Reconfiguration of rs-functional connectivity in NVLD

The present study reports a substantial reconfiguration of resting state connectivity of the spatial attentional networks in NVLD. We propose here that in NVLD slow rs-EEG connectivity in the delta band, predicting visuospatial performance in the NVLD group, may index immature functional interregional coupling, as a consequence of a primary white matter abnormality in the right dorsal attentional network, specialized for active aspects of visuospatial processing compromised in the disorder, while that the bilateral increased gamma connectivity in the DAN, combined to the more diffuse (less modular) gamma connectivity in the frontal nodes, may represent secondary plastic changes in an ineffective attempt to compensate for the primary connectivity dysfunction. Similarly, reconfiguration of functional connections in the VAN in NVLD may constitute an additional compensatory mechanism recruiting more the right TPJ, typically activated by exogenous, bottom-up, more automatic spatial orienting to targets [45].

2.4.6 Hemispheric differences

Our behavioral prediction findings provide evidence that an abnormality at the level of the right dorsal attentional network explains visuoconstructive and visuospatial deficits in NVLD, bringing some support to the centrality of intact right hemisphere white matter fibers in this neurodelopmental disorder [1]. Mind however that the machine learning approach for the rs-connectivity measures found that NVLD and neurotypical children were discriminated by differential gamma band and beta band

connectivity patterns *bilaterally* in the DAN and VAN. Moreover, gamma band connectivity at rest in the *left* DAN strongly predicted visuospatial performance in the neurotypical group.

A body of evidence in the literature suggests that the verbal/left visuospatial/right dichotomy may be too simplistic, especially when considering visuospatial working memory in its active, effortful, manipulation aspects. First, an in-vivo structural MRI study in brain injured patients using voxel-based lesion symptom mapping approach, reported that visuospatial working memory, measured by visuospatial span, was most impaired for lesions in the *left* hemisphere centered on areas of the fronto-parietal network, such as the FEF [72]. This confirmed earlier lesion correlation evidence reporting both verbal and visuospatial short term memory deficits in left-hemisphere patients with aphasia [73–75].

Second, several neuroimaging studies in healthy volunteers reported increased activity in the DAN bilaterally (both frontal and parietal nodes) in both verbal and visual working memory tasks [76–78], and another study reported activation of left prefrontal areas in a short-term memory task involving abstract objects [79]. The latter, influential study brought the authors to argue against the established left vs. right hemisphere specialization for verbal and nonverbal/visuospatial processing, respectively. Based on the evidence mentioned above, our findings of a prediction of functional connectivity in the *left* DAN to visuospatial performance for the TD children are therefore not in contrast with extant literature.

2.5 Caveats and future directions

Because of their relevance to the visuospatial processing symptoms in NVLD, and since we wanted to avoid issues related to significance of multiple comparisons, the present study used a network of interest approach, only exploring resting state functional connectivity in cortical networks clearly associated to spatial attention (i.e., DAN and VAN). However, other cortical networks may be relevant in the association

to NVLD symptoms. One of them may be the Default Mode Network (DMN), which has been associated with social cognition and whose abnormality may explain the mild deficits of NVLD individuals in social abilities. A previous resting state fMRI study comparing NVLD, reading disorder (RD) and TD children found reduced connectivity in NLVD only among posterior DMN regions of both hemispheres [30].

Second, it would be important to test the specificity of the present EEG connectivity abnormalities in NVLD, extending the same approach to other neurodevelopmental disorders and, first of all, to autism spectrum disorder, which shares some symptoms in the social domain, albeit of different extent/severity. Finally, it would be important to include measures of social ability such as perspective taking or theory of the mind [80,81] in order to assess differences between the two clinical groups.

Lastly, since our study strongly implicates a primary functional connectivity abnormality in the right DAN, it may be important to conduct in NVLD children a MRI Diffusion Tensor (DTI) tractography study of the superior longitudinal fasciculus in the right hemisphere, particularly the superior longitudinal fasciculus II component connecting the caudal inferior parietal cortex to the dorsolateral prefrontal cortex (dlPFC), providing dlPFC with parietal cortex information regarding perception of visual space [82,83], since this is the primary white matter tract we suspect to be compromised in NVLD.

2.6 Conclusions

The above limitations notwithstanding, this is the first study to assess resting state functional connectivity measures in the DAN and VAN using a state-of-the-art graph metrics approach providing important clues about the topology of functional maps in NVLD and TD groups as a function of the hemisphere, to report that such rs-FC measures can discriminate between children with and without NVLD, and to discover an association between visuospatial processing and the dorsal attentional

network in the right hemisphere of NVLD children as a confirmation that the visuospatial deficit is linked to a right hemisphere pathological process, while an opposite left hemisphere association is present in neurotypical developing children.

2.7 Appendix A

Table A1. Bayesian Independent Samples T-Test (BF = Bayes Factor) and Group Descriptive (Mean, SD = Standard Deviation, and SE = Standard Error) of the Non-Verbal Learning Disability (NVLD) and the control (TD) groups. The dependent variable is the connectivity *degree* in the *Dorsal Attentional Network* in both beta and gamma frequency bands.

Beta frequency band	**BF_{10}**	**Group**	**N**	**Mean**	**SD**	**SE**
L S_intrapariet_and_P_trans	40.70	NVLD	16	1.44	1.03	0.26
		TD	16	0.31	0.60	0.15
R S_intrapariet_and_P_trans	10.46	NVLD	16	1.44	0.89	0.22
		TD	16	0.38	1.03	0.26
Gamma frequncy band	**BF_{10}**	**Group**	**N**	**Mean**	**SD**	**SE**
R G_front_sup	3.52	NVLD	16	1.81	0.83	0.21
		TD	16	1.00	0.97	0.24
L S_intrapariet_and_P_trans	218.12	NVLD	16	1.06	0.85	0.21
		TD	16	0.06	0.25	0.06
R S_intrapariet_and_P_trans	20.93	NVLD	16	1.25	0.86	0.21
		TD	16	0.25	0.78	0.19
R S_interm_prim-Jensen	8.12	NVLD	16	0.50	0.73	0.18
		TD	16	1.25	0.68	0.17

Table A2. Bayesian Independent Samples T-Test (BF = Bayes Factor) and Group Descriptive (Mean, SD = Standard Deviation, and SE = Standard Error) of the Non-Verbal Learning Disability (NVLD) and the control groups (TD). The dependent variable is the connectivity *strength* in the *Dorsal Attentional Network* in both beta and gamma frequency bands.

Beta frequency band	**BF_{10}**	**Group**	**N**	**Mean**	**SD**	**SE**
L S_intrapariet_and_P_trans	35.45	NVLD	16	0.95	0.73	0.18
		TD	16	0.18	0.41	0.10
L S_front_middle	31.10	NVLD	16	0.68	0.63	0.16
		TD	16	1.40	0.46	0.12
R S_intrapariet_and_P_trans	39.96	NVLD	16	0.99	0.72	0.18
		TD	16	0.16	0.51	0.13
Gamma frequency band	**BF_{10}**	**Group**	**N**	**Mean**	**SD**	**SE**
G_front_sup R	1528.70	NVLD	16	1.36	0.53	0.13
		TD	16	0.45	0.44	0.11
S_intrapariet_and_P_trans L	83.33	NVLD	16	0.70	0.66	0.17
		TD	16	0.02	0.07	0.02
S_front_middle L	5.10	NVLD	16	0.71	0.61	0.15
		TD	16	1.26	0.52	0.13

G_front_sup L	7.69	NVLD	16	1.39	0.70	0.17
		TD	16	0.72	0.57	0.14
S_intrapariet_and_P_trans R	12.70	NVLD	16	0.83	0.66	0.17
		TD	16	0.15	0.53	0.13
G_front_middle R	4.45	NVLD	16	0.61	0.65	0.16
		TD	16	1.10	0.34	0.08

Table A3. Bayesian Independent Samples T-Test (BF = Bayes Factor) and Group Descriptive (Mean, SD = Standard Deviation, and SE = Standard Error) of the Non-Verbal Learning Disability (NVLD) and the control groups (TD). The dependent variable is the *clustering coefficient* in the *Dorsal Attentional Network* in the beta frequency bands.

Beta frequency band	**BF_{10}**	**Group**	**N**	**Mean**	**SD**	**SE**
R G_front_sup	3.72	NVLD	16	0.04	0.17	0.04
		TD	16	0.28	0.33	0.08
L S_front_middle	17.6	NVLD	16	0.02	0.07	0.02
		TD	16	0.30	0.32	0.08

Table A4. Bayesian Independent Samples T-Test (BF = Bayes Factor) and Group Descriptive (Mean, SD = Standard Deviation, and SE = Standard Error) of the Non-Verbal Learning Disability (NVLD) and the control groups (TD). The dependent variable is the *clustering coefficient* in the *Ventral Attentional Network* in both beta and gamma frequency bands.

Beta frequency band	**BF_{10}**	**Group**	**N**	**Mean**	**SD**	**SE**
L G_front_inf-Opercular	4.39	NVLD	16	0.01	0.04	0.01
		TD	16	0.21	0.30	0.08
Gamma frequency band	**BF_{10}**	**Group**	**N**	**Mean**	**SD**	**SE**
R G_temp_sup-G_T_transv	7.26	NVLD	16	0.33	0.30	0.08
		TD	16	0.07	0.19	0.05
R G_temp_sup-Lateral	11.29	NVLD	16	0.35	0.30	0.07
		TD	16	0.07	0.19	0.05

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Chapter 3 Visuospatial and Social functioning in NVLD and ASD without Intellectual Disability: A Resting-State EEG study

Nonverbal Learning Disorder (NVLD) and Autism Spectrum Disorder (ASD) without Intellectual Disability display overlapping symptoms in the areas of visuospatial functioning and social abilities. Characterizing brain connectivity patterns related to visuospatial and social functioning can provide confirmatory evidence that NVLD and ASD are indeed distinct neurodevelopmental disorders. Therefore, the first aim was to investigate if the networks related to the cognitive domains of interest contained information able to distinguish between the groups: Dorsal and Ventral Attention Networks for visuospatial, and Defaults Mode and Salience Networks for social. The second aim was to better characterize this information with a graph-theory approach. Finally, we were interested to investigate the link between these functional connectivity maps and the cognitive performance in the visuospatial and social domains. Here we report preliminary results concerning the first goal of the present study pointing to interesting findings about the discriminability of the groups of interest, since the rest of the analysis is still ongoing.

3.1 Introduction

The main aim of the present project is to identify brain biomarkers of two neurodevelopmental disorders, e.g., Nonverbal Learning Disability (NVLD) and Autism Spectrum Disorder (ASD) without Intellectual Disability (ID), which show a

partial overlap in their behavioral presentations, creating a challenge for their diagnosis and consequently for their treatment.

To date, only few studies have investigated structural and functional brain differences between these two disorders using MRI. In a first paper, Semrud-Clikeman, Fine, Bledsoe, and Zhu [1] used structural Magnetic Resonance Imaging (MRI morphometry) to address volume differences in a-priori defined key brain regions for socioemotional functioning between three groups of children: ASD without ID, NVLD, and typically developing (TD) controls. Significantly larger bilateral volumes of the amygdala and the hippocampus were found in the ASD group relative to both the NVLD and the TD group. In contrast, significantly smaller bilateral volumes of the anterior cingulate cortex (ACC) were observed both in children with ASD without ID and in those with NVLD relative to TD children. This was the first neuroimaging evidence showing shared (ACC) as well distinct (amygdala, hippocampus) structural abnormalities between children with ASD without ID and NVLD [1]. The differential effect in ASD is important, in view of the fact that limbic emotion recognition nodes (e.g., the amygdala) mature earlier then corticolimbic emotion nodes of the prefrontal regions (including ACC), involved in social cognition, such as empathy and perspective-taking [2]. A limitation of the study was that it could not determine connectivity between key structures for emotional and social processing. Recent progress in techniques mapping brain connectivity, particularly resting state fMRI (rs-fMRI), has allowed to reliably identify a number of large-scale cognitive networks formed by highly interconnected nodes which level of activity varies in synchrony for each individual during the resting state.

A very recent study employed rs-fMRI to address functional connectivity within and between networks involved in the processing of social information, i.e., the salience network (SN) [3] and the default mode network (DMN) [4]. Margolis, Pagliaccio, Thomas, Banker & Marsh in 2019 [5] collected MRI scans from NVLD and TD

children, and compared their data with those of ASD children from a Brain Imaging exchange database. Within the SN, children with NVLD showed reduced connectivity between the anterior insula and both ACC and rostral prefrontal cortex [rPFC]), whereas children with ASD showed greater connectivity between the supramarginal gyrus of the parietal lobe and rPFC relative to the other groups. They concluded that the social deficits common across children with NVLD and ASD may derive from distinct alterations in functional connectivity between nodes within the salience network involved in aspects of social processing [5]. One limitation of the study is that its comparisons between ASD and NVLD groups should be considered exploratory at best, given that the ASD data were derived from an exchange database, therefore the patient groups may have not had been ideally selected and matched, and the MRI scanners were different across institutions.

Concerning visuospatial abilities, the only available study is that of Fine, Musielak & Semrud-Clikeman in 2014 [6], a structural MRI study comparing the volume of an a-priori selected region, the corpus callosum (CC, the main white matter structure connecting left and right hemispheres) in groups of children with ASD without ID, NVLD, Attention Deficit-Hyperactivity disorder (ADHD), and typical developing controls. The key finding was that the NVLD group only was observed to have significantly smaller volume of the splenium compared to all other groups- the posterior CC portion conveying somatosensory and visual information between the two halves of the parietal and occipital lobes. Critically, smaller splenium volume was associated, in the NVLD group only, with lower Performance IQ scores but not Verbal IQ scores. However, this important study left open the critical question of how, in terms of structural and functional network connectivity, a smaller splenium can result in the differential impairments in nonverbal intelligence present in NVLD individuals. Since regions of the parietal cortex and dorsolateral prefrontal cortex, particularly in the right hemisphere, are known to be crucial substrates of spatial cognition, spatial attention and visuospatial working memory, investigating the functional and structural connectivity

among nodes of the dorsal and ventral attention networks (DAN and VAN) [7], particularly in the right hemisphere in NVLD children, appears particularly promising. Abnormalities in functional and structural connectivity involving nodes in these cognitive networks could be associated and better explain the visuospatial performance deficits in NVLD as well the greater severity of visuospatial impairments in NVLD children relative to ASD children without ID.

The main goal of the present project was to compare EEG measures of rs-FC in networks supporting social and visuospatial processing in ASD without ID, NVLD and TD controls, and to determine the association between performance levels in behavioral measures of social perspective taking and visuospatial processing with functional EEG measures at rest. Specifically, the first aim was to discriminate between the three groups only by looking at rs-FC maps in four networks: two involved in visuospatial processing, that is the Dorsal and Ventral Attention, and the other two implicated in social processing that is the Salience and the Default mode network.

The second aim was to better characterize the differences in FC-maps employing state-of-the-art global and nodal graph measures.

The third and last aim was to predict performances in visuospatial and social domains starting from FC-maps within the networks of interest. The scientific impact of the project comes from the possibility to differentiate the ASD and NVLD profiles through new neuroimaging biomarkers of illness that could be measured through EEG which is a less expensive and time-consuming tool compare to MRI.

In order to reach our goal of discriminating two clinical populations from each other and relative to neurotypical children, we have employed the same approach used in chapter two, to discriminate NVLD from typically developing children. Here I will report preliminary results on only the first previously presented aim and selectively for

the DMN and the SN. Furthermore, in discussion section I will briefly talk about the results and the next analysis we are going to perform on the dataset.

3.2 Methods

3.2.1 Participants

A total of 48 participants, aged 8 to 16 years old, were selected to take part in the present study. The experimental group included participants diagnosed with NVLD (n = 16; age in months = 157.19 ± 21.78) or ASD without ID (n = 16; age in months = 159.12 ± 36.61), and participants without any diagnosis (not diagnosed), and for whom a typical development was assumed (TD, n = 16; age in months = 155.44 ± 23.09). Only children who achieved a standard score of 80 or above on the full-scale IQ on the Wechsler Intelligence Scale (WISC-IV) [8] were included in the sample. All participants were native Italian speakers and had normal or corrected-to-normal vision and hearing.

Children with NVLD had previously received an independent clinical diagnosis by private psychologists or child psychiatrists at clinical specialized centers, following recommendations from the literature [9], while children in the TD group were recruited via local schools or community contacts.

3.2.2 Visuospatial domain

The Rey–Osterrieth complex figure test (ROCFT) [10] assesses visuo-constructive abilities and visuospatial memory. Participants are asked to copy a complex geometrical figure as accurately as possible. After 3 minutes, they are requested to reproduce it from memory. Accuracy is determined by scoring each element based on its presence, accurate reproduction, positioning and respect for proportions [10].

3.2.3 Social domain

The social perception abilities were measured through the test of the Theory of Mind (ToM) included in the NEPSY-II Battery [11]. The test is divided into two parts: the verbal and the contextual. The first part evaluates the ability to understand mental processes such as beliefs, emotions, deceits, fantasies and the ability to understand that others have their own thoughts, ideas, and feelings which could be different from our own. The contextual version assesses the ability to understand how emotions are connected to the social context and which emotions are appropriate depending on various contexts.

3.2.4 EEG Resting-State recording

For each participant, the rs HD-EEG activity was recorded before the active tasks (not reported here) in a 4-minute eyes closed session. We used a Geodesic high-density EEG System (EGI® Net Amp GES-400) with a pre-cabled 256-channels, through NetStation EEG Software. The elastomer structure of the EEG net is formed by polyvinyl alcohol sponges that are housed within the HydroCel Hydrating Skin interface chamber. The sampling rate of the recording was set to 500 Hz with an automatic alignment of real time EEG.

3.2.5 EEG preprocessing

The preprocessing was performed in MATLAB (v2019b) using functions from the EEGLab (v.2020.048) Toolbox. Continuous data were down-sampled to 256 Hz, high-pass filtered at 0.01 Hz, and re-referenced to the average of all channels. Following, the clean_artifacts routine in EEGLab was used with default parameters to detect bad channels and exclude them from further processing. A lowpass filter was applied at 30 Hz and excluded channels were interpolated. Finally, Independent

Component Analysis (ICA) was performed, and artifact components were marked with ICLabel and manually discarded.

3.2.6 EEG Source Modeling and Connectivity Analysis

The processing phase was carried out with Brainstorm and Matlab (MathWorks, Inc.). In order to model the source activity, a forward model was calculated with the BEM, a three-layer boundary element method, and the source was estimated with the weighted Minimum Norm Estimation (wMNE) method. This inverse solution was then downsampled to 148 cortical parcels defined by the Destrieux Atlas [12]. The connectivity matrices were calculated with the Magnitude Squared Coherence (MSC; equation 1), which describes the linear relationship (covariance) between two signals in the frequency domain and it is calculate as follows:

$$| C_{xy}(f) |^2 = \left(\frac{| S_{xy}(\mathrm{f}) |}{\sqrt{S_{xx}(\mathrm{f}) S_{yy}(\mathrm{f})}} \right)^2 \quad (1)$$

$S_{xy}(\mathrm{f})$: Cross-spectrum

$S_{xx}(\mathrm{f})\ and\ S_{yy}(\mathrm{f})$: Auto-spectra or power spectral density

Thus, the MSC (C) between two signals (x and y) is estimated by the square of the coherence value between x and y divided by the square root of the coherence of x with x multiplied by the cohere of y with y.

3.2.7 Discrimination between groups: A machine learning approach

After EEG signal preprocessing, source-reconstructed cortical activity and whole-brain resting state functional connectivity (rs-FC) were computed. Subsequently, phase coherence values were extracted from the parceled cortex (Destrieux atlas, 148 ROIs) [12] to estimate individual rs-FC in the DAN, VAN, SN and DMN. A machine-learning approach (i.e., support vector machine) was applied in order to investigate whether group membership could be predicted from rs-FC maps in each hemisphere and frequency band (Delta: 2-4 Hz, Theta: 5-7 Hz, Alpha: 8-12 Hz, Beta: 15-29 Hz, Gamma: 30-59 Hz). The objective of this first analysis was to investigate whether there was such information, within the coherence maps of the selected networks of interest, able to discriminate between groups. In order to test this hypothesis, we used a machine learning approach based on the SVM classifier. The Matlab functions *svmtrain* and *svmclassify*, respectively, were used in order to train a linear SVM model (with default parameters) for discriminating between groups, starting from the functional connectivity matrices. Matlab function *cvpartition* was employed, at each run, for implementing the *leave one subject out* cross-validation scheme. The prediction accuracy was computed at the end of the cross-validation loop on the corrected predicted classes (one per each test subject, at each run).

3.3 Results

There was a high accuracy in discriminating children with ASD and NVLD in the left-hemisphere DMN (alpha: 56.25, beta: 65.63, delta: 81.25; gamma: 81.25). In addition, we found that the same pattern of results was evident in the discrimination between ASD and TD (alpha: 71.88, delta: 81.25, gamma: 78.13, theta: 53.13) further supporting that the discrimination was driven by differences in the ASD group. These effects were found in at least 4 out of 5 frequency bands highlighting the consistency of the results. When looking at the discrimination of NVLD from TD, such an effect was

diminished in the left DMN having an above chance level of accuracy only in the alpha (62.50) and beta bands (65.63). Figure 1 summarize the results found in both hemispheres of the DMN.

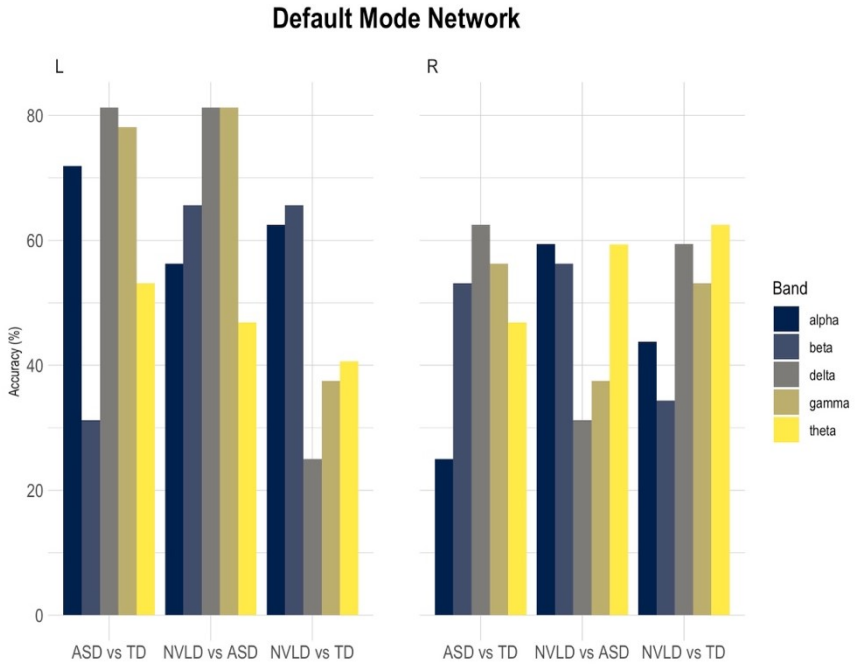

Figure 1. Accuracy of the Support Vector Machine in the Default Mode Network for the right and left hemisphere. The x-axis presents all the contrasts between the studied populations.

Conversely the SN network seemed more implicated in NVLD since when we try to discriminate it from ASD, we found that the left SN was accurate in this discrimination in 4 out if 5 frequency bands (alpha: 68.75, beta: 68.75, delta: 71.88; gamma: 78.13). These results were found also in the discrimination between NVLD and TD among all frequency bands (alpha: 62.50, beta: 65.63, delta: 59.38; gamma: 65.63, theta: 71.88). Furthermore, in the analysis of discrimination between ASD and TD, none of the frequency bands of the left SN was able to discriminate between the two groups. Figure 2 summarize the results found in both hemispheres of the SN.

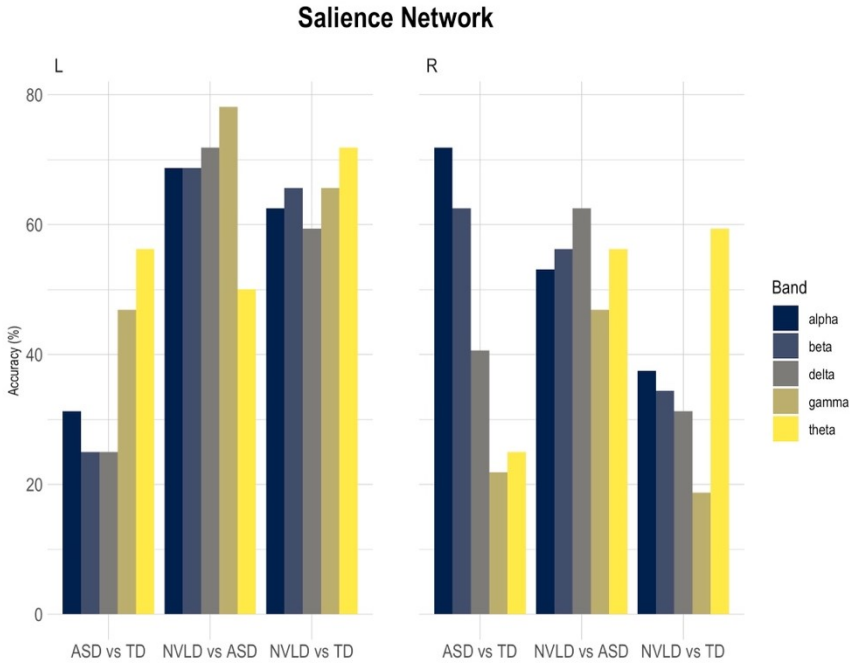

Figure 2. Accuracy of the Support Vector Machine in the Salience Network for the right and left hemisphere. The x-axis presents all the contrasts between the studied populations.

3.4 Discussion

The evidences are pointing toward a peculiarity in the connectivity patterns of the left Salience network in NVLD, while in the left Default Mode network there was information uniquely present in the group with Autism without intellectual disability. For ASD, there is plethora of findings related to DMN and also specifically linking this network to the social domain and the Theory of Mind [13–16]. Nonetheless, our main goal was to find differences between the two clinical population to substantiate how the cognitive deficits are differentially expressed in brain connectivity patterns.

It remains to better characterize these findings in terms of increased or decreased connections in the studied groups by use of the graph theory measures. Furthermore, the analysis linking performance in the social domain and functional connectivity maps will help shading light on the different social and visuospatial deficits found in ASD

without ID and NVLD while giving important information related to the interpretability of these results.

In fact, the next step of the present research is to better characterize the differences in the three groups by looking at nodal and global graph-based measures within the networks of interest (DAN, VAN, SN, DMN). At the global level, we will investigate segregation and integration properties of each network at rest; At the nodal level, we analyzed how each node of the networks is organized in terms of number and strength of connections. By applying graph theoretical analysis, we are able to study whether network topology properties, can be a significant marker for discriminating between NLD, ASD and TD groups, and if they can be predictive of individual performance during the behavioral assessment. In order to link the functional connectivity information with behaviors, we will apply a Support Vector Regression (SVR) between DAN/VAN and the visuospatial performance, between SN/DMN and the social scores. This phase is an important step toward a better understanding of findings that conjugate neuropsychological and electrophysiological data and therefore the clinical and the neuroscientific approaches.

3.5 Conclusion

Our preliminary analysis with the Support Vector Machine confirmed that the selected networks contained information able to discriminate between the groups. The results from the other two analyses need to be considered in order to better understand how the networks differ between the groups and how their functional connectivity maps are predictive of the visuospatial and social domains.

Furthermore, in the broader project it was included the electroencephalographic (EEG) recording during the execution of two tasks in the domains of interest: Theory of mind task [17] and Visuospatial Working Memory task [18]. This last piece of

information will make use of the high temporal resolution of the EEG to further explore how the selected brain networks dynamically adapt in response to the tasks and if this information is able to discriminate between NVDL, ASD and TD. Finally, we can compare the accuracy in the discrimination between groups at rest and in active tasks to assess which one is the best approach highlighting the different information extracted from both.

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Conclusion

The present project represents a joint effort of clinical and neuroscientific researchers to accumulate evidences regarding Non-Verbal Learning Disability: a neurodevelopmental condition not yet recognized by main diagnostic systems. The first aim of the project was to estimate the prevalence of the disability and its neural correlates, and to better characterize the core deficits of NVLD.

The estimated prevalence of NVLD was found to be 8% of a sample of 11 876 children of 9/10 years old collected in 21 different sites in the United States; Therefore we can broadly estimate that around 65.000 of children in the US can suffer of this learning disability (from a total of 24.5 millions 6-11 years-old children counted in 2019 by the U.S. Census Bureau). Given the relevance of these results, it appears essential to establish the best diagnostic criteria, involving both behavioural and brain indices to improve the treatment options and quality of life in this disorder. Therefore, after estimating the prevalence, we asked the question of whether the core deficits in visuospatial processing found in NVLD were linked to abnormalities of the cerebral White Matter (WM). Indeed, we found that the cognitive profile of NVLD was associated to an increased disorganization of WM fibers, especially in the right hemisphere, as previously postulated by Rourke's model of brain dysfunction in NVLD.

Second aim of the project was to determine whether EEG resting state functional connectivity (rs-FC) patterns in the traditional visuospatial attentional networks (Dorsal and Ventral Attention Networks - DAN and VAN) were able to distinguish NVLD children from children with typical development (TD), and whether such rs-FC patterns

could predict visuospatial functioning. We found that the FC maps in the networks of interest were able to accurately discriminate between the two groups. Furthermore, while rs-FC of the left DAN predicted visuospatial scores for TD children, in the NVLD group rs-FC of the right DAN predicted impaired visuospatial performance, confirming that NVLD is a disorder with a predominant dysfunction in connectivity patterns of the right hemisphere.

Finally, preliminary findings were presented from a group of children with Autism Spectrum disorder (ASD) without Intellectual disability (ID), with the inclusion of the social domain, both for behaviour (Theory of Mind) and cortical networks (Salience and Default Mode Networks), since ASD presents overlapping symptoms with NVLD in terms of visuospatial and socioemotional processing making it difficult to differentiate between them in clinical settings. These preliminary findings confirm that there are important information discriminating the groups in socioemotional networks. Further analysis extended to the visuospatial attention networks, ands on the directions of these differences throughout the graph-theory approach, in addition to the link between rs-FC and behaviours in visuospatial and social domains, will provide important insight for the differential diagnosis between the two clinical groups while also offering more information about how children with NVLD differs from typically developing children.

In conclusion, these evidences taken together represent the first attempt to conjugate clinical and neuroscientific findings in order to describe the core deficits found in NVLD, test the WM model, and investigate the differences between NVLD and ASD without ID.

Whether or not the NVLD will be included as a distinct disorder in the main diagnostic systems, it is an emerging category of symptoms that is found in clinical settings and as such it is worth investigating the condition to accumulate more conclusive empirical evidence coming from interdisciplinary approaches.