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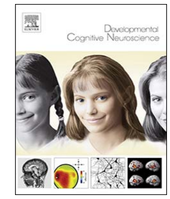

Functional hyperconnectivity vanishes in children with developmental dyscalculia after numerical intervention

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ABSTRACT

Developmental dyscalculia (DD) is a developmental learning disability associated with deficits in processing numerical and mathematical information. Although behavioural training can reduce these deficits, it is unclear which neuronal resources show a functional reorganization due to training. We examined typically developing (TD) children (N = 16, mean age: 9.5 years) and age-, gender-, and handedness-matched children with DD (N = 15, mean age: 9.5 years) during the performance of a numerical order task with fMRI and functional connectivity before and after 5-weeks of number line training. Using the intraparietal sulcus (IPS) as seed region, DD showed hyperconnectivity in parietal, frontal, visual, and temporal regions before the training controlling for age and IQ. Hyperconnectivity disappeared after training, whereas math abilities improved. Multivariate classification analysis of task-related fMRI data corroborated the connectivity results as the same group of TD could be discriminated from DD before but not after number line training (86.4 *vs.* 38.9%, respectively). Our results indicate that abnormally high functional connectivity in DD can be normalized on the neuronal level by intensive number line training. As functional connectivity in DD was indistinguishable to TD's connectivity after training, we conclude that training lead to a re-organization of inter-regional task engagement.

1. Introduction

The term "developmental dyscalculia" (DD) was first introduced in 1968 (Cohn, 1968) to describe a learning disability in basic numerical and mathematical operations, such as addition or subtraction deficits. Similar to dyslexia, DD affects about 3–6% of the population (Badian, 1999; Gross-Tsur et al., 1996; Kosc, 1974), and recent findings suggest that slightly more girls are affected than boys (Fischbach et al., 2013). One interesting observation is that individuals with DD cannot master mathematics despite normal cognitive abilities in other domains. Children with DD also show a persistent inability to commit basic arithmetic information to long-term memory, to understand, or access magnitudes associated with number words and Arabic numerals, as well as a delay in the learning of arithmetical procedures (Butterworth et al., 2011; Geary, 1993; Jordan et al., 2003; Rousselle and Noël, 2007; Mazzocco et al., 2011).

Several behavioural and neuroimaging studies examined children with DD relative to typically developing (TD) children to identify the role of confounding behavioural factors and underlying neuronal substrates associated with DD. Behavioural studies predominantly focused on tasks involving addition problems and could show that children with DD rely more on counting strategies relative to TD (Geary et al., 2004). Structural and functional MRI studies revealed that DD show structural disparities from TD, particularly in the parietal and frontal cortex (Rotzer et al., 2008; Rykhlevskaia et al., 2009), as well as altered "activation" (*i.e.* fMRI signal changes) in parietal and frontal regions during various arithmetic tasks (Ashkenazi et al., 2012; Kucian et al., 2006a; Berteletti et al., 2014; Morocz et al., 2012; Molko et al., 2003; Kucian et al., 2011a; Mussolin et al., 2010a; Kucian et al., 2011b; Dinkel et al., 2013; Kaufmann et al., 2009a; Kaufmann et al., 2011). Although these findings are based on different numerical tasks, ranging from rather basic number sense (non-symbolic distance effect (Price et al., 2007)) to the understanding of ordinality (Kucian et al., 2011b), symbolic number comparison (Mussolin et al., 2010b), arithmetic (multiplication or approximate addition) (Kucian et al., 2006a; Berteletti et al., 2014), and non-numerical skills that are processed by overlapping networks (spatial working memory) (Rotzer et al., 2009), coincident reductions of brain activation has been reported in the parietal lobe. These findings suggest that children with demonstrate functional demonstrate deficits in the core region for number proces-

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sing in the parietal lobe, which might be seen as a direct neuronal correlate of math difficulties (for review see Kucian, 2016).

However, it is known that functional changes in DD affect multiple distributed brain networks including different subparts of the frontal and parietal cortex but also areas in the (ventral) temporo-occipital cortex and subcortical regions (Ashkenazi et al., 2012; Mussolin et al., 2010a; Kaufmann et al., 2011; Kucian and von Aster, 2015). Commonly, activity is less modulated in DD (comparing simple and complex task situations) in the bilateral inferior parietal sulcus (IPS), irrespective of the overall difference in signal level, indicating that the IPS is not just under-activated but also lacks the ability to generate distinct neuronal responses for arithmetic problems with different task-complexities. Pinel et al. found that regions whose activation was modulated by numerical distance were located in the bilateral IPS, the precuneus, and the left precentral gyrus. Activation decreased quasi-monotonically as the numerical distance increased in each of these brain regions (Pinel et al., 2001). Some fMRI studies using numbers larger than 10 in a number comparison task (Le Clec et al., 2000; Rickard et al., 2000) reported bilateral or unilateral activation of the IPS or angular gyrus. More recently, Berteletti and colleagues demonstrated that DD children could only engage numerical processing regions (*i.e.* reflected in fMRI signal changes of the IPS) when solving small problems, suggesting an impaired functionality of the IPS particularly in complex mathematical operations. In a longitudinal study, Dumontheil and Klingberg reported that working memory related activation of the (left) IPS predicts arithmetical outcome better than behavioural measures alone (Dumontheil and Klingberg, 2012), providing further evidence for the apparent link between the IPS and mathematical reasoning abilities.

To our knowledge, only two studies investigating changes in brain activation after intervention in children with DD have been conducted so far (Kucian et al., 2011b; Iuculano et al., 2015). In the study by Kucian and co-workers, children with and without DD completed a 5-week training program with the aim of improving number representations and strengthening the link between numbers and spatial processing on the internal mental number line (Kucian et al., 2011b). Results indicated that, after completion of the training, functional circuitry was positively influenced, *i.e.* the pattern of brain activation altered from atypical towards more typical brain activation. Iuculano and colleagues demonstrated that cognitive tutoring induced widespread neuroplasticity and restores brain function in children with mathematical learning disabilities (MLD) using an addition (verification) task (Iuculano et al., 2015). Typically, the definition of MLD is based on a slightly more liberal cut-off criterion (1 SD below norm) with respect to math abilities than usually conducted in DD (1.5 SD below norm). The authors found non-discriminable fMRI brain activity only after cognitive tutoring. In the identical sample, Supekar et al. (2015) additionally demonstrated a remediation of (childhood) math anxiety and associated neuronal circuits through cognitive tutoring.

To study development-related cognitive deficits, it is important to consider not only alterations in local brain responses but also neuronal network properties, especially since arithmetic processing is thought to rely on multiregional coordination (Rosenberg-Lee et al., 2011). Fast and accurate connections between the different brain regions are crucial for efficient transfer and maintenance of information during number processing and calculation. Little is currently known concerning specific impairments of brain connections that are probable neuronal correlates of DD (for review see Kucian, 2016). Nevertheless, existing diffusion tensor imaging (DTI) studies provide evidence of impaired fiber tracts connecting different brain areas of the fronto-parietal network known to be responsible for number processing and calculation. More specifically, Rykhlevskaia et al. reported long-range white matter projection fibres linking the right fusiform gyrus with temporo-parietal white matter as a specific source of vulnerability in children with DD. More recent results of Kucian et al. (Kucian et al., 2013a) highlighted a deficit in children with DD of the superior longitudinal fasciculus, a fibre tract that connects parietal, frontal, and temporal areas. In particular, the superior longitudinal fasciculus seemed to be affected in parts that are adjacent to key areas for number processing, namely the IPS. Few functional connectivity (FC) studies, using fMRI, examined inter-regional communication in DD (*i.e.* children with mathematical disabilities) during either addition and subtraction tasks (Rosenberg-Lee et al., 2015) or a rest (eyes closed) condition (Jolles et al., 2016). Rosenberg-Lee and colleagues selected a subtraction task as it is subtraction which is thought to draw more on an internal representation of quantity (Dehaene et al., 2003) and the execution of calculation procedures (Campbell and Xue, 2001). The authors reported greater fMRI signal changes in the IPS and other visuo-parietal brain regions in DD compared to TD (Rosenberg-Lee et al., 2015). For the (effective) FC analysis, the authors focused on the IPS, due to its important role in numerical processing and arithmetic deficits in DD (for a review see: Kucian (Ashkenazi et al., 2012; Kucian et al., 2006a; Kucian et al., 2011a)), and since it is known that greater structural connectivity is linked to better math ability (Emerson and Cantlon, 2012; Kucian et al., 2013b). One possible explanation for the hyperconnectivity in DD could be due to its spatial specificity, present in the Default Mode Network (DMN) and Task Positive Network (TPN). Specifically, and consistent with observations from another fMRI study (Davis et al., 2009), children with DD seem to be unable to deactivate core regions of the DMN (such as the posterior cingulate cortex and ventromedial prefrontal cortex) during subtraction or addition. Alternatively, hyperconnectivity in the TPN might reflect prefrontal compensation (as children with DD showed stronger fMRI responses across tasks in part of this network).

Based on these previous studies, it is not yet known whether hyperconnectivity in DD is also observable during other tasks, such as numerical ordinality judgement. Secondly, it is unknown if number line training leads to normalization of this hyperconnectivity, especially of prefronto-parietal regions in DD, similar to the reported neuronal normalization using cognitive tutoring. Different approaches have been proposed, in order to assess task-related neuroplasticity changes on the level of connectivity measures. For example, some studies applied (spontaneous) background FC analysis to extract task-related FC (Fair et al., 2007; Ghisleni et al., 2015; Norman-Haignere et al., 2012). Although the strength of FC is constrained by structural connectivity, it is modulated by mental states and current context, such that intrinsic activity constitutes the brain's internal context for processing external information and generating behaviour (Fontanini and Katz, 2008; Sadaghiani et al., 2010a; Sadaghiani et al., 2010b). Hence, background FC captures "general task engagement", as it removes some portion of the FC differences that are simply due to co-activation as a response to the task but the remaining residual signal still contains task effects. Alternatively, Psycho-Physical Interaction (PPI) analysis informs about the interaction between a psychological state (*e.g.* ordinality processing) and the functional coupling between two (or more) brain areas (Friston et al., 1997).

In this study, we applied background and PPI FC analysis in order to asses if a 5-week number line training (Kucian et al., 2011b) can normalize aberrant neuronal connectivity patterns in DD relative to TD who also received the same intervention. We then applied a multivariate classification analysis to assess if brain activity before and after number line training can dissociate TD from DD. Based on previous findings (Kucian et al., 2011b; Iuculano et al., 2015; Rosenberg-Lee et al., 2015; Supekar et al., 2015) we hypothesize that number line training leads to a neuronal normalization (*i.e.* comparable FC to TD) of fronto-parietal brain regions and increased task performance in DD. To test this hypothesis, we selected a numerical order task for fMRI, as it has been demonstrated that children with DD show altered activation in the bilateral IPS and various frontal (superior frontal and insular) regions during ordinality judgement (Kucian et al., 2011b).

2. Methods

2.1. Participants

The participants were chosen from a former study (Kucian et al., 2011b) including originally 16 TD children and 16 DD. We had to exclude data from one DD child (from the original sample used in (Kucian et al., 2011b)) because the log-file of the fMRI paradigm (post training) was not readable anymore. This resulted in a total of 31 children between 7.8 and 11.8 years of age, which could be included in the present study, of whom 15 children fulfilled the clinical criteria of DD and 16 were age-, gender-, and handedness-matched TD children with age-appropriate calculation performance.

This sample size is similar to previously published studies of children with MLD or DD (Kucian et al., 2011a; Price et al., 2007; Iuculano et al., 2015; Rosenberg-Lee et al., 2015; Kaufmann et al., 2009b). According to the DSM-5 (Association, 2013), criteria for DD were met (and applied in this study) if a child's performance in the Neuropsychological Test Battery for Number Processing and Calculation in Children (ZAREKI-R (Kucian et al., 2006b)) was below the 10th percentile in at least three subtests or in the total score, and estimated intelligence quotient lay in the normal range.

None of the participants had neurological or psychiatric disorders, was on medication, or had exclusion criteria for MRI such as braces. A detailed list (including demographical and cognitive measures) of all volunteers is provided in Table 1. Ethics approval was obtained from the cantonal ethics-commission Zurich based on guidelines from the World Medical Association's Declaration of Helsinki (WMA, http://www.wma.net/en/30publications/10policies/b3/). The parents of all participants gave written informed consent prior to the study.

2.2. Cognitive assessment

2.2.1. Number processing

Numerical abilities were assessed using the standardized Neuropsychological Test Battery for Number Processing and Calculation in Children (ZAREKI-R (Kucian et al., 2006b)). This neuropsychological battery examines basic skills in calculation and arithmetic and aims to identify and characterize the profile of mathematical abilities in children with DD. It is composed of 12 subtests: 1. counting dots, 2. counting backwards, 3. writing Arabic digits, 4. mental calculation (addition, subtraction, and multiplication), 5. reading Arabic digits, 6. number line I (allocation of a number word or an Arabic digit to one of four given marks on a number line 0–100), and number line II (mark the position of a number word or an Arabic digit on a number line 0–100), 7. digit span forward and backward, 8. number word comparison, 9. perceptive magnitude judgement (estimation of number of dots, balls, cups shown for 2 or 5 s), 10. cognitive contextual magnitude evaluation (*e.g.* four fridges in a kitchen – is this few, normal, a lot?), 11. word problems, 12. Arabic digit comparison. According to the ZAREKI-R instructions for diagnosis, criteria for DD are met if a child's performance in the ZAREKI-R is below the 10th percentile in the total score or in at least three of the following subtests: writing Arabic digits, reading Arabic digits, mental calculation addition, mental calculation subtraction, mental calculation multiplication, number word comparison, Arabic digit comparison, cognitive contextual magnitude evaluation, number line I, number line II. However, ZAREKI does not use results for counting backwards, digit span forwards and backwards, and hence working memory related tests are not considered by this type of DD diagnosis.

Table 1
Demographic and behavioural data of children with developmental dyscalculia (DD) and typically developing children (TD).

	DD	TD	Statistics p-value
Subjects (N)	15	16	–
Age (Mean (SD) years)	9.5 (0.7)	9.5 (0.8)	0.611
Gender (male/female)	6/9	7/9	0.833[a]
Handedness (right/ambidextrous/left)	14/1/0	12/4/0	0.165[a]
Training (Mean (SD) days)	23.9 (2.2)	24.6 (2.0)	0.319
Number processing[b] (Mean (SD) percentile rank)	17.4 (24.3)	75.3 (20.3)	**0.000**
Intelligence[c d] (Mean (SD) IQ)	101.3 (8.4)	110.6 (6.8)	**0.002**

[a] Pearson-Chi-square tests were used because of nominal data input. (For all other statistical comparisons between groups, two-sample *t*-tests were performed).
[b] Number processing and calculation skills are based on total reached percentile rank in the ZAREKI-R (von Aster et al., 2006).
[c] Mean IQ is based on the mean of three verbal (vocabulary, arithmetic, similarities) and two performance subtests (picture arrangement, block design) of the Wechsler Intelligence Scale for Children (WISC-III) (Wechsler, 1999).
[d] IQ values were normal distributed in both groups (TD: $p = 0.24$, DD: $p = 0.06$; one-sample Shapiro-Wilk test).

2.2.2. Number line performance

The spatial representation of numbers was measured by a paper-and-pencil number line task. Children had to indicate on a left-to-right oriented number line from 0 to 100 the location of 20 Arabic digits, results of 20 additions and 20 subtractions (task conditions), or the estimated number of 10 different dot arrays (control condition). First, a card with an Arabic digit was shown to the child. Then, the child marked with a pencil the location of the number on the number line, at which point the next card was shown and the child indicated the location on the next number line template. After 20 Arabic digits, 20 cards with additions were presented, which the child had to solve and indicate the location of the result on the number line. The same procedure was repeated for 20 subtraction problems. Finally, 10 cards were shown for only 3 s, which contained randomly arranged dots. The child had to estimate the number of dots and mark the location on the number line. The error rate of the paper-and-pencil number line task was evaluated by measuring the distance in percent (% distance) relative to the position of the correct number for each trial. Mean% distance was then calculated over all trials (Arabic digits, additions, subtractions, dots), but only correctly calculated addition and subtraction problems were included. A detailed description of the task is described in one previous publication of our group (Kucian et al., 2011b).

Children performed balanced different versions of this task immediately before starting the training and after finishing the training period of 5 weeks to examine domain specific effects of the number line intervention.

2.2.3. Arithmetic

Arithmetic performance was assessed from the mean number of correctly solved addition and subtraction problems solved in the number line task. In total 40 arithmetic problems had to be solved (20 additions, 20 subtractions) in the number range 0–100. Difficulty levels of additions and subtractions were balanced. Arithmetical skills were measured before and after the training by paralleled versions of the task to evaluate possible transfer effects of an approximate number line intervention to exact arithmetical skills.

2.2.4. Intelligence

Intelligence was measured with three verbal (Vocabulary, Arithmetic, Similarities) and two performance subtests (Picture Arrangement, Block Design) of the Wechsler Intelligence Scale for Children (WISC-III) (Wechsler, 1992).

2.2.5. Spatial memory span & spatial working memory

Spatial memory span was measured with the CORSI-Block Tapping test (Schellig, 1997), which requires participants to repeat the sequences of touched cubes on a wooden board in the same order as that demonstrated by the examiner. While the sequences gradually increase in length, the number of cubes last tapped in two consecutively correct sequences is defined as the maximum span. Spatial working memory was assessed by the Block Suppression Test (Beblo et al., 2004). This test is based on the CORSI-Block tapping test, and subjects need to reproduce every second block in a given sequence. Both, spatial memory span and spatial working memory were assessed before and after training to test if the numeric intervention has also an effect on general cognitive abilities.

2.2.6. Handedness

Handedness was determined by the Edinburgh Handedness Inventory (Oldfield, 1971).

2.3. Training

All TD and DD received the same training. By this, we could track (1) if DD children overcome their difficulties in numerical and mathematical operations (see behavioural results) and (2) if TD benefit from the training as well. Children trained at home 15 min a day, 5 days a week for 5 weeks with the computer-based intervention software "Rescue Calcularis" (Kucian et al., 2011b). A timer controls the daily training time, which is always visible during the game. After completing the 15 min training session, the program is automatically blocked until the next day. The program consists of a number line training program, which aims to improve the spatial representation of numbers and automated access to the internal mental number line, including an improved association between representations of numbers and space, the understanding of ordinality of numbers, estimation, and arithmetical skills. Therefore, the evaluation of possible training effects included a variety of numerical skills ranging from number line performance and ordinality judgements to arithmetic. The training consists of a number line 0–100 that is displayed at the bottom of the screen, while from the top of the screen a spaceship appears carrying an Arabic digit, an addition or subtraction problem, or a set of dots. The player is asked to steer the spaceship to the exact location on the number line corresponding to the Arabic digit, the result of the addition or subtraction problem, or the estimated number of dots displayed on it. If the child lands within a range of ± 10 of the correct position, the challenge is rated as successful. Immediately after landing, the exact position within the range of ± 10 is given as feedback. The training consists of 30 levels with increasing difficulty. Each level is built of 75 trials, resulting in 2250 trials for all levels. The next level can be reached when each problem on the current level has been solved correctly. Incorrectly solved tasks are repeated until they are solved successfully to support learning. Thus, a major virtue of the training is that it works in an adaptive way and each child trains at her or his individual performance level and speed. To sustain motivation and focus attention, the rocket flies with a speed that can be accelerated or decelerated to the initial velocity, and motivating feedback appears when the child has performed very well or very badly. For further description of the training procedure, please see Kucian et al. (Kucian et al., 2011a).

2.4. Brain imaging

2.4.1. Functional MRI paradigm

To investigate possible effects of the number line training on brain function, children performed a numerical order judgement task in the scanner, since the mental representation of ordinal sequences is spatially organized and therefore related to the internal mental number line representation (e.g. Gevers et al., 2003; Rubinsten and Sury, 2011; Goffin and Ansari, 2016). The fMRI task was comprised of two conditions: numerical order and control. In both conditions three single-digit Arabic numbers were presented horizontally via video goggles (MRI Audio/Video System, Resonance Technology, Inc., USA) and button press answers were recorded by means of the response box (LUMINA, Cedrus Corporation, San Pedro, USA). In the order condition, children were instructed to judge whether the three numbers were in ascending or descending order. In the control task, they had to distinguish whether one of the digits was a "2" or not. The contrast between order and control condition was selected to map numerical ordinality. The paradigm was programmed on E-Prime (E-Prime, Psychology Software Tools Inc.). It lasted 10.5 min and consisted of four epochs of the order and four epochs of the control condition. Epochs of order and control tasks were presented in a counter-balanced manner between subjects. A fixation cross was displayed for 20 s between any epoch. Each epoch included 10 trials, each of which was presented for 2 s, followed by a blank screen. The inter-stimulus-interval was jittered between 3 and 5 s. For further details, please see (Kucian et al., 2011b).

2.4.2. FMRI data acquisition

FMRI data was collected on a 3 T GE scanner (GE Medical Systems, Milwaukee, WI, USA), equipped with an 8-channel head coil. Thirty-six slices were acquired parallel to the anterior commissure – posterior commissure line. Others parameters were: slice thickness: 3.4 mm, matrix size: 64 × 64, field of view: 220 mm × 220 mm, flip angle: 45°, echo time: 31 ms, and repetition time: 2100 ms. Three-dimensional anatomical images of the entire brain were obtained with a T1-weighted gradient echo pulse sequence (number of slices: 172, slice thickness: 1.0 mm, repetition time: 9.972 ms, echo time: 2.912 ms, field of view: 240 mm × 240 mm, flip angle: 20°, and matrix size: 256 × 192). Cushions were placed around participants' heads to minimize head movement.

2.5. Data analyses

2.5.1. Behavioural data

Statistical analysis of behavioural data was performed with IBM SPSS Statistics Version 22. A repeatedmeasures general linear model (GLM) analysis was conducted to evaluate training effects (pre-/post-training) as within-subject factor and group (DD/TD) as between-subject factor. Effects of training, training x group interaction, as well as tests of between group effects are reported. In the case that one of these effects turned out to be significant, post-hoc *t*-tests were conducted. In addition, partial eta-squared (η2) effect sizes are reported, whereas effects 0.01–0.06 are interpreted as small effects, 0.06–0.14 as medium effects, and > 0.14 as large effects (Cohen, 1988). Pearson-Chi-squared tests were used for nominal data comparisons between groups.

2.5.2. FMRI data pre-processing

SPM5 (Wellcome Department of Cognitive Neurology, London, UK) was used for all prepossessing steps. The steps included head motion realignment, coregistration of the individual T1-images to the first motion-corrected functional image of each subject, normalisation of the T1-images an age-matched paediatric template (CCHMC paediatric brain template, http://www.irc.cchmc.org/ped_brain_templates.htm), transformation of normalisation parameters to the realigned functional images, and spatial smoothing (6 mm Gaussian kernel). All children fulfilled the inclusion criteria of (1) fMRI task performance above chance level, (2) mean scan-to-scan displacement < 1 mm (for the pre- and post-training fMRI run) and (3) head rotation below 1° in pitch, jaw or roll direction. Movement parameters did not differ between DD and TD between the pre- and post-training sessions (all $p > 0.13$). Within each group, no differences in movement parameters were seen between the pre and post training session (all $p > 0.16$). No differences were seen in the mean scan-to-scan displacement between groups and session (all $p > 0.21$).

2.5.3. Functional connectivity (FC) analysis

All FC analyses (using the pre-processed fMRI data) were performed with the Conn toolbox (version 15b, Whitfield-Gabrieli and Nieto-Castanon (Whitfield-Gabrieli and Nieto-Castanon, 2012)). Additionally, data were temporally filtered (0.01–0.1 Hz) as is common for fMRI FC analysis (Biswal et al., 1995). In addition to the six motion parameters, white matter- and cerebrospinal fluid signals were used as covariates of no interest to reduce variance unlikely to reflect functional connectivity-related neuronal activity (Fair et al., 2007; Villalobos et al., 2005). Only the white matter and cerebrospinal fluid signals were removed to avoid any bias introduced by removing the global signal (*i.e.*, grey matter) (Behzadi et al., 2007; Murphy et al., 2009). This denoising step should 'normalize' the distribution of voxel-to-voxel connectivity values as effectively as including the global signal as a covariate of no interest, but without the potential problems of the latter method. Although we did not record respiration and cardiac responses, it has been demonstrated that non-neuronal physiological noise (*e.g.*, cardiac and respiratory signal) can successfully be removed by the CompCor algorithm (Behzadi et al., 2007), as implemented in the Conn toolbox. After the denoising step, we visualized the distribution of voxel-to-voxel connectivity for each step. Participants were only included if the values were normally distributed.

For each individual, the fMRI time-series were extracted for the seed region of interest using MarsBaR (Brett, Anton (Brett et al., 2002); http://marsbar.sourceforge.net/). As seed, we selected the bilateral IPS with identical coordinates (left: −28, −64, 42, right: 32, −60, 44) to a previous FC study in DD (Rosenberg-Lee et al., 2015). The size of each seed was determined as a 6-mm-diameter sphere.

First, we investigated FC related to spontaneous background activity to assess FC differences related to the general task engagement. For each subject, we defined one regressor that spanned the whole time interval of the fMRI run. The effect of task conditions (*i.e.*, pre and post ordinality blocks) was regressed out by moving them to the first-level covariates list (Fair et al., 2007). On the second level, we first estimated the main effects (F-tests) of group (TD or DD) and time (before and after training) using a 2 × 2 analysis of variance. We also calculated group x time interaction effects. In case of significant main effects, we applied planned contrasts (using *t*-tests) to assess the within and between group differences prior and after number line training. This results in eight group comparisons: TD pre *vs.* TD post (and vice versa), DD pre *vs.* DD post (and vice versa), TD pre *vs.* DD pre (and vice versa), and TD post *vs.* DD post (and vice versa). Further, we compared DD post *vs.* TD pre to have further estimate of abnormal FC pattern in DD. In a subsequent analysis, we included IQ as nuisance regressor to elucidate if any TD vs. DD FC difference (before and after training) was modulated by IQ, as IQ differs between TD and DD. As it has been argued that parametric analysis of fMRI data are not always warranted (Eklund et al., 2012), we repeated the analysis for the two main effects of interest: TD pre *vs.* DD pre (and vice versa) and DD post *vs.* TD pre (and vice versa), using non-parametric tests with a voxel threshold of $p < 0.001$ (uncorrected) and a cluster-mass correction of $k > 32$ voxels to achieve a $p < 0.05$ (corrected).

In addition, and similar to Rosenberg and colleagues, we applied a PPI analysis for the two contrasts of interests (*i.e.* TD > DD and DD > TD) prior and after number line training. In our case, PPI measures the temporal relation between the IPS seed regions and all other brain voxels after accounting for the common driving influence of task activity on both the IPS seed and target voxel (Friston et al., 1997). We used a generalized form of PPI (gPPI) as implemented in Conn (McLaren et al., 2012). The gPPI method has the flexibility of estimating task-dependent FC for more than one task condition, as it models all condition effects and interactions simultaneously in a single model instead of using a separate model for each task condition tested. Simulation and empirical studies have shown that gPPI is more powerful than the standard PPI implementation in SPM, and is especially suited to assessing FC in block design experiments (Cisler et al., 2014). At the individual participant level, we included: (1) a psychological variable representing the two types of conditions (control condition and numerical order condition), (2) one physiological variable (*i.e.* the time course in the seed region), and (3) a PPI term (*i.e.* the cross product of the first two regressors). As for the background FC analysis, we used the bilateral IPS as seed region to determine between-group differences.

FC comparisons are initially calculated at a voxel threshold of $p < 0.001$ (uncorrected for multiple comparisons, $t > 3.2$). We additionally applied a cluster-correction (using AlphaSim, afni.nimh.nih.gov/afni/doc/manual/AlphaSim) of $k > 32$ voxels to achieve $p < 0.05$, corrected, and these results are reported in the paper. The voxel-threshold is identical to a previous report examining FC in DD (Rosenberg-Lee et al., 2015).

2.5.4. Multivariate classification analysis

In order to validate the FC results, we applied statistical pattern recognition analysis in order to test if (task-related) brain activity can be used to discriminate DD from TD (before and after number line training). Brain activity maps (ordinal task – control, $p < 0.001$, $t > 3.2$ uncorrected) were taken from the fMRI results described in our previous publication (Kucian et al., 2011b). The (freely-available) software PRoNTo (http://www.mlnl.cs.ucl.ac.uk/pronto/) (Schrouff et al., 2013) was used to perform the multivariate classification analysis with a classification algorithm using a linear model. Leave-One-Out cross-validation was applied and contained the following steps: (1) dividing the data in n-folds (*i.e.* number of subjects), (2) using n-1 folds as training data (the remaining fold is the test data), (3) rotate which fold is the test data, (4) estimate the result for each fold and an overall result. The pre- and post-training brain activity maps were used as input feature sets to a pattern based classifier. Classification accuracy (see below) was estimated in % and accuracies for each subject were combined at the group level in an unpaired *t*-test and were compared to chance level. Chance level was 50%, since we had a two-class problem (TD and DD before training, and TD and DD after training).

The accuracy is the total number of correctly classified test samples divided by the total number of test samples N, irrespective of class. The accuracy is exactly equivalent to:

$$\text{Accuracy} = 1 - \frac{1}{N}\Sigma_n l01(\mathrm{y_n},\ \mathrm{f/x_n})$$

where $l01(y_n, f/x_n)$ is a 0–1 loss function that counts each classification error as costing 1 and each classification success as costing 0. The fMRI data was mean corrected (z-transformed) before computing the classification analysis, in order to ensure that group differences were independent of differences in task-related activity levels.

2.5.5. Bayesian power estimation analyses and "classical" power analysis

In terms of sample size, we used Bayesian power estimation analysis, which provide full distributions of credible values for group means and their differences (Kruschke, 2013). Specifically, it was tested if a pre-training between-group difference −based on the available fMRI data- was seen using our sample size. We additionally performed a "classical" power analysis (based on the means' and SDs of each sample) for a mask including all significantly activated clusters (see Table 4 in (Kucian et al., 2011b)) showing an fMRI group difference (TD *vs*. DD) using a confidence interval of 95% (alpha error rate of 5%). This analysis was repeated for all individual clusters for this comparison too (Kucian et al., 2011b).

3. Results

3.1. Demographic and cognitive measures

Groups were matched for age, gender, and handedness. All children completed more than 19 training days within 5 weeks and groups did

Table 2
Training effects of children with developmental dyscalculia (DD) and typical developing children (TD).

	DD		TD		Training effects	
Training:	pre	post	pre	post	p-value	Partial eta-squared
fMRI task accuracy (Mean (SD) %)	69.7 (15.7)	77.8 (16.0)	83.9 (12.2)	86.5 (10.0)	n.s.	–
fMRI task reaction time (Mean (SD) ms)	1722.0 (398.1)	1788.4 (431.5)	1829.8 (326.7)	1790.0 (457.6)	n.s.	–
Number line[a] (Mean (SD) % distance)	10.2 (3.1)	7.0 (1.8)	7.8 (2.0)	6.0 (1.1)	< **0.001**	**0.469**
Arithmetic accuracy[b] (Mean (SD) %)	74.7 (18.4)	82.5 (11.7)	92.0 (4.3)	94.4 (6.0)	< **0.005**	**0.380**
Spatial memory span[c] (Mean (SD) total score)	4.7 (0.6)	4.8 (0.7)	4.9 (1.0)	5.2 (0.9)	n.s.	–
Spatial working memory[d] (Mean (SD) total score)	2.2 (0.4)	2.3 (0.5)	2.8 (0.9)	3.1 (0.9)	n.s.	–

[a] Number line performance is based on mean percent distance of digits, additions, subtractions, and dots between exact and indicated location on a number line 0–100.
[b] Arithmetic is based on percent correctly solved additions and subtractions of totally 40 arithmetical problems.
[c] Spatial memory span is based on total score of CORSI-Block-Tapping test.
[d] Spatial working memory is based on total score of CORSI-Block-Suppression test.

not differ with respect to the amount of training days. DD children's numerical and calculation abilities based on the ZAREKI-R were significantly lower compared to TD children ($p < 0.001$). IQ values were normally distributed (TD: $p = 0.24$, DD: $p = 0.06$; one-sample Shapiro-Wilk test) and were in the normal range for both groups, but differed significantly between groups ($p < 0.01$). Please see Table 1.

3.2. Training effects

3.2.1. fMRI task performance

Neither TD nor DD showed ceiling effects with regard to fMRI task performance before the intervention, leaving enough room for improvement after the training (Table 2). 100% task accuracy was only achieved by one TD (before training) and one DD (after training). A repeated-measures GLM analysis with mean accuracy rate or reaction time of the numerical order task as within-subject factor (pre-/post-training) and group as between subject factor (DD/TD) showed no significant training effects or interaction for the number of correctly solved trials or mean reaction time of correct solved items during the fMRI examination (Table 2). Tests of between group effects revealed significant group differences in accuracy ($F(1, 22) = 5.630$, $p < 0.05$, $\eta^2 = 0.204$), but not for reaction time. Post-hoc tests for accuracy between groups indicated lower performance of DD compared to TD before training ($p < 0.01$) and after training ($p < 0.05$).

3.2.2. Number line performance

Significant training effects and group differences were found for the number line performance. Repeated-measures GLM analysis with percent distance between exact and indicated location on a number line 0–100 as within-subject factor (pre-/post-training) and group (DD/TD) as between-subjects factor revealed a significant main effect of training ($F(1, 29) = 25.65$, $p < 0.001$, $\eta^2 = 0.469$) and no interaction between training x group. Hence, children were significantly more accurate in the number line task after training. Test of between group effects was significant as well ($F(1, 29) = 8.458$, $p < 0.01$, $\eta^2 = 0.226$). Post-hoc analyses for group differences pre and post training indicated that DD children performed less accurately before the training compared to TD ($p < 0.05$), but this significant difference was no longer evident after the training.

3.2.3. Arithmetic

Significant improvements in arithmetic after training and group differences were found. Repeated-measures GLM analysis, using the mean of correctly solved addition and subtraction trials as within-subject factor (pre-/post-training) and group as between subjects factor (DD/TD), revealed a significant main effect of training ($F(1, 23) = 14.12$, $p < 0.005$, effect size partial eta-squared = 0.380). This indicates that both groups increased in arithmetical performance after training (Table 2), but the interaction between training x group was not significant. Test of between group effects was significant ($F(1, 23) = 13.689$, $p < 0.005$, $\eta^2 = 0.373$). Post-hoc analyses between groups showed that TD outperformed DD children in arithmetic before ($p < 0.005$) and after training ($p < 0.005$).

3.2.4. Spatial memory span and spatial working memory

The training had no effect on spatial memory span or spatial working memory. Repeated-measures GLM revealed no significant training effects or interactions between groups and task performance for both general cognitive memory skills (spatial memory span and spatial working memory). Please see Table 2. Test of between group effects showed significant group differences for spatial working memory ($F(1, 29) = 10.123$, $p < 0.005$, $\eta^2 = 0.259$), but not for spatial memory span. Subsequent *t*-test analyses showed that DD children performed worse in spatial working memory before ($p < 0.05$) and after the training ($p < 0.01$).

3.2.5. Background FC analysis

Fig. 1 shows IPS-related within-group FC for the two examined groups (TD and DD) before (left columns) and after the training (right columns). Already this visual illustration indicates hyperconnectivity in the DD pre-training group relative to the TD pre-training group, which drops after number line training. Indeed, we observed a main effect of group ($F(1, 29) = 3.83$, $p < 0.001$) in several brain regions. To examine this in more detail, we calculated between-group FC differences before training (Fig. 2 and Table 3A) as well as within (Fig. 3) and between FC group (Fig. 4 and Table 3B) differences after training. Since IQ significantly differed between TD and DD, we performed FC group analyses (comparing DD − TD before and after training) using IQ values as a nuisance variable in the statistical model. FC findings did not lead to qualitative changes, *i.e.* hyperconnectivity was evident in the same brain regions in DD (relative to TD) and this pattern disappeared after training (TD – DD, $p > 0.05$, two-sided *t*-tests; results not shown). The same pattern of FC group differences was observed using age as a nuisance variable, since the age range was wide (7.8–11.8 years) but not significantly different between groups.

a) Between-group differences before number line training

DD demonstrated hyperconnectivity relative to TD in parietal, frontal, visual, cerebellar, and temporal brain regions (Fig. 2). A full list of between-group differences before number line training is listed in Table 3A.

- Training effects

The 2 × 2 analysis of variance revealed a main effect of time ($F(1,29) = 3.41$, $p < 0.001$) as well as a group x time interaction ($F(1,29) = 3.72$, $p < 0.001$) in numerous brain regions, the latter indicating that number line training mediates between-group FC differences. Comparing pre *vs.* post number line training effects in

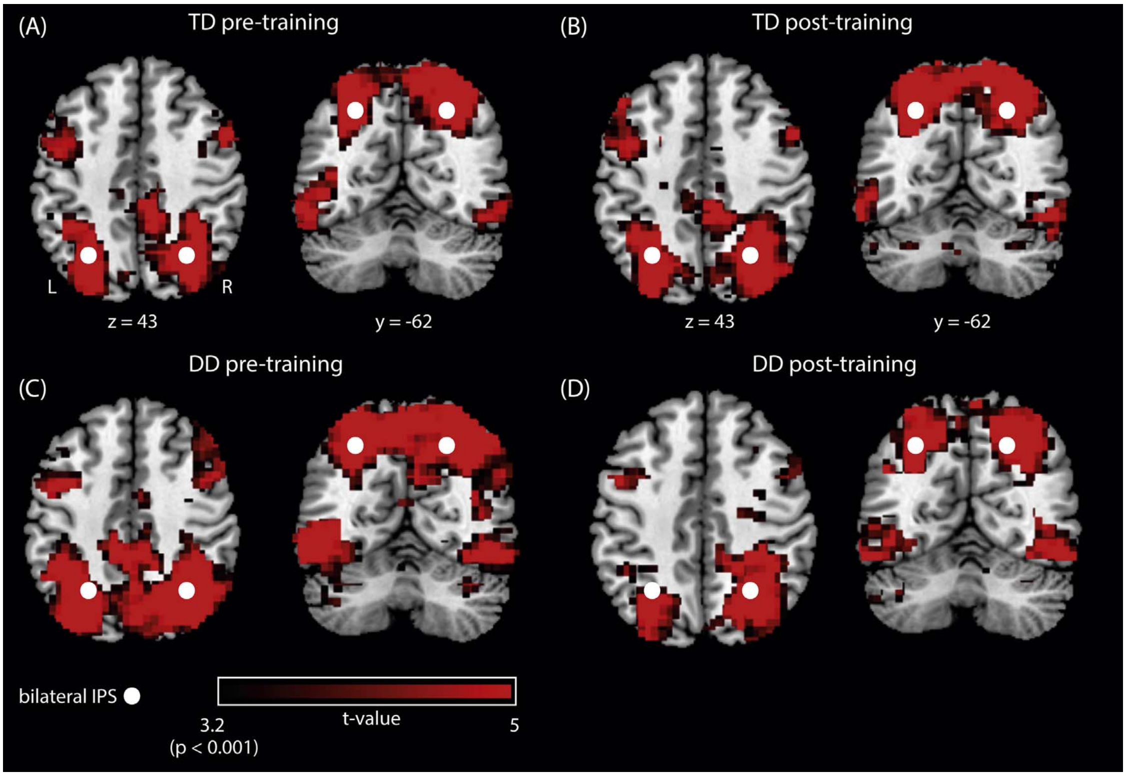

Fig. 1. Functional connectivity results before (A and C) and after (B and D) number line training for typically developing (TD) children (top row) and children with developmental dyscalculia (DD) (bottom row). The seed for the background FC analysis was placed bilaterally in the IPS (highlighted by the white dots in all panels). All results are shown at $p < 0.001$ (uncorrected, $t > 3.2$).

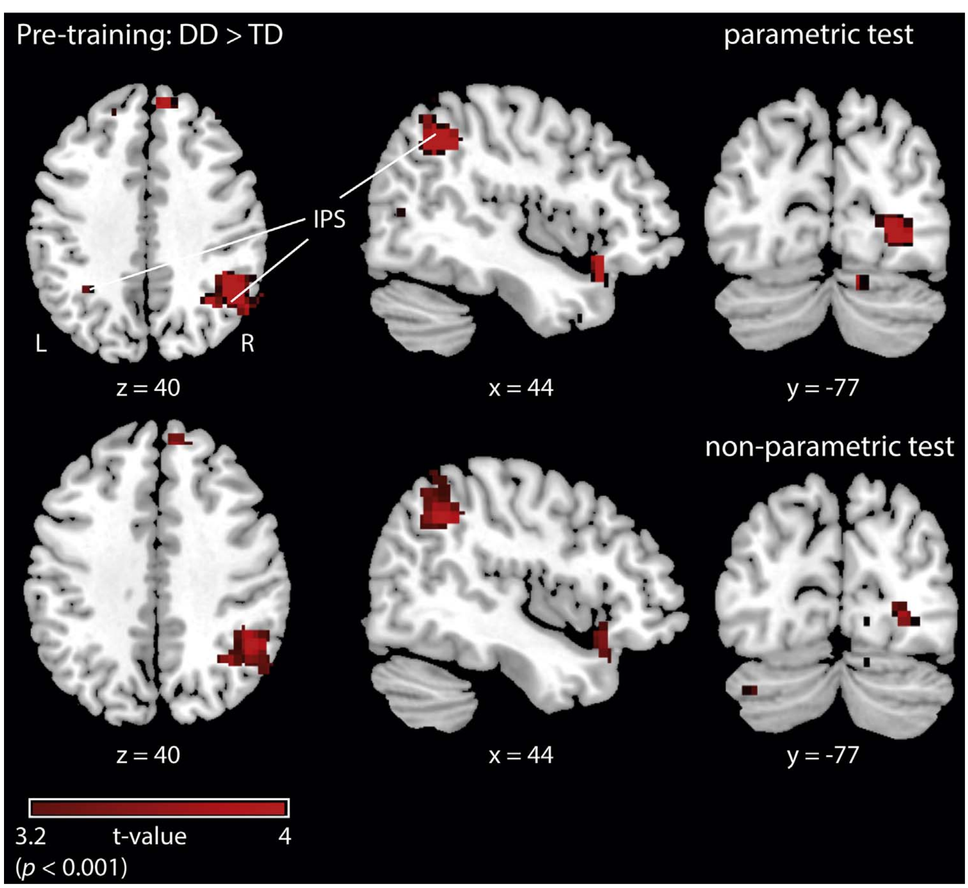

Fig. 2. Background FC maps before number line training. DD showed hyperconnectivity in frontal, parietal, and visual areas relative to TD. For example, DD showed higher FC in the bilateral parietal cortex, such as the intraparietal sulcus (IPS). Results for both contrasts (TD > DD and DD > TD) are summarized in Table 3. All results are shown at $p < 0.001$ (uncorrected, $t > 3.2$). The seed was placed in the bilateral IPS.

Table 3
Background functional connectivity analysis. Summary of within- and between-group functional connectivity differences before and after number line training. All effects are reported at a height threshold of $p < 0.001$ with additional cluster correction of $k > 32$ voxels (corresponding to $p < 0.05$, corrected). The seeds were placed in the bilateral intraparietal sulcus (left: −28, −64, 42, right: 32, −60, 44). DD: developmental dyscalculia, TD: typically developing children. We did not observe significant differences in FC for the following contrasts: TD pre > TD post, DD post > DD pre, TD post > DD post, and DD post > TD post.

Pre-Training effects

TD pre > DD pre

Region	MNI coordinate	Cluster size	t-value
Left putamen	−21 12 9	38	3.6
Right paracentral lobe	6–28 80	37	4.2
Left anterior cingulum	−2 18 26	36	3.2
Left anterior insula	−30 23 12	33	3.2

DD pre > TD pre

Region	MNI coordinate	Cluster size	t-value
Right inferior parietal lobule	48 −50 39	188	4.6
Left inferior parietal lobe	−28–51 40	41	3.6
Right precuneus	17 −66 30	61	3.8
Right cuneus	20 −70 18	43	3.4
Right superior frontal gyrus	17 46 48	84	3.5
Right inferior frontal gyrus/insula	43 19 −12/44 16 −9	198	3.3
Left inferior frontal gyrus/insula	−37 18 −19	83	3.3
right inferior (anterior) temporal lobe	40 5 −45	133	3.7
Right inferior temporal lobe	57 −46 −17	96	3.6
Left inferior temporal lobe	−57 −20 −23	50	3.4
Right cerebellum (Crus1)	15 −80 −22	56	3.4
Left cerebellum (Crus 1)	−32 −79 −32	82	3.9
Left cerebellum (Crus 2)	−6 −88 −32	378	3.4
Right fusiform gyrus	29 −48 −4	34	3.7
Right fusiform gyrus	30 −77 −1	33	3.9
Left fusiform gyrus	−30 −75 −11	56	3.7
Left parahippocampus	−17 −21 −14	55	3.5
Left inferior occipital gyrus	−32 −76 −7	66	3.6
Right lingual gyrus	23 −63 −5	32	3.7

Post-Training effects

TD post > TD pre

Region	MNI coordinate	Cluster size	t-value
Left thalamus	−13–26 6	44	3.6
Right inferior temporal lobe	61 −23 −22	83	3.2
Right cerebellum (culmen)	28 −56 −33	33	3.4
Left cerebellum	−3 −62 −37	97	3.4
Left insular cortex	−43 −17 0	39	4.8
Right inferior frontal gyrus	52 15 4	91	4.7

DD pre > DD post

Region	MNI coordinate	Cluster size	t-value
Left lingual gyrus	−16–66 −9		3.6
	−10 −71 3	40	3.3
Left cuneus	−13 −84 33	33	3.2
Right precuneus	27 −80 39	46	3.9
Left precuneus	−19 −80 43	251	3.6
Left precentral gyrus	−43 −2 38	42	3.7

DD post-training > TD pre-training

Region	MNI coordinate	Cluster size	t-value
Right inferior parietal lobe	40–56 40	78	3.2

TD, no brain regions survived the statistical threshold (Fig. 3A). However, completion of number line training in TD induced subtle increases in FC in the left thalamus, bilateral insular cortex, cerebellum, and right inferior temporal lobe (Fig. 3B, Table 3B). In contrast, DD showed stronger FC prior to number line training relative to TD in several areas such as the insular cortex, superior and inferior frontal cortex, inferior and parietal and temporal lobe (Fig. 3C, Table 3A). After number line training, no brain region showed hyperconnectivity in DD *versus* TD. Within the DD group, hyperconnectivity was present in the pre-training condition relative to post-training (Table 3B). A direct comparison of the two groups indicated that none of the brain regions showed significantly different FC after number line training.

To illustrate the effect of training in DD in more detail, Fig. 4 shows brain regions with higher FC in DD before and after the training relative to the TD FC pre-training map on an inflated brain template. Remarkably, only a small cluster in the right IPS showed hyperconnectivity in DD after the training relative to TD pre-training map. After the training, no significant FC differences were evident between groups, such that the contrasts TD post > DD post and DD post > TD post showed no significant differences, at the preselected voxel-threshold of $p < 0.001$.

The overlap of FC maps for DD and TD after number line training is shown in Fig. 5. Interestingly, TD and DD share common FC in most of the correlated brain regions, including frontal and parietal brain areas.

We repeated the analysis using non-parametric testing (see Methods) for the two main contrasts of interests: TD pre vs. DD pre (and vice versa) and DD post vs. TD pre. Connectivity maps show no qualitative differences, *i.e.* the same regions reveal connectivity differences for both types of analyses. The only difference was that the size and number of clusters differ with respect to the type of analysis. For example, the IPS-related connectivity to the cerebellum involved other sub-parts of the cerebellum too using non-parametric testing. One region that showed a difference in functional connectivity (comparing DD pre and TD pre) comparing parametric and non-parametric testing was the right inferior temporal cortex (evident during non-parametric testing).

As seen for the results using parametric tests, comparing DD post vs. TD pre revealed the lack of any connectivity differences using non-parametric testing.

3.2.6. gPPI analysis

The gPPI results mostly resembled the background FC results. We observed main effects for group ($F(1,29) = 4.2$, $p < 0.001$) and time ($F(1,29) = 3.5$, $p < 0.001$), and a group x time interaction ($F(1,29) = 3.9$, $p < 0.001$). The main contrast of interest (DD pre > TD pre) is shown Fig. 6 and summarized in Table 4. In contrast to the background FC analysis, task related FC before number line training was especially enhanced in DD in prefrontal rather than in parietal and visual brain regions. For the contrast DD post – TD post, no significant differences were seen (results not shown). This was also true – and hence identical to the background FC analysis – for the contrasts TD post > TD pre and DD post > DD pre.

3.2.7. Power analysis and multivariate classification analysis

Before running the multivariate classification analysis, we examined if the sample size was large enough to detect between-group differences using the available fMRI data. The Bayesian analysis indicated that before number line training, between-group (*i.e.* TD > DD) differences in brain activation could be identified with a confidence interval of 95% using our sample size. Furthermore, the "classical" power analysis showed that sample size of even n = 14 (for both TD and DD) was required to achieve a power of 80% (alpha error of 5%) to detect a group difference. In our analysis, we used an n = 16 (TD) and n = 15 (DD), respectively, which results in an overall power of 93.9% (alpha error of 5%). We also extracted the effect sizes of each region reported in the paper by (Kucian et al., 2011b) separately to check if the power is > 80% for different clusters. We found that 9 out of 13 regions showed a power of > 80% (mean 91.2%), whereas the mean power across all 13 regions was 82.5%. This suggests that the selected sample size was sufficiently large to report the detected group differences for the connectivity analysis.

The multivariate classification analysis revealed a classification accuracy to differentiate TD from DD was 86.3% ($p = 0.003$) before number line training and 38.3% ($p > 0.05$, *i.e.* below chance level) after training (Fig. 7). This result confirmed the FC results, reflected by a neuronal normalization after number line training on the task-related brain activity level.

4. Discussion

Our study provides three central neuroimaging findings: First, we found increased functional connectivity (using both background and PPI based FC measures) in frontal, parietal, temporal, and visual brain regions in children with DD prior to number line training relative to TD which cannot be explained by group differences in IQ. Second, no significant hyperconnectivity was evident in DD participants after training, and FC in DD after training was non-distinguishable from FC observed in TD (prior to training). Third, classification analysis revealed a high classification rate before but not after number line training.

4.1. Behavioural results

The training had positive effects on several behavioral measurements, which are underpinned by obtained effect sizes that can all be interpreted as large effects according to Cohen (Cohen, 1988). In particular, children improved significantly on number line performance and arithmetic. Hence, children were more accurate in the paper and pencil number line task and solved more addition and subtraction trials correctly after the training. The goal of the training was to improve the mental number representation and for instance never trained explicitly arithmetic skills. Therefore, our results nicely show a transfer effect of trained number line performance to arithmetical performance.

fMRI task accuracy or reaction time showed no training effects, but TD solved more trials correctly compared to DD before training, whereas after training equal numbers of trials were correctly solved by both groups. Since fMRI task accuracy was not at the ceiling level before and after training in any of the examined groups, we conclude that the effects reflect not simply test-retest effects or regression to the mean but are rather effects of the training intervention.

Regarding spatial working memory span and spatial working memory, the training did not influence these cognitive skills, which strengthens the idea that the training improves domain-specific abilities and not domain-remote cognitive skills, such as working memory.

As expected, DD children performed generally lower compared to TD (accuracy rate of numerical order fMRI task pre and post training, paper and pencil number line task pre training, arithmetic, and spatial working memory). However, as demonstrated, DD children were able to improve specifically their numerical skills significantly due to intervention and could even catch up to the level of TD children in number line performance.

These behavioral improvements might be the consequence of a functional reorganization, *e.g.* re-balanced FC (see next paragraphs).

4.2. FC before number line training

Using the IPS as seed region, we noted stronger (background and task-related gPPI) FC in the DD group than in TD. This finding is consistent with results from two recent studies comparing DD and TD in a similar age range during a subtraction task (Rosenberg-Lee et al., 2015) and during rest (Jolles et al., 2016). Similar to the study by Rosenberg and colleagues we observed hyperconnectivity in DD in prefrontal and parietal brain regions. These regions have been discussed

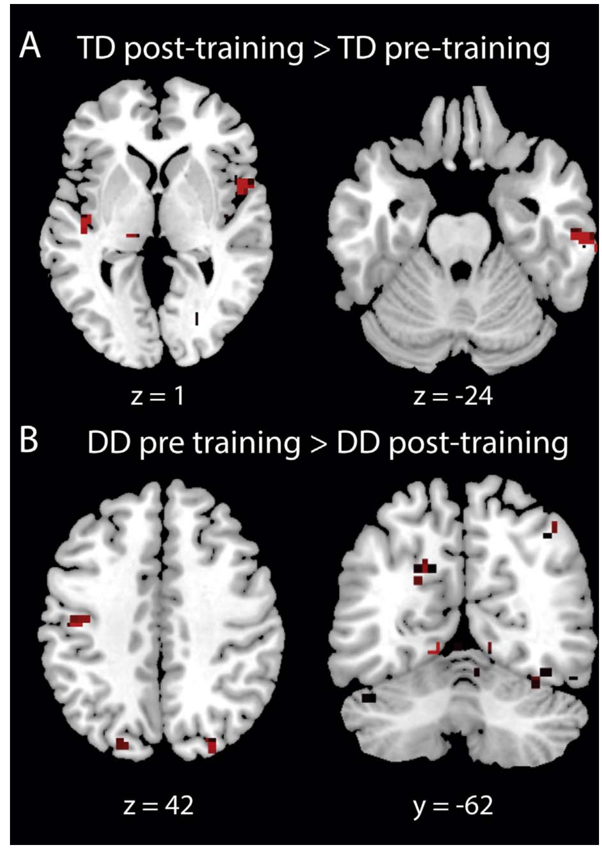

Fig. 3. Within-group background FC maps for number line training effects. (A) TD post- vs. TD pre-training revealed connectivity differences ($p < 0.001$, uncorrected, $t > 3.2$) in the insular and inferior frontal cortex as well in the cerebellum (not shown), left posterior thalamus, and inferior temporal gyrus. (B) DD showed higher FC before than after training in visual and precentral brain regions.

as core regions in numerical operations (Menon, 2014), such as numerical magnitude processing and mental arithmetic. Aberrant functional responses in these areas have been reported in several studies in children with DD and MLD (Kucian et al., 2011b; Iuculano et al., 2015; Supekar et al., 2015). In general, hyperconnectivity (prior to training) in DD can occur in three ways. First, it can be present as a spatially unspecific pattern, *i.e.* increases in connectivity are observable in both task-relevant (*i.e.* prefrontal and parietal brain regions) and task-irrelevant brain regions. Second, it can occur only in non-task related brain regions. Third, it can be present only in task-relevant areas. Our data rather support the first notion as we found hyperconnectivity in and outside task-relevant areas before training (Fig. 3). However, after training, FC in task-irrelevant areas disappeared and the remaining FC overlaps with task-related fMRI signal responses. We conclude that functional aberrations in DD most probably reflect the need for greater neuronal resources during numerical ordinality judgement, rather than the inability to activate task relevant areas, as we observed numerous FC increases outside task-related brain regions.

We observed hyperconnectivity not only in prefrontal and parietal but also in visual brain regions in DD compared to TD. Background connectivity captures general task engagement rather than condition-specific connectivity (Norman-Haignere et al., 2012). Consequently, DD appears to be involved differently during the time course of task procedure than TD. Using fMRI, we previously reported different responses not only in the IPS but also in the fusiform gyrus during a non-symbolic distance task (Kucian et al., 2011a), similar to that used in the present study. In addition, a recent study found that hyperconnectivity in the fusiform gyrus (and fronto-parietal regions) normalized after training in children with high math anxiety (Supekar et al., 2015). Hence, we suggest that number line training minimizes any task-

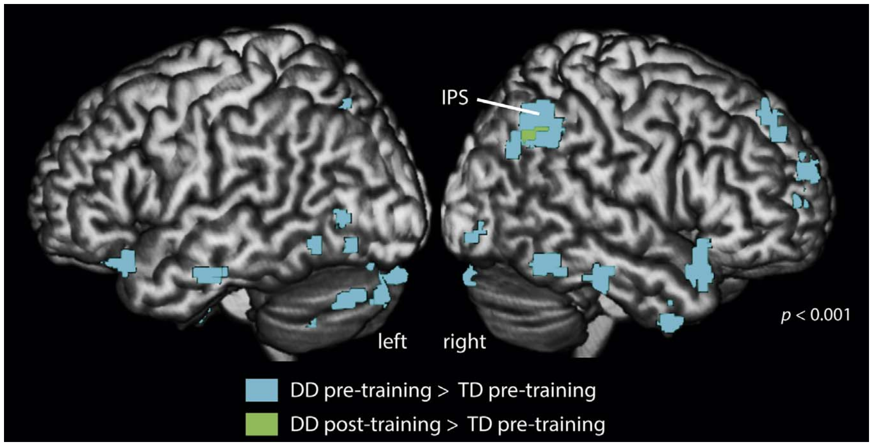

Fig. 4. Number line training FC results (before and after number line training) on a render brain. Stronger FC in the DD group is evident in right frontal, bilateral temporal, visual, cerebellar, and parietal regions (blue spots) relative to TD. After number line training, hyperconnectivity disappeared and is only present in a small, right-hemispheric parietal region (green spot). All results are shown at $p < 0.001$ ($t > 3.2$). The seed was placed in the bilateral IPS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

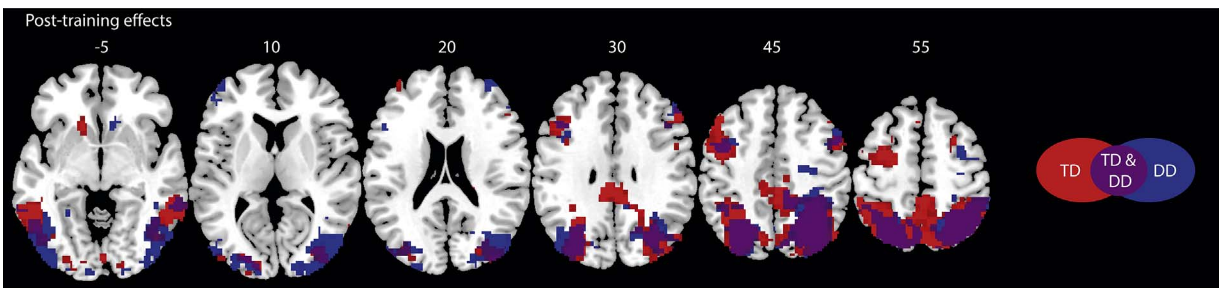

Fig. 5. Overlap between post-training FC connectivity after in TD (red) and DD (blue) on different axial slices. The overlap is highlighted in purple. All results are shown at $p < 0.001$ ($t > 3.2$). The seed was placed in the bilateral IPS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

irrelevant FC during the ordinality judgement task.

In addition, DD in our study showed stronger pre-training FC in various temporal brain regions. The medial temporal lobe plays a pivotal role in memory encoding, and lower fMRI signal responses in this region have been linked to brain maturation (Menon et al., 2005). However, it has also been argued that – amongst the prefrontal and parietal cortex – the temporal lobe is involved in number processing (Serra-Grabulosa et al., 2010). For example, children with MLD display reduced involvement of verbal medial temporal lobe, IFG, superior temporal gyri, and numerical (IPS) brain regions when solving multiplication problems (Berteletti et al., 2014). Conversely, TD children showed a modulation of activation with problem size in verbal regions. This suggests that TD children were effectively engaging verbal mechanisms for easier problems.

In summary, our results – on the pre-training level – give further support for the notion that DD (as MLD) is associated with functional alterations in multiple brain regions in addition to the IPS.

4.3. FC after number line training

In our study, the pre-training hyperconnectivity in DD was present in brain regions that have been previously linked to numerical problem solving (Kucian and von Aster, 2015; Fias et al., 2014; Kucian et al., 2015). A 5-week training of number line skills not only increased post-training number line and arithmetic skill performance but also diminished hyperconnectivity in the DD group. This result was verified in several analyses. First, FC maps do not differ between TD and DD (after training) even at an unconventional low statistical threshold ($p < 0.01$ uncorrected). Second, hyperconnectivity was seen in the DD pre *versus* post-training FC map. This was not the case (in any brain region) for the reversed contrast. Third, the "DD post-training map" was non-differentiable from the "TD pre-training map", indicating a normalized FC pattern in DD. This is a novel finding, as no study has yet compared the influence of repetitive number line training on brain connectivity in DD. Our result are consistent with those from Iuculano et al., which

Table 4
Generalized PPI functional connectivity analysis. Summary of between-group pre- and post-training number line training effects on functional connectivity. All effects are reported at a voxel height threshold of $p < 0.001$ ($t > 3.2$) with additional cluster correction of $k > 32$ voxels (corresponding to $p < 0.05$, corrected). The seeds were placed in the intraparietal sulcus (left: −28, −64, and 42, right: 32, −60, and 44). DD: developmental dyscalculia, TD: typically developing children.

Pre-Training effects			
DD pre > TD pre			
Region	MNI coordinate	Cluster size	t-value
Right insular cortex	41 12 −7	50	3.6
Left superior frontal cortex	−10 41 32	36	3.4
Right superior frontal cortex	30 42 38	72	3.7
Left inferior frontal gyrus	−39 18 −12	33	3.3
Right inferior parietal lobe (IPL)	57–41 41	55	4.1
Right inferior temporal cortex	52 −16 −20	135	3.3
Right hippocampus	33 −14 −12	42	3.7
Left cerebellum	−4 −57 −34	47	3.4
TD post > DD post			
Region	MNI coordinate	Cluster size	t-value
n.s.			

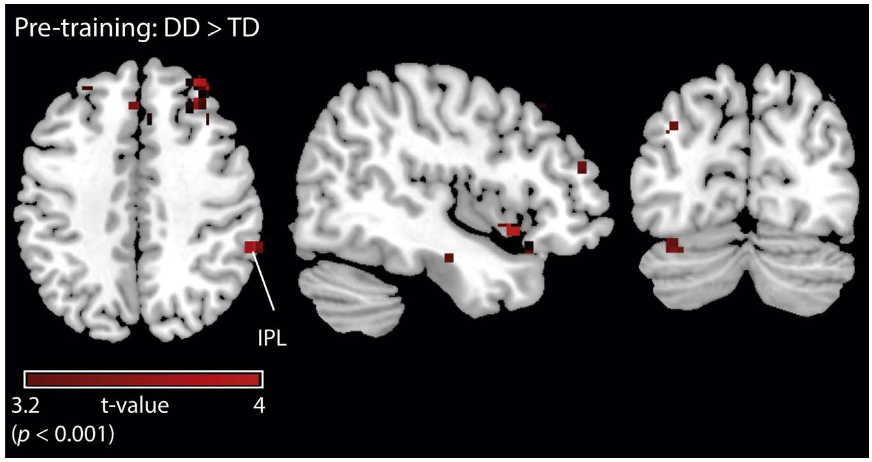

Fig. 6. Summary of pre-training results using a generalized psychophysical interaction (gPPI) FC analysis. DD showed hyperconnectivity relative to TD in frontal, parietal, temporal, and cerebellar brain regions. A full list of gPPI FC results is given in Table 4. IPL: inferior parietal lobe.

demonstrated neuronal normalization in children with MLD after eight weeks of 1:1 cognitive tutoring (Iuculano et al., 2015). Also in our study, pre-training hyperconnectivity in parietal, frontal, visual, temporal, and cerebellar regions vanished after training.

Our results are also consistent with a school-based study that reported performance normalization after first-grade number knowledge tutoring (Fuchs et al., 2013), as well as with reports from other studies using cognitive tutoring (Fuchs et al., 2008; Fuchs et al., 2009; Powell et al., 2009) or computer-based intervention (Rauscher et al., 2016; Käser et al., 2013), as we noticed – in addition to FC normalization – significantly increased number line performance and arithmetical skill performance in DD after number line training.

In summary, our results demonstrate a reduction of FC after training which was not limited to fronto-parietal brain regions but also present in visual and temporal brain regions. Hence, the observed neuronal change with number line training in primary and secondary brain regions involved in ordinality processing suggests that number line training induces widespread changes across distributed brain systems encompassing multiple stages of information processing required for successful judgements of numerical ordinality. As the statistical FC maps were comparable between DD post and TD pre (and TD post), we argue that our results reflect a normalization of neuronal activity and efficiency rather than a persistent neuronal aberration (present even after number line training). This assumption is also supported by results from a recent study using rigorous cognitive tutoring inMLD (Iuculano et al., 2015).

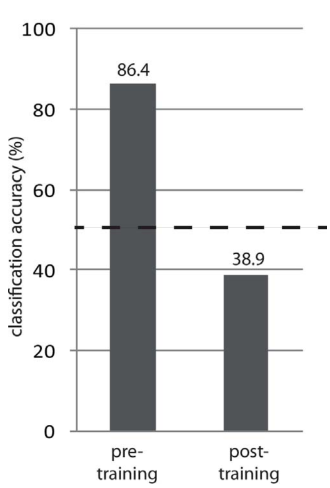

Fig. 7. Result of the multivariate classification analysis. TD could be separated from DD with a significant ($p = 0.003$) classification accuracy of 86.4% before cognitive intervention (5-week number line training), which was not the case after this intervention (classification accuracy: 38.9%, $p > 0.05$). The dashed line indicates chance level.

While DD participants showed decreased FC after training, TD showed increased post-training FC relative to pre-training FC. Regions showing post-training effects were located in the frontal cortex, thalamus, cerebellum, and inferior temporal lobe and might point towards a temporary boost in neuronal processing capacity for the numerical order task.

4.4. Classification analysis

A multivariate classification analysis corroborated our FC findings. In the examined sample, task-related brain activity could be used to discriminate DD from TD before but not after number line training. This means that only the pre-training activity was significantly different between the two groups but was indistinguishable after training. We conclude that both the FC and the classification analysis indicate a neuronal normalization after number line training.

5. Limitations

DD is a heterogeneous learning disorder with many potential causal factors. Therefore different attributes of DD may potentially explain the observed elevated FC in DD, but have not been considered in this study, such as comorbidities like dyslexia or attention-deficit-hyperactivity disorder, or possible causes of DD including genetic factors (Carvalho et al., 2014), environmental factors (*e.g.* schooling), developmental effects (*e.g.* preterm birth (Jaekel and Wolke, 2014; Simms et al., 2015) a) or prenatal alcohol exposure (Meintjes et al., 2010; Woods et al., 2015). Therefore, future studies might reveal a more detailed picture of aberrant FC in DD by investigation of larger cohorts and differentiation into more homogenous subgroups or by taking a multidimensional parametric approach which systematically tests an extended network of cognitive functions (Szűcs, 2016).

Although subjects were carefully selected to have normal IQ, detailed examination revealed differences in estimated IQ between children with and without DD. IQ measures are known to be not fully independent from measures of math ability, and the present sample therefore reflects the typically observed cognitive pattern in DD, even though all children showed IQ scores in the normal range. Consequently, our results cannot be explained by substandard intelligence levels in DD, but are attributed to profound deficits in numeracy in DD. Moreover, we want to emphasize that the FC results did not change if IQ values were considered in the analysis.

Third, confirmation of specific training effects in future studies will require results to be compared not only to a rest period, but also to another intervention. Yet, both groups completed the training successfully and did not differ in the number of training days. Furthermore, as the present number line training had specific positive effects on the numerical domain (number line performance and arithmetic), but showed no influence on general cognitive skills like spatial memory span and working memory, we conclude that the completed training specifically induced behavioural improvements in number processing and calculation and reduced hyperconnectivity in DD children. We would argue that the change in FC in DD after training is unlikely to be driven by strategies acquired during training but is rather the result of neuroplastic adaptations, as we (1) did not observe a 'task x group' interaction, and (2) because the FC pattern in DD was not separable after training relative to TD (Fig. 5). If TD and DD subjects used differing strategies to solve the task, we would expect that one or more brain regions would have shown a post-training group effect.

6. Conclusion

Our study highlights that children with developmental dyscalculia not only profit from a 5 week number line training with respect to numerical skills but additionally show training induced functional brain plasticity which leads to a reduction in aberrant functional hyperconnectivity relative to that seen in typically developing children.

Compliance with Ethical Standards

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest

The authors have no (financial, personal, or any other) conflict of interest.

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