MAX PLANCK INSTITUTE FOR HUMAN COGNITIVE AND BRAIN SCIENCES LEIPZIG

Michael A. Skeide: The Brain Basis of Developmental Dyslexia. Leipzig: Max Planck Institute for Human Cognitive and Brain Sciences, 2018 (MPI Series in Human Cognitive and Brain Sciences; 194)

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Impressum

Max Planck Institute for Human Cognitive and Brain Sciences, 2018

Druck: Sächsisches Druck- und Verlagshaus Direct World, Dresden

Titelbild: © Clara Sophia Skeide, 2018

ISBN 978-3-941504-80-6

The brain basis of developmental dyslexia

HABILITATIONSSCHRIFT

zur Erlangung der Lehrbefähigung für das Fach Psychologie

vorgelegt dem Rat der Lebenswissenschaftlichen Fakultät
der Humboldt-Universität zu Berlin

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Berlin, 09.11.2017

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ACKNOWLEDGEMENTS

I thank
Angela D. Friederici, Rasha Abdel Rahman, Elke van der Meer, Werner Sommer, Nadine Gaab, Thomas Lachmann, Reinhard Beyer, Ulrike Lüken, Alexander Engen, Johannes Boltze, Falk Huettig, Vinod Menon, Bob Berwick, Erich Schröger, Axel Mecklinger, Christian Fiebach, Arnulf Deppermann, Klaus-Peter Konerding, Alfred Anwander, Gerald Hahn, Dan Abrams, Ed Mei, Tanya Evans, Pierre-Louis Bazin, Robert Trampel, Indra Kraft, Ulrike Kuhl, Bent Müller, Frank Emmrich, Holger Kirsten, Arndt Wilcke, Katharina von Kriegstein, Alexander Schäfer, Claudia Männel, Jantje Willems, Eva Goedel, Aaron Doliana, Rob Schwartz, Katharina Wehrmann, Micha Vollmann, Nadine Bobovnikov, Antje Viehweger, Liane Dörr, Franzi Stock, Simone Lehmann, Margarete Störel, Micha Schwarz, Doreen Bennemann, Maria Wohlfahrt, Lea Hermann, Uttam Kumar, Ramesh Mishra, Viveka Tripathi, Anupam Guleria, Jay Singh, Frank Eisner, Jan Schreiber, Riccardo Cafiero, Riccardo Metere, Gesa Schaadt, Jens Brauer, Katja Paasche, Verena Müller, Christina Schröder, Ingrid Schmude, André Pampel, Thom Gunter, Maaike Vandermosten, Aniela França, Ana Paula da Silva Passos Jakubów, Margund Greiner, Melanie Trümper, Andrea Gast-Sandmann, Heike Schmidt-Duderstedt, Käpt'n Freitag, Bundesministerium für Bildung und Forschung, Max Planck Gesellschaft, Dr. med Helene-Charlotte Wolf Stiftung, Niedersächsische Akademie für Leseförderung, Stadt Salzgitter, Organization for Human Brain Mapping, Deutsche Gesellschaft für Klinische Neurophysiologie, Deutsche Gesellschaft für Neuropsychologie, and
my family and friends.

Book cover artist: Clara Sophia Skeide

CONTENTS

1. INTRODUCTION

Developmental dyslexia[1] is characterized by severe deficits in literacy learning (ICD-10 (WHO, 2016); DSM-5 (APA, 2016)). Like all learning disorders, it is assumed to originate from a complex interplay between (epi-) genetic and environmental factors and their effects on brain development (Peterson & Pennington, 2015; Ozernov-Palchik et al., 2016). With a heritability rate of about 40-60% (Christopher et al., 2013; Logan et al., 2013), dyslexia occurs across cultures in all educational systems studied (Peterson & Pennington, 2015). Prevalence rates vary considerably, however, as a function of the writing system of a certain language. Diagnostic criteria are met by about 3% of the population in *transparent* orthographies (such as Italian), in which letter-sound links are relatively unambiguous (Barbiero et al., 2012), but by about 7% in *intransparent* orthographies (like English) (Shaywitz et al., 1990).[2] Nevertheless, despite critical voices even questioning its very existence (e.g. (Elliott & Grigorenko, 2014)), dyslexia is internationally considered as one of the most common of all learning disorders.

Literacy impairment is often not only a life-long burden for affected individuals (Klassen et al., 2013), but also leaves a substantial amount of intellectual potential unexploited by society. Moreover, it is a substantial burden for governments since, without proper support, dyslexics are more likely to become unemployed or criminally offensive than unaffected individuals (Rack, 2005; Elbeheri et al., 2009). Taking the United Kingdom as an example and considering also educational and healthcare resources, the annual cost of dyslexia has been estimated to be as high as 1.6 billion pounds (KPMG, 2006). It is therefore not surprising that scientific research projects on this topic have received considerable amounts of funding. For example, between the years 2000 and 2009, the National Institutes of Health (NIH) have spent more than 107 million dollars to support studies on dyslexia (Bishop, 2010).

In this thesis, I report a series of experiments that I have conducted together with several collaborators to explore the neural origins of dyslexia from the

[1] henceforth I use the terms "developmental dyslexia" and "dyslexia" synonymously
[2] German stands roughly in between with about 4-5% (Moll et al., 2014a)

perspective of a biological psychologist. Clearly, an understanding of *impaired* literacy learning requires an understanding of *unimpaired* literacy learning. Accordingly, *CHAPTER 2 ("BECOMING LITERATE")* comprises an overview of reading- and writing[3]-related neuroplasticity. This blueprint is informed by the results of an experiment in which collaborators from Nijmegen taught illiterate adults in Northern India how to read and write so that we could examine the effect of becoming literate using functional magnetic resonance imaging (fMRI) (**Skeide et al., 2017**). *CHAPTER 3* is then focused on dyslexia itself. The overarching question of *Section 3.1* is *how dyslexia manifests itself in brain function and brain structure*. To provide new answers to this old question, I walk the reader through two new research avenues. Namely, I suggest that dyslexia might reveal itself by spontaneous activity patterns that are self-generated by the neural network underlying literacy (**Skeide et al., 2015**) and by subtle anatomical differences in the architecture of cortical layers within literacy-relevant regions (**Skeide et al., 2018**). Cross-sectional experiments with adults or school children (as described in *Section 3.1*) are a valuable tool for understanding the various forms of appearance of dyslexia. However, they do not allow to disentangle *potential causes* from the *consequence* that dyslexic individuals gain less literacy experience (both in terms of quantity and quality) compared to unaffected individuals (Goswami, 2015). In *Section 3.2 ("Predisposition")*, I report the results of three effortful studies overcoming this limitation. In these studies (carried out together with my former Ph.D. student Indra Kraft) we examined preliterate children *before systematic instruction in school* (**Kraft et al., 2015**) and followed them longitudinally to assess their literacy outcome (**Kraft et al., 2016**; **Skeide et al., 2016c**). Finally, in *Section 3.3 ("Clinical perspectives for early diagnosis and treatment")*, I outline potential future applications of my findings following a recent review article in which I argued that MRI might help to *predict the risk for dyslexia before school enrollment* (**Skeide, 2017**).

[3] referring to orthographic processing (German: "Rechtschreibung"), not to the motor act of writing (also note footnote 7 in this context)

2. BECOMING LITERATE

In contrast to language, we do not *implicitly* acquire the ability to read and write. Instead, literacy requires systematic instruction and intensive practice and is thus mostly (but not entirely) learned *explicitly*. A classical model (Frith, 1986) postulates that a learner goes through three stages of proficiency: a logographic, an alphabetic and an orthographic stage. In the *logographic* stage, print is processed like any other visual stimulus so that meaning is (unreliably) associated with *global* visual features (such as color, overall size or font). The transition to the *alphabetic* stage requires "*phonological awareness*", i.e. the ability to attentively segment the continuous speech stream into discrete units (*phonemes*). This ability usually emerges with early cultural experience (e.g. rhymes in children's songs) at around age 5 (Wagner & Torgesen, 1987) and refines with literacy experience (Castles et al., 2011). Learning the links between phonemes and letters (with their specific *local* features) is the first challenge. Next, the learner must grasp that single letters can be combined in a certain order to form a sequence. With this knowledge, letter strings and the corresponding phonemes can be merged into first words. Further refinement is eventually necessary to be able to tie certain phonemes to groups of letters (*graphemes*), such as [ʃ] to 'sch' in German. The final *orthographic* stage is entered when first grapheme strings are stored in memory as visual word forms to build up the *orthographic lexicon*. In the course of further development, *phoneme-grapheme mapping* gets progressively replaced by *whole-word recognition*. As a consequence, meaning can be accessed more efficiently via an interface between the *orthographic* and the *semantic lexicon*. These multiple milestones of literacy constitute a learning challenge that requires recombination, coordination and automatization of visual and language skills (Lachmann & van Leeuwen, 2014).

Learning how to read and write is a prime example of neuroplasticity. It crucially depends on the flexibility of the brain to reorganize itself in response to environmental influences. To investigate the neuroplastic emergence of alphabetic and orthographic skills, two gold-standard designs can be applied. One option is to examine *preliterate children* and follow them longitudinally over the school years. The other option is to instruct *illiterate*

adults and assess longitudinal changes caused by the intervention. To the best of my knowledge, up to now, only three MRI studies have successfully implemented these very resource-intensive longitudinal designs: the study of Silvia Brem and colleagues (Brem et al., 2010), the study of Kanwisher and colleagues (Saygin et al., 2016) and the study that I have conducted together with my colleague Falk Huettig (**Skeide et al., 2017**)[4].

In their groundbreaking project, Brem et al. (2010) let preliterate 6-year-old kindergarten children practice letter-sound links at home with a computer game over an 8-week period totaling about 3.6 hours (20 minutes per week). At the behavioral level, the children almost tripled their response accuracy in the letter-sound matching game (from 5% to 14% on average). At the neural level, the training induced significant changes of functional reactivity. In particular, the amplitude of the BOLD response to visually and/or auditorily presented words increased significantly after training in parts of the visual system. One of the effects was obtained in the dorsal cuneus, i.e. an area in the *dorsal* visual "where" *stream* (Goodale & Milner, 1992) supporting visuospatial processing (Vossel et al., 2014). The other (stronger) effect was obtained in the ventral temporo-occipital cortex, i.e. an area in the *ventral* visual "what" *stream* (Goodale & Milner, 1992) supporting the recognition of complex objects, particularly faces (Kanwisher et al., 1997). This significant increase of functional responsivity to print in the ventral temporo-occipital cortex was later replicated in an independent sample followed from a preliterate kindergarten age (5 years) to second grade (8 years) (Saygin et al., 2016). Left temporo-parietal and particularly the left ventral temporo-occipital cortices, thus play a central role for reaching the *alphabetic* stage of literacy.

An additional central role of the ventral temporo-occipital cortex for reaching the *orthographic* stage of literacy was illuminated in an independent set of experiments published a few months later (Dehaene et al., 2010). The authors of this study demonstrated cross-sectionally that BOLD amplitudes to written sentences were significantly higher in literate compared to illiterate adults, namely, in a left fusiform subregion partly overlapping with the

[4] but note the longitudinal work on samples with dyslexia (*Section 3.2.*)

cluster reported by Brem et al. (2010). This region is now well-known as the "visual word form area" (Cohen et al., 2000). Moreover, it turned out that literate, but not illiterate adults were able to activate core regions of the *auditory* language network via the visual word form area and vice versa. Accordingly, when becoming literate, parts of the ventral temporo-occipital cortex transform from a seemingly unimodal visual processing area into an *interface* between the visual system and the auditory language system in the temporo-parietal cortex.[5]

Another neuroplastic effect of literacy is a permanent enhancement of BOLD responses to simple visual patterns, such as checkerboards, and also to complex visual objects such as faces, tools or houses. Interestingly, this change can not only be observed in the fusiform cortex but even in lower-level processing regions within the primary visual cortex (V1) (Dehaene et al., 2010). Our recent work **(Skeide et al., 2017**) suggests that such literacy-induced functional reorganization of the visual system is even more far-reaching than previously thought. We have shown that only 6 months of formal reading and writing instruction leads to functional neuroplasticity in the mature brain of illiterate adults that 1. is detectable even further upstream of V1 in the earliest subcortical computation centers and 2. reflects self-generated, spontaneous activity of neuronal populations (not task-induced reactions to external stimulation). Specifically, after literacy instruction, resting-state BOLD timecourses turned out to be significantly more strongly correlated in a cortico-subcortical network including the right occipital cortex (V1-V4), the bilateral pulvinar nuclei of the thalamus and the right superior colliculus of the brainstem. In addition, individual slopes of cortico-subcortical functional connectivity were significantly positively associated with individual gains in letter knowledge and word reading skills. Based on the results of experiments with rodents and nonhuman primates it has long been speculated that the superior colliculi might support literacy learning, but this has never been shown in humans before. In particular, it is assumed that the superior colliculi *amplify* signals directly fed in from direc-

[5] Note, however, that the "visual word form area" is not necessarily a strictly bimodal, but potentially a multimodal interface that can develop even without visual experience, e.g. as an audio-tactile interface in congenitally blind Braille readers (Reich et al., 2011).

tion-selective retinal ganglion cells (Shi et al., 2017). Our findings thus might reflect the encoding and/or consolidation of direction detection mechanisms presumably needed for efficiently navigating fixations through letter strings (Lewis et al., 2009; Gregory et al., 2014). The pulvinar nuclei receive direct input from the superior colliculi (Tamietto & de Gelder, 2010) and likely act as a first *filter* preselecting signals for further processing in the occipital cortex (Yantis et al., 2002; Kastner et al., 2004). Interestingly, the pulvinar nuclei have been recently related to dyslexia (Jednoróg et al., 2015), but we will later see that thalamic anomalies should currently be seen as a consequence rather than a possible cause of the disorder.

Finally, it must be noted that, at the behavioral level, it is well-documented that literacy learning refines not only visual, but also higher-order auditory processing, in particular phonological awareness (Castles et al., 2011). While, to my knowledge, compatible longitudinal evidence at the neural level is currently not available, one cross-sectional positron emission tomography experiment underlines this notion (Castro-Caldas et al., 1998). Here, the authors were able to demonstrate that literate adults (with high phonological awareness) outperformed illiterate adults (with low phonological awareness) in a pseudoword repetition task requiring controlled phoneme discrimination. Crucially, literate participants activated the left temporoparietal cortex significantly more strongly than illiterate participants while performing this task. Accordingly, functional adaptation of the left temporoparietal cortex to advanced phonological processing demands seems to be another neural milestone of becoming literate.

Integrating these findings and adding further work, I introduce a blueprint of how reading and writing skills emerge in the brain (**Figure 1**). Thereby, I distinguish the finetuning of the *bilateral* visual system (**Figure 1A**) from the emergence of a *left-hemisphere* interface between the visual system and the auditory language system (**Figure 1B**).[6]

As described before, literacy learning tailors low-level visuospatial computation to the characteristics of print. It optimizes bottom-up signal prepro-

[6] The interface with a subportion of the motor system needed for handwriting (i.e. Exner's area (Roux et al., 2009)) is beyond the scope of this blueprint.

cessing in the superior colliculi of the brainstem (*amplification*) and in the pulvinar nuclei of the thalamus (*filtering*) before the information is fed forward into the occipital cortices for stimulus direction detection (**Figure 1A**) (Shi et al., 2017; **Skeide et al., 2017**). It remains to be shown if becoming literate also has an impact on saccade initiation which is related to a network directly connecting the superior colliculi with the frontal eye fields as parts of the dorsal attention system (Dorris et al., 1997). Evidentially, literacy learning also influences higher-order visuospatial processing in the bilateral antero-superior cuneus (as part of the dorsal visual stream) which might be under top-down attentional control (Brem et al., 2010).

Another consequence of becoming literate is an increased response to complex visual objects in the ventral temporo-occipital cortex (as part of the ventral visual stream; **Figure 1A**) (Dehaene et al., 2010). Several skills are associated with this effect in the literature, in particular refined discrimination of shape and orientation (Sigman et al., 2005; Pegado et al., 2014). Furthermore, increased response to complex visual objects is assumed to specifically indicate the formation of abstract graphematic representations enabling us to identify visually distinct letter notations (e.g. a handwritten “L” and an “L” in Times New Roman font) as a common conceptual entity (Vinckier et al., 2007).

Similarly, in the auditory domain, literacy learning leads to an increased response to phonemes in the left temporo-parietal cortex whenever phoneme access is under attentional control (Castro-Caldas et al., 1998). The left temporo-parietal and left inferior frontal cortex form the backbone of phonological awareness (**Figure 1B**).

An additional major neurocognitive transformation that takes place when learning how to read and write is that orthographic knowledge is stored in the ventral temporo-occipital cortex – initially in the form of graphemes (Brem et al., 2010) and finally also in the form of whole words (Glezer et al., 2009) (**Figure 1B**). The growing orthographic lexicon can be seen as an interface to the three core domains of auditory language processing: phonology, semantics and syntax (Hagoort, 2013; Skeide & Friederici, 2016b).

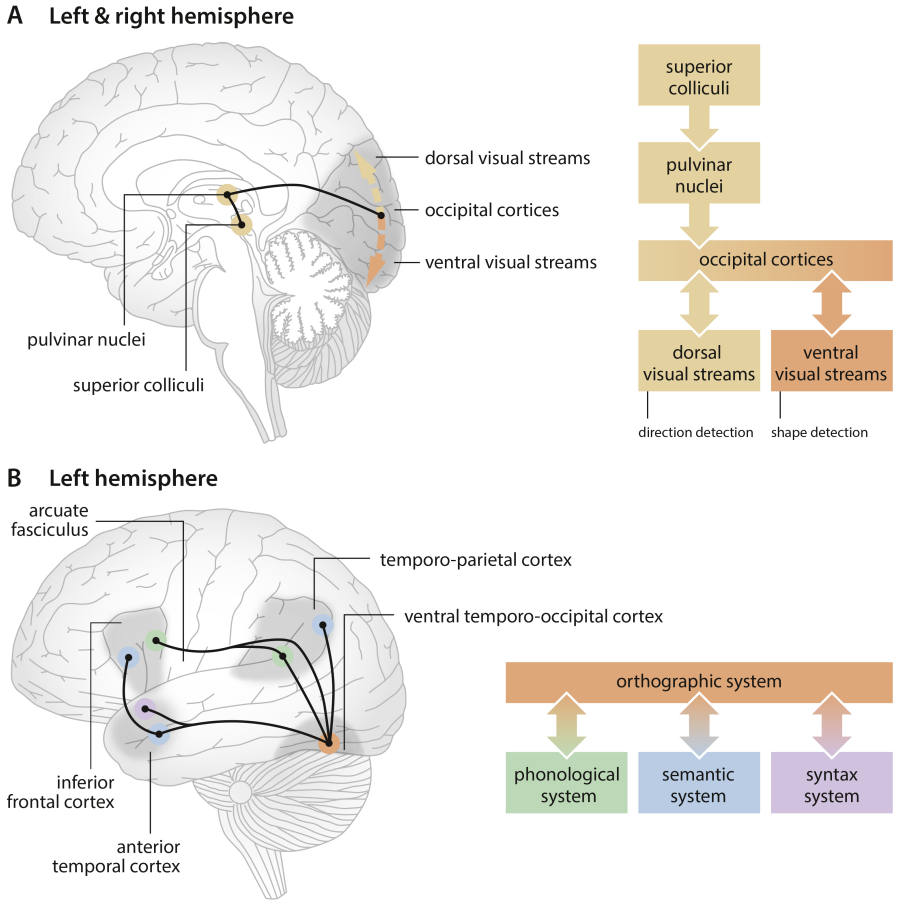

Figure 1. Literacy-induced neuroplastic reorganization. (A) Text recognition requires refined direction detection mechanisms necessary for efficient visual navigation through letter strings. The brain adapts to these demands by adjusting preprocessing of signals in the superior colliculi of the brainstem and the pulvinar nuclei of the thalamus that are later fed into the dorsal visual stream (*ocher*). Moreover, the ventral visual stream becomes more sensitive to subtle differences in shape characterizing letters (*orange*). (B) Practising letter-sound links triggers several other reconfiguration processes. The ventral temporo-occipital cortex transforms into the orthographic system that creates abstract representations of letters and later entire words (*orange*). At the same time, controlled phonological processing in left temporo-parietal and inferior frontal cortices is further fine-tuned so that the phonological system becomes more sensitive to subtle differences between speech sounds (*green*). Both systems quickly wire together into the orthography-phonology interface (*orange-green up-down arrow*). With further literacy experience, the orthographic system connects with the semantic system (parietal, anterior temporal and inferior frontal cortices; *orange-blue up-down arrow*) and the syntactic system (anterior temporal cortex; *orange-purple up-down arrow*). As a consequence, semantic and syntactic representations can be directly accessed via letter strings without taking a detour via the phonological system.

The orthography-phonology interface between the left ventral temporo-occipital cortex, the left temporo-parietal cortex (extending from the superior temporal to the supramarginal gyrus) (Linkersdörfer et al., 2015) and the left inferior frontal cortex (i.e. the posterior pars opercularis of Broca's area) (Boets et al., 2013) (**Figure 1B**) is established via the left arcuate fasciculus (Thiebaut de Schotten et al., 2014). This network constitutes the neural basis for phoneme-grapheme conversion (Preston et al., 2016). It is therefore a crucial computational resource in the alphabetic stage of literacy and remains important in the orthographic stage of literacy whenever infrequent or foreign-language words or pseudowords are encoded or decoded.

The interface between the orthographic lexicon and the semantic lexicon is assumed to comprise a ventral pathway including left anterior temporal and inferior frontal cortices and also a dorsal pathway including the left angular gyrus (Carreiras et al., 2014) (**Figure 1B**). However, given that semantic information is stored in widely distributed cortical repositories (Huth et al., 2016), it is likely that there are multiple other dorsal and ventral semantic streams converging in the ventral temporo-occipital cortex.

Morpho-orthographic processing is an integral part of efficient reading and writing (Grainger & Ziegler, 2011). Orthographic codes for morphemes facilitate word categorization. The German suffix "-ung", for example, unambiguously marks a noun. Accordingly, morpho-orthographic processing strongly speeds up word recognition (Hasenäcker et al., 2017). Currently available data suggest that the neural underpinnings of this orthography-syntax interface are the ventral temporo-occipital cortex and the left anterior temporal cortex (Dikker et al., 2009; Zweig & Pylkkänen, 2009) (**Figure 1B**) connected by a ventral pathway.

Taken together, the data discussed in this chapter allowed us to draw a coherent picture of the brain basis of literacy learning. It has become clear that both the visual and the auditory system undergo neuroplastic change when we become literate. In the visual domain, direction detection in the dorsal stream and shape detection in the ventral stream is finetuned such that grapheme strings can be recognized efficiently. In the auditory domain, the left temporo-parietal cortex reacts more sensitively to differences between phonemes. Finally, grapheme-phoneme mapping is implemented in a cross-

domain network consisting of left ventral temporo-occipital, temporo-parietal and inferior frontal cortices.

Longitudinal studies allowing for a direct look at the neuroplasticity of literacy learning are still very scarce. Accordingly, there are numerous open questions only some of which can be mentioned here. For example, follow-up longitudinal studies should test the hypothesis that subcortical neuroplasticity cannot only be found in the visual, but also in the auditory pathway (see *Section 3.1.*). Additionally, although not shown yet, it is likely that literacy does not only reorganize the functional responses of our visual and our phonological system, but also of our semantic and syntactic system given that our semantic lexicon grows and our syntax skills refine substantially during primary school (Skeide et al., 2016a). Finally, like many other colleagues in the field, I assume that the brain accommodates *writing* skills in the same network that underlies *reading* skills, but this hypothesis needs to be directly confirmed.[7]

3. DEVELOPMENTAL DYSLEXIA

Reading and writing skills are most adequately represented as continuous variables following an approximate normal distribution in the population. Accordingly, to make the diagnosis "developmental dyslexia", a predefined arbitrary cutoff must be set to split these variables into two categories: impaired vs. unimpaired performance (Peterson & Pennington, 2015). Actual diagnostic criteria used vary considerably in educational practice. To the best of my knowledge, to date, no country has established a national standard procedure. In Germany, for example, diagnostic guidelines are often not even the same within federal states – with the exception of Saxony (Steinbrink & Lachmann, 2014).

The only constant in *scientific* studies on dyslexia is that children with below-average domain-general abilities (IQ < 85) and/or sensory dysfunction are usually excluded. By definition, following ICD-10 and DSM-V, any specific learning disorder must not be explicable by such basic deficits (WHO, 2016; APA, 2016). Apart from that, acquisition and selection of

[7] The methodological challenge here is to avoid that writing tasks introduce motion artifacts compromising MRI data quality.

diagnostic data, and also applied cutoff values differ substantially between studies. Some authors rely on an "official diagnosis" by professionals which is usually not only based on quantitative psychometric assessment (mostly reading, writing, IQ, audition, vision and language) but also on qualitative evaluation (e.g. parental anamnesis or school report). Others report the results of standardized literacy and IQ measures acquired in the laboratory. A combination of both, i.e. an independent validation, is rarely found. Furthermore, while reading speed (number of words or pseudowords read in a certain time) is most often taken into account, additional measures, such as reading accuracy (conversion of printed text into corresponding speech), reading comprehension (decoding meaning from print), or writing (after dictation) accuracy, are not consistently reported. Finally, cutoffs for impaired performance characterizing dyslexia usually vary from 1.67 to 0.67 standard deviations (SD) below the mean of the corresponding age group. Some authors even argue that individuals with above-average IQ (>115) should receive a diagnosis if their literacy performance is less than 0.67 SD below the mean in case it is at least 1SD below their individual IQ (Schulte-Körne, 2010). These authors point out that individuals with high ability but discrepant poor literacy skills should not be excluded from intervention (Peterson & Pennington, 2015). Remarkably, at the neural level, conservative and more liberal diagnostic criteria have revealed surprisingly similar results that are summarized below in the subsequent *Section 3.1*. In fact, the most consistently reported differences are quantitative rather than qualitative in nature. This observation supports the notion that dyslexia is not a categorical construct. Instead, it represents the lower extreme of a continuum related to a consistent phenotype across the whole distribution.

3.1. Manifestation

Systematic empirical research on dyslexia beginning in the 1960s has led to a remarkable diversity of behavioral findings. Observations range from memory deficits over high-level executive function deficits to low-level sensory processing deficits. A small selection of clearly delimitable results marking the spectrum of the field is provided in **Table 1**. A complete summary of the behavioral results is far beyond the scope of the present thesis.

Table 1. Dyslexia-specific behavioral deficits

General domain	*Specific function affected*	*First empirical evidence*
declarative and procedural long-term memory	"rapid automatized naming" -link visual object to corresponding phonological word form	(Denckla, 1972)
procedural long-term memory	visuomotor coordination	(Nicolson & Fawcett, 2000)
short-term memory	store phonemes	(Liberman et al., 1977)
	store visuospatial information	(Ben-Yehudah et al., 2001)
	store auditory frequency information	(Ahissar et al., 2006)
executive function	phonological awareness -distinguish and/or compare phonemes while suppressing irrelevant (e.g. semantic) information	(Bradley & Bryant, 1978)
oculomotor control	navigate fixation through print	(Frank & Levinson, 1973)
vision	contrast sensitivity	(Lovegrove et al., 1980)
	motion sensitivity	(Cornelissen et al., 1995)
visual attention	attentional dwell time (letters)	(Hari et al., 1999)
	visual search (form, color)	(Vidyasagar & Pammer, 1999)
audition	temporal processing (tones and phonemes)	(Tallal, 1980)
	spectral processing (frequency discrimination)	(Ahissar et al., 2000)
	rhythm detection	(Goswami et al., 2002)
	prosodic stress detection	(Goswami et al., 2010)
auditory attention	attentional dwell time (click sounds)	(Hari & Kiesila, 1996)

Similarly, numerous theoretical approaches have been developed to integrate behavioral dimensions of dyslexia into a coherent conceptual framework. While first attempts are largely based on monocausal explanations considering dyslexia as a visual processing deficit, recent models emphasize the multifactorial nature of a very complex learning disorder. A good exam-

ple is Lachmann's "functional coordination deficit" hypothesis. The central claim made here is that dyslexia could not only result from sensory dysfunction, including auditory modification deficits (e.g. phonological categorization) and visual modification deficits (e.g. mirror invariance suppression[8]), but also from any other deficit in cognitive coordination (e.g. phoneme-grapheme mapping), even if it emerges at a later stage of literacy acquisition (e.g. whole-word recognition) (Lachmann, 2002). Pennington's "multiple deficit model" is another influential contemporary contribution (Pennington, 2006) and has recently undergone substantial refinement by Gaab and colleagues (Ozernov-Palchik et al., 2016). Considering all explanatory levels, including genetic, environmental, neural and behavioral dimensions, the authors distinguish not only risk but also protective factors. Moreover, they do not only acknowledge that these factors interact in a complex fashion, but also point out that this interplay can undergo considerable changes as it underlies strong developmental dynamics.

Following the scope of the current thesis, I focus my summary on theoretical accounts that make specific predictions regarding the brain basis of developmental dyslexia (**Table 2**). It is thus unavoidable that several important and influential concepts, like Coltheart's "double deficit theory" (Coltheart et al., 2001) or the claims made in Seidenberg's "connectionist model" (Seidenberg, 2012), cannot be considered here.

In contrast to numerous behavioral observations of dyslexia-specific deficits and the resulting variety of explanatory approaches, to date, only three major theories of the brain basis of dyslexia have been put forward explicitly. It is surprising to see that already in 1877 German neurologist Kussmaul made specific predictions in the framework of Wernicke's theory of aphasia, although he never put them to the empirical test. Kussmaul was convinced that dyslexia ("word blindness") is a disconnection syndrome caused by a disruption of one or more white matter fiber tracts connecting retinal neurons and downstream relay stations of the visual pathway with Wernicke's speech area in the left temporo-parietal cortex (Kussmaul, 1877).

[8] i.e. learning to recognize a mirror image as a different object to distinguish e.g. between "d" and "b"

Table 2. Theoretical accounts of the brain basis of dyslexia

Account	*Specific main claims/observations*	*First formulation*
word blindness	fiber pathways originating from retinal neurons and terminating in Wernicke's area are defect	(Kussmaul, 1877)
cerebellar deficit	-reduced nerve cell sizes in the right cerebellum (Rae et al., 1998) -hypoactivation of the right cerebellum and hyperactivation of the medial prefrontal cortex while learning a sequence of finger presses (Nicolson et al., 1999)	(Frank & Levinson, 1973)
magnocellular deficit	-reduced nerve cell sizes in magnocellular layers of the lateral geniculate nucleus of the thalamus (Livingstone et al., 1991) -reduced nerve cell sizes in magnocellular layers of the medial geniculate nucleus of the thalamus (Galaburda et al., 1994) -reduced BOLD response to randomly moving dots in visual area 5 (Eden et al., 1996) -dysfunction of the "posterior parietal cortex" (Brodmann areas 5, 7, 39 and 40) (Stein & Walsh, 1997) -reduced BOLD response to phoneme changes in left medial geniculate nucleus of the thalamus (Díaz et al., 2012)	(Livingstone et al., 1991)

Almost a century later, Frank & Levinson came up with the cerebellar deficit hypothesis. Assessing a large sample of 6- to 14-year-old children with dyslexia (N = 115), they argued that 97% percent of these individuals showed behavioral signs of cerebellar dysfunction known from patients with lesions in the cerebellum, including difficulties in oculomotor control (fixation) (Frank & Levinson, 1973). First neural evidence in support of this claim was later provided by a magnetic resonance spectroscopy study sug-

gesting reduced nerve cell sizes in the right cerebellum of adult dyslexic men (Rae et al., 1998). In line with this, several MRI experiments, also mostly involving male subjects, revealed reduced gray matter volume in the right cerebellum of individuals suffering from dyslexia (Brown et al., 2001; Eckert et al., 2003; Brambati et al., 2004; Eckert et al., 2005; Pernet et al., 2009). Corresponding functional evidence is scarce. The first study reporting hypoactivation of the right cerebellum in dyslexics vs. controls while learning a sequence of finger presses (Nicolson et al., 1999) did not provide any direct association of this measure with literacy skills. The same limitation applies to a later study reporting reduced functional connectivity between the right cerebellum and middle/superior frontal gyrus during a phonological awareness task and another study vaguely describing more diffuse cerebellar activation patterns during a covert word generation task (Stanberry et al., 2006; Baillieux et al., 2009).

The magnocellular deficit hypothesis was initially grounded on two small-scale post mortem studies. Nissl staining of the thalami of 11 adult (10 male) brains indicated that nerve cell bodies were significantly reduced in size in 5 dyslexic cases compared to 6 control individuals. This difference was found both in the lateral and in the medial geniculate nucleus and anatomically confined to the magnocellular layer known to consist of larger cell bodies compared to the parvocellular layer of the thalamus (Livingstone et al., 1991; Galaburda et al., 1994). Based on these observations, Galaburda and colleagues hypothesized that dyslexia might arise from impaired low-level processing in subcortical computational cores of the visual (lateral geniculate nucleus) and the auditory pathway (medial geniculate nucleus). To date, there is only sporadic in-vivo evidence supporting this notion. It cannot be excluded, however, that this lack of support goes back to technical limitations of currently available methods. Structural MRI at 3 Tesla does not provide sufficient resolution to reconstruct thalamic layers. Moreover, pulsating veins of the brainstem can decrease the sensitivity of subcortical functional MRI in surrounding thalamic nuclei (Guimaraes et al., 1998). So far, one fMRI study suggests that hemodynamic activity in the medial geniculate body of the left thalamus is reduced in adult dyslexic individuals compared to controls during phonological processing in an auditory syllable

discrimination task (Díaz et al., 2012). The authors of another recent structural MRI study have reported a significant volume reduction and shape alteration of the left lateral geniculate nucleus of dyslexics compared to controls (Giraldo-Chica et al., 2015). Further indirect evidence in favor of the magnocellular deficit hypothesis comes from a small-scale fMRI experiment in which 6 adult subjects with dyslexia and 8 matched controls judged the velocity of randomly moving visually presented dots. BOLD responses turned out to be significantly reduced in the bilateral middle temporal visual area (V5), a part of the ventral attention system known to be directly connected with the lateral geniculate nucleus (Eden et al., 1996).[9]

The "phonological deficit hypothesis" in its classical version already framed in the late 1970s (see **Table 1**, *phonological awareness*) stands out as the conceptual framework that is most strongly in line with the currently available brain data. Numerous studies have identified a high-level phonological processing system comprising left temporo-parietal and inferior frontal cortices that is connected via the arcuate fasciculus as the neural substrate of dyslexia (Paulesu et al., 1996; Klingberg et al., 2000; Shaywitz et al., 2002; Hoeft et al., 2006; Blau et al., 2010; Vandermosten et al., 2012a; Boets et al., 2013). As a consequence, dyslexia-specific hypoactivation, reduced gray matter volume and reduced white matter fractional anisotropy[10] are consistently found within this network in MRI metaanalyses (Richlan et al., 2009; Vandermosten et al., 2012b; Richlan et al., 2013). My own work (**Skeide et al., 2015**) seamlessly fits into this view and at the same time adds an additional explanatory dimension to it. Given that reading-related tasks in previous fMRI studies had already consistently revealed reduced responses in left temporo-parietal and inferior frontal cortices of dyslexics vs. controls (Richlan et al., 2009), we were not interested in replicating these results. Instead, following earlier groundwork (Lohmann et al., 2010; Koyama et al., 2011) we tested the hypothesis that dyslexia-relevant functional alterations in this network can even be detected in the resting brain. To this end, we focused on spontaneous BOLD signals that were not task-based, but self-

[9] but see direct counterevidence reported later by the same group (Olulade et al., 2013)
[10] a measure of water diffusion direction in white-matter fibers that is unspecifically related to myelin concentration, axon size and fiber density (Scholz et al., 2009; Paus, 2010)

generated without any external linguistic stimulation. Moreover, despite the known heritability of dyslexia, only very few earlier studies had combined genetic and fMRI data before (Cope et al., 2012; Pinel et al., 2012). Accordingly, our second goal was to shed new light on the associations between dyslexia risk variants and brain function. The single nucleotide polymorphism rs11100040, a modifier of the dyslexia risk gene *SLC2A3*, emerged as an ideal test case. It was already known to be related to phonological processing in German-speaking children and to regulate neuronal glucose transport (Roeske et al., 2011). As expected, we observed that 9- to 12-year-old children who carried a risk variant of rs11100040 (compared to non-carriers) showed significantly reduced resting-state functional connectivity between the left inferior frontal and the left superior temporal cortex. In addition, individual functional connectivity indices were significantly associated with individual fractional anisotropy values of the interconnecting fiber pathway (i.e. the arcuate fasciculus) which in turn were significantly related to individual phonological awareness skills. These results are corroborated by independent studies supporting the notion that a fronto-temporal network including the left temporo-parietal and inferior frontal cortices forms the backbone of phonological awareness and is thus closely linked to dyslexia (Saygin et al., 2013; Myers et al., 2014; Vandermosten et al., 2015).

In addition to the classical phonological deficit hypothesis, which, in my opinion, should rather be labeled as the "high-level phonological deficit hypothesis", a second line of comprehensive evidence can be considered as what I call the "low-level phonological deficit hypothesis". Advocates of the high-level phonological deficit hypothesis (see e.g. (Boets et al., 2013)), on the one hand, would argue that acoustic-phonological representations of dyslexic individuals are *intact*, *but cannot be accessed quickly enough* to ensure efficient reading and writing. The reason for this deficit is seen in that top-down access of the left inferior frontal cortex to phonemes stored in the left temporo-parietal cortex is impaired by faulty functional and structural connectivity of these areas via the arcuate fasciculus. Advocates of the low-level phonological deficit hypothesis, on the other hand, would argue that acoustic-phonological representations of dyslexic individuals are *not*

intact. In the last years, various possible explanations for this phenotype have been given, three of which are now briefly summarized. Kraus and colleagues, for example, have demonstrated that dyslexic individuals might build up noisy acoustic-phonological representations already in the inferior colliculus of the auditory brainstem, even before any information reaches the auditory cortex (Hornickel & Kraus, 2013). Another account is given by the already mentioned study also supporting the magnocellular deficit hypothesis (Díaz et al., 2012). The argument made here is that, in dyslexia, responses of the medial geniculate body of the thalamus are not properly modulated by the auditory cortex during phonological processing. This kind of cortico-subcortical feedback is believed to be vital for efficient reading and writing. Finally, there is also evidence for reduced responses to sound transitions at phoneme-specific frequencies of 30 Hz in the dyslexic auditory cortex presumably pointing to phonological short-term memory problems (Lehongre et al., 2011).

These three accounts are supported and specified by a small-scale study (6 dyslexic adults vs. 6 controls) that I have conducted (**Skeide et al., 2018**). Here, we acquired structural images with a resolution of 400 micrometers isotropic using ultra-high field MRI at 7 Tesla. These data allowed us to develop a detailed reconstruction of the cortex profiles in phonological processing areas (**Figure 1B**, green regions). Accordingly, we could very precisely measure cortical thickness (Bazin et al., 2014) and accurately estimate myelin concentration from the T1 signal (Stüber et al., 2014). It turned out that these indices did not distinguish dyslexic cases from controls neither in the left inferior frontal nor in the temporo-parietal cortex. Instead, only the core region of the left auditory cortex revealed an atypically and significantly increased myelin concentration in the sample of dyslexics. Interestingly, this difference was most pronounced in a sampling point within layer IV. In contrast to other cortical layers, layer IV of the auditory cortex is known to receive input from both the medial geniculate body of the thalamus and the inferior colliculus of the brainstem via afferent fibers (Sakata & Harris, 2009). These data, together with the data of the three studies mentioned shortly before, suggest faulty functional and structural connectivity within low-level processing units of the auditory pathway, including mesencephalic

and diencephalic nuclei upstream of the core auditory cortex. This might lead to impaired firing of neuronal populations in layer IV (**Skeide et al., 2018**) and/or disrupted oscillatory activity generated in layers II/III of the auditory cortex (Giraud & Ramus, 2013). Follow-up studies combining ultra-high field MRI with time-sensitive electrophysiological techniques are necessary to confirm these scenarios.

Given its crucial role for becoming literate (as seen in *Section 2*), it is not surprising that the left ventral temporo-occipital cortex has also emerged as an often identified major candidate region (see metaanalysis of (Richlan et al., 2009)). In this area, samples of dyslexic individuals usually show under-activation compared to controls in reading-related tasks (Paulesu et al., 2001; Shaywitz et al., 2002; Maurer et al., 2007; van der Mark et al., 2009; van der Mark et al., 2011). While this system did not receive much attention in the mentioned major modeling attempts, it plays an outstanding role in the blueprint of the neural origins of dyslexia that I outline in *Section 3.2*.

In sum, in this chapter, we have seen that classical concepts of the brain basis of dyslexia have received relatively little empirical support so far. Instead, the existing evidence speaks in favor of the "phonological deficit hypothesis", particularly in its "high-level" version focusing controlled language processing, but also in its "low-level" version centered around the early auditory pathway. Nevertheless, both the currently less and the more evident forms of manifestation deserve further investigation. Their relative contributions remain to be precisely determined to diminish the number of puzzle pieces that have to be put together to develop a comprehensive model. The direct way to reach this goal would be a systematic evaluation of ideally all phenotypes[11] in one consistent series of experiments run on a single sample. As soon as we can fully answer the question "What are the neural signatures of dyslexia?", we are also in a better position to answer the question: "What might cause dyslexia?" This perspective now brings us to the next chapter.

[11] many of which could not be mentioned here

3.2. Predisposition

Despite the fact that dyslexia is a *developmental* learning disorder, an overwhelming majority of neuropsychological studies has not taken a *developmental perspective* on this topic. Cross-sectional experiments elucidating the outcome of dyslexia in the brain of adults or school children form the by far largest part of the currently available body of literature. Undoubtedly, this core chapter of the thesis would not even exist without this precious groundwork. However, this type of experimental design is severely limited with respect to the endeavor of finding the neural origins of dyslexia. Specifically, whenever we start investigating individuals only after they already underwent literacy instruction, we have no experimental control over a crucial confound: Namely, we cannot rule out that participants with dyslexia have trained their brains *less efficiently* than participants without difficulties (Goswami, 2015). Affected individuals strongly tend to avoid practicing because reading and writing is coupled with negative experiences in school and at home (e.g. missing sense of achievement, desperate parents, being bullied by peers etc.) (Valas, 1999; Goldston et al., 2007). Coming back to the two longitudinal studies discussed above in *Section 2* (Brem et al., 2010; **Skeide et al., 2017**), there is good reason to assume that the impact of this training deficit is large. Even a couple of weeks of reduced experience likely lead to significant differences in brain development. Examining children "…before reading skills have been *extensively* trained" (Perrachione et al., 2016) thus does not resolve this issue. Theoretically, a better solution would be to test dyslexic and typically developing children with comparable reading experience. Monitoring training times, however, is difficult, if not impossible, in practice, so that several investigators have come up with a compromise solution, a "reading-level matched design". In this type of experiment, brain data of children with dyslexia are compared against data of younger unimpaired children who have attained similar literacy levels (Goswami, 2003). Although it is unclear how well performance levels correspond to actual experience levels, it is safe to assume that this correspondence is far from perfect. This might explain why such a design, so far, has revealed inconsistent effects not reaching statistical significance at the

whole-brain level (Hoeft et al., 2006; Hoeft et al., 2007; Olulade et al., 2013; Krafnick et al., 2014).

Obviously, the only way to control for the confounding factor of literacy experience, is to start examining children *before* they receive literacy instruction. Only research on preliterate children allows to separate neural signatures that qualify to play a *causal* role for dyslexia from those that must be seen as a *consequence* of the disorder (i.e. a training deficit). One way to narrow down the search for potential neural predispositions is to test preliterate children with a family history of dyslexia. This approach takes into account that dyslexia is heritable, so that 40-60% of all children carry their parents' problems further (Gilger et al., 1996; Ziegler et al., 2005). The first MRI experiments implementing such a design were run in the laboratory of Nadine Gaab at Harvard University. In their seminal study, Gaab and colleagues compared structural MRI data of 5-6-year old English-speaking children who had at least one first degree relative with a clinical diagnosis of developmental dyslexia (N = 10) to children that did not carry this burden (N = 10). Both samples were matched for age, sex and IQ. The authors made a remarkable discovery. Namely, statistically strongest reductions in gray matter volume in individuals with a familial risk were observed in the left temporo-occipital cortex and the left temporo-parietal cortex (Raschle et al., 2011). In other words, neuromaturational deviation in children at risk was localized in brain areas that later transform into core components of the reading and writing network (*see Section 2*; **Figure 1**) and that is also affected after years of literacy instruction in adults (*see Section 3.1*). Strikingly, together with my Ph.D. student Indra Kraft, I was able to precisely replicate this finding in a larger sample of 4-6-year old German-speaking children (N = 25 at risk vs. N = 28 without risk). Here we focused on cortical thickness, a measure that tightly relates to gray matter volume (Hutton et al., 2009). Cortical thickness turned out to be significantly reduced again in the left temporo-occipital cortex and the left temporo-parietal cortex at the whole-brain level. These differences could not be explained by differences in age, sex, IQ and parental education level (**Kraft et al., 2015**).

Significantly smaller cortical surface area and more sulcal basins of smaller size are also reported for the left temporo-parietal and the ventral temporo-

occipital region in addition to several domain-general regions (Hosseini et al., 2013; Im et al., 2016). Effect sizes of these features, however, seem to be smaller compared to gray matter volume given that the corresponding findings did not reach significance at the whole-brain level.

Interestingly, gray matter volume reduction and hypoactivation during high-level phonological processing (*see Section 3.1*) converge in left temporo-parietal and temporo-occipital cortices of the risk population. This important piece of evidence comes from a follow-up functional MRI study, Gaab and colleagues conducted on a larger sample of 5-6-year old children with a family history of dyslexia that was compared with matched controls (N = 18 vs. N = 18) (Raschle et al., 2012).

Familial risk studies on preliterate children are also impressively consistent from a network perspective. As we have seen in the preceding sections, the arcuate fasciculus is the structural backbone of the later orthography-phonology interface connecting temporo-parietal, temporo-occipital and inferior frontal cortices. Exactly in this white matter pathway, two very recent studies have now reported significant and anatomically specific fractional anisotropy reductions for children with a family history of dyslexia. Remarkably, this difference is not only present at a kindergarten age (Wang et al., 2017) but already in 6- to 17-month old infants (Langer et al., 2017). Accordingly, an aberrant maturation of the arcuate fasciculus might affect risk carriers long before literacy instruction and must therefore be seen as a strong candidate for a neurobiological predisposition of dyslexia. This view is corroborated by the fact, that arcuate fasciculus anisotropy has been so frequently found to be significantly related to phonological awareness (*see Section 3.1*). Namely, large-scale studies have shown that phonological awareness at a preliterate age is the best behavioral predictor of later literacy skills (Ziegler et al., 2010; Moll et al., 2014b).

What exactly might go wrong during the maturation of the arcuate fasciculus in children at familial risk for dyslexia? Unfortunately, fractional anisotropy analyses cannot answer this question. Fractional anisotropy is not only unspecific with respect to the underlying neurobiology (*see footnote 6*), but also influenced by geometric features of a fiber such as differences in axon coherence (Mezer et al., 2013). To tackle this issue, we set up a study in

which we computed myelin concentration (see *Section 3.1*) within a reconstruction of the arcuate fasciculus passing the two clusters derived from our previous study (**Kraft et al., 2015**) and the left inferior frontal cortex. Myelin concentration was significantly lower in risk carriers compared to controls in an anterior segment of the arcuate fasciculus connecting the left temporo-parietal and inferior frontal cortex (**Kraft et al., 2016**). This result points to a more specific potential neural predisposition for dyslexia. Namely, molecular work in rodents indicates that myelin might act as a consolidator of efficient information flow. In particular, it stabilizes white matter pathways between neuronal populations that coactivate in response to certain stimuli by preventing these pathways from forming new sprouts (Nave, 2010). Accordingly, being at familial risk for dyslexia might disturb the consolidation of finetuned cross-talk within an inferior frontal and temporo-parietal circuit and thus hinder the emergence of an intact high-level phonological processing system. Whether such a myelination defect occurs first in the fibers and then also affects cortical structure and function or vice versa remains to be found out.

While the literature on school-instructed dyslexics suggested that these individuals carry many different neural phenotypes, a review of cross-sectional experiments involving preliterate children at risk for dyslexia allowed us to carve out a relatively small set of consistent core patterns. However, the reported comparisons have limited explanatory power since they are only indirect in nature. Directly disentangling temporal cause from consequence requires the most effortful type of design: longitudinal studies following one and the same participants from preliteracy to literacy. To date, to my knowledge, three such longitudinal MRI analyses are published. The study from our laboratory that I have just discussed in the previous passage (**Kraft et al., 2016**) belongs in this circle. A subsample of 35 out of 53 children could be assessed for reading and spelling skills either at the end of first or second grade. 12 of these 35 children met diagnostic criteria for dyslexia, i.e. they performed below the 10th percentile rank of the corresponding age group either in a reading comprehension, reading fluency or spelling accuracy test. The final control group comprised 21 children with an at least low average performance above the 25th percentile in all three tests. Diagnostic

group membership (dyslexia vs. typical performance) was then entered as a dependent variable into a hierarchical binary logistic regression analysis. Not only brain measures (cortical thickness and myelin concentration) but also high-level phonological processing test data were included in the model. The rationale behind this selection of independent variables was to determine whether the neural indices could make a specific contribution to forecasting dyslexia prior to literacy instruction on top of the best known behavioral predictor.[12] While the behavioral model alone yielded a classification accuracy of 63%, myelin concentration of the left anterior arcuate fasciculus emerged as a statistically significant predictor raising classification accuracy of the combined model to 80%. Remarkably, 90% of all school children with dyslexia were correctly identified based on their arcuate fasciculus myelination and their phonological skills at a kindergarten age. These results are highly consistent with electrophysiological work in which a reduced phonemic mismatch negativity[13] turned out as a significant predictor of dyslexia not only at a kindergarten age (Maurer et al., 2009), but already in infancy at around 2 months of age (van Zuijen et al., 2013; Schaadt et al., 2015). Source-localization of the phonemic mismatch negativity has repeatedly revealed areas interconnected by the arcuate fasciculus, in particular inferior frontal and temporo-parietal cortices, both in kindergarteners (Maurer et al., 2009) and infants (Mahmoudzadeh et al., 2013).

To our surprise, the authors of an earlier study drew a completely different picture of the neural predisposition for dyslexia. Namely, based on their comprehensive MRI experiments, Clark and colleagues made the claim that "… primary neuroanatomical abnormalities that precede dyslexia are not in the reading network itself, but rather in lower-level areas responsible for auditory and visual processing and core executive functions." (Clark et al., 2014). How did the authors come to this conclusion? They computed cortical thickness maps of 17 Norwegian children at age 6-7 when they had not

[12] Both groups did not differ in terms of age, sex and parental education. A trend towards significance, however, was detected for non-verbal IQ which therefore was modeled as a covariate.

[13] which occurs later and reflects attentively controlled auditory processing (Wetzel et al., 2006; Roeske et al., 2011) in contrast to the early mismatch negativity reflecting preattentive auditory processing (Näätänen et al., 2007)

yet undergone formal literacy instruction in school. 7 of these children met the authors' liberal criterion for dyslexia[14] in grade 6 (age 11-12) while the remaining 10 children acquired typical literacy skills. Comparing these data at the whole-brain level, cortical thickness in the group of future dyslexics was considered significantly reduced (at an unacceptably liberal threshold[15]) in left auditory and visual cortices, cingulate gyrus and prefrontal cortices. However, a closer look at the participants indicates that the inferences made are questionable. Although the children seemingly had not yet undergone *formal* literacy training in school, many of them must have already acquired considerable letter knowledge and word reading skills *informally*. In fact, the 10 children in the control group were (on average) already able to correctly identify 34 out of 48 lower and uppercase letters and to correctly read aloud 8 words from a list of 34 words. Accordingly, these children had literacy skills comparable with the literacy skills of "ex-illiterate" adults after 6 months of intense training (*see Section 2*). The future dyslexics, however, only knew 15 letters and did not read any word correctly. In other words, differences in literacy experience (although acquired *before* school entry) might have drastically driven the effects.

In another set of experiments (**Skeide et al., 2016c**), we were able to overcome the limitations of the work by Clark et al. Moreover, we established a genetic framework to come up with a more mechanistic answer to the question whether gray matter maturation points to potential neural predispositions for dyslexia. At the time when we genotyped[16] our participants, a selection of 19 risk genes emerged from the literature reporting dyslexia/reading-relevant gene-behavior associations. Cellular functions of most of these genes were already relatively well-described. Many of them are known to play a role for early cortical maturation processes. In particular, some of them regulate sprouting and branching of dendrites and axons (neurites), while others regulate the formation of cortical layers by guiding neu-

[14] Dyslexia was quite unusually defined as scoring below the 25th percentile in 2 out of 4 accuracy-based tests: letter decoding, spelling, reading and writing after dictation.

[15] Combining a statistical height threshold of $p < 0.05$ with a spatial extent threshold of $p < 0.05$ does not reduce the risk of false positives in a satisfactory way, at least in voxel-based MRI data (Eklund et al., 2016).

[16] i.e. examined their DNA extracted from skin cells in saliva

ronal migration (Mascheretti et al., 2017). Downregulation of these genes leads to cortical malformation in rodents that is remarkably similar to the apparently life-long cortical malformations identified post mortem in dyslexic individuals (Galaburda et al., 2006). Given the persistent nature of gray matter abnormalities induced by dyslexia risk genes, we tested for gene-brain associations in a sample with a large age range of 3-12 years so that we could collect 141 datasets. To factor out general maturational variance, the effects of age, handedness, sex and total intracranial volume were statistically removed. Moreover, our aim was to account for evidence that risk genes might lead to both overgrowth[17] and diminished growth[18] at the same time (Gabel et al., 2010). To this end, we set up a multivariate model capturing clusters of both increased and decreased gray matter volume as a function of carrying the risk variant of a gene. Statistically significant associations were found in regions with previously described links to literacy learning, including the cerebellum and the prefrontal cortex (Nicolson et al., 1999), the angular gyrus (Carreiras et al., 2014), the cuneus and the left ventral temporo-occipital cortex (Brem et al., 2010). Our next and decisive step was to explore whether the gray matter volume profile of each of these clusters can statistically distinguish between children with and without dyslexia independently of general factors (age, sex, handedness, non-verbal IQ, parental education). Initially, we took a cross-sectional perspective and classified a subsample of 9-12-year olds (N = 34, N = 17 with a diagnosis of dyslexia). The discovery made was noticeably in line with the literature reported in the previous sections: Of all reported regions in which significant gene-brain links were found, only the cluster in the left ventral temporo-occipital cortex (associated with the gene *NRSN1*) allowed us to separate cases from controls significantly above chance (accuracy: 73.53%). Corresponding longitudinal data were available for 20 kindergarteners (age 5-6), 10 of which later developed dyslexia and 10 of which turned out to be typical learners at the end of first grade. These data gave us the unique oppor-

[17] i.e. axons/dendrites excessively sprouting/branching and/or neurons overshooting target layers
[18] i.e. axons/dendrites poorly sprouting/branching and/or neurons getting stranded in deep layers

tunity to identify gene-brain-behavior links possibly pointing to the biological predisposition for developing dyslexia. Our discovery was stunning. Aside from the angular gyrus cluster, only the left ventral temporo-occipital cortex cluster (also associated with the gene *NRSN1*) came to the fore as a significant predictor of future dyslexia before literacy (accuracy: 75%; sensitivity: 77%; specificity: 73%). Together, these analyses made it possible to sketch a coherent and complex scenario of the biological origin of dyslexia. Namely, independent of general brain maturation, general cognitive ability and parental education background, the gene *NRSN1* might disrupt sprouting and branching of cortical dendrites and axons in left ventral temporo-occipital regions and thus distorts computational processes supporting literacy learning.

While in the last years we have got a first grasp of potential neural predispositions for developing dyslexia, it must be noted that several important points are still open. At the genetic level, the field is confronted with the problem that the size of samples recruited at a single site usually does not offer sufficient statistical power to exclude false negative findings (Medland et al., 2014). In other words, although it is clear that dyslexia is polygenic, i.e. caused by many genes (Kere, 2014), the relative brain-maturational contribution of each variant cannot currently be determined. Moreover, it is puzzling that dyslexia risk genes seem to act upon anatomically very confined regions despite their general role for cortex growth (Plomin & Kovas, 2005). *NRSN1*, as an example, shows an expression gradient with maximum expression levels in posterior parts of the cortex (developmental transcriptome atlas at *www.brainspan.org*). Still, it is not clear why the effect of an *NRSN1* risk variant would be most severe in ventral temporo-occipital regions.

At the neural level, areas that are most often identified in the context of dyslexia are still broadly defined. The only exception is the inferior frontal region which is usually confined to Brodmann Area 44 (van der Mark et al., 2011; Boets et al., 2013). Effects in temporo-parietal regions reach from the posterior superior temporal cortex (Raschle et al., 2012; **Skeide et al., 2015**) up into the supramarginal gyrus (Raschle et al., 2011; **Kraft et al., 2015**). Similarly, effects in ventral temporo-occipital regions extend from the pos-

terior inferior temporal gyrus (**Kraft et al., 2015**) to the posterior ventrolateral fusiform cortex at the boarder with the occipital cortex (Raschle et al., 2012; **Skeide et al., 2016c**). What determines the exact location of dyslexia-specific differences? The answer to this question requires a network perspective leading us to several stimulus-specific anterior-to-posterior processing gradients. Coming to temporo-parietal regions first, it seems that anterior effects in the superior temporal cortex reflect deficient phonological processing at the sublexical level (Blau et al., 2010; Boets et al., 2013) and that posterior effects in the supramarginal gyrus reflect deficient phonological processing at the lexical level where semantic information comes into play and more short-term memory resources are needed (**Kraft et al., 2016**). Considering the temporo-occipital cortex, anterior effects in the posterior inferior temporal gyrus are related to decoding visual objects with links to phonological and/or semantic entities while effects in the posterior ventrolateral fusiform cortex are related to decoding visual objects without such links (van der Mark et al., 2009). How these gradients map onto white matter pathways, i.e. the several subbranches and termination zones of the arcuate fasciculus, is currently not understood.

As explained before, current structural MRI findings can be linked to cortical maturation mechanisms identified in animal studies. We are only beginning to understand, however, what the functional MRI findings, particularly hypoactivation during phonological processing, might mean at a mechanistic level. Interestingly, rats might represent a valid animal model for phonological processing since their phoneme discrimination thresholds seem to be nearly identical to human thresholds. Knockdown of the dyslexia risk gene *KIAA0319* in rat auditory cortex was recently found to result in delayed and inconsistent neuronal responses to human speech (Centanni et al., 2014)[19]. Such altered firing cascades might decrease BOLD signaling (Rees et al., 2000; Nir et al., 2008), but most likely also disturb the timing of oscillatory activity (Giraud & Ramus, 2013). Multimodal longitudinal studies ideally starting right after birth are necessary to determine when, where and how

[19] but see recent null findings for *DCDC2 (Centanni et al., 2016)*

noise comes into neural networks that form the brain basis of literacy (Hancock et al., 2017).

Finally, while inferior frontal and temporo-parietal effects in preliterate future dyslexics can be easily reconciled with behavioral data indicating that phonological awareness is the best predictor of literacy skills, it is less clear what the behavioral counterpart of the reported ventral temporo-occipital effects could be. Mapping visual objects to phoneme strings would be a plausible candidate, but this remains to be shown. A reported relation to "rapid automatized naming" (*see Table 2*), the second best behavioral predictor (Moll et al., 2014b), is driven by group differences but not by individual differences (Raschle et al., 2011). This underlines the plausibility of the view that rapid automatized naming most strongly challenges long-term memory and thus might contribute rather indirectly to literacy outcome.

To conclude, the data available up to this point make it possible to construct a clear yet coarse blueprint of the neural origins of dyslexia (**Figure 2**). At the heart of this blueprint is the observation that atypical architecture and faulty functioning of the later reading and writing network presage dyslexia in the first years of life, long before literacy training. From infancy on, future dyslexic individuals might have less myelin in the left arcuate fasciculus (**Kraft et al., 2016**; Langer et al., 2017). In addition, from age 5 on, at the latest, they show signs of cortical malformation, conceivably neurite growth and/or neuronal migration defects in left temporo-parietal cortices (**Kraft et al., 2015**). As a potential consequence, the consolidation of stable signal exchange between left inferior frontal and temporo-parietal cortices might get hampered (Nave, 2010; Boets et al., 2013). This connectivity problem then probably leads to deficits in attentive phonological processing (Maurer et al., 2009; Schaadt et al., 2015) which later make it hard to get phonemes under executive control (see *Section* 2: phonological awareness) (Raschle et al., 2012) and map them onto letters. Finally, not later than 5 years of age, children who later struggle with literacy also show signs of cortical malformation (most likely neurite growth but possibly also neuronal migration defects) in left ventral temporo-occipital cortices (**Skeide et al., 2016c**). I hypothesize that, as a result, this area cannot establish a coordinated connection with left temporo-parietal (and maybe also left inferior

frontal) areas. Therefore, the brain presumably fails to create a fully functioning interface between the visual system and the phonological system (Brem et al., 2010). Alphabetic learning, i.e. learning to map letters onto phonemes (see *Section 2*) thus becomes difficult.

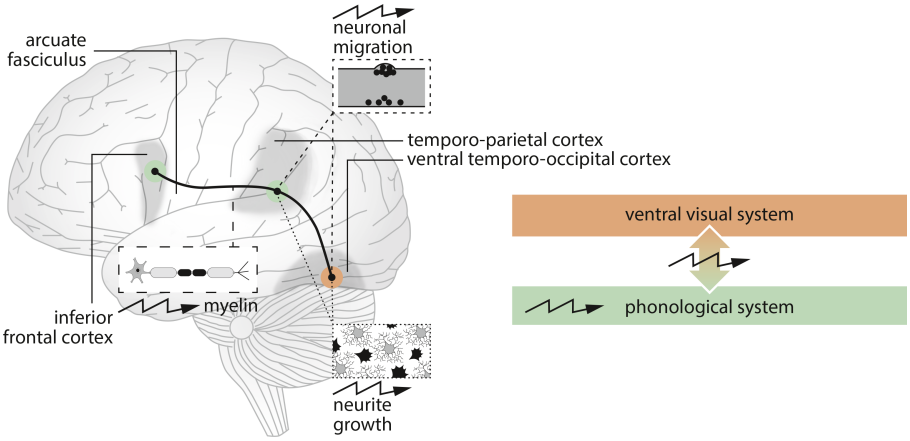

Figure 2. Neural predispositions for developing dyslexia. Three phenotypes could play a causal role for dyslexia, namely, reduced myelin in the left arcuate fasciculus (*long dashed lines*) as well as neurite growth defects (*short dashed lines*) and neuronal migration defects (*dotted lines*) both in left temporo-parietal and ventral temporo-occipital cortices. Two potential problems might arise from these types of atypical maturation. First, a malfunctioning network including left inferior frontal and temporo-parietal cortices (connected by the arcuate fasciculus) might impair attentive phonological processing from birth on (*flash in green box*). As a consequence, affected individuals might not reach a level of phonological awareness necessary for learning how to map phonemes onto letters. Finally, a malfunctioning left ventral temporo-occipital cortex does not seem to hamper visual processing per se, but learning how to link visual to phonological (auditory) representations (*flash on orange-green up-down arrow*).

3.3. Clinical perspectives for early diagnosis and treatment

Written forms of communication increasingly influence our private and professional lives in the digital age, from emails over chats to social networks. If untreated, however, dyslexia-specific reading and writing difficulties usually persist throughout life (Shaywitz, 1998; Gabrieli, 2009). Accordingly, new generations of affected individuals have to face a massive psychological strain. The disorder often leads to lower educational attainment than expectable based on IQ (Richardson & Wydell, 2003) and thus causes frustration and limited self-esteem (Humphrey & Mullins, 2002).

Even worse, negative learning experiences in school are often coupled with reduced peer acceptance (Goldston et al., 2007). It is therefore not surprising that individuals with dyslexia have a significantly increased risk of coming to clinical attention with anxiety disorders and depression (Klassen et al., 2013) and to attempt committing suicide (Daniel et al., 2006).

With these issues in mind, I have recently argued that now, more than ever, it is our responsibility as a society to help dyslexic people to compensate for their deficits as early as possible (**Skeide, 2017**). In Germany, and in almost all other countries, affected children usually receive professional help only after several years in school. While there is evidence that therapeutic opportunities are still good when starting with systematic deficit-oriented remediation in grade 3 (Lachmann, 2002), there is good reason to assume that an earlier start (when the brain is even more plastic) would yield even better results. Actually, considering not only educational but also individual and social perspectives, it would be desirable to be able to *prevent* dyslexia. Prevention promises not only to equalize educational chances at school enrollment but also to protect children from years of suffering. In Germany, practitioners are confronted with the problem that a promising prevention program for the last kindergarten year exists (Plume & Schneider, 2004; Küspert & Schneider, 2008), but that a corresponding diagnostic tool (Jansen et al., 2002) does not reliably predict the individual risk for dyslexia. Unfortunately, it captures not even half of the future-dyslexic kindergarteners (Marx & Weber, 2006), which is astonishing given a test duration of 30 minutes. In contrast, as we have seen in *Section 3.2*, the predictive power of MRI seems to be much better. In fact, gray matter profiles reconstructed from a 7-minute MRI scan correctly identify around 77% of kindergarteners that later develop literacy deficits in school (**Skeide et al., 2016c**). Nevertheless, these first results should not be taken as evidence that MRI can fully replace psychometric testing. Instead, our other work (**Kraft et al., 2016**) indicates that a combination of MRI and psychometrics together might reveal best predictive values. I am currently evaluating a 15-minute procedure consisting of MRI and a well-designed phonological awareness test in an exploratory sample. If this procedure yields a sensitivity of more than 90%

also in a representative replication sample, it would be worth to consider integrating it into standard pediatric screenings[20] (**Skeide, 2017**).

There is an incredible diversity of scientifically published recommendations for dyslexia therapy. Approaches include hypnotherapy (Johnson et al., 1981), osteopathy (Bull, 2007), wearing colored glasses (Mitchell et al., 2008) and following diet plans[21] (Stein, 2014), but also musical training (Flaugnacco et al., 2015) and finally deficit-oriented tutoring training phonological awareness (Alexander et al., 1991; Wolff, 2011) as well as letter-sound mapping (Fraga Gonzalez et al., 2015). Which type of intervention most efficiently targets dyslexia-specific deficits, cannot be properly answered until a comprehensive comparative evaluation of ideally all major approaches in a single large-scale study becomes available. Nevertheless, a metaanalysis of bias-free randomized controlled trials indicates that, *at a school age*, only tutoring programs focusing on letter-sound mapping significantly ameliorate reading and spelling deficits (Galuschka et al., 2014). To the best of my knowledge, the therapeutic impact of preliteracy interventions has not yet been assessed in a randomized controlled trial. I hypothesize that phonological awareness training complemented by letter-sound mapping exercises in the last months before school[22] is the most promising candidate. This remains to be demonstrated in future studies, ideally in combination with MRI, because neuroplastic mechanisms of action are poorly understood. Unfortunately, the two existing (non-randomized) MRI studies on school children did not find any significant direct links between intervention, neuroplastic change and reading outcome (Temple et al., 2003; Keller & Just, 2009).

4. CONCLUSION

In this thesis, I have given an outline of brain-functional and brain-structural mechanisms underlying typical literacy acquisition and specific literacy learning deficits (developmental dyslexia). We have seen (*CHAPTER 2*) that

[20] e.g. German "Kindervorsorgeuntersuchung U9"
[21] e.g. intake of omega-3 fatty acid supplements to ameliorate putative visual problems
[22] as implemented, for example, in the German "Würzburger Trainingsprogramm" developed by Schneider and colleagues (Plume & Schneider, 2004; Küspert & Schneider, 2008)

literacy training leads to a reorganization of visual computation already at lowest subcortical preprocessing levels (**Skeide et al., 2017**). Further downstream, the left ventral temporo-occipital cortex transforms into a multimodal interface linking orthographic representations to the core components of the auditory language system (phonology, semantics, syntax) (Dikker et al., 2009; Dehaene et al., 2010; Carreiras et al., 2014). Difficulties in becoming literate are related to multifaceted neural phenotypes (*CHAPTER 3.1*). While the visual system seems intact in dyslexia, the auditory pathway might be affected by malformation and abnormal response of the auditory cortex (Lehongre et al., 2011) (**Skeide et al., 2018**) or even further upstream by thalamus and brainstem dysfunction (Díaz et al., 2012; Hornickel & Kraus, 2013). However, high-level phonological deficits related to possibly inherited inefficient cross-talk between left inferior frontal and temporo-parietal cortices are better documented (Boets et al., 2013; **Skeide et al., 2015**). Moreover, crucially, as seen in *CHAPTER 3.2*, reduced myelin in the white matter pathway connecting these areas precedes literacy instruction and thus might indicate a predisposition for developing dyslexia (**Kraft et al., 2016**; Langer et al., 2017). The same likely causal role can be ascribed to the left ventral temporo-occipital cortex. Genetically associated malformation in this area predicts dyslexia at a preliterate age and points to potential early problems in mapping visual to phonological entities (Raschle et al., 2011; **Kraft et al., 2015**; **Skeide et al., 2016c**). Consequently, in *CHAPTER 3.3*, phonological awareness and letter-sound mapping have emerged as the most effective targets for dyslexia prevention (Galuschka et al., 2014; **Skeide, 2017**).

5. FUTURE DIRECTIONS

Instead of taking up again the numerous open issues that I have already mentioned, I briefly sketch some further key topics that should guide future research. First, very little is known about neurochemical signatures of dyslexia (Pugh et al., 2014). Spectroscopy studies targeting the core reading network could greatly enhance our understanding of dyslexia-related alterations in neurotransmitter concentration. Second, well-powered longitudinal studies (ideally beginning in infancy) that combine genotyping and MRI

with "home literacy monitoring" are needed to find out how complex gene-environment interplay shapes the dyslexic brain (Ozernov-Palchik et al., 2016). Finally, at least every second child with dyslexia also suffers from other comorbid learning disorders, in particular developmental dyscalculia and specific language impairment (Lewis et al., 1994; Bishop, 2013). A unified explanatory framework for the "Big Three" learning disorders remains to be designed. It would be a great advance if we would be able to determine individual risk combinations before school, e.g. by complementing behavioral assessment with MRI.

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LIST OF OWN PUBLICATIONS (in descending chronological order)

Study I

Skeide MA, Bazin P, Trampel R, Schäfer A, Männel C, von Kriegstein K, Friederici AD (2018) Hypermyelination of the left auditory cortex as a novel biomarker of developmental dyslexia. *Neurology* 90:e492–e497.

Study II

Skeide MA (2017). MRT-basierte Bestimmung des Risikos für die Lese-Rechtschreib-Störung im Vorschulalter. *Klinische Neurophysiologie* 48:164–167.

Study III

Skeide MA, Kumar U, Mishra RK, Tripathi V, Guleria A, Singh J, Eisner F, Huettig F (2017) Learning to read alters cortico-subcortical crosstalk in the visual system of illiterates. *Science Advances* 3:e1602612.

Study IV

Kraft I, Schreiber J, Cafiero R, Metere R, Schaadt G, Brauer J, Neef N, Müller B, Kirsten H, Wilcke A, Boltze J, Friederici AD, **Skeide** MA (2016) Predicting early signs of dyslexia at a preliterate age by combining behavioral assessment with structural MRI. *Neuroimage* 143:378–386.

Study V

Skeide MA, Kraft I, Müller B, Schaadt G, Neef NE, Brauer J, Wilcke A, Kirsten H, Boltze J, Friederici AD (2016) *NRSN1* associated grey matter volume of the visual word form area reveals dyslexia before school. *Brain* 139:2792–2803.

Study VI

Skeide MA, Kirsten H, Kraft I, Schaadt G, Müller B, Brauer J, Wilcke A, Emmrich F, Boltze J, Friederici AD (2015) Genetic dyslexia risk variant is related to neural connectivity patterns underlying phonological awareness in children. *Neuroimage* 118:414–421.

Study VII

Kraft I, Cafiero R, Schaadt G, Brauer J, Neef N, Müller B, Kirsten H, Wilcke A, Boltze J, Friederici AD, **Skeide** MA (2015) Cortical differences in preliterate children at familiar risk of dyslexia are similar to those observed in dyslexic readers. *Brain* 138:e378.

Study I

Skeide MA, Bazin P, Trampel R, Schäfer A, Männel C, von Kriegstein K, Friederici AD (2018) Hypermyelination of the left auditory cortex as a novel biomarker of developmental dyslexia. *Neurology*.

ARTICLE

Hypermyelination of the left auditory cortex in developmental dyslexia

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Neurology® 2018;90:e492-e497. doi:10.1212/WNL.0000000000004931

Abstract

Objective

Cortical malformations are documented postmortem in speech processing areas of the dyslexic human brain. The goal of this pilot study was to find out if such anatomic anomalies can be detected noninvasively and in vivo.

Methods

We developed a reconstruction of left perisylvian cortex profiles at a resolution of 400 μm using T1 data acquired with ultra-high-field MRI at 7T. Cortical thickness, myelinated cortical thickness, and layer-wise myelination were then compared in 6 men with developmental dyslexia and 6 healthy controls matched for age, sex, handedness, education level, and nonverbal IQ.

Results

Compared to healthy controls, dyslexic individuals showed comparable cortical thickness ($t[1,10] = 1.98$, $p = 0.311$) but significantly increased myelinated cortical thickness ratio ($t[1,10] = 3.85$, $p = 0.013$, familywise error–corrected, Cohen $d = 2.03$), resulting in an area under the receiver operator characteristic curve of 0.944 ($p = 0.010$, standard error 0.067, 95% confidence interval 0.814–1). Moreover, T1 relaxation, especially in layer IV of the left auditory cortex, was also significantly increased ($t[1,10] = 3.32$, $p = 0.043$, familywise–error corrected, Cohen $d = 1.67$).

Conclusions

Our findings provide critical insights into the neurobiological manifestation of the most common learning disorder and suggest that our approach might also shed new light on other neurodevelopmental disorders associated with cortical abnormalities.

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Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Glossary

FWE = familywise error; **MP2RAGE** = magnetization-prepared 2 rapid acquisition gradient echoes; **ROI** = region of interest; **TA** = acquisition time; **TE** = echo time; **TR** = repetition time.

Developmental dyslexia is the most common learning disorder, occurring across cultures in every orthographic system.[1]

Specific reading and spelling deficits have been linked to various sensory and cognitive domains, but phonologic processing deficits, which are already present in infancy and persist throughout life, remain the most consistent result across writing systems.[1] In line with this, phonologic processing circuits in the left superior temporal lobe have been repeatedly found to show atypical functional responses and structural features in dyslexic samples.[1]

Genetic association studies revealed several dyslexia risk genes, with *KIAA0319*, *DYX1C1*, and *DCDC2* representing the most frequently replicated variants.[1] Knockdown of these genes in rodents disrupts the migration of nerve cell populations during intrauterine formation of the neocortex and results in gray or white matter heterotopias.[2] Gray matter heterotopias are already documented ex vivo in the perisylvian cortex of dyslexic individuals.[3]

Recently, it was proposed that dyslexia might originate from faulty neuronal migration in left auditory cortex.[4] Migration defects in layers II and III likely alter local functional interactions between layers and their global crosstalk with remote interconnected areas.[4] In addition, migration defects in layer IV are assumed to affect firing responses to auditory stimuli.[4] These microcircuitry anomalies could be related to the hallmark phonologic deficits in dyslexia.[4]

Here we reconstructed the cortical ribbon at a resolution of 400 μm isotropic to compute cortical thickness and estimate myelin concentration. Subsequently, we computed the proportional thickness of the myelinated part of the cortex in relation to its overall thickness, i.e., myelinated cortical thickness ratio. The resulting indices were then compared in a sample of dyslexic adults and healthy controls matched for age, sex, handedness, education level, and nonverbal IQ (table). We hypothesized that cortical thickness or myelinated cortical thickness ratio is significantly increased in the left superior temporal cortex of dyslexic compared to healthy controls. In addition, we predicted that increased myelination is most pronounced in layers II, III, or IV.

Methods

Participants

A pilot sample of 6 dyslexic individuals and 6 healthy controls (age range 25–32 years) were recruited in 2014 from the institute's database. All participants were native German speakers and had no history of neurologic or psychiatric conditions and normal IQ (≥85), hearing, and vision. Individuals with dyslexia received an official diagnosis during childhood by a qualified professional educator or speech therapist. Moreover, their phonologic and literacy deficits persisted into adulthood (table). Participants reported no official diagnosis of comorbid auditory processing disorder, specific language impairment, or attention-deficit/hyperactivity disorder.

Standard protocol approvals, registrations, and patient consents

All participants gave written informed consent. The study was approved by the Ethics Committee of the University of Leipzig, Germany.

MRI acquisition

Participants were scanned in 2014 on a 7T Siemens Magnetom magnetic resonance scanner with a 1-channel transmit/24-channel receive NOVA head coil.

Whole-brain T1-weighted images were acquired using a magnetization-prepared 2 rapid acquisition gradient echoes (MP2RAGE) sequence (TI1/TI2 900/2,750 ms, repetition time [TR] 5.000 ms, echo time [TE] 2.45 ms, flip angle α1/α2 5°/3°, bandwidth 250 Hz/px, GRAPPA acceleration factor 2, voxel size 700 μm isotropic, acquisition time [TA] 10:57 minutes). An adiabatic inversion pulse was implemented and dielectric pads were placed around the participants' heads to minimize sensitivity to B1 inhomogeneity.

Additional T1-weighted slabs of the temporal lobes were acquired in axial orientation (MP2RAGE: TI1/TI2 900/2,750 ms, TR 5.000 ms, TE 4.16 ms, flip angle α1/α2 5°/3°, bandwidth 240 Hz/px, voxel size 400 μm isotropic, TA 23:42 minutes).

MRI analysis

Whole-brain images were skull-stripped, rigidly aligned to Montreal Neurological Institute space, resampled to 400 μm isotropic, and segmented using a multiple object geometric deformable model. Corresponding slabs were denoised with a total variation algorithm and aligned to the whole brain images using scanner coordinates as priors. Cortical boundary surfaces were extracted using implicit surface evolution.

Volume-preserving representations of cortical depth were estimated, and myelin-sensitive cortical T1 relaxation profiles[5] were obtained from 11 equidistant points ranging from the

Table Demographic and behavioral data

	Patients with dyslexia	Healthy controls	Δ[a]
Age, y, mean ± SD	29.00 ± 2.61	27.33 ± 2.25	$t(1,10) = 1.19$, $p = 0.264$
Sex, female/male	0/6	0/6	$t(1,10) = 0.00$, $p = 1.000$
Handedness, left/right/ambidextrous	0/5/1	1/4/1	$\chi(2) = 3.11$, $p = 0.211$
Education level[b,c]	3.17 ± 0.41	2.83 ± 0.41	$\chi(2) = 2.00$, $p = 0.368$
Nonverbal IQ[c]	95.33 ± 14.10	103.33 ± 14.12	$z = 0.80$, $p = 0.485$
Verbal working memory[d] (phonologic loop)	12.00 ± 4.20	19.50 ± 4.20	$t(1,8) = 3.53$, $p = 0.008$
Text reading comprehension[e]	48.67 ± 5.61	60.67 ± 12.93	$t(1,10) = 2.09$, $p = 0.064$
Text reading speed[e]	38.83 ± 4.58	59.67 ± 10.05	$t(1,10) = 4.62$, $p < 0.001$
Spelling accuracy[f]	84.00 ± 6.03	107.00 ± 9.90	$t(1,10) = 4.86$, $p < 0.001$
Pseudoword reading accuracy[g]	12.17 ± 9.28	2.33 ± 1.51	$z = 2.51$, $p = 0.009$
Pseudoword reading speed[h]	119.52 ± 43.52	87.21 ± 29.11	$t(1,10) = 1.51$, $p = 0.166$
Phonologic awareness[i]	55.00 ± 11.87	68.25 ± 3.30	$t(1,8) = 2.59$, $p = 0.041$
Rapid automatized naming[h]	21.67 ± 2.88	16.47 ± 3.35	$t(1,10) = 2.88$, $p = 0.017$

Values are n or mean ± SD.
[a] Group difference (independent samples *t* test, Mann-Whitney *U* test, or χ^2 test).
[b] 1 = Partial high school; 2 = high school graduate; 3 = partial college; 4 = college graduate; 5 = graduate degree.
[c] Raven matrices, standard scores: mean 100, SD 15.
[d] Wechsler Adult Intelligence Scale (forward digit span), raw scores (correct repetitions).
[e] German reading speed and reading comprehension test, standard scores: mean 50, SD 10.
[f] German spelling test, standard scores: mean 100, SD 10.
[g] Raw scores (number of errors).
[h] Raw scores (seconds).
[i] German Basiskompetenzen für Lese-und Rechtschreibleistungen, raw scores (maximum 74 points).

white/gray matter boundary to the gray matter/CSF boundary. The proportional thickness of the myelinated part of the cortex in relation to its overall thickness (myelinated cortical thickness) was computed with a fuzzy classification technique combining information about radial and tangential fibers. Partial volume effects are consistent across gyri and sulci. Accordingly, local averages converge to underlying T1 values at the same cortical depth, even in strongly curved areas. Preprocessing was performed using the Medical Image Processing, Analysis, and Visualization toolbox (https://mipav.cit.nih.gov/) within the framework of the Java Image Science Toolkit (https://www.nitrc.org/projects/jist/) and the Cognitive and Brain Sciences Tools (http://www.cbs.mpg.de/institute/software/cbs-tools).

Indices were extracted from 4 regions of interest (ROIs) (core, medial belt, lateral belt, and parabelt of the left auditory cortex) defined in an independent sample of 210 healthy young adults and available in the Brain Analysis Library of Spatial Maps and Atlases (https://balsa.wustl.edu/). We transferred the annotation of each region from FreeSurfer template space into native individual space, created labels from annotations, and finally transformed each label into a volume using FreeSurfer (http://surfer.nmr.mgh.harvard.edu/). Assignment of cortical profile points to anatomic layers was based on histologic layer thickness measures of area supratemporalis simplex magnocellularis specified in the cytoarchitectonic atlas of von Economo and Koskinas.[6p672] Layer thickness was modeled as average across the entire ROI. Eleven points regularly spaced across the surface in perpendicular direction were chosen to form 10 sections sampling 10 full voxels across the average thickness of the 4 ROIs (3.98 mm).

Statistics

Continuous values were ranked to optimize normality of distribution and homogenize variance. Then independent-samples *t* tests were carried out. Mann-Whitney *U* tests were used instead whenever the data violated the normality or homogeneity assumption according to Kolmogorov-Smirnov and Shapiro-Wilk tests. Categorical dependent variables were analyzed with χ^2 tests. Two-sided *p* values are provided for demographic and behavioral data. Two-sided *p* values (familywise error [FWE]–corrected for number of regions) are reported for comparisons of cortical thickness and myelinated cortical thickness ratio. One-sided *p* values (FWE-corrected for number of layers) are reported for post hoc comparisons of layer-wise T1 values. Area under the ROC curve was computed nonparametrically. Post hoc power was calculated using G × Power (gpower.hhu.de/). All other statistical tests were run with SPSS 22.1 (IBM, Armonk, NY).

Results

Cortical thickness within the core, the medial belt, the lateral belt, and the parabelt of the left auditory cortex (figure 1A) was comparable in the dyslexic and the healthy control group (core: $t[1,10] = 1.98$, $p = 0.311$, FWE-corrected; medial belt: $t[1,10] = 0.31$, $p = 0.765$; lateral belt: $t[1,10] = 1.74$, $p = 0.311$, FWE-corrected; parabelt: $t[1,10] = 1.98$, $p = 0.311$, FWE-corrected) (figure 1B).

In contrast, myelinated cortical thickness ratio of the dyslexic individuals was significantly higher compared to healthy controls in the left auditory core region ($t[1,10] = 3.85$, $p = 0.013$, FWE-corrected, Cohen $d = 2.03$, Hedges $g = 1.88$, power 0.95). This difference was not significant in the medial belt ($t[1,10] = 1.53$, $p = 0.632$, FWE-corrected), the lateral belt ($t[1,10] = 0.79$, $p = 0.450$), and the parabelt region ($t[1,10] = 1.53$, $p = 0.632$, FWE-corrected) (figure 1C). Moreover, the effect was not found in the core region of the right hemisphere ($t[1,10] = 1.14$, $p = 0.283$). Myelinated cortical thickness ratio of the left auditory core distinguished cases from healthy controls with a sensitivity of 0.833 and a specificity of 1, resulting in an area under the receiver operator characteristic curve of 0.944 ($p = 0.010$, standard error 0.067, 95% confidence interval 0.814–1) (figure 1D).

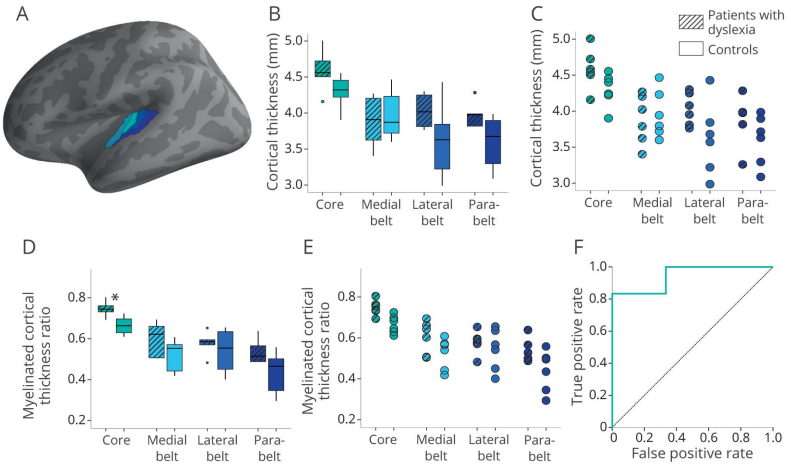
To further bolster the anatomic specificity of our results, we carried out an additional control analysis in the granular visual region (V5) and did not find a group difference ($t[1,10] = 0.63$, $p = 0.545$).

Our final step was to explore in which layers of the auditory cortex the dyslexia-specific increases in myelination originated. For this purpose, we divided the left core region into 10 sections sampling at 11 profile points. T1 value reductions in dyslexic participants vs healthy controls were significant in point 6 ($t[1,10] = 3.32$, $p = 0.043$, FWE-corrected, Cohen $d = 1.67$, Hedges $g = 1.54$, power 0.85) and point 7 ($t[1,10] = 3.32$, $p = 0.043$, FWE-corrected, Cohen $d = 1.61$, Hedges $g = 1.49$, power 0.83). Considering tissue and layer boundaries specified in the von Economo and Koskinas cortex atlas,[6] these points covered layer IV of Heschl gyrus (figure 2).

Discussion

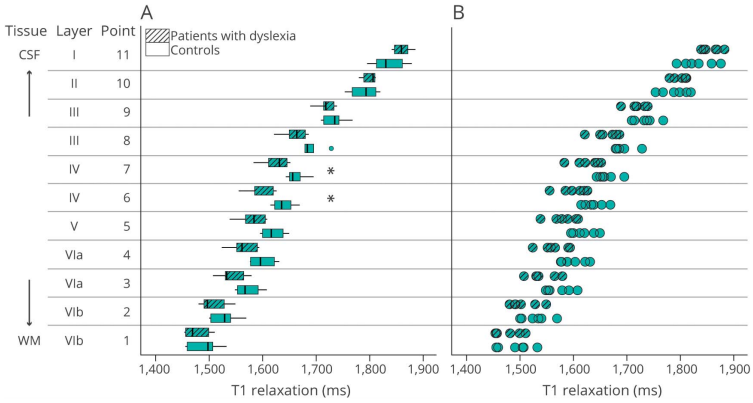
Our findings support the assumption that dyslexia is characterized by neuronal migration defects in the auditory cortex. Specifically, when neuronal populations migrate beyond their target layers, they also carry along their axons into upper layers of the cortex where they get myelinated and form white matter heterotopias reported in rodents after deactivation of

Figure 1 Dyslexia-specific intracortical hypermyelination of the left auditory core region



(A) The 4 regions of interest in the left superior temporal cortex. (B, D) Cortical thickness and myelinated cortical thickness ratio of each region plotted separately for dyslexic individuals and healthy controls. Horizontal lines within the bars represent the group median. Vertical lines at the top and the bottom of the bars depict the SD. Dots mark single cases more than 1.5 SDs away from the group mean. Asterisks indicate significant differences between groups at a familywise error–corrected threshold of $p < 0.05$. (C, E) Cortical thickness and myelinated cortical thickness ratio of each region plotted separately for dyslexic individuals and healthy controls. Dots mark individual values of each participant. (F) Receiver operating characteristic curve quantifying the accuracy of the case–control classification based on the myelinated cortical thickness ratio of the core region of the left auditory cortex. The y-axis represents the rate of correctly identified dyslexic individuals. The x-axis represents the rate of correctly identified healthy controls.

Figure 2 Layer IV origins of left auditory hypermyelination in dyslexia



The cortex was divided into 10 equally spaced sections ranging from the white matter/gray matter boundary to the gray matter/CSF boundary. Eleven profile points marking the boundaries of the sections were assigned to cortical layers based on layer thickness data available from the von Economo and Koskinas[6] human cytoarchitectonic atlas. T1 values in each profile point are plotted separately for dyslexic individuals and healthy controls. Note that faster T1 relaxation (i.e., lower T1 values) indicates higher myelin content. (A) Vertical lines within the bars represent the group median. Horizontal lines on each side of the bars depict the SD. Dots mark single cases more than 1.5 SD away from the group mean. Asterisks indicate significant differences between groups at a familywise error–corrected threshold of $p < 0.05$. (B) Dots mark individual values of each participant.

genes regulating layer formation.[2] Currently available data suggest that heterotopic myelin is most dense and expanded over the center of the cortex and less pronounced in superficial layers.[7] At the macroscopic level captured by MRI, this leads to an expansion of the myelinated part of the cortex and thus to an increased cortical thickness ratio in dyslexic compared to healthy control individuals.

It would be of interest to find out whether this effect is consequential or causal by applying our method to preliterate children and follow them longitudinally to assess their literacy outcome. This would also help clarify why a cortical thickness reduction in the left auditory cortex, previously described as a persistent feature of preschool children who later developed dyslexia,[8] has not been found yet in adult samples, including ours.

Myelination increase in the dyslexic group was most pronounced in layer IV, not in layers II and III, of the left core region. This has important implications for the interpretation of the effect, since it has been proposed that layers II and III generate oscillatory activity whereas layer IV regulates stimulus-driven firing during phonologic processing.[4] Interestingly and in line with our finding, delayed and inconsistent neuronal responses to speech were recently induced by knockdown of the dyslexia risk gene *KIAA0319* in the primary auditory cortex of rats, whose phoneme discrimination thresholds are nearly identical to human thresholds.[9] Impaired firing of layer IV neurons in the core region of the left auditory cortex might lead to poor phoneme distinction in dyslexia by a disrupted thalamocortical feedback mechanism. This is suggested by a previous fMRI experiment including a subset of our participants showing that blood oxygenation level–dependent activity in the medial geniculate body of the left thalamus is reduced in dyslexic individuals during phonologic processing.[10] Faulty top-down modulation of the medial geniculate body via efferent fiber connections that are densest in layer IV of the auditory cortex might hinder the auditory system from fine-tuning its responses to phonemes.[10]

Despite a small sample size, the risk of having detected false-positive effects is low given the very large conservatively estimated effect sizes and the conservative correction for multiple comparisons. It should be noted as a limitation that we cannot rule out additional intracortical anatomy differences in other regions that are beyond the resolution of our approach. Moreover, the generalizability of our results depends on their replicability in logographic readers.

Author contributions

Michael A. Skeide: study concept and design, data acquisition and analysis, data interpretation, manuscript draft, figure composition. Pierre-Louis Bazin: data analysis, critical review of manuscript for intellectual content. Robert Trampel: imaging data acquisition, critical review of manuscript for intellectual content. Andreas Schäfer: development of imaging protocols. Claudia Männel: behavioral data acquisition,

critical review of manuscript for intellectual content. Katharina von Kriegstein: data interpretation, critical review of manuscript for intellectual content. Angela D. Friederici: project supervision, data interpretation, critical review of manuscript for intellectual content.

Study funding

K.v.K. is supported by the Max Planck Society and by the European Research Council (ERC Consolidator Grant 647051 SENSOCOM).

Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

Received June 28, 2017. Accepted in final form October 27, 2017.

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Study II

Skeide MA (2017). MRT-basierte Bestimmung des Risikos für die Lese-Rechtschreib-Störung im Vorschulalter. *Klinische Neurophysiologie* 48:164–167.

Alois-Kornmüller-Preis 2017 Thieme

MRT-basierte Bestimmung des Risikos für die Lese-Rechtschreib-Störung im Vorschulalter

Predicting the Risk for Developmental Dyslexia before School Age with MRI

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Schlüsselwörter
Lese-Rechtschreib-Störung (LRS), Magnetresonanztomografie (MRT), Gyrus fusiformis, *NRSN1*

Key words
developmental dyslexia, magnetic resonance imaging (MRI), fusiform gyrus, *NRSN1*

Bibliografie
DOI http://dx.doi.org/10.1055/s-0043-105960
Online-Publikation: 11.5.2017
Klin Neurophysiol 2017; 48: 164–167

ISSN 1434-0275

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ZUSAMMENFASSUNG

Die Lese-Rechtschreib-Störung (LRS) gilt als die häufigste aller Lernentwicklungsstörungen überhaupt. Etwa 5 % der deutschen Bevölkerung leidet unter den psychischen und sozialen Folgen schwerwiegender umschriebener Probleme beim Erlernen des Lesens und Schreibens. LRS entsteht aus dem komplexen Zusammenspiel von genetischen Faktoren und Umweltfaktoren (z. B. sprachliche Lernvoraussetzungen im Elternhaus). In zahlreichen vorangegangenen Magnetresonanztomografie (MRT) Studien wurde zudem gezeigt, dass der linke gyrus fusiformis (FFG, sogenanntes „visuelles Wortformareal") des Gehirns eine entscheidende Rolle für den Schriftspracherwerb spielt. Die hier vorgestellte Arbeit legt nahe, dass die kortikale Plastizität des FFG bei LRS durch das Tragen einer Risikovariante des Gens *NRSN1* eingeschränkt sein könnte, dessen Proteine u. a. das Wachstum von Dendriten steuern. *NRSN1* erwies sich als signifikant mit dem Volumen des linken FFG assoziiert, welches mithilfe von voxelbasierter Morphometrie (VBM) auf Grundlage von MRT Aufnahmen gemessen wurde. Anhand der durch genetische Assoziation bestimmten volumetrischen Profile von Kindern, die sich etwa 10 Monate vor Schuleintritt befanden, konnte die spätere Ausprägung einer LRS mit einer Klassifikationsgenauigkeit von 75 % vorhergesagt werden. Diese Daten lassen hoffen LRS in Zukunft so früh feststellen zu können, dass betroffene Kinder in der Lage sind ihre Defizite vor der Einschulung mithilfe von Frühförderungsmaßnahmen zu kompensieren.

ABSTRACT

Developmental dyslexia (DD) is considered to be the most common among all learning disorders. About 5 % of the population in Germany and 7 % in the USA suffer from the psychological and social consequences of severe deficits in learning how to read and spell. DD arises from the complex interplay of genetic and environmental factors (e. g. home literacy environment). Moreover, numerous previous magnetic resonance imaging (MRI) studies have shown that the left fusiform gyrus (FFG, "visual word form area") of the brain plays a crucial role in literacy acquisition. The present work suggests that the cortical plasticity of the FFG might be limited in individuals with DD because they carry a risk variant of the gene *NRSN1* that codes proteins regulating neurite growth. *NRSN1* turned out to be significantly associated with the volume of the left FFG that was estimated by conducting a voxel-based morphometry (VBM) analysis of MR images. Using volumetric profiles determined by genetic association in children, DD could be predicted 10 months before school entry with a classification accuracy of 75 %. These data might make it possible in the future to diagnose DD so early that affected children might be able to compensate their deficits before school enrollment by making use of early intervention programs.

Einleitung

Die Lese-Rechtschreib-Störung (LRS) ist eine schwerwiegende umschriebene Beeinträchtigung der schriftsprachlichen Entwicklung [ICD-10-GM]. Das Störungsbild tritt weitgehend unbeeinflusst von kulturellen Faktoren in jedem Bildungssystem auf. Die tatsächliche Prävalenz hängt jedoch davon ab wie eindeutig die Zuordnung von Buchstaben und Sprachlauten im orthografischen System der jeweiligen Sprache geregelt ist. Mit einer Prävalenz von etwa 5 % in Deutschland und etwa 7 % in den USA gilt die LRS als häufigste aller Lernentwicklungsstörungen [1, 2]. Betroffene Individuen kämpfen nicht nur mit erschwerten Bedingungen für den Bildungserfolg, sondern entwickeln auch signifikant gehäuft Angststörungen bzw. depressive Störungen [3].

Aus Familienstudien ist bekannt, dass der schriftsprachliche Lernerfolg etwa jeweils zur Hälfte von genetischen Faktoren und von Umweltfaktoren wie den sprachlichen Lernvoraussetzungen im Elternhaus beeinflusst wird [4]. In hoch gebildeten Elternhäusern, die eine optimale sprachliche Lernumgebung bieten, kann der Anteil genetischer Einflüsse auf die schriftsprachliche Entwicklung jedoch auf über 70 % ansteigen [5]. Anhand psychometrischer Testdaten konnten in zahlreichen Linkage- und Assoziationsstudien bisher mehr als 20 Kandidatengene der LRS identifiziert werden. Über welche intermediären neuronalen Phänotypen diese Gene zur Ausprägung der LRS führen könnten, ist allerdings nur im Ansatz verstanden. Bisherige Untersuchungen, in denen Genotypisierungsdaten mit magnetresonanztomografischen (MRT) Aufnahmen des Gehirns in Verbindung gebracht wurden, waren auf bereits beschulte und betroffene Kinder und Erwachsene ausgerichtet [6–9]. Mit dieser Herangehensweise können jedoch potenziell prädispositionale neuronale Risikofaktoren nicht von dem Folgeeffekt der LRS getrennt werden, dass betroffene Individuen geringere schriftsprachliche Erfahrungen sammeln, indem sie sich sowohl qualitativ als auch quantitativ weniger intensiv mit dem Lesen und Schreiben befassen als unbelastete Individuen [10]. Das Ziel der vorliegenden Studie war diese Einschränkung zu überwinden, indem Kinder im Vorschulalter längsschnittlich bis zum Abschluss der ersten Klasse untersucht werden. Dabei haben wir zunächst Assoziationen zwischen Volumenprofilen der grauen Substanz mit Kandidatengenen der LRS in einer Gesamtstichprobe von 141 Kindern im Alter von 3 bis 12 Jahren bestimmt. Anschließend haben wir überprüft, ob anhand der dabei identifizierten Areale eine Untergruppe von 9–12-jährigen Individuen mit LRS korrekt klassifiziert werden kann. Und schließlich sind wir der entscheidenden Frage nachgegangen, ob mit diesen Daten auch vorhergesagt werden kann, ob eine weitere Untergruppe 5–6-jährige Kindergartenkinder später in der Schule Merkmale einer LRS ausgeprägt hat oder nicht. Unser Fokus lag hierbei auf dem Volumen der grauen Substanz, weil aus molekulargenetischen Experimenten am Tiermodell bereits bekannt war, dass ein Großteil der Kandidatengene der LRS an Wachstumsprozessen der Großhirnrinde, insbesondere an neuronaler Migration und dendritischer Expansion, beteiligt ist [11, 12].

Kinder mit einer familiären Vorbelastung für LRS zeigen schon vor ihrer Einschulung sowohl funktionale als auch strukturelle Auffälligkeiten in temporo-okzipitalen und temporo-parietalen Hirnarealen, die sich im Verlauf der Schulbildung zu Kernregionen der schriftsprachlichen Verarbeitung entwickeln. Im Vergleich zu unbelasteten Kindern sind diese Areale in Risikogruppen bei der Verarbeitung sprachlicher Laute unteraktiviert und weisen zudem eine geringere kortikale Dicke auf [13–16]. Dementsprechend war zu erwarten, dass temporo-okzipitale und/oder temporo-parietale Hirnareale als intermediäre neuronale Phänotypen der LRS in Frage kommen.

Methodik

In unserer Studie wurden insgesamt 141 neurologisch unauffällige Kinder untersucht. Dabei wurde DNA aus Speichelproben entnommen, um 69 Einzelnukleotidpolymorphismen (SNPs) 19 verschiedener Gene zu genotypisieren. Für all diese SNPs waren in der Literatur bereits Zusammenhänge mit psychometrischen Testdaten zu Lese- und Rechtschreibfähigkeiten dokumentiert. Zusätzlich haben alle Kinder an einer etwa 6-minütigen MRT Messung teilgenommen, die eine hochauflösende 3-dimensionale Rekonstruktion des gesamten Gehirns ermöglichte. Aus diesen Daten konnte mithilfe verschiedener standardisierter Auswertungsverfahren im Rahmen einer voxelbasierten Morphometrie (VBM) das Volumen der grauen Substanz berechnet werden. Psychometrische Testungen wurden ebenfalls durchgeführt. Neben dem non-verbalen IQ wurden alle Kinder im Schulalter hinsichtlich ihrer Lesefähigkeiten (Leseverständnis und Lesegeschwindigkeit) und ihrer Rechtschreibfähigkeiten (Diktat) untersucht.

In einem ersten Auswertungsschritt wurden unter Verwendung der Methode von Ge et al. [17] statistische Zusammenhänge zwischen den 19 LRS-relevanten Genen und den volumetrischen Profilen für die Gesamtstichprobe ermittelt. Die signifikant assoziierten Hirnareale wurden dann als „regions of interest“ für ein Klassifizierungsverfahren verwendet, das mit dem Ziel durchgeführt wurde Individuen mit und ohne LRS statistisch voneinander zu unterscheiden. Diese Analyse wurde zunächst querschnittlich in einer Untergruppe von 34 Kindern (17 mit LRS, 12 Mädchen) im Alter von 9–12 Jahren durchgeführt. Anschließend wurde überprüft, ob eine Untergruppe von 20 Kindern (10 mit LRS, 10 Mädchen) anhand ihrer im Alter von 5–6 Jahren (im Kindergarten) erhobenen Daten bereits dahingehend unterschieden werden können, ob sie später im Alter von 7–8 Jahren (am Ende der 1. Klasse) Merkmale einer LRS aufweisen oder nicht. Eine ausführliche Beschreibung aller methodischen Details findet sich bei Skeide et al. [18].

Ergebnisse

Korrigiert signifikante Zusammenhänge mit dem Volumen der grauen Substanz konnten für die 3 Gene *NRSN1*, *FOXP2* und *COL4A2* gefunden werden. Dabei wurden die Effekte der Altersunterschiede sowie individueller Unterschiede in der Schädelgröße als Kovariaten statistisch entfernt. Die insgesamt 5 identifizierten Areale sind alle im Zusammenhang mit LRS in der Literatur dokumentiert, wobei nur eines dieser Areale, der linke gyrus fusiformis (FFG), als Kernregion des schriftsprachlichen Verarbeitungssystems bekannt ist.

Die korrigierte Klassifizierungsleistung jedes einzelnen Areals wurde dann jeweils in einem separaten Modell für die jeweilige Untergruppe bestimmt. Die 9–12-jährigen Kinder konnten nur auf Grundlage des FFG, nicht jedoch auf Grundlage der 4 anderen Areale, überzufällig genau als Individuen mit bzw. ohne LRS klassifiziert werden. Dabei lag die Trefferquote bei 73.53 %. Ein ähnliches

Bild ergab sich schließlich auch für die Vorhersage von LRS-relevanten Merkmalen bei 7–8-jährigen Kindern anhand der Hirndaten, die im Alter von 5–6 Jahren erhoben worden waren. Erneut stellte sich heraus, dass anhand des FFG eine überzufällig genaue Klassifikation erzielt werden konnte, wobei die Trefferquote hier bei 75 % lag. Unsere Hypothese konnte in diesen Analysen also durchgehend bestätigt werden. Zu erwähnen ist noch, dass alle Individuen ohne LRS für diese Analysen so ausgewählt wurden, dass sie sich von den Individuen mit LRS im Hinblick auf Alter, non-verbalen IQ und Bildungsstand der Eltern nicht signifikant unterschieden.

Diskussion

Lesen und Schreiben zu lernen erfordert intensive langjährige Übung, in deren Folge sich das Gehirn reorganisieren muss, um sprachliche Verarbeitungsprozesse auf Grundlage visuell dargestellter Symbole in Gang setzen zu können. Diese Schnittstelle zwischen dem visuellen System und dem Sprachsystem bildet der FFG, der in der Literatur als „visuelles Wortformareal" bekannt ist [19–21]. In mehreren vorhergehenden Untersuchungen konnte bereits nachgewiesen werden, dass der FFG bei LRS sowohl im Hinblick auf seine funktionale Reaktivität als auch auf seinen strukturellen Aufbau beeinträchtigt ist [22–24]. Unsere Studie legt nun erstmalig nahe, dass die kortikale Plastizität dieses Areals und damit seine Anpassungsfähigkeit an kognitive Herausforderungen schriftsprachlichen Lernens genetisch begrenzt sein könnte. Der Zusammenhang zwischen dem Volumen der grauen Substanz des FFG und einer Risikovariante des Gens *NRSN1* deutet auf einen möglichen Pathomechanismus hin, wonach LRS auf gestörte dendritische Wachstumsprozesse in einer Kernregion der schriftsprachlichen Verarbeitung zurück zu führen sein könnte. Allerdings sollte die vorliegende Untersuchung trotz ihres längsschnittlichen Aufbaus in Bezug auf den genetischen Zusammenhang als korrelativ, nicht aber als kausal interpretiert werden. Darüber hinaus gilt es als unwahrscheinlich, dass ein komplexer Phänotyp wie die LRS auf einen einzigen Pathomechanismus zurückgeführt werden kann [25]. Insbesondere Defizite bei der Verarbeitung sprachlicher Laute wurden bei Individuen, die später eine LRS ausprägten, mehrfach sogar schon im Säuglingsalter nachgewiesen [26, 27].

Die hier vorgestellten Ergebnisse müssen in Folgestudien unabhängiger Forschungsgruppen bestätigt werden, um ihre Reliabilität abschließend bewerten zu können. Einschränkend muss außerdem noch erwähnt werden, dass falsch negative Befunde bei genetischen Assoziationsstudien grundsätzlich nicht ausgeschlossen werden können. LRS ist polygen, d. h. *NRSN1* ist nur eines von derzeit über 20 verschiedenen Kandidatengenen, die mit der Störung in Zusammenhang stehen. Um gemeinsame Effekte aller beteiligten Varianten zu entschlüsseln, sind weitaus größere Stichproben nötig. Solch umfangreiche Datensätze können nur durch gemeinsame Anstrengungen mehrerer Forschungszentren gewonnen werden.

Welchen Beitrag könnten die hier vorgelegten Befunde für die klinische Praxis leisten? Zunächst liefern sie einen ersten Anhaltspunkt dafür, dass eine sehr kurze MRT Aufnahme einen hohen diagnostischen Nutzwert für die LRS Früherkennung haben könnte. Das ist besonders bedeutsam, weil das rein psychometrische „Bielefelder Screening zur Früherkennung von Lese-Rechtschreibschwierigkeiten" (BISC) [28], als bisher einziges Frühdiagnoseverfahren nur eine Klassifikationsgenauigkeit von etwa 57 % erzielt. In Zukunft ist es denkbar eine MRT Untersuchung mit demjenigen Untertest des BISC zu kombinieren, der die größte Vorhersagekraft hat. Die Erhebung dieser Daten würde zusammen genommen weniger als 15 Minuten in Anspruch nehmen und könnte z. B. in die Vorsorgeuntersuchung U9 aufgenommen werden. Abzuwarten bleibt, welche Klassifikationsgenauigkeit mit diesem kombinierten Diagnoseinstrument erreicht werden kann.

An unsere Beobachtung schließt sich aus therapeutischer Perspektive die Forschungsfrage an, ob die eingeschränkte Plastizität des linken FFG bereits im Vorschulalter durch Training ausgeglichen werden kann. In Form des Würzburger Buchstaben-Laut-Trainings steht ein Interventionsprogramm bereit, das für den Einsatz im letzten Kindergartenjahr konzipiert wurde, um die Fähigkeit zu verbessern Lautstrukturen eines Buchstabens mit dessen visueller Repräsentation zu verbinden [29]. Wir nehmen an, dass diese 10-wöchige, täglich 15-minütige Intervention genau diejenigen neuronalen Systeme gezielt ansprechen sollte, die später schriftsprachliche Verarbeitungsaufgaben übernehmen. Allerdings ist nicht davon auszugehen, dass kompensatorische Effekte auf den FFG beschränkt sind. Bekannt ist z. B., dass der präfrontale Kortex der rechten Hemisphäre mit dem schriftsprachlichen „outcome" bei LRS zusammenhängt und somit ebenfalls eine kompensatorische Funktion haben könnte [30]. Denkbar ist auch, dass das homologe Areal des FFG in der rechten Hemisphäre eine entsprechende Rolle spielt.

Den Kosten der hier diskutierten Diagnose- und Therapieverfahren ist deren enormes gesellschaftliches Verwertungspotenzial gegenüberzustellen. Arbeitsbeschaffungsmaßnahmen und Heilbehandlungen für Menschen mit LRS verursachen jährliche Kosten in Milliardenhöhe. Ein Präventionsprogramm könnte diese Ausgaben erheblich senken und sollte gleichzeitig über die frei werdenden Mittel finanzierbar sein, wobei die Produktivität der vorbelasteten Individuen deutlich anstiege. Nicht zuletzt wäre eine wirksame Prävention der LRS ein wichtiger Schritt auf dem Weg zur Angleichung der individuellen Bildungschancen.

Über den Autor

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Dr. Skeide hat an der Universität Heidelberg Linguistik und an der Harvard University Neurobiologie studiert. Seine Promotion im Fach Psychologie schloss er an der Universität Leipzig mit einer Arbeit zu den neuroplastischen Dynamiken der Entwicklung höherer sprachlicher Verarbeitungsfunktionen ab. In der Arbeitsgruppe Neuropsychologie von Prof. Dr. Dr. h.c. Angela D. Friederici untersucht er die neuronalen Grundlagen des normalen und gestörten (Schrift-) Spracherwerbs. Dr. Skeide war zudem Gastwissenschaftler am Cognitive and Systems Neuroscience Laboratory der Stanford University.

Interessenkonflikt

Der Autor gibt an, dass kein Interessenkonflikt besteht.

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Study III

Skeide MA, Kumar U, Mishra RK, Tripathi V, Guleria A, Singh J, Eisner F, Huettig F (2017) Learning to read alters cortico-subcortical crosstalk in the visual system of illiterates. *Science Advances* 3:e1602612.

SCIENCE ADVANCES | RESEARCH ARTICLE

NEUROSCIENCE

Learning to read alters cortico-subcortical cross-talk in the visual system of illiterates

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Learning to read is known to result in a reorganization of the developing cerebral cortex. In this longitudinal resting-state functional magnetic resonance imaging study in illiterate adults, we show that only 6 months of literacy training can lead to neuroplastic changes in the mature brain. We observed that literacy-induced neuroplasticity is not confined to the cortex but increases the functional connectivity between the occipital lobe and subcortical areas in the midbrain and the thalamus. Individual rates of connectivity increase were significantly related to the individual decoding skill gains. These findings crucially complement current neurobiological concepts of normal and impaired literacy acquisition.

INTRODUCTION

Learning to read is a profound cultural experience requiring systematic instruction and intensive practice over months or years (*1*). Yet, hemodynamic brain activity induced by perceiving printed words changes after only a few weeks of training letter-sound links (*2*). Enhanced functional selectivity to print emerges in parts of the visual system, that is, the bilateral occipital cortices (*3*), and in a multimodal symbol processing region located in the left temporo-occipital fusiform cortex (*2*, *4*, *5*). These findings have revealed the important insight that literacy-related learning triggers cognitive adaptation mechanisms manifesting themselves in increased blood oxygen level–dependent (BOLD) responses during print processing tasks (*6*, *7*). However, it remains elusive whether reading acquisition also leads to an intrinsic functional reorganization of neural circuits.

Here, we used resting-state functional magnetic resonance imaging (fMRI) as a measure of spontaneous neuronal activity to capture the impact of reading acquisition on the functional connectome (*8*). In a controlled longitudinal intervention study, we taught 21 illiterate Hindi-speaking adults how to read Devanagari script for 6 months. The goal was to compare the changes in resting-state fMRI data before and after learning of the sample taught to read with those of a sample of nine Hindi-speaking illiterates who did not undergo such instruction. Participants were recruited from the same societal community in two villages of a rural area near the city of Lucknow in North India and matched for the most relevant cognitive, demographic, and socioeconomic variables.

Given that becoming literate goes along with widely distributed modulations of cortical responses to print, we assumed that the effects of our intervention could be best captured with a two-step procedure. First, we performed an unbiased network centrality analysis to explore functional connectivity between each voxel and all other voxels of the brain without predefining seed regions. The cluster of the most strongly connected voxels was then used as a post hoc seed region to identify the specific network driving the global change in functional connectivity.

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RESULTS

Behavioral effects of practicing Devanagari script on letter knowledge and word-reading skills

The behavioral effectiveness of the literacy instruction was reflected in significant group (reading-trained individuals versus untrained illiterates) by time (before versus after intervention) interactions of letter knowledge [$F_{1,28}$ = 17.80, P < 0.001, η^2 = 0.39; 2 × 2 mixed analysis of variance (ANOVA)] and word reading ($F_{1,28}$ = 15.96, P < 0.001, η^2 = 0.36; 2 × 2 mixed ANOVA). Both interactions were driven by significant improvements of the trained group (letter knowledge: z = 4.20, P < 0.001, r = 0.65; word reading: z = 3.83, P < 0.001, r = 0.59; Wilcoxon signed-rank tests) that were not observed in the untrained group (letter knowledge: z = 0.41, P = 0.684; word reading: z = 0.37, P = 0.715; Wilcoxon signed-rank tests) (Table 1).

Resting-state network centrality changes in the bilateral pulvinar nuclei and the right superior colliculus

Initially, we investigated in a voxel-wise fashion at the whole-brain level whether the experience of becoming literate modifies network nodes of spontaneous hemodynamic activity. Therefore, we compared training-related differences in the degree centrality of BOLD signals between the groups (*9*). A significant group by time interaction (t_{max} = 4.17, P < 0.005, corrected for cluster size) was found in a single coherent cluster (k = 35 voxels; voxel size 3 × 3 × 3 mm^3) extending from the right superior colliculus of the brainstem [MNI (Montreal Neurological Institute) coordinates: +6, −30, −3] to the bilateral pulvinar nuclei of the thalamus (MNI coordinates: +6, −18, −3; −6, −21, −3) (Fig. 1). This interaction was characterized by a significant mean degree centrality increase in the trained group ($t_{1,20}$ = 8.55, P < 0.001, d = 1.31; paired t test) that did not appear in the untrained group, which remained at the baseline level ($t_{1,8}$ = 0.14, P = 0.893; paired t test) (Fig. 1). To establish the reliability of the training-induced increase in subcortical network centrality, we performed a confirmatory leave-one-out cross-validation analysis. A linear binary support vector machine classification revealed that the experimental and control groups are not statistically distinguishable before the training (accuracy, 54.76%; P = 0.272), but do show a statistically significant difference after the training (accuracy, 76.98%; P = 0.017).

Increasing temporal coupling of spontaneous BOLD activity in the subcortical visual nuclei and the visual cortex

The cluster obtained from the degree centrality analysis was then used as a seed region in a voxel-wise functional connectivity analysis (*10*).

Table 1. Participant demographic information and behavioral performance.

	Trained group	Untrained group	Group difference
n	21	9	—
Age (years)	31.57 ± 4.90*	31.78 ± 5.47*	$z = 0.21$, $P = 0.837$
Gender (female/male)	20/1	8/1	—
Monthly income (Rupees)	2313.50 ± 629.15*	2500 ± 433.01*	$z = 0.96$, $P = 0.375$
Literate family members	2.95 ± 1.54*	2.86 ± 1.46*	$z = 0$, $P = 1$
Raven test	13.29 ± 2.67*†	11.67 ± 2.60*†	$z = 1.42$, $P = 0.164$
Letter knowledge pretest	10.38 ± 12.50*‡	7.22 ± 10.12*‡	$z = 0.98$, $P = 0.341$
Letter knowledge posttest	33.81 ± 7.11*‡	5.44 ± 9.84*‡	$z = 4.21$, $P < 0.001$
Word reading pretest	0.57 ± 1.57*‡	1.56 ± 2.65*‡	$z = 1.41$, $P = 0.301$
Word reading posttest	7.10 ± 8.53*‡	1.56 ± 2.35*‡	$z = 2.61$, $P = 0.009$
Days between tests	189.76 ± 22.74*	171.22 ± 63.85*	$z = 1.31$, $P = 0.193$

*Mean ± SD. †Raven test raw scores (maximum 60 points). ‡Raw scores.

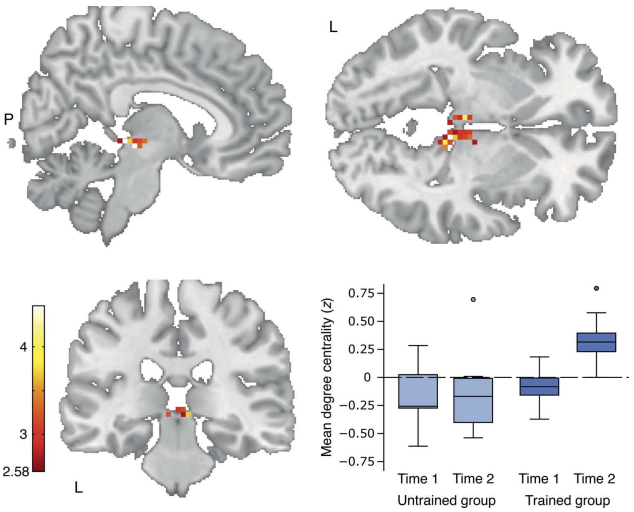

Fig. 1. Learning to read modifies subcortical network centrality. Whole-brain degree centrality map thresholded at $z = 2.58$ ($P < 0.005$, corrected for cluster size) with corresponding color bar indicating the range of *z* scores. The effect of literacy instruction is depicted as a group (reading-trained individuals versus untrained illiterates) by time (before versus after intervention) interaction. The significant cluster stretches from the right superior colliculus of the brainstem (MNI coordinates: +6, −30, −3) to the bilateral pulvinar nuclei of the thalamus (MNI coordinates: +6, −18, −3; −6, −21, −3). The box plot resolves the interaction by separately showing the individual mean *z* values for each factor level. Mean degree centrality values of the untrained group did not differ significantly from zero (time 1: $t_{1,8} = 1.76$, $P = 0.116$; time 2: $t_{1,8} = 1.10$, $P = 0.302$; one-sample *t* tests).

Our aim was to identify brain areas whose BOLD time courses became more strongly coupled to those of the right superior colliculus and the bilateral pulvinar nuclei as a consequence of learning to read. A significant group by time interaction (t_{max} = 4.45, P < 0.005, corrected for cluster size) emerged as a single coherent cluster in the areas V1, V2, V3, and V4 of the right occipital cortex (k = 48 voxels; voxel size 3 × 3 × 3 mm^3; MNI coordinates: +24, −81, +15; +24, −93, +12; +33, −90, +3) (Fig. 2). The cortico-subcortical mean functional connectivity got significantly stronger in the group that took part in the reading program (z = 3.77, P < 0.001, r = 0.58; Wilcoxon signed-rank test) but not in the group that remained illiterate (z = 0.77, P = 0.441; Wilcoxon signed-rank test).

Stronger functional coactivation in the early visual pathway and the individual gain in letter and word knowledge

Finally, we wanted to find out whether there was a relation between the detected neural alterations and the behavioral improvements at the individual level. To this end, we derived an index for the growth of brain-functional connectivity [correlation coefficient of the BOLD time courses of each of the two regions of interest (ROIs) after minus before the intervention] and two indices for the increase of literacy (letter knowledge/word-reading skills after minus before the intervention). Individual slopes of cortico-subcortical connectivity were significantly associated with improvement in letter knowledge (r = 0.40, P = 0.014; one-sided Pearson's correlation) and with improvement in word-reading ability (r = 0.38, P = 0.018; one-sided Spearman's rank correlation).

DISCUSSION

We used resting-state fMRI to examine the specific effects of learning Devanagari script on the functional connectome of illiterate Hindi-speaking Indian adults within the framework of a controlled longitudinal design. Network centrality of spontaneous hemodynamic activity significantly increased with training in the bilateral pulvinar nuclei of the thalamus and the right superior colliculus of the brainstem.

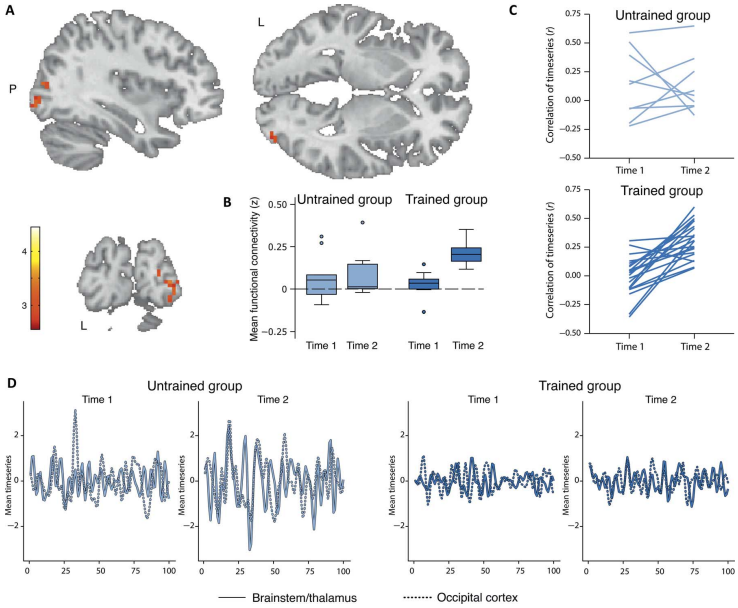

Fig. 2. Learning to read strengthens cortico-subcortical functional connectivity. (**A**) Voxel-wise functional connectivity map derived from seeding in the significant degree centrality cluster. The image is thresholded at z = 2.58 (P < 0.005, corrected for cluster size). The color bar indicates the range of z scores. Becoming literate goes along with increased coupling of BOLD signal time courses between mesencephalic/diencephalic visual nuclei and a single cluster spanning the areas V1, V2, V3, and V4 of the right occipital cortex (MNI coordinates: +24, −81, +15; +24, −93, +12; +33, −90, +3). (**B**) The group (reading-trained individuals versus untrained illiterates) by time (before versus after intervention) interaction becomes evident from the box plot, indicating that the functional connectivity is strongly and specifically enhanced in the group that underwent reading instruction. (**C**) Line graphs depicting the coefficients of the correlations between the hemodynamic time series separately for each individual subject, each group, and each time. (**D**) Mean time series of the BOLD signal for each group and each time.

Moreover, BOLD signal time courses of these subcortical structures were significantly more strongly coupled with the areas V1 to V4 of the right occipital cortex after acquiring basic literacy skills. Individual gains in intrinsic functional connectivity turned out to be significantly associated with individual gains in letter identification and word-reading skills.

Currently existing neurobiological models of reading assume that literacy boosts low-level hemodynamic responses to complex visual objects in areas V1 to V4 of the occipital cortex (*6*). Here, we provide the first evidence for functional neuroplasticity in mesencephalic and diencephalic nuclei upstream of V1 as a consequence of reading acquisition. These results call for a reconceptualization of the neural basis of reading by expanding the experimental perspective from one focused solely on the cortex to one that also includes the subcortical areas associated with oculomotor control and selective visuospatial attention.

Nonhuman primate experiments on visual motion perception suggest that the superior colliculi support the initiation of saccadic eye movements (*11*). Accordingly, the observed increase in connectomic centrality of the right superior colliculus in the course of literacy training might reflect the fine-tuning of oculomotor activity necessary for guiding fixations through printed text. An explanation for the effect in the bilateral pulvinar nuclei can be derived from numerous studies in humans highlighting the central role of these thalamic structures for selectively allocating attentional resources to visual stimuli (*12*–*16*). This is in line with several independent studies suggesting a causal role of visuospatial attention skills for reading acquisition. Namely, it has been repeatedly shown in preliterate children that visuospatial skills predict reading outcome (*17*, *18*). Moreover, there is evidence that reading abilities can be improved by training with an action video game that challenges visual attention (*19*).

If interpreted in light of recent nonhuman primate work, enhanced functional connectivity between the subcortical nuclei and the right occipital cortex detected after reading intervention indicates that the pulvinar is involved in synchronizing information transmission across the visual cortex (*20*, *21*). Signal exchange between these structures is hypothesized to be located anatomically along the long-distance white matter fiber tract that directly connects them (*22*, *23*).

Literacy-driven functional modulations of the right occipital cortex were not restricted to V1 and V2, as one would expect for alphabetic writing systems (*24*), but extended into V3 and V4. This might be explained by the nature of the Devanagari script, which is visually more complex than alphabetic writing systems. Devanagari is written from left to right and used for over 100 languages other than Hindi (for example, Bengali, Nepali, and Tibetan) and by hundreds of millions of people. It is an alpha-syllabic writing system comprising the so-called *aksharas* that represent sound simultaneously at the syllable and phoneme level. Vowels and consonants are, thus, not ordered sequentially as independent letter units in words. Devanagari is similar to alphabetic writing systems in that symbols mostly convey a word's phonology (that is, distinct units that correspond to individual phonemes rather than syllables or words). However, Devanagari is also similar to logographic writing systems (for example, Japanese, Chinese) in that it also consists of visually complex symbols that are larger than phonological units and that are indivisible in that some of the component parts (for example, diacritic signs) cannot stand alone. In line with our finding in Devanagari, fMRI effects in V3 and V4 during print processing are known from Chinese readers (*25*). Right-lateralized manifestations of functional plasticity in the occipital cortex after training reading-related decoding skills have been repeatedly found especially in comparable samples of illiterate adults reaching modest performance levels but remain to be illuminated in future studies (*3*, *4*).

Previous task-based fMRI experiments have associated the process of learning to read with increasing BOLD responses in the so-called "visual word form area" (VWFA) of the left temporo-occipital fusiform cortex (*2*, *4*). We hypothesize that the high visual processing demands arising from the complex visuospatial arrangement of Devanagari characters might have induced a strong training effect in low-level visual areas (*26*), and that the potentially more subtle effect of symbolic learning in the VWFA would not reach statistical significance. Follow-up studies combining event-related fMRI paradigms with resting-state fMRI are necessary to confirm this hypothesis. However, we did not expect to be able to identify the VWFA when seeding in subcortical nuclei of the visual pathway to examine their resting-state functional connectivity. The VWFA has been shown repeatedly to be functionally connected to the dorsal attention network and not to lower-level visual areas when examining BOLD signals at the low-frequency sampling range covered in resting-state fMRI (*27*, *28*).

Recent cross-sectional MRI studies on adults and school-age children have reinvigorated the long-standing view that functional deficits and structural disruptions of the thalamus might play a role in developmental dyslexia, the most common learning disorder characterized by severe difficulties in learning how to read and spell (*29*–*32*). Our results indicate that the functional connectivity profile of the thalamus can change substantially even after 6 months of reading instruction in adulthood. Hence, beginning readers appear to train their subcortical sensory and attentional systems intensively. Therefore, one of the core challenges for the field is to determine whether thalamic abnormalities are a potential causal factor for developmental dyslexia or just a consequence of the impoverished reading experience of dyslexic individuals. Recent behavioral work suggests that visual motion processing skills are causally related to literacy acquisition. Specifically, dyslexic individuals perform such tasks more poorly than age-matched and reading level–matched controls (*33*, *34*). This could mean that a disruption of the underlying neural pathway connecting the lateral geniculate nucleus of the thalamus with V5 might be a contributing cause of dyslexia. Whether a similar role can be ascribed to the pathway connecting the pulvinar nuclei of the thalamus with the occipital cortex must be determined in follow-up studies. In particular, longitudinal studies following preschool children are needed to disentangle physiological causes from consequences arising from impaired literacy acquisition in scripts carrying both alphabetic and logographic features (*35*).

Learning-induced changes in coupling of spontaneous functional responses support the encoding or consolidation of novel experiences (*36*–*38*). Specifically, increased connectivity of functionally distinct areas might reflect the synchronization of excitability states of different neuronal populations (*39*). Future work on animal models combining resting-state fMRI and electrophysiological recordings is needed to confirm this hypothesis.

Note that the size of the sample investigated, though comparable to recent fMRI studies of literacy acquisition (*4*), is nevertheless small. Another limitation is that the training effects of the intervention group were compared with a passive, but not an active, control group. Accordingly, it remains to be shown whether the results reported here are literacy-specific or a general effect of visual training involving intricate symbols.

In conclusion, we have shown that only 6 months of learning to read leads to massive macroscopic functional reorganization processes in the mature human brain. When becoming literate in adulthood,

spontaneous hemodynamic activity of mesencephalic and diencephalic nuclei is strongly coupled with hemodynamic activity of the occipital cortex. These findings crucially complement current neural concepts of reading by suggesting that literacy experience reshapes the earliest visual computation centers even before reaching the primary visual cortex. It remains to be shown whether deficits in these subcortical structures are a consequence of the reduced literacy experience of dyslexic individuals or a potential cause of their disorder.

MATERIALS AND METHODS

Participants

Participants were recruited from two villages near the city of Lucknow in the northern Indian state of Uttar Pradesh as part of a study that was approved by the ethics committee of the Center of Biomedical Research, Lucknow. After giving informed consent, 51 healthy right-handed human volunteers without a known history of psychiatric disease or neurological condition took part in the reading training and in the resting-state fMRI experiment. For unknown reasons, 18 participants did not complete the scanning sessions and were therefore excluded from further analysis (see "Demographic and behavioral data" for more details). Three additional participants were disregarded because their fMRI data did not pass our quality control procedure (see "MRI data" for more details). Accordingly, 30 participants (mean age, 31.63 years; two males; Table 1) were included in the final behavioral and neural analyses. At the beginning of the study, all of them self-reported that they were never taught how to read, spell, or write and never attended school. Subsequently, they were first assessed for their actual letter (*akshara*) knowledge and word-reading skills (Table 1) and then underwent MRI scanning. Not one of them was able to read more than eight simple words at the beginning of the study. The participants were randomly assigned either to the group that received reading instruction ($n = 28$ at the beginning of the study; $n = 21$ included in the final analysis) or to the group that did not receive any instruction ($n = 23$ at the beginning of the study; $n = 9$ included in the final analysis). Final sample sizes were similar to recent fMRI studies of literacy acquisition (*4*). Group assignment was based on the following order: The first subject was assigned to the training group, the next subject to the control group, the third subject to the training group, and so on. For organizational reasons, all investigators knew the group allocation during acquisition and analysis of the data. The instructor was a professional teacher who followed the locally established method of reading instruction. During the first month of instruction, reading and writing of the 46 primary Devanagari characters were taught simultaneously. The practice of *aksharas* was followed by the practice of two-syllable words. Approximately 200 words were taught in the first month. During the second month, participants were taught to read and write simple sentences containing mostly two-syllable words. In the third month of instruction, the participants started to learn three-syllable words and continued to practice reading and writing of simple sentences. For the remaining 3 months of the program, more complex words and some basic grammar rules were taught. For example, the participants learned about the differences between nouns, pronouns, verbs, proverbs, and adjectives and also about basic rules of tense and gender. At the end of the study, that is, approximately 6 months later (mean gap, 184 days), participants were first scanned and then tested again on the same day for their *akshara* letter knowledge and word-reading skills. The pretest items (used before the intervention) and posttest items (used after the intervention) were identical. We cannot exclude the possibility that the participants—as a side effect of literacy—were more frequently exposed to complex pictures (for example, in magazines).

Demographic and behavioral data

Participants were matched for age, gender, handedness, income, number of literate family members, and nonsymbolic intelligence (Table 1). Each variable revealed a significant result either in a Kolmogorov-Smirnov test or in a Shapiro-Wilk test for normality of distribution, so that nonparametric Mann-Whitney *U* tests were run to compare the groups. No significant differences were found for any of the variables (all $z < 1$; Table 1). The 18 excluded participants who did not complete the scanning sessions were significantly younger ($z = 2.97$, $P = 0.003$; Mann-Whitney *U* test), performed significantly better in the test of nonsymbolic intelligence ($z = 2.17$, $P = 0.030$; Mann-Whitney *U* test), and had significantly fewer literate family members ($z = 2.54$, $P = 0.011$; Mann-Whitney *U* test) compared to the included 30 participants who completed the sessions. The groups showed no significant difference either in letter knowledge ($z = 0.47$, $P = 0.638$; Mann-Whitney *U* test) or word-reading ($z = 0.62$, $P = 0.538$; Mann-Whitney *U* test) ability at the beginning of the study (see below for details regarding these measures). Information on age, income, and number of literate family members was obtained by personal interview. Right-handedness was also verified in an interview by asking the participants which hand they used for common activities (for example, drawing). Raven's Progressive Matrices were administered to test for nonverbal abilities.

Two measures of literacy were taken, namely, letter identification (knowledge of the 46 primary Devanagari characters) and word-reading ability (knowledge of 86 words of varying syllabic complexity). The effects of literacy instruction on behavioral performance were statistically evaluated using SPSS (www.ibm.com/software/de/analytics/spss/) to calculate a 2 × 2 mixed-design ANOVA with time [test performance before the (non-)intervention versus test performance after the (non-)intervention] as a within-subjects factor and group (illiterates who underwent intervention versus illiterates who did not undergo intervention) as a between-subjects factor. ANOVA is an appropriate test here because it has been repeatedly demonstrated to yield valid results independent of the assumption of normality of data distribution (*40*, *41*), which was violated here according to the Kolmogorov-Smirnov and Shapiro-Wilk tests. Post hoc, nonparametric Wilcoxon signed-rank tests were run to compute within-subject–level changes in performance.

MRI data

MRI examination was conducted with a 3.0-Tesla Siemens MAGNETOM Skyra (Siemens AG) whole-body magnetic resonance scanner using a 64–radio frequency–channel head coil.

For anatomical localization, T1-weighted three-dimensional magnetization-prepared rapid-acquisition gradient echo images were acquired using a pulse sequence with repetition time (TR) = 1.690 ms, echo time (TE) = 2.60 ms, inversion time (TI) = 1.100 ms, field of view (FOV) = 256 × 256, matrix size = 256 × 256 × 192, and voxel size = 1.0 × 1.0 × 1.0 mm^3.

For resting-state fMRI (eyes closed, no active stimulation, and no explicit task), 150 T2*-weighted gradient echo echo-planar imaging volumes covering 38 slices were collected by applying a pulse sequence with TR = 2.400 ms, TE = 30 ms, FOV = 224 × 224, matrix size = 64 × 64 × 38, and voxel size = 3.5 × 3.5 × 3.0 mm^3.

The T1 images were visually inspected for artifacts and then segmented into gray matter, white matter, and cerebrospinal fluid using the

DARTEL algorithm (*42*) implemented in SPM8 (www.fil.ion.ucl.ac.uk/spm/software/spm8/). These segmentations served to create individual tissue masks and a sample-specific template in MNI space.

The fMRI data were preprocessed using the SPM8 software package (www.fil.ion.ucl.ac.uk/spm/software/spm8/) and the DPARSF toolbox (www.restfmri.net). First, the first four volumes of each scan were discarded to allow for signal equilibration. Second, the images were slice time–corrected by interpolation and resampling to the slice at the mid–time point of each TR. Third, the images were motion-corrected by realigning them to the first acquired volume. Fourth, additional motion correction was carried out by regressing out three translational and three rotational motion parameters of each volume and its preceding volume as well as the square of each of these values (*43*). Mean signals of the white matter and the cerebrospinal fluid and linear and quadratic trends were also included in this model to control for physiological noise induced by respiration and pulsating veins. Fifth, each time series was temporally bandpass-filtered (0.01 to 0.1 Hz) using an ideal rectangular filter. Sixth, the images were resampled to a spatial resolution of $3.0 \times 3.0 \times 3.0$ mm^3 and normalized to the sample-specific template in MNI space. Finally, the images were spatially smoothed with a 4-mm full width at half maximum Gaussian kernel, resulting in an average smoothness of $7.0 \times 6.9 \times 7.0$ mm^3.

To account for the confounding effect of residual head motion, we calculated the framewise displacement (FD) of each individual time series following the approach introduced by Power *et al.* (*44*). Of 33 data sets, 30 did not exceed a single-volume threshold of 0.5981 at both acquisition time points when determining the 100 volumes with the lowest FD values. The three data sets violating this criterion were removed from the further analyses. The mean FD of the least motion-distorted 100 volumes included in the final analyses was as low as 0.1036 (SD, 0.0443) for the first time point and 0.1193 (SD, 0.0600) for the second time point. Of 6000 volumes, 5394 revealed an FD < 0.2.

Whole-brain functional connectivity was computed using the degree centrality algorithm developed by Zuo *et al.* (*9*), which quantifies connectivity by counting the number of correlations of each voxel with all voxels at a threshold of $r > 0.25$ and then assigns this number as a centrality value to each voxel. This analysis was carried out in MNI space using a group-average gray matter mask of 67.441 voxels. The resulting degree centrality images were Fisher's *r*-to-*z*–transformed and then statistically analyzed in the framework of the flexible factorial design implemented in SPM8 running a 2 × 2 mixed-design ANOVA with time [test performance before the (non-)intervention versus test performance after the (non-)intervention] as a within-subjects factor and group (illiterates who underwent intervention versus illiterates who did not undergo intervention) as a between-subjects factor. Mean FD values did not differ significantly within groups between time points (trained individuals: $z = 0.92$, $P = 0.357$; untrained illiterates: $z = 0.53$, $P = 0.594$; Wilcoxon signed-rank tests) and also not between groups (time point 1: $z = 0.11$, $P = 0.934$; time point 2: $z = 1.15$, $P = 0.263$; Mann-Whitney *U* tests) but were nevertheless entered as a nuisance covariate of interest into the ANOVA to remove any potential relations between residual head motion and the effects of interest (*45*). When testing for statistical significance, signal variance of the two groups was not assumed to be equal because group sizes were different. Accordingly, *P* values were Greenhouse-Geisser–corrected to account for potential nonsphericity of the data. Clusters, that is, connected voxels sharing at least a corner (26 voxels), were multiple-comparison–corrected by combining a type I error threshold of $P < 0.005$ with a spatial extent threshold of $P < 0.05$. The latter threshold was determined by running 10,000 iterations of a Monte Carlo simulation as implemented in the AlphaSim tool (http://afni.nimh.nih.gov/), which revealed a minimum cluster size cutoff of $k = 35$ voxels (for the 67.441 gray matter voxels). Note that the size and the smoothness of the image were determined with SPM8 rather than AlphaSim to avoid overestimating the level of significance (*46*). Individual mean *z* values of the significant clusters were extracted with the REX toolbox (https://www.nitrc.org/projects/rex/) and then plotted separately across the factor levels with SPSS to resolve the effects characterizing the interaction. A confirmatory leave-one-out cross-validation analysis was carried out by training a linear support vector machine classifier (with the goal of distinguishing group membership before and after the training) first on a random subject before quantifying its performance on the remaining data sets. In accordance with the number of subjects in the sample, this procedure was repeated 30 times, each time with a new assignment of subjects and leaving aside each of the already given observations. Classification performance was estimated by averaging the indices obtained on the different repetitions. Statistical significance was determined nonparametrically by running 10,000 iterations of a permutation test.

The seed-based voxel-wise functional connectivity analysis (*10*) was carried out by extracting the individual means of the BOLD signal time series from the significant cluster identified with the degree centrality approach and then calculating their brain-wide correlation maps, which were finally Fisher's *r*-to-*z*–transformed. The procedure of statistical testing was identical to the procedure applied to the degree centrality maps.

Anatomical identification of all significant clusters was based on the Harvard-Oxford Subcortical Structural Atlas and the Juelich Histological Atlas implemented in FSL (*47*).

Seed-based ROI-wise functional connectivity analyses (*10*) were run by extracting the individual means of the BOLD signal time series from the two significant clusters obtained from the previous analyses and by correlating them with each other. Subsequently, the individual correlation coefficients of the BOLD time courses of each of the two ROIs obtained before the (non-)intervention were subtracted from the coefficients obtained after the (non-)intervention. In addition, the individual letter identification and word-reading test scores, respectively, acquired before the (non-)intervention were subtracted from the scores obtained after the (non-)intervention. The resulting index of increase of functional connectivity was correlated separately with the index of increase of letter identification skills (normally distributed data; Pearson's product-moment correlation coefficient) and the index of increase of word-reading performance (not normally distributed data; Spearman's rank correlation coefficient) in SPSS. One-sided *P* values are reported because the analyses were carried out under the a priori assumption that better literacy skills would go along with stronger functional connectivity.

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Acknowledgments: F.H. would like to thank A. Cutler. **Funding:** This project was funded by the Max Planck Society. **Author contributions:** F.H. designed the research; U.K., R.K.M., V.N.T., A.G., and J.P.S. recruited the participants and collected data; M.A.S. performed the analyses; and M.A.S., F.E., and F.H. wrote the paper. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper. Additional data related to this paper may be requested from F.H.

Submitted 22 October 2016
Accepted 23 March 2017
Published 24 May 2017
10.1126/sciadv.1602612

Citation: M. A. Skeide, U. Kumar, R. K. Mishra, V. N. Tripathi, A. Guleria, J. P. Singh, F. Eisner, F. Huettig, Learning to read alters cortico-subcortical cross-talk in the visual system of illiterates. *Sci. Adv.* **3**, e1602612 (2017).

Study IV

Kraft I, Schreiber J, Cafiero R, Metere R, Schaadt G, Brauer J, Neef N, Müller B, Kirsten H, Wilcke A, Boltze J, Friederici AD, **Skeide** MA (2016) Predicting early signs of dyslexia at a preliterate age by combining behavioral assessment with structural MRI. *Neuroimage* 143:378–386.

NeuroImage 143 (2016) 378–386

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/neuroimage

Predicting early signs of dyslexia at a preliterate age by combining behavioral assessment with structural MRI

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ARTICLE INFO

Article history:
Received 5 April 2016
Accepted 2 September 2016
Available online 5 September 2016

Keywords:
Developmental dyslexia
Arcuate fascicle
Cortical thickness
Quantitative T1
Diffusion-weighted imaging
Reading

ABSTRACT

Background: Recent studies suggest that neurobiological anomalies are already detectable in pre-school children with a family history of developmental dyslexia (DD). However, there is a lack of longitudinal studies showing a direct link between those differences at a preliterate age and the subsequent literacy difficulties seen in school. It is also not clear whether the prediction of DD in pre-school children can be significantly improved when considering neurobiological predictors, compared to models based on behavioral literacy precursors only.

Methods: We recruited 53 pre-reading children either with (N=25) or without a family risk of DD (N=28). Quantitative T1 MNI data and literacy precursor abilities were assessed at kindergarten age. A subsample of 35 children was tested for literacy skills either one or two years later, that is, either in first or second grade.

Results: The group comparison of quantitative T1 measures revealed significantly higher T1 intensities in the left anterior arcuate fascicle (AF), suggesting reduced myelin concentration in preliterate children at risk of DD. A logistic regression showed that DD can be predicted significantly better ($p=.024$) when neuroanatomical differences between groups are used as predictors (80%) compared to a model based on behavioral predictors only (63%). The Wald statistic confirmed that the T1 intensity of the left AF is a statistically significant predictor of DD ($p<.05$).

Conclusions: Our longitudinal results provide evidence for the hypothesis that neuroanatomical anomalies in children with a family risk of DD are related to subsequent problems in acquiring literacy. Particularly, solid white matter organization in the left anterior arcuate fascicle seems to play a pivotal role.

1. Introduction

Five to seven percent of all children have developmental dyslexia (DD), a specific learning disorder that is characterized by severe difficulties in the acquisition of reading and spelling despite adequate cognitive abilities and effective classroom instruction (Moll et al., 2014; Peterson and Pennington, 2012). Studies on families and twins indicate that DD is moderately to highly heritable. A prevalence of 34–77% is observed in children with a dyslexic parent or sibling (Lyytinen et al., 2004; Snowling et al., 2003; Pennington and Lefly, 2001; Gallagher et al., 2000; DeFries et al., 1987; Hallgren, 1950).

At the microstructural level, certain DD-associated genetic variations are related to disruptions of neuronal migration during neocortical development (Tammimies et al., 2013; Szalkowski et al., 2012; Gabel et al., 2011, 2010; Wang et al., 2011; Burbridge et al., 2008; Rosen et al., 2007). It is suggested that these structural

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http://dx.doi.org/10.1016/j.neuroimage.2016.09.004
1053-8119/

anomalies in the cortical layers of the perisylvian brain regions lead to a functional disruption of the local microcircuitry (Giraud and Ramus, 2013; Galaburda, et al., 2006; Galaburda and Kemper, 1979). Consistent with this assumption, Darki et al. (2014) found a significantly increased cortical thickness (CortT) of the left temporo-parietal regions, the angular and supramarginal gyri (SMG) in readers with DD-related genetic polymorphisms. Several functional magnetic resonance imaging (fMRI) studies also described hypoactivations during reading and reading-related tasks in individuals with DD in temporo-parietal brain regions as well as in superior temporal, inferior parietal, and in inferior frontal brain regions (pars opercularis of the inferior frontal gyrus, IFG) (for a review, see Richlan et al., 2009). These brain regions belong to the dorsal sublexical reading network and are activated during reading of pseudowords. Further functional hypoactivations were observed in the ventral lexical reading network, which is activated in reading of orthographically irregular words, and particularly in occipito-temporal brain regions including the fusiform gyrus and posterior inferior and middle temporal regions (Richlan et al., 2009). Consistent with these fMRI findings, structural MRI studies reported neuroanatomical differences in the gray matter of the dorsal reading network, including the left superior temporal cortex (Richlan et al., 2013), the left inferior parietal cortex (Darki et al., 2014; Hoeft et al., 2007; Eckert et al., 2005) and the ventral reading network, including the left fusiform gyrus (Skeide et al., 2016; Altarelli et al., 2013).

Recent studies described disruptions of interregional connectivity besides the local findings in specific brain regions as another main problem in dyslexia. Altered structural (Skeide et al., 2015; Vandermosten et al., 2012; Yeatman et al., 2011; Rimrodt et al., 2010) as well as functional connectivity (Schurz et al., 2015; Finn et al., 2014; Norton et al., 2014; van der Mark et al., 2011) has been reported in the dorsal and ventral reading network. Skeide et al. (2015) reported disrupted connectivity in the dorsal reading network in children with DD. The study revealed decreased fractional anisotropy (FA) in the arcuate fascicle (AF), a fiber tract connecting temporo-parietal brain regions with frontal regions. Catani et al. (2005) identified three separate segments of the AF and mapped them on different aspects of phonological processing. The long segment of the AF, directly connecting temporal and frontal brain regions, has been associated with phoneme awareness (Vandermosten et al., 2012; Yeatman et al., 2011), which is relevant for DD. The anterior segment is suggested to be involved in segmental phonological processing (Rimrodt et al., 2010; Fiez and Petersen 1998), while the posterior AF circuit is associated with grapheme-to-phoneme mapping (Thiebaut de Schotten et al., 2014). Furthermore, disrupted connectivity was also reported in the ventral reading network, including anomalies within the inferior fronto-occipital fascicle (IFOF). The IFOF is a long-distance fiber tract which has been related to visual word form recognition (Vandermosten et al., 2012; Jobard et al., 2003).

It is largely unknown whether such anomalies exist before literacy is acquired at school, or, alternatively, whether they are consequences of impaired reading. First indirect evidence in favor of the former hypothesis comes from cross-sectional studies which described disruptions in the connectivity of the dorsal and ventral reading network in pre-school children with a family risk of DD (Vandermosten et al., 2015; Hosseini et al., 2013). Additionally, a longitudinal study reported disruptions in the connectivity of the dorsal reading network in kindergartners who were at risk of DD because of their poor phonological skills (Saygin et al., 2013).

Most of the previous studies investigating the white matter connectivity of the reading network focused on FA (Basser and Pierpaoli, 1996; Basser 1995), a parameter of water diffusion which is based on diffusion tensor imaging (Basser et al., 1994). FA has been characterized as a measure which depends on microstructural fiber tract features such as myelination (Paus et al., 2012), although such interpretation of tensor-derived parameters is still debated. The tensor model is based on the assumption that the diffusion of the water molecules is Gaussian. However, this assumption poorly reflects the more complex non-uniform fiber architecture with crossing, kissing and fanning fibers (Tardif et al., 2015a). The fact that 63–90% of voxels in the brain contain crossing fibers impairs a specific connection of diffusion indices such as FA to physiological consequences (Jones et al., 2013).

Given the fact that FA is a neurobiologically unspecific measure which combines many biological and geometric properties of white matter in a single index, we looked for an alternative, potentially more specific index. Recent studies showed that T1 relaxation time strongly correlates with myelin concentration in the white matter of the brain (Tardif et al., 2015a, 2015b; Sereno et al., 2013; Dick et al., 2012). Accordingly, we analyzed T1 intensity maps as an inverse measure of myelination within fiber pathways relevant for literacy in the present study. Probabilistic tractography (Mori and van Zijl, 2002) was performed to investigate the myelination within fiber tracts of the dorsal and ventral reading network. The network was defined by selecting seed and target regions based on the results of our previous CortT study showing reduced CortT in preliterate children with a family risk of DD compared to children without such risk in the left SMG, the left inferior temporal gyrus (ITG) and in the left superior and transversal occipital sulci (SOS/TOS) (see Figs. S1 and S2; further details of the cross-sectional CortT analysis are provided in Kraft et al., 2015 and Supporting Information S1).

In addition to the cross-sectional investigations of white matter connectivity in the dorsal and ventral reading network in preliterate children at risk of DD, we also performed a longitudinal follow-up assessment in a subsample of 35 children.

The empirical evidence for the neuroanatomical predictability of DD is sparse compared to the intensively investigated behavioral predictors. Phonological development, rapid automatized naming (RAN) and visual attention were identified as reliable predictors for literacy proficiency (Franceschini et al., 2012; Caravolas et al., 2012; Lervåg et al., 2009). Nevertheless, the predictability of DD based on behavioral literacy predictors alone does not provide sufficient specificity when distinguishing between individuals with and without DD (Steinbrink et al., 2010; Marx and Weber, 2006). The involvement of further risk factors such as early variations in the development of specific brain structures might improve the prediction of DD.

The present study addressed one research question from a cross-sectional perspective and two other ones from a longitudinal perspective. Cross-sectionally, we investigated whether preliterate children with a family risk of DD show differences in quantitative T1 measurements in the dorsal and/or ventral reading network compared to children without a family risk of DD. Longitudinally, we investigated (i) whether children with structural brain anomalies at pre-school age will subsequently develop poorer reading abilities and (ii) whether prediction of DD can be improved when neurobiological anomalies are taken into consideration in addition to behavioral precursors of reading.

2. Methods

2.1. Design

The present study relied on two time points of data acquisition. At time point 1, we measured children from kindergartens who did not receive literacy instruction. We acquired structural, quantitative, and diffusion-weighted brain imaging data in children with and without a family risk of DD to extract a potential

neuroanatomical predictor for DD. We investigated behavioral precursors of reading abilities in children with a family risk of DD and compared them to age-, sex and intelligence-matched controls. Regression analyses were also controlled for the potentially confounding effect of intelligence, age and sex. A subsample of the previously examined children was invited back at time point 2 to test their reading and spelling abilities after one or two years of school education.

2.2. Participants

In total, 71 native German-speaking children were recruited from kindergartens on a voluntary basis. The following exclusion criteria were applied: (i) a history of neurological, psychiatric, hearing or vision disorders (based on parental report in the parental questionnaire), (ii) left-handed children (laterality quotient ≤ 0 according to the Edinburgh Handedness Inventory, which was modified into a child-appropriate version for the present study; Oldfield, 1971), (iii) multilingualism (parental questionnaire), (iv) children with second degree relatives with DD (parental questionnaire), (v) termination of the test session in the mock-up scanner because of extensive movement or distress, and (vi) termination of the measurement in the MRI scanner because of extensive movement or distress. From the remaining 59 children, six had to be excluded from the study due to poor image quality of the structural MRI data (i.e. blurring and ringing artifacts, which do not allow correct identification of the gray/white matter border; for more details see Supporting information S1). The finally included 53 children presented an age range from 4 years, 9 months to 6 years, 3 months (mean: 5 years 5 months, SD=0.4; 27 females). Twenty-five of the recruited children were at genetic risk of DD based on their family history. All of these children had one or more first-degree relatives with DD as reported in a parental questionnaire. The 28 children of the control group had neither first nor second degree relatives with DD (demographic information is provided in Table 1). A subgroup of 35 children out of the 53 children that took part in the baseline measurement was tested longitudinally (age range: 7 years, 0 months to 8 years, 9 months; mean: 7 years, 8 months, SD=7.2; 18 females). Eighteen participants were unable to attend follow-up sessions at the end of the school year. Ten participants had finished second grade and 25 had finished first grade at the time of investigation. The returning group was spread over 2 years because the 5-year-old children of the baseline measurement started school one year later than children who were 6 at the time of baseline measurement. Twelve of the 35 children originated from the group of dyslexic children (for classification details, see next paragraph 2.3). There were no statistically significant group differences between the dyslexic children and the control children with respect to age ($t(31)=.845$, $p=.441$), sex ($\chi^2(1)=.038$, $p=.845$) or intelligence ($t(30)=-.831$, $p=.271$). All experimental procedures of the study were approved by the ethics committee of the University of Leipzig.

Table 1
Demographic information.

	Risk	Control	Significance (*p*-value, univariate analysis of variance)
N	25	28	
Age[a]	5;7 ± 0;4	5;6 ± 0;4	.227
Sex[b]	14/11	16/12	.933[d]
Non-verbal IQ[c]	99 ± 13	104 ± 12	.098

[a] years;months, MRI-scan age, mean ± standard deviation.
[b] male/female.
[c] mean ± standard deviation.
[d] chi-square test.

2.3. Psychometric assessment

We investigated six literacy precursor abilities at kindergarten age: quality of phonological representations (PR), phonological awareness on the syllable/rhyme level (PA), rapid automatized naming (RAN), visual attention and verbal working memory. PR was assessed using the pseudoword repetition subtest of the SETK 3–5 (a developmental German language test for children between 3 and 5 years of age; Grimm et al., 2001). Note that only phonemic failures but not failures resulting from articulation disorders (such as sigmatism) are taken into account in this subtest of the SETK 3–5 (Grimm et al., 2001). Verbal working memory was used as a covariate to exclude the potentially confounding effect of verbal working memory on pseudoword repetition in all analyses including PR. The decision for using this task was based on a systematic review of longitudinal studies in German-speaking countries (Pfost, 2015), which revealed that phonological awareness tasks at the syllable or rhyme level were less related to reading and spelling abilities than tasks on the phoneme level. PA was assessed using syllable segmentation and rhyme identification tasks of the BISC (Bielefeld screening of literacy precursor abilities; Jansen et al., 2002). RAN was assessed using the corresponding subtest of the BISC. Visual attention was assessed using the symbol comparison subtest of the BISC. Verbal working memory was assessed using the digit span subtest of the German version of the Kaufman Assessment Battery for Children, third edition (Kaufman et al., 2009). Furthermore, we investigated parental education using a self-constructed parental questionnaire and non-verbal intelligence using the Wechsler preschool and primary scale of intelligence (Wechsler et al., 2009). Note that visual attention, rapid automatized naming, verbal working memory and non-verbal intelligence data were not available for one participant, who was therefore excluded from the data analysis presented in Sections 3.1, 3.2, 3.4 and 3.5.

We investigated reading skills to obtain follow-up information regarding the actual acquisition of literacy abilities using the reading fluency subtest of the SLRT-II (Salzburg test of reading and spelling, second edition; Moll and Landerl, 2010) and the reading comprehension test ELFE 1-6 (Reading comprehension test for 1st to 6th grade; Moll et al., 2006). Furthermore, we investigated spelling skills using the DERET (German spelling test; Stock and Schneider, 2008), a writing test after a dictation of words. We finally assessed phoneme awareness using the BAKO (Test of basic reading and spelling skills; Stock et al., 2003). Spelling and phoneme awareness data were not available for one participant, who was therefore excluded from the final DD prediction analysis (see Section 3.5).

The diagnostic status of DD was defined based on the individual performance in tasks assessing reading fluency, reading comprehension and spelling accuracy. Twelve (9 children with a family risk of DD and 3 without such risk) of the 35 children were assigned to the group of dyslexic children based on poor task performance. These children performed below the 10th percentile rank of the population performance in at least one of the tests (reading fluency, reading comprehension and/or spelling accuracy). Eleven children of the dyslexic group showed reading comprehension deficits (performance below 10^{th} percentile in reading comprehension test). Moreover, 80% of all children of the dyslexic group showed additional reading fluency and/or spelling deficits (performance below 10^{th} percentile in reading fluency and/or spelling accuracy test). To ensure good reading skills in the control group and that there is no overlap between groups, the two children between the 10^{th} and 25^{th} percentile were excluded from the analysis in Section 3.5 (Shaywitz et al., 2002).

2.4. MRI data acquisition

Children were invited to a training session conducted using a mock scanner in order to familiarize them with the MRI procedure. Both the training and the experimental session were adapted for young children and set up as interesting games. These games increased the cooperation of the children and facilitated familiarization with the experimental procedures.

MRI was performed on a 3 T Siemens TIM Trio (Siemens AG) magnetic resonance scanner with a 12 channel radio-frequency head coil. We used the magnetization-prepared 2 rapid acquisition gradient echo (MP2RAGE; Marques et al., 2010) method to acquire T1 maps with the following parameters: TR=5000 ms; TI1, TI2=700, 2500 ms; α1, α2=4°, 5°; FOV=250 × 219 × 187 mm; matrix size=192 × 168 × 144; voxel size1.3 mm isotropic; GRAPPA factor=3 (with 32 ref. lines). We also performed simulations of the Bloch equations for these acquisition parameters, to ensure accurate T1-MP2RAGE maps, and particularly robustness against typical (for our MRI system) B1+ variations, within the observed T1 range for white matter (see Fig. S3). The MP2RAGE sequence was validated for T1 estimation against an inversion recovery sequence (Marques et al., 2010), and it was recently shown that this validation approach ensures accurate estimation of T1 relaxation times (Stikov et al. 2015). The T1 relaxation time captured by these images strongly correlates with myelin content in the white matter of the brain (Tardif et al. 2015a, 2015b; Sereno et al., 2013; Dick et al., 2012).

Diffusion-weighted imaging (DWI) data were acquired using the echo planer imaging method with the following parameters: TR=8 s, matrix size=100 × 100, voxel size=1.9 mm isotropic and 66 axial slices. We used 60 diffusion-encoding gradient directions with a b-value of 1000 s/mm^2. Acquisition time was 32 min.

2.5. Data processing

2.5.1. MP2RAGE

The brain images from all uniform T1-weighted volumes of the MP2RAGE sequence were extracted using Freesurfer (http://surfer.nmr.mgh.harvard.edu/). These were then rigidly aligned to the MNI coordinate system and interpolated to 1-mm isotropic voxel size. The same transformation was applied to the quantitative T1 maps.

2.5.2. DWI

Before pre-processing the DWI data, a semi-automatic method (Schreiber et al., 2014) was used to identify DWI volumes corrupted by movement by plotting the average signal intensities for every axial slice of each volume. Signal drop-outs due to subject motion led to noticeably decreased average signal intensity and warranted manual inspection of those volumes. Two directions were removed on average per participant. The spherical deconvolution local model was computed using an order of 8, which requires at least 45 independent diffusion directions. Keeping at least 45 diffusion directions and checking that no more than 3 of these directions were neighboring ensured a sufficient over-determination of the system. The remaining data were also visually inspected to verify the results of this automatic method (Soares et al., 2013; Tournier et al., 2011). The DWI data were then corrected for motion and eddy currents as well as for susceptibility-induced distortions using the Topup tool (Andersson et al., 2003) as implemented in FSL (Smith et al., 2004), aligned to the uniform T1-weighted data and interpolated to 1-mm voxel size. All these procedures were performed with a single step of interpolation to preserve high data quality. Further preprocessing included the separation of background from diffusion data by applying the T1 brain mask and the computation of FA maps using the "dtifit" tool from the FSL software package.

2.6. Differences in quantitative T1 values within literacy relevant fiber pathways

The clusters showing statistically significant differences between children with and without a family risk of DD from a previous whole-brain CortT analysis (Kraft et al., 2015) were used as seed and target regions for tractography (Mori and van Zijl, 2002; Koch et al., 2002). These clusters were located in the left SMG, the left ITG and the left SOS/TOS. All ROIs were projected onto the adjacent gray/white matter border and extended by one voxel to ensure streamline connectivity within the white matter. Two additional target regions were defined in the IFG pars opercularis and IFG pars orbitalis to reconstruct the dorsal and ventral long distance fiber tracts of the reading network. The IFG pars opercularis was drawn in each subject's T1 image because of the high intersubject variability in this region's anatomy. The criteria of prior studies were used for the exact determination of the anatomical borders of the IFG pars opercularis (Amunts et al., 2010; Keller et al., 2007). Rostrally, the IFG pars opercularis was demarcated by the anterior ascending ramus of the Sylvian fissure, caudally by the inferior precentral sulcus and dorsally by the inferior frontal sulcus. For the extraction of IFG pars orbitalis, the *G_front_inf-Orbital* map from the Destrieux Atlas 2009 (https://surfer.nmr.mgh.harvard.edu/fswiki/Destrieux/AtlasChanges) was transformed to individual structural space of each subject. The subsequent visual inspection of each individual mask confirmed successful transformation in each case.

To reconstruct the fiber bundles connecting the aforementioned regions, fiber orientation density functions were computed in every voxel using constrained spherical deconvolution as implemented in MRtrix (Tournier et al., 2007). In contrast to the diffusion tensor (Basser et al., 1994), this model allows distinguishing multiple fiber orientations in each voxel (Zhao et al., 2016; Vanderauwera et al., 2015). Probabilistic tractography (Tournier et al., 2012) based on constrained spherical deconvolution was used to define the connecting pathways between ROIs. For this analysis, 500 000 streamlines were started in the seed locations to propagate to the target regions considering the orientational uncertainty described by the fiber orientation density functions. Probabilistic tractography was used to trace the following specific white matter tracts: connection between the CortT ROI of the SMG and the IFG pars opercularis (anterior AF), connection between the SMG-ROI and the ITG-ROI (posterior AF), connection between the ITG-ROI and the IFG pars opercularis (long AF), and connection between the ITG-ROI and IFG pars orbitalis (IFOF). Streamlines were seeded in the SMG-ROI to reconstruct both the anterior and posterior segment of the AF. Streamlines leading to a hub in the IFG pars opercularis were selected for the anterior segment and only streamlines leading to a hub in the ITG for the posterior one. We seeded in the statistically significant ITG-ROI, and selected streamlines leading to a hub in the IFG pars opercularis to reconstruct the long AF. Finally, we seeded in the statistically significant ITG-ROI, and selected streamlines leading to a hub in the IFG pars orbitalis to reconstruct the IFOF. Furthermore, streamlines were restricted to those fibers running between seed and target ROI.

Finally, mean T1 intensity values were calculated within each tract of interest by using the number of streamlines passing each voxel as weighting factors. This procedure has two major advantages. First, regions at the edge of gray and white matter, which could be contaminated by partial volume effects and contain fewer streamlines, contribute less to the average of an individual fiber

tract. Second, the central regions of the fiber tract with the highest densities of streamlines are more strongly weighted.

2.7. Statistical analyses

2.7.1. White matter

The FSL tool for nonparametric permutation tests within the framework of the general linear model ("Randomize"; Winkler et al., 2014), was used to estimate group effects (risk group versus control group) of averaged T1 intensities at each fiber tract. Results were corrected for family-wise error rate (FWE).

2.7.2. Behavioral test scores

A univariate analysis of variance was conducted for the group comparison of the behavioral data.

2.7.3. Structural MRI measures and precursor abilities

Multiple regression analysis was used to investigate the effect of structural brain anomalies, identified in children with a family risk of DD, on the development of phonological and visual precursor abilities. Gray matter anomalies from our recently published cross-sectional study were used (see Kraft et al., 2015 and Supporting information S1) in addition to predictors derived from the white matter analysis described above. We included a measure of the quality of phonological representations (PR) to investigate phonological precursor abilities and used a visual attention task to investigate visual precursor abilities. The multiple regression analysis was performed while controlling for age, sex and intelligence. Multiple regression analysis, which was used to investigate the effect on the PR, was run controlling verbal working memory in addition to age, sex and intelligence. This approach was used to exclude the potentially confounding effects of working memory on the pseudoword repetition subtest of the SETK.

2.7.4. Prediction of DD

We ran a hierarchical binary logistic regression analysis implemented in SPSS (SPSSInc., Chicago, IL, USA) to investigate whether the gray and white matter differences identified in children with a family risk of DD could predict the disorder better than the literacy precursors and general cognitive development. The independent variables were selected from the longitudinal correlation analysis (provided in Tables S1 and S2) and consisted of all statistically significant predictors of literacy ($p < .05$). During this analysis, three different DD prediction models were specified and hierarchically introduced to the analysis. In order to control for the potential confounding effect of general cognitive development, the first model included the intelligence score as the only predictor. In a second step, the effect of literacy precursors was investigated by adding PR and working memory as further predictors. The third model acknowledged statistically significant structural brain differences as an additional predictor.

3. Results

3.1. Behavioral group comparisons at a preliterate age

There was a statistically significant difference between the risk and control group in RAN ($F(1, 50)=6.84$, $p=.012$) after controlling for the effects of intelligence, age and sex. No statistically significant differences were found for PR ($F(1, 51)=1.07$, $p=.306$), PA ($F(1, 51)=0.38$, $p=.542$), visual attention ($F(1, 50)=0.61$, $p=.439$), working memory ($F(1, 50)=0.02$, $p=.903$) and parental education ($F(1, 51)=0.13$, $p=.722$). Mean raw scores and standard deviations are reported in Table 2.

Table 2
Results of behavioral group statistics: literacy precursor abilities.

	Risk (mean ± SD)	Control (mean ± SD)	Significance (*p*-value, univariate analysis of variance)
N	25	28	
PR	7.88 ± 3.80	8.96 ± 3.90	.306
PA	32.00 ± 6.84	30.93 ± 5.88	.542
RAN	**5.17 ± 2.68**	**6.61 ± 1.07**	**.012***
Visual attention	8.96 ± 1.92	8.43 ± 2.81	.908
Working memory	9.28 ± 2.92	9.37 ± 2.37	.903
Parental education[a]	16.32 ± 5.12	15.79 ± 5.69	.439

* $p < .05$.
[a] questionnaire-derived, single cumulative score per participating child computed by adding the sum of 2 scores (one per parent) for school education (4-point scale; no degree: 1 point; German "Abitur": 4 points) and the sum of 2 scores (one per parent) for further education (9-point-scale; no degree: 1 point; German "Habilitation": 9 points), mean ± standard deviation.

3.2. Behavioral group comparisons after one or two years of literacy education at school

There was a statistically significant difference between the risk and control groups in the reading score (additive score based on results in reading fluency test and reading comprehension test) ($F(1, 31)=7.25$, $p=.011$) and phoneme awareness ($F(1, 25)=1.96$, $p=.002$). The group difference in spelling did not reach statistical significance ($F(1, 30)=3.42$, $p=.074$). Mean scores and standard deviations are reported in Table 3.

3.3. White matter connectivity group comparisons at a preliterate age

The group comparison of averaged T1 intensities within the anterior segment of the left AF revealed significantly higher T1 intensities in pre-school children with a family risk of DD compared to pre-school children without a family risk of DD ($t(51)=1.82$, $p=.034$, Cohen's d=.501 FWE-corrected, Fig. 1). In all other tracts, no statistically significant differences were found between the risk and control group: posterior segment of the left AF ($t(51)=0.52$, $p=.296$, Cohen's d=.143), long segment of the AF ($t(51)=0.34$, $p=.361$, Cohen's d=.094) and IFOF ($t(51)=0.26$, $p=.741$, Cohen's d=.072). We also tested whether similar differences in the left anterior AF could be found when using FA as the dependent variable, but the difference between groups was not statistically significant ($t(51)=0.86$, $p=.194$).

3.4. Link between structural brain differences and literacy precursors

We then investigated the link between structural brain differences in the gray matter (Kraft et al., 2015; Supporting information

Table 3
Results of behavioral group statistics: literacy abilities.

	Risk (mean ± SD)	Control (mean ± SD)	Significance[+] (p-value, univariate analysis of variance)
N	15	20	
Reading score	**32.74 ± 30.75**	**59.19 ± 24.99**	**.011***
Spelling	27.64 ± 26.51	45.85 ± 23.23	.074
Phoneme awareness	**26.35 ± 27.01**	**58.40 ± 24.33**	**.002****

* $p < .05$.
** $p < .005$.

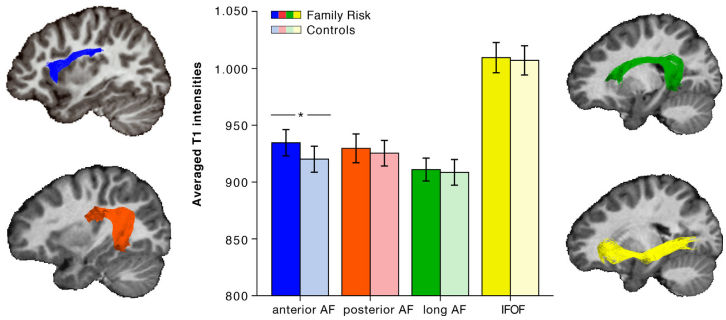

Fig. 1. Comparison of the averaged T1 intensities in the anterior, posterior and long segment of the arcuate fascicle (AF) as well as in the inferior fronto-occipital fascicle (IFOF) in preliterate children. Compared to children without a family risk of DD (N=28), children with a family risk of DD (N=25) showed significantly increased T1 intensities indicating reduced myelin concentration in the left anterior AF ($p < .05$ FWE-corrected, Cohen's d=.501). In all other tracts, no statistically significant differences were found between the risk and the control group. Error bars indicate standard errors of mean (SEM). The anterior segment of the AF connects the supramarginal gyrus (seed region) with the pars opercularis of the inferior frontal gyrus (target region). The posterior segment of the AF connects the supramarginal gyrus (seed region) with the inferior temporal gyrus (target region). The long segment of the AF connects the inferior temporal gyrus (seed region) with the pars opercularis of the inferior frontal gyrus. The IFOF connects the inferior temporal gyrus (seed region) with the pars orbitalis of the inferior frontal gyrus (target region). All tracts are shown for a single representative subject in native space.

Table 4
Results of hierarchical logistic regression analysis: coefficients of the model predicting whether an individual will have DD.

	β	Standard error	Wald	df	p	Exp(β)
Model 1						
Intelligence	−.062	.036	3.030	1	.082	.940
Model 2						
Intelligence	−.046	.040	1.273	1	.259	.955
PR	−.173	.162	1.144	1	.285	.841
Working memory	.005	.220	.001	1	.981	1.005
Model 3						
Intelligence	−.105	.059	3.193	1	.074	.900
PR	−.126	.172	.532	1	.466	.882
Working memory	−.012	.308	.001	1	.970	.988
Cortical thickness SMG	−2.864	1.926	2.212	1	.137	.057
T1 intensities $AF_{anterior}$	**.052**	**.025**	**4.266**	**1**	**.039***	**1.054**

* $p < .05$.

S1), white matter and literacy precursor abilities. Multiple regression analyses were performed to investigate the associations between structural brain differences identified in children at risk of DD and phonological (first analysis) and visual (second analysis) literacy precursor abilities. The results revealed a statistically significant association between the CortT in the left SMG and PR (β=.24, $t(50)=2.07$, $p=.044$) while no such effect was found for the CortT of the left ITG (β=−.14, $t(50)=-1.05$, $p=.301$) or the T1 intensity of the left anterior AF (β=.12, $t(50)=1.00$, $p=.328$). No statistically significant relations were found between the ITG (β=−.11, $t(49)=-.67$, $p=.509$), the SMG (β=−.04, $t(49)=-.26$, $p=.795$), the anterior AF (β=−.08, $t(49)=-.51$, $p=.615$) and visual attention (for more detailed information, see Tables S3 and S4).

3.5. Prediction of DD

Finally, a hierarchical binary logistic regression analysis was computed to investigate the role of behavioral and brain measures at pre-school age as DD predictors. For this, CortT measures were used as described previously (Kraft et al., 2015). The first model investigating the effect of general cognitive development (intelligence) on predictability of DD revealed no statistically significant improvement compared to the null model (Chi-square=3.553, $p=.059$). The second model adding the effect of behavioral literacy precursors (PR and working memory) on predictability of DD failed to detect a statistically significant improvement compared to the first model (Chi-square=1.66, $p=.436$). However, the third model adding the effect of structural brain differences on predictability of DD was statistically significant, demonstrating that inclusion of specific structural brain differences of the model significantly improves prediction of DD (Chi-square=7.482, $p=.024$). Nagelkerke's R^2 was .472, with an overall prediction success of 80% (90% for participants with DD and 64% for participants without DD), in contrast to 63% prediction success of the model including only behavioral predictors. The Wald statistic revealed that the T1 intensity left anterior AF was a statistically significant predictor of DD ($p=.039$) while the gray matter of the left SMG did not reach statistical significance ($p=.137$). The PR ($p=.466$), working memory ($p=.970$) and IQ ($p=.074$) did not significantly predict DD. The beta values, their standard errors and the statistical significance values of all coefficients of the model are reported in Table 4.

4. Discussion

We observed distinct brain structure profiles for children who have a family risk of DD compared to children without such risk. The children with a family risk of DD showed increased T1 intensities in the dorsal reading network, particularly in the left anterior AF, in addition to reduced CortT in previously described brain regions (Kraft et al., 2015). The observed structural brain differences together with behavioral literacy precursor measures showed a DD prediction success of 80%, thereby improving the prediction success based on behavioral literacy precursor measures alone by 17%. The best predictor of DD was an increase in T1 intensities indicating reduced myelin concentration in the left anterior AF.

The left anterior AF belongs to the dorsal reading network, which has been associated with segmental reading of regular words and pseudowords and in particular with phoneme-to-

grapheme mapping (Roux et al., 2012; Jobard et al., 2003). Previous studies investigating school-aged children and adults with DD reported reduced FA in the white matter of the dorsal reading network (e.g. Rimrodt et al., 2010; Deutsch et al., 2005; Klingberg et al., 2000). So far, however, no such anomalies have been found in preliterate children with a family risk of DD (Vandermosten et al., 2015). To our knowledge the present study is therefore the first one showing that such differences in the white matter of the dorsal reading network in children with a family risk of DD (compared to controls) exist before reading is taught at school. This result is in line with another study investigating pre-school children with poor phonological development without taking their family risk of dyslexia into account (Saygin et al., 2013). Saygin and colleagues showed a statistically significant relation between development of the dorsal reading network and the development of phonological skills (PR tasks, e.g. pseudoword repetition).

Given the fact that FA is a neurobiologically unspecific measure which combines many microstructural properties of white matter in a single index, the aim of the present study was to measure myelination using a more specific method. Indeed, the chosen quantitative T1 measure, which was previously shown to reflect myelin content in the white matter of the brain (Tardif et al., 2015a, 2015b; Sereno et al., 2013; Dick et al., 2012), revealed increased T1 intensities. This indicated reduced myelination in the left anterior AF in children at risk of DD (compared to controls), but this effect could not be replicated when we investigated differences in the FA of the left anterior AF. FA is more related to structure than to myelin (Beaulieu, 2002) and hence, quantitative T1 measurement might be a more sensitive method to investigate changes related to myelination in the developing brain.

In contrast to previous studies investigating school-aged individuals and adults with DD (Vandermosten et al., 2012; Yeatman et al., 2011), no anomalies were found in the long and posterior segment of the AF. It might be that the changes in these segments of the AF are not present in preliterates, but only emerge with the beginning of grapheme acquisition (Dehaene et al., 2015). Such reading-associated differences in the posterior AF were previously reported in a study comparing adult beginner readers (ex-illiterates) with illiterates (Thiebaut de Schotten et al., 2012).

The present study revealed no anomalies in the IFOF in preliterate children at risk of DD compared to children with no such risk. This result is in contrast to the result of the previous FA study in pre-reading children with a family risk of DD, which reported reduced FA in the left IFOF in children at risk of DD (Vandermosten et al., 2015). The divergent results might indicate that the difference in FA in preliterate children, which was observed by Vandermosten et al. (2015), is not exclusively driven by myelination but could alternatively be explained by other tissue properties, such as axonal density (Scholz et al., 2009) and axonal diameter (Paus, 2010) or fiber orientation (Beaulieu, 2002). Zhao et al. (2016) found reduced leftward asymmetry of the IFOF using hindrance-modulated oriented anisotropy (HMOA, Dell'Acqua et al., 2013) in school-aged children. Based on our hypotheses and the results of our CortT study (whole brain analysis revealed reduced CortT in left-hemispheric but not in right-hemispheric regions), no further investigation of the left-right-hemispheric asymmetry was performed in the present study. Such asymmetry analysis would nevertheless be an interesting question for future studies in order to investigate the lateralization of the dorsal and ventral reading network pathways in pre-school children with a family risk of DD.

The individual assessment of family risk of DD is an efficient approach to determine the genetic predisposition for DD. Previous research showed that 34% of first-degree relatives of individuals with DD develop DD compared to a rate of only 6% in individuals with no family risk (Pennington and Lefly, 2001). Nevertheless, we are aware of the difficulty of estimating a family risk of DD (Clark et al., 2014). Of the 35 subjects recruited to the longitudinal group in our study, 15 had a family history of DD nine of which (60%) developed dyslexia. Only three of the children without a family history of DD developed dyslexia (15%). This outcome shows that, besides a family history of DD (genetic factor), other factors seem to play a role, which confirms the observation that DD is a heterogeneous disorder with many determinants.

Another goal of the present study was to identify neuroanatomical measures that significantly improve the predictability of DD compared to a prediction model solely based on behavioral precursors. The observed results revealed that only the model considering neuroanatomical predictors was able to classify participants into dyslexics and non-impaired individuals, while the model including only the behavioral predictors was not. The model including neuroanatomical and behavioral predictors revealed a prediction accuracy of 80%, while the prediction based only on behavioral precursors reached a prediction accuracy of 63%. Until now, the main focus of the literature about the predictability of DD has been on behavioral precursors. The identified behavioral precursor abilities, however, could not provide sufficient specificity with regard to separation of individuals with and without DD (Steinbrink et al., 2010; Marx and Weber, 2006). The result of the multifactorial approach introduced here shows that the prediction of DD can be significantly improved by considering neuroanatomical measures. This result is in line with a previous study reporting high prediction accuracy ($>90\%$) for long-term reading abilities in school children when considering brain measures in contrast to a model including only behavioral measures (Hoeft et al., 2011).

5. Limitations

It should be noted that our sample size is relatively small and future studies with larger sample sizes should be warranted to investigate the robustness of our results. The objective of this study was to trace connections from regions where the children with a family risk of DD showed reduced CortT to subregions of Broca's area that are relevant for the development of literacy. Thus, it might be that the tracts reconstructed in this study do not display the complete tracts as they were defined in adult anatomical atlases (e.g. Wakana et al., 2007). Given the recent report of tract specific lateralization differences in school children with DD based on the orientation-specific measure HMOA (Zhao et al., 2016), future studies should include HMOA to investigate such lateralization differences already in pre-school children. Moreover, our findings need to be corroborated by further measurement points in stages of advanced literacy.

6. Conclusion

The present longitudinal study goes beyond previous studies by demonstrating that biologically informative neuroanatomical profiles in children with a family risk are related to subsequent reading difficulties. Moreover, we provide evidence that these profiles can be used to optimize the distinction between children with and without DD. Solid white matter organization in the left anterior arcuate fascicle seems to play a pivotal role in typical literacy acquisition. Future studies employing multimodal quantitative MRI techniques are necessary to better characterize brain tissue microstructure of the developmental trajectories of DD.

Acknowledgments

This work was supported by the Max Planck Society and the Fraunhofer Society (Grant number M.FE.A.NEPF0001). RM was partly funded by EU through the 'HiMR' Marie Curie ITN (FP7-PEOPLE-2012-ITN-316716).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.neuroimage.2016.09.004.

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Study V

Skeide MA, Kraft I, Müller B, Schaadt G, Neef NE, Brauer J, Wilcke A, Kirsten H, Boltze J, Friederici AD (2016) *NRSN1* associated grey matter volume of the visual word form area reveals dyslexia before school. *Brain* 139:2792–2803.

doi:10.1093/brain/aww153 BRAIN 2016: Page 1 of 12 | 1

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NRSN1 associated grey matter volume of the visual word form area reveals dyslexia before school

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Literacy learning depends on the flexibility of the human brain to reconfigure itself in response to environmental influences. At the same time, literacy and disorders of literacy acquisition are heritable and thus to some degree genetically predetermined. Here we used a multivariate non-parametric genetic model to relate literacy-associated genetic variants to grey and white matter volumes derived by voxel-based morphometry in a cohort of 141 children. Subsequently, a sample of 34 children attending grades 4 to 8, and another sample of 20 children, longitudinally followed from kindergarten to first grade, were classified as dyslexics and controls using linear binary support vector machines. The *NRSN1*-associated grey matter volume of the 'visual word form area' achieved a classification accuracy of ~ 73% in literacy-experienced students and distinguished between later dyslexic individuals and controls with an accuracy of 75% at kindergarten age. These findings suggest that the cortical plasticity of a region vital for literacy might be genetically modulated, thereby potentially preconstraining literacy outcome. Accordingly, these results could pave the way for identifying and treating the most common learning disorder before it manifests itself in school.

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Keywords: dyslexia; visual word form area; NRSN1; imaging genetics; voxel-based morphometry

Abbreviations: SNP = single nucleotide polymorphism; VWFA = visual word form area

Received November 19, 2015. Revised April 13, 2016. Accepted May 8, 2016.

Introduction

The acquisition of reading and spelling skills requires thorough instruction and intensive training. As a consequence of this experience, an extended network of cortical areas is strongly reshaped. Functional specialization and structural transformation in the course of literacy acquisition have been demonstrated repeatedly for the left temporo-occipital cortex (Brem *et al.*, 2010; Dehaene *et al.*, 2010; Monzalvo *et al.*, 2012; Langer *et al.*, 2015), the left superior temporal cortex (Maurer *et al.*, 2007, 2009; Dehaene *et al.*, 2010; Monzalvo *et al.*, 2012; Linkersdorfer *et al.*, 2015), the left temporo-parietal cortex (Myers *et al.*, 2014; Linkersdorfer *et al.*, 2015), and the parieto-occipital cortex (Carreiras *et al.*, 2009). Unsurprisingly, the same regions are also affected in individuals suffering from developmental dyslexia (Paulesu *et al.*, 2001; Hoeft *et al.*, 2006, 2007; Blau *et al.*, 2010; Frye *et al.*, 2010; Lehongre *et al.*, 2011; Altarelli *et al.*, 2013; Finn *et al.*, 2014; Im *et al.*, 2016), a severe impairment of literacy acquisition considered as the most common learning disorder with a prevalence of 5 to 7% in the population (Peterson and Pennington, 2012; Moll *et al.*, 2014).

However, the variance in literacy achievement is not entirely explained by environmental factors. Instead, family studies have shown that there is also a heritable component (Hallgren, 1950; Bakwin, 1973; Gilger *et al.*, 1996; Harlaar *et al.*, 2005). By now, numerous single nucleotide polymorphisms (SNPs) on multiple genes and intergenic regions spanning several chromosomes have been found to be linked to literacy in association studies (see references provided in Supplementary Table 1). Moreover, there is MRI evidence that some of these SNPs are related to the haemodynamic functionality of dyslexia-relevant areas in left inferior frontal, superior temporal, and temporo-parietal cortices as well as their structural interconnections (Meda *et al.*, 2008; Cope *et al.*, 2012; Darki *et al.*, 2012; Pinel *et al.*, 2012; Wilcke *et al.*, 2012; Skeide *et al.*, 2015).

The goal of the present study was to identify potential relations between dyslexia candidate genes and brain macrostructure to investigate if such gene–brain association clusters can not only separate dyslexics from controls but also predict dyslexia before literacy onset. Dyslexia is defined here as significant difficulties in reading or spelling revealed by psychometric testing instead of by a formal diagnosis.

As a first step, we selected a set of 69 SNPs on 19 candidate genes that were previously reported to be linked with reading, spelling or other literacy-related language traits. To carry out a biologically valid analysis of the joint effects of SNPs located in the same gene, we set up a multi-locus model assessing gene-level associations with voxel-based morphometry measures in a sample of 141 children. Thereby, grey and white matter volume images were not reduced to predefined regions of interest to ensure an unbiased investigation of dyslexia risk gene effects on the whole brain. We were particularly interested in volumetric indices because of their suitability to capture potential dyslexia endophenotypes such as dendritic growth (Araki *et al.*, 2002), axonal growth (Araki *et al.*, 2002; Yue *et al.*, 2006), or dysregulated neuronal migration into cortical target layers (Galaburda and Kemper, 1979; Threlkeld *et al.*, 2007; Penagarikano *et al.*, 2011).

While it has been demonstrated that all genes analysed in this study are abundant as RNA in the brain (see the literature cited in Supplementary Table 1 and the developmental transcriptome database at http://www.brainspan.org/), the precise spatial distribution of their neural expression profiles is not yet fully explored. Accordingly, our hypothesis regarding the localization of the gene–brain association clusters was broad. We expected effects in multiple regions linked to literacy and dyslexia. For grey matter, these regions comprise the already mentioned left temporo-occipital cortex, the left superior temporal cortex, the left temporo-parietal cortex, the parieto-occipital cortex, and, additionally, the left inferior frontal cortex (Shaywitz *et al.*, 1998; Frye *et al.*, 2010; Boets *et al.*, 2013), the thalamus (Diaz *et al.*, 2012; Jednorog *et al.*, 2015), the cerebellum (Nicolson *et al.*, 1999), and the brainstem (Chandrasekaran *et al.*, 2009). For white matter, these regions comprise the local white matter next to the aforementioned grey matter areas (Klingberg *et al.*, 2000; Rimrodt *et al.*, 2010; Darki *et al.*, 2012) as well as the arcuate fasciculus (Vandermosten *et al.*, 2012; Yeatman *et al.*, 2012; Thiebaut de Schotten *et al.*, 2014) and the inferior fronto-occipital fasciculus (Vandermosten *et al.*, 2012).

As a second step, we used a linear binary support vector machine algorithm to classify children as dyslexic individuals and controls based on the genetically associated volumetric clusters. This subsample comprised two age groups. One group (the advanced literacy group) consisted of 34 participants that underwent MRI after at least 3 years of schooling between age 9 to 12 (grades 4 to 6) and were tested on average 1.7 years later, i.e. between age 10 to 14 (grades 4 to 8) for their reading and spelling skills to determine diagnostic status. The other group (the beginning literacy group) consisted of 20 participants, aged 5 to 6 years, from kindergartens not providing literacy instruction that underwent MRI at least 10 months before school entry to ensure that they had at best sporadic knowledge of letter-sound correspondences. The latter children were followed longitudinally to measure their reading and spelling performance at the end of the first grade. Individual measurement time points of the entire subsample are provided in Supplementary Table 2.

There is growing evidence that preliterate children at familial risk of dyslexia already show functional and structural alterations in temporo-parietal and temporo-occipital regions similar to those observed in diagnosed dyslexics (Raschle *et al.*, 2011, 2012; Hosseini *et al.*, 2013; Kraft *et al.*, 2015). Therefore, we predicted distinct morphometric signatures between dyslexic individuals and controls in the same cortical areas, not only in the literate brain at age 9 to 12 but also in the preliterate brain at age 5 to 6.

Table 1 Demographic information and psychometric performance of the cohort

	All	$Dys_{9\text{-}13}$ [a]	Con_{Match} [b]	Δ [c]	$Dys_{5\text{-}6}$ [d]	Con_{Match} [b]	Δ [c]
n	141	17	17	-	10	10	-
Age[e]	6.4 ± 2.7 (3.0–12.2)	10.4 ± 0.6 (9.4–11.4)	10.6 ± 0.8 (9.2–12.2)	$z = 0.55$ $P = 0.586$	5.6 ± 0.4 (5.1–6.3)	5.8 ± 0.2 (5.4–6.1)	$z = 1.217$ $P = 0.247$
Gender[f]	57/84	4/13	8/9	-	4/6	6/4	-
Handedness[g]	127/6/8	16/1/0	16/0/1	-	9/1/0	9/0/1	-
Parental education[h,i]	15 ± 4 (5–24)	13 ± 4 (6–23)	16 ± 5 (5–24)	$z = 2.23$ $P = 0.026^*$	13 ± 4 (7–20)	15 ± 3 (10–19)	$F = 1.167$ $P = 0.294$
Non-verbal IQ[h]	105 ± 14 (66–139)	114 ± 7 (100–126)	114 ± 9 (86–125)	$z = 0.59$ $P = 0.563$	110 ± 15 (90–137)	111 ± 13 (96–135)	$F = 0.002$ $P = 0.961$
Reading comprehension[h,j]	-[k]	23.0 ± 16.0 (9.0–56.0)	59.3 ± 17.2 (33.0–94.0)	$F = 25.753$ $P < 0.001^*$	10.4 ± 6.7 (2.1–21.5)	71.7 ± 24.0 (29.8–99.0)	$z = 3.585$ $P < 0.001^*$
Reading speed[h,j]	-[k]	21.0 ± 8.4 (8.0–32.0)	56.6 ± 20.2 (27.0–81.0)	$z = 3.671$ $P < 0.001^*$	31.4 ± 18.4 (0.5–50.5)	82.4 ± 17.6 (48.0–96.0)	$F = 36.074$ $P < 0.001^*$
Spelling accuracy[h,j]	-[k]	25.5 ± 27.4 (0.0–82.0)	65.9 ± 23.1 (32.0–99.0)	$z = 3.451$ $P < 0.001^*$	17.9 ± 13.7 (5.0–49.0)	57.0 ± 22.3 (31.0–88.0)	$z = 3.293$ $P < 0.001^*$

Dys = dyslexic; Con = control.

[a] Dyslexic individuals MRI scanned between age 9–12 and psychometrically diagnosed between 10–14 years of age.

[b] Controls matched according to MRI scan age.

[c] Statistic and *P*-value of the compared variable (asterisks indicate significant differences). *F* indices were derived from one-way ANOVAs (data normally distributed). *z* indices were derived from Mann-Whitney U-tests (data not normally distributed).

[d] Dyslexic individuals were MRI scanned at 5–6 years of age and psychometrically diagnosed at 7–8 years of age.

[e] MRI scan age in years, mean ± SD (minimum–maximum).

[f] Female/male.

[g] Right handers/left handers/ambidextrous [according to customized Edinburgh Handedness Inventory (Oldfield, 1971) laterality quotient (LQ)] [left-handedness defined as $LQ < -28$, i.e. the first decile value; right-handedness defined as $LQ > 48$, i.e. the first decile value; ambidexterity: $-28 < LQ < +48$)].

[h] Mean ± SD (minimum–maximum).

[i] Questionnaire-derived, single cumulative score per participating child computed by adding the sum of 2 scores (one per parent) for school education (4-point scale; no degree: 1 point; German 'Abitur': 4 points) and the sum of 2 scores (one per parent) for further education (9-point scale; no degree: 1 point; German 'Habilitation': 9 points).

[j] Literacy data are presented as standardized scores (percentile ranks).

[k] Literacy data of the entire cohort are not provided, because they were unavailable for 67 participants and because 20 additional participants could not be matched to the dyslexic individuals and were therefore not included in the MRI classification analyses which require equal sample sizes.

Materials and methods

Participants

One hundred and forty-one children were tested as part of the LEGASCREEN project (www.legascreen.de). Detailed demographic information of this cohort can be found in Table 1. Participants were recruited mainly from the Leipzig metropolitan area but also from other parts of Germany through our homepage, newspaper announcements, magazine articles, a television documentary and talks in local schools and speech therapy centres. Families with a history of developmental dyslexia were particularly encouraged to participate. All parents completed a questionnaire revealing that no participant had a history of neurologically or psychiatrically relevant diseases. All children that met these two selection criteria, and of whom high-quality MRI scans could be taken, were included in the present study. All parents gave written informed consent while their children gave documented verbal assent to participate in the study. All experimental procedures were approved by the University of Leipzig Ethical Review Board.

Psychometric data acquisition

Non-verbal IQ

IQ scores of all children MRI-scanned at age 6 or below were determined using the performance subscale of the Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III) (Wechsler, 2009). IQ scores of all children MRI-scanned at age 9 or above were determined using the Kaufman Assessment Battery for Children (K-ABC) (Kaufman et al., 2003). For additional details, see Supplementary material.

Reading comprehension and reading speed

In children between grade 1 and 6, reading comprehension was tested with the 'Ein Leseverständnistest für Erst- bis Sechstklässler' (ELFE 1-6; translation: Reading comprehension test for grades 1 to 6) (Lenhard and Schneider, 2006) and reading speed was tested with the 'Weiterentwicklung des Salzburger Lese- und Rechtschreibtests' (SLRT-II; translation: Improved Salzburg reading and orthographic writing test) (Moll and Landerl, 2010). In children attending grade 7 or 8, both reading comprehension and reading speed were tested with the 'Lesegeschwindigkeits- und -verständnistest für die Klassen 6-12' (LGVT; translation: Reading speed and reading comprehension test for grades 6 to 12) (Schneider *et al.*, 2007).

Spelling accuracy

Performance in spelling (writing after dictation) was assessed with the grade-appropriate versions of the 'Deutscher Rechtschreibtest' (DERET; translation: German spelling test) (Stock and Schneider, 2008*a*, *b*).

Criterion for the diagnosis of developmental dyslexia

Following current German guidelines, we applied dual diagnostic criteria of developmental dyslexia. Individuals were categorized as being dyslexic if they scored equal to or below the 15th percentile rank of the population performance either in the reading comprehension, the reading speed, or the spelling accuracy test, given that their IQ was not more than 1 standard deviation (SD) below the population average (≥ 85). In addition, individuals were also categorized as being dyslexic if their score lay within the 25th percentile rank in one of the mentioned tests and was at least 1 SD below the level expected based on the child's IQ according to a regression criterion (Schulte-Korne, 2010). Our approach was more liberal than the clinical practice guideline as participants had to perform below threshold only in one but not in all of the tests in order to meet the diagnostic criterion. However, we consider this an appropriate compromise as our approach is still more conservative than a frequently used criterion requiring sub-25th percentile performance in a single subtest (Tanaka *et al.*, 2011; Finn *et al.*, 2014).

The inclusion of spelling as a sufficient criterion in the diagnosis of dyslexia might have inflated the proportion of cases in the beginning literacy group. However, in this subsample there was only 1 of 10 dyslexic individuals that only had spelling accuracy deficits but neither reading comprehension nor reading speed deficits. Accordingly, the risk of having identified a false-positive case seems to be limited to a single participant. Nine of ten dyslexic individuals in the beginning literacy group showed reading comprehension deficits, which seems to be the primary sign of dyslexia after the first year of school instruction. It remains open, if spelling accuracy deficits alone represent a reliable diagnostic criterion of dyslexia in German first graders.

Literacy achievement data were only available for 74 of 141 participants. First, 27 participants (17 individuals of the advanced literacy group and 10 individuals of the beginning literacy group) were identified as dyslexics. An equal number of 27 control participants scoring above threshold was selected from the remaining sample of 47 participants for which literacy data were available (advanced literacy group: 20 participants; beginning literacy group: 27 participants) to best match the cases in terms of age, gender, handedness, IQ, and parent education. Group differences according to these variables yielded a significance threshold of $P > 0.2$ with the exception of parental education in the older group (Table 1).

Genotyping

Genotypic information was collected for 69 SNPs documented in the literature as significantly associated with reading, spelling, phonological processing, articulation, and vocabulary (Supplementary Table 1). DNA was extracted from saliva applying standard procedures (Quinque *et al.*, 2006) or using Oragene DNA Genotek Kits (Kanata). Two different techniques were used. Initially, 59 SNPs were genotyped with the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry system iPLEX® (Agena). This set was complemented with eight SNPs from the bead chip Infinium HumanCoreExome Psych Chip. The bead chip genotyping was performed according to the manufacturer's protocols and data were processed using the GenomeStudio Genotyping Module (Illumina). Two SNPs not covered by the iPLEX® technique or by the bead chip were replaced with proxy SNPs revealing the largest linkage to the original candidate (Supplementary Table 1). Only SNPs with a minor allele frequency (MAF) >0.10 and a call-rate $>97\%$ were included for analysis. SNPs were not allowed to violate Hardy-Weinberg-Equilibrium (HWE) ($P > 0.05$, family-wise error corrected) and each genotype had to be present in at least five individuals. Individuals with a call-rate $<95\%$ were excluded and non-relatedness among all individuals was ensured by principal component analyses of the kinship (identity by state) measures between the participants. The maximum accepted identity by state was set to 0.125. All quality control parameters were estimated using GenABEL (Aulchenko *et al.*, 2007) and R (http://www.r-project.org/). The final set of 69 SNPs on 19 genes covering eight chromosomes together with the corresponding numbers of participants in each genotypic category and the MAF are listed in Supplementary Table 1.

Voxel-based morphometry analysis

The T_1 images acquired in the present study (see Supplementary material for details) passed a two-stage quality assessment procedure. As a first step, each image was visually inspected for coarse artefacts. As a second step, the exact covariance between all volume images was calculated to include only those images with a covariance coefficient within 2 SD of the mean.

For the voxel-based morphometry analysis, we used version 8 of the Voxel-based Morphometry Toolbox (http://dbm.neuro.uni-jena.de/vbm.html) implemented in the Statistical Parametric Mapping 8 software (http://fil.ion.ucl.ac.uk/spm/). First, the images were normalized to an age-specific template in Montreal Neurological Institute (MNI) space that was directly derived from the sample by employing the Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) algorithm (Ashburner, 2007). Second, the images were segmented into grey matter, white matter, CSF, dura, non-brain soft tissue and air. Tissue probability maps used as priors for the segmentation were customized using the Template-O-Matic Toolbox Version 8 (https://irc.cchmc.org/software/tom/downloads.php) to reflect age and gender of the present sample. As the toolbox only provides reference data in the age range of 5–18 years, all 38 children from our sample that are below this age were treated as 5-year-old children. Tissue probability maps of 5-year-old children are considered as sufficiently reliable priors for the segmentation of 3-year-olds' brain images. Despite the proven superior brain tissue differentiation of MP2RAGE scans compared to conventional MPRAGE scans (Marques *et al.*, 2010), we applied a tissue probability threshold of 0.15, which is slightly more conservative than the commonly used threshold of 0.1. The rationale behind this choice was to minimize the risk of voxel misclassification at tissue boundaries while at the same time keeping as many voxels as possible in the analysis. Grey matter and white matter maps were modulated for non-linear effects to preserve local volumetric values while accounting for individual differences in total intracranial volume. Finally, the images were smoothed with an 8 mm full-width at half-maximum Gaussian kernel.

Cluster-wise gene association analysis

Relations between genes and volume images were explored as previously described (Ge *et al.*, 2012). In short, the approach

combines cluster-wise statistical inference within brain images based on the random field theory with a multivariate non-parametric genetic model based on least-squares kernel machines. *P*-values are estimated accurately with a time-efficient permutation procedure based on parametric tail approximation. The method ensures statistical validity as it models the nonlinearity of SNP effects with high sensitivity and as it is robust to missing SNP data. A multi-locus model was set up for each brain tissue class to test for the joint effect of SNPs in a gene. Age, gender, handedness and parental education were included as covariates of no interest in the model.

Clusters were defined as connected voxels sharing at least a corner (i.e. 26 voxels) and assessed for significance by applying a 3-step multiple comparison correction procedure: First, a type I error threshold was set to $P < 0.001$. Moreover, the sizes of the remaining clusters were adjusted according to the local smoothness of the data to avoid potential type I and type II errors caused by non-stationarity, i.e. non-isotropic smoothness, of the volumetric images (Worsley *et al.*, 1999). Second, spatial extent thresholds at $P < 0.001$ were obtained running 10 000 iterations of a Monte Carlo Simulation as implemented in the AlphaSim tool (http://afni.nimh.nih.gov/). This procedure revealed minimum cluster size cut-offs of $k = 322$ voxels (for 449 972 grey matter voxels) and $k = 249$ (for 212 870 white matter voxels). Finally, a family-wise error correction for the 19 tested genes was performed. We favoured a cluster-based over a voxel-wise approach because the latter is agnostic to any spatial correlation between voxels and thus might decrease power to detect regions that show valid effects. Images were visualized using the Mango toolbox (http://ric.uthscsa.edu/mango/). The anatomical labels, sizes, MNI coordinates and maximum *P*-values of all surviving clusters can be found in Supplementary Tables 3 and 4.

Multivariate pattern classification analysis

The Pattern Recognition for Neuroimaging Toolbox (http://www.mlnl.cs.ucl.ac.uk/pronto) was used to classify a subsample of 54 children (34 participants that underwent MRI between 9 to 12 years of age and 20 participants that underwent MRI between 5 to 6 years of age) into dyslexic individuals and controls using the nine volume clusters that showed a significant association with a gene. Nine separate classifiers were trained with a linear binary support vector machine, one on each of the nine regions of interest (i.e. the images containing all in-mask volume-labelled voxels for each participant). We applied a 10-fold cross-validation, so that each classifier was first trained on a random subset of 90% of the images and then tested for its performance on the remaining 10% of the images. All images were mean-centred during cross-validation.

Post hoc, we tested to which degree the individual volumetric profile within a region of interest is related to the individual level of parental education. For this purpose, a kernel ridge regression analysis was carried out on a random subset of 90% of the mean-centred region of interest images before assessing the accuracy on the remaining 10%. The same approach was applied to the grey matter volume images when testing for the region of interest- and whole-brain level correlates of literacy skills, i.e. percentile ranks of reading comprehension, reading speed, and spelling accuracy.

For all statistical models, *P*-values were determined non-parametrically via permutation tests iteratively running 10 000 permutations. The *P*-values obtained from the classification were family-wise error corrected for all region of interest-wise tests within each modality (separately for grey matter and white matter volume). The *P*-values obtained from the regression analyses were family-wise error corrected for the three types of literacy skills tested.

Tests of associations between dyslexia candidate genes and literacy skills

To investigate the joint effects of all SNPs of each individual gene on the individual dyslexia diagnosis and on literacy skills, respectively, we constructed two models (separately for each gene). The first model only included the intercept and the covariates parental education and age. The second model included the same covariates and, in addition, all SNPs of each gene of interest. Finally, both models were compared using a likelihood ratio test to capture the additional effects of the SNPs using the package 'testing linear regression models' implemented in the R software (https://cran.r-project.org/web/packages/lmtest/). All *P*-values derived from these analyses were family-wise error corrected for the number of genes tested.

Results

Dyslexia candidate gene associations with grey and white matter volume

Significant associations with grey matter volume at a threshold of $P < 0.001$ (corrected) were found for 3 of 19 genes, namely *NRSN1*, *FOXP2*, and *COL4A2*. Effects of *NRSN1* were distributed over three clusters located in the right dorsal parieto-occipital cortex (MNI coordinates: 42, −18, 53 / 35, −71, 41 / 29, −48, 57), the left lateral occipital cortex (−9, −83, 42) and the left temporo-occipital fusiform cortex (−33, −63, −18), also known as the 'visual word form area' (VWFA). *FOXP2* showed an association in the left medial superior frontal gyrus (−3, 38, 53). *COL4A2* was found to be related to a cluster in the right cerebellum (17, −77, −54) (Fig. 1A, B, and Supplementary Fig. 1).

NRSN1, *CNTNAP2* and *CMIP*, i.e. 3 of the 19 genes, revealed significant associations with white matter volume ($P < 0.001$, corrected). *NRSN1* was related to a cluster in the local white matter of the left postcentral cortex (−45, −23, 60), *CNTNAP2* was related to the left cerebral and cerebellar peduncles (−20, −27, −8 / −11, −41, −45), and *CMIP* was related to bilateral portions of cerebellar white matter (−9, −83, 42 / 32, −68, −36) (Fig. 1C, D and Supplementary Fig. 2).

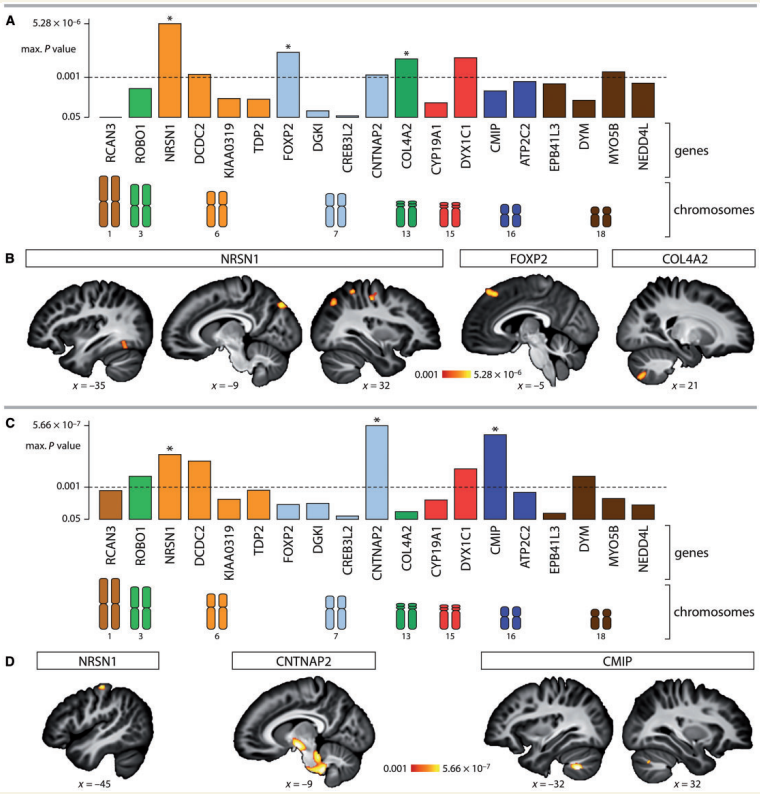

Figure 1 Associations of dyslexia candidate genes with grey and white matter volume. The *x*-axes of the diagrams depict 19 candidate genes colour-coded for the eight chromosomes they belong to. These genes are known from the literature to be significantly associated with reading, spelling or other language-related behavioural traits. The *y*-axis in **A** represents the maximum *P*-values of the associations between the genes and the grey matter volume images. The *y*-axis in **C** represents the maximum *P*-values of the associations between the genes and the white matter volume images. The dashed line represents the corrected threshold of $P = 0.001$. Asterisks indicate genetically associated clusters that remain significant after correction for type I error, spatial extent and number of genes tested. Note that bars crossing the dashed line without receiving an asterisk only passed the type I error but not the spatial extent correction and thus were not considered significant. (**B**) *P*-value images showing all grey matter volume clusters that revealed a significant association with the genetic variants *NRSN1*, *FOXP2* and *COL4A2*. (**D**) *P*-value images showing all white matter volume clusters that revealed a significant association with the genetic variants *NRSN1*, *CNTNAP2* and *CMIP*. The letter 'x' indicates the MNI coordinate of the sagittal cut plane. The colour bars indicate the range of the *P*-values. All presented *P*-values are corrected for multiple testing. The exact *P*-values of the most significant voxels within the clusters as well as the *P*-value images from transversal and coronal perspectives can be found in Supplementary Figs 1 and 2.

The models were adjusted for the effects of age and total intracranial volume to capture specific genetic associations with volumetric profiles independent of general maturational trajectories. Furthermore, the effect of parental education was also removed from the data to focus on gene–brain associations that are not mediated by factors reflecting literacy-related experiences in the early home environment. Cluster sizes and exact *P*-values are listed in Supplementary Tables 3 and 4.

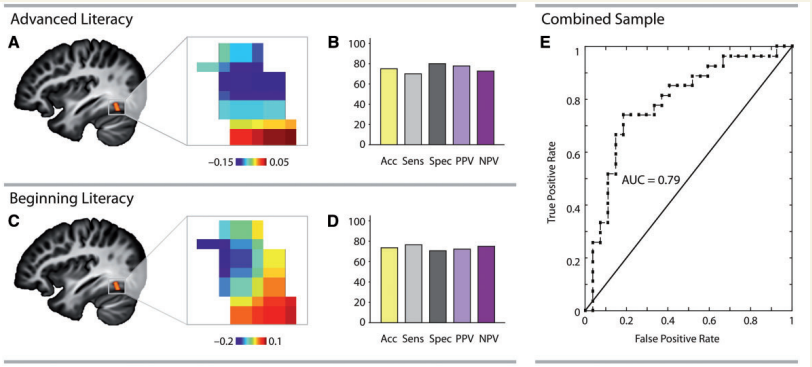

Figure 2 Classification of dyslexics and controls with the genetically associated grey matter volume profile of the visual word form area. Binary support vector machine classification weight maps are presented separately for two subsamples. (**A**) Thirty-four children in a stage of advanced literacy, MRI-scanned and psychometrically diagnosed for dyslexia in grades 4 to 7 (17 dyslexics versus 17 controls). (**C**) Twenty children in a beginning stage of literacy, MRI-scanned at a kindergarten age before literacy instruction and psychometrically diagnosed for dyslexia at the end of first grade (10 dyslexics versus 10 controls). The colour bars indicate the range of classification weights. Five classification indices are displayed separately on the *x*-axis for **B** the advanced literacy group, and for **D** the beginning literacy group (Acc = total classification accuracy; Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value). The *y*-axis displays the classification performance (0 to 100%). All classification indices for all regions of interest including the remaining eight grey and white matter volume clusters are provided in Supplementary Figs 3 and 4. (**E**) Receiver operating characteristic curve illustrating the performance of the classifier in the combined sample of 27 dyslexic participants and 27 controls. The *y*-axis represents the true positive rate, i.e. the rate of individuals that were correctly identified as cases, and the *x*-axis represents the false positive rate, i.e. the rate of individuals that were correctly identified as controls. The overall performance of the classifier is quantified as the area under the receiver operating characteristic curve (AUC).

Classification of dyslexics and controls with the genetically associated volume profiles

Initially, case-control classification performance was tested separately for each of the significant clusters derived from the association analysis in the sample of 9- to 12-year-old children. Of the five grey matter and four white matter volume clusters, only the grey matter volume cluster located in the VWFA performed significantly above chance classifying the participants into dyslexic and control individuals (total classification accuracy: 73.53%, $P = 0.031$, corrected) (Fig. 2A and B). Subsequently, this analysis was also carried out in the sample of 5- to 6-year-old children to evaluate if their structural brain data at a preliterate age had the distinctive power to identify young dyslexics and controls after one school year of literacy instruction. Again, the classification performance of the grey matter volume cluster located in the VWFA reached significance (total classification accuracy: 75%, $P = 0.035$, corrected) (Fig. 2C and D) in addition to the grey matter volume cluster located in the left lateral parieto-occipital cortex (total classification accuracy: 80%, $P = 0.028$, corrected). The detailed classification performance of all clusters can be found in Supplementary Figs 3 and 4. In the combined sample, the classifier trained on the VWFA region of interest distinguished cases from controls with a true positive rate of 0.74 and a false positive rate of 0.81, revealing an area under the receiver operator characteristic curve of 0.79 (Fig. 2E). Moreover, a kernel ridge regression within the region of interest revealed a significant association with reading comprehension ($R^2 = 0.07$, $P = 0.025$, corrected) and reading speed ($R^2 = 0.06$, $P = 0.047$, corrected), but not with spelling accuracy ($R^2 = 0.01$, $P = 0.480$, corrected).

Finally, as it was not possible to match dyslexics and controls in the advanced literacy group with respect to parental education, we evaluated to which degree this variable was related to the volumetric profile of the VWFA. However, a kernel ridge regression within the VWFA failed to detect any volumetric spatial pattern that explained variance in parental education, both in the advanced literacy group ($R^2 = 0.00$, $P = 0.904$) and in the beginning literacy group ($R^2 = 0.00$, $P = 0.981$).

Whole-brain grey matter volume correlates of individual literacy skills

We also aimed to identify brain areas related to individual variation in literacy skills independent of the genetic

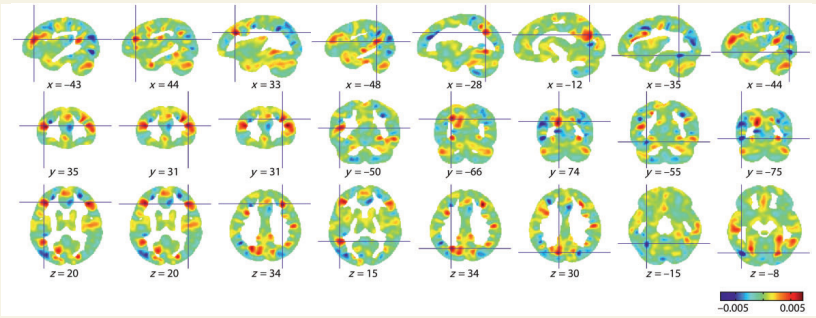

Figure 3 Whole-brain grey matter volume correlates of individual reading speed. Depicted are kernel ridge regression weight maps with cross-hairs over regions where weights exceeded the threshold of −0.005 (blue) or 0.005 (red). The colour bar indicates the range of the regression weights. The letters *x*, *y* and *z* specify the MNI coordinates of the sagittal, coronal and transversal cut planes, respectively. Non-significant grey matter volume associations with individual reading comprehension performance and with individual spelling accuracy are provided in Supplementary Fig. 5.

variants tested. Therefore, we ran whole-brain-level kernel ridge regression analyses to determine to which degree each voxel in each grey matter volume image is related to each of the 54 participants' performance in the reading comprehension, reading speed, and spelling accuracy tests, respectively. A significant association was found for reading speed ($R^2 = 0.15$, $P = 0.035$, corrected). Voxels with the highest positive relative weight were located in the bilateral middle frontal gyri (−43, 35, 20 / 44, 31, 20 / 33, 31, 34), the left posterior superior temporal gyrus (−48, −50, 15), and the left parieto-occipital cortex (−28, −66, 34 / −12, 74, 30). Clusters in the VWFA (−35, −55, −15) and the left visual cortex (−44, −75, −8) revealed the highest negative weights (Fig. 3). No significant whole-brain-level associations were found for reading comprehension ($R^2 = 0.03$, $P = 0.2916$, corrected) and spelling accuracy ($R^2 = 0.05$, $P = 0.2715$, corrected) (Supplementary Fig. 5).

Associations between dyslexia candidate genes and individual literacy skills

To further support the current evidence from the literature that the SNPs rs9356928, rs4285310 and rs3178 on *NRSN1* are related to dyslexia, we computed their joint association with the individual diagnostic status (dyslexic versus control) of each participant. This effect reached statistical significance [$\chi(3) = 8.18$, $P = 0.042$].

We also observed significant associations between the individual reading comprehension performance and the genes *NRSN1* [$\chi(3) = 14.54$, $P = 0.002$], *KIAA0319* [$\chi(3) = 7.86$, $P = 0.049$], *CNTNAP2* [$\chi(6) = 13.77$, $P = 0.032$], and *CMIP* [$\chi(5) = 18.69$, $P = 0.002$]. Moreover, individual reading speed was significantly associated with *KIAA0319* [$\chi(3) = 9.77$, $P = 0.021$] and *TDP2* [$\chi(1) = 4.378$, $P = 0.036$]. The association between *NRSN1* and reading comprehension turned out to remain significant when family-wise error correcting for the number of all tested genes. Full results of all association tests are provided in Supplementary Table 5.

Discussion

Here, we investigated associations between 19 candidate genes reported to be linked to literacy skills and the relative volume of the grey and the white matter in a cohort of 141 children ranging from age 3 to 12. The genes *NRSN1*, *FOXP2*, and *COL4A2* turned out to be significantly related to grey matter regions known to support functions that play a role for literacy. The genes *NRSN1*, *CNTNAP2*, and *CMIP* were found to be significantly related to white matter regions known to be part of the structural network underlying literacy proficiency. Within a grey matter cluster in the VWFA that was significantly associated with *NRSN1*, we detected volumetric patterns that classified dyslexic individuals, defined as not being formally diagnosed, but as showing substantial difficulties in psychometric tests of reading or spelling, and control individuals with significant above-chance performance. These patterns were found in a sample of 17 dyslexics and 17 control subjects MRI-scanned at age 9 to 12 (grades 4 to 6) and assessed for literacy skills on average 1.7 years later at age 10 to 14 (grades 4 to 8), and, moreover, in a sample of 10 dyslexics and 10 control subjects MRI-scanned at age 5 to 6 (attending kindergarten) and assessed for literacy skills

at age 7 to 8 (end of the first grade). In the latter sample, an additional significantly classifying volumetric pattern was found in the left lateral occipital cortex that was also associated with *NRSN1*. All effects were statistically independent of the participants' age, gender and handedness, as well as the educational level of their parents. The grey matter volume of the VWFA was significantly associated with reading comprehension and reading speed, but not with spelling accuracy. A significant association with reading speed, but neither with reading comprehension nor with spelling accuracy, was also found at the whole-brain level in several grey matter regions known to support reading acquisition. In line with previous evidence from the literature (Deffenbacher *et al.*, 2004; Couto *et al.*, 2010), *NRSN1* was significantly related to the individual dyslexia diagnosis and the individual reading comprehension skills. Further significant associations with reading comprehension were observed for the genes *KIAA0319*, *CNTNAP2* and *CMIP*. Reading speed was significantly related to *KIAA0319* and *TDP2*.

Our observation that *NRSN1* was related to both grey and white matter volume is corroborated by *in vitro* evidence indicating that the protein encoded by this gene is involved in neurite extension by transporting vesicles to the growing ends of dendrites and axons (Araki *et al.*, 2002). A similar role during early brain development is also ascribed to *FOXP2* (Vernes *et al.*, 2011), which revealed an association with grey matter volume in the present study. The neuromolecular mechanisms regulated by *COL4A2* and its potential link to the grey matter, however, are currently unclear and require further investigation (Verbeek *et al.*, 2012). Supporting previous studies, the relation between *CNTNAP2* and white matter volume revealed by our study is in line with the finding that its protein product contributes to the clustering of potassium channels at juxtaparanodes of axons which is vital for intact neuronal signalling (Rodenas-Cuadrado *et al.*, 2014). Finally, *CMIP* was also associated with white matter volume, but its microstructural functions in the maturing brain are not yet uncovered (Wang *et al.*, 2015).

The neurobiological validity of the genetic association clusters with respect to the transcriptome of the dyslexic brain might be best evaluated on the basis of future post-mortem work shedding light on the neural expression landscape of the dyslexia candidate genes. Nevertheless, all effects were localized in brain areas that have been linked to literacy or dyslexia in previous studies. The VWFA is part of the ventral visual stream that becomes increasingly sensitive to print when reading and spelling is learnt (Dehaene *et al.*, 2015). A dorsal functional network including parieto-occipital and superior frontal cortices is assumed to influence how well readers can allocate top-down attentional resources to the visual discrimination of letters (Finn *et al.*, 2014). The cerebellum and also pre- and postcentral cortices are thought to support the automatization of both explicitly and implicitly learned skills, which is crucial for fluent reading and spelling (Nicolson *et al.*, 1999; Menghini *et al.*, 2006, 2008). There is evidence that the auditory brainstem plays a role for encoding basic acoustic features of speech sounds and thus affects the quality of phonological representations (Chandrasekaran *et al.*, 2009). The left cerebellar peduncle could be a pathway over which basic acoustic information is propagated from the brainstem to the thalamus for further acoustic processing. This hypothesis should be tested in follow-up studies. Finally, the relation of the left cerebral peduncle to *CNTNAP2* and its possible contribution to literacy also remains to be further assessed.

It has been argued that it is almost impossible to isolate structural brain changes underlying childhood literacy acquisition owing to unspecific maturational changes and uncontrollable environmental differences (Carreiras *et al.*, 2009). Indeed, existing data on volumetric differences between dyslexic and control individuals have been ambiguous so far. On the one hand, there are studies suggesting that higher grey matter volume in temporo-parietal and temporo-occipital regions relate to higher literacy skills (Silani *et al.*, 2005; Hoeft *et al.*, 2007). On the other hand, there are studies suggesting that lower grey matter volume in temporo-parietal regions relate to higher literacy skills (Darki *et al.*, 2012, 2014). Here we resolved this ambiguity by sidestepping this dichotomy and accommodating the possibility that cortical disparities between dyslexics and controls might be averaged out when simply testing for mean differences between them. Instead, we argue that the disparities follow rather complex spatial distributions that are more adequately represented by volumetric patterns. The specificity of our results is additionally bolstered by the fact that we removed the effects of total intracranial volume and age in our models to account for interindividual and age-related differences in maturation, particularly synaptic pruning.

Our knowledge about the role of the environment in literacy acquisition is still limited. Nevertheless, there is evidence that genetic contributions to dyslexia increase while environmental contributions decrease the higher the level of parental education (Friend *et al.*, 2008). It is assumed that the educational level of parents is related to the language and literacy skills of their children. These skills are in turn considered protective factors of dyslexia for children in the home literacy environment (Lyytinen *et al.*, 2004; Peterson and Pennington, 2012; van der Leij *et al.*, 2013). Importantly, we ruled out the plausible possibility that the effect of *NRSN1* on the VWFA could be explained by parental education as we controlled for this factor in our models. Furthermore, there was no indication that the case–control classification performance of the VWFA could have been blurred by latent variables related to parental education. It is certain that the present study does not allow us to estimate how much variance in literacy phenotypes can be explained by genetic relations to the VWFA compared to environmental influences on the VWFA. Nevertheless, it allows us to reason that the association of *NRSN1* with the VWFA specifically explains a certain portion of variance in dyslexia independently of parental education, the variable that best reflects the most proximate

environmental mediator currently known. An exact quantification of the unique and shared contributions of genetic and environmental factors to the literate brain remains as a major future challenge for the field.

We acknowledge that the regions affected in dyslexic individuals might also vary as a function of their cognitive deficits (Heim and Grande, 2012) and of their age. The latter aspect is supported by our observation that a pattern in the left lateral parieto-occipital cortex significantly separated dyslexics from controls before literacy instruction but not after at least 3 years of schooling. At the same time, we emphasize that our results provide evidence for an endophenotypic continuum of *NRSN1* polymorphisms in relation to volumetric features of the VWFA. Further experiments are needed to corroborate the view that fluctuating and stable endophenotypes co-occur in developmental dyslexia.

Limitations

It should be noted that our sample of 141 participants is considered small for a genetic association analysis. Moreover, the current study does not include an external replication sample. The feasibility of larger follow-up analyses depends on the possibility to combine the data of our cohort with data from other cohorts from populations that are comparable in terms of orthographic transparency and genetic homogeneity. Furthermore, it should be acknowledged that the original *NRSN1*-related SNP rs4285310 was not covered by the genotyping techniques used and therefore replaced by the proxy SNP rs10946673 in the present analyses (Supplementary Table 1).

To provide a reliable literacy assessment after only 1 year of schooling, we made sure that all participants were familiar with the core German alphabet and fully understood all tasks (Supplementary material). Moreover, the reliability of the diagnostic categorization is bolstered by the fact that the classification of dyslexics and controls with the genetically associated volume profiles revealed a comparable performance in the first-graders compared to an independent sample of children with more advanced literacy skills. Nevertheless, we acknowledge that there are potential other sources that might decrease the reliability of diagnosing dyslexia after the first school year.

Conclusion

The present study sheds new light on the interplay of 'nature and nurture' during literacy acquisition. Justifiably, the VWFA is a prime example of how learning-induced cortical plasticity leads to an expansion of the human cognitive repertoire. Here, we have shown, however, that there seems to be a genetic limit to the adaptivity of this region to literacy-related skills. The grey matter volume of the VWFA was found to be related to *NRSN1*, a gene assumed to regulate neurite growth from early maturation stages on. Moreover, the *NRSN1*-associated cluster in the VWFA robustly distinguished dyslexics and controls not only after several years of schooling, but also already at a kindergarten age before literacy instruction had actually begun. There was no indication that these effects could have been mediated by environmental influences reflecting parental education levels. Nevertheless, the genetic and environmental dynamics underlying the pivotal role of the VWFA for literacy acquisition require further investigation in large-scale future studies.

Funding

This work was funded by the Max Planck Society and the Fraunhofer Society (Grant number: M.FE.A.NEPF0001).

Supplementary material

Supplementary material is available at *Brain* online.

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Study VI

Skeide MA, Kirsten H, Kraft I, Schaadt G, Müller B, Brauer J, Wilcke A, Emmrich F, Boltze J, Friederici AD (2015) Genetic dyslexia risk variant is related to neural connectivity patterns underlying phonological awareness in children. *Neuroimage* 118:414–421.

NeuroImage 118 (2015) 414–421

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Genetic dyslexia risk variant is related to neural connectivity patterns underlying phonological awareness in children

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ARTICLE INFO

Article history:
Received 10 December 2014
Accepted 6 June 2015
Available online 12 June 2015

ABSTRACT

Phonological awareness is the best-validated predictor of reading and spelling skill and therefore highly relevant for developmental dyslexia. Prior imaging genetics studies link several dyslexia risk genes to either brain-functional or brain-structural factors of phonological deficits. However, coherent evidence for genetic associations with both functional and structural neural phenotypes underlying variation in phonological awareness has not yet been provided. Here we demonstrate that rs11100040, a reported modifier of SLC2A3, is related to the functional connectivity of left fronto-temporal phonological processing areas at resting state in a sample of 9- to 12-year-old children. Furthermore, we provide evidence that rs11100040 is related to the fractional anisotropy of the arcuate fasciculus, which forms the structural connection between these areas. This structural connectivity phenotype is associated with phonological awareness, which is in turn associated with the individual retrospective risk scores in an early dyslexia screening as well as to spelling. These results suggest a link between a dyslexia risk genotype and a functional as well as a structural neural phenotype, which is associated with a phonological awareness phenotype. The present study goes beyond previous work by integrating genetic, brain-functional and brain-structural aspects of phonological awareness within a single approach. These combined findings might be another step towards a multimodal biomarker for developmental dyslexia.

Introduction

Phonological awareness is the central precursor skill of reading and spelling and thus, the best predictor of the word recognition difficulties that characterize developmental dyslexia, one of the most common learning disorders (Gabrieli, 2009; Peterson and Pennington, 2012).

It is assumed that phonological deficits strongly depend on genetic factors, but a link between the best validated dyslexia risk genes, *KIAA0319*, *DCDC2* and *DYX1C1*, and phonological awareness, which takes intermediate neural phenotypes into account has not been established yet (Galaburda et al., 2006; Giraud and Ramus, 2013). In a recent genome-wide screening, however, two variants on chromosome 4 affecting expression levels of *SLC2A3* were found to be specifically associated with a late left-lateralized auditory mismatch negativity (MMN) component peaking around 300 to 600 ms (Roeske et al., 2011) that is considered a specific functional neural marker of phonological processing deficits (Korpilahti et al., 1995; Stoodley et al., 2006).

The available neuroimaging literature provides converging evidence that difficulties in phonological processing are characterized by a reduced hemodynamic reactivity and functional connectivity of left superior temporal, inferior parietal and inferior frontal cortices (Boets et al., 2013; Koyama et al., 2011; Raschle et al., 2012). These functional differences are corroborated by structural findings in the same regions indicating an altered gray matter morphometry (Silani et al., 2005) and white matter fractional anisotropy (FA) (Klingberg et al., 2000). Furthermore, the left arcuate fasciculus, which forms the connection of these cortical areas, was also linked to phonological processing (Vandermosten et al., 2012a; Saygin et al., 2013; Myers et al., 2014).

The picture emerging from the literature is that variation in phonological processing skills is based on brain-structural and brain-functional factors, which in turn depend on genetic factors (Peterson and Pennington, 2012). However, although a few studies revealed relations between subsets of these factors (Darki et al., 2012, 2014; Pinel et al., 2012), no experiment so far has succeeded in integrating all explanatory levels within a single approach. To provide an integrative and comprehensive analysis

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http://dx.doi.org/10.1016/j.neuroimage.2015.06.024
1053-8119/

of normal variation in phonological awareness, we genotyped 34 children aged 9 to 12 years for two single nucleotide polymorphisms (SNPs) acting on the gene *SLC2A3*, which is known to regulate neural glucose transport (Maher et al., 1994; Maher and Simpson, 1994; McCall et al., 1994). Furthermore, they underwent functional and structural MRI and were tested behaviorally for their phonological awareness as well as reading and spelling skills. Additionally, results of a dyslexia screening conducted when the participants were 5 to 6 years of age were available. The children were separately grouped either as carriers or as non-carriers of the two dyslexia risk variants rs11100040 and rs4234898 decreasing the expression of *SLC2A3* in individuals that have at least one risk (T) allele. These two particular SNPs were selected because they are currently the only variants that were specifically linked to phonological processing at the genome wide level (Roeske et al., 2011).

It is considered highly informative to explore the influence of genetic risk variants on a normal population, but not dyslexics since the phenotype of interest, i.e. phonological awareness, can be reliably related to dyslexia as previously shown (Saygin et al., 2013; Myers et al., 2014). An imaging genetics approach was taken in the present study because the effect size of genetic variants on intermediate neural phenotypes is known to be higher compared to behavioral phenotypes. This increases the power to detect statistically significant effects in small samples (Meyer-Lindenberg and Weinberger, 2006; Mier et al., 2010). As a first step, we investigated if the children's genetic risk profiles were related to their resting state functional connectivity profiles. Second, we tested the hypothesis that there was an association between the children's genetic risk profile and their structural connectivity profiles. Third, we hypothesized that the individual functional and structural connectivity indices were correlated with the individual performance in the phonological awareness test. Finally, we expected associations between phonological awareness, the retrospective dyslexia screening and the reading and spelling tests. The results section is organized relative to the order of these hypotheses.

Materials and methods

Participants

Data from 34 right-handed 9- to 12-year-old children, randomly selected from the cohort of the German Language Development Study (Friedrich and Friederici, 2004), were included in the final analyses (Table 1). Initially, the sample consisted of 36 children but datasets from two children were disregarded because they did not meet our MR data quality criterion (see below for details). All parents completed a questionnaire revealing that no participant had a history of neurologically relevant diseases. None of the children has been diagnosed with developmental dyslexia. Based on an informative briefing regarding study aims and methodology, parents were asked to give written informed consent while children gave documented verbal assent to participate in the study. All experimental procedures were approved by the University of Leipzig Ethical Review Board.

Genotyping

Genotyping and DNA extraction were carried out on saliva using standard procedures (Quinque et al., 2006) or using Oragene DNA Genotek saliva kits (Kanata, Ontario, Canada). The alternative protocol was performed according to the user-developed protocol of the DNeasy® Blood & Tissue Kit (Purification of total DNA from animal saliva using the DNeasy® Blood & Tissue Kit). The following changes were applied: Centrifugation in steps 6, 7 and 8 was executed with 15,700 times gravity. The final elution was completed with 100 μl AE buffer. At least 0.75 ml of saliva was collected per subject. Two SNPs, rs11100040 (T) and rs4234898 (T) (Roeske et al., 2011) (risk alleles in parentheses) were genotyped by the mass spectrometry based technique GenoSNIP (Bruker Daltonics, Bremen, Germany) as described elsewhere (Kirsten et al., 2007) with minor modifications. PCR primers (MWG-Biotech AG, Ebersberg, Germany) were:

5′-ACGTTGGATGAACAGTAAGGAAAATGACAGT-3′
and 5′-ACGTTGGATGGATGAAACACAGTTGTTTACA-3′
as well as 5′-ACGTTGGATGTGGATCCTACACCTACACA-3′
and 5′-ACGTTGGATGGTTTTCAGATTCTGCCAT-3′, respectively.
The sequence of the single base extension primer (Biotez, Berlin, Germany) was
5′-bioAACGTTTACATTTLATCACACTTTCTTA-3′
and 5′-bioTGTGTTCLCTGGCCTCTGGA-3′,
respectively, where "bio" is a biotin residue and L is a photo cleavable linker (Wenzel et al., 2003). Additionally, the variant rs11100040 was verified by genotyping using iPlex (Sequenom, Hamburg, Germany). No inconsistencies were found. Both SNPs had a call rate of 100% and did not violate Hardy–Weinberg equilibrium among all 34 individuals. Genetic risk was assigned according to the number of risk

Table 1
Demographic information, genotyping results and psychometric assessment results.

	Measure	N	Mean	Range
Demographic information	Age		10 years 05 months	09 years 00 months to 12 years 02 months
	Gender	20 male, 14 female		
Genotyping results	rs11100040	17 non-risk, 17 risk		
	rs4234898	26 non-risk, 8 risk		
Psychometric assessment results	IQ		111.22	86–126
	Speech therapy	23 not treated, 11 treated		
	Musical instrument instruction	18 not instructed, 16 instructed		
	Attention deficit disorder	29 without suspicion, 5 diagnosed		
	Phonological awareness test	22 non-risk, 12 risk[a]	49.47[c]	19–71[c]
	Reading test* (acquired at ages 11 to 14)	23 non-risk, 11 risk[a]	50.8[c,d]	27–79[c,d]
			48.15[c,e]	38–80[c,e]
	Spelling test	25 non-risk, 9 risk[a]	47.28[f]	3–99[f]
	Preschool dyslexia screening (acquired at ages 5 to 6)	23 non-risk, 11 risk[b]	2.59[g]	0–6[g]

[a] 25th percentile rank.
[b] 15th percentile rank.
[*] Available for 24/34 children.
[c] *T* values.
[d] Accuracy.
[e] Speed (number of words read in a time interval of 4 min).
[f] Percentile ranks.
[g] Risk score (1 risk point assigned when performance in a subtest below 15th percentile rank; 10 subtests; 4 or more risk points indicate at risk status).

alleles per SNP with '0' for 0 risk alleles and '1' for 1 or 2 risk alleles (Lewis, 2002) (Table 1).

MR data acquisition

MRI was conducted on a 3.0-T Siemens TIM Trio (Siemens AG) whole-body magnetic resonance scanner using a 12-radiofrequency-channel head coil.

For anatomical localization, T1-weighted three-dimensional magnetization-prepared rapid-acquisition gradient echo (MP2RAGE) pulse sequences with TR = 5.000 ms, TE = 2.82 ms, TI_1 = 700 ms, TI_2 = 2.500 ms, FOV = 256 × 240, matrix size = 250 × 219 × 144 and voxel size = 1.3 × 1.3 × 1.3 mm^3 were acquired.

For resting state fMRI, a T2*-weighted gradient-echo echo-planar imaging (EPI) sequence comprising 100 volumes was applied to the participants (closed eyes, no active stimulation) using 28 slices with TR = 2 s, TE = 30 ms, FOV = 192 mm, matrix size = 64 × 64 voxels and voxel size 3.0 × 3.0 × 3.0 mm^3.

Diffusion-weighted MR images were collected as a twice-refocused spin EPI sequence (Reese et al., 2003) with TE = 83 ms, TR = 8000 ms, matrix size = 100 × 100 voxels, voxel size = 1.9 × 1.9 × 1.9 mm^3, 66 axial slices covering the whole brain. We used 60 isotropically distributed diffusion-encoding gradient directions with a b-value = 1000 s/mm^2. Seven anatomical reference b0 images without diffusion weighting were acquired at the beginning of the sequence and after each block of 10 diffusion-weighted images for off-line motion correction. Fat saturation was applied together with 6/8 partial Fourier imaging and generalized auto-calibrating partially parallel acquisitions (GRAPPA) with an acceleration factor of 2 (Griswold et al., 2002). Random noise was reduced by averaging two acquisitions. All images were visually checked for motion artifacts (signal losses). As mentioned above, 2 out of 36 participants were removed from the study because they exceeded the cut-off quality criterion of maximum 5 head-motion-corrupted image directions in the entire dataset (Brauer et al., 2013). The final sample therefore consisted of 34 participants.

Resting state fMRI data analysis

The functional resting state images were slice-time-corrected, realigned, motion-corrected, normalized to a group-specific template, and spatially smoothed with a 4 mm FWHM Gaussian kernel using the DPARSF 2.3 software package (http://www.restfmri.net/forum/DPARSF). Framewise displacement was less than 0.5 mm in all participants. To control for head motion and nuisance, realignment parameters, global signals, white matter signals and cerebrospinal fluid signals were entered as regressors into the first-level model. Time courses of hemodynamic gray matter signals within a low-frequency range of 0.01 to 0.1 Hz were extracted from three seed regions (r = 6 mm) in MNI space including the left inferior frontal gyrus (IFG) (−51, 10, 10), left posterior superior temporal gyrus (pSTG) (−53, −31, 9), and left temporo-parietal junction (TPJ) (−59, −45, 15) using DPARSF 2.3 (http://rfmri.org/DPARSF). These areas were chosen as regions of interest (ROIs) as they have been found to support phonological processes during word reading in previous meta-analyses of event-related fMRI studies and as they have been used in a previous resting-state fMRI study (Koyama et al., 2011). Finally, individual Pearson's correlation coefficients of the BOLD time courses (mean $r = 0.41$, $SD = 0.2$, range: −0.11 to 0.77) were computed and then entered separately in 3 one-way ANOVAs (for rs11100040, equal group sizes) and 3 Mann–Whitney U tests (for rs4234898, unequal group sizes) to compare all combinations of ROI pairs. These statistical tests were carried out using PASW 18 (http://www.spss.com.hk/statistics/).

All ROI-wise seed based correlation analyses were adjusted for the effects of age, gender, IQ, speech therapy, musical instrument instruction, and attention deficit disorder (ADD) entering these variables as covariates into the models. The rationale for controlling for speech therapy and musical instrument instruction in these analyses was to avoid possible confounds introduced by these two environmental factors with respect to their potential to induce compensatory mechanisms altering brain structure and function and performance (Goswami et al., 2011). Attention deficits, a comorbidity of developmental dyslexia, were covaried out to account for possible indirect effects of attention on phonological processing, reading and writing (Kibby et al., 2009).

All parametric and nonparametric tests resulting in a $P < 0.0083$ which equals a Bonferroni corrected $P < 0.05$ (divided by 6 for the three ROI pairs analyzed for each of the two SNPs) were considered significant. The Pearson correlation coefficients were normally distributed in each of the two rs11100040 risk groups (0 risk alleles vs. 1 or 2 risk alleles) and the three corresponding ROI pairs according to Kolmogorov–Smirnov and Shapiro–Wilk tests (no risk: IFG-pSTG: $P = 0.2/0.923$; *IFG-TPJ: P = 0.2/0.183; pSTG-TPJ: P = 0.23/0.272*; risk: $P = 0.2/0.768$; *IFG-TPJ: P = 0.2/0.314; pSTG-TPJ: P = 0.2/0.684*). For the rs4234898 risk groups it was not necessary to assess these distributions since the data were passed to non-parametric Mann–Whitney U tests. These statistical tests were carried out using PASW 18 (http://www.spss.com.hk/statistics/).

DTI data analysis

Motion correction parameters for the diffusion-weighted images were combined with a global rigid-body registration (Jenkinson et al., 2002) to the individual skull-stripped T1-weighted structural image using the FSL linear image registration tool (flirt, http://www.fmrib.ox.ac.uk/fsl). The gradient direction for each volume was corrected with the rotation parameters (Leemans and Jones, 2009). In the registration process, the images were interpolated to an isotropic voxel resolution of 1 mm before the FA was computed. Note that the registration to the T1 anatomical image with 1 mm isotropic resolution was preferred over an analysis in the diffusion space to reduce smoothing artifacts introduced by several interpolation steps included in standard procedures and to reduce the smoothing bias to the different directions by registration to an independent image.

All single-subject FA images were then mutually aligned on each other by nonlinear registration to determine the anatomically most typical template image and all individual FA images were registered to this target image. Subsequently, all FA images averaged and skeletonized using the FSL tract based spatial statistics (TBSS) (Smith et al., 2006) toolbox. The skeleton was masked with an FA threshold of 0.25 which is slightly higher than the commonly used default threshold (0.2). It represented the best trade-off between reducing as much cross-subject variability as possible by disregarding peripheral branches of the skeleton while at the same time keeping as much information as possible.

Finally, the mean FA skeletons entered a voxel-wise one-way analysis of variance (ANOVA) separately for each SNP including age, gender, IQ, speech therapy, musical instrument instruction, and attention deficit disorder (ADD) as covariates for the reasons provided in the Resting State fMRI Data Analysis section. Cross-subject variance was estimated separately for each genetic risk group to account for unbalanced distributions of risk alleles across the sample potentially resulting in unequal variances. These analyses were carried out using FSL FEAT (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT).

We corrected for multiple comparisons within the entire FA skeleton on a cluster level based on the Gaussian random field theory (GRFT). In order to meet the assumptions of the GRFT with respect to the two-dimensionality of the white matter skeleton we used a threshold of $P < 0.01$ which delivers an optimal approximation to a quadratic representation of the cluster (Hagler et al., 2006). The minimum number of contiguous voxels showing a statistically significant difference at $P < 0.01$ was simulated by a Monte Carlo simulation taking into account the voxel resolution of 1 × 1 × 1 mm^3 and the intrinsic smoothness of

the data which was x = 1.37 mm, y = 1.78 mm and z = 1.59 mm. Clusters were considered significant if they exceeded a threshold of k = 17 controlling for the chance of ever reporting a false-positive finding to be less than 0.0025 which equals a Bonferroni corrected $P < 0.005$ (divided by 2 for the two single nucleotide polymorphisms under investigation).

Individual FA values were extracted from the cluster revealed by the analysis described in the previous passage (k = 36, MNI coordinates: −34, −16, 34). According to the ICBM-DTI-81 white matter labels atlas (Mori et al., 2008), 76% of this cluster was located in a subregion of the left superior longitudinal fasciculus which we visually identified as the arcuate fasciculus based on previous literature (Makris et al., 2005).

Psychometric assessment

We used the BAKO test (test for basal competences for reading and spelling abilities) to assess the children's phonological awareness (Stock et al., 2003). The BAKO comprises valid measures for phonological processing skills both at the phoneme level (phoneme categorization, phoneme deletion, phoneme permutation, vowel length assignment, and vowel replacement) and the word level (word inversion and pseudoword segmentation). Additionally, we tested the children's spelling skills using running text-dictations from the DERET (German spelling test) (Stock and Schneider, 2008). Retrospective data of an early dyslexia screening (BISC—Bielefeld screening for early recognition of reading and spelling deficits) (Jansen et al., 1999) acquired 10 months before school enrollment (5 to 6 years of age) were available for all participants. In an additional assessment 2 years later (age of the children: 11 to 14 years), reading performance was assessed using a reading accuracy and speed test (Schneider et al., 2007). *T* scores were used for further statistical analyses with the exception of the spelling test, for which only percentile ranks were available, and the dyslexia screening, for which only risk scores were available. Note that higher scores in the dyslexia screening indicate worse performance.

IQ scores were determined using the German version of the Kaufman Assessment Battery for Children (K-ABC) (Melchers and Preuss, 2009). Missing IQ values for seven children (four children at risk, three non-risk children) were imputed using multiple imputation as implemented in PASW 18 (http://www.spss.com.hk/statistics/). Based on a parental questionnaire, we assessed if the children had undergone speech therapy, if they had learned how to play a musical instrument, and if they had a medically diagnosed ADD (Table 2).

In order to relate the individual behavioral performance measures (see Table 1, Psychometric assessment results) to each other and to the brain-structural and brain-functional measures, respectively, we computed non-parametric partial Spearman's rho correlations removing the effects of age, gender, IQ, speech therapy, musical instrument instruction, and ADD for the reasons provided in the Resting State fMRI Data Analysis section. All *P* values for the direct and indirect correlation analyses between FA, the functional connectivity indices and phonological awareness were Bonferroni corrected for the four statistical tests conducted. Correlation coefficients were directly compared running Meng's *z* test (Meng et al., 1992).

Results

rs11100040 is associated with fronto-temporal functional connectivity at resting state

We compared pair-wise temporal correlations of low-frequency BOLD signal fluctuations in three ROIs, the left inferior frontal gyrus (IFG), the left posterior superior temporal gyrus (pSTG), and the left temporo-parietal junction (TPJ), which are known to support phonological processes during word reading (Koyama et al., 2011).

Children without any risk allele at rs11100040 ($n = 17$) showed significantly stronger temporal correlations of the BOLD signals induced at rest in the left IFG and left pSTG than children carrying at least one risk allele ($n = 17$) ($F_{1, 33} = 2.81$, $P < 0.05$, Bonferroni corrected) (Fig. 1). However, no significant effects could be detected between the left IFG and the left TPJ ($F_{1, 33} = 0.9$, $P = 0.524$) or the left pSTG and the left TPJ ($F_{1, 33} = 0.69$, $P = 0.682$). Children with a risk allele at rs4234898 ($n = 8$) did not differ significantly from children carrying at least one risk allele ($n = 26$) in all three pairs of ROIs (IFG-pSTG: $U = 67$, $P = 0.133$; IFG-TPJ: $U = 91$, $P = 0.618$; pSTG-TPJ: $U = 103$, $P = 0.968$).

rs11100040 is associated with the fractional anisotropy of the arcuate fasciculus

Given that rs11100040 was related to the functional resting state connectivity of fronto-temporal cortices involved in phonological processing, we hypothesized that the observed brain functional effect should be reflected in the fractional anisotropy of their structural white matter fiber connection via the arcuate fasciculus.

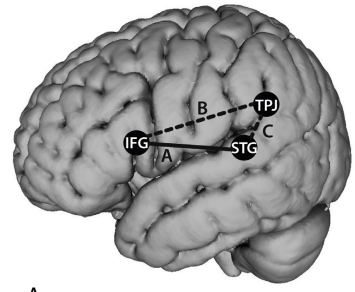
Tract-based spatial statistics revealed that children who carried at least one risk allele at the *SLC2A3* modifier rs11100040 had significantly reduced FA values in a cluster located in the left arcuate fasciculus

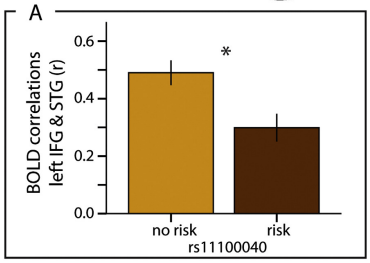
Table 2
Effects of potential confounders on analyzed variables.

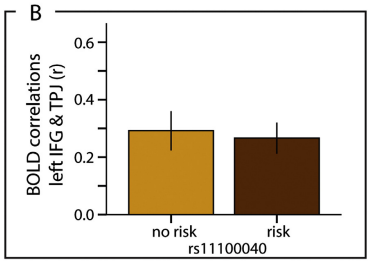
Variable	Potential confounder	*P*	Effect size (SE)	R^2
FA values of the arcuate fasciculus	Age	0.549	0.105 (0.173)	0.011
	Gender	0.964	0.008 (0.174)	0.000
	IQ	0.221	0.249 (0.198)	0.057
	Speech therapy	0.163▪	0.241 (0.169)	0.058
	ADD	0.579	−0.097 (0.173)	0.009
	Musical instrument	0.415	0.142 (0.172)	0.020
Temporal correlation coefficients left IFG and pSTG	Age	0.21	−0.217 (0.17)	0.047
	Gender	0.263	−0.195 (0.171)	0.038
	IQ	0.955	0.01 (0.178)	0.000
	Speech therapy	0.029*	0.369 (0.162)	0.136
	ADD	0.041*	0.347 (0.163)	0.120
	Musical instrument	0.993	−0.002 (0.174)	0.000
Phonological awareness test score	Age	0.006**	0.458 (0.155)	0.210
	Gender	0.865	−0.03 (0.174)	0.001
	IQ	0.055▪	0.383 (0.19)	0.135
	Speech therapy	0.032*	−0.364 (0.162)	0.132
	ADD	0.042*	−0.345 (0.163)	0.119
	Musical instrument	0.651	0.079 (0.174)	0.006

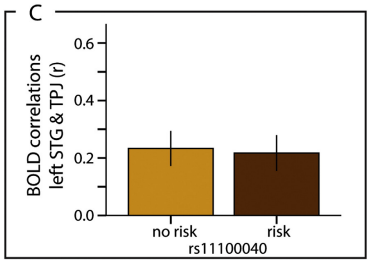
Effect size (SE) = effect size beta in a linear regression model; SE = standard error of effect size; R^2 = variance of the variable explained by the potential confounder.
**$P < 0.01$; *$P < 0.05$; ▪$P < 0.2$.

compared to non-carrier children ($k = 36$, MNI coordinates: −34, −16, 34, $P < 0.01$, cluster size Bonferroni corrected to $P < 0.01$) (Fig. 2). We did not find any effects on the white matter skeletons for the other SNP. The reported cluster was the only one withstanding multiple comparison correction at $P < 0.01$ and $k > 17$. The individual FA values were correlated with the individual functional connectivity indices (partial $r_s = 0.6$, $P < 0.005$).









Relations between the neural phenotypes and phonological awareness

The individual FA values of the cluster detected in the arcuate fasciculus were related to the performance of the children in the phonological awareness tasks (partial $r_s = 0.44$, $P < 0.05$, Bonferroni corrected) when controlling for the influence of age, gender, IQ, and of external factors including speech therapy, musical instrument instruction, and ADD (Fig. 3 and Table 2). The strength of this correlation decreased after inclusion of the functional connectivity indices as a covariate into this model (partial $r_s = 0.31$, $P = 0.181$). This indicates that the correlation between FA and phonological awareness was partly explained by functional connectivity. Phonological awareness was not significantly associated with the resting state functional connectivity indices (partial $r_s = 0.09$, $P = 0.6537$).

Relations between phonological awareness and other dyslexia-relevant behavioral phenotypes

Given that phonological awareness was related to the FA of the arcuate fasciculus which in turn was related to rs11100040 we finally aimed to confirm that phonological awareness was also related to other dyslexia-relevant behavioral measures. The individual phonological awareness scores at ages 9 to 12 were predicted retrospectively by the individual risk scores in an early screening for DD (BISC) at ages 5 to 6 (partial $r_s = -0.42$, $p = 0.049$) when controlling for the influence of age, gender, IQ, and of external factors including speech therapy, musical instrument instruction, and ADD. Additionally, the individual phonological awareness at ages 9 to 12 was associated with the individual spelling performance at the same age (partial $r_s = -0.38$, $p = 0.03$). However, it did neither predict the individual reading accuracy (partial $r_s = 0.47$, $p = 0.15$) nor reading speed (partial $r_s = 0.41$, $p = 0.218$) at ages 11 to 14.

Discussion

Numerous task-based fMRI and PET studies have previously identified altered functional responses in left inferior frontal and temporo-parietal cortices as playing a central role for the phonological deficit of dyslexic individuals (Hoeft et al., 2007; McCrory et al., 2005; Paulesu et al., 2001; Raschle et al., 2012). Rather than providing a replication of these results, we followed the hypothesis that a differential hemodynamic interplay between these cortices can already be detected at resting state in the default language network in the absence of any linguistic stimulation (Lohmann et al., 2010). Our main finding was that the temporal correlations of low frequency BOLD signal fluctuations between inferior frontal and superior temporal cortices were significantly reduced in children carrying risk alleles at rs11100040. As this gene variant was found to be associated with phonological deficits in a genome-wide association study (Roeske et al., 2011), our finding extends recent studies linking

Fig. 1. Group differences between pair-wise temporal correlations of low frequency BOLD signals at resting state in three seed regions (left inferior frontal gyrus (IFG): −51, 10, 10, left posterior superior temporal gyrus (pSTG): −53, −31, 9, and left temporo-parietal junction (TPJ): −59, −45, 15) known to support phonological processing. (A) Children without any risk allele at the *SLC2A3* modifier rs11100040 showed significantly higher correlations between the left IFG and the left pSTG compared to children with a genetic risk (solid line) ($F_{1, 33} = 2.81$, $P < 0.05$, Bonferroni corrected). (B and C) The groups did not differ significantly from each other in the two other seed pairs (dashed lines) (left IFG and left TPJ: $F_{1, 33} = 0.9$, $P = 0.524$; left pSTG and left TPJ: $F_{1, 33} = 0.69$, $P = 0.682$). Children without a risk allele at rs4234898 did not differ significantly from children carrying risk alleles in all three pairs of ROIs.

the functional resting state connectivity of these regions to reading phenotypes in children and adults (Koyama et al., 2010, 2011). We introduced rs11100040 as a genetic variant potentially playing a role for the development of fronto-temporal functional connectivity profiles. This observation is supported by a considerable body of literature providing evidence that *SLC2A3* is a glucose transporter strongly expressed in neurons of the cortical gray matter (Maher et al., 1994; Maher and Simpson, 1994; McCall et al., 1994).

In order to link the brain-functional differences to brain-structural differences, we analyzed the relation between rs11100040 and FA following a whole brain white matter approach in order not to limit our analyses a priori only to a subset of the various candidate tracts discussed in the literature (Vandermosten et al., 2012b). Using this approach we made the observation that rs11100040 is associated with the FA of the arcuate fasciculus, a long-distance white matter fiber tract essential for language processing (Friederici, 2011). Since this result is strongly supported by recent studies reporting a link between the arcuate fasciculus and phonological processing (Vandermosten et al., 2012a; Boets et al., 2013; Saygin et al., 2013; Myers et al., 2014), our study extends these findings by suggesting a possible contribution of rs11100040 to the development of this fiber tract. Currently, the actual neurobiological foundation of the FA is a matter of ongoing scientific debate. On the one hand, FA provides several microstructural characteristics of the white matter related to its maturity including the overall number of fibers within a tract, their degree of myelination (Friederici, 2012), their axonal caliber (Paus, 2010), and the amount of surrounding glia cells (Wandell and Yeatman, 2013). On the other hand, FA is also influenced by the orientation of fibers and by fiber crossings. It is likely that both microstructure and coherence of the fibers have an influence on the observed FA differences. Future studies will have to clarify this issue.

The present study goes beyond the results of previous studies by detecting a strong correlation between fronto-temporal functional connectivity and arcuate fasciculus FA suggesting a tight explanatory relation between both neural markers. Still, given that a recent study did not find a correlation between an effective, i.e. task-based, functional connectivity measure and the FA of the arcuate fasciculus (Boets et al., 2013), further research is needed to corroborate the link between functional and structural connectivity indices of the phonological processing network.

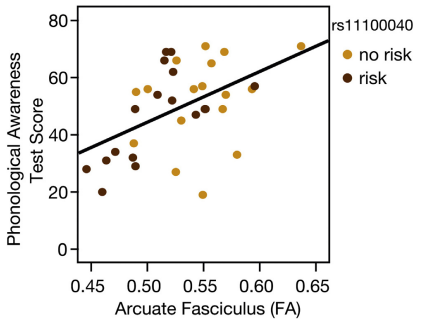


Fig. 3. FA in the left arcuate fasciculus was correlated with phonological processing performance. Individual FA values in a central sub-portion of the left arcuate fasciculus were significantly associated with the individual phonological awareness scores (partial $r_s = 0.44$, $P < 0.05$, Bonferroni corrected).

We were able to link brain structural variance to behavior by showing that the FA of the left arcuate fasciculus was associated with phonological awareness. This extends results from previous studies demonstrating that FA is a sensitive neuroanatomical correlate of phonological processing abilities in the arcuate fasciculus (Vandermosten et al., 2012a; Saygin et al., 2013; Myers et al., 2014). Individual phonological awareness, however, and individual functional connectivity were not significantly related when directly correlated. This observation is in line with data from a recent resting state fMRI study on dyslexia, in which brain–behavior correlations were only found in fronto-parietal attention areas but not

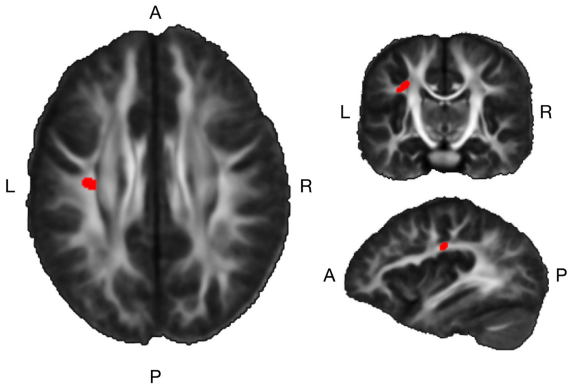


Fig. 2. Fractional anisotropy (FA) differences between children either with or without risk alleles at rs11100040. Children who did not carry any risk allele had significantly higher FA values in a cluster located in the left arcuate fasciculus ($k = 36$, MNI coordinates: −34, −16, 34, $P < 0.01$, cluster size Bonferroni corrected to $P < 0.01$). Depicted are axial, coronal, and sagittal views of the cluster within the group average white matter skeleton (A: anterior, P: posterior, L: left, R: right). No significant FA difference was observed between risk carriers and non-risk carriers at rs4234898.

in the temporo-parietal cortices selectively supporting phonological processing (Koyama et al., 2013). Thus, it remains to be demonstrated whether resting state functional connectivity can function as a direct measure for the individual phonological awareness, or whether brain structural measures are the more sensitive measure.

Phonological awareness at ages 9 to 12 was predicted significantly by retrospective behavioral risk scores in an early dyslexia screening administered at ages 5 to 6 and it was also significantly related to spelling performance measured at the same age. These findings corroborate numerous studies indicating that phonological awareness is a central correlate of literacy skills across development (Ziegler et al., 2010; Peterson and Pennington, 2012). The observation that phonological awareness was not significantly associated with reading performance might be specific to German as a language with an orthography that is relatively transparent with respect to its high grapheme–phoneme correspondence (Moll et al., 2014). Due to this high correspondence, reading acquisition is less dependent on phonological skills in German compared to other languages. Several previous investigations suggest that the impact of phonological awareness on reading is stronger in less transparent orthographies such as English or French (Ziegler et al., 2010; Peterson and Pennington, 2012; Moll et al., 2014).

We did not detect a significant difference between the genetic risk groups with respect to phonological awareness as determined by a one-way ANOVA adjusted for the confounders ($F_{1,\ 33} = 0.987$, $P = 0.33$). The most likely reason for this effect might be that the group difference did not reach significance due to the small effect size of the SNP on behavioral data. Therefore, this study focused on brain functional and brain structural endophenotypes where genetic effects can be much higher as we see in the present study. Our results are similar to those of other genetic imaging studies, where no correlation could be reported with the behavioral phenotype but a significant association was found with the brain-functional or the brain-morphological endophenotype (e.g. Mier et al., 2010; Darki et al., 2012).

The validity of our findings is bolstered by additional facts. First, it is known that effect sizes of a genetic variant in functional neuroimaging data are higher compared to other brain-functional measures like EEG (Mier et al., 2010). Second, it has been shown that FA variance in the arcuate fasciculus has one of the highest heritability rates of all white matter structures (Jahanshad et al., 2013), thereby strongly suggesting effects of genetic modulators. Third, all analyses were controlled for possible compensatory mechanisms induced by speech therapy and musical instrument instruction (Goswami et al., 2011) as well as for attention deficits as a relevant comorbidity (Kibby et al., 2009). The latter may potentially interact with the fronto-parietal phonological processing system via the fronto-parietal attention system located in the immediate vicinity (Koyama et al., 2013).

In principle, we cannot exclude a minor contribution of the other SNP analyzed in our experiment (rs4234898) to the analyzed structural and functional measures, particularly since Roeske et al. (2011) reported an association of this SNP with the auditory MMN by itself. We tested for effects of SNP rs4234898 on both neural measures but did not obtain any significant results. Therefore, we assume that if there was an effect of rs4234898 it should be considerably smaller than the effect of rs11100040. In this work, we did not recruit a replication sample to reproduce the observed effects. Accordingly, follow-up studies are necessary to disentangle the specific contributions of rs11100040, rs4234898 and the haplotype including both SNPs, to both electrophysiological and neuroimaging measures.

The challenge for future imaging genetics studies on phonological awareness will be to collect data from larger samples in order to validate all variables internally and to detect further direct linkage between the individual genome, neural markers, and the core behavioral phenotypes characterizing developmental dyslexia. This will not only augment our understanding of developmental neural plasticity, but may also allow a more reliable and earlier diagnosis of phonological deficits, potentially paving the way for a more effective treatment.

Conclusions

The present study integrates genetic, brain-functional, brain-structural and behavioral measures suggesting a complex association between a dyslexia risk genotype and two closely linked neural phenotypes, the fractional anisotropy of the left arcuate fasciculus, and the functional resting state connectivity of its termination areas in the inferior frontal and posterior superior temporal cortex. The fractional anisotropy of the left arcuate fasciculus is related to a phonological awareness phenotype in 9- to 12-year-old children which in turn is related to prereading dyslexia risk scores as well as to spelling skills. These findings call for a need to combine biomarkers from genetic and neural domains to optimize potential diagnostic tools for developmental dyslexia. Future work will have to show if such a multimodal neurogenetic biomarker can be applied to predict the risk to be affected by dyslexia before school entry so that existing preschool intervention tools can be used more efficiently.

Acknowledgments

This work was funded by the Max Planck Society and the Fraunhofer Society (grant number M.FE.A.NEPF0001). We thank Alfred Anwander for providing his expertise in diffusion-weighted magnetic resonance imaging.

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Study VII

Kraft I, Cafiero R, Schaadt G, Brauer J, Neef N, Müller B, Kirsten H, Wilcke A, Boltze J, Friederici AD, **Skeide** MA (2015) Cortical differences in preliterate children at familiar risk of dyslexia are similar to those observed in dyslexic readers. *Brain* 138:e378.

doi:10.1093/brain/awv036 BRAIN 2015: 138; 1–3 | e378

BRAIN
A JOURNAL OF NEUROLOGY

LETTER TO THE EDITOR

Cortical differences in preliterate children at familiar risk of dyslexia are similar to those observed in dyslexic readers

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Sir,

In their recent report in *Brain*, Clark *et al.* (2014) presented cortical thickness data obtained from a cohort of 27 children that were compared longitudinally at three time points (first grade: ages 6–7, third grade: ages 8–9, sixth grade: ages 11–12) categorized as either dyslexic or not according to their reading outcome in sixth grade. Based on their observations, the authors conclude that the neuroanatomical precursors of developmental dyslexia are found predominantly in primary sensory cortices and that structural abnormalities in the reading network only emerge after children have learned how to read and write. This study is indeed invaluable as it follows preliterate children longitudinally until the disorder is diagnosed, providing a unique picture of structural cortical changes in dyslexic and non-dyslexic children during this time. However, there are a number of discrepancies between the presented findings and results from other groups including our own. These differences might be explained by the relatively low statistical power of the analyses carried out by Clark and colleagues. Moreover, because genetic and environmental factors are not included in their analyses, it remains unclear how the data can be integrated into a comprehensive account of developmental dyslexia.

The first limitation is based on the experimental design of the study. Although the subsamples compared by Clark *et al.* (2014) are small for a neuroimaging study [MRI time point 1: children who later were identified as dyslexic ($n = 7$) and those who were not ($n = 10$); MRI time point 3: children who were identified as having dyslexia ($n = 11$) and those who were not ($n = 13$), male dyslexic children ($n = 5$) and male control children ($n = 8$), female dyslexic children ($n = 6$) and female control children ($n = 5$)], the authors do not report results from a pretest power analysis. Hence, it is hard to determine whether the observations are truly significant or whether the effects were randomly detected and might not be reproducible in larger samples. The chosen whole-brain significance threshold of $P < 0.05$ (cluster size corrected to $P < 0.05$) is the most liberal confidence level possible in a neuroimaging study. The consequences of a potential power problem may be aggravated by substantial subsample size variations across measurement points. In particular, at MRI time point 1, <64%

Advance Access publication February 20, 2015

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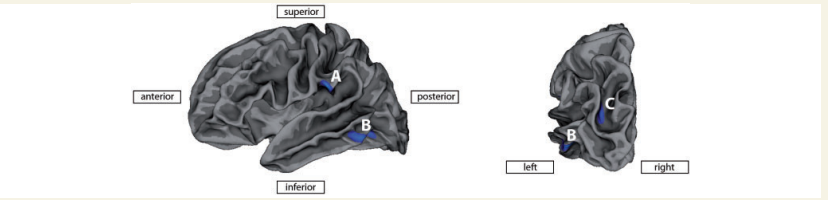

Figure 1 Cortical thickness differences between 5-year-old preliterate children at familial risk of developmental dyslexia and non-risk controls. Compared to children without a family history of developmental dyslexia, individuals with a family history of developmental dyslexia showed a significantly reduced cortical thickness ($P < 0.05$, false discovery rate corrected) in the left supramarginal gyrus (**A**), the left inferior temporal gyrus (**B**), and the left superior and transversal occipital sulci (**C**), but not in right-hemispheric regions. The opposite contrast (non-risk children versus at-risk children) did not reveal any significant results. Depicted are z-transformed cortical thickness values on the white matter surface of the group-averaged left hemisphere.

of the data about the children with dyslexia and <77% of the control data were available compared to MRI time point 3. We understand that such variations are almost unpreventable as they primarily emerge from the complex logistics of longitudinal surveys enrolling children in the given age range. Nevertheless, these variations compromise the longitudinal comparability of the data, even given very good scan-rescan reliability, and would therefore have deserved more detailed discussion.

The second limitation relates to the conceptual framework for interpreting the results. Developmental dyslexia is moderately to highly heritable with rates of inheritance ranging from 30% in families with low levels of parental education to 70% in families with high levels of parental education (Scerri and Schulte-Körne, 2010). Unfortunately, the authors accounted for neither the impact of genetic nor environmental variance, particularly parental education, in their analyses. This is limiting because previous imaging genetics studies indicate that the direct effect of dyslexia susceptibility genes on cortical thickness phenotypes is stronger than on behavioural phenotypes such as reading level (Darki *et al*., 2014). Accordingly, the statement from the authors that their 'results are specific to dyslexia *per se* rather than a family history of dyslexia' remains vague. Clark and colleagues do not provide an alternative account of the current best-supported integrative model of dyslexia introduced by Giraud and Ramus (2013). This model assumes that a certain set of genetic risk variants alters neuronal precursor migration to their cortical target layers *in utero*, which, in turn, leads to acoustic-phonological deficits detectable in newborns predicting the later reading and writing outcome (Giraud and Ramus, 2013).

The third limitation is that the authors categorize the participants according to their reading outcome at MRI time point 3, but do not directly relate the corresponding reading and spelling data to the cortical thickness data. Additionally, as no behavioural correlates of the brain measures obtained at MRI time points 1 and 2 are provided, it remains unclear whether the distinct cortical thickness patterns have any behavioural implications with respect to phonological awareness or reading and spelling.

Recently, we compared whole-brain cortical thickness in 53 pre-reading children (mean age: 5 years 5 months, range 4 years 9 months to 6 years 3 months) either with ($n = 25$, 11 females) or without ($n = 28$, 12 females) a familial risk of developmental dyslexia defined as having one or more first-degree relatives with dyslexia. This analysis revealed a significantly reduced cortical thickness in the left supramarginal gyrus and the left occipito-temporal cortex ($P < 0.05$, false discovery rate corrected) in children with a familial risk compared to non-risk children (Fig. 1), whereas the inverse contrast did not reveal any significant differences. The participants were tightly matched for their parents' education and profession and did not differ significantly ($P = 0.694$) with respect to this environmental factor most substantially contributing to the development of dyslexia (Peterson and Pennington, 2012). This supports the assumption that genetic factors but not parental education and profession are an important source of variance for explaining the observed cortical differences.

The anatomical confinement of these effects to temporo-parietal and occipito-temporal cortices is not only in line with the adult literature (Peterson and Pennington, 2012) but also supports all other comparably powered studies investigating brain structure and function in preliterate children at risk of dyslexia. Both regions were identified cross-sectionally with respect to familial risk in a functional MRI study on phonological processing at a pre-reading age (Raschle *et al*., 2012). Additionally, the arcuate fasciculus as the long-distance white matter fibre tract connecting temporo-parietal cortical areas with temporal and frontal areas was not only shown cross-sectionally to be related to phonological awareness (Saygin *et al*., 2013) but was also shown to predict reading outcome at third grade (Myers *et al*., 2014).

In conclusion, the current literature and our own results obtained in larger samples suggest an endophenotypic developmental continuum of genetic risk factors affecting

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temporo-parietal and occipito-temporal cortical maturation. This is assumed to be present in pre-reading children as well as in young and adult readers, which is in contrast to the results reported by Clark and colleagues. Given the relatively small sample size and longitudinal group variations in this study, it cannot be excluded that the absence of differences in several cortical areas, which form the later reading network, might be obscured by limited statistical power to detect such effects, whereas effects in other areas might be overestimated. Crucially, Clark and colleagues had only 57% power in their sample at MRI time point 1 to detect the clusters in the left supramarginal gyrus and the left occipito-temporal cortex identified in our analyses (effect size = 0.93; effect size in Clark *et al.* = 0.53; effect size is defined as the mean difference divided by common standard deviation). Despite the significant value of the longitudinal study by Clark and colleagues for the field, larger and statistically more powerful studies may be required to reveal ultimately which of the contrary hypotheses best approximates reality. This could comprise international collaborations to investigate larger samples collected from populations being comparable with respect to orthographic regularity and genetic background.

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SELBSTSTÄNDIGKEITSERKLÄRUNG

Herewith I declare that the present thesis is the result of my own work. All contributions of co-authors and all citations of external sources are designated as such in the text.
*Hiermit erkläre ich, dass ich die vorgelegte Arbeit eigenständig angefertigt habe. Sämtliche Beiträge von Mitautor*innen und Inhalte aus fremden Quellen sind als solche kenntlich gemacht.*

Leipzig, 09.11.2017
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