A Comprehensive Study of Whole-Brain Functional Connectivity in Children and Young Adults

Dietsje D. Jolles1,2,3, Mark A. van Buchem1,3, Eveline A. Crone1,2 and Serge A.R.B. Rombouts1,2,3

1Leiden Institute for Brain and Cognition, Leiden University, 2300 RC Leiden, the Netherlands, 2Institute of Psychology, Leiden University, 2333 AK Leiden, the Netherlands and 3Department of Radiology, Leiden University Medical Center, 2300 RC Leiden, the Netherlands

Address correspondence to Dietsje Jolles, Department of Radiology, Leiden University Medical Center, Postzone C2-S, PO Box 9600, 2300 RC Leiden, the Netherlands. Email: d.d.jolles@lumc.nl.

Over the past decade, examination of functional connectivity using functional magnetic resonance imaging has become an important tool to investigate functional changes in patient populations, healthy aging, and recently also child development. Most prior developmental studies focused on functional connectivity between brain regions important for cognitive or emotional control and the so-called “default-mode network.” In the present study, we investigated whole-brain functional connectivity in children (11–13 years; N = 19) and young adults (19–25 years; N = 29), without a priori restrictions to specific regions. We found similar patterns of functionally connected regions in children and young adults, but there were differences in the size of functionally connected regions (i.e., the number of voxels), as well as in the strength of functional connectivity (i.e., the correlation value) between brain regions. This indicates that functional connectivity continues to change during adolescence. Developmental differences were found across the whole brain, but the effects differed for functional connectivity patterns associated with higher cognitive or emotional functions and functional connectivity patterns associated with basic visual and sensorimotor functions. Finally, we showed that the majority of functional connectivity differences could not be explained on the basis of gray matter density alone.

Keywords: development, functional connectivity, MRI, resting-state, voxel-based morphometry

Introduction

The development of cognitive, social, and emotional functioning is accompanied by changes in the magnitude and the extent of activation in the neural systems underlying these functions (e.g., Casey et al. 2008; Blakemore 2008; Luna et al. 2010). Recently, some studies have shown that also the “functional connectivity” between brain regions changes throughout childhood and adolescence (Thomason et al. 2008; Fair et al. 2009; Kelly et al. 2009; Supekar et al. 2009). Functional connectivity is defined as the “temporal correlation of a neurophysiological index measured in different brain areas” (Friston et al. 1993) and can be studied by analyzing correlations of spontaneous blood-oxygen level-dependent (BOLD) signal fluctuations between brain regions obtained from functional magnetic resonance imaging (fMRI; for a review, see Fox and Raichle 2007). The correlation patterns of these spontaneous fluctuations show close correspondence to task-related activation patterns, even in a task-free setting (Smith et al. 2009; Biswal et al. 2010). Although functional connectivity patterns are broadly consistent with anatomical

connectivity (e.g., Bullmore and Sporns 2009), strong BOLD correlations have also been found between regions with no direct anatomical connections (Koch et al. 2002; Vincent et al. 2007; Zhang et al. 2008; Honey et al. 2009).

One of the most used methods to investigate functional connectivity is to calculate the correlation of the BOLD time course from a specific “seed region of interest” with the time courses from all other voxels in the brain (Fox and Raichle 2007). With this method, Kelly et al. (2009) investigated developmental changes in functional connectivity with the anterior cingulate cortex. They demonstrated that children showed more diffuse functional connectivity patterns and increased functional connectivity with regions close to the seed region, as compared with adults, who showed more focal functional connectivity patterns and increased functional connectivity with regions at long distances from the seed region. These findings indicate that functional brain development is characterized by a transition from large undifferentiated systems to specialized neural networks (e.g., Fair et al. 2009) and they are in agreement with developmental differences in functional connectivity between other brain regions (Fair et al. 2007, 2008, 2009). However, prior studies focused mainly on functional connectivity between brain regions important for cognitive or emotional control and the so-called “default-mode network” (Raichle et al. 2001). It is currently not clear whether these functional connectivity differences can be found for other functional domains. Furthermore, it is unknown to what extent observed developmental differences in functional brain connectivity could be explained by changes in local gray matter density.

In the present study, 1) we investigated voxel-wise whole-brain functional connectivity in children (11-13 years) and young adults (19–25 years), without a priori restriction to specific seed regions, and 2) we corrected the results for differences in gray matter density. We used an independent component analysis (ICA)-based approach, in which the entire BOLD data set is decomposed into distinct “functional networks” (defined as brain regions with strong interregional functional connectivity), based on their different temporal characteristics (Fox and Raichle 2007). This approach allows studying the full repertoire of functional networks including visual, auditory, and sensorimotor networks, the default-mode network, and networks associated with higher cognitive functions (Smith et al. 2009). In general, we expected to find more diffuse patterns of functional connectivity in children, although developmental effects might differ across functional networks depending on their functional domain.


Material and Methods

Participants

Twenty-nine young adults (age 19.3–25.3, M = 22.2, standard deviation [SD] = 1.67, 16F) and 20 children (or young adolescents) participated in the study. Data from one child were excluded due to scanner artifacts, resulting in a group of 19 children (age 11.5–13.3, M = 12.5, SD = 0.51, 10F). Sex distributions did not differ between the age groups, χ²(1, N = 48) = 0.30, *p* = 0.863. The participants were right-handed according to self-report. They were screened for MRI using a comprehensive medical questionnaire to exclude participants with contraindications for MRI and to ensure that participants did not have a history of psychiatric or neurologic illness. All participants gave written informed consent for participation in the study. Parents of children that participated in the study gave written informed consent as well. Young adults received financial compensation for participation. Children received a gift and their parents received a monetary compensation for travel costs. The experiment was approved by the Central Committee on Research involving human subjects in the Netherlands.

Image Acquisition

Scanning was performed with a standard whole-head coil on a 3-T Philips Achieva MRI system in the Leiden University Medical Center. First, a resting-state scan was acquired. During this scan, all participants were instructed to lie still with their eyes closed and not to fall asleep. A total of 160 T₁-weighted whole-brain echo planar images (EPIs) were acquired, including 2 dummy scans preceding the scan to allow for equilibration of T₁; saturation effects (time repetition [TR] = 2.2 s; time echo [TE] = 30 ms, flip angle = 80 degrees, 38 transverse slices; 2.75 × 2.75 × 2.72 mm + 10% interslice gap). In addition, a high-resolution EPI scan was obtained (for registration purposes) as well as a T₁-weighted anatomical scan (EPI scan: TR = 2.2 s; TE = 30 ms, flip angle = 80 degrees, 84 transverse slices, 1.964 × 1.964 × 2 mm; 3D T₁-weighted scan: TR = 9.717 ms; TE = 4.59 ms, flip angle = 8 degrees, 140 slices, 0.875 × 0.875 × 1.2 mm, field of view (FOV) = 224.000 × 168.000 × 177.333). In accordance with Leiden University Medical Center policy, all anatomical scans were reviewed and cleared by a radiologist from the Radiology department. No anomalous findings were reported.

Functional Connectivity Data Analysis

For the functional connectivity analyses, we used an ICA-based approach (using multivariate exploratory linear decomposition into independent components [MELODIC]), in combination with a "dual regression technique" (see also Filippini et al. 2009; Biswal et al. 2010). This technique allows voxel-wise comparisons of functional connectivity between groups, using Randomise implemented in FSL (FMRIB's software library, www.FMRIB.ox.ac.uk/fsl; Smith et al. 2004).

The following prestatistics processing was applied: motion correction (Jenkinson et al. 2002), nonbrain removal (Smith 2002), spatial smoothing using a Gaussian kernel of full-width at half-maximum 4.0 mm, grand-mean intensity normalization of the entire 4D data set by a single multiplicative factor, highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 50.0 s). To register fMRI scans to standard space, functional scans of an individual were registered to the corresponding high-resolution EPI images, which were registered to standard Montreal Neurological Institute (MNI) space (Jenkinson and Smith 2001; Jenkinson et al. 2002).

The dual regression approach included 3 stages (see also Filippini et al. 2009; Biswal et al. 2010). The first stage involved the decomposition of all data in separate functional networks. For that purpose, time series of all young adults and children were temporally concatenated into a single 4D time series. This 4D time series was separated in 25 components using ICA in MELODIC, with automatic dimensionality estimation (i.e., the number of components to extract was determined by MELODIC). One advantage of the ICA technique is that it automatically isolates noise-related signal fluctuations such as head motion (Damoiseaux et al. 2006; Fox and Raichle 2007). This can be especially relevant in children. We selected 9 components based on spatial similarity to functional networks described before (Damoiseaux et al. 2006; Supplementary Fig. 1S: A–I) network A: visual system; network B: sensorimotor system; network C: default-mode network; network D: auditory system; network E: ventral stream; network F: executive control system; network G: dorsal attention system; network H: frontoparietal network (left hemisphere); network I: frontoparietal network (right hemisphere). In addition, we selected 4 other components that were potentially relevant functional networks (Supplementary Fig. 1S: J–M): network J: anterior default-mode network; network K: occipitoparietal network; network L: insula/operculum-cingulate network; network M: superior parietal network.

The assemblies of brain areas that constituted these functional networks are described in the Supplementary Material (Supplementary Fig. 1S). The other 12 components were related to white matter, cerebrospinal fluid, head movement, and other (nonneuronal) noise.

The second stage involved the identification of subject-specific component maps. First, individual time series were extracted for each component, using the 25 component maps in a (spatial) regression against the individual data. The resulting time series matrices were then entered in a second (temporal) regression against the associated data to estimate 25 spatial component maps for each individual.

In the final stage of the analysis, we used one sample nonparametric *t*-tests to obtain group averages and 2-sample *t*-tests to obtain group differences for each of the 13 selected functional networks. Voxel-wise nonparametric permutation testing was performed using Randomise in FSL (with 5000 permutations; Nichols and Holmes 2002). All statistical maps were family-wise error (FWE) corrected using *p* < 0.05, based on the threshold-free cluster enhancement (TFCE) statistic image (Smith and Nichols 2009). Group comparisons were masked by group main effects (i.e., voxels that fell within the group map of the children and/or the group map of the young adults, thresholded at *p* < 0.05, FWE corrected for multiple comparisons using the TFCE technique).

We studied developmental differences in the size of functional networks, as well as in the strength of functional connectivity in all regions within these networks. Changes in the size of functional networks were examined by calculating the average number of voxels with a Z > 3.1 (corresponding to a *p* < 0.001, uncorrected) for each network in each group. When a group showed a significantly larger number of voxels in a particular functional network, this was referred to as "more widespread functional connectivity." Changes in the strength of functional connectivity were examined by using a voxel-wise comparison of correlation values between children and young adults. Higher correlation values in a specific area correspond to stronger involvement of that area in the functional network. When a group showed higher correlation values within a particular functional network, this was referred to as "increased functional connectivity." In contrast to seed-based analyses, the present method is not well suited to calculate developmental changes in the distance of functional connections.

Correction for Gray Matter Differences

Some additional analyses were carried out to determine whether the observed differences in functional connectivity were influenced by underlying differences in gray matter density or registration error (Oakes et al. 2007). First, a voxel-based morphometry (VBM) analysis was performed to highlight regions with differences in gray matter density between children and young adults, using FSL-VBM with default settings (Ashburner and Friston 2000; Good et al. 2001). The following prestatistics processing was applied: nonbrain removal (Smith 2002), tissue-type segmentation (Zhang et al. 2001), and nonlinear registration to MN152 standard space (Andersson et al. 2007a, 2007b). A study-specific template was created by averaging structural images from 19 children and 19 (randomly selected) young adults. Then, the native gray matter images were nonlinearly reregistered to this template map. The registered partial volume images were then modulated to correct for local expansion or contraction. The resulting images were spatially smoothed with an isotropic Gaussian kernel with a sigma of 3 mm. Finally, group maps for children and young adults were compared by voxel-wise nonparametric permutation testing (with 5000 permutations; Nichols and Holmes 2002), correcting for multiple comparisons.
across space (thresholded at $P < 0.05$, FWE corrected) using the TFCE technique (Smith and Nichols 2009).

Second, the fMRI data were reanalyzed using gray matter density information of each participant as a voxel-dependent covariate (see also Filippini et al. 2009). By including structural information into the functional connectivity analysis, the results are corrected for differences in gray matter density and the effects of possible misregistrations are accounted for (Oakes et al. 2007). One- and 2-sample non-parametric $t$ests were performed to obtain group averages as well as group differences for all functional networks. Voxel-wise nonparametric permutation testing was performed using Randomise in FSL (with 1000 permutations due to computational burden; Nichols and Holmes 2002). The statistical maps were thresholded at $P < 0.05$, FWE corrected for multiple comparisons using the TFCE technique (Smith and Nichols 2009). Group comparisons were masked by group main effects (thresholded at $P < 0.05$, FWE corrected for multiple comparisons using the TFCE technique).

Results

Functional Connectivity

During the first stage of the analysis, resting-state fMRI data from the whole group were decomposed into 25 separate patterns (or groups) of functionally connected regions, defined as functional networks. Hence, a functional network is characterized by strong functional connectivity between regions within the network. Nine of these networks were selected based on spatial similarity to functional networks described before (Damoiseaux et al. 2006; Supplementary Fig. 1S: A–I). In addition, we selected 4 other functional networks that seemed functionally relevant (Supplementary Fig. 1S: J–M). Inspection of the spatial patterns of group main effects revealed overlapping functional networks in children and young adults (Fig. 1A). Core regions of all 13 functional networks were found in both groups ($P < 0.05$, FWE corrected, based on the TFCE statistic image). To examine whether functional networks were more widespread in children, we calculated for both groups the average number of voxels with a $Z > 3.1$ (corresponding to a $P < 0.001$, uncorrected; Fig. 1B). A Mann–Whitney test showed that network D, F, J, L, and M were significantly larger in children, while activity differences from gray matter density effects or registration error. To this end, gray matter information was added as a voxel-dependent covariate in the functional connectivity analysis (Oakes et al. 2007; Filippini et al. 2009). Despite the gray matter correction, we still found significant functional connectivity differences in all 11 functional networks that initially showed group differences (Table 1; Supplementary Figs 3S and 4S; all $P < 0.05$, FWE corrected, based on the TFCE statistic image). However, some effects were reduced and a few regions were no longer significantly different. In other words, in these regions it was not possible to distinguish functional connectivity differences from gray matter density effects or registration error.

Discussion

Functional connectivity is defined as the temporal correlation between BOLD fluctuations from different parts of the brain (Friston et al. 1993; Fox and Raichle 2007) and is organized in the brain in a number of functional networks (defined as brain regions with strong interregional functional connectivity). In the present study, we examined whole-brain functional connectivity in children and young adults. We found similar functional networks in children and young adults. That is, core regions of all functional networks were present in both groups. This is in agreement with developmental task-fMRI studies that have demonstrated that core task-related regions can already be detected early in development (e.g., Casey et al. 1997; Gaillard et al. 2000; Holland et al. 2001; Passarotti et al. 2003). However, we found differences in the size of functional networks (i.e., the number of voxels in a functional network), as well as in the strength of functional connectivity in specific areas within these networks (i.e., the correlation value). These findings suggest that while the basic configuration of functional networks in the brain has been established by the age of 12, the fine-tuning or specialization of functional networks may continue during adolescence. This is consistent with the hypothesis that large-scale anatomical networks are prespecified, while activity-dependent processes might be crucial for functional specialization of these networks (Johnson 2005; Raichle 2006; Rakic et al. 2009).

Functional Connectivity Differences

The majority of functional networks (i.e., 8 of 13) showed regional increases of functional connectivity in children and for these functional networks functional connectivity was often
more widespread. This is in agreement with prior studies of functional connectivity (Kelly et al. 2009) and task activation patterns in children (Holland et al. 2001; Casey et al. 2002; Moses et al. 2002; Tamm et al. 2002; Konrad et al. 2005; Durston et al. 2006). In addition, it has been demonstrated that children show more functional connectivity “between” functional networks (Stevens et al. 2009) and lower levels of hierarchical functional organization (Supekar et al. 2009). Taken together, these developmental differences indicate that functional networks in children are less specialized or efficient (Johnson and Munakata 2005; Durston et al. 2006; Fair et al. 2007, 2009).

We specifically found increased functional connectivity in functional networks associated with complex cognitive or emotional functions, such as the executive control system, the dorsal attention system, and the default-mode network. Increased functional connectivity was also found in the auditory network. Although this network is associated with auditory perception, it is also involved in higher cognitive functions related to language (Smith et al. 2009). Surprisingly, functional networks associated with basic visual or sensorimotor functions (i.e., the sensorimotor system, the visual system, and the ventral stream) showed the opposite effect. These networks involved regions with reduced functional connectivity in children compared with young adults. Although most prior studies did not specifically focus on functional connectivity in basic visual and sensorimotor networks, one study demonstrated reduced functional connectivity in a motor control network in children and adolescents (8–12 and 13–17 years) compared with young adults (Kelly et al. 2009). Thus, the present results suggest qualitatively different developmental trajectories for functional connectivity between regions associated with complex cognitive or emotional functions and between regions associated with basic visual or sensorimotor functions.

**Correction for Gray Matter Density**

In agreement with prior studies, we found extensive gray matter differences between children and adults. The majority of cortical areas showed increased gray matter density in children (Sowell et al. 2001, 2003; Giedd 2004; Gogtay et al. 2004), whereas reduced gray matter density was found in anterior hippocampus and amygdala (Giedd et al. 1996; Guo
et al. 2007; Ostby et al. 2009), cerebellum (Sowell et al. 2002; Konrad et al. 2005), and occipital cortex (Giedd et al. 1999). Despite the extensive gray matter differences, a functional connectivity analysis using gray matter density as a voxel-dependent covariate revealed still significant functional connectivity differences in all 11 networks that initially showed group differences. These results suggest that the majority of functional connectivity differences are not simply explained by gray matter density effects.

The Underlying Anatomy and Physiology of Developing Functional Connectivity

One remaining question is how to relate changes of functional connectivity to anatomical and physiological changes in the developing brain. Structural brain maturation involves a multitude of complex and overlapping processes (Johnson 2005; Uylings 2006; Stiles 2008), and from the present data we cannot conclude directly which underlying mechanisms contribute to the development of functional connectivity. However, some parallels exist between the development of functional connectivity and anatomical, histological, and neurochemical processes described elsewhere. For example, we found similar connectivity patterns in children and young adults, which seems consistent with the fact that major pathways are in position by the age of 12 and the peak of dendritic development has been reached (e.g., LaMantia and Rakic 1990; Mrzljak et al. 1990; Petanjek et al. 2008). The majority of functional networks showed regional increases of functional connectivity in children, which seems consistent with the increased number of synaptic contacts in children (Huttenlocher 1979; Bourgeois and Rakic 1993; Bourgeois et al. 1994; Huttenlocher and Dabholkar 1997) and the high levels of glucose metabolism and cerebral blood flow (Chiron et al. 1992; Chugani 1998, 2002). Development of functional connectivity might be guided by selective elimination (or “reorganization”; Kostovic 1990) of synapses, which enhance the specificity and efficiency of information processing (Changeux and Danchin 1976; Goldman-Rakic 1987; Chechik et al. 1998). In addition, myelination and/or increases in axon diameter (Yakovlev and Lecours 1967; LaMantia and Rakic 1990; Benes et al. 1994; Paus et al. 1999) may increase the speed of neuronal signal transmission and modulate the synchrony of neuronal firing across functional networks (Fields 2008; Paus 2010). Finally, the efficiency of communication across functional networks might be further modulated by the protracted development of neurotransmitter systems (Kostovic 1990; Benes 2001). Taken together, the functional connectivity differences that were found in the present study might reflect a combination of factors, including myelination, synaptic reorganization, changing levels of neurotransmitters, and decreasing glucose metabolism and cerebral blood flow, all of which should be investigated further in future research. To this end, investigations into the development of functional connectivity should be combined with MRI measures of anatomical connectivity, electrical measures of brain activity (e.g., electroencephalography or local field potential recordings), and/or postmortem histological data (Fox and Raichle 2007).

Conclusion

The results of the present study indicate that although the basic configuration of functional networks in the brain has been established by the age of 12, functional networks continue to change during adolescence (or young adulthood) depending on the functional domain. In addition, we showed that the majority of functional connectivity differences could not be explained on the basis of gray matter density alone. In future studies, it is important to replicate the present results across a wider age range and to identify the underlying
anatomical and neurophysiological mechanisms that cause these functional connectivity differences. Finally, the age period between 12 and 25 is characterized by important changes in neocognitive skills and psychosocial functioning. These changes are dependent upon the rapid accumulation of experiences and are accompanied by a changing (social) environment in which significant others (e.g., peers, parents, and teachers) play an important role. Development of functional connectivity in the brain may be a prerequisite for the proper development of psychological functions. On the other hand, functional connectivity might also be shaped by experience and develop in relation to the environmental demands (Sporns et al. 2004; Raichle 2006). The interplay between the development of functional connectivity and cognitive, social, and emotional maturation is therefore an important direction for future research.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org.

Funding
The Netherlands Organisation for Scientific Research (VIDI grant 91786368 and 45207011 to S.A.R.B.R. and E.A.C.); LUF Gratama stichting to E.A.C.

Notes
The authors thank Dr Christian Beckmann for developing and providing the dual regression analysis technique, and Joram van Driel and Dewi Douwes for their assistance in the data collection. We thank Prof. Dr Harry Uylings and Dr Cédric Koolschijn for reading (parts) of the manuscript. Conflict of Interest: None declared.

References
Fox MD, Raichle ME. 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci. 8:700–711.


