Age-Related Increase in Inferior Frontal Gyrus Activity and Social Functioning in Autism Spectrum Disorder

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Background: Hypoactivation of the inferior frontal gyrus during the perception of facial expressions has been interpreted as evidence for a deficit of the mirror neuron system in children with autism. We examined whether this dysfunction persists in adulthood, and how brain activity in the mirror neuron system relates to social functioning outside the laboratory.

Methods: Twenty-one adult males with autism spectrum disorders and 21 typically developing subjects matched for age, sex, and IQ were scanned in three conditions: observing short movies showing facial expressions, performing a facial movement, and experiencing a disgusting taste. Symptom severity and level of social adjustment were measured with the Autism Diagnostic Observation Schedule and the Social Functioning Scale.

Results: Inferior frontal gyrus activity during the observation of facial expressions increased with age in subjects with autism, but not in control subjects. The age-related increase in activity was associated with changes in gaze behavior and improvements in social functioning. These age-related neurocognitive improvements were not found in a group of individuals with schizophrenia, who had comparable levels of social functioning.

Conclusions: The results of this cross-sectional study suggest that mirror neuron system activity augments with age in autism and that this is accompanied by changes in gaze behavior and improved social functioning. It is the first demonstration of an age-related neurocognitive improvement in autism. Increased motor simulation may contribute to the amelioration in social functioning documented in adolescence and adulthood. This finding should encourage the development of new therapeutic interventions directed at emotion simulation.

Key Words: Aging, autism, emotion, mirror neuron system, schizophrenia, social functioning

Autism is a lifelong disorder defined by impairments in social and communicative functioning and by pronounced behavioral rigidities (1,2). Autism spectrum disorders (ASD) have a strong genetic component, but no biological marker is available to date. An influential (3–6) but controversial (7,8) theory holds that the core social difficulties in ASD originate from a dysfunction of the putative mirror neuron system (MNS). Mirror neurons are found in macaques in the ventral premotor and inferior parietal regions involved in action execution. Single-cell recordings demonstrate that these neurons fire when the monkeys perform an action and when they observe a similar action (9–12). The discovery of this mirroring property challenges the distinction between action and perception and suggests motor programs may play a role in action understanding (13). A subset of ventral premotor neurons triggering mouth actions also fire to the observation of similar mouth actions, including communicative gestures (14). Single-cell (15), functional magnetic resonance imaging (fMRI) (16–19), and transcranial magnetic stimulation (TMS) (20,21) studies show that a similar system exists in humans. The motor simulation mechanism implemented in the human MNS may contribute to understanding the intentions behind the actions of others (22). This also seems to be true for emotional facial expressions (23), which trigger an increase of activity in the precentral motor face area of the observer (24–29) associated with facial mimicry (27). The observer (unconsciously) mimics the emotion in a muscle-specific manner (30–32), which can facilitate emotion recognition (33–35). Adopting emotion-specific postures triggers the corresponding emotion (36), whereas motor interference modifies the subjective experience of observed emotions (37). The interaction between emotion perception and motor simulation might be instantiated by the inferior frontal gyrus (IFG; Brodmann's area [BA] 44/45) and the anterior insular cortex (24,38), which are anatomically connected (39). The anterior insular cortex, thought to represent bodily sensations (40), may serve as a relay between the premotor cortex and the limbic system (24,38,41). Activity in the IFG during the perception of a disgusted expression indeed seems to cause increased activity in the anterior insular cortex (42). High empathizers activate these regions more strongly (38,43) and mimic more (44), which underlines the importance of motor simulation for emotion recognition and empathy (23).

In this context, the finding that children and adolescents with ASD fail to activate the IFG normally during the perception and the imitation of emotional facial expressions (41,45–47) suggests an MNS dysfunction that can potentially affect social comprehension. The first experiment tested children aged 12 $\pm$ 2 years and found a significant (negative) correlation between IFG activity and symptom severity (41). In fact, at the group level, children with ASD did not show any significant IFG activity during the observation of emotional facial expressions. Three subsequent investigations with children and adolescents produced similar findings on tasks in which the subjects had to match upright and inverted faces (45), recognize themselves on a set of morphed pictures (46), or judge their own emotional response while empathizing with a face on the screen (47). Previous investigations with adults have provided mixed results, with two out of three studies failing to show signifi-

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cant group differences in the IFG for face perception (48–50). However, the sample size in these studies was small (approximately 10 subjects per group), and groups were not matched on critical variables. Here, using fMRI and dynamic facial expressions, we examine the relationship among IFG activity, autistic symptoms, and social behavior outside the laboratory in an adult population of 21 males with ASD, who are pair-matched on age and IQ with 21 typically developing (TD) males.

Methods and Materials

Participants

Twenty-one adult males with ASD (mean age = 30.6 years, SD = 10.09, range 18–54 years) were recruited via local mental health institutions and mailing lists. All subjects were diagnosed with autism, Asperger syndrome, or pervasive developmental disorder—not otherwise specified by a clinical psychologist or psychiatrist according to DSM-IV-TR criteria (51). Clinical diagnoses were verified with the Autism Diagnostic Observation Schedule (ADOS) (52). One of the subjects scored below the communication domain cutoff; his diagnosis was confirmed by the Autism Diagnostic Interview—Revised (53). The subjects were considered high-functioning by their clinicians, and none had an IQ score below 70 (mean IQ = 102.5, SD = 14.81) on the Groninger Intelligence Test 2 (54). The control group consisted of 21 TD men (mean age = 30.5 years, SD = 9.85, range 18–53 years), who were pair-matched on age and IQ (mean IQ = 101.5, SD = 17.40) with the subjects in the ASD group (Table S1 in Supplement 1). The presence of major psychiatric disorders was ruled out with the Dutch version of the Schedules of Clinical Assessment in Neuropsychiatry (version 2.1) (55). In addition, they were interviewed to verify that first-degree relatives did not have a pervasive developmental disorder or a history of psychosis. All subjects had normal or corrected-to-normal hearing and vision, were eligible for MRI research, and gave written informed consent to participate in the study, which was approved by the Institutional Review Board of the University Medical Center Groningen.

Behavioral Measures

We assessed each subject's current level of social adjustment through the Social Functioning Scale (SFS) (56), which has originally been developed for schizophrenia. The SFS, which is filled out by both the subject (SFS—Client) and an informant (e.g., a parent, SFS—Other), is preeminently a measure of current social adjustment in people with known social difficulties, because it is a continuous measure that taps those areas that are crucial to community maintenance (e.g., prosocial activities, independent living skills, employment). For the ASD group, we additionally used the social domain of the ADOS as a measure of symptom severity.

fMRI Tasks

The study of mirror mechanisms requires measuring brain activity not only when subjects perceive, for instance, the emotion of another individual, but also when they themselves feel or express an emotion. Therefore, subjects first performed an observation task, followed by two control tasks: facial movement execution, and emotion experience through a disgusting taste (Supplement 1).

Observation of Dynamic Facial Expressions. The observation task comprised two visual runs, during which subjects were asked to watch short movies of facial expressions carefully (3 sec, 14° × 18°). Each run consisted of the same 60 movies presented in random order, which showed 1) actors making a disgusted, pleased, or neutral facial expression (i.e., blowing up the cheeks) or 2) actors responding as naturally as possible to one of three tastes: water (neutral condition), lemon juice (disgust condition), or a sweet juice (pleasure condition). In these cases, the actors responded with a clear emotional facial expression after tasting the liquid through a straw (Figure 1A). For each stimulus type, there were eight actors (male and female), who were recruited from a local professional theater company and a youth theater school. The movies were validated and used in two previous experiments (28,38). Movies were separated by a red fixation cross (1° × 1°) with an intertrial interval that varied randomly between 5 and 12 sec (baseline). A still image of the background against which the actors were filmed was presented at the beginning of each run and served as a background for the fixation crosses to improve stability of the eye tracking signal (Supplement 1) by keeping the pupil size constant. Stimuli were presented using Presentation software (Neurobehavioral Systems, Albany, California).

Magnetic Resonance Images Acquisition and Preprocessing. See Supplement 1.

Subject Level Analysis. Statistical analyses were implemented using the Statistical Parametric Mapping software package (SPM2, Wellcome Department of Cognitive Neurology, London, United Kingdom; http://www.fil.ion.ucl.ac.uk) and the region of interest (ROI) toolbox MarsBaR (http://marsbar.sourceforge.net). Time series were high-pass filtered at 385 sec for the visual runs to remove low-frequency noise and slow drifts in the signal. At the subject level, separate predictors were used as boxcar functions convolved with the hemodynamic response function for the six movie types (disgust, pleasure, and neutral, either with or without a cup).

Group Analyses. Dapretto and colleagues (41) found that the strongest difference between children with autism and typically developing children during the imitation of facial expressions was located in the pars opercularis of the right IFG (BA44) around peak coordinates (10, 16, 57). To examine activity in this region (Figure 1B) in adults, we first created a spherical ROI centered on the corresponding Montreal Neurological Institute coordinates (Figure 1C). In the absence of information on the cluster size of the activated region, we used a 5-mm radius sphere for our analyses. We checked whether our ROI had mirror properties (Figure 1C) by examining its activity in the control group during facial expression execution and during emotion experience (Supplement 1). Next, contrast estimates for all six movie types were extracted from the ROI at the subject level and subjected to a mixed-model analysis of covariance with factors Emotion × Context × Group, including IQ and Age as covariates. Because the effect of group did not interact with factors Context or Emotion, we then averaged the contrast estimates per subject over emotion and context to compute the general effect of watching facial expressions. Subsequently, we set up a multiple regression analysis in MarsBaR with six columns in the design matrix: one constant for each group, and separate IQ and Age covariates for each group to account for the broad age and IQ range in both groups (18–54 years, 73–133 IQ points). This analysis was repeated after the removal of two outliers, the BA44 activity of which was more than two standard deviations apart from the group mean. To explore whether the effects found in the ROI were spatially limited, we repeated this analysis for all voxels in the brain using SPM (without removing the outliers).

To examine whether there was hypoactivity in the ROI for the younger subjects with ASD, we selected the eight youngest and eight oldest subjects of each group and ran two independent-sample *t* tests. A sample size of eight is enough to enable parametric statistics, while preserving sufficient difference in age between the subgroups.

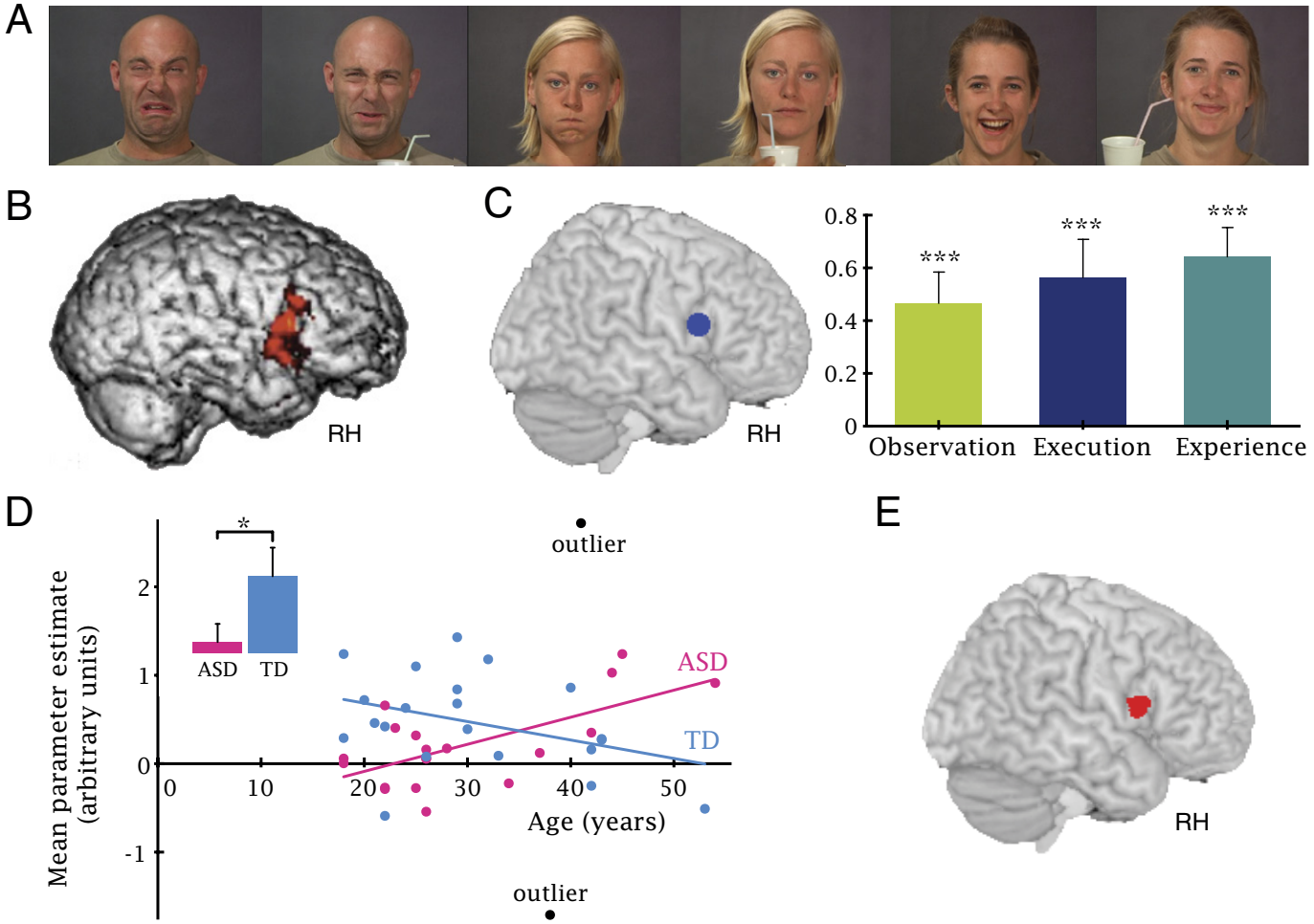

Figure 1. Region of interest (ROI) definition, stimuli, and functional magnetic resonance imaging results. **(A)** Still frames at maximum intensity of the disgusted, neutral, and happy facial expressions with and without the presence of a gustatory stimulus. Neutral movies involved movement of the face to make them more comparable to the emotional facial expressions (third from left: blowing up of the cheeks, fourth from the left: tasting and lip movements). **(B)** Hypoactivation found in children with autism spectrum disorders (ASD) (41). **(C)** Mirror neuron system ROI (5-mm sphere) based on peak coordinates in right Brodmann's area (BA) 44 where a group difference was reported in children (Montreal Neurological Institute coordinates 56, 10, 14). In the typically developing (TD) group, the ROI was significantly active not only during the observation of facial emotional expressions but also during the execution of a facial expression and during emotional experience (*** uncorrected $p < .001$). This suggests that the ROI in our sample of TDs has mirror properties. **(D)** Scatterplot of the Age × Group interaction in the BA44 ROI: the older the subjects with ASD (pink), the stronger the activity and vice versa for the TDs (blue). The bar graph in the top left shows the activity in BA44 for the youngest adults with ASD (pink, $n = 8$, mean age = 21.9) compared with the youngest TDs (blue, $n = 8$, mean age = 21.3), $p < .05$. **(E)** Whole-brain analysis showed that the interaction between age and group is maximal in BA44 ($k = 94$).

Large variability in brain responses to a stimulus reduces the information that a region can provide about that stimulus. It was recently proposed that in ASD, premotor regions show more variable responses to the vision of action (57), which challenges their contribution to social perception. To examine whether premotor responses were less consistent in the ASD group during facial expression observation, we calculated in our ROI each subject's correlation between the modeled and measured time courses across the two perception runs. The correlations were analyzed across participants using a multiple regression analysis in MarsBaR with a single entry per participant, separate constants for the ASD and TD groups, and covariates for IQ and age.

Finally, because in children with ASD BA44 activity predicts symptom severity (41) and social competence (43), we examined the link among social symptoms, social adjustment, and brain activity in adults with ASD. To this end, we calculated the linear pairwise regressions of BA44 activity, age, ADOS (social domain), and SFS scores and compared the regression slopes with those of the control group if applicable and with those of a group of participants with a diagnosis of schizophrenia (Supplement 1). The variability in SFS scores was too low in the TD group to perform regression analyses.

Results

Social Functioning Scale

The SFS scores were significantly lower in the ASD group compared with the TD group [SFS—Client: $t(22.8) = -6.234$, $p = .000$, SFS—Other: $t(23.4) = -7.205$, $p = .000$]. The variability in SFS scores was low in the TD group, reflecting a ceiling effect [TD: $\sigma^2 = 9$, ASD: $\sigma^2 = 107$; $p < .005$].

Movie Ratings

The ratings collected after scanning for the different emotions are summarized in Figure S1 in Supplement 1.

fMRI Group Comparison

During the observation of dynamic facial emotional expressions, high-functioning adults with ASD activated a similar neural network as TD subjects, including BA44 (Tables S2 and S3 in Supplement 1). Compared with the TD group, the ASD group did not show reduced activity in any region of the brain using a standard threshold ($t = 3.33$, uncorrected $p = .001$, $k = 20$, Figure S2 in Supplement 1), nor was there any group difference in the BA44 ROI [one-tailed $t(40) = 1.16$, $p = .13$]. To examine further whether there was an effect of emotion (Disgust, Neutral, vs. Pleasure) or the presence of a context (Cup vs. No Cup) on group difference, we analyzed the signal measured in these six conditions in the ROI using an Emotion (3) × Context (2) × Group (2) mixed-model analysis of covariance including IQ and age as nuisance variables. This analysis confirmed the absence of a main effect of Group [$F(1,38) = 1.07$, $p = .31$] and found no evidence for interactions of Group × Emotion [$F(2,37) = 1.66$, $p = .20$], Group × Context [$F(1,38) = .78$, $p = .38$], or Group × Emotion × Context [$F(2,37) = 1.41$, $p = .25$]. Accordingly, we examined the average activity across all six stimulus types in all further analyzes.

Age Effect on Brain Activity

The regression analysis in the predefined BA44 ROI suggests that age may be a critical factor in determining BA44 activity: although there was no main effect of Age ($F = 0.33$, $p = .57$), or IQ ($F = .03$, $p = .87$), there was a significant interaction for IQ × Group ($F = .4.73$, $p = .04$) and a highly significant interaction for Age × Group ($F = 10.55$, $p = .003$). After the removal of two outliers (see Methods and Materials), the Age × Group interaction became even more significant ($F = 15.93$, $p = .000$), whereas the interaction of IQ × Group disappeared ($F = 2.51$, $p = .13$). As shown in Figure 1D and Figure 2A and 2B, activity in BA44 during emotion perception increased with age for the ASD group ($n = 19$, slope = 3.1, $t = 2.89$, $p = .003$), but not for the TD group ($n = 21$, slope = –2.9, $t = –2.75$, $p = .99$) with the slopes being significantly different ($p = .003$). The whole-brain analysis did not reveal any regions showing a significant IQ × Group interaction. In contrast, the Age × Group was significant in a single region of the brain: right BA44 (Talairach coordinates 58, 12, 12), which matches the area of hypoactivation in children with ASD perfectly (Figure 1E). Selection of the eight youngest subjects in each group showed that young adults with ASD (mean BA44 = .01, mean age = 21.9, mean IQ = 93.6) activated the BA44 ROI significantly less than their TD peers [Figure 1D, mean BA44 = .53, mean age = 21.3, mean IQ = 89.9, $F(1,13) = 6.16$, $p = .03$]. For the oldest subjects, there was no significant difference between the groups, $F(1,13) = .47$, $p = .51$.

If a group has higher variability in brain response, the predicted brain response (i.e., time course of the task convolved with the hemodynamic response) should correlate less with the measured brain response. We found no significant difference in this correlation in our BA44 ROI between the ASD and TD group (one-tailed $t = -.37$, $p = .64$). However, there was a differential effect of age in the two groups ($F = 6.99$, $p < .01$): the correlation increased (i.e., unexplained variance decreased) with age in the ASD group (slope = .3, $t = 2.41$, $p = .01$), but not in the TD group (slope = −.2, $t = -1.34$, $p = .91$).

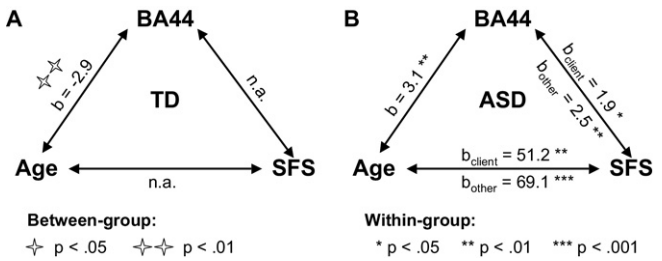

Figure 2. Regression diagram. The diagrams show linear pairwise regressions among Brodmann's area (BA) 44 activity, age, and social functioning (if applicable) in the **(A)** typically developing (TD) group and **(B)** autistic spectrum disorder (ASD) group. Regression slopes are expressed in arbitrary units (× 100) per year/Social Functioning Scale (SFS) point and are reported in combination with their respective significance levels (*$p < .05$, **$p < .01$, ***$p < .001$). Regression slopes that are significantly different from the ASD group are marked by stars in panel A. SFS—client refers to the questionnaire that was filled out by the subject, SFS—other refers to the version that was filled out by an informant (e.g., parent or caretaker).

Social Functioning and BA44 Activity

To examine the behavioral significance of our findings, we investigated the relationship among age, BA44 activity, a measure of autistic symptoms (ADOS social domain), and a measure of social adjustment (SFS) that assesses the subject's engagement in activities that are crucial to community maintenance. In the ASD group, age, BA44 activity, and SFS scores were significantly and positively associated (Figure 2): older subjects not only activated BA44 more, they were also more socially adjusted than younger individuals. In contrast, the social domain of the ADOS was not significantly correlated with age, BA44 activity, or SFS scores (all $ps > .24$), suggesting that age-related changes in BA44 activity were associated with social adjustment as measured using the SFS but not with the remission of autistic symptoms as measured using the ADOS. Because the TD group is characterized by high social functioning and little variation in SFS scores, we cannot assess whether the link between age and SFS and between IFG activity and SFS is specific to autism or whether it would be observed in any population with social functioning deficits. To disentangle these possibilities, we tested a group of individuals with schizophrenia having predominantly negative symptoms, which are frequently associated with social deficits (58) and autistic-like symptoms (59,60) in schizophrenia. These analyses demonstrate that age-related increases in BA44 activity and social functioning seen in ASD do not occur in schizophrenia (Results and Figure S3 in Supplement 1).

Discussion

In this cross-sectional study, we measured brain activity during the observation of dynamic facial expressions in a group of adults with ASD compared with pair-matched control subjects. Although three previous investigations with children aged approximately 12 years had consistently found significant hypoactivity of the IFG (41,45,46), in our relatively large sample of adults, both groups activated this location to the same extent, even when the analysis was restricted to the region of hypoactivity in children. This confirms the results of two other studies reporting whole-brain analyses for an adult population in which no group difference was found involving the IFG (48,50; but see [49]). The discrepancy between findings in children and adults is intriguing. Our study demonstrates that age might be a critical factor determining IFG activity in ASD: activity increased with age in the autism group but not in the control group, so that by age 30, individuals with ASD no longer differed from typically developing individuals. In addition, the within-subject variance decreased with age in the ASD group. This suggests that neural "noise" in the IFG (57) decreases with age in ASD, which may indicate improved functioning of the MNS. Our results suggest prima facie that motor simulation of facial expressions follows a developmental trajectory with a deficit affecting individuals with ASD during their first years of life, and vanishing some-

where in late adolescence or early adulthood. Importantly, we found that the age-related increase of activity in pars opercularis of the IFG (BA44) in ASD was associated with improvements in social functioning. Increased simulation of facial expressions in the IFG is likely to affect emotion recognition and enhance the ability of some adults with ASD to share the feelings of others as noted earlier in this article. This probably has a positive impact on social affiliation and plays a positive role in the construction of a tissue of social relationships (61,62). It is well established that there is a certain degree of abatement in the behavioral difficulties experienced by individuals with ASD throughout adolescence and adulthood (63–67). Improvements are mostly seen in high-functioning individuals (67,68) and concern social behaviors, as well as language, repetitive and stereotyped behaviors, and emotional responsiveness to other's distress. Here, we found an improvement of social functioning (as measured using the SFS) with age, but autistic symptoms (as measured using the ADOS) did not change significantly. This could be the consequence of a selection bias because we selected only participants who scored above cut-off on the ADOS. Alternatively, it could indicate that although autistic symptoms predominantly persist, the way individuals with ASD cope with their social difficulties improves with age. Although speculative, this would be consistent with a longitudinal study showing that age significantly predicts a decline of maladaptive behaviors such as withdrawal and inattentiveness but not of autistic symptoms (67). The relationship between IFG activity and social functioning could not be investigated in typically developing individuals, because they showed little variation in scores on the social functioning measure. To examine whether the association between IFG activity, age, and social functioning was specific to autism, we included a group of individuals with schizophrenia (Supplement 1). Although the scores on the social functioning scale were comparable to those in the ASD group, IFG activity was not associated with age or social functioning in schizophrenia, suggesting that the developmental pattern might be unique to ASD. Here, it is important to note that the SFS has not been age-normed in an older adult population ($>$ 30 years). However, the fact that we found no evidence of increased SFS scores with age in individuals with schizophrenia argues against the idea that the age-related increase of SFS scores in the ASD group reflects an inherent property of the SFS measure. Instead, our findings are compatible with the idea that increased motor simulation could lead to the documented age-related improvements in social functioning in autism and the improved responsiveness to other's distress evidenced throughout adolescence (64).

Further research is necessary to determine the origin of the increased activity in the IFG during face perception, but the analyses conducted on the available eye tracking data (Supplement 1) suggest that eye gaze behavior might be determinant (69–74). On a group level, eye gaze behaviors in our study did not differ between the individuals with ASD and TDs. This could mean that motor simulation is normal in adults with ASD as long as they pay attention to the same aspects of the face as control subjects (the same conclusion has also been reached for the fusiform gyrus during face processing) (71). Again, age seems to play a critical role. In normal aging, time spent looking at the eyes decreases, whereas fixations to the lower part of the face increase (75,76). We found that in adults with ASD the amount of time spent on the lower half of the face also increases with age. The associated increase in BA44 activity suggests that this could be a beneficial strategy for individuals with ASD. Individuals with ASD reach higher levels of accuracy on emotion recognition (73) and familiar face recognition tasks (77) when presented with information from the lower regions of the face, particularly the mouth region, compared with the eye region. Perhaps high-functioning individuals with ASD, while growing older, learn to look more at the parts of the face that are most relevant for them to decode emotional facial expressions. Further research is necessary to investigate this hypothesis and its implications. For instance, increased fixations to the lower part of the face might lead to better recognition of some (e.g., disgust) but not other (e.g., fear) emotions not tested in this experiment (75).

If there is an actual improvement of facial simulation abilities in ASD during adolescence and early adulthood (78,79) and if it contributes to social adjustment as suggested by our study, therapeutic interventions targeting the same mechanism should be experimentally tested in children. Some recently developed training methods produce significant improvements for face recognition (80) as well as emotion recognition (81,82), but the generalization from training material to real life is not guaranteed (81,82). We are not aware of any study reporting the effect of training motor simulation of facial expressions. The MNS is flexible and learning is possible even in adulthood (83,84). Furthermore, expertise in a motor domain is associated with increased activity in the MNS during the observation of similar movements (85–87). Therefore, children with ASD might particularly benefit from imitation training.

In conclusion, activity in the IFG during the perception of dynamic facial expressions increases with age in autism, and this is associated with improved social functioning. This is the first published evidence of an age-related neurocognitive improvement in autism and suggests that individuals with ASD may learn to improve their social skills over the course of life. There was no significant age-related change in a group of individuals with schizophrenia with comparable levels of social functioning, suggesting that our findings might be specific to autism. Because autism is a developmental pathology with changes occurring over the life span, researchers should examine how individuals with ASD develop to deal with their initial dysfunctions and how therapeutic interventions aimed at promoting motor simulation of emotional expressions can support this process.

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Supplementary material cited in this article is available online.

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