Functional connectivity alterations associated with literacy difficulties in early readers

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Abstract

The link between literacy difficulties and brain alterations has been described in depth. Resting-state fMRI (rs-fMRI) has been successfully applied to the study of intrinsic functional connectivity (iFc) both in dyslexia and typically developing children. Most related studies have focused on the stages from late childhood into adulthood using a seed to voxel approach. Our study analyzes iFc in an early childhood sample using the multivariate pattern analysis. This facilitates a hypothesis-free analysis and the possible identification of abnormal functional connectivity patterns at a whole brain level. Thirty-four children with literacy difficulties (LD) (7.1±0.69yr.) and 30 typically developing children (TD) (7.43±0.52yr.) were selected. Functional brain connectivity was measured using an rs-fMRI acquisition. The LD group showed a higher iFc between the right middle frontal gyrus (rMFG) and the default mode network (DMN) regions, and a lower iFc between the rMFG and both the bilateral insular cortex and the supramarginal gyrus. These results are interpreted as a DMN on/off routine malfunction in the LD group, which suggests an alteration of the task control network regulating DMN activity. In the LD group, the posterior cingulate cortex also showed a lower iFc with both the middle temporal poles and the fusiform gyrus. This could be interpreted as a failure in the integration of information between brain regions that facilitate reading. Our results show that children with literacy difficulties have an altered functional connectivity in their reading and attentional networks at the beginning of the literacy acquisition. Future studies should evaluate whether or not these alterations could indicate a risk of developing dyslexia.

Key words: reading and writing difficulties, literacy difficulties, MVPA**,** rs-fMRI, dyslexia, default mode network, early childhood.

1. INTRODUCTION

Over the past two decades, significant efforts have invested in understanding the neurobiology of reading, from infancy to adulthood (Horowitz-Kraus and Hutton 2015). Recent reading models suggest that reading is a process regulated by multiple brain areas working as a complex network (Dehaene 2009). Starting at visual areas, information spreads over the brain—mainly in the left hemisphere—following two routes that supplement each in the reading process. First is the dorsal route related to phonology-based reading processes; and secondly, the ventral system related to memory-based visual-orthographic word recognition. Other areas of the brain also play a critical role in the reading process: the frontoparietal attention network is needed to initiate and maintain the task of reading, while the left-sided temporal and frontal areas encode meaning, sound patterns and articulation.

Learning to read is a complex process, and not all children are successful. An elevated percentage of children (5-17%) have severe reading difficulties and suffer from developmental dyslexia (DD) (Habib and Giraud 2013). Individuals with DD show poor phonological and orthographic skills, with slow and inaccurate reading despite having adequate instruction, intelligence and intact sensory abilities (Lyon et al. 2003). With dyslexia working memory and other cognitive abilities are also often impaired (Habib and Giraud 2013). However, reading and writing difficulties have a high co-occurrence with other disorders, such as ADHD (attention-deficit/hyperactivity disorder) (Sexton et al., 2012) and developmental language disorders (Adlof and Hogan, 2018).

Multiple studies have proven that children with reading difficulties at primary school will likely continue to struggle with reading until adulthood. In addition to having negative effects on their academic performance, these difficulties could have an adverse impact on their social relationships, and emotional development (Ferrer et al., 2015). Therefore, major efforts have been invested in recent years to identify risk factors for reading difficulties (Hulme and Snowling 2016). One line of research has focused on detecting functional and structural brain alterations related to reading difficulties. Neuroimaging has been used widely to improve our knowledge of brain areas linked to reading difficulties and DD. Magnetic resonance imaging (MRI) studies have revealed altered structural and functional brain reading networks in DD (Xia et al., 2017). To date, most papers studying the neural functional connectivity of reading difficulties have been limited to individuals in late childhood, adolescence or adults. It is interesting to

note that a recent task-related fMRI study focused on developmental changes in effective connectivity between the ages of 6 (preliterate children) and 12 years old (fluent readers) in children with and without reading difficulties (Morken, Helland, Hugdahl, & Specht, 2017). These authors showed that at the early stages of learning to read, children at risk of dyslexia showed a delay on effective cerebral connectivity in areas of the reading network. Surprisingly, at age 12, these differences disappeared; nevertheless, the reading abilities in the dyslexia group were significantly lower than in the typical readers group. These results pointed out the importance of longitudinal studies (Black et al., 2017).

Although multiple studies focus on the functional brain characteristics of dyslexia, most of these are task-related. Thus, the specific brain areas with over- or under-activation showing differences between typically developing children (TD) and children with DD may differ from one study to another. Certain factors such as task election (Pugh et al. 2000; Price and Mechelli 2005), the variation of stimulus rate and duration (Price et al. 1996; Maisog et al. 2008), in addition to age (Richlan et al. 2011) and language (Siok et al. 2004) could contribute to different results and inconsistency between studies (Xia et al., 2017).

To avoid the constraints of task related studies, this study proposes the use of resting state functional resonance imaging (rs-fMRI). Resting state functional connectivity (rs-FC) has proven to be a new tool to avoid the high levels of variability found in task-related studies, thus facilitating a better understanding of functional networks in certain neurological disorders (Biswal et al. 1995; Raichle et al. 2001). Resting state fMRI (rs-fMRI) measures spontaneous low-frequency fluctuation in the blood-oxygen-level dependent (BOLD) signal; this facilitates research in the functional architecture of the brain without the constraint of task limitation. Some researchers have successfully applied rs-FC to study reading networks by selecting reading seeds from the meta-analysis and seeking out functional connectivity between these seeds and every location in the brain (seed-to-voxel analysis) (Vogel et al. 2013; Martin et al. 2015). Koyama (2010) found a relationship between the task-based activity pattern and the FC associated with word reading related seeds. More recently (Finn et al., 2014) used data-driven brain parcellation to compare connectivity profiles of dyslexic *versus* non-impaired readers, suggesting that non-impaired readers perform better in tasks where they have to integrate visual information and modulate their attention to visual stimuli. In this sense, Zhang (2014) found that rs-FC between reading areas and the

default mode network (DMN) is negatively correlated with reading abilities. It has been hypothesized that DMN supports internal mentation and monitors the external environment when focused attention is relaxed (Buckner et al. 2008), when active in emotional processing (ventromedial prefrontal cortex), self-referential activity (dorsomedial prefrontal cortex), and the recollection of prior experiences (posterior elements of the DMN) (Raichle 2015). More recently, dyslexic children were found to have deficits in their visual network and prefrontal modulation by using an rs-FC paradigm (Zhou et al., 2015).

The main objective of this study is to explore the alterations of cerebral networks associated with literacy difficulties in early childhood. For that, we selected a highly homogeneous sample of young children with literacy difficulties. The functional connectivity of the brain was analyzed by means of rs-FC, with a multivariate pattern analysis (MVPA); this is a novel agnostic data-driven approach that has never been used before in reading or literacy difficulty studies. This method provides a hypothesis-free analysis, without aprioristic assumptions, to detect putative abnormal iFC patterns on a whole brain scale. Therefore, the entire brain is examined; there is no bias as to the selection of any particular area. Knowledge of functional brain connectivity alterations could provide us with a better understanding of brain development in children with literacy difficulties.

2. MATERIALS AND METHODS

2.1. Participants

Participants were right-handed (Oldfield, 1971) native Catalan or Catalan-speaking primary school children in 1st or 2nd grade. The sample included a group with literacy difficulties (n=34, LD group) (7.1±0.69yr.) and an age and gender matched group of typically developing children (n=30, TD group) (7.43±0.52yr.). Inclusion in the LD group was established by a cut-off of 1.5 SD below the mean age in three reading and writing subtests. Exclusion criteria included having an IQ below 85, a history of chronic disorders or mental illness, diagnostic or signs of attention-deficit/hyperactivity disorder or of a developmental language disorder, being fluent in Catalan, having motor or sensorial deficits that might interfere with neuropsychological evaluation. All participants were assessed individually. Participants who met the inclusion criteria were selected for MRI scan at the *Hospital Clínic de Barcelona*. Literacy

performance measures, compared between groups, are shown on Table 1. After providing a complete description of the study to all participants, written and verbal informed consent was obtained from a parent and affirmed assent was obtained from the children. The research ethics committee Institutional Review Board (IRB00003099) of the University of Barcelona (Spain) approved the study.

2.2. Neuropsychological Assessment

All study participants were assessed individually by a trained neuropsychologist (S.F.).

Measures

IQ estimation. The Vocabulary subtest of WISC-IV (Wechsler, 2005) was used to obtain an estimation of verbal IQ (VIQ), and the Block design subtest to obtain an estimation of the performance IQ (PIQ).

Attention/verbal short- term memory. This measure was assessed by Digit span (WISC-IV; Wechsler, 2005). The task was to repeat sequences of digits (spanning from two to eight digits) in the correct order. Each correctly repeated span was scored.

Working memory. This measure was assessed using Digit span (WISC-IV; Wechsler, 2005). The task was to repeat digits (spanning from two to eight digits) backwards in the correct order. Each correctly repeated span was scored.

Phonetic and semantic fluencies. These measures were used as an assessment of executive function and verbal fluency. First, children were asked to generate words that began with letters F, A, and S in a 60-seconds interval per letter (total number of words was used as a measure of verbal phonetic fluency). Next, children were asked to generate as many animal names as possible within a one-minute interval (total number of names was used as a measure of semantic verbal fluency) (Straus, Sherman, & Spreen, 2006).

Naming speed task. Rapid automatized naming for letters and colors was used as a naming speed measure (Wolf & Denckla, 2005). The total time in seconds for naming letters and colors was registered for each child.

Reading. This measure was assessed using the standardized Catalan reading skills tests TALE-C (Cervera, Toro, Gratacós, De la Osa, & Pons, 1991) and PROLEC-R (Cuetos, Rodríguez, Ruano, & Arribas, 2007). Reading speed and accuracy measures were obtained from the letters, syllables, words

and text subtests of TALE-C, and from the pseudowords subtest of the PROLEC-R. Text comprehension was assessed by the TALE-C text comprehension test.

Spelling. Natural and arbitrary orthography were measured by the TALE-C writing subtest (Cervera et al. 1991).

Behavior. To assess behavior and signs of ADHD, the Conners' Teacher and Parent Rating Scales (Farré-Riba & Narbona, 1997) were used.

2.3. Image Acquisition and Pre-processing

All participants were examined on a 3T MRI scanner (Magnetom Trio Tim, Siemens Medical Systems, Germany) at the *Centre de Diagnòstic per la Imatge in the Hospital Clínic of Barcelona*. Magnetic resonance imaging acquisition included the following sequences: a high-resolution 3D structural dataset (T1-weighted magnetization prepared rapid gradient echo (MPRAGE), sagittal plane acquisition (TR = 2300 ms, TE = 3 ms, 240 slices, slice thickness = 1 mm, FOV = 244 mm, matrix size = 256 x 256), and a resting state fMRI sequence (T2*-weighted GE-EPI sequence, TR = 2500, TE = 29 ms, 40 slices per volume, slice thickness = 3 mm, FOV = 240 mm, matrix size = 80 x 80) that lasted 10 min 07 sec (240 volumes).

Pre-processing of MR images were performed using SPM12 software (SPM12, Welcome Trust Center for Neuroimaging, University College London, UK) and the Conn-fMRI toolbox 15h for SPM (Whitfield-Gabrieli and Nieto-Castanon 2012). Slice-timing, realignment and reorientation steps were processed with SPM12. Structural and functional images were normalized to the Montreal Neurological Institute (MNI) template, which was found to be appropriate for children age 5 and above (Altaye et al. 2008), and spatially re-sliced into 2mm isotropic voxels. Normalization, segmentation and smoothing into 8-mm FWHM Gaussian kernel were performed with the default parameters of the Conn toolbox.

2.4. Resting State Functional Connectivity Analysis

Data analysis was carried out using the Conn-fMRI toolbox (Cognitive and Affective Neuroscience Laboratory, Massachusetts Institute of Technology, Cambridge, USA) (Whitfield-Gabrieli and Nieto-

Castanon 2012; http://www.nitrc.org/projects/conn). Subject-specific regressors pertaining to white matter and cerebrospinal fluid signals were included as nuisance covariates, as well as six (6) motion parameters and scrubbing parameters for outlier volumes. Movement outliers were detected performing ArtRepair, with default cut-off score (frame wise displacement > 0.5mm, or signal intensity changes > 3 SD) (Mazaika et al. 2009). Five LD children were excluded from analysis because they did not surpass this quality threshold. Band-pass filtering ([0.1–0.008] Hz) was performed on functional volumes, simultaneously, with regressors (Hallquist et al. 2013). Additional steps included performing BOLD signal linear detrending and before-regression despiking. No differences were found between groups on mean frame wise displacement o invalid scans.

An MVPA (Multi-voxel Pattern Analysis) separately creates a pairwise connectivity map for each voxel of the brain template. It identifies the connectivity pattern between one voxel and the rest of the brain voxels and separately performs a Principal Component Analysis (PCA) of the variability in connectivity patterns across all subjects for each voxel. PCA uses a low number of spatial components to maximize the explained inter-subject variability in the resulting patterns (Flodin et al. 2016). First-level analysis computes each voxel average BOLD time series between every pair of voxels. The connectivity matrix of each participant was concatenated for all participants into a matrix of M (number of participants) x N (number of voxels in the brain) for each single voxel. The dimensions of these multivariate patterns were then reduced with a principal component analysis, which maximizes the proportion of inter-participant variance explained by fewer components. PCA component signals allow multivariate analysis of functional connectivity patterns. Thus, the matrix of voxel-to-voxel bivariate correlation coefficients is computed. This matrix is characterized by its eigenvectors and associated eigenvalues. This process produced a matrix for previously selected “number of participants” x “appropriate number of components.” To maintain an approximate 5:1 ratio between subjects and number of components, we computed 10 components (Whitfield-Gabrieli and Nieto-Castanon, 2012). Since the optimal size of the region of interest (ROI) is still not well defined (Korhonen et al. 2017), only clusters with 100 or more voxels were selected, in an effort to keep similar size as most reproduced ROIs found in rsfMRI literature (radius 6mm3 = 113 voxels).

After this first-level analysis, each of these measures could then be entered into a second-level general linear model (GLM) to obtain population-level estimates and inferences. This second-level analysis consisted of an omnibus test to identify the main effect of the variables of interest. Therefore, post-hoc general linear model analyses were required to determine specific connectivity patterns in the data. A seed-based correlation analysis was performed using voxel clusters which showed between-group connectivity differences on MVPA, treating them as ROIs (Figure 1). Those clusters that did not surpass a minimum size of 100 voxels were discarded. To analyze the ROI-to-voxel correlation mapping of functional connectivity, the program extracts the average time course from non-smoothed rs-fMRI data for each ROI and participant. Next, the temporal correlation between each time course extracted and all other brain voxels is computed using a General Linear Model (GLM) approach. To avoid false-positive results, as pointed out in a recent publication by Eklund (2016), all results were thresholded at voxel-wise height threshold $p<0.001$ and false discovery rate (FDR) corrected to $p<0.05$. Connectivity results were labeled with the Harvard-Oxford Atlas implemented in CONN.

(Figure 1 goes about here)

3. RESULTS

Neuropsychological evaluation showed that LD children had a significant lower performance. They were slower and less accurate in all reading tasks. Additionally, they exhibited a poorer performance in naming tasks, as well as in verbal fluency and spelling (Table 1).

(Table 1 goes about here)

An MVPA second level analysis showed significant differences between both groups with regards to connectivity profiles. Specifically, these significant differences were observed in components 1 and 2 ($p < 0.05$ FDR corrected, two-sided) (Figure 2).

Component 1 (C1) included 2 clusters of interest. The first one (C1_1) is located on the posterior cingulate cortex (pCC) ($p=0.003$, $k=276$) and the second (C1_2) on the right middle frontal gyrus (rMFG) ($p=0.026$, $k=158$).

Component 2 (C2) included one significant cluster on the left fusiform gyrus (FFG) (p=0.020, k=108). Intra group analysis of each component is displayed on the supplementary material (Figure 1S).

(Figure 2 goes about here)

3.1. Between-group Comparative Analysis

Statistical comparisons between TD and LD groups showed significant differences (Table 2 and Figure 3). An analysis of cluster C1_1 showed that the TD group has greater rs-FC between the pCC and both temporal lobes (including middle and inferior temporal gyrus), the medial frontal cortex (MedFC) and the precuneus (Prec)/pCC area than the LD group.

The comparison of cluster C1_2 between the two groups showed a greater rs-FC between the bilateral insular cortex (IC) and the anterior SMG among the TD group in comparison with the LD group, as well as between the IC and the supplementary motor area (SMA), the right posterior cingulate gyrus (postCG) and the left cerebellum (Cereb45). On the other hand, rMFG also revealed that the LD group had a stronger rs-FC with clusters that included the precentral and the posterior cingulate gyrus, the paracingulate gyrus (PaCiG)/FP, and the bilateral middle temporal gyrus (MTG)/inferior temporal gyrus (ITG) and angular gyrus (AG) than the TD group.

Between-group comparison of C2 displayed a greater connectivity between the left FFG and the pCC among the TD group.

(Table 2 goes about here)

(Figure 3 goes about here)

No significant correlations were found between the neuropsychological tests and the rs-FC patterns in any of the groups.

4. DISCUSSION

The main objective of our study was to evaluate brain connectivity differences between typically developing children (TD) and children with literacy difficulties at the early stages of the literacy acquisition. We used an rs-fMRI paradigm, which measures spontaneous low-frequency fluctuations in the BOLD signal, using a methodology that avoids aprioristic assumptions.

In our study, the reading network was found to be partially altered in the LD group. It is interesting to highlight that, in addition to the alteration of the reading network, our results suggest an alteration of the attentional system and, more specifically, of the default mode network, which is related to the resource allocation in directed goal activities (Buckner et al. 2008). Differences in connectivity patterns from the anterior and posterior cingulate cortex and from the medial frontal gyrus support this suggestion; this is consistent with previous studies suggesting that the alteration of attentional mechanisms can contribute to reading difficulties (Shaywitz and Shaywitz 2008).

Firstly, LD children showed weak functional connectivity between the pCC and the bilateral middle and inferior temporal lobe. The posterior cingulate cortex is a region that integrates information from different brain regions and networks (Leech et al. 2012), with connections that facilitate reading (Finn et al. 2014) and comprehension (Smallwood et al. 2013). In line with our results, previous studies have found an altered brain connectivity of the pCC in children with reading difficulties (Shaywitz et al. 2002). Moreover, an abnormal pattern of activity in the pCC has also been related to dyslexia, thus suggesting that this pCC alteration could reflect a pre-attentive processing deficit for reading (Stoitsis et al. 2008). It should be noted that some studies have showed that there is an increased activation on pCC after remediation (Meyler et al. 2008; Gebauer et al. 2012; Barquero et al. 2014).

Another region that exhibited a different connectivity pattern in LD children is the rMFG. It showed a weaker bilateral functional connectivity in areas of the IC, SMG/SMA and cerebellum. Moreover, rMFG had a greater iFC within areas of frontal and temporal cortex in children with LD when compared to TD children. This emphasizes the complexity of the alteration. MFG acts as a gateway between top-down and bottom-up attention control (Japee et al. 2015). It is a crucial region of the frontoparietal attention network, and in dyslexia, it has been found to have functional (Richards and Berninger 2008; Siok 2008; Zhang 2013; Olulade 2015; Martin et al. 2016; Feng 2017) and structural (Krafnick et al. 2014)

alterations. Interestingly, Yamada (2011) found that following an intervention program, children with LD exhibited increased levels of activation in the rMFG region.

A weaker rs-FC in children with LD between rMFG and SMG and IC could reflect a functional alteration of the dorsal reading system (Sandak et al. 2004). Previous studies have linked reading difficulties to structural (reduced grey matter) (Kronbichler et al. 2008; Linkersdörfer et al. 2012) and functional (lower activation) alterations of the SMG (Simos et al. 2000). In close relationship with the insula, this region is described as a core area for both speech and language processing, acting as a relay between cognitive aspects of language and the motor preparation in the basal ganglia and cerebellum (Eickhoff et al. 2009). Previous studies have described that the functional alteration of the insula is related to difficulties in speech-language processing (Adank 2012; Oh et al. 2014). Furthermore, insula alterations have also been found in dyslexic children (Gaab et al. 2007). It is interesting to note that the Gaab study described the effects of remediation on brain plasticity, as they found increased brain activation in insula after the remediation.

Our results also showed a decreased iFC between the rMFG, the SMA and the aCC in children with LD. As Price et al. (1994) reported, the SMA is one of the key areas of lexical decision. In their meta-analysis, Paulesu (2014) described the left posterior SMA as an area associated with phonological manipulation, motoric or visuo-spatial perception/attention. Therefore, this weaker connectivity among children with LD could be explained, in part, by the decoding difficulties they have. On the other hand, it has been suggested that the aCC plays a role and is an essential part of the task control network (TCN), together with the bilateral anterior insula (Dosenbach et al. 2006). This network is functionally important, as it initiates and maintains task-level control, selects appropriate sensorimotor mapping, and suppresses irrelevant distracting information (Kerns et al. 2004; Dosenbach et al. 2006; Fan et al. 2011). As Wen and colleagues proposed (Wen et al. 2013), top-down control of the TCN regulates the activity of the DMN, which enhances behavioral performance. On the other hand, bottom-up control from the DMN to the TCN interferes with task control, to possibly act as internal noise and leading to degraded behavioral performance.

Reading is a complex process that requires the correct function of attentional mechanisms. It has been suggested that attentional mechanisms could be critical in reading and that disruption of such mechanisms could play a causal role in reading difficulties (Shaywitz & Shaywitz, 2008).
Our results suggest that literacy difficulties could be related to an alteration of the DMN, reflected by a stronger rs-FC in the LD group between the rMFG and areas belonging to this network: the left and right AG and the MTG, the frontal pole and the precuneus/pCC. Being that MFG acts as a gateway between top-down and bottom-up control of attention (Japee et al. 2015), a higher connectivity with the DMN in the LD group could indicate an unbalance of attentional control areas, thus interfering with reading.

Our results agree with previous studies that have also found connectivity alterations between the DMN and the reading-related areas (Schurz et al. 2014; Cao et al. 2017), thus suggesting a reduced segregation between the language network and the default mode network in children with reading difficulties. In line with these studies, and in a block design study, Finn et al. (2014) also described an alteration of DMN in dyslexic children; they found an alteration in the connectivity of the pCC in their dyslexic group when compared to typically developing children. In their study, the pCC appeared better synchronized with other areas of the DMN while exhibiting a poorer synchronization with areas related to reading.

Along this same line, Smallwood and colleagues (2013) highlighted the importance of DMN in the reading process. They found that higher internal connectivity of DMN was related to a higher task focus and a better reading comprehension. More recently, in a Chinese sample of children with difficulties in reading and/or math, authors found an alteration of the connectivity between the DMN and prefrontal areas, suggesting a deficit in executive function (Weng et al., 2018).

Since the DMN is characterized by being most active when the brain is at rest, or during mind-wandering (Shulman et al. 1997; Raichle 2015), the hyper connectivity of the DMN with the rMFG in LD children in our study could interfere with a goal-directed activity such as reading. These results, together with the lower rs-FC between the rMPC and TCN areas in the LD group, suggest a joint activity imbalance between both networks could interfere with the reading process.

On the other hand, altered patterns of rs-FC between the rMFG and the cerebellum were found in the LD group; this could be consistent with the cerebellar theory of dyslexia (Nicolson et al., 2001; Alvarez and Fiez 2018). This theory postulates that based on the role of the cerebellum in motor control and automatization of overlearned tasks, an alteration in the cerebellum and its connections could cause deficient phonological representations and a more delayed learning of the grapheme–phoneme correspondence (Ramus et al. 2003). Previous studies have reported increased activation of the left cerebellum in dyslexic children (Yang et al. 2013; Feng et al. 2017). Moreover, structural alterations of the cerebellum have been related to reading difficulties (Yang et al. 2016). Taken together, these results could support the cerebellar theory of dyslexia.

Finally, our results showed an alteration of the left FFG brain connectivity pattern. Specifically, we found a greater iFC between the left FFG and the pCC in the TD versus the LD group. The fusiform gyrus is a hub of the visual word form area (VWFA), which has been suggested to play a key role during memory-based orthographic word recognition (Cohen et al. 2002; Cohen and Dehaene 2004). It could also play a role in object naming (McCrory et al. 2005) and phonological decoding (Dietz et al. 2005; Desroches et al. 2010). The reading difficulty meta-analysis also described functional and structural alterations of this area (Elnakib et al. 2014).

Functional activation and connectivity pattern alterations in this area have also been extensively identified in dyslexia (Brambati et al. 2006; Richlan et al. 2010; Tanaka et al. 2011; Olulade et al. 2012). Van der Mark (2011) found that dyslexic children have a reduced rs-FC between the VWFA and the left IPL and, in contrast, an increased rs-FC between the VWFA and the left occipito-temporal area, left STG and left insula. Furthermore, Olulade (2015) found a significant pattern of FC between the left FFG and the left inferior frontal gyrus (IFG), a key area for language comprehension and production belonging to the articulatory network in typically developing children, but not in dyslexic children. Schurz (2014) found the same increased FC in between-groups comparison. In concordance with our results, Finn (2014) found increased FC between the pCC and the left fusiform gyrus and dorsal visual pathway in non-impaired readers. As the cited authors note in their research, these results could reflect a better integration and cognitive control of visual information. Taking into account structural abnormalities, two different cortical thickness (CT) studies found abnormalities in the FFG, while Ma et al. (2015) found that the left

FFG had significantly increased cortical thickness in dyslexia; Altarelli (2013) detected a dyslexia-related CT reduction. There are other controversial results on this subject, but the structural alteration of the fusiform gyrus in dyslexic children seems to have been well established.

Our study has a number of limitations. The limited knowledge regarding neural interactions and their relation to individual skills in the rs-FC analysis should be noted. Additionally, we interpreted our results, in part, by comparing data obtained using an rs-fMRI paradigm with data from previous studies that used a task paradigm. Thus, the interpretation of results has to be considered with reservations. The sample size could also be considered a limiting factor for our research. Although our study did not start with a small sample, and all children were evaluated in-depth at a cognitive level, with strict exclusion and inclusion criteria, it has been pointed out that the robustness of neuroimage results increases with the sample size (Thirion et al. 2007). This fact could also lead to correlations between clinical symptoms and the functional connectivity in both the TD and LD groups. Finally, future studies should increase in-scan length to improve reliability across subjects and sessions (Birn et al. 2013).

In summary, this current study investigates the neural functional connectivity alterations of literacy difficulties in the early childhood by using a hypothesis-free analysis. Our results indicate that literacy difficulties during literacy acquisition are associated with a pattern of brain functional connectivity that differs from typically developing children. This alteration affects the ventral, dorsal, and articulatory reading systems, in addition to the DMN and TCN networks, which emphasizes the importance of the interaction of multiple systems in reading, as described previously (Dehaene, Cohen, Morais, & Kolinsky, 2015). This failed coordination between the reading, attentional and task-control networks suggests that an optimal communication between these areas is needed for the literacy acquisition to be efficient in early childhood. On the other hand, the use of an MVPA approach facilitates a better understanding of the dynamics of brain connectivity, as it is not limited to a concrete group of regions of interest in its evaluation of functional connectivity. As stated above, most of the areas where iFC differences were found to be altered correspond to children with dyslexia. Since we studied a group of children with literacy difficulties at the early stages of the reading learning process, these iFC alterations could represent brain markers for dyslexia.

Although more studies need to be conducted to confirm our results, these highlight the importance of researching brain networks at the beginning of the reading learning process. It could help to improve our understanding of the neurobiology behind literacy difficulties, to then implement intervention programs based on brain alteration patterns. These results offer a snapshot of brain functional connectivity in 6-7 year-old children with literacy difficulties, and highlight the need for longitudinal studies that evaluate the effects of development and remediation strategies on these functional alterations, and their relationship with future literacy skills.

COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of potential conflicts of interest: The authors declare that they have no conflict of interest.
Research involving Human Participants and/or Animals: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.
Informed consent: Informed consent was obtained from all individual participants included in the study.

REFERENCES

Adank, P., (2012). The neural bases of difficult speech comprehension and speech production: Two Activation Likelihood Estimation (ALE) meta-analyses. *Brain and Language*, 122, 42–54. doi: 10.1016/j.bandl.2012.04.014.

Adlof, S.M., Hogan, T.P. (2018). Understanding Dyslexia in the Context of Developmental Language Disorders. *Language, speech, and hearing services in schools,* 49(4):762-773. doi:10.1044/2018_LSHSS-DYSLC-18-0049.

Altarelli, I., Monzalvo, K., Iannuzzi, S., et al. (2013). A Functionally Guided Approach to the Morphometry of Occipitotemporal Regions in Developmental Dyslexia: Evidence for Differential Effects in Boys and Girls. *Journal of Neuroscience*, 33, 11296–11301. doi: 10.1523/JNEUROSCI.5854-12.2013.

Altaye, M., Holland, S. K., Wilke, M., Gaser, C. (2008). Infant brain probability templates for MRI

segmentation and normalization. *Neuroimage*, 43, 721–730. doi: 10.1016/j.neuroimage.2008.07.060.

Alvarez, T. A., Fiez, J. A. (2018). Current perspectives on the cerebellum and reading development. *Neuroscience & Biobehavioral Reviews*, 92, 55–66. doi: 10.1016/j.neubiorev.2018.05.006.

Barquero, L. A., Davis, N., Cutting, L. E. (2014). Neuroimaging of Reading Intervention: A Systematic Review and Activation Likelihood Estimate Meta-Analysis. *PLoS One*, 9:e83668. doi: 10.1371/journal.pone.0083668.

Birn, R. M., Molloy, E. K., Patriat, R., et al. (2013). The effect of scan length on the reliability of resting-state fMRI connectivity estimates. *Neuroimage*, 83, 550–558. doi: 10.1016/j.neuroimage.2013.05.099.

Biswal, B., Yetkin, F. Z., Haughton, V. M., Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magnetic Resonance in Medicine*, 34, 537–541.

Black, J. M., Xia, Z., & Hoeft, F. (2017). Neurobiological bases of reading disorder part II: The importance of developmental considerations in typical and atypical reading. Language and Linguistics Compass. doi: 10.1111/lnc3.12252

Brambati, S. M., Termine, C., Ruffino, M., et al. (2006). Neuropsychological deficits and neural dysfunction in familial dyslexia. *Brain Research*, 1113, 174–185. doi: 10.1016/j.braiTRes.2006.06.099.

Buckner, R. L., Andrews-Hanna, J. R., Schacter, D. L. (2008). *The Brain's Default Network. Annals of the New York Academy of Sciences*, 1124, 1–38. doi: 10.1196/annals.1440.011.

Cao, F., Yan, X., Wang, Z., et al. (2017). Neural signatures of phonological deficits in Chinese developmental dyslexia. *Neuroimage*, 146, 301–311. doi: 10.1016/j.neuroimage.2016.11.051.

Cohen, L., Dehaene, S. (2004). Specialization within the ventral stream: the case for the visual word form area. *Neuroimage*, 22, 466–476. doi: 10.1016/j.neuroimage.2003.12.049.

Cohen, L., Lehéricy, S., Chochon, F., et al. (2002). Language-specific tuning of visual cortex? Functional properties of the Visual Word Form Area. *Brain*, 125, 1054–1069.

Cuetos, F., Rodríguez, B., Ruano, E., & Arribas, D. (2007). *PROLEC-R: Batería de Evaluación de los Procesos Lectores, Revisada*. Barcelona: TEA Ediciones.

Dehaene, S. (2009). *Reading in the brain*. Penguin Viking.

Dehaene, S., Cohen, L., Morais, J., & Kolinsky, R. (2015). Illiterate to literate: Behavioural and cerebral changesinduced by reading acquisition.Nature Reviews. Neuroscience,16(4), 234–244. https://doi.org/10.1038/nrn3924

Desroches, A. S., Cone, N. E., Bolger, D. J., et al. (2010). Children with reading difficulties show differences in brain regions associated with orthographic processing during spoken language processing. *Brain Research*, 1356, 73–84. doi: 10.1016/j.braiTRes.2010.07.097.

Dietz, N. A. E., Jones, K. M., Gareau, L., et al. (2005). Phonological decoding involves left posterior fusiform gyrus. *Human Brain Mapping*, 26, 81–93. doi: 10.1002/hbm.20122.

Dosenbach, N. U. F., Visscher, K. M., Palmer, E. D., et al. (2006). A Core System for the Implementation of Task Sets. *Neuron*, 50, 799–812. doi: 10.1016/j.neuron.2006.04.031.

Eickhoff, S. B., Heim, S., Zilles, K., Amunts, K. (2009). A systems perspective on the effective connectivity of overt speech production. *Philosophical Transactions of the Royal Society A*, 367, 2399–2421. doi: 10.1098/rsta.2008.0287.

Eklund, A., Nichols, T. E., Knutsson, H. (2016). Cluster failure: Why fMRI inferences for spatial extent have inflated false-positive rates. *Proceedings of the National Academy of Sciences U S A*, 113, 7900–7905. doi: 10.1073/pnas.1602413113.

Elnakib, A., Soliman, A., Nitzken, M., et al. (2014). Magnetic resonance imaging findings for dyslexia: a review. *Journal of Biomedical Nanotechnology*, 10, 2778–2805.

Fan, J., Gu, X., Liu, X., et al. (2011). Involvement of the anterior cingulate and frontoinsular cortices in rapid processing of salient facial emotional information. *Neuroimage*, 54, 2539–2546. doi: 10.1016/j.neuroimage.2010.10.007.

Farré-Riba, A., Narbona, J. (1997). Conners' rating scales in the assessment of attention deficit disorder with hyperactivity (ADHD). A new validation and factor analysis in Spanish children. *Revista de Neurología*, 25(138), 200-204.

Feng, X., Li, L., Zhang, M., et al. (2017). Dyslexic Children Show Atypical Cerebellar Activation and Cerebro-Cerebellar Functional Connectivity in Orthographic and Phonological Processing. *Cerebellum*, 16, 496–507. doi: 10.1007/s12311-016-0829-2.

Ferrer, E., Shaywitz, B. A., Holahan, J. M., Marchione, K. E., Michaels, R., & Shaywitz, S. E. (2015). Achievement gap in reading is present as early as first grade and persists through adolescence. The Journal of Pediatrics, 167(5), 1121–1125. e1122. https://doi.org/10.1016/j.jpeds.2015.07.045

Finn, E. S., Shen, X., Holahan, J. M., et al. (2014). Disruption of Functional Networks in Dyslexia: A Whole-Brain, Data-Driven Analysis of Connectivity. *Biological Psychiatry*, 76, 397–404. doi: 10.1016/j.biopsych.2013.08.031.

Flodin P, Martinsen S, Altawil R, et al (2016) Intrinsic Brain Connectivity in Chronic Pain: A Resting-State fMRI Study in Patients with Rheumatoid Arthritis. *Frontiers in Human Neuroscience*, 10, 107. doi: 10.3389/fnhum.2016.00107.

Gaab, N., Gabrieli, J. D. E., Deutsch, G. K., et al. (2007). Neural correlates of rapid auditory processing are disrupted in children with developmental dyslexia and ameliorated with training: an fMRI study. *Restorative Neurology and Neuroscience*, 25, 295–310.

Gabrieli, J. D. E. (2009). Dyslexia: A New Synergy Between Education and Cognitive Neuroscience. *Science*, (80-), 325, 280–283. doi: 10.1126/science.1171999.

Gebauer, D., Fink, A., Kargl, R., et al. (2012). Differences in Brain Function and Changes with Intervention in Children with Poor Spelling and Reading Abilities. *PLoS One*, 7:e38201. doi: 10.1371/journal.pone.0038201.

Habib, M., Giraud, K. (2013). Dyslexia. *Handbook of Clinical Neurology*, 111, 229–235. doi: 10.1016/B978-0-444-52891-9.00023-3.

Hallquist, M. N., Hwang, K., Luna, B. (2013). The nuisance of nuisance regression: spectral misspecification in a common approach to resting-state fMRI preprocessing reintroduces noise and obscures functional connectivity. *Neuroimage*, 82, 208–225. doi: 10.1016/j.neuroimage.2013.05.116.

Horowitz-Kraus, T., Hutton, J. S. (2015). From emergent literacy to reading: how learning to read changes a child's brain. *Acta Paediatrica*, 104, 648–656. doi: 10.1111/apa.13018.

Hulme, C., Snowling, M. J. (2016). Reading disorders and dyslexia. *Curren Opinion in Pediatrics*, 28, 731–735. doi: 10.1097/MOP.0000000000000411.

Japee, S., Holiday, K., Satyshur, M. D., et al. (2015). A role of right middle frontal gyrus in reorienting of attention: a case study. *Frontiers in Systems Neuroscience*, 9, 23. doi: 10.3389/fnsys.2015.00023.

Kawagoe, T., Onoda, K., Yamaguchi. S. (2019). Subjective memory complaints are associated with altered resting-state functional connectivity but not structural atrophy. *Neuroimage Clinical*, 21:101675. doi: 10.1016/j.nicl.2019.101675.

Kerns, J. G., Cohen, J. D., MacDonald, A. W., et al. (2004). Anterior Cingulate Conflict Monitoring and

Adjustments in Control. *Science*, 303(5660), 1023–1026. doi: 10.1126/science.1089910.

Korhonen, O., Saarimäki, H., Glerean, E., et al. (2017). Consistency of Regions of Interest as nodes of fMRI functional brain networks. *Network Neuroscience*, 1, 254–274. doi: 10.1162/NETN_a_00013.

Koyama, M. S., Kelly, C., Shehzad, Z., et al. (2010). Reading networks at rest. *Cerebral Cortex*, 20, 2549–2559. doi: 10.1093/cercor/bhq005.

Krafnick, A. J., Flowers, D. L., Luetje, M. M., et al. (2014). An investigation into the origin of anatomical differences in dyslexia. *Journal of Neuroscience*, 34, 901–908. doi: 10.1523/JNEUROSCI.2092-13.2013.

Kronbichler, M., Wimmer, H., Staffen, W., et al. (2008). Developmental dyslexia: Gray matter abnormalities in the occipitotemporal cortex. *Human Brain Mapping*, 29, 613–625. doi: 10.1002/hbm.20425.

Leech, R., Braga, R., Sharp, D. J. (2012). Echoes of the Brain within the Posterior Cingulate Cortex. *Journal of Neuroscience*, 32, 215–222. doi: 10.1523/JNEUROSCI.3689-11.2012.

Lezak, M. (2004). *Neuropsychological assessment*. New York: Oxford Univ Press, 4º edition.

Linkersdörfer, J., Lonnemann, J., Lindberg, S., et al. (2012). Grey matter alterations co-localize with functional abnormalities in developmental dyslexia: an ALE meta-analysis. *PLoS One*, 7:e43122. doi: 10.1371/journal.pone.0043122.

Lyon, G.R., Shaywitz, S.E., Shaywitz, B.A. (2003). A definition of dyslexia. Annals of Dyslexia, 53, 1-14.

Ma, Y., Koyama, M. S., Milham, M. P., et al. (2015). Cortical thickness abnormalities associated with dyslexia, independent of remediation status. *NeuroImage Clinical*, 7, 177–186. doi: 10.1016/j.nicl.2014.11.005.

Maisog, J. M., Einbinder, E. R., Flowers, D. L., et al. (2008). A Meta-analysis of Functional Neuroimaging Studies of Dyslexia. *Annals of the New York Academy of Sciences*, 1145, 237–259. doi: 10.1196/annals.1416.024.

Martin, A., Kronbichler, M., Richlan, F. (2016). Dyslexic brain activation abnormalities in deep and shallow orthographies: A meta-analysis of 28 functional neuroimaging studies. *Human Brain Mapping*, 37, 2676–99. doi: 10.1002/hbm.23202.

Martin, A., Schurz, M., Kronbichler, M., Richlan, F. (2015). Reading in the brain of children and adults: a meta-analysis of 40 functional magnetic resonance imaging studies. *Human Brain Mapping,* 36,

1963–1981. doi: 10.1002/hbm.22749.

Mazaika, P. K., Hoeft, F., Glover, G. H., Reiss, A. L. (2009). Methods and Software for fMRI Analysis for Clinical Subjects. *In: Human Brain Mapping Conference*. Florence, Italy.

McCrory, E. J., Mechelli, A., Frith, U., Price, C. J. (2005). More than words: a common neural basis for reading and naming deficits in developmental dyslexia? *Brain*, 128, 261–267. doi: 10.1093/brain/awh340.

Meyler, A., Keller, T. A., Cherkassky, V. L., et al. (2008). Modifying the brain activation of poor readers during sentence comprehension with extended remedial instruction: a longitudinal study of neuroplasticity. *Neuropsychologia*, 46, 2580–2592. doi: 10.1016/j.neuropsychologia.2008.03.012.

Morken, F., Helland, T., Hugdahl, K., Specht, K. (2017). Reading in dyslexia across literacy development: A longitudinal study of effective connectivity. *Neuroimage*, 144(Pt A):92-100. doi: 10.1016/j.neuroimage.2016.09.060.

Oh, A., Duerden, E. G., Pang, E. W. (2014). The role of the insula in speech and language processing. *Brain and Language*, 135, 96–103. doi: 10.1016/j.bandl.2014.06.003.

Oldfield, R.C. (1971). The assessment and analysis of handedness: The Edinburgh Inventory. *Neuropsychologia*, 9, 97-113.

Olulade, O. A., Flowers, D. L., Napoliello, E. M., Eden, G. F. (2015). Dyslexic children lack word selectivity gradients in occipito-temporal and inferior frontal cortex. *NeuroImage Clinical*, 7, 742–754. doi: 10.1016/j.nicl.2015.02.013.

Olulade, O. A., Gilger, J. W., Talavage, T. M., et al. (2012). Beyond Phonological Processing Deficits in Adult Dyslexics: Atypical fMRI Activation Patterns for Spatial Problem Solving. *Developmental Neuropsychology*, 37, 617–635. doi: 10.1080/87565641.2012.702826.

Paulesu, E., Danelli, L., Berlingeri, M. (2014). Reading the dyslexic brain: multiple dysfunctional routes revealed by a new meta-analysis of PET and fMRI activation studies. *Frontiers in Human Neuroscience*, 8, 830. doi: 10.3389/fnhum.2014.00830.

Peterson, R.L., Pennington, B.F. (2015). Developmental Dyslexia. *Annual Review of Clinical Psychology, 11*, 283-307. doi: 10.1146/annurev-clinpsy-032814-112842.

Price, C. J., Mechelli, A. (2005). Reading and reading disturbance. *Current Opinion in Neurobiology*, 15, 231–238. doi: 10.1016/j.conb.2005.03.003.

Price, C. J., Moore, C. J., Frackowiak, R. S. J. (1996). The Effect of Varying Stimulus Rate and Duration

on Brain Activity during Reading. *Neuroimage*, 3, 40–52. doi: 10.1006/nimg.1996.0005.

Price, C. J., Wise, R. J., Watson, J. D., et al. (1994). Brain activity during reading. The effects of exposure duration and task. *Brain*, 117, 1255–1269.

Pugh, K. R., Mencl, W. E., Shaywitz, B. A., et al. (2000). The Angular Gyrus in Developmental Dyslexia: Task-Specific Differences in Functional Connectivity Within Posterior Cortex. *Psychological Science*, 11, 51–56. doi: 10.1111/1467-9280.00214.

Raichle, M. E. (2015). The Brain's Default Mode Network. *Annual Review of Neuroscience*, 38, 433–447. doi: 10.1146/annurev-neuro-071013-014030.

Raichle, M. E., MacLeod, A. M., Snyder, A. Z., et al. (2001). A default mode of brain function. *Proceedings of the National Academy of Sciences, U S A*, 98, 676–682. doi: 10.1073/pnas.98.2.676.

Ramus, F., Rosen, S., Dakin, S. C., et al. (2003). Theories of developmental dyslexia: insights from a multiple case study of dyslexic adults. *Brain*, 126:841–865. doi: 10.1093/brain/awg076.

Richards, T. L., Berninger, V. W. (2008). Abnormal fMRI Connectivity in Children with Dyslexia During a Phoneme Task: Before But Not After Treatment . *Journal of Neurolinguistics*, 21, 294–304. doi: 10.1016/j.jneuroling.2007.07.002.

Richlan, F., Kronbichler, M., Wimmer, H. (2011). Meta-analyzing brain dysfunctions in dyslexic children and adults. *Neuroimage*, 56, 1735–4172. doi: 10.1016/j.neuroimage.2011.02.040.

Richlan, F., Sturm, D., Schurz, M., et al. (2010). A Common Left Occipito-Temporal Dysfunction in Developmental Dyslexia and Acquired Letter-By-Letter Reading? *PLoS One*, 5:e12073. doi: 10.1371/journal.pone.0012073.

Sandak, R., Mencl, W. E., Frost, S. J., et al. (2004). The neurobiology of adaptive learning in reading: a contrast of different training conditions. *Cognitive, Affective, & Behavioral Neuroscience*, 4, 67–88. doi: 10.3758/CABN.4.1.67.

Schurz, M., Wimmer, H., Richlan, F., et al. (2014). Resting-State and Task-Based Functional Brain Connectivity in Developmental Dyslexia. *Cerebral Cortex*, doi: 10.1093/cercor/bhu184.

Sexton, C.C., Gelhorn, H.L., Bell, J.A., Classi, P.M. (2012). The co-occurrence of reading disorder and ADHD: epidemiology, treatment, psychosocial impact, and economic burden. *Journal of Learning Disabilities,* 45(6):538-564. doi:10.1177/0022219411407772

Shaywitz, B. A., Shaywitz, S. E., Pugh, K. R., et al. (2002). Disruption of posterior brain systems for reading in children with developmental dyslexia. *Biological Psychiatry*, 52, 101–110.

Shaywitz, S. E., Shaywitz, B. A. (2008). Paying attention to reading: the neurobiology of reading and dyslexia. *Development and Psychopathology*, 20, 1329–1349. doi: 10.1017/S0954579408000631.

Shulman, G. L., Fiez, J. A., Corbetta, M., et al. (1997). Common Blood Flow Changes across Visual Tasks: II. Decreases in Cerebral Cortex. *Journal of Cognitive Neuroscience*, 9, 648–663. doi: 10.1162/jocn.1997.9.5.648.

Simos, P. G., Breier, J. I., Fletcher, J. M., et al. (2000). Brain activation profiles in dyslexic children during non-word reading: a magnetic source imaging study. *Neuroscience Letters*, 290, 61–65.

Siok, W. T., Niu, Z., Jin, Z., et al. (2008). A structural-functional basis for dyslexia in the cortex of Chinese readers. *Proceedings of the National Academy of Sciences U S A*, 105, 5561–6. doi: 10.1073/pnas.0801750105.

Siok, W. T., Perfetti, C. A., Jin, Z., Tan, L. H. (2004). Biological abnormality of impaired reading is constrained by culture. *Nature*, 431, 71–76. doi: 10.1038/nature02865.

Smallwood, J., Gorgolewski, K. J., Golchert, J., et al. (2013). The default modes of reading: modulation of posterior cingulate and medial prefrontal cortex connectivity associated with comprehension and task focus while reading. *Frontiers in Human Neuroscience*, 7, 734. doi: 10.3389/fnhum.2013.00734.

Stoitsis, J., Giannakakis, G. A, Papageorgiou, C., et al, (2008). Evidence of a posterior cingulate involvement (Brodmann area 31) in dyslexia: A study based on source localization algorithm of event-related potentials. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 32, 733–738. doi: 10.1016/j.pnpbp.2007.11.022.

Tanaka, H., Black, J. M., Hulme, C., et al. (2011). The Brain Basis of the Phonological Deficit in Dyslexia Is Independent of IQ. *Psychological Science*, 22, 1442–1451. doi: 10.1177/0956797611419521.

Thirion, B., Pinel, P., Mériaux, S., et al. (2007). Analysis of a large fMRI cohort: Statistical and methodological issues for group analyses. *Neuroimage*, 35, 105–120. doi: 10.1016/j.neuroimage.2006.11.054.

Toro, J., Cervera, M. (2011). *TALE. Test de Análisis de Lecto-Escritura*. Barcelona: TEA Ediciones, 8º edition.

van der Mark, S., Klaver, P., Bucher, K., et al. (2011). The left occipitotemporal system in reading: Disruption of focal fMRI connectivity to left inferior frontal and inferior parietal language areas in

children with dyslexia. *Neuroimage*, 54, 2426–2436. doi: 10.1016/j.neuroimage.2010.10.002.

Vogel, A. C., Church, J. A., Power, J. D., et al. (2013). Functional network architecture of reading-related regions across development. *Brain and Language*, 125, 231–243. doi: 10.1016/j.bandl.2012.12.016.

Wechsler, D. (2005). *WISC-IV: escala de inteligencia Wechsler para niños IV*. Madrid: TEA.

Wen, X., Liu, Y., Yao, L., Ding, M. (2013). Top-down regulation of default mode activity in spatial visual attention. *Journal of Neuroscience*, 33:6444–53. doi: 10.1523/JNEUROSCI.4939-12.2013.

Weng, J. C., Wang, N. Y., Jui Li, C., and Sharon Wang, H. L. (2018). Resting-State Functional Connectivity within Default Mode Network in Chinese-speaking Children with Specific Learning Disabilities. *Neuropsychiatry*, *08*(03). https://doi.org/10.4172/neuropsychiatry.1000414.

Whitfield-Gabrieli, S., Nieto-Castanon, A. (2012). *Conn* : A Functional Connectivity Toolbox for Correlated and Anticorrelated Brain Networks. *Brain Connectivity*, 2, 125–141. doi: 10.1089/brain.2012.0073.

Wolf, M., Denckla, M. (2005). *The Rapid Automatized Naming and Rapid Alternating Stimulus Tests*. Austin: Pro-Ed Examiner's.

Xia, Z., Hancock, R., & Hoeft, F. (2017). Neurobiological bases of reading disorder Part I: Etiological investigations. Language and Linguistics Compass. doi: 10.1111/lnc3.12239

Yamada, Y., Stevens, C., Dow, M., et al. (2011). Emergence of the neural network for reading in five-year-old beginning readers of different levels of pre-literacy abilities: an fMRI study. *Neuroimage*, 57, 704–713. doi: 10.1016/j.neuroimage.2010.10.057.

Yang, Y-H., Yang, Y., Chen, B-G., et al. (2016). Anomalous Cerebellar Anatomy in Chinese Children with Dyslexia. *Frontiers in Psychology*, 7, 324. doi: 10.3389/fpsyg.2016.00324.

Yang, Y., Bi, H-Y., Long, Z-Y., Tao, S. (2013). Evidence for cerebellar dysfunction in Chinese children with developmental dyslexia: an fMRI study. *International Journal of Neuroscience*, 123, 300–310. doi: 10.3109/00207454.2012.756484.

Zhang, M., Li, J., Chen, C., et al. (2014). Resting-state functional connectivity and reading abilities in first and second languages. *Neuroimage*, 84, 546–553. doi: 10.1016/j.neuroimage.2013.09.006.

Zhang, Y., Whitfield-Gabrieli, S., Christodoulou, J. A., Gabrieli, J. D. E. (2013). Atypical Balance between Occipital and Fronto-Parietal Activation for Visual Shape Extraction in Dyslexia. *PLoS One*, 8:e67331. doi: 10.1371/journal.pone.0067331.

Zhou, W., Xia, Z., Bi, Y., & Shu, H. (2015). Altered connectivity of the dorsal and ventral visual regions

in dyslexicchildren: A resting-state fMRI study.Frontiers in Human Neuroscience,9, 495. https://doi.org/10.3389/fnhum.2015.00495

Table 1: Reading performance measures of normal reading (NR) and reading difficulties (RD) groups.

	NR group mean (s.d.)	RD group mean (s.d.)	Student t-test	*p*	Effect size (d)
NAMING					
Objects	50.05 (5.83)	40.38 (7.97)	4.71	<0.001	0.57
Colors	46.50 (5.96)	35.58 (9.13)	4.81	<0.001	0.58
SPEED READING					
Words	59.82 (3.46)	48.12 (6.45)	7.87*	<0.001	0.75
Non-words	50.41 (7.76)	27.50 (7.80)	9.98	<0.001	0.83
Text	59.00 (4.02)	41.80 (12.40)	6.55*	<0.001	0.68
READING ACCURACITY					
Words	61.68 (2.73)	38.76 (9.31)	11.75*	<0.001	0.86
Non-words	51.64 (7.37)	25.48 (7.32)	11.95	<0.001	0.87
Text	59.68 (1.89)	36.63 (12.68)	8.43*	<0.001	0.79
TEXT COMPREHENSION	51.23 (10.45)	42.83 (11.90)	2.53	0.015	0.35
SPELLING					
Arbitrary orthography	59.09 (8.37)	52.13 (10.98)	2.40	0.021	0.34
Natural orthography	64.18 (4.52)	40.79 (11.60)	9.15*	<0.001	0.80
VERBAL FLUENCY					
Phonemic	52.86 (9.69)	43.46 (9.17)	3.45	0.001	0.45
Semantic	61.00 (11.64)	45.93 (9.65)	4.96	<0.001	0.58

* After correction (Levene's test $p < 0.05$)

s.d: standard deviation; NR: Normal reading; RD: Reading difficulties

Table 2. Significant group differences in functional connectivity for each cluster region

CLUSTER	x	y	z	Area	k	cluster p-FDR
C1_1	2	-42	38	pCC	276	0.003
NR > RD	-48	-23	-33	MTG_l	3082	< 0.001
	56	-14	-20	MTG_r	1370	0.001
	-2	30	-30	SubCalC / MedFC	1125	0.003
	-8	-57	26	Prec / pCC	1037	0.003
	11	-42	8	pCC	522	0.049
C1_2	**38**	**32**	**41**	**rMFG**	**158**	**0.026**
NR > RD	-38	-5	2	IC_l	1952	< 0.001
	-56	-24	20	PO_l / aSMG_L	1903	< 0.001
	53	-24	24	aSMG_r / PO_r	1440	0.001
	38	-17	2	IC_r	1240	0.002
	-6	-51	-18	Cereb45	1197	0.002
	-6	-2	47	SMA_l & r / AC	815	0.011
	18	-29	47	PostCG_r / SPL_r	727	0.015
RD > NR	-6	-47	42	Prec / pCC	2353	< 0.001
	48	2	-35	MTG_r	2237	< 0.001
	-50	2	-23	aMTG_l	1667	< 0.001
	-44	-53	32	AG_l	1532	< 0.001
	12	54	17	PaCiG_l / FP_r	1443	< 0.001
	-62	-35	0	pMTG_l	575	0.025
	56	-53	29	AG_r	506	0.032
C2_1	**-33**	**-30**	**-21**	**FFG_l**	**108**	**0.020**
NR > RD	2	-47	27	pCC	1465	< 0.001

Regions are labeled based on the locations of the largest number of voxels within the significant cluster, as identified and labeled in the CONN-toolbox. *k: Number of voxels; L: Left; R: Right; NR: Normal reading; RD: Reading difficulties; pCC: Posterior cingulate cortex; MTG: Middle temporal gyrus; SubCalC: Subcallosal cortex; MedFC: Medial frontal cortex; Prec: Precuneus; MFG: Middle frontal gyrus; IC: Insular cortex; PO: Parietal operculum; aSMG: Anterior supramarginal gyrus; ; Cereb: Cerebellum; SMA: Superior motor area; AC: Anterior cingulate gyrus; PostCG: Posterior cingulate gyrus; SPL: Superior parietal lobe; AG: Angular gyrus; PaCiG: Paracingulate gyrus; FP: Frontal pole; FFG: Fusiform gyrus; a: Anterior; p: Posterior.*

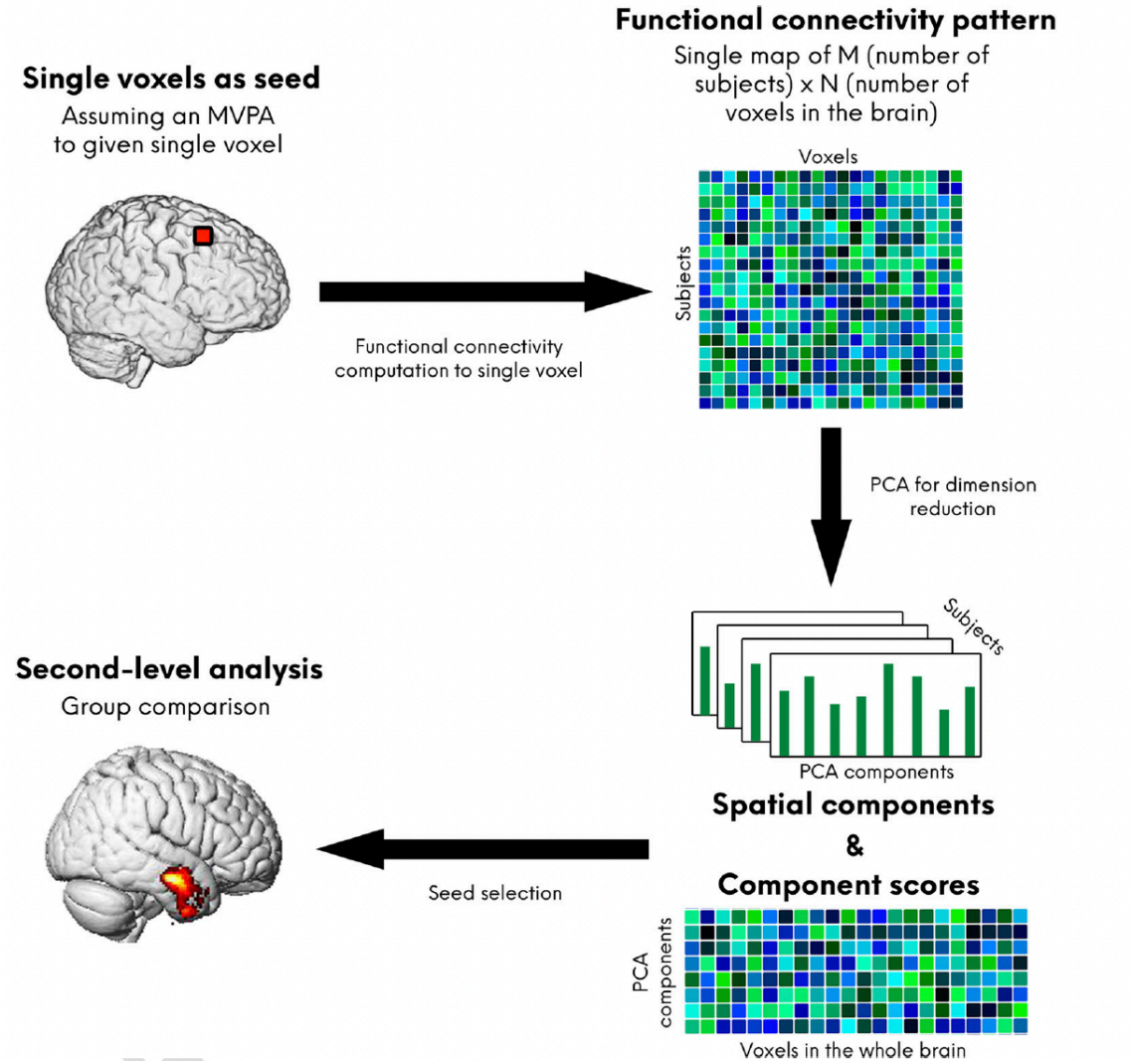

Figure 1. Illustration of resting-state multivariate pattern analysis (MVPA) procedure for a single voxel.

Adapted from Kawagoe et al. (2018).

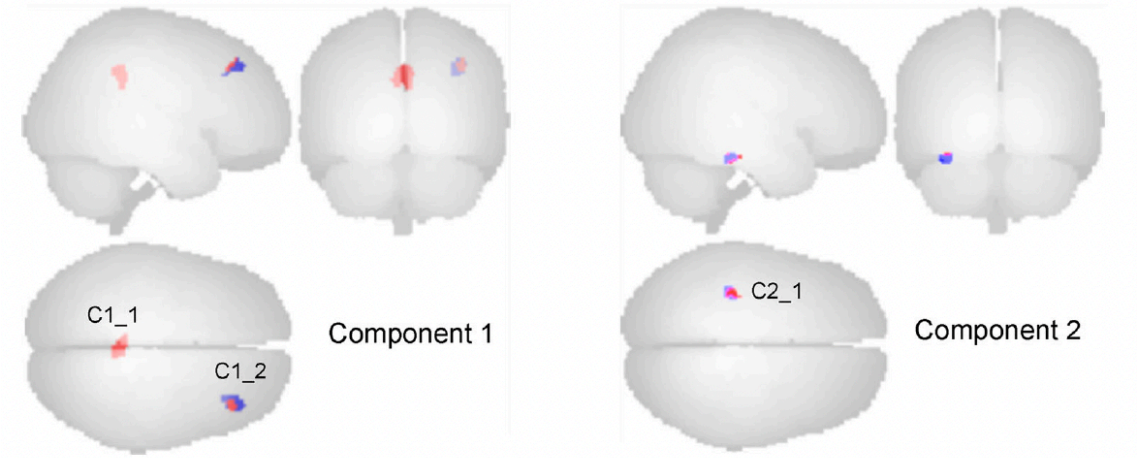

Figure 2. MVPA group differences. Component 1 contains clusters located on the pCC (C1_1) and the right MFG (C1_2); Component 2 contains cluster located on the left FFG (C2_1). Maps are shown at a voxel-wise threshold of P<0.001 (uncorrected) and a cluster extent threshold of P<0.05 FDR (corrected). pCC: Posterior cingulate cortex; MFG: Middle frontal gyrus; FFG: Fusiform gyrus; iFC: Intrinsic functional connectivity.

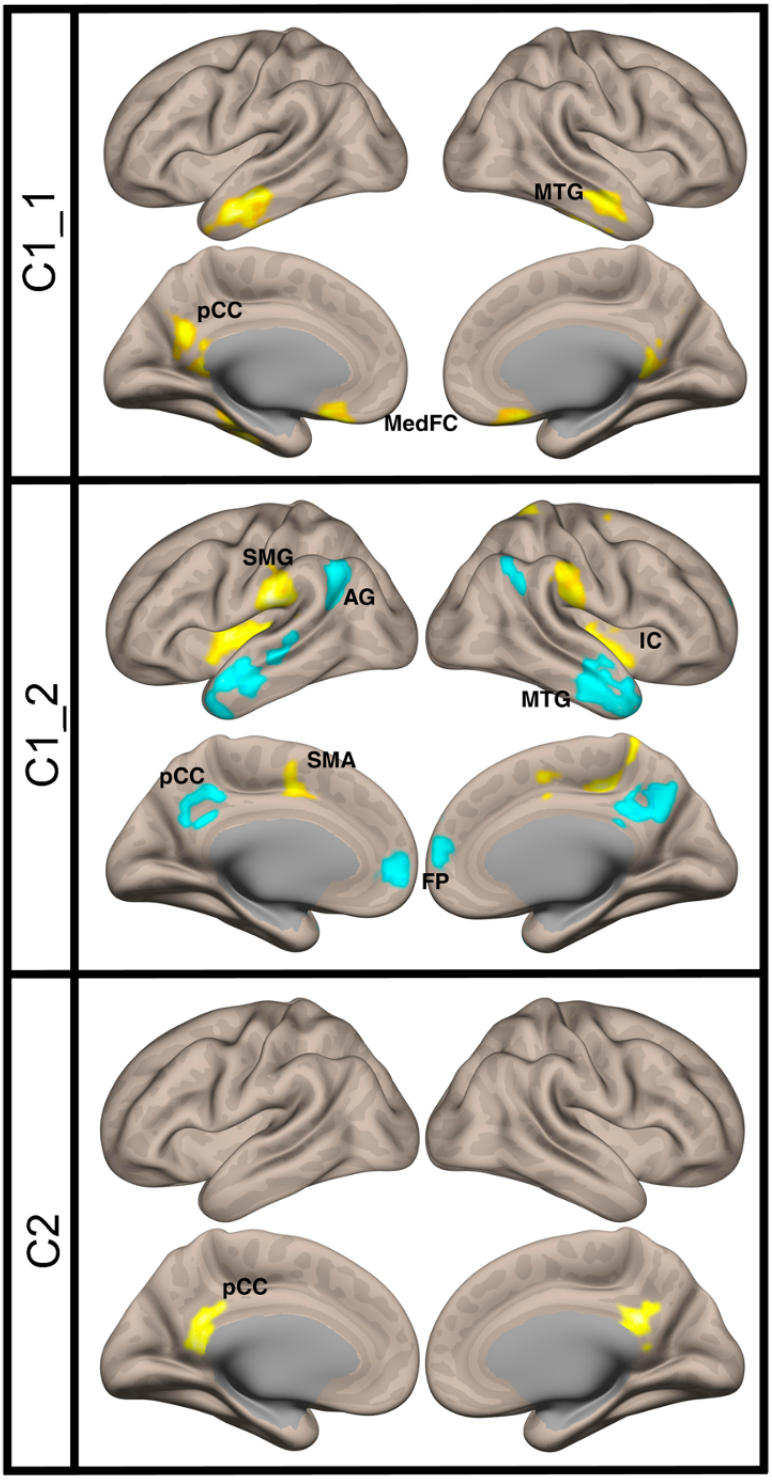

Figure 3. Between-group differences in functional connectivity for each cluster region. Group differences are shown in terms of main outcomes; greater iFC in the TD group is shown in red, greater iFC for the LD group is shown in blue. Maps are shown at a voxel-wise threshold of P<0.001 (uncorrected) and a cluster extent threshold of P<0.05 FDR-corrected. iFC: Intrinsic functional connectivity; TD: typically developing children; LD: literacy difficulties.