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The Impact of Puberty and Social Anxiety on Amygdala Activation to Faces in Adolescence

Jamie Ferri Jennifer N. Bress Nicholas R. Eaton Greg Hajcak Proudfit

Stony Brook University, Stony Brook, N.Y., USA

Key Words
Puberty · Social anxiety · Adolescence · Faces · Amygdala

Abstract
Adolescence is associated with the onset of puberty, shifts in social and emotional behavior and an increased vulnerability to social anxiety disorder. These transitions coincide with changes in amygdala response to social and affective stimuli. Utilizing an emotional face-matching task, we examined amygdala response to peer-aged neutral and fearful faces in relation to puberty and social anxiety in a sample of 60 adolescent females between the ages of 8 and 15 years. We observed amygdala activation in response to both neutral and fearful faces compared to the control condition but did not observe differential amygdala activation between fearful and neutral faces. Right amygdala activity in response to neutral faces was negatively correlated with puberty and positively correlated with social anxiety, and these effects were statistically independent. Puberty and social anxiety did not relate to amygdala activation in response to fearful faces. These findings suggest that emotional differentiation between fearful and neutral faces may arise during later pubertal development and may result from decreasing sensitivity to neutral faces rather than increasing sensitivity to threatening faces. Furthermore, these findings highlight the importance of considering individual differences in social anxiety when examining the neural response to social stimuli in adolescents.

Introduction

The beginning of adolescence roughly corresponds to the onset of puberty, which initiates drastic changes in hormone levels and a cascade of physical changes in the body and the brain [1]. During adolescence, there are also dramatic shifts in motivation, social behavior and rates of psychopathology, particularly for girls [2–5]. One of the most notable social changes during adolescence is increasing independence from parental figures and corresponding reliance on close friendships and romantic relationships with peers; by the 7th grade peer and parent relationships become equally important to adolescents, and by the 10th grade peer relationships become primary [3, 6].

Both the value of social relationships and the facility for processing social stimuli increase during adolescence. For example, the ability to identify faces [7, 8] and to rec-

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Jamie Ferri, MA
Department of Psychology, Stony Brook University
Stony Brook, NY 11794-2500 (USA)
E-Mail jamie.ferri@stonybrook.edu

ognize and remember emotional expressions [9–12] continues to develop throughout adolescence. Adolescence may also be a period during which individuals are beginning to extract new information from faces, such as attractiveness, trustworthiness, social status and dominance, especially from peers [13].

Increasing sensitivity to emotional and social cues coincides with changes in neural structures, particularly the amygdala [14, 15]. Indeed, the amygdala is one of the few regions of the brain that contains both estrogen and androgen receptors [16–18], indicating that its function may be directly influenced by hormonal changes during puberty. Recent studies have demonstrated that directly administered sex hormones can increase amygdala response to emotional faces [19]. Consistent with these data, pubertal development has been linked to increased amygdala reactivity to faces [20, 21]; in fact, some studies have reported greater amygdala activation to faces in adolescents compared to adults [22–24].

In adults, amygdala activation is typically enhanced for emotional compared to neutral faces [25]. However, numerous studies across childhood and adolescence have failed to find differentiation between emotional and neutral faces [15, 26–28] and some studies have even reported greater activation to neutral compared to fearful faces [29–31].

Although it seems clear that pubertal development is associated with changes in amygdala activation to faces, it is unclear whether pubertal development impacts the increased response of the amygdala to emotional compared to neutral facial expressions. Existing studies on this topic span a wide range of ages (7–17 years), and lack of emotional differentiation in amygdala response, or an increase in response to neutral faces, is more common in studies on younger [28, 30] compared to older adolescents [22, 23]. One possibility is that only adolescents advanced in pubertal development demonstrate adult-like amygdala differentiation between emotional and neutral faces – an effect that could be due to either increasing or decreasing response to threatening or neutral faces, respectively.

However, there are two additional factors that may impact the relationship between pubertal development and amygdala reactivity to emotional faces. First, existing studies have primarily probed amygdala activation using pictures of adult faces. Initial evidence suggests that children may process peer and adult faces differently; in a recent study with adolescents, greater amygdala activation was observed in response to neutral adult compared to peer faces, and preferential amygdala activation was observed in response to happy peer faces and angry adult faces [32]. Thus, failure to find differential amygdala reactivity to emotional compared to neutral faces may reflect, to some degree, the stimuli used. If social focus shifts from parents to peers over the course of adolescence [3, 33], emotional expressions of peers may become more relevant with pubertal development. The neural response to peer stimuli, particularly in regions like the amygdala that are influenced by sex hormones, may better index relevant changes in social and pubertal maturation.

Second, it is also important to consider the potential impact of trait-like individual differences that covary with pubertal development – such traits might impact the relationship between puberty and amygdala activity to emotional faces [34]. Specifically, symptoms of social anxiety increase during adolescence for girls [2, 35], and anxiety in general has been shown to impact amygdala response to facial stimuli [29, 34, 36–40]. Social phobia, in particular, is associated with enhanced amygdala activation to emotional faces [34] and, even among healthy adolescents, social anxiety symptoms positively correlate with amygdala activation to emotional faces [40]. Thus, it is possible that pubertal effects on amygdala response to faces could reflect developmental increases in social anxiety. However, no studies have simultaneously assessed the impact of pubertal development and social anxiety symptoms on amygdala response to faces.

In summary, adolescence is a period characterized by increased amygdala reactivity to facial stimuli – an increase that may relate to pubertal development and the increased salience of social signals. Whereas adults consistently show differential amygdala activation to fearful compared to neutral faces, findings in adolescents are mixed. Variability across studies could be potentially due to small sample sizes, failure to account for pubertal stage or confounding factors such as gender or anxiety symptomatology; moreover, the majority of these studies have not utilized age-appropriate facial stimuli.

Accordingly, the goal of this study was to employ adolescent facial stimuli to examine the effect of puberty on amygdala activation to neutral and fearful faces in a relatively large sample (n = 60) of females between the ages of 8 and 15 years, when pubertal changes initially begin to arise. Age and puberty are often highly correlated; however, neurodevelopment, particularly in sex hormone-rich areas of the brain like the amygdala, may be more closely tied to measures of puberty than to age, and some studies have even found age and puberty to have dissociable effects [41]. In addition, we examined relationships between amygdala activation and social anxi-

ety; because the effects of social anxiety may interact with puberty and confound the amygdala response to faces, we examined the independence of these effects.

Methods

Participants

A total of 75 girls between the ages of 8 and 15 years participated in this study. Participants were part of the larger Impact of Puberty on Affect and Neural Development across Adolescence (iPANDA) study at Stony Brook University. Subjects were recruited using a commercial mailing list of families in the Stony Brook area with daughters in the targeted age range, through posted flyers in locations likely to be frequented by families with children including grocery stores, libraries, and medical offices, through an online advertisement on Craigslist and, finally, through references from other participating families. Brief phone screens were conducted with families that expressed interest, and eligible participants were invited to participate in the study.

Participants who did not complete all puberty measures (n = 5) or participants who had excessive motion during the fMRI portion of the study (n = 10) were excluded from analysis. The final sample for this study included 60 female participants (mean age = 12.49 years, SD = 1.89; see table 1 for demographic details).

Table 1. Demographic characteristics of the sample

	Mean	SD
Age, years	12.49	1.89
MASC social anxiety	8.78	5.79
PDS:P	2.55	0.85
PDS:SR	2.52	0.86
PBIP:P	3.05	1.37
PBIP:SR	3.34	1.22

Table 2. Correlations among puberty measures

	PDS:SR	PDS:P	PBIP:SR	PBIP:P
PDS:SR		0.900***	0.865***	0.855***
PDS:P			0.814***	0.914***
PBIP:SR				0.824***

Correlations represent Pearson's r values. *** $p < 0.001$.

Pubertal Assessments

Pubertal Development Scale

The Pubertal Development Scale (PDS) [42] is a questionnaire version of a scale originally designed to be administered to children and adolescents in interview format [43]. In the version administered to girls, development is assessed across five physical domains: growth spurt, body hair, changes in skin, breast development, and menstruation. All items except for menstruation are rated on a scale from 1 ('not yet started') to 4 ('seems complete'); the menstruation item is rated either 1 ('no') or 4 ('yes'). An overall puberty score is calculated as the mean of the five items.

Participants and their parents completed computer-based versions of the self-rated and parent versions of the PDS (PDS:SR and PDS:P, respectively). Child-parent agreement on mean PDS is relatively high in 5th- and 6th-grade girls (Spearman's r of 0.71 and 0.80, respectively), as is agreement on PDS pubertal stage score (Spearman's r of 0.70 and 0.82, respectively) [42]. Internal consistency of the PDS:SR (Cronbach's α of 0.67–0.70) and PDS:P (Cronbach's α of 0.68–0.78) is moderate to high [42]. In the current study, Cronbach's α was 0.84 for the PDS:SR and 0.88 for the PDS:P. Correlations between parent and child ratings of the PDS in the current study are reported in table 2.

Picture-Based Interview about Puberty

The Picture-Based Interview about Puberty (PBIP) [44] is a two-item measure assessing pubertal development on a scale from 1 to 5; ratings are anchored by pictures and accompanying verbal descriptions of each stage. The PBIP correlates highly with other measures of puberty, including the PDS (r of 0.72–0.81) and a physical examination (r of 0.75–0.88) [45]. For correlations between the PBIP and PDS in the current study, see table 2. The PBIP was designed to be administered by an interviewer. However, in order to increase consistency of administration and to decrease potential discomfort of participants, subjects in the current study were given a fully automated computer-based interview in which a recorded voice provided a verbal description of the developmental stages. This recording was timed to correspond to pictures of each stage that appeared on-screen. No interviewer was present in the room. As participants viewed the automated interview, they filled out a paper response-rating sheet on which they indicated their level of development on each of the items. The slideshow-based PBIP was administered in a private room to participants (PBIP:SR) and their parents (PBIP:P), separately.

Latent Puberty Factor Score

Participant and parent ratings on both puberty measures were highly correlated, with correlation coefficients ranging from 0.81 to 0.91; see table 2 for all correlations. In order to estimate common variance across measures of puberty, the pubertal measures (PDS:SR, PDS:P, PBIP:SR and PBIP:P) were modeled as observed indicators of a dimensional puberty latent variable in a confirmatory factor-analytic framework. The latent puberty factor scores for participants were estimated and used in subsequent analyses. This latent variable modeling was conducted using Mplus software with a robust maximum likelihood estimator to account for observed variables with nonnormal distributions [46]. A one-factor model was estimated, and examination of model-implied correlations suggested the presence of a reporter-based measurement effect – that is, the self-report measures correlate in part due to being self-report. As such, the residuals of the two self-report measures (PDS:SR and PBIP:SR) were allowed to correlate to model this source of covariance. Model identification precluded the inclusion of a second correlated residual for the two parent report measures.

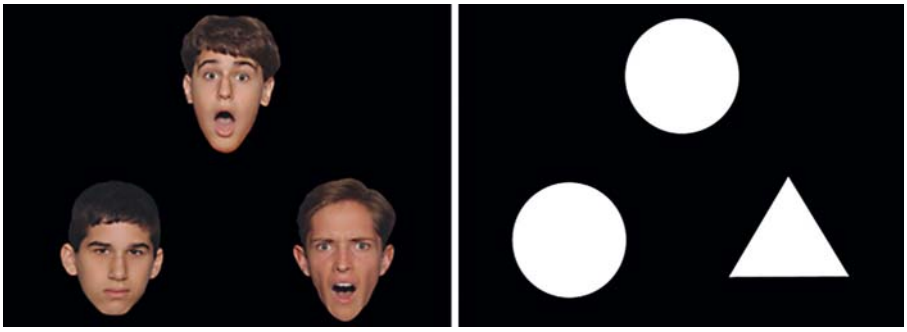

Fig. 1. A trial from the fearful face-matching condition (left) and the shape-matching condition (right). Each block consisted of 4 trials displayed for 5 s each; participants were instructed to select the face or shapes at the bottom that matched either the emotional expression or the shape that was displayed at the top.

This one-factor model of the four puberty indicators produced an excellent fit on the comparative fit index (0.98), surpassing the common threshold of >0.95 suggestive of a good fit [47]. Similarly, the Tucker Lewis Index (0.91) surpassed the common threshold of 0.90, suggesting an acceptable fit. The root mean square error of approximation (0.23) did not reach the common threshold of <0.06, however, suggesting some areas of misfit appearing to result from residual correlations between parent report measures. In further support of our model, all four indicators loaded significantly ($p < 0.001$) and with relatively high magnitude on the latent factor. Additionally, resultant factor scores were very highly determined (98.5%), indicating that factor score indeterminacy was not an analytic concern. The R^2 values for the four indicators were as follows: 0.846 for PDS:SR, 0.947 for PDS:P, 0.747 for PBIP:SR and 0.881 for PBIP:P, indicating that our single latent factor accounted for between 75 and 95% of the variance of each of our four indicators.

Assessment of Anxiety Symptomatology

The Multidimensional Anxiety Scale for Children

Participants completed a computer-based version of the Multidimensional Anxiety Scale for Children (MASC) [48]. The MASC is a 39-item self-report questionnaire assessing symptoms over the course of the week prior to assessment. Items cover a wide range of anxiety symptoms, which are divided into four subscales: physical symptoms (e.g. 'I get dizzy or faint feelings'), harm avoidance (e.g. 'I check to make sure things are safe'), social anxiety (e.g. 'I worry about other people laughing at me'), and separation anxiety (e.g. 'I try to stay near my mom or dad'). Responses are rated on a scale from 0 ('never true about me') to 3 ('often true about me') and are summed to create an overall anxiety score. The current analyses focus on the social anxiety subscale of the MASC.

The MASC has generally strong psychometric properties. Internal reliability in both clinical and nonclinical samples of children is high, with Cronbach's α ranging from 0.87 to 0.93 [49–53]. In the current sample, Cronbach's α was 0.86. In unselected children and adolescents, 3-week test-retest reliability is strong with a single-case intraclass correlation of 0.78 [48]; 1-year test-retest reliability is moderate ($r = 0.52$) [49]. In 8- to 16-year-olds diagnosed with anxiety disorders or attention deficit hyperactivity disorder, 3-week test-retest reliability is satisfactory (single-case intraclass correlation of 0.65), and 3-month test-retest reliability is excellent with a single-case intraclass correlation of 0.87 [53]. The MASC shows both convergent and divergent validity in clinical samples [49, 50, 52, 53]. The social anxiety subscale of the MASC is also psychometrically robust, with Cronbach's α of 0.82–0.90 [49, 52, 53] and moderate-to-strong test-retest reliability in clinical and nonclinical samples over a range of time delays [48, 49, 53]. In the current study, Cronbach's α was 0.85 for the social anxiety subscale.

Paradigm

Participants completed an emotional face-matching task adapted from Hariri et al. [54], using happy, fearful, sad, and neutral faces selected from the NIMH Child Emotional Faces Picture Set [55]. Selected facial stimuli were from subjects ranging in age from 10 to 16 years with a mean age of 13.42 years. All faces had a direct gaze, and an equal number of male and female faces were presented. Only neutral and fearful faces were analyzed for this study. Based on the ratings provided by Egger et al. [55] from 20 adult volunteers, there were no significant differences between fearful and neutral faces on inter-rater agreement concerning the expression of the face ($t = 0.338$, $p = 0.737$) or overall goodness of the stimuli ($t = 0.989$, $p = 0.328$), but fearful faces were rated as significantly more intense ($t = -2.055$, $p = 0.046$) and more representative of the expression ($t = 4.504$, $p = 0.001$) than neutral faces. Shape matching was used as a baseline control condition.

During each trial, a single target face or shape was presented at the top of the screen and two additional faces or shapes were presented at the bottom of the screen. Participants were instructed to select the face or shape on the bottom of the screen that matched either the emotional expression of the target face or the target shape that was displayed at the top. During emotional face-matching trials, the nonmatching facial expression was always neutral. During neutral face-matching trials, the nonmatching facial expression was fearful in 50% of trials and happy in the other 50%. Each trial type was presented a total of 16 times in four 20-second blocks. Each block consisted of 4 trials displayed for 5 s each. Blocks were counterbalanced and alternated between face- and shape-matching conditions. A schematic diagram of the design is presented in figure 1.

fMRI Data Acquisition and Analysis

Images were acquired on a whole-body 3-tesla Siemens Trio-Trim scanner (Siemens AG, Erlangen, Germany) with a 12-channel head coil. An echo planar imaging sequence was used to acquire 324 T2*-weighted whole-brain volumes for analysis of BOLD signal. Scanning parameters were as follows: TR = 2.1 s, TE = 23 ms, flip angle = 83°, and slices = 37 (3.5 mm interleaved slices parallel to the AC-PC).

Data analysis was performed using SPM8. Standard preprocessing procedures were applied, including slice time correction, realignment for motion correction, coregistration, and normalization to standard Montreal Neurological Institute space and spatial smoothing using an 8-mm FWHM gaussian kernel. Additional motion correction was applied using ArtRepair [56] for 16 participants with >2 mm (but <5 mm) of movement. For these participants, spikes in motion were corrected by interpolating volumes in excess of the motion cutoff; no more than 10% of scans were interpolated for any given participant. Data for those participants were then reprocessed using corrected volumes. Participants were excluded from analysis if they had >5 mm of motion or if they required interpolation on more than 10% of scans. Participant SPMs were created at the first level from a model that specified the onset of each face- and shape-matching condition. For participants whose data was motion-corrected with ArtRepair, first-level analyses were deweighted to account for interpolated volumes. Random-effects analyses were then conducted at the second level to test for statistical differences between fearful faces and shapes, neutral faces and shapes, and fearful and neutral faces, using contrasts created at the first level for each individual. A height threshold was set to 0.05 correcting for family-wise error (FWE) to correct for multiple comparisons.

The Automated Anatomical Labeling atlas [57] from WFU PickAtlas was used to create a mask for the right and left amygdala. Eigenvariates representing right and left amygdala activation were then extracted and imported in SPSS for analysis. In SPSS, Pearson's correlations and partial correlations were used to assess relationships between variables.

Procedures

Upon arrival at the laboratory, participants and their parents were introduced to a member of the research team who was trained in consenting procedures. In a private room, the study was explained to both the participant and the parent; written informed consent was then obtained from the parent, and written informed assent was obtained from the participant. Pubertal assessments and fMRI scans were conducted in the context of other questionnaires and tasks, which were randomized over the course of the visit.

Before participating in the fMRI scans, all participants underwent a 20- to 30-min session in a mock fMRI scanner to acclimate to the environment of the scanner and to become familiar with task procedures. The mock scanner is similar in size and appearance to the actual scanner and is equipped with speakers and a computer screen to simulate the noises and computer tasks used in the actual machine. In the mock scanner, participants were trained to reduce head motion using MoTrak software, which allowed them to view their head movement against a bull's-eye with a crosshair superimposed. Participants viewed a short cartoon, and playback paused whenever the tracking cursor moved out of the bull's-eye. Participants were then introduced to the emotional face-matching task and the other fMRI tasks and completed short practice versions of each.

Families were paid USD 20 per hour for their participation in the study, and participants were given an additional USD 20–29 in earnings from other tasks with monetary incentives; thus, in total, participants and their families were paid approximately USD 100–130. Participants were also offered a choice of small prizes such as candy and stickers for completing each study task. This study was formally approved by the Institutional Review Board of Stony Brook University.

Results

Correlations between Age, Puberty and Social Anxiety

There were no significant correlations between social anxiety scores on the MASC and age ($r = -0.014$, $p = 0.914$) or the latent puberty measures ($r = 0.061$, $p = 0.664$). Puberty and age were strongly correlated ($r = 0.830$, $p < 0.001$).

Neutral Face Matching Compared to Shape Matching

Whole-brain analysis for the effect of neutral face matching compared to shape matching revealed peak activation in the bilateral occipital gyrus [Brodmann's area (BA) 17], right fusiform gyrus (BA 37), right amygdala, bilateral middle prefrontal gyrus (BA 8/9/46), left medial prefrontal gyrus (BA 6), bilateral precentral gyrus (BA 6), right superior parietal lobe (BA 7), and right precuneus (BA 7). Table 3 and figure 2 (top) display peak activations associated with this contrast.

Fearful Face Matching Compared to Shape Matching

Whole-brain analysis for the effect of fearful face matching compared to shape matching revealed enhanced BOLD response to fearful faces in the bilateral occipital gyrus (BA 18), right fusiform gyrus (BA 37), bilateral precentral gyrus (BA 6/9), bilateral middle frontal gyrus (BA 9/46), left inferior frontal gyrus (BA 13), right thalamus, right amygdala, right superior parietal lobe (BA 7), and right precuneus (BA 7). Results from this contrast are presented in table 4 and figure 2 (bottom).

Fearful Face Matching Compared to Neutral Face Matching

Whole-brain analyses contrasting fearful face matching with neutral face matching showed increased BOLD responses to fearful faces in the bilateral occipital cortex (BA 17/18), right precuneus (BA 7) and thalamus. Peak and cluster activations are listed in table 5 and displayed in figure 3.

Amygdala Correlations

Table 6 and figure 4 present correlations between average amygdala activity and age, puberty and social anxiety scores on the MASC. Right amygdala activity during neutral face matching compared to shape matching was negatively correlated with age and puberty and positively correlated with social anxiety. Left amygdala activity during neutral face matching compared to shape matching was not significantly related to age, puberty or social anxiety. Neither right nor left amygdala activation during

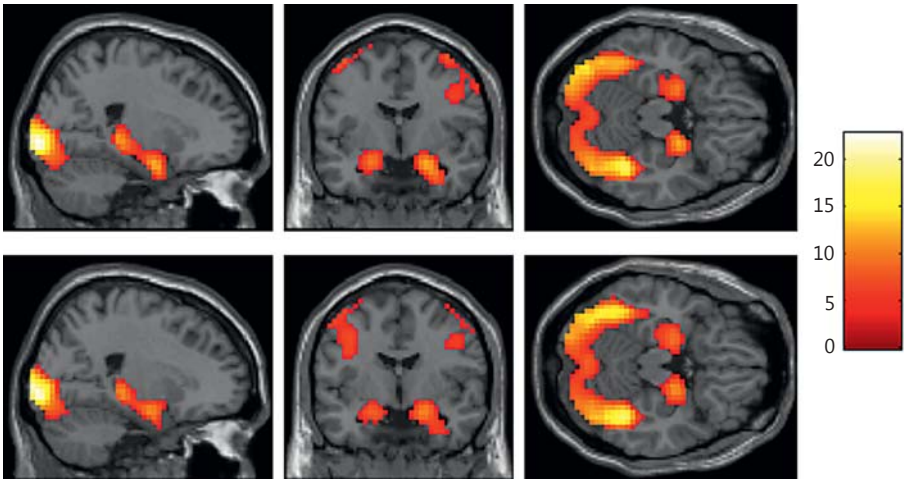

Fig. 2. Regions of the brain more active for neutral face matching compared to shape matching (top); regions of the brain more active for fearful face matching compared to shape matching (bottom).

Table 3. Peak activations for neutral face matching compared to shape matching

Cluster size	T	Z	Talairach coordinates				Region	BA
			x	y	z			
1,596	22.83	>8	19.13	−91.13	−5.83	R	inferior occipital gyrus	17
	21.67	>8	−17.84	−90.59	−10.01	L	inferior occipital gyrus	17
	17.9	>8	37.94	−45.12	−15.57	R	fusiform gyrus	37
383	12.91	>8	−21.38	−27.22	−4.07	L	lateral geniculate body	
	12.32	>8	23.35	0.37	−18.71	R	amygdala	
	11.81	>8	19.54	−4.03	−11.99	R	amygdala	
334	10.02	7.62	41.22	6.58	32.62	R	precentral gyrus	9
	7.99	6.55	52.24	9.55	40.3	R	middle frontal gyrus	8
	7.9	6.5	33.36	−11.42	63.22	R	precentral gyrus	6
182	8.91	7.07	−36.52	3.26	30.99	L	precentral gyrus	6
	7.89	6.5	−40.09	18.88	25.21	L	middle frontal gyrus	46
	6.74	5.78	−51.29	18.24	32.16	L	middle frontal gyrus	9
46	7.86	6.47	−40.63	−10.69	58.43	L	precentral gyrus	6
	6.46	5.59	−33.34	−18.88	64.98	L	precentral gyrus	6
	6.3	5.49	−29.62	−11.45	65.75	L	precentral gyrus	6
20	7.14	6.04	26.06	−58.09	40.65	R	superior parietal lobule	7
14	5.87	5.19	3.99	−60.65	29.23	R	precuneus	7
15	5.65	5.03	−3.54	0.64	56.53	L	medial frontal gyrus	6

All cluster and peak activations are significant at $p < 0.05$, FWE corrected. R = Right; L = left.

fearful face matching compared to shape matching related to age, puberty or social anxiety scores.

Partial correlations were then conducted to examine the relationship between right amygdala activation, age and puberty while controlling for social anxiety. The negative correlations between right amygdala activation during neutral face matching compared to shape matching and age ($r = -0.0304$, $p = 0.019$) and puberty ($r = -0.343$, $p = 0.008$) remained significant after controlling for social anxiety. The positive relationship between social anxiety

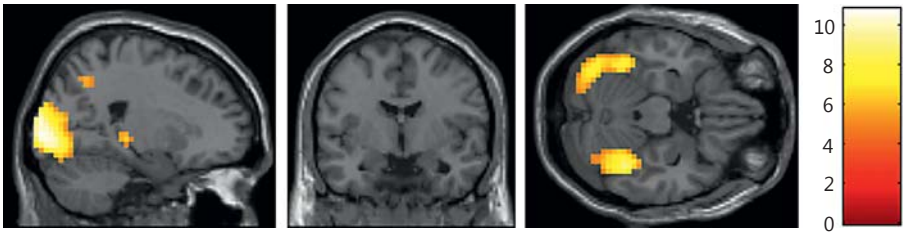

Fig. 3. Regions of the brain more active for fearful face matching compared to neutral face matching.

Table 4. Peak activations for fearful face matching compared to shape matching

Cluster size	T	Z	Talairach coordinates				Region	BA
			x	y	z			
1,782	23.52	>8	−21.54	−90.57	−10.07	L	fusiform gyrus	18
	22.93	>8	22.88	−90.8	−9.34	R	inferior occipital gyrus	18
	19.6	>8	37.94	−45.12	−15.57	R	fusiform gyrus	37
410	12.09	>8	41.22	6.58	32.62	R	precentral gyrus	9
	9.23	7.23	45.11	26.24	23.74	R	middle frontal gyrus	46
	8.94	7.08	48.43	1.42	46.67	R	precentral gyrus	6
431	10.81	>8	−51.35	14.16	35.38	L	middle frontal gyrus	9
	9.64	7.44	−36.53	−0.47	30.64	L	precentral gyrus	6
	7.26	6.11	−43.58	27.74	11.57	L	inferior frontal gyrus	13
409	10.72	>8	−21.38	−27.22	−4.07	L	lateral geniculate body	
	10.6	>8	19.29	−27.78	0.17	R	thalamus	
	10.59	>8	19.53	−7.76	−12.34	R	amygdala	
23	7.39	6.19	26.02	−58.44	44.22	R	superior parietal lobule	7
10	6.1	5.35	3.99	−60.65	29.23	R	precuneus	7

All cluster and peak activations are significant at $p < 0.05$, FWE corrected. R = Right; L = left.

Table 5. Peak activations for fearful face matching compared to neutral face matching conditions

Cluster size	T	Z	Talairach coordinates				Region	BA
			x	y	z			
1,788	10.84	>8	15.38	−91.46	−2.33	R	lingual gyrus	17
	10.12	7.67	−10.54	−95.05	−3.1	L	lingual gyrus	17
	9.7	7.47	26.62	−75.92	−7.87	R	lingual gyrus	18
53	7.09	6	22.35	−61.79	40.24	R	precuneus	7
	6.09	5.34	26.18	−68.22	28.89	R	precuneus	7
13	6.24	5.45	19.29	−27.78	0.17	R	thalamus	

All cluster and peak activations are significant at $p < 0.05$, FWE corrected. R = Right; L = left.

and right amygdala activation during neutral face matching compared to shape matching also remained significant when controlling for puberty ($r = 0.378$, $p = 0.003$) or age ($r = 0.360$, $p = 0.005$).

Discussion

This study used a recently developed set of adolescent facial stimuli to examine changes in the neural response

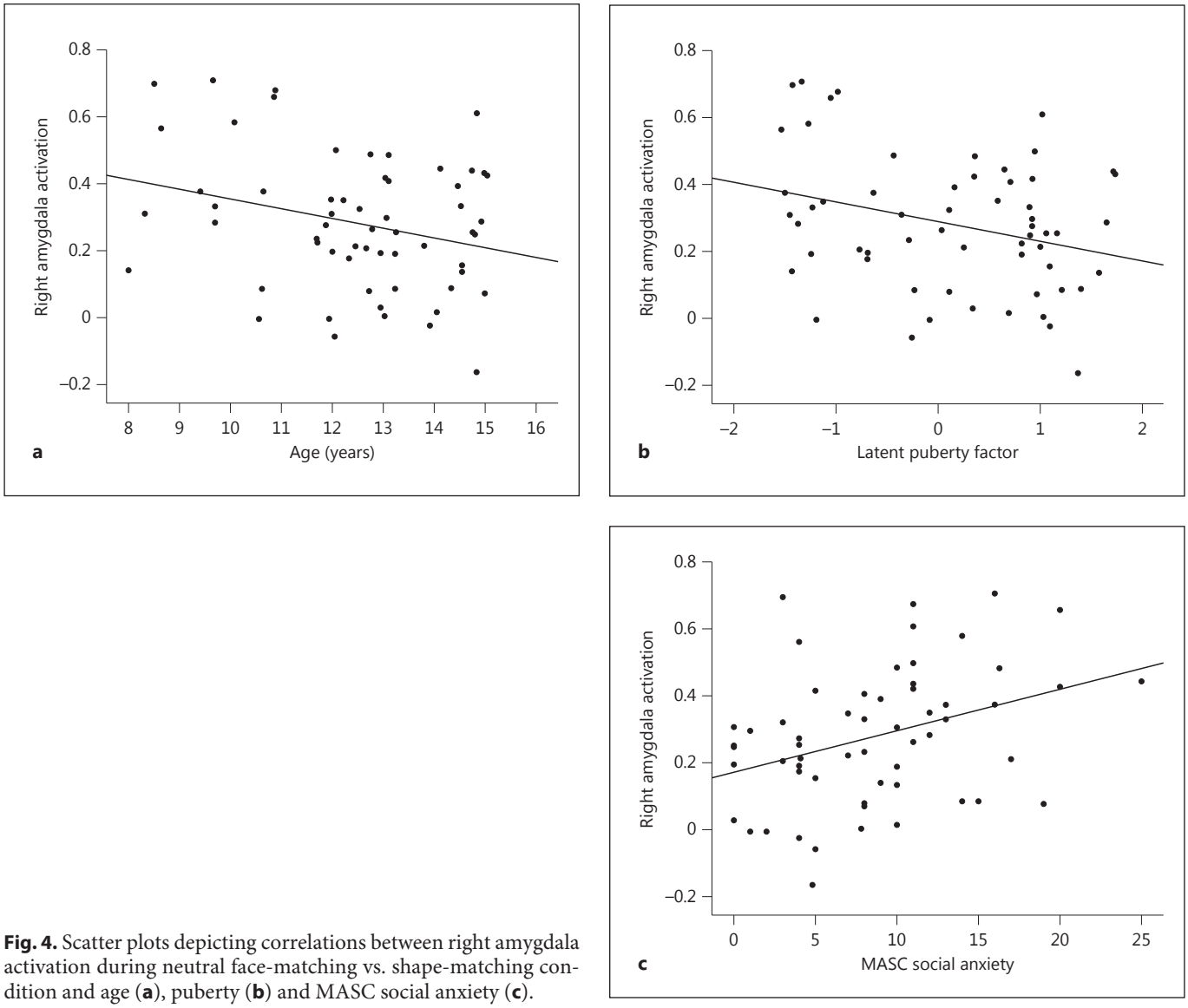

Fig. 4. Scatter plots depicting correlations between right amygdala activation during neutral face-matching vs. shape-matching condition and age (**a**), puberty (**b**) and MASC social anxiety (**c**).

Table 6. Correlations between average amygdala activation (neutral face matching vs. shape matching and fearful face matching vs. shape matching) and age, puberty and MASC social anxiety

	Neutral vs. shape		Fear vs. shape	
	left amygdala	right amygdala	left amygdala	right amygdala
Age	–0.109	–0.280*	–0.088	–0.217
Puberty	–0.027	–0.301**	0.063	–0.048
MASC social anxiety	0.135	0.342***	–0.115	–0.046

Correlations represent Pearson's r values. Correlations with p values <0.025 remained significant after correcting for multiple comparisons. * $p < 0.05$, ** $p < 0.025$, *** $p < 0.01$.

to neutral and fearful faces in relation to puberty and social anxiety symptoms across adolescence. Neutral and fearful faces both elicited increased amygdala activation compared to shapes. Right amygdala activation to neutral, but not fearful, faces was negatively related to both age and puberty and positively related to social anxiety. Thus, greater amygdala activation to neutral faces was characteristic of less pubertally developed and more socially anxious adolescent girls. The negative relationship between puberty and amygdala activation to neutral faces compared to shapes remained significant even when controlling for social anxiety – increasing slightly in magnitude. When controlling for puberty, the positive relationship between amygdala activation to neutral faces compared to shapes and social anxiety also remained significant. Thus, puberty and social anxiety had independent and opposing effects on amygdala response to neutral faces.

We did not, however, observe differential amygdala activity when comparing fearful to neutral faces. This replicates findings from studies using adult face sets with participants in a similar pubertal range [15, 28] and extends these findings by demonstrating that a lack of differentiation is not specific to adult faces. The current results suggest that amygdala differentiation between fearful and neutral faces may only emerge during the latest stages of pubertal development. Furthermore, in the current study, a puberty-related decline in amygdala activation was specific to neutral faces, suggesting that later-developing emotional differentiation may result from decreasing sensitivity to neutral faces rather than increasing sensitivity to threatening faces.

This puberty-related decline in amygdala response to neutral faces is consistent with a recent study demonstrating that participants in mid-to-late puberty have reduced amygdala activation to neutral faces compared to those in early puberty [15]. Amygdala activation is thought to reflect salience [58], indicating that reduced amygdala activation to neutral faces across puberty may reflect changes in the relative meaning of neutral social cues. A decline in amygdala reactivity to neutral stimuli, therefore, might reflect the increasing ability of adolescents to extract salient emotional cues and discriminate emotionality – and neutral peer faces may become less ambiguous over the course of pubertal development. As children mature, and their ability to discriminate between emotional and neutral social stimuli increases, they may be better able to categorize neutral faces as nonthreatening or irrelevant stimuli. This ability may stem from an increased focus on interactions with peers, which provide opportunities to practice the evaluation and interpretation of ambiguous facial expressions. This reduced reactivity to neutral facial expressions, then, may facilitate social exploration in unfamiliar or ambiguous social situations and allow for the formation of new peer relationships.

Though we only focused on female participants, and therefore cannot generalize these findings to males, studies with mixed-gender samples in both childhood and mid-to-late adolescence have also observed reductions in amygdala response to neutral faces as a function of age and/or puberty or a lack of amygdala differentiation between emotional and neutral faces [15, 28]. Thus, the current results are broadly consistent with studies that include both male and female participants.

Whereas amygdala response to neutral faces was negatively associated with puberty, it was positively related to social anxiety symptoms. A limited number of studies that have specifically examined social phobia in adolescence using adult face sets have found increased activation in response to emotional faces [34, 40]. The current findings are more consistent with studies in adult social anxiety, which report increased amygdala activation to neutral faces [59]. Neutral peer faces may be particularly ambiguous or threatening for more socially anxious adolescents [60]. While social anxiety symptoms and puberty were not significantly correlated in this sample [2, 61], controlling for social anxiety strengthened the negative relationship between puberty and amygdala activation to neutral faces, suggesting that relationships between the neural response to social stimuli and puberty may be masked to some degree by the opposing impact of social anxiety symptoms.

Although the current study focused on activation of the amygdala, it is likely that changes in the affective processing of social stimuli are reflected in broader networks that involve the amygdala, particularly frontal and parietal networks implicated in emotion regulation and attentional control [62, 63]. It will be important for future research to examine whether activation within and across these networks relates to changes in social and affective processing over the course of puberty. Furthermore, it will be important to examine other individual difference factors such as emerging symptoms of depression, relative reliance on parental figures and peers as well as romantic relationships that may also impact neural activation to peer social stimuli. In addition, although the focus of the current study was on neutral and fearful faces, changes in the processing of other emotional faces, particularly happy faces, may also reflect important developmental changes. One advantage of the current study is the

use of peer-aged facial stimuli; however, this stimulus set is relatively new and future studies might further validate the classification of these facial expressions as well as valence and arousal ratings in an adolescent sample; ratings were not obtained from participants in the current study. Finally, as we did not track eye movements during this task, we cannot rule out the possibility that the results of the current study are partially due to puberty- and anxiety-related changes in attention to the nonmatching emotional faces during neutral face-matching trials rather than the processing of neutral faces.

In conclusion, the current study suggests that reductions in amygdala reactivity to neutral peer faces may be a critical change that occurs over the course of pubertal development and may explain, in part, why amygdala differentiation between neutral and emotional faces is found more consistently in adult than adolescent and child populations. In addition, increased social anxiety symptoms predicted greater amygdala activation to neutral faces. Thus, pubertal development and social anxiety symptoms exert opposing effects on amygdala activation to neutral social stimuli. These results highlight the importance of assessing both pubertal development and social anxiety when evaluating amygdala response to social stimuli during adolescence.

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