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Effects of age and MAOA genotype on the neural processing of social rejection

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Adolescents are often sensitive to peer rejection, a factor that might contribute to the risk of affective disorder in this age group. Previous studies suggest a significant overlap among socioaffective brain regions involved in the response to social rejection, regions continuing to develop functionally during adolescence and regions influenced by monoamine oxidase A (MAOA) polymorphism. The current study investigated whether the neural response to social rejection is functionally immature in adolescents compared with adults, and whether these responses are modulated by MAOA genotype. Blood-oxygen-level-dependent response was measured with functional magnetic resonance imaging during a rejection-themed emotional Stroop task in 19 adolescents (aged 14–16) and 16 adults (aged 23–28) genotyped for MAOA polymorphism. Similar numbers of MAOA-L and MAOA-H carriers were recruited to maximize power to detect genotype effects. Main effects of rejection stimuli (relative to neutral and acceptance control stimuli) were seen in predicted socioaffective brain regions. Adolescents did not show the adult pattern of modulation by rejection stimuli in the right ventrolateral prefrontal cortex, suggesting continued functional maturation of this regulatory region during adolescence. Age and genotype interacted in the left amygdala, in which the predicted effect of genotype on responses to rejection stimuli was seen in the adults, but not in the adolescents. The data suggest continued functional development of the circuitry underlying the processing of social rejection between adolescence and adulthood, and show that the effects of MAOA genotype on neural responses may vary with age.

Keywords: Adolescence, brain development, emotional Stroop, fMRI, MAOA, rejection

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Functional magnetic resonance imaging (fMRI) studies investigating the neural bases of social rejection have implicated several regions involved in socioaffective cognition, including the dorsal, ventral and subgenual anterior cingulate cortex (ACC), right ventrolateral prefrontal cortex (VLPFC), amygdala and insula (Eisenberger *et al*. 2003, 2007a; Somerville *et al*. 2006; Kross *et al*. 2007; Masten *et al*. 2009). One recent study (Eisenberger *et al*. 2007b) found that a functional polymorphism in the X-linked monoamine oxidase A (MAOA) gene, associated with differing MAOA expression levels (Sabol *et al*. 1998), may influence neural response to social rejection. This study reported greater dACC activity during social exclusion in MAOA-low individuals compared with MAOA-high individuals, regardless of sex. This polymorphism also influences individual differences in socioaffective neural function more broadly: MAOA-low alleles have been associated with increased activation to affective stimuli in the amygdala (Meyer-Lindenberg *et al*. 2006, Lee & Ham, 2008), decreased activation in prefrontal cognitive control regions (Fan *et al*. 2003; Passamonti *et al*. 2006) and aberrant functional connectivity between these regions (Buckholtz *et al*. 2008).

Evidence that this polymorphism may interact with childhood environment to influence behavioural outcome (Caspi *et al*. 2002; Kim-Cohen *et al*. 2006) suggests that it is important to understand the influence of this gene at different developmental stages. However, studies have not yet investigated whether the functional effects of MAOA polymorphism on the developing brain are the same as those seen in adult studies. A stable relationship between genetic polymorphism and brain function across development cannot necessarily be assumed. For example, Lau *et al*. (2009) studied the effects of a length polymorphism in the serotonin transporter gene, and found greater amygdala activation to emotional faces in anxious/depressed adolescents who were homozygous for the 'L' allele than in 'S' carriers, in contrast to previous adult studies.

Adolescence is an interesting time to study the neural response to rejection, as neuroimaging studies have shown structural (Gogtay *et al*. 2004) and functional (Blakemore 2008) development of brain regions underlying social cognition during this time. Behavioural studies have also shown hypersensitivity to social rejection in adolescent females (O'Brien & Bierman 1988; Sebastian *et al*. 2010), whereas a recent fMRI study of social rejection in adolescents (Masten *et al*. 2009) suggests differential engagement of regions such as the subgenual ACC between adolescence and adulthood (although adolescents and adults were not compared directly).

The current study used a rejection-themed emotional Stroop task to investigate developmental differences in the

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neural processing of social rejection between adolescence and adulthood in females. This task activates a network of socioaffective brain regions similar to those influenced by MAOA genotype including perigenual cingulate cortex (Whalen *et al*. 1998), subgenual ACC (Haas *et al*. 2007) and amygdala (Isenberg *et al*. 1999). These regions additionally develop during adolescence, and form part of a 'socioaffective scaffold' comprising amygdala, perigenual cingulate cortex and ventromedial PFC, which may be influenced by MAOA genotype during development (Buckholtz & Meyer-Lindenberg 2008). This task was therefore particularly suitable for investigating both adolescent development in socioaffective circuitry, and potential modulatory effects of MAOA genotype.

Main effects were predicted in regions known to mediate socioaffective cognition, and in particular the neural response to social rejection (perigenual and subgenual ACC, VLPFC, amygdala). Predictions for age-group differences in these regions were based on previous studies showing functional development of socioaffective brain circuitry between adolescence and adulthood. For genotype, it was predicted that MAOA-low carriers would show hyper-reactivity in affective regions (e.g. amygdala), and hypoactivity in prefrontal regulatory regions, compared with MAOA-high homozygotes. The possibility that the effect of genotype on neural response might vary with age was also investigated in an exploratory analysis.

Method

Participants

The study included 35 IQ-matched, native-English speaking female participants: 19 adolescents (mean IQ = 114.00, SD = 12.19) and 16 adults (mean IQ = 120.56, SD = 9.24); see Table 1. Participants were Caucasian to avoid possible population stratification confounds (Cardon & Palmer 2003), and had no history of psychiatric, neurological or neurodevelopmental disorder. Procedures were approved by the local ethics committee, and all participants (or their parents/guardians) gave written informed consent. Only females were included as previous studies have found sex differences in brain development trajectory during adolescence (Giedd *et al*. 1999). Averaging across males and females might therefore yield an inaccurate picture and reduce statistical power. Females were chosen as they have been reported to be more sensitive to social rejection than males (Crick *et al*. 2002).

Genotyping

Adults were recruited from a database and genotyped using a pre-existing DNA sample. Forty-nine adolescents from local schools provided saliva samples using the Oragene collection system (DNA Genotek, Ottawa, Canada). Of these, 19 were selected to create genotype groups matched on age/IQ variables. Similar numbers of females were recruited to MAOA-high and MAOA-low groups to increase the statistical power of the imaging genetics analysis.

The polymorphism of interest consists of a 30 bp variable number tandem repeat (VNTR) located in the promoter region. The presence of 3.5 or 4 repeats is associated with higher MAOA expression (MAOA-high) than 3 or 5 repeats (MAOA-low) (Sabol *et al*. 1998). Participants with one or more MAOA-low allele were placed in an MAOA-low group, whereas an MAOA-high group comprised individuals who were MAOA-high homozygous, in line with previous studies (Meyer-Lindenberg *et al*. 2006; Buckholtz *et al*. 2008). No suitable MAOA-low homozygote adults were able to attend a scanning session. Therefore, the adult/low group contained only heterozygotes, whereas two of the adolescent/low group were homozygous. In total, there were eight participants in each of the adult/high, adult/low and adolescent/high groups, and 11 participants in the adolescent/low group. More adolescents were scanned in the latter group to enable the exclusion of two MAOA-low homozygous participants without matched counterparts in the adult/low group. However, including these participants did not alter the results, and so their data are included below. The researcher conducting the fMRI scanning was blind to genotype.

DNA was extracted using the standard protocol on the Oragene extraction kit and normalized by dilution to a concentration of 100 μM after measurement by UV spectrophotometry (NanoDrop 2000, Thermo Scientific, USA). Samples were genotyped by polymerase chain reaction (PCR) amplification using the following primers:

5′FAM-ACAGCCTGACCGTGGAGAAG-3′

5′-GAACGGACGCTCCATTCGGA-3′

Table 1: Participant variables and behavioural data in Age × Genotype groups. Mean (*SD*) values are displayed

	Adult		Adolescent		
Age group	High	Low	High	Low	
Genotype group	$n = 8$	$n = 8$	$n = 8$	$n = 11$	
Age	30.22 *(4.6)*	27.18 *(2.51)*	15.45 *(0.76)*	15.43 *(0.87)*	
WASI IQ	122.00 *(9.72)*	119.13 *(9.14)*	115.00 *(9.34)*	113.27 *(13.96)*	
Tanner stage	—	—	3.41 *(0.33)*	3.56 *(0.42)*	
RT (millisecond)					Total
Rejection	758.47 *(90.98)*	689.26 *(102.91)*	738.84 *(78.63)*	780.87 *(67.04)*	744.15 *(87.95)*
Acceptance	760.50 *(87.93)*	716.02 *(109.87)*	752.30 *(69.89)*	771.43 *(47.48)*	751.32 *(79.35)*
Neutral	731.86 *(77.75)*	691.90 *(86.96)*	741.57 *(84.23)*	787.45 *(83.36)*	741.09 *(86.87)*
Error (%)					
Rejection	1.39 *(1.82)*	2.26 *(1.81)*	3.47 *(2.46)*	5.56 *(4.73)*	3.37 *(3.46)*
Acceptance	2.08 *(2.78)*	3.30 *(1.27)*	3.47 *(2.10)*	4.42 *(2.31)*	3.41 *(2.27)*
Neutral	2.08 *(1.96)*	3.13 *(2.07)*	4.86 *(3.15)*	4.55 *(3.57)*	3.73 *(2.95)*
Missed trials (%)					
Rejection	1.74 *(2.20)*	2.98 *(4.64)*	3.17 *(2.86)*	2.78 *(2.36)*	2.65 *(2.96)*
Acceptance	1.39 *(1.48)*	2.78 *(3.59)*	3.77 *(2.62)*	2.22 *(2.72)*	*2.47 (2.68)*
Neutral	1.74 *(1.23)*	2.18 *(2.10)*	3.57 *(4.53)*	3.89 *(1.83)*	2.91 *(2.65)*

WASI IQ was measured with the two-subtest (vocabulary and matrix reasoning) full-scale version.

The first primer was fluorescently labelled with FAM on its 5′ end and the primer pair was blasted with the *in-silico* PCR application on the UCSC genome browser (Kent *et al*. 2002). Amplification was performed in a final volume of 25 μl, 25 ng genomic DNA, 200 μM dNTP, 10 pmol of each primer and 0.25 U Taq DNA polymerase (Molzym, Germany) in the standard buffer at 1.5 mM $MgCl_2$. Cycling was optimized and was performed at 30 s at 92°C denaturation, 1 min at 62°C annealing and 1 min at 72°C extension for 35 cycles with a final extension of 72°C for 7 min (Sabol *et al*. 1998). In each well, 2 μl of the PCR product was added to 2.5 μl of formamide and 0.5 μl of 500XL ROX size standard before denaturation for 5 min at 95°C and being placed on ice. The size of the PCR amplicon in the final mixture was determined by GeneScan on the 3730xl and analysed by GeneMapper (Applied Biosystems, UK) by an independent blind investigator.

Experimental task

The experimental task consisted of a rejection-themed emotional Stroop. Participants were asked to indicate with a button press the ink colour in which stimulus words were presented. Previous studies have shown that emotional Stroop tasks can induce reaction time (RT) interference (Williams *et al*. 1996) and activation of affective circuitry (e.g. Whalen *et al*. 1998) in response to negative emotional words compared with neutral and positive words. In the present study, stimulus words were divided into three valence categories; rejection, acceptance and neutral, with 12 words in each. Valenced words (rejection and acceptance) were themed around social rejection (e.g. 'pathetic') and inclusion (e.g. 'admired'). Neutral stimuli consisted of household objects and neutral adjectives. Stimuli were matched for frequency, length, number of syllables, part of speech and arousal levels. Where available, normed valence ratings (scored 1–9) were taken from the Affective Norms for English Words (Bradley & Lang 1999). Mean valence ratings of stimulus categories were 2.49 for rejection, 5.45 for neutral and 7.81 for acceptance, with significant differences between all valences (Ps $< .001$).

Blocks of each stimulus type were presented in a permuted design, with each block presented six times in total. A fixation cross appeared for 15 seconds every third block. Within each block, the order and colour (red, green, blue, yellow) of the 12 words were pseudorandomized. Stimuli were presented using Cogent in Matlab 6.5, and were projected onto a screen using a mirror mounted on the headcoil. Words appeared in Arial 80 pt font on a black background at the centre of the screen for 1500 milliseconds until an interstimulus interval of 500 milliseconds. Button box responses to the colours were made using the index and middle fingers of both hands. Participants practiced these responses before the fMRI session with neutral stimuli not seen in the main experiment. RTs, error rates and missed trials were recorded.

The task was validated in a behavioural pilot with 74 female IQ-matched participants: 26 adults (mean age = 27.37, SD = 6.19), 23 young adolescents (mean age = 12.74, SD = .60) and 25 mid-adolescents (mean age = 15.00, SD = .53). For RT, a Group × Valence analysis of variance(ANOVA) showed a main effect of Valence: $F_{2,142} = 6.91$, $P = .001$, with Bonferroni-corrected comparisons showing significantly slower RTs for rejection words (mean = 625.29 milliseconds, SD = 66.72) than for both neutral (mean = 609.51, SD = 69.43; $P = .009$) and acceptance (mean = 609.64, SD = 66.95; $P = .007$) words. Acceptance and neutral RTs did not differ ($P > .99$). The young adolescents were significantly slower than the other two groups across all three word valances, so this group was not included in the fMRI study. There was no interaction between age and valence. There were no significant results involving error rates, with mean errors across all participants at 3.92% (SD = 3.39). This pilot study concluded that rejection-related stimuli were capable of inducing emotional conflict (RT interference) in both adolescents and adults.

fMRI data acquisition

A 1.5 T Siemens Sonata MRI scanner was used to acquire 12 min 3D T1-weighted structural images, and multislice T2*-weighted echo planar volumes with BOLD contrast. The T2* EPI sequence was optimized to reduce dropout in the orbitofrontal cortex (Weiskopf *et al*. 2006), and the following acquisition parameters were used: thirty-three 2 mm slices acquired in a descending trajectory with a 1 mm gap, TE = 50 milliseconds; TR = 90 milliseconds; slice tilt = −30° (T>C); flip angle = 90°; field of view = 192 mm; matrix size = 64 × 72. Functional data were acquired in a single scanning session of approximately 9 min, in which 183 volumes were acquired.

Data analysis

RT, error and missed trial data were analysed using 3 × 2 × 2 mixed model ANOVAs with within-subjects factor valence (rejection, acceptance, neutral), and between-subjects factors age (adult, adolescent) and genotype (MAOA-High, MAOA-Low). Participant outliers were defined as >2.5 SD above the sample mean.

Imaging data were analysed with SPM5 (www.fil.ion.ucl.ac.uk/spm). The first five images from each run were discarded to allow for T1 equilibrium effects, leaving 178 image volumes per participant. Pre-processing included rigid-body transformation, normalization into standard space defined by the Montreal Neurological Institute (MNI) template with a voxel size of 3 × 3 × 3 mm, and smoothing with a Gaussian filter of 8 mm.

Each word valence block was modelled as a boxcar function convolved with a canonical haemodynamic response function. The six realignment parameters were modelled as effects of no interest, to account for variance as a result of head movement. In addition, distorted scans caused by excessive movement (>1.5 mm between scans) were removed (one scan for each of two adolescents only). Data were high-pass filtered at 128 seconds to remove low-frequency drifts. First-level analysis was conducted on two rejection-related contrasts of *a priori* theoretical significance: rejection>neutral and rejection>acceptance (and their reverse). At the second level, age and genotype served as between-subject variables in a factorial design. *t* statistics were calculated for the main effects of valence (e.g. rejection>neutral) across all participants, for two-way interactions (Valence × Age and Valence × Genotype) and for three-way interactions between valence, age and genotype.

For completeness, main effects of valence (rejection>neutral, rejection>acceptance and their reverse) are displayed in Table 2 at a level of $P < .001$, uncorrected, with a threshold extent of 10 voxels. Regions showing an interaction with age and genotype factors at $P < .001$, uncorrected, $k \geq 10$, are displayed in Table 3. Regions reported in the text and in Table 4 are those for which there was an *a priori* hypothesis (ACC, VLPFC, amygdala) and which survived family-wise error (FWE) correction for multiple comparisons at $P < .05$, adjusted for small volume with spheres of 8 mm radius, centred around a peak voxel obtained from previously published studies. Co-ordinates for small volume correction (SVC) were obtained from three previous studies that used stimuli of close relevance to the current study. Amygdala co-ordinates were those used by Phillips *et al*. (2001) in their investigation of amygdala response to social threat (fearful>neutral facial expressions). Right VLPFC and subgenual ACC co-ordinates were taken from the social rejection fMRI papers by Eisenberger *et al*. (2003) and Masten *et al*. (2009), respectively.

In FWE-corrected regions showing an interaction, *post hoc* analyses were conducted using *t* tests. Simple main and interaction effects were considered significant at a nominal threshold of $P < .001$. To maximize the information displayed, two-way interactions are shown relative to baseline fixation. Therefore, some of the 'activations' discussed appear as 'deactivations' relative to fixation. However, this does not change the interpretation of differences between word valence conditions. For clarity, difference values are plotted for three-way interactions.

Results

Participant variables

Demographic and behavioural data are presented in Table 1. Full-scale IQ was measured with the two subtest,

Table 2: Main effects for the contrasts rejection>neutral, rejection>acceptance and their reverse

Brain region	BA	L/R	Peak voxel (*xyz*)			*k*	*z*
Main effect of rejection>neutral							
Temporal pole (ext. inferior frontal gyrus)	38	L	−42	21	−18	67	4.57
			−51	18	−12		
			−54	24	−3		
Dorsomedial PFC (superior frontal gyrus)	9	L	−6	54	36	27	4.48
Amygdala*	—	L	−15	3	−9	14	3.92
Thalamus (midline nucleus)	—	L	−6	−18	18	14	3.74
			−12	−12	24		
Subgenual anterior cingulate cortex*	25	L	−3	15	−9	11	3.67
Main effect of neutral>rejection							
Putamen	—	R	24	18	3	11	4.10
Putamen	—	L	−18	18	0	10	4.07
Main effect of rejection>acceptance							
Thalamus (pulvinar)	—	L	−9	−30	15	37	4.92
Premotor cortex (precentral gyrus)	6	L	−48	−6	21	16	4.74
Superior temporal sulcus	22	R	60	−39	15	23	4.30
			54	−45	9		3.36
Visual cortex (middle occipital gyrus)	18	R	33	−90	−6	12	3.95
Main effect of acceptance>rejection							
Visual cortex (middle occipital gyrus)	18	L	−12	−84	−15	19	4.18
Medial PFC (superior frontal gyrus)	10	L	−30	63	9	13	3.81
Visual cortex (cuneus)	17	R	12	−81	6	10	3.81

Regions displayed are significant at $P < .001$, uncorrected, with a threshold extent of $k \geq 10$. BA, Brodmann area; L/R, left/right; peak voxel, peak voxel co-ordinates in MNI space; *k*, cluster size (3 × 3 × 3 mm voxels: for empty cells, activations are part of above clusters); *z*, *z*-value for peak voxel.
*Results in predicted regions surviving small volume correction.

Table 3: Interactions with age and genotype for the contrasts rejection>neutral and rejection>acceptance

Contrast	Brain region	BA	L/R	Peak voxel (*xyz*)			*k*	*z*
Rejection>neutral								
Age: Adult>adolescent	—							
Adolescent>adult	—							
Genotype: high>low	Thalamus (medial dorsal nucleus)	—	L	−6	−15	12	29	4.14
	Caudate	—	L	−9	0	18	11	3.73
Low>high	—							
Age × Genotype interaction	Amygdala (ext. parahippocampal gyrus)*	—	L	−18	3	−18	12	3.41
Rejection>acceptance								
Age: adult>adolescent	Ventrolateral PFC (inferior frontal gyrus)*	47	R	45	30	−6	10	3.73
Adolescent>adult	—							
Genotype: high>low	Orbitofrontal cortex	11	R	42	42	−18	19	3.73
Low>high	—							
Age × Genotype interaction	Cerebellum	—	R	18	−84	−42	26	4.62

Display details as for Table 2. Note that the effect of age group in the right VLPFC for rejection>neutral is displayed only in Table 4 because the cluster size was less than 10 voxels.

full-scale version of the Wechsler Abbreviated Scale of Intelligence, with no group differences found (Ps > .1). For adolescents, mean Tanner stage (based on an adapted puberty questionnaire (Carskadon & Acebo 1993) was 3.5 (SD = .04) (mid-late puberty). Tanner stage did not differ between genotype groups: $t(17) = -.85$, $P = .41$.

Behavioural data

RTs from one adolescent/low participant were excluded as an outlier. For the missed trials analysis, data from three participants were excluded because of a button box malfunction identified at the time of testing. The recorded data for these three participants did not otherwise differ

Table 4: Predicted regions from the rejection>neutral and rejection>acceptance contrasts reaching significance at $P < .05$ (FWE-corrected) with small volume spheres of 8 mm radius based on similar previous studies

Brain region	BA	L/R	Peak voxel (*xyz*)			*k*	*z*	Co-ordinate used for SVC			Paper of origin	FWE-corrected *P*-value
Rejection>neutral												
Main effect of condition (rejection>neutral)												
Amygdala	—	L	−15	3	−9	14	3.92	−21	−6	−15	Phillips *et al*. (2001)	.004
Subgenual anterior cingulate cortex	25	L	−3	15	−9	11	3.67	−6	22	−12	Masten *et al*. (2009)	.007
Condition × Age interaction (adult>adolescent)												
Ventrolateral PFC (inferior frontal gyrus)	47	R	45	30	−12	4	3.49	42	27	−11	Eisenberger *et al*. (2003)	.019
Condition × Age × Genotype interaction												
Amygdala (ext. parahippocampal gyrus)	—	L	−18	3	−18	12	3.41	−21	−6	−15	Phillips *et al*. (2001)	.044
Rejection>acceptance												
Condition × Age interaction (adult>adolescent)												
Ventrolateral PFC (inferior frontal gyrus)	47	R	45	30	−6	10	3.73	42	27	−11	Eisenberger *et al*. (2003)	.008

BA, Brodmann area; L/R, left/right; peak voxel, peak voxel co-ordinates in MNI space; *k*, cluster size (3 × 3 × 3 mm voxels: for empty cells, activations are part of above clusters); *z*, *z*-value for peak voxel; SVC, small volume correction; FWE, family-wise error.

from the rest of the group, and so were included in the relevant analyses. RT and missed trial rates showed no main effects or interactions involving valence, age or genotype (all Ps $> .1$). Adolescents made more errors than did adults across the whole task: $F_{1,31} = 7.12$, $P = .012$, $\eta_p^2 = .19$, but importantly for the subsequent interpretation of fMRI contrasts (e.g. rejection-neutral), there were no interactions between valence and age/genotype (Ps $> .20$).

fMRI data

Main effects for the contrasts rejection>neutral and rejection>acceptance (and their reverse) at the whole brain level ($P < .001$, uncorrected, $k \geq 10$) are presented in Table 2. Interactions with age and genotype at the whole brain level ($P < .001$, uncorrected, $k \geq 10$) are presented in Table 3. Main effects and interactions surviving SVC in predicted regions are presented in Table 4.

Rejection> neutral results in predicted regions

Main effect of valence

Significantly increased BOLD responses to rejection stimuli relative to neutral were seen in the left amygdala and subgenual ACC.

Two-way interactions: Valence × Age

There was a significant Valence × Age interaction in the right VLPFC driven by significantly greater BOLD response to rejection stimuli relative to neutral in the adults: $t(31) = 4.45$, $P < .001$, but no difference between word valence in the adolescents (Fig. 1a).

Three-way interaction: Valence × Age × Genotype

There was a significant Valence × Age × Genotype interaction in the left amygdala extending into the parahippocampal gyrus. The interaction was driven by a marginally greater response to rejection words relative to neutral in the adult/low group compared with adult/high: $t(31) = -2.62$, $P = .007$, but a greater response in the adolescent/high group than in adolescent/low for the same contrast: $t(31) = 4.97$, $P < .001$ (Fig. 2). For the within-subject simple effects (rejection-neutral in each of the four age/genotype groups), there was a marginally greater response to rejection words relative to neutral in the adolescent/high group, $t(31) = 2.83$, $P = .004$. No other simple effects were significant.

There were no significant voxels in predicted regions, even at $P < .001$, uncorrected, for the reverse contrast (neutral>rejection).

Rejection> acceptance results in predicted regions

Main effect of valence

Regions showing a main effect for this contrast are displayed in Table 2, as none fell in predicted regions.

Two-way interaction: Valence × Age

There was a significant Valence × Age interaction in the right VLPFC. *Post hoc* analysis showed marginally significantly greater BOLD response to rejection relative to acceptance words in the adults: $t(31) = 3.22$, $P = .002$, but the opposite response in the adolescents: $t(31) = 3.00$, $P = .003$ (Fig. 1b). The region and direction of the Valence × Age interaction are the same as found for the rejection-neutral contrast above.

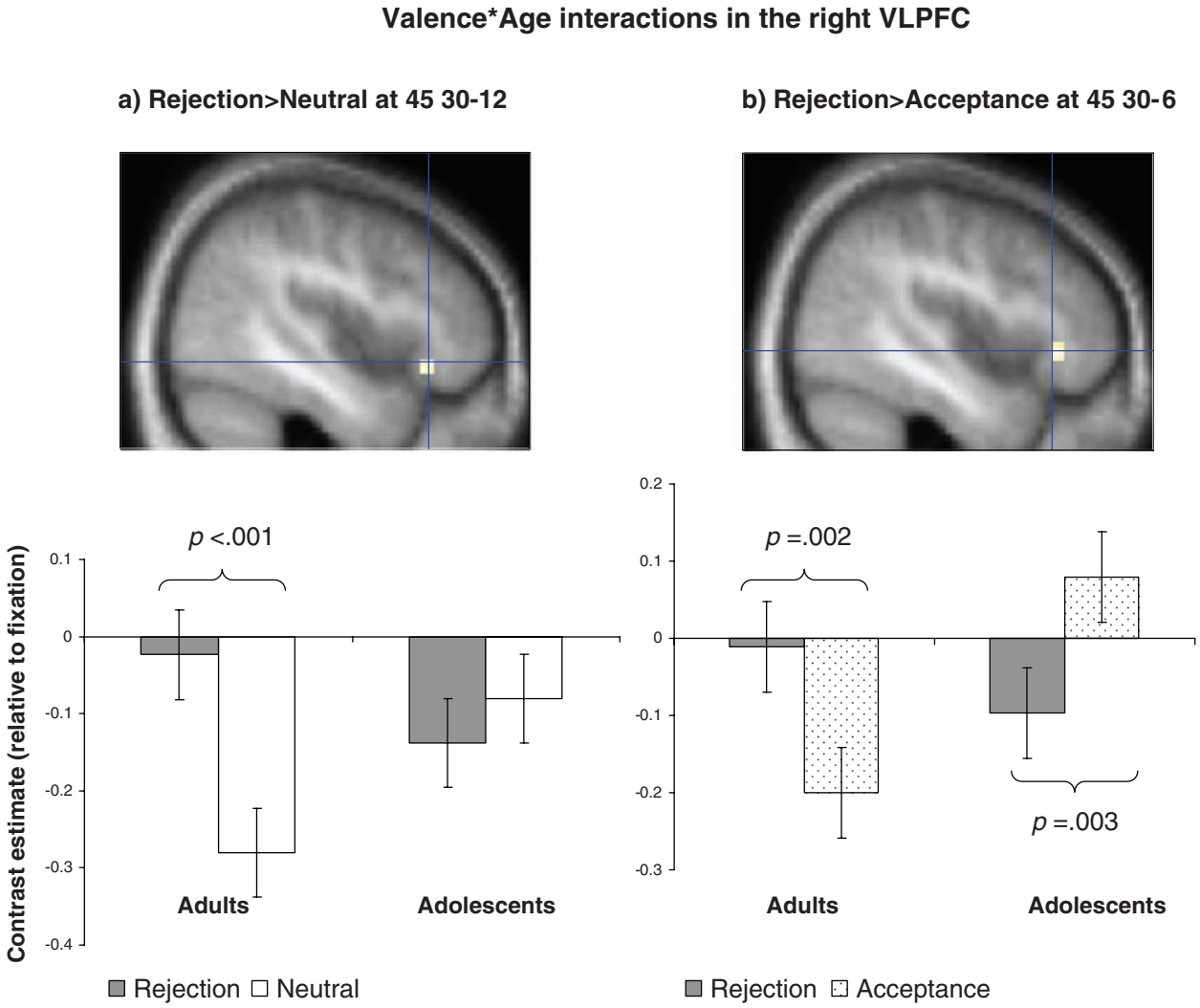

Figure 1: Top: Valence × Age interaction for (a) rejection>neutral and (b) rejection>acceptance in the right VLPFC. Significant voxels at $P < .001$ are overlaid on a coregistered average T1 image from all 35 participants. Bottom: adults showed a typical regulatory response, with greater response in rejection than in neutral and acceptance conditions. However, the adolescents did not show this pattern of response. Contrast estimates are shown relative to baseline fixation. Error bars indicate the standard error of the difference for rejection−neutral (a) and rejection−acceptance (b).

There were no significant three-way interactions for the rejection>acceptance contrast. There were no significant voxels in predicted regions at $P < .001$, uncorrected, for the reverse contrast (acceptance>rejection).

Discussion

This study explored the effects of age and MAOA genotype on neural responses to rejection-related information. Effects of Age on BOLD response to rejection stimuli were seen in the right VLPFC (relative to both neutral and acceptance words). In addition, there was a Valence × Age × Genotype interaction in the amygdala for the rejection>neutral contrast. This suggests that the effect of MAOA polymorphism on the neural response to rejection-related stimuli may be influenced by developmental stage.

Main effects of rejection stimuli

The use of both neutral and acceptance control stimuli enabled the characterization of neural responses to rejection in comparison with both non-social stimuli and stimuli matched for social/emotional content. Consistent with previous fMRI studies of the emotional Stroop (e.g. Whalen *et al*. 1998), the RT interference effect for rejection words

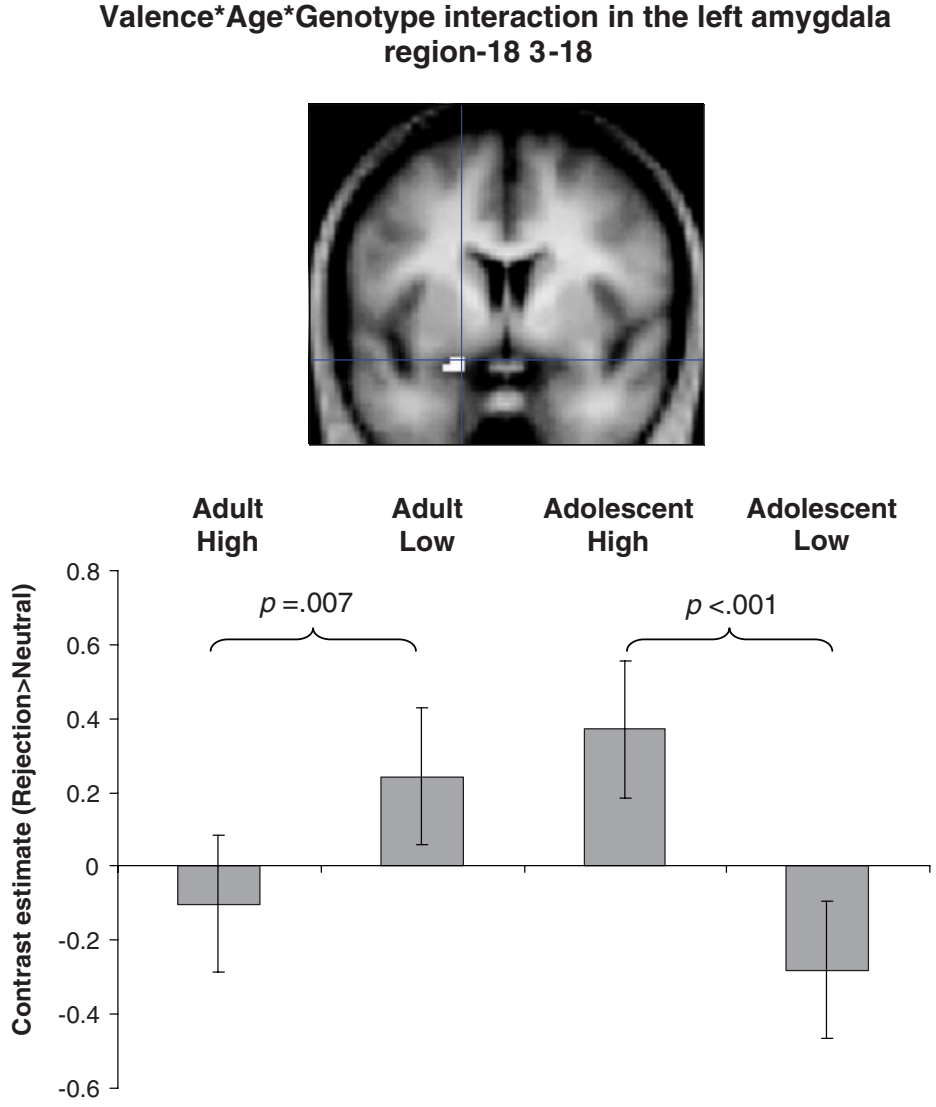

Figure 2: Top: Valence × Age × Genotype interaction for rejection>neutral in the left amygdala region. Significant voxels at $P < .001$ are overlaid on a coregistered average T1 image from all 35 participants. Bottom: contrast estimates for the difference between rejection and neutral parameter estimates. The adult/low group showed greater BOLD response for the contrast rejection>neutral than the adult/high group, whereas the opposite pattern was seen in the adolescent groups.

was not significant. However, RT interference was found in the pilot study (reported above), probably because of greater statistical power. This suggests that the task was capable of inducing emotional conflict in both adolescents and adults.

Imaging main effects for the rejection>neutral contrast showed activations in two predicted regions (amygdala and subgenual ACC), both of which have been shown in previous studies to respond to social rejection (Eisenberger *et al*. 2007a; Masten *et al*. 2009). These previous social rejection studies used an explicit social rejection game scenario. The current data suggest that similar circuitry is also activated in response to the implied threat of social rejection. The data further show that, as with physical threat (e.g. Whalen *et al*. 1998) social threat words can also induce an affective neural response in the context of an emotional Stroop paradigm.

Interestingly, activation in these regions was not seen for the rejection>acceptance contrast. The lack of main effects in predicted regions for this contrast may result from conceptual similarities between rejection and acceptance words. Both refer to abstract social concepts with strong affective salience, whereas neutral words lacked both of these qualities. The rejection>neutral contrast might

therefore have had greater power to show activation in predicted regions. This should not imply, however, that the brain treats rejection and acceptance words in the same way. In addition to main effects outside predicted regions listed in Table 2 (including socioaffective brain regions such as the posterior superior temporal sulcus), this contrast also yielded the largest difference between age groups, as discussed in the next section. This suggests that the more subtle rejection>acceptance contrast may be particularly useful in highlighting regions of relative functional immaturity during mid-adolescence.

Effects of age on responses to rejection stimuli

Valence × Age interactions were seen in the same region of right VLPFC for both rejection>neutral and rejection>acceptance contrasts. This was driven by greater activation to rejection than control words in the adults, but either no difference between conditions (rejection>neutral) or the reverse pattern (rejection>acceptance) in the adolescents. The right VLPFC is involved in affect regulation, including regulation of distress caused by social exclusion (Eisenberger *et al*. 2003), and in stop-signal inhibitory control (Aron *et al*. 2004). The absence of greater engagement in this region for rejection stimuli in the adolescents may indicate a failure to deploy this regulatory region flexibly in response to task demands, in line with previous findings (Eshel *et al*. 2007). This could reflect continuing structural brain development, or the development of functional connections between brain regions involved in socioaffective cognition. Lower levels of regulatory control in response to rejection-related stimuli may contribute to the heightened sensitivity to social rejection seen during adolescence in everyday life (O'Brien & Bierman 1988; Kloep 1999) and in experimental settings (Sebastian *et al*. 2010). However, it should be noted that age differences in the experience of social rejection might also contribute to differences in the way the brain processes this rejection-related task.

Interaction between valence, age and MAOA genotype

No predicted brain regions showed a Valence × Genotype interaction independent of age (although it should be noted that the right orbitofrontal cortex and left thalamus and caudate showed a greater BOLD response to rejection stimuli in the MAOA-high group compared with MAOA-low across age groups at $P < .001$ uncorrected). However, there was a three-way interaction between valence, age and genotype in the left amygdala extending into the parahippocampal gyrus for the rejection>neutral contrast. The adult/low group showed a greater response in this region for rejection stimuli relative to neutral than did adult/high, in line with previous studies (Meyer-Lindenberg *et al*. 2006; Lee & Ham 2008). In contrast, the reverse pattern was seen in the adolescents. Studies investigating functional activation of the amygdala during adolescence have found contradictory results, with some showing reduced activation to negative stimuli in adolescents compared with adults (Thomas *et al*. 2001), and others showing the opposite effect (Hare *et al*. 2008). In the current study, an effect of age group (across genotype) was not seen in this region, suggesting that age differences in amygdala response to rejection-related stimuli may interact with individual differences such as MAOA genotype. The need to take into account the role of individual difference variables such as MAOA genotype across development may partially explain inconsistent results in previous studies comparing amygdala response between adolescents and adults.

A differential effect of genotype between adolescent and adult groups is not without precedent (e.g. Lau *et al*. 2009). However, the mechanism underlying such an effect is unknown, and therefore the possibilities discussed here are speculative. It is perhaps most likely that this effect is a product of the functional reorganization of socioaffective circuitry during adolescence. The 'socioaffective scaffold' described by Buckholtz and Meyer-Lindenberg (2008) as being particularly sensitive to MAOA genotype comprises top-down regulation of the amygdala by prefrontal regions such as perigenual cingulate cortex and ventromedial PFC. Developmental mismatch models of adolescent brain development (Casey *et al*. 2008; Steinberg 2008) suggest that this prefrontal regulation of the amygdala is less effective in adolescence because of the functional immaturity of top-down connections (e.g. Hare *et al*. 2008). It may be that the immaturity of PFC–amygdala circuitry during adolescence obscures the predicted pattern of MAOA genotype effect. As Buckholtz *et al*. (2008) have shown, MAOA genotype does not influence amygdala response in isolation, but affects the quality of PFC–amygdala connectivity. If these connections are not fully mature in adolescence, it is perhaps less surprising that this should manifest as a differential effect of MAOA genotype at different developmental stages. Future studies could address whether the 'reversed' genotype effect found in adolescents in the current study is characteristic of adolescence, or whether this represents just one of several ways in which the predicted effect of polymorphism in the adult brain may be obscured by continued brain maturation in adolescence. Such studies could also investigate changing connectivity profiles between these regions over development, and their interaction with MAOA genotype.

Outside fMRI, a number of studies suggest a changing relationship between gene and brain between adolescence and adulthood. Gene expression patterns and monoamine metabolism are known to vary over the course of development (Kruesi *et al*. 1988; Weickert *et al*. 2009), whereas structural MRI studies have shown increasing heritability of cortical thickness in PFC and temporal regions during adolescence (Lenroot *et al*. 2009). In addition, behavioural genetic studies have shown an increasing genetic influence on the development of affective disorder during this time (Lau & Eley 2006). In line with the current findings, these studies highlight the importance of taking a developmental approach to cognitive genetics (Scerif & Karmiloff-Smith 2005). This is particularly crucial for candidate genes such as MAOA, which is known to interact with environmental factors to influence behavioural outcome over development (Kim-Cohen *et al*. 2006).

Owing to the relatively small sample sizes in each Age × Genotype cell, the three-way interaction data should be regarded as preliminary. However, while the data would benefit from replication with a larger sample, previous imaging genetic studies involving MAOA have employed similar sample sizes (Fan *et al*. 2003; Passamonti *et al*. 2006; Eisenberger *et al*. 2007b). The direction of the effect in the adults also represents a replication of two previous fMRI studies of this MAOA polymorphism in adults, showing increased left amygdala reactivity to negative emotional stimuli in L-carriers (Lee & Ham 2008 (females); Meyer-Lindenberg *et al*. 2006 (males)). In addition, the present study had the advantage of pre-selecting participants by MAOA genotype, so that there were roughly equal numbers of participants in each cell. This served to maximize the statistical power of our analyses. However, larger studies will be needed in order to obtain a true estimate of the size of such an effect (Green *et al*. 2008).

Another point to consider is that the MAOA-low group consisted almost entirely of MAOA heterozygotes. Although it has been suggested that MAOA escapes X-chromosomal inactivation, it is possible that random X-inactivation of MAOA alleles in females (Nordquist & Oreland 2007) increases individual variance in the heterozygote group and thus decreases sensitivity to differences in our comparison of genotypes. This is because of a random and relatively variable ratio in the expression of one chromatid of the X-chromosome, and thus one of the MAOA alleles vs. the other, across the brains of heterozygotes. However, previous studies have shown that BOLD response in heterozygous women is intermediate to that seen in high and low hemi- and homozygotes (Meyer-Lindenberg *et al*. 2006; Eisenberger *et al*. 2007b) in the socioaffective regions of interest studied.

Future studies could extend the findings to males, and to female MAOA-low homozygotes. It would also be interesting to conduct a longitudinal study to explore the trajectory of changes in the effects of specific polymorphisms known to influence socioaffective cognition across development. It might also be fruitful to investigate whether MAOA genotype alters the risk for adolescent-onset affective disorders. The specificity of the effect for social threat stimuli (i.e. rejection) relative to general threat could also be explored. Individual differences in exposure to social rejection may also modulate the influence of MAOA polymorphism on neural responses to rejection-related stimuli.

Conclusions

The current data suggest functional change between adolescence and adulthood in the neural processing of rejection-related information in females. Right VLPFC did not show the typical 'adult' pattern of response in the adolescent group to rejection stimuli, supporting previous studies showing late functional maturation of this region. Although predicted effects of MAOA genotype were seen in the amygdala in the adult group, the reverse pattern was seen in the adolescent group. This study provides preliminary evidence that continued adolescent development of socioaffective brain circuitry may interact with the effects of MAOA polymorphism, and that effects of MAOA polymorphism on emotional brain circuitry may not be fixed by mid-adolescence.

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