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Working memory network alterations in high-functioning adolescents with an autism spectrum disorder

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Aim: People with autism spectrum disorder (ASD) typically have deficits in the working memory (WM) system. WM is found to be an essential chain in successfully navigating in the social world. We hypothesize that brain networks for WM have an altered network integrity in ASD compared to controls.

Methods: Thirteen adolescents (one female) with autistic disorder (n = 1), Asperger’s disorder (n = 7), or pervasive developmental disorder not otherwise specified (n = 5), and 13 typically developing healthy control adolescents (one female) participated in this study. Functional magnetic resonance imaging (MRI) was performed using an n-back task and in resting state.

Results: The analysis of the behavioral data revealed deficits in WM performance in ASD, but only when tested to the limit. Adolescents with ASD showed lower binary global efficiency in the WM network than the healthy control group with n-back and resting-state data. This correlated with diagnostic scores for total problems, reciprocity, and language.

Conclusion: Adolescents with higher-functioning autism have difficulty with the WM system, which is typically compensated. Functional MRI markers of brain network organization in ASD are related to characteristics of autism as represented in diagnostic scores. Therefore, functional MRI provides neuronal correlates for memory difficulties in adolescents with ASD.

Key words: autism spectrum disorder, functional magnetic resonance imaging, higher functioning, network analysis, working memory.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with a complex brain etiology, for which many causes have been proposed (including genetic factors), but a full understanding is incomplete. Consequently, the diagnosis is made based on behavioral assessments. ASD is characterized by persistent deficits in social communication and social interaction across multiple contexts, and restricted, repetitive patterns of behavior, interests, or activities. These symptoms must be present in the early developmental period and cause clinically significant impairments in social, occupational, or other important areas of current functioning (DSM-5). Additionally, cognitive, and especially executive function, problems often co-occur. Symptoms can change within one person over the course of development; furthermore, other disorders, such as mental
retardation, epilepsy, anxiety, and mood disorders, frequently co-occur with ASD and may modify the threshold for presentation of symptoms. Finally, due to the shift from autism as a categorical disorder to a dimensional spectrum, more subtle features have been included in the definition of autism. For social interaction to be successful, it is necessary to understand another person’s emotions, intentions, beliefs, and knowledge. This information is needed to predict another person’s behavior and adjust one’s own behavior accordingly. The ability to impute and understand the mental states of others, and recognize that these states may differ from your own is called ‘mentalizing’ or having a theory of mind.

The ability to mentalize depends on a range of both lower-level mechanisms, such as face and emotion processing, gaze direction and the detection of animacy, as well as higher executive function mechanisms, such as attention and working memory (WM). Of these, the human face and its emotional expressions in particular play an important role in mentalizing, as the face constitutes an important source of information about a person’s inner state. High-functioning adolescents with ASD are expected to have impaired mentalizing abilities.

Executive functions are a group of higher cognitive functions necessary for the regulation of voluntary planned behavior and the inhibition of task-inappropriate responses. Therefore, they are central to human cognition. WM is a core component of these executive functions in its role as a temporary storage system under attentional control. And, although WM processes have always been related with abstract information processing, recent research shows that WM is, in addition to the socio-affective processes, also essential for successfully navigating in the social world. Accordingly, WM deficits are often found in several developmental psychopathologies, including ASD.

Phillips and colleagues have provided evidence for the involvement of WM in processing social information. They explored the role of verbal WM in decoding emotions and found that the process of labeling the emotions portrayed on facial expressions places high demands on WM resources. These demands increase as the number of labels that are available increases, as is the case in real-life situations: decoding social cues from faces, bodies, and verbal material all involve substantial investment of WM for accurate performance. Furthermore, Bankó and colleagues found evidence that humans can store fine-grained information related to facial emotions and identity in short-term memory with high precision. Such high-fidelity short-term memory processing is crucial for the ability to monitor emotional expressions efficiently. As a result, impairment of high-precision short-term memory storage of emotional information might (partly) underlie the deficits of emotional processing found in psychiatric disorders, including autism. Through these processes, WM not only plays a central role in processing complex cognitive information, but also an essential role in social cognition and interpersonal interactions.

In this study, we investigated adolescents with high-functioning autism and healthy controls (HC), thus excluding individuals with autism who have intellectual deficits. By assessing these two groups, the potential influence of intellectual impairment rather than ASD is removed. Furthermore, adolescents with high-functioning autism are more prone to psychosocial stress than their low-functioning counterparts, as they are more aware of opinions and evaluations of peers.

It has been suggested that during adolescence, a complex frontal-parietal network of brain regions is involved in WM processes (for review, see Barendse et al.). Functional magnetic resonance imaging (fMRI) is a neuroimaging technique that allows the assessments of correlates of brain activity, by measuring changes in blood oxygenation levels that occur in response to the metabolic requirements of active neurons. In addition to the localization of which brain regions are involved in certain cognitive processes, fMRI also allows the assessment of the integrity of functional connections between brain regions. Using fMRI, one can investigate the WM network and assess whether it is abnormal in high-functioning adolescents with ASD, which might explain its impact on cognitive and social functioning.

We hypothesize that brain networks for WM have altered network integrity outcomes in ASD compared to HC, possibly underlying social difficulties in adolescents with ASD. To investigate this, we compared a group of high-functioning adolescents with a normally developing HC group using fMRI of an n-back WM task and during resting state, in which we focused on activation patterns (n-back) and WM network efficiency (n-back and resting state).
METHODS

Participants

Fifteen participants with ASD were recruited from the special secondary education school, de Ber- kenschutse (located in Heeze, the Netherlands), as detailed previously.22 All high-functioning adolescents with ASD fulfilled established diagnostic criteria according to the DSM-IV, as well as the autism algorithm cut-offs on the Autism Diagnostic Observation Schedule (ADOS).26 The HC group consisted of 18 normally developing adolescents who were recruited through advertisements in newspapers and most were living in other parts of the country.

Participants of both groups were aged 12–18 years. Adolescents in the HC group were excluded if they and/or one of their siblings and/or parent(s) had had a diagnosis of ASD. Further exclusion criteria for both groups were: (i) a comorbid psychiatric disorder; (ii) a significant hearing or visual impairment; (iii) an inability to speak/understand the Dutch language; and/or (iv) a comorbid central neurologic or other somatic disorder.

Two adolescents with ASD and five HC were excluded from the analysis: one because of a scanner malfunction and six because of an uncorrectable susceptibility artifact (braces). Eventually, 13 adolescents (one female) with autistic disorder (n = 1), Asperger’s disorder (n = 7), or pervasive developmental disorder not otherwise specified (n = 5), and 13 normally developing HC adolescents (one female) participated in this study. Four adolescents with ASD used medication. Three used methylphenidate, but discontinued medication at least 20 h before scanning, allowing for complete washout. Methylphenidate is often used in treatment of attention deficit hyperactivity disorder (ADHD), however in this case it was used against impaired executive functioning, which is not restricted to ADHD.27 One adolescent with ASD used pipamperone (an antipsychotic drug) and did not discontinue medication. None of the HC adolescents used medication. None of the adolescents with ASD had a diagnosis for other comorbid psychological disorders or psychiatric diseases (including ADHD) as formulated in the DSM-IV-TR. All adolescents had an estimated full-scale IQ (FSIQ) over 105 (range ASD: 107–124; range HC: 107–135), as measured with a short version of the Wechsler Intelligence Scale for Children (WISC-III).28–30 The two groups did not differ from each other with respect to sex (P = 0.567), age (P = 0.102), Verbal Comprehension Index of the WISC-III (P = 0.986), Freedom of Distractibility Index (P = 0.670), FSIQ (P = 0.179), or the ability to recognize emotions (P = 0.414) as measured with the Emotion Recognition Task31 (Table 1).

The groups differed on WM abilities in daily life as reported by parents on the Behavior Rating Inventory of Executive Function Working Memory subscale (P = 0.001), with parents of adolescents with ASD reporting more WM problems in daily situations (Table 1). Furthermore, the ASD group showed significantly higher scores on the Child Behavior Checklist (CBCL) than the age-matched HC (Table 1).

The study protocol was approved by the Medical Ethical Commission of the Maastricht University Medical Center. In accordance with the Declaration of Helsinki, informed consents were obtained from all adolescents and their parents or caregivers.

Experimental paradigm

During fMRI, the adolescents performed a specific WM task, the visual n-back task, with two experimental conditions: 1-back and 2-back (Fig. 1). In the 1-back condition, participants were instructed to press the ‘yes’ response button when the same picture (house or face) was presented twice in a row; if not, they were instructed to press the ‘no’ response button. In the 2-back condition, they had to press the ‘yes’ response button when the picture on the screen matched the one that had been represented two pictures ago, otherwise they had to press the ‘no’ response button. The stimuli were 80 color pictures of faces (male and female) from the Radboud Faces Database32 and 80 color pictures of houses, presented with the E-PRIME presentation software (Psychology Software Tools, Sharpsburg, PA, USA), which also recorded the adolescents’ behavioral performance. Each adolescent practiced for both experimental conditions in a separate practice session approximately 30 min before scanning.

The experiment in the scanner utilized a blocked design, with four epoch houses stimuli and four epoch faces stimuli for each of the experimental conditions (16 epochs total). Each epoch contained 15 pictures and three, four, six, or seven targets occurring at a random order per epoch. At the beginning of an experimental block, a visual instruction was given for 5500 ms, followed by a fixation cross for 500 ms. Each stimulus picture was shown for 1500 ms, followed by a fixation cross for 500 ms.
After 15 stimuli, at the end of an epoch, the fixation cross was shown for 30,000 ms (see picture 1). The experimental conditions were presented in a fixed order, with a 7-min resting-state MRI first, followed by the 1-back condition, then another 7-min resting-state MRI, followed finally by the 2-back condition. The total scanning time per experimental condition was 8 min and the total scanning time, including the anatomic and resting-state MRI scanning, was 60 min.

Group comparisons on the behavioral data were made according to generalized linear model (GLM) multivariate analyses of covariance using SPSS version 20.0 (SPSS, Chicago, IL, USA) for both experimental conditions separately. The dependent variable was the error rate as measures with the Total number of errors, Total number of negative errors or misses ( ‘no’ response when the correct response was ‘yes’), Total number of positive errors or false alarms ( ‘yes’ response when the correct response was ‘no’), and Total number of error omissions (non-response). As previous research has shown a relation between intelligence level and WM performance, we included FSIQ as a covariate in the analyses.

### Functional imaging

The fMRI data were acquired with a 3.0-Tesla unit (Achieva; Philips Medical Systems, Best, The Netherlands). An echo-planar imaging (EPI) sequence was used with the following parameters: repetition time (TR) = 2 s, echo time (TE) = 35 ms, flip angle = 90°.
31–32 slices, transverse orientation, slice thickness = 4 mm, in-plane resolution = 2 mm × 2 mm, and volumes per acquisition: 236 (n-back) and 210 (resting state). Additionally, for anatomical reference, a transverse orientated T1-weighted dataset was acquired with the following parameters: TR = 8.233 s, TE = 3.77 ms, flip angle = 8°, and 150 slices.

fMRI preprocessing
The fMRI data were processed with MATLAB R2012b (MathWorks Inc., Natick, MA, USA) and SPM8 (Wellcome Trust Centre for Neuroimaging, UCL, London, UK). The functional images were realigned, resliced, and slice-time corrected. The mean image of the functional images was co-registered to the T1-weighted image. The T1-weighted image was segmented using SPM8. Functional images were normalized to the segmented image with a final voxel size of 2 mm × 2 mm × 2 mm, and subsequently smoothed with a kernel of 8-mm full-width at half maximum.

For n-back activation mapping, a GLM model was fitted that included two conditions: rest and picture assessment. Differences between the two conditions were assessed through a first-level analysis. The average activation per group was assessed by a one-sample t-test with a family-wise error threshold of $P < 0.05$. The differences between the two groups were assessed by a two-sample t-test with a family-wise error threshold of $P < 0.05$.

Regions of interest definition
FREESURFER (Martinos Center of Biomedical Imaging, Boston, MA, USA) software was used to segment the T1-weighted images of each subject into 84 cortical and subcortical regions. Additionally, a frontotemporal subnetwork, including 16 areas related to WM, was created, consisting of left and right lateral premotor cortex, dorsal cingulate and medial premotor cortex, dorsolateral and ventrolateral prefrontal cortex, frontal poles, and lateral posterior parietal cortex and hippocampus (Fig. 2).

The Brodmann areas found in the research by Owen et al. were matched to their corresponding FREESURFER areas. The hippocampus was added, as previous research has demonstrated that this is part of the WM of adolescents.

Functional connectivity
Preprocessed images of the resting state and the n-back tasks were realigned, co-registered to the atlas of the cortical and subcortical regions, and smoothed with a kernel of 8-mm full-width at half maximum. The images were then band-pass filtered with a frequency window of 0.01–0.1 Hz. To correct for physiological noise, linear regression with time series from the cerebrospinal fluid and white matter signals was applied. Also, correction with linear regression for the moving parameters was applied. The sum of moving parameters in the x, y, and z directions was computed. No differences between
the groups were found ($P > 0.22$) (resting state, $P = 0.22$, mean ASD: 0.41, mean HC: 0.35; 1-back, $P = 0.09$, mean ASD: 1.09, mean HC: 0.50; 2-back, $P = 0.29$, mean ASD: 1.67, mean HC: 0.80).

Pearson’s linear correlation coefficients between the region-averaged time-series of all pairs of Freesurfer regions were computed. This resulted in an $84 \times 84$ connectivity matrix (whole brain) and a $16 \times 16$ connectivity matrix (WM) for each subject. A Fisher’s $r$-to-$z$ transformation was applied at both connectivity matrices.37,38

Negative correlations were set to zero.39 Subsequently, a two-sample $t$-test was applied with Bonferroni corrected $P$-value threshold for multiple comparisons. Additionally, permutation tests were performed to increase statistical power.40 The subjects were randomly divided in two groups, which was repeated 1000 times. Subsequently, for every connection, the difference between the two groups was compared to the random realization using a $t$-test.

Subsequently, the correlation matrix for each subject was made sparse. A percentage ranging from 20 up to 80% of connections with the highest correlation coefficients was included.41,42 Two different methods were applied to determine the sparse correlation matrices. In the first method, the average correlation matrix of all the subjects was used to create a sparse ‘common’ mask matrix, which was applied at each subject. This ensured that the sparse matrix for every subject contained the same connections. In the second method, the sparse matrix was determined for every subject separately, which yielded a unique sparse matrix on an individual level.

All connections greater than zero were set to one to create a binary matrix. Subsequently, with the Brain Connectivity Toolbox,43 four network metrics were calculated for the sparse connectivity matrices of each subject. We included the characteristic path length ($L$), global efficiency ($E_{\text{global}}$), modularity ($Q$), and clustering coefficient ($C$), both the binary as the weighted variants. A rank sum test was applied to investigate group differences using a $P$-value threshold of 0.05. The network metrics were correlated with CBCL scores (the total score and scores for internalizing and externalizing problems) for the groups separately and for the whole group, and ADOS (communication, reciprocal social interactions, and total) for the ASD group. In order to determine whether the networks had small-world

Figure 2. Selection of areas of the working memory network, including lateral premotor cortex, dorsal cingulate and medial premotor cortex, dorsolateral and ventrolateral prefrontal cortex, frontal poles and lateral posterior parietal cortex and hippocampus (both left and right areas are included).
properties, the values for the characteristic path length and cluster coefficient were scaled to values from generated random networks. Random networks with small-world properties are characterized by the characteristic path length close to random ($\lambda = L/L_{\text{random}} \approx 1$) and the clustering coefficient higher than random ($\gamma = C/C_{\text{random}} > 1$). One hundred random networks were generated with the Brain Connectivity Toolbox.

**RESULTS**

**Behavioral performance**

In the 1-back condition, the ASD and HC groups showed similar behavioral performance, with high levels of accuracy. GLM analysis revealed no significant main effects on error rates of: group, $F(3, 18) = 0.936, P = 0.444$; FSIQ, $F(3, 18) = 1.790, P = 0.185$; or main interaction effect of group and FSIQ, $F(3, 19) = 0.917, P = 0.452$. In the 2-back condition, we found main effects on the error rates of: group, $F(3, 19) = 3.923, P = 0.025, \eta_p^2 = 0.382$; FSIQ, $F(3, 19) = 4.449, P = 0.016, \eta_p^2 = 0.413$; and an interaction effect of group and FSIQ, $F(3, 19) = 3.814, P = 0.027, \eta_p^2 = 0.376$. Subsequent analyses of variance showed a significant group effect on the Total number of negative errors, $F(1, 21) = 8.194, P = 0.009, \eta_p^2 = 0.281$. Adolescents with ASD made more negative errors than HC on the 2-back task (Table 2).

A significant interaction effect was found between group and FSIQ on Total number of negative errors, $F(1, 21) = 7.874, P = 0.011, \eta_p^2 = 0.273$, and subsequent Pearson correlation analysis per group revealed in the ASD group a significant negative correlation between FSIQ and Total number of negative errors ($r = -0.664, P = 0.007$). This significant relation was not present in the HC group.

**fMRI data analysis**

**Differences in activation**

No significant differences in activation were found for the two groups.

**Functional connectivity**

Resting-state and n-back tasks data did not yield any significant differences in correlation on matrix element level, either for the whole or the WM network. Additionally, the permutation tests also failed to yield any significant differences.

The individual method for the determination of the sparse matrices yielded the following results: the $E$ global binary was significant in the sparsity range of $0.41-0.77$ (resting state) (Fig. 3) and $0.59-0.7$ (1-back) (Fig. 4). After correction for random networks, the $F_{\text{global}}$ binary showed a significant

<table>
<thead>
<tr>
<th>Table 2. Behavioral data: 1-back and 2-back conditions</th>
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<tbody>
<tr>
<td><strong>1-Back</strong></td>
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<tr>
<td>Sex (male/female)</td>
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<tr>
<td>M (SD)</td>
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<tr>
<td>Total no. errors</td>
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<td>Total no. negative errors</td>
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<td>Total no. positive errors</td>
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<td>Total no. errors omission</td>
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<tr>
<td><strong>2-Back</strong></td>
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<tr>
<td>Sex (male/female)</td>
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<td>M (SD)</td>
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<td>Total no. errors</td>
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<td>Total no. negative errors</td>
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<td>Total no. positive errors</td>
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<td>Total no. errors omission</td>
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<td><strong>Group comparison</strong></td>
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1Missing data due to registration errors.

ASD, autism spectrum disorder; HC, healthy controls; M, mean.

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difference for sparsities from 0.5 up to 0.52 (resting state) and 0.55 up to 0.57 (1-back). Networks of the adolescents with ASD showed lower scores for $E_{\text{global}}$.

The 2-back task and other network metrics did not show any significant difference. No significant results were found with the common mask method to calculate sparse matrices.

The global efficiency binary (resting state) correlated significantly with the CBCL total problems (sparsities 0.65–0.78) with correlations between −0.42 and −0.56 ($P$-values of 0.003–0.032), ADOS reciprocity (sparsities 0.71–0.8) with correlations between −0.62 and −0.76 ($P$-values of 0.003–0.025), and ADOS total (sparsities) with correlations between −0.61 and −0.78 ($P$-values of 0.002–0.028).

The global efficiency binary (1-back) correlated significantly with ADOS language/communication (sparsities 0.63–0.69) with correlations between 0.55 and 0.65 ($P$-values of 0.027–0.049).

Network metrics corrected by the random networks yielded significant correlations for $\lambda$ (binary) in the sparsity range of 0.77–0.79 (resting state) and 0.55–0.57 (1-back), $\gamma$ (binary) in the range of 0.40–0.41 (resting state) and $E_{\text{global}}$ (binary) for sparsities 0.50–0.52 (resting state). For $E_{\text{global}}$ binary, overlapping sparsities were found for the uncorrected and corrected networks.

Analysis with females or patients on medication excluded yielded similar results.

**DISCUSSION**

The analysis of the behavioral data confirmed deficits in WM performance in ASD, but only when tested to the limit. Statistically significant differences were not found on the 1-back task, but they did occur in the 2-back task. Adolescents with ASD made more negative errors than HC on the 2-back task. Moreover, in the ASD group, a significant negative correlation was found between FSIQ and Total number of negative errors. This significant relation was not present in the HC group. We have to acknowledge, though, that it is possible that the small group size underlies the lack of an association. The adolescents with ASD seemed to rely more on their general intellectual capacities to solve more complex WM tasks than typically developing adolescents, which is an indication that compensation...
occurs, likely related to the use of cognitive reserve capacity in these youngsters.17

In a brief report, Silk and colleagues found a dysfunction of the frontostriatal network in high-functioning adolescents with ASD,44 which is in line with behavioral studies that reported executive and WM problems in ASD. It has further been hypothesized that the functional connectivity between and within several neural networks is altered in individuals with ASD, due to immature or aberrant developmental processes.55,46 Evidence for hypo- and hyper-connectivity have also been suggested in other fMRI studies that investigated WM functioning in individuals with ASD, focused on broad age groups.17

The two findings combined show a subtle neurological correlate showing weaknesses in WM processing that are largely compensating using cognitive reserve capacity. Thus, adolescents with ASD seem to rely more on their general intellectual capacities to solve more complex WM tasks than typically developing adolescents.

As anticipated, activation mapping revealed no differences between the groups. Pathology or changed brain function seldom leads to changed patterns (localization, severity) of activated areas in reaction to a task.47 However, resting-state fMRI and n-back tasks data did not yield any significant differences in functional connectivity either for the whole or the specific WM network. This latter, much more sensitive, method was expected to yield differences in the organization of networks. No differences were found for network metrics in the 2-back fMRI scan between the two groups. A possible explanation for this might be the fact that the 2-back task was administered at the very end of the imaging protocol, and the volunteers might have experienced some fatigue during this task, which can lead to more unwanted head motion and a poorer focus.

The individual method for the determination of the sparse matrices yielded the following results:

1 Adolescents with ASD showed lower binary global efficiency in the WM network than the HC group with resting-state data. This correlated with CBCL (total problems) and ADOS (reciprocity and ADOS total) outcomes.
2 Adolescents with ASD showed lower binary global efficiency in the WM network than the HC group with 1-back data. This correlated with ADOS language/communication scores.

3 Global efficiency refers to the ability of a network to transmit information at the global level. An important metric that couples with network efficiency is ‘network costs,’ which indicates how much effort is needed to maintain a network.41 The loss of global efficiency suggests an increase of network costs, which may be a sign of the same compensatory process that is also seen in the behavioral data.48 Since the fMRI efficiency measures correlate with the behavioral data indicative of the severity of the disorder (lower efficiency is related to more social problems), compelling evidence is provided that links connectivity with the behavioral phenotype of the disorder.

The current study has some limitations. First, the total number of included subjects was relatively small, which might limit the general applicability of the findings. Second, a possibility exists that some individuals with ASD from this study have comorbid ADHD. But, as the DSM-IV-TR criteria were used, this could unfortunately not be confirmed. Lastly, a medication effect cannot be excluded entirely, but it is expected to be negligible.

We can conclude that adolescents with higher-functioning autism have difficulty with the WM system, which is typically compensated. Resting-state fMRI and task-related fMRI showed lower global efficiency parameters of brain network organization that were related to characteristics of autism as represented in the CBCL and ADOS scores. Thus, fMRI provides a neuronal correlate of social deficits in high-functioning adolescents with an autism spectrum disorder.

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DISCLOSURE STATEMENT

The authors have no conflicts of interest to declare.
AUTHOR CONTRIBUTIONS


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