

# A longitudinal analysis of neural regions involved in reading the mind in the eyes

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**The ability to perceive social intentions from people's eyes is present from an early age, yet little is known about whether this skill is fully developed in childhood or that subtle changes may still occur across adolescence. This fMRI study investigated the ability to read mental states by using an adapted version of the Reading the Mind in the Eyes task within adolescents (aged 12–19 years) over a 2-year test–retest interval. This longitudinal setup provides the opportunity to study both stability over time as well as age-related changes. The behavioral results showed that participants who performed well in the mental state condition at the first measurement also performed well at the second measurement. fMRI results revealed positive test–retest correlations of neural activity in the right superior temporal sulcus and right inferior frontal gyrus for the contrast mental state > control, suggesting stability within individuals over time. Besides stability of activation, dorsal medial prefrontal cortex showed a dip in mid-adolescence for the mental state > control condition and right inferior frontal gyrus decreased linearly with age for the mental state > control condition. These findings underline changes in the slope of the developmental pattern depending on age, even in the existence of relatively stable activation in the social brain network.**

**Keywords:** social brain; longitudinal design; mentalizing; adolescence; development

## INTRODUCTION

Understanding what someone else is thinking or feeling is an important component of reading intentions, beliefs and needs of others (Baron-Cohen *et al.*, 1999). A term that is often used to describe this ability is mentalizing, which is defined as a certain type of mental state reasoning in order to define how social cues are interpreted in the complex world of social interactions (Baron-Cohen *et al.*, 1997). In reading the mental states of others, at least two processes are involved: a social perceptual process and a social cognitive process (Adams *et al.*, 2009); both part of the social information-processing network (SIPN; Nelson *et al.*, 2005). The social perceptual process refers to 'reading' someone's mental state by direct observation of non-verbal social cues (Sabbagh, 2004; Adams *et al.*, 2009). The social cognitive process tends to be more complex, as this process enables mental reasoning about others in order to estimate intentions and its associated goal-directed behavior. A key question concerns how reading mental states develops in children and adolescents. In this study, we use a longitudinal design to study the ability to infer the mental states of others by seeing only the eye region of faces across adolescence, and the brain regions involved in this process.

A task which has been used previously in adults to test brain regions involved in reading mental states is the 'Reading the Mind in the Eyes task' (RMET) developed by Baron-Cohen and colleagues (2001a). The task displays the eye region of faces, which can signal basic (e.g. happy) or complex (e.g., confused) mental states whereupon the participant needs to select the correct answer from an array of choices (Baron-Cohen *et al.*, 2001b). As such, the task requires both social perceptual and social cognitive processes, and has been referred to as a social mentalizing task (Dal Monte *et al.*, 2014). With an absence of ceiling effects even in typically developing adults, this social

mentalizing task can be applied in studies including participants of varying ages and in both clinical and typical developing groups (Baron-Cohen *et al.*, 1997; Gunther Moor *et al.*, 2012).

With regard to neural activation, a set of studies in adults has reported robust activation in posterior superior temporal sulcus (pSTS), anterior temporal cortex (ATC) and inferior frontal gyrus (IFG) when reading mental states compared to a control condition which required gender and/or age judgments (Baron-Cohen *et al.*, 1997; Castelli *et al.*, 2010; Gunther Moor *et al.*, 2012). These regions have been related to different social cognitive processes (Blakemore and Mills, 2014). That is, the STS has been found to be important for predicting intentional behavior based on biological motion, i.e. non-verbal body language and facial expressions. Therefore, this brain region has been associated with mentalizing and theory of mind (Blakemore, 2008; Carter *et al.*, 2012). The ATC, also denoted as temporal pole, is labeled as being involved in relating an emotional response to social processes involving memory, such as social recognition (Nelson *et al.*, 2005; Pfeifer and Peake, 2012). The IFG, an area which is implicated in a wide variety of tasks, has been found to be involved in reading action tendency based on non-verbal and verbal cues (Rizzolatti and Craighero, 2004; Sebastian *et al.*, 2010; Liakakis *et al.*, 2011), in understanding social situations (Carter *et al.*, 2012) and in semantic working memory (Dal Monte *et al.*, 2014). These brain areas together are often described as part of the 'social brain' network, which are linked to both social perception and cognition. The areas concerning the social brain network, which are implicated in Reading the Mind in the Eyes (STS, ATC and IFG), are still developing during childhood and adolescence as was shown in several mental state reasoning studies (reviewed in Blakemore, 2008).

A prior cross-sectional study reported that 10- to 12-year-olds and 14- to 15-year-olds showed robust activation in posterior STS, ATC and IFG when performing the RMET (Gunther Moor *et al.*, 2012). This finding was interpreted to suggest that the human system for social perception is already tuned to read the intentions of others from the eyes at a young age, consistent with the notion that children from the age of 10–12 years were already able to perform well on the RMET (Baron-Cohen *et al.*, 2001b; Gunther Moor *et al.*, 2012). However, 10- to 12-year-old children showed additional activation

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in medial prefrontal cortex (mPFC), bilateral IFG and the right temporal pole compared with adults, despite similar performance levels. The elevated level of activation in mPFC during early adolescence has also been reported in other studies using different social cognitive tasks (Burnett and Blakemore, 2009; Van den Bos et al., 2011). The researchers explained this elevated early-adolescence age effect as a refining period in which structural and functional development is still ongoing. This leads to the question whether reading mental states is a stable characteristic which develops early (as was suggested for pSTS activity; Gunther Moor et al., 2012), or whether the neural regions involved in mentalizing undergo further specialization in adolescence (as was suggested for medial PFC, IFG and ATC; Gunther Moor et al., 2012).

The current study tested stability vs change of mentalizing while performing the RMET in adolescents using behavioral and brain measures by means of a longitudinal design. That is, longitudinal designs are imperative for testing stability because even though cross-sectional results are informative for detecting general developmental patterns, the question of whether activation is stable within individuals can only be tested using longitudinal assessment (Plichta et al., 2012; Van den Bulk et al., 2013). For example, a pattern may seem stable across time, but could still be driven by large variability within groups and across sessions.

In sum, the goal of the current fMRI study was to test whether the regions of the social brain network related to mental state reasoning (Blakemore, 2008) are consistently active within adolescents across time or whether regions in this network change during development. In this study, we retested adolescents who had previously participated in the study of Gunther Moor et al. (2012) after a 2-year interval. Participants between ages 10 and 16 years at the first measurement were tested again between 12 and 19 years and completed the RMET in the scanner on both occasions. Brain regions of a priori interest concerning stability over time were bilateral STS, determined based on the results of the first measurement (Gunther Moor et al., 2012). Brain regions of a priori interest concerning changes over time were mPFC, right temporal pole and bilateral IFG (Gunther Moor et al., 2012).

## METHODS

### Participants

This longitudinal fMRI study spanned a time period of 2 years (min = 1.83; max = 2.42; s.d. = 0.15), with a subset of the 10- to 23-year-olds who participated in the first measurement (see Gunther Moor et al., 2012). Data from the first measurement have been published before and focused on the cross-sectional developmental trajectories of brain regions involved in social mentalizing abilities. In this study, a total of 37 right-handed adolescents in the age of 12 to 19 (Mean Age = 15.49, s.d. = 2.08; 23 females) participated again in the second measurement. For the current study, we selected all adolescents ( $N = 39$ , ages 10–16) who took part in the original study of whom 77% of the participants were scanned in the follow-up (T2). The eight participants (six 14- to 16-year-olds and two 16- to 18-year-olds) who did not participate at the second time point (T2) were excluded based on braces or because they did not return our calls and/or e-mails. In addition to the sample reported by Gunther Moor and colleagues (2012), 10 additional participants were included in the analyses of both T1 and T2. All participants followed the exact same scanning protocol. Because we focused specifically on developmental changes across adolescence, no adults were recruited for the longitudinal study.

Intelligence scores were determined during the first scanning session by two subscales of the Wechsler Intelligence Scale for Children (WISC): the subscales similarities and block design (Wechsler, 1991, 1997), and were not significantly correlated with age. Child Behavior

Checklist (CBCL; Achenbach, 1991) scores on the first time point indicated no clinical thresholds and therefore did not indicate exclusion of participants at the second measurement. As a result of technical problems during the second scanning session, data from five adolescents were lost, which led to a total remaining number of 32 participants (who participated at both time points) in the final analysis (Mean Age T1 = 13.2, s.d. = 1.95; Mean Age T2 = 15.37, s.d. = 2.01, 20 females).

Participants provided informed consent, and for minors, parents gave informed consent. Depending on the age of the participant, a fixed amount was paid to either the parent or the participant. The Institutional Review Board of the University Medical Center approved all procedures.

### Experimental task

Participants performed an adapted version of the child version of the RMET (Baron-Cohen et al., 2001a). In this task, participants were presented with photographs of the eye region of faces during the performance of two task conditions: a mental state condition and an age/gender condition presented as a mixed block/event-related design. This design (mixed block/event-related design) is used—instead of an event-related design—in order to ensure that no executive functioning was measured because of enforced attention shifts due to a random presentation of different conditions (Baron-Cohen et al., 1997).

The task conditions were presented in four blocks of 14 trials and contained 28 different black and white photographs (each photograph was displayed once in each condition) that were randomized within each block for each participant. The four blocks alternated between a mental state condition (A) or an age/gender condition (B), which were presented in an ABAB or BABA design (counterbalanced across participants). Depending on the task condition, participants were asked to either judge what the person on the photo was thinking or feeling (mental state condition) or to judge the age and gender of the person on the picture (age/gender condition). Both task conditions required participants to select one of the four simultaneously presented words that best described the photograph. In the mental state condition, the words included both basic emotion terms such as sadness, anger or happiness and more complex emotion terms like thinking, joking or being sure about something. These mental state terms were translated to Dutch by the help of native Dutch speakers (Gunther Moor et al., 2012).

The age/gender condition served as a control condition and consisted of the same pictures as shown in the mental state condition. Participants were instructed to determine whether the presented person on the picture was—(i) either younger than 60 or older than 60, and (ii) a man or a woman. Based on this assessment, participants could choose from the following four response options: ‘younger male’, ‘younger female’, ‘older male’ or ‘older female’. The location of these phrases differed randomly across trials, to avoid that participants responded automatically without reading the possible answers (see Gunther Moor et al. 2012, for a complete description of the procedure).

The age judgments used in the RMET were validated by 10 adults and led to a discrepancy in age judgment on three of the 28 trials. Therefore, in task performance these trials were scored as being correct in cases where the participants labeled only gender accurately.

In between trials, a jittered fixation cross was presented in the center of the screen (varying between 600 and 8000 ms). After each fixation cross, a facial stimulus was presented, accompanied by the four simultaneous presented words (see Figure 1). All stimuli remained visible for 9 s, though participants were required to give an answer within 8 s. Responses that were not made within this time frame elicited the



**Fig. 1** Example of a mental state condition trial ('Reading the Mind in the Eyes Task'; Baron-Cohen *et al.*, 2001b).

feedback 'Too slow' for 1 s, signaling the end of the trial. Responses could be made by pressing a button with the index and middle fingers of the right and left hand. The position of the response buttons mapped to the location of the four response options on the screen. Following the response, the word that was selected by the participant was displayed for the remaining length of the trial.

### Procedure

Prior to scanning, participants practiced both task conditions by performing eight practice trials. In addition, they were asked to read a list of words to make sure they understood all of the mental state terms that were used in the experiment. At the first measurement, six children between ages 10 to 12 years indicated that they did not understand one or two words on the list. Analyses showed that four of these six children made incorrect judgments on a total of five trials (one or two trials per person), despite the explanation of the words before the fMRI session started. These trials have been excluded from further analysis to ensure that these incorrect judgments were not the result of a lack of understanding of the words. At the second measurement, a similar procedure was used and none of the participants indicated that they did not understand the meaning of a word.

During scanning, participants completed two runs of 28 trials and switched between conditions once within a run (e.g. AB-short break-AB). Both runs started with a short instruction display, explaining participants whether they would start with condition A or B. After 14 trials, a display with the text 'SWITCH' was presented for 5 s to indicate the switch between task conditions.

### Data acquisition

Prior to scanning, participants were familiarized with the scanner environment using a mock scanner. Scanning was performed using a 3.0-Tesla Philips Achieva scanner at the Leiden University Medical Center. Foam inserts that surrounded the head restricted head motion. Functional data were acquired using T2\*-weighted Echo-Planar Images (EPI) (TR = 2.2 s, TE = 30 ms, slicematrix = 80 × 80, FOV = 220, 35 2.75 mm transverse slices with 0.28 mm gap) during two functional runs of 153 volumes each. The first two volumes of each run were discarded to allow for equilibration of T1 saturation effects. After the functional scanning, high-resolution T2\*-weighted images and high-resolution T1 anatomical images were obtained.

### fMRI data analysis

Data were analyzed using SPM8 (Wellcome Department of Neurology, London, UK). For each participant, the T1-weighted image was

segmented and spatially normalized using the default parameters. The fMRI data were corrected for motion, coregistered with the T1 anatomical image and normalized to a T1 template. Templates were based on the MNI305 stereotaxic space (Cocosco *et al.*, 1997). The normalization algorithm used a 12-parameter affine transformation together with a non-linear transformation involving cosine basic functions, and resampled the volumes to 3 mm cubic voxels. Data were then spatially smoothed with a 8 mm FWHM isotropic Gaussian kernel. Translational movement parameters never exceeded 1 voxel (<3 mm) in any direction for any subject or scan. The participants who participated in both scanning sessions had a mean and maximum head movement of 0.08 and 2.52 mm at the first measurement and a mean and maximum head movement of 0.1 and 2.32 mm at the second measurement. Hence, head movement never exceeded 3 mm at both scanning sessions. Images were corrected for differences in timing of slice acquisition, followed by rigid body motion correction.

Statistical analyses were performed on individual participants' data using the general linear model in SPM8. The fMRI time series data were modeled by a series of events convolved with a canonical hemodynamic response function (HRF). The onset of each presented stimulus was modeled as a separate event and was labeled as mental state or as age/gender (i.e., control) condition. The duration of the separate events was fitted based on length of the reaction time (RT) on each trial. Trials in which participants responded too slow (not within 8 s), or incorrect, were modeled separately as covariates of no-interest, and removed from further analysis. The modeled events based on correctly performed trials were used as covariates of interest in a general linear model along with a basic set of cosine functions that high-pass filtered the data and a covariate for run effects. The least-squares parameter estimates of height of the best-fitting canonical HRF for each condition were used in pair-wise contrasts.

The first-level analyses were group averaged at the second level using a fully flexible factorial design, with the factors: subject, time (T1 and T2) and condition (mental state and control). In this random effects model, we allowed for violations of sphericity by modeling non-independence across images from the same subject and unequal variances between conditions and subjects as implemented in SPM8. This analysis allowed studying main effects of Time (T1 and T2) and Condition (mental state and control) and possible interactions between Time and Condition on a whole-brain level. Task-related responses were considered to be significant at a threshold of  $P < 0.05$  using FDR correction, with a minimum extent of 10 voxels. All brain coordinates are reported in MNI atlas space (see Table 1).

We used the Marsbar toolbox for use with SPM8 (Brett *et al.*, 2002) to perform region of interest (ROI) analyses to further investigate patterns of activation for the two different time points. ROI analyses focused on brain regions of a priori interest (stability vs change) and were based on regions that were identified in the functional mask of the whole-brain analyses at T1 (mental state vs control). Subsequently, activation in these regions was tested for stability between T1 and T2 and age-related changes in activation (see Koolschijn *et al.*, 2011 for a similar procedure). A whole-brain threshold of  $P < 0.05$  (FDR corrected) resulted in activation in the seven task-related areas, which were activated in the mental state > control contrast at T1 ( $N = 32$ ). It appeared that the left STS, the left IFG and the left temporal pole were interconnected, as well as the right STS, the right IFG and the right temporal pole. In order to separate the overlapping brain areas, a threshold of  $P < 0.05$  (FWE corrected) was used for ROI extraction of these regions (see Table 2).

The first set of analyses focused on stability within ROIs over time by correlating activity for the mental state > control condition at both time points for each region. When correlations were present, these

**Table 1** All brain coordinates based on the whole-brain contrast mental state > control for  $N = 32$  in a flexible factorial design; interconnection of subjects on T1 and T2 (FDR corrected,  $P > 0.05$ ; 10 contiguous voxels)

Contrast	Region	MNI (x, y, z) coordinates	Z-value	Volume <sup>1</sup> (=k <sub>ε</sub> value in SPM)
All participants: mental > control				
Frontal	L inferior frontal gyrus	-48, 24, -3	>8	2194
	L inferior frontal gyrus	-54, 18, 18	>8	
	L inferior frontal gyrus	-54, 24, 6	>8	
	Dorsal medial prefrontal cortex	-9, 54, 36	5.67	
Frontal temporal	Dorsal medial prefrontal cortex	9, 57, 33	4.96	906
	R superior temporal sulcus	48, -39, 3	6.88	
Temporal	R inferior frontal gyrus	57, 24, 6	6.81	116
	L temporal pole	-57, -6, -12	7.15	
	R temporal pole	51, 12, -24	5.87	

**Table 2** All brain coordinates for the seven task-related ROIs based on the whole-brain contrast mental state > control for  $N = 32$  on T1 (FWE corrected  $P > 0.05$ ; 10 contiguous voxels: bilateral STS, bilateral IFG, bilateral temporal pole; FDR corrected,  $P > 0.05$ ; 10 contiguous voxels: dmPFC)

Contrast	Region	MNI (x, y, z) coordinates	Z-value	Volume <sup>1</sup> (=k <sub>ε</sub> value in SPM)
All participants: mental > control				
Frontal	L inferior frontal gyrus	-51, 21, 18	6.22	183
	R inferior frontal gyrus	54, 24, 3	5.25	24
	Dorsal medial prefrontal cortex	-9, 51, 36	4.13	139
Temporal	L superior temporal sulcus	-51, -42, 0	6.32	197
	R superior temporal sulcus	48, -36, 0	4.87	14
	L temporal pole	-57, -6, -12	5.44	40
	R temporal pole	51, -9, -21	5.20	37

were followed up by post hoc comparisons for the mental state > fixation baseline and control > fixation baseline conditions.

The second set of analyses assessed the effects of age on neural activation in which we used a linear mixed-effect model approach. This approach is a type of regression model that is able to account for the nested nature of the longitudinal data (participants measured multiple times). These analyses were performed using the lme4 package in R (Bates et al., 2011).  $P$  values were determined using likelihood ratio tests as implemented in the mixed() function in the afex package (Singmann, 2013). These analyses were performed on ROI activation for the contrasts mental state > control, mental state > fixation, control > fixation. For behavioral data, similar analyses were performed to test for effects of age on percentage correct and RT in the mental state and control condition. In all models, a fixed intercept, a fixed effect of Age (linear) and a fixed effect of Age<sup>2</sup> (quadratic) as predictors to test different patterns of change across development were used. These predictor-variables were mean-centered. The nested nature of the data was modeled by including a random intercept per participant.

## RESULTS

### Behavioral data

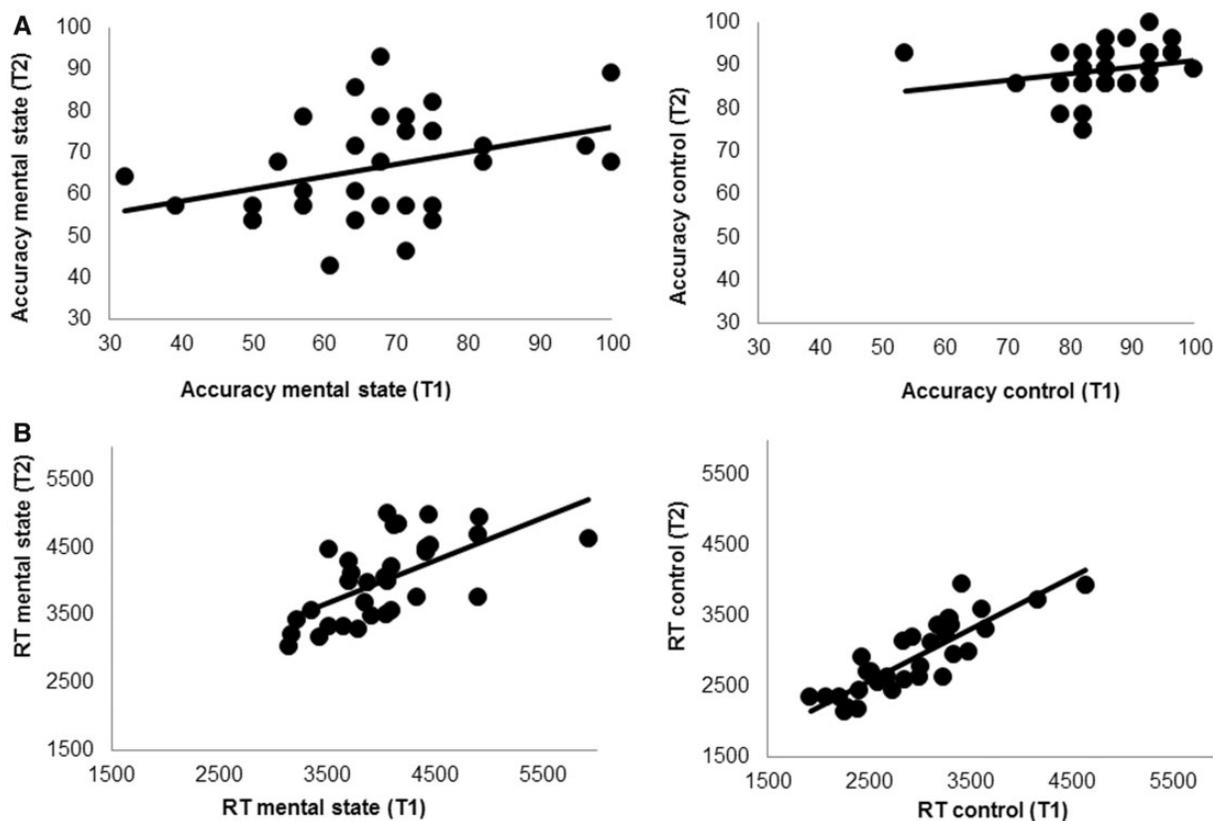
The 2 (Condition: mental state vs control) × 2 (Time: T1 vs. T2) repeated-measures ANOVA for percentage correct resulted in a main effect of Condition [ $F(1, 31) = 101.32, P < 0.001$ ], but showed no main effect of Time or an interaction effect between Time and Condition. The main effect of Condition showed that percentage correct was higher in the control condition ( $M = 87.33\%$ ,  $s.d. = 5.67$ ) than in the mental state condition ( $M = 66.96\%$ ,  $s.d. = 11.52$ ). Even though percentage correct was generally lower on the mental state condition, participants performed well above chance level (25%).

To test whether there was stability in performance across sessions, we computed correlations between percentages correct in the mental state condition at T1 and T2. The analysis for mental state trials revealed a significant positive correlation between T1 and T2 ( $r = 0.37, P = 0.037$ ; see Figure 2A), showing that those participants who performed well at the first measurement also performed well at the second measurement. There was no significant correlation between T1 and T2 for both the control condition ( $r = 0.25, P = 0.16$ ), as well as for the difference score in accuracy between the mental state and the control condition.

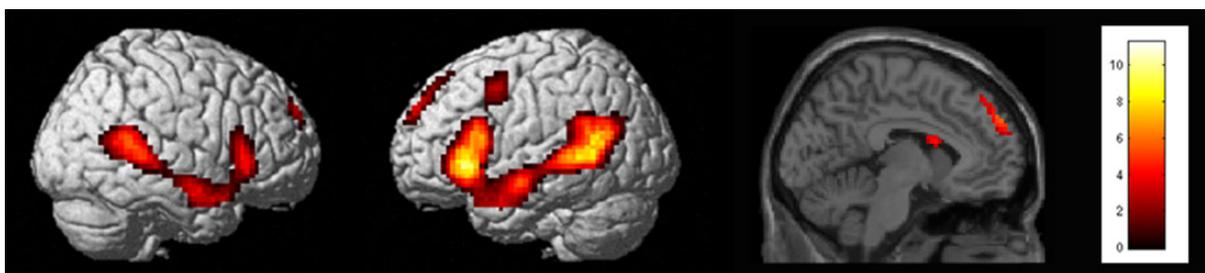
Next, we tested performance changes by testing RT differences between conditions on correctly performed trials. For this purpose, a 2 (Condition: mental state vs control) × 2 (Time: T1 vs T2) repeated-measures ANOVA was performed. This resulted in a main effect of Condition [ $F(1, 31) = 387.13, P < 0.001$ ], but no main effect of Time nor an interaction effect between Time and Condition. The main effect of condition showed that participants responded slower in the mental state condition ( $M = 4.03$  s,  $s.d. = 5.37$ ) than in the control condition ( $M = 2.94$ ,  $s.d. = 5.42$ ).

Correlations between RT in the mental state condition at T1 and T2 revealed a significant positive correlation ( $r = 0.62, P < 0.001$ , see Figure 2B). The same correlation analysis for the control condition also showed a significant positive correlation between T1 and T2 ( $r = 0.86, P < 0.001$ ). These results show that those participants who responded fast at the first measurement also responded fast at the second measurement for both the mental state as the control condition. The correlation between T1 and T2 was not significant for the difference scores in RT between the mental state and control condition.

Finally, age-related changes were tested with a mixed-model approach including behavioral indices from T1 and T2. Results showed no significant age effects on performance (percentage correct answers) for both the mental state and the control condition ( $P > 0.1$ ). Also RT



**Fig. 2** (A) The correlation of the percentage correct responses in the mental state condition between T1 and T2, and the correlation of the percentage correct responses in the control condition between T1 and T2. (B) The correlation of the mean RT on the correct trials in the mental state condition between T1 and T2, and the correlation of the mean RT on the correct trials in the control condition between T1 and T2.



**Fig. 3** Whole-brain contrast (flexible factorial design) for the main effect of mental state > control for  $N = 32$ ; constellation of subjects on T1 and T2 (FDR corrected,  $P < 0.05$ ; > 10 contiguous voxels).

in the mental state and the control condition showed no significant age effects ( $P > 0.1$ ).

**fMRI analyses**

**Whole-brain contrasts**

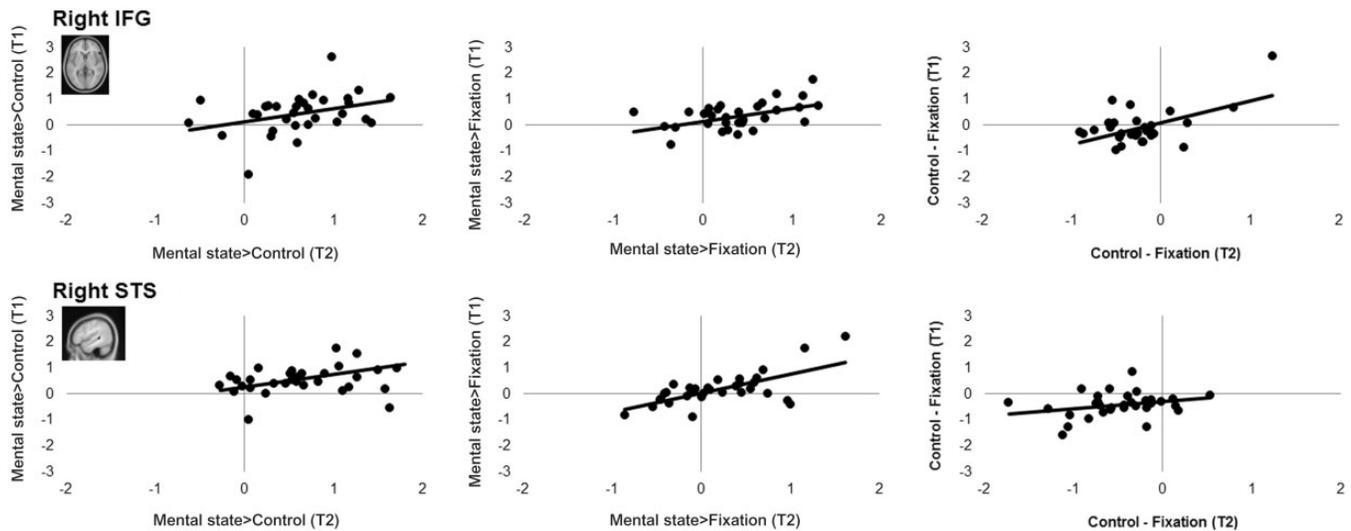
The whole-brain contrast involved a 2 (Condition: mental state vs control)  $\times$  2 (Time: T1 vs. T2) ANOVA using a flexible factorial design. In the analysis, the factors ‘subjects’ (independence = yes, variance = equal), ‘time’ (independence = no, variance = equal) and ‘condition’ (independence = yes, variance = equal) were included to investigate the effect of time. The main effect of Condition revealed more activation in the bilateral STS, bilateral IFG, dorsal medial PFC and bilateral temporal pole for mental state > control condition (see Figure 3 and Table 1). There was no main effect of Time and no Condition  $\times$  Time interaction (note that even when the threshold was lowered to a lenient threshold of  $P < 0.001$ , 10 contiguous

voxels, uncorrected for multiple comparisons, there were no significant effects). Thus, on a whole-brain level, we find merely stability of activation over time within these mentalizing areas.

In order to further investigate patterns of stability vs change we focused on ROI analyses for the regions activated by the mental > control contrast at T1 and correlated those ROIs with activity at T2. Additionally, these ROIs were submitted to analyses testing for age-related changes in neural activity across T1 and T2.

**A test for stability**

First, we tested for stability by correlating activation between T1 and T2 for the ROIs extracted from the mental state > control contrast (see Table 2). A correlation analysis for mental state > control between T1 and T2 resulted in significant correlations in two areas (see Figure 4): right STS (rSTS) ( $r = 0.4$ ,  $P = 0.025$ ; not Bonferroni corrected) and right IFG (rIFG) ( $r = 0.36$ ,  $P = 0.046$ ; not Bonferroni



**Fig. 4** Scatterplots of the correlation between T1 and T2 for the neural activation in the right IFG (54, 24, 3) and the right STS (48, -36, 0) for the contrast mental state > control, mental state > fixation and control > fixation.

corrected). In order to determine to what extent the stability in rSTS and rIFG in the contrast mental state > control is driven by the mental state condition or the control condition, post hoc analyses were performed by correlating mental state > fixation and control > fixation at T1 and T2. Correlating mental state > fixation at both time points resulted in significant correlations in both rSTS ( $r=0.67$ ,  $P=0.001$ ) and rIFG ( $r=0.5$ ,  $P=0.004$ ). A correlational analysis for the control > fixation contrast between T1 and T2 resulted in a significant correlation in the rIFG ( $r=0.55$ ,  $P=0.001$ ). Together, these results indicate that activation in the right STS showed stability over time only for the mental state condition. The right IFG showed stability over time for both the mentalizing and control conditions. The remaining post hoc tests that did not follow from the mental state > control correlation analyses are included in the [Supplementary Material](#) (see [Appendix A](#)).

#### A test for change

Additionally, we tested age-related changes in neural activation over time. That is to say, even though neural activation is stable over time, smaller differences between participants may appear dependent on age.

The mixed models were fitted separately for each ROI. Results for the contrast mental state > control showed significant age effects in the right IFG and the dmPFC. Activation in the right IFG for the mental state > control contrast showed a linear decrease with age ( $P=0.02$ ; not Bonferroni corrected; see [Figure 5](#)). Activation in the dmPFC showed a curvilinear pattern with age, being lowest around mid-adolescence ( $P<0.02$ ; not Bonferroni corrected; see [Figure 5](#)). No age effects were found for the remaining areas.

Next, we performed post hoc analyses in order to determine to what extent these age effects in rIFG and dmPFC in the contrast mental state > control were driven by the mental state or the control condition. For right IFG, the mental state > fixation activation showed no significant age-related changes. Results for the contrast control > fixation showed a linear increase with age ( $P=0.04$ ; not Bonferroni corrected; see [Figure 5](#)). Activation in the dmPFC for the contrast mental state > fixation showed a linear decrease with age ( $P=0.05$ ; not Bonferroni corrected; see [Figure 5](#)). The control > fixation contrast did not show significant age-related changes.

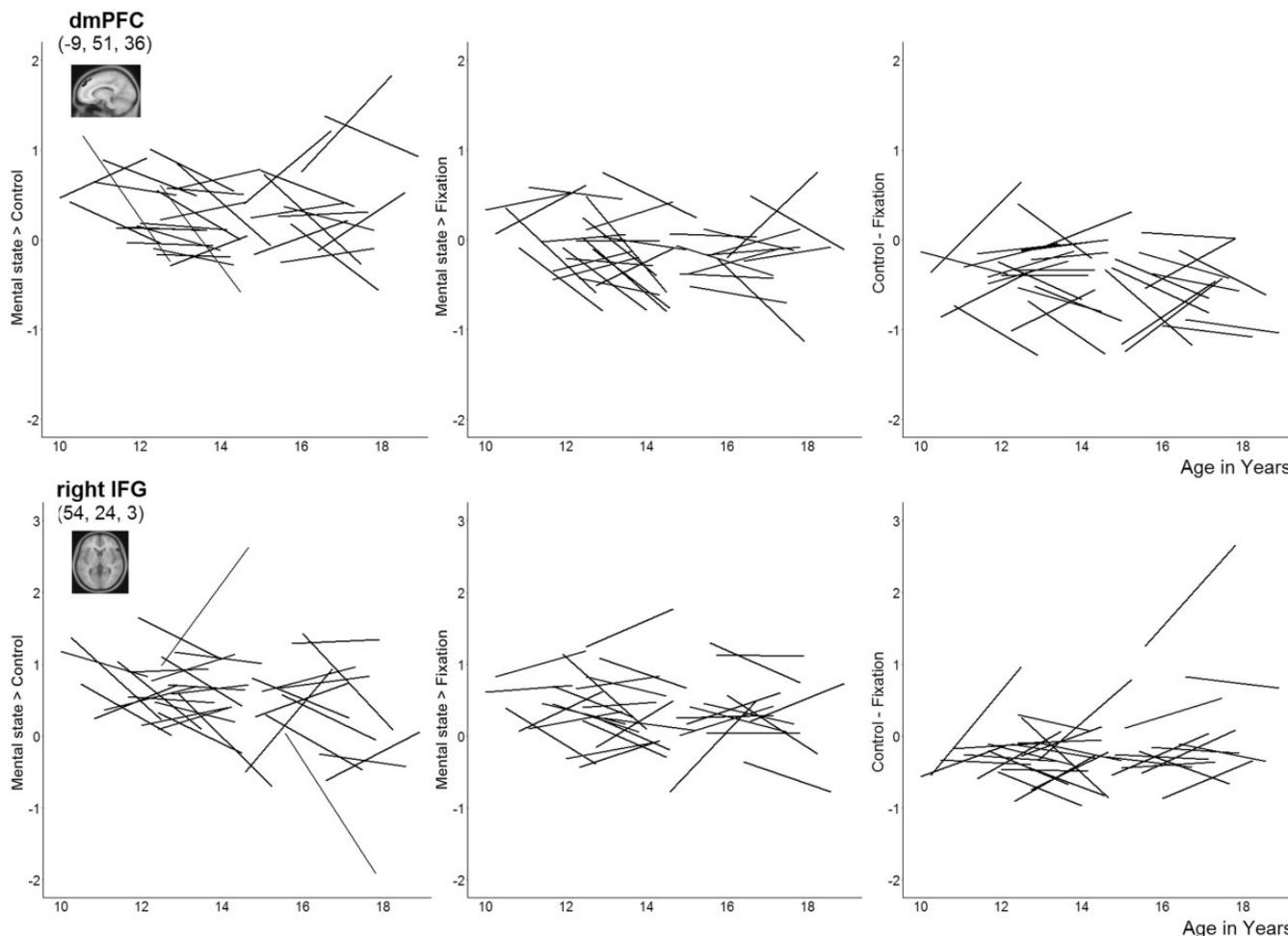
See [Supplementary Appendix B](#) for the remaining post hoc tests that did not follow from the mental state > control contrast.

## DISCUSSION

In this study, we addressed the question whether reading mental states is a stable or changing characteristic during adolescence across a 2-year interval. The analyses resulted in three important findings. First, behavioral results for performance on the RMET demonstrated stability in the mental state condition. This indicates that mentalizing is a relatively stable characteristic within individuals over time. Second, brain-imaging comparisons revealed stability over time in STS and IFG, two regions which have been consistently reported in neuroimaging studies using the RMET ([Adams et al., 2009](#); [Castelli et al., 2010](#); [Gunther Moor et al., 2012](#); [Dal Monte et al., 2014](#)). Third, developmental comparisons revealed age-related decreases in dmPFC and IFG, two regions that have been found to become less active across adolescence when performing social mentalizing tasks ([Gunther Moor et al., 2012](#); [Nolte et al., 2013](#)). As such, the findings show that a social mentalizing task can expose subtle developmental changes within relatively stable social perceptual and cognitive processes.

#### Stable activity in rSTS and rIFG

An important question in research on the neural mechanisms of social information processing concerns whether the neural patterns we observe represent trait-like characteristics that are stable across time or whether these patterns are sensitive to fluctuating emotional states. The neuroimaging studies to date which have examined longitudinal patterns have shown that activity in subcortical brain regions associated with processing emotional phases is varying over time ([Van den Bulk et al., 2013](#)) and changes during pubertal developmental ([Pfeifer et al., 2011](#)). In addition, also in the frontal medial cortex longitudinal comparisons associated with thinking about traits of self and others are associated with change over time ([Pfeifer et al., 2013](#)). However, little is known about the test-retest reliability of the social information processing network including the cortical areas STS, IFG and the temporal poles. The current study found evidence for stability in the right STS and the right IFG over time, suggesting that these



**Fig. 5** Contrast values at T1 and T2 for the mental state > control, mental state > fixation and control > fixation contrasts for dmPFC (−9, 51, 36) and right IFG (54, 24, 3). The connected lines are the activation values at T1 and T2 for each participant separately. The dmPFC shows a significant quadratic dip for mental state > control and an age-related decrease for mental state > fixation. The right IFG shows a significant age-related decrease for mental state > control and age-related increase for control > fixation.

regions are a reliable index of individual differences in mentalizing, at least as measured by the RMET.

In case of the right STS, post hoc tests showed that stability was specific for the mental state, but not for the control condition, confirming that right STS is an important region for performing the RMET (Gunther Moor *et al.*, 2012; Nolte *et al.*, 2013). Overall, the STS region, which has previously been identified as a component of the social detection area of the brain, is important for detecting other people’s mental states and is therefore a basic component of social information processing (Nelson *et al.*, 2005).

For the right IFG, stability was found for both the mentalizing condition and the control condition (mental state > control condition, mental state > fixation condition and control condition > fixation condition). The IFG spans a large area of the lateral prefrontal cortex and has previously been related to a variety of functions such as language processing, motor control and social understanding (Fusar-Poli *et al.*, 2009; Carter *et al.*, 2012). A recent study that compared normally developing adults with traumatic brain injured (TBI) patients showed that TBI patients performed less well on the RMET and especially the left IFG was found to be crucial for performing the task. In addition, tasks that made an appeal on the semantic working memory system correlated positively with performance on the RMET, which led the authors to conclude that the IFG plays an important role in the semantic memory components of the RMET (Dal Monte *et al.*, 2014).

Even though stability was specifically found in the right STS and the right IFG, it should be noted that post hoc comparisons focusing on the mentalizing condition relative to fixation (instead of mentalizing relative to the control condition, presented in the supplement) revealed also stability of neural activation in left lateralized areas. Prior whole brain comparisons showed that the mental state > control contrast resulted in bilateral activation in both STS and IFG (see also Adams *et al.*, 2009; Castelli *et al.*, 2010; Gunther Moor *et al.*, 2012). Thus, the current findings do not provide conclusive evidence with respect to potential lateralization effects, and the study should be replicated in the future using various samples and larger sample sizes.

**Age-dependent changes in rIFG and dmPFC**

Although the ability to judge someone’s mental state is present from an early age (Nelson *et al.*, 2005), the influence of hormonal changes, structural brain changes and environmental factors can influence subtle developmental changes related to social cognition (Blakemore and Mills, 2014). Therefore, we tested whether there were regions in the social information-processing network that continued to change during adolescence. Based on previous research on adolescents, our expectation was to find age-related changes in mPFC, right temporal pole and bilateral IFG (Gunther Moor *et al.*, 2012, see also Blakemore, 2008; Blakemore and Mills, 2014). Longitudinal analyses are particularly useful for addressing this question because the increase in power

by testing the same participants over time allowed us to detect also small within-person changes.

The first region showing age-related change was the right IFG. As was described in the previous section, this region showed relative stability over time, but age comparisons revealed also subtle developmental changes. The main contrast of mental state > control resulted in a developmental decrease over time. As such, the IFG showed the same linear age effect as was shown in the cross-sectional study of Gunther Moor and colleagues (2012). Post hoc tests, however, showed that activity in the control condition increased over time, whereas activity in the mental condition did not change. Therefore, it is important to have a better understanding of the processes involved in the control condition. Several aspects of the control condition deserve attention in future studies. First, the mentalizing condition displayed different words for each trial, whereas the control condition used the same words. Even though the words were presented at different locations on each trial to avoid pre-decisions, the reading demands were much lower than in the mental state condition. Second, behavior is less stable for the control condition over time. Third, adults may have more experience with making age or gender judgments than children. Therefore, different groups may use different strategies when making age or gender judgments. Future studies disentangling the processes involved in the control condition may further reveal these possible strategy changes.

The second region that showed an age-related change was the mPFC. In accordance with previous studies that demonstrated a developmental decrease in mPFC involvement with increasing age (Blakemore et al., 2007; Burnett and Blakemore, 2009; Gunther-Moor, 2012), this study confirmed a developmental change in mPFC recruitment. However, the pattern of change was observed in two directions. The mental state > control contrast showed a quadratic pattern with a dip in mid-adolescence, but the post hoc comparisons revealed an age-related decrease for the mental condition and a non-significant pattern for the control condition. The finding of an age-related decrease in mPFC has interesting parallels with structural changes in this area of the brain from childhood to adulthood (age 8–23; e.g. Mills et al., 2014). The structural changes, indicated by a cubic decreasing trajectory for gray matter development and surface area, and a linear decrease of cortical thickness, could be an indicator for the functional decrease. Although no study up till now has investigated the link between functional and structural brain imaging concerning the social brain in relation to age, larger scale longitudinal studies are necessary to test these relations. Moreover, functional studies concerning mentalizing abilities have consistently found age-related decreases in mPFC activation (Blakemore, 2008), although it is currently unclear which process accounts for this decrease in activity. Perhaps, young adolescents make more use of complex, higher-order processes for mentalizing whereas older adolescents use more automatic social perceptual processes (Blakemore, 2008). Overall, this could indicate a strategy shift from childhood to adulthood (Blakemore et al., 2007). Note that some of the stimuli in the RMET task include direct gaze, which has previously been related to increased mPFC activation (Kuzmanovic et al., 2009). Although this was not the main manipulation in the current experiment, future studies should focus on the role of mPFC in direct and diverted gaze.

A limitation of this study is that the sample size was relatively small to detect more complex developmental patterns, and future studies are necessary with three of four longitudinal measurements to fit growth models. Despite this limitation, this study adds to a growing number of studies showing that longitudinal change in neural regions involved in social cognition can be detected by comparing the same individuals over two time points (Pfeifer et al., 2011, 2013; Van den Bulk et al., 2013) and provides important information about stability and change which cannot be detected in cross-sectional comparisons.

## CONCLUSION

Taken together, several studies have been performed in order to map the neural development of mentalizing abilities from childhood to adulthood and resulted in inconsistent findings. The current study used a longitudinal design and revealed stability over time in the extended social detection network (rSTS and rIFG). In addition, the current study proved that longitudinal measures are able to detect small subtle changes in activation patterns in brain regions that are involved in mentalizing, including the dmPFC and the right IFG. Although interpreting the exact processes of mentalizing that are developing during adolescence is challenging, it is likely that more complex social cognitive processes are still ongoing during adolescence.

## SUPPLEMENTARY DATA

Supplementary data are available at SCAN online.

## Conflict of Interest

None declared.

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